MONOAMINE OXIDASE INHIBITORS IN AMAZONIAN HALLUCINOGENIC PLANTS: ETHNOBOTANICAL, PHYTOCHEMICAL, AND PHARMACOLOGICAL INVESTIGATIONS

By

DENNIS JON MCKENNA

B. A., University of Colorado, 1973
M.Sc., University of Hawaii, 1979

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

(Department of Botany)

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

February 1984

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Botany

The University of British Columbia
1956 Main Mall
Vancouver, Canada
V6T 1Y3

Date 26 April, 1984

ABSTRACT

Ethnobotanical, phytochemical, and pharmacological investigations of two Amazonian hallucinogens are presented.

The extant literature on the botany, chemistry, and ethnopharmacology of the Malpighiaceous and Myristicaceous hallucinogens is reviewed. The hallucinogenic beverage ayahuasca is prepared from the woody liana Banisteriopsis caapi (Malpighiaceae) and various admixture plants which strengthen or modify the effect. The genus Virola (Myristicaceae) is the source of the other hallucinogens investigated; the cambial resin of certain Virola spp. is made into hallucinogenic snuffs by some Amazonian tribes, while others prepare an orally-ingested drug from the resin. Although derived from entirely different botanical sources, both ayahuasca and the Virola drugs owe their hallucinogenic activity to indole alkaloids, viz., tryptamines and β-carbolines. The major tryptamines found in these preparations are N,N-dimethyltryptamine and/or 5-methoxy-N,N-dimethyltryptamine; both are potent hallucinogens but are inactive when ingested orally, presumably due to oxidative deamination by visceral monoamine oxidase (MAO). The β-carboline alkaloids, although having limited activity as hallucinogens, are potent reversible inhibitors of MAO; thus they may protect the tryptamines from visceral MAO and render them orally active. This mechanism may underly the oral activity of ayahuasca and the orally-ingested Myristicaceous drugs. Ayahuasca is an integral part of mestizo folk medicine among the lower socio-economic classes living in semi-urban Amazonian centers, but the utilization of Virola spp. as the source of hallucinogenic

preparations is confined to a few indigenous Amazonian tribes, and even among some of these groups is a rapidly disappearing practice.

The pharmacology and biochemistry of tryptamine and β-carboline derivatives is reviewed (Chapter II). Their biosynthesis, distribution, structure/activity relationships, interactions with MAO inhibitors, endogenous synthesis and degradative metabolism in mammals, hallucinogenic properties, and other biological activities are reviewed.

Results of ethnographic and ethnobotanical fieldwork in the Amazon Basin are presented (Chapter III). Herbarium voucher collections are documented (Appendix II), and the methods used in the collection of drug samples and plant materials for chemical analysis are described. Ethnographic observations on the use of ayahuasca and the orally-ingested Virola pastes are presented, and the methods used in their preparation are documented. The folk-medical use of ayahuasca by an ayahuasquero living near Pucallpa, Peru, is described. The procedures followed by Bora and Witoto informants in the preparation of the orally-ingested Virola pastes are also described. Observations on the biological activity of ayahuasca and the Virola pastes in self-experiments are included. The contemporary use of ayahuasca in mestizo folk medicine is compared to the use of the oral Myristicaceous drugs, which has remained ethnologically restricted to the Bora and Witoto tribes. The possibility is raised that the somewhat unreliable pharmacological activity of the Myristicaceous drugs, a reflection of their chemical variability, may have contributed to a decline in the use of the

pastes.

The alkaloids of a number of ayahuasca brews, cultivars of B. caapi, and admixture plants were qualitatively and qualitatively analyzed using thin-layer chromatography (TLC), high-pressure liquid chromatography (HPLC) and gas chromatography/mass spectrometroy (GC/MS) The ayahuasca samples contained insufficient levels of β-carbolines to account for their hallucinogenic properties at the doses typically used; however in most samples the concentration of DMT was well above the threshold level, assuming that it is orally activated by the blockade of visceral MAO. Different batches of ayahuasca had similar alkaloid compositions, however the concentrations of total alkaloids and the proportions of individual constituents varied considerably in batches of ayahuasca prepared by different ayahuasqueros in various parts of Peru. Different batches prepared by the same practitioner were remarkably consistent both in total alkaloid concentration and in the proportions of constituents. Considerable variation in alkaloid concentration in several recognized cultivars of B. caapi were found but may be due to environmental factors rather than genetic differences. Substantial concentrations of DMT were found in all Psychotria viridis samples analyzed, and in one collection of Diplopterys cabrerana but DMT was not detected in Psychotria carthagenensis. DMT was the single major base detected in these admixtures; only traces of other alkaloids were detected. Several uncommon admixture plants were screened for alkaloids, but only one, Abuta grandifolia, (Menispermaceae) gave an unambiguously positive reaction.

Alkaloids in twenty-eight Myristicaceous bark and leaf samples were qualitatively and quantitatively determined using TLC, GC, precipitation tests, and GC/MS. Sixteen of the twenty-eight samples contained detectable alkaloids. DMT and 5-MeO-DMT were the major bases, with much smaller amounts of NMT and/or tryptamine also present in most samples. Detectable levels of β-carbolines were not found in the bark and leaf samples. Fourteen of the eighteen Virola samples contained alkaloids; none of the six Iryanthera species contained detectable alkaloids. An indolic base, identified as N-methyl-tryptophan methyl ester, was found in Osteophloem platyspermum. Seven samples of orally-ingested drugs made from Virola spp. were analyzed. All but one contained substantial amounts of tryptamines, but the types and proportions varied greatly between samples. Samples of Virola snuff including various admixtures were analyzed and all but one contained tryptamines. Drug samples with the highest concentrations of alkaloids contained 15-20 mg/g d wt; the bark and leaf samples had concentrations ranging from 0.04 to 0.25 mg/g d wt. Only two Virola paste samples contained detectable levels of β-carbolines, which were identified as MTHβC and DMTHβC. The β-carbolines were trace constituents.

Methods were devised for the in vitro assay of rat-liver monoamine oxidase (MAO) using ^{14}-C-serotonin as substrate. Structure/activity relationships of various tryptamine and β-carboline derivatives as MAOI were determined. The MAOI activity of β-carboline derivatives was several orders of magnitude greater than the activity measured with tryptamine derivatives. DMT was the most active MAOI of the tryptamines tested while

harmine was the most active of the β-carbolines. Multi-component mixtures of β-carbolines were not significantly more effective than the single most active component, indicating an additive rather than a synergistic mechanism of action. Samples of ayahuasca were highly active as MAOI even when diluted by several orders of magnitude. The activity was comparable to mixtures of β-carbolines having similar concentrations and proportions. Samples of orally-ingested Virola pastes were less effective than ayahuasca as MAOI. The inhibition which they elicited was closely matched by mixtures of tryptamine standards having comparable proportions and concentrations. An alkaloid-free paste sample and a crude lignan fraction from V. elongata elicited only a slight degree of non-specific inhibition at the highest concentrations. These observations indicate that the limited MAOI activity of the pastes is due primarily to the tryptamines; the traces of β-carbolines or non-nitrogenous inhibitors present probably do not contribute significantly to the total inhibition. These results suggest that the inhibition of peripheral MAO by β-carbolines may explain the oral activity of ayahuasca, but it is unlikely that this mechanism can account for the oral activity of the Myristicaceous pastes. Some alternative mechanism must therefore be considered. One possible alternative is that Virola spp. may contain non-alkaloid constituents which are active as inhibitors of hepatic microsomal mixed-function oxidases (MFOs). Experimental evidence is reviewed which indicates that these enzymes, rather than hepatic MAO, may actually be more important in the peripheral metabolism and inactivation of orally-administered DMT and

related compounds.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

An interdisciplinary research project of the sort reported here owes its successful completion to the co-operation, encouragement, and assistance of numerous individuals. The fieldwork formed an indispensable prelude to the laboratory investigations; it was carried out with the permission of the Peruvian government and but for their co-operation could not have been completed at all. The fact that this research has been accomplished is testament to that spirit of international goodwill that is one of the most exemplary characteristics of the scientific endeavor in free societies. Accordingly, I am indebted to many people, in Canada, the United States, and Peru, for helping to make this work possible. Only some can be mentioned by name but to all I give my deepest thanks.

In Lima we were assisted by Dr. Ramon Ferreyra, Director of the Museo Historia Natural "Javier Prado". Use of the herbarium facilities of the Museo and Dr. Ferreyra's help in obtaining collection permits are greatly appreciated. Srs. Elias Mucha, formerly of the Faculty of Forestry at UBC, and Jose Purisaca of the Ministerio de Agricultura were also very helpful in guiding two inexperienced gringos through the maze of Peruvian bureaucracy, and we thank them for their patience. In Pucallpa we were received hospitably and helped in numerous ways by the members of the Instituto Linguistico de Verano, particularly Ted Long and his family, Dick Rutter, and Wes Theison. We were filled with good food, good wine, and good stories on several occasions by Conner and Mary Nixon, owners of La Brisa at Yarina Cocha. Nicole Maxwell became a valued informant, an enjoyable traveling companion, and an excellent friend; she was

instrumental in helping us to accomplish many of our goals, and no thanks can possibly repay her adequately. On my second stay in Pucallpa, I enjoyed the hospitality of Sr. Francisco Montes Shuna and his brother Pablo Shuna and I thank them for their kindness.

In Iquitos we were greatly assisted by Dr. Franklin Ayala Flores, Dean of Qualificación and director of the Herbario Amazonense, Universidad Nacional Amazonia Peruana (UNAP). Dr. Ayala's extensive knowledge of the Rio Ampiyacu area made it possible to successfully pursue our fieldwork there. At Dr. Ayala's invitation, we made free use of the herbarium facilities at UNAP. The excellent taxonomic field assistance of Sr. Juan Ruiz and Sr. Jose Torres contributed greatly to our collecting efforts. We owe all of these men a sincere thanks; their generosity and expertise has contributed significantly to this work. No less a contribution was made by Sra. Adriana Loyaza, head of the Iquitos office of the Amazon Natural Drug Company; she was instrumental in helping us obtain phytosanitary permits for the export of live specimens, and allowed us to hold our live collections in the garden of the ANDC until they could be safely airlifted to Canada. Adriana also provided our introduction to Alfredo Moreno, one of our most valuable informants at Puco Urquillo. Adriana's many favors, quiet support, and good humor were all invaluable and we are very grateful for the time and effort she put in on our behalf. Other people in Iquitos who helped out in some way are Dr. Al Gentry, assistant curator of the Missouri Botanical Gardens; Mr. Wade Davis, of the Harvard Botanical Museum, who was instrumental to the successful completion of our fieldwork in the Rio Ampiyacu

area; and the crew of the RV Heraclitus, who provided partial logistical support for that portion of the expedition; the contribution of all of these individuals or groups, however great or small, is very much appreciated. Several employees of the Ministerio de Agricultura in Iquitos also assisted us with the export of live plants and herbarium vouchers; among them Ing. Carlos Salazar Marchand, and Ing. Emilio Villar Castro. We are grateful for their co-operation.

Many people in North America also assisted, advised, or helped us in some way. Among them are Dr. Timothy Plowman of the Chicago Field Museum; not only has Dr. Plowman encouraged and supported every aspect of this project, from its initial conception to its completion, he has also identified most of the herbarium voucher collections, a time-consuming and monumental task which is far beyond the capabilities of this researcher. His efforts in this respect and every other deserve the warmest acknowledgement. Other people at the Field Museum and elsewhere contributed to the voucher determinations, including Penny Matakaitis, Drs. W. R. Anderson and Bronwen Gates of the University of Michigan, Al Gentry at Missouri, and Dr. W. A. Rodrigues of the Instituto Pesquisas do Amazonense(INPA) in Manaus. We thank them all for taking time away from more pressing and important matters to devote to these determinations. Dr. Bo Holmstedt of the Karolinska Institute, Prof. R. E. Schultes of the Harvard Botanical Museum, and Dr. Laurent Rivier of the Université de Lausanne, all provided support and encouragement for the project through correspondence, useful suggestions, and sage advice; we sincerely thank them for their informed insights and for the

example they set for aspiring ethnopharmacologists everywhere.

Completion of various technical phases of this work depended on the co-operation and goodwill of several people; among them Dr. C.-K. Wat and Felipe Balza, who provided excellent technical assistance in the GC/MS analyses of various samples; Dr. G. Bellward, who permitted the use of her radioisotope laboratory facilities for the development of the methods used in the MAO assays; and Malcolm Finlayson, who gave useful advice on the work-up of the rat-liver enzyme preparation. Their contributions to these aspects of the work are gratefully acknowledged.

I would also like to thank several other people or institutions who have contributed to the success of this project through some form of support, either moral, emotional, spiritual, or financial. High on this list is Don MacRae, the other half of the pronoun "we" used in these acknowlegements. He was a steadfast traveling companion in Peru and always kept his head level and his sense of humor engaged whatever the prevailing psychological weather; there were many times when it was reassuring to have someone like that around. He is a fine friend and an excellent colleague and I wish him luck in whatever he undertakes in the future, knowing he will handle it easily and well. Other people I wish to thank for their many tangible and intangible contributions to this project are Sheila Humphrey, Luis Luna, Terence McKenna, and Joe McKenna; each of you knows how much you have given to make it all worth it. I would like to express my appreciation to the Faculty of Graduate Studies at UBC for financial support in the form of UBC Graduate Fellowships; and to the Department of Botany for support in the

form of a Teaching Assistanceship. I would also like to thank the members of my graduate research committee who have contributed considerable time and effort to the review of this thesis. Finally, but certainly not least of all, I wish to thank my friend, who also happens to be my supervisor, Dr. Neil Towers. His enthusiasm for this project has been unstinting to the very end; and his support has gone far beyond the mundane financial variety (although he has furnished plenty of that, too). To each and all of these people, and also to anyone I have overlooked inadvertently who deserved to be mentioned, I give my warmest and sincerest thanks.

ABBREVIATIONS USED IN TEXT

TLC - thin-layer chromatography

GC - gas chromatography

GC/MS - gas chromatography/mass spectrometry

UV - ultra-violet

HPLC - high pressure liquid chromatography

SAR - structure/activity relationships

MAO - monoamine oxidase

MAOI - monoamine oxidase inhibitor

MFO - mixed function oxidase

CNS - central nervous system

MTHF - 5-methyltetrahydrofolate

SAM - S-adenosyl-methionine

INMT - indole-N-methyl transferase

TA - tryptamine

NMT - N-methyltryptamine

DMT - N,N-dimethyltryptamine

DMT-NO - N,N-dimethyltryptamine-N-oxide

5-MeO-TA - 5-methoxy-tryptamine

5-MeO-DMT -5-methoxy-N,N-dimethyltryptamine

5HT - 5-hydroxy-tryptamine (serotonin)

5HO-DMT - 5-hydroxy-N,N-dimethyltryptamine (bufotenine)

IAA - indole acetic acid

DET - N,N-diethyltryptamine

DPT - N,N-dipropyltryptamine

DIPT - N,N-diisopropyltryptamine

DAT - N,N-diallyltryptamine

DBT - N,N-dibutyltryptamine

THH - tetrahydroharmine

6-MeO-THH - 6-MeO-tetrahydroharman

6-MeO-MTHβC - 6-methoxy-2-methyl-tetrahydro-β-carboline

6-MeO-DMTHβC - 6-methoxy-1,2-dimethyl-tetrahydro-β-carboline

THβC - tetrahydro-β-carboline

MTHβC - 2-methyl-tetrahydro-β-carboline

DMTHβC - 1,2-dimethyl-tetrahydro-β-carboline

THH3C - tetrahydroharman-3-carboxylate

βCC - β-carboline-3-carboxylate

βCEE - β-carboline-3-carboxylate ethyl ester

This thesis is respectfully dedicated to
the indigenous folk healers of the Amazon,
whose knowledge of botanical medicine constitutes
a legacy of value for all of humanity.

PART I: INTRODUCTION AND LITERATURE REVIEW

CHAPTER I: THE MALPIGHIACEOUS AND MYRISTICACEOUS* HALLUCINOGENS OF SOUTH AMERICA

I. Introduction

During the last three decades our scientific knowledge of the botany and chemistry of plant hallucinogens has expanded enormously. This has been accomplished through the co-operative efforts of ethnobotanists, working in the field to identify and collect the source-plants utilized by aboriginal peoples, and phytochemists working in the laboratory, who have in many cases succeeded in isolating and characterizing the biodynamic constituents responsible for these properties [1,2]. The number of higher plant species is estimated at between 400,000 and 800,000; of these, only an insignificant number--somewhat fewer than 100--are known to be exploited as hallucinogens throughout the world, and less than twenty of these species may be described as major [2]. Most hallucinogenic plants owe their properties to alkaloidal constituents, and since at least 5000 species of higher plants contain alkaloids, there is a strong likelihood that many more hallucinogenic species remain undiscovered [2].

Scientific interest in the botanical and chemical sources

---

* Note: Usage of the terms 'Malpighiaceous' and 'Myristicaceous' in this thesis follows that established by Schultes [7,8]; used in reference to plant specimens or plant material they refer to members of the Malpighiaceae and Myristicaceae, respectively; used in reference to drug samples or hallucinogens, they refer to drugs derived primarily from source-plants in the Malpighiaceae or Myristicaceae.

of hallucinogens has partly followed upon, and partly resulted from, the increased "recreational" use of such compounds in our own society. While the use of hallucinogens, or "psychedelics," as they are sometimes called, is not as widespread a practice today as it was ten or twelve years ago [3], hallucinogens nevertheless continue to be used in contemporary society. This has given rise to the misconception that they are something relatively new. In fact, hallucinogens have been known and valued in human cultures at least since the late Paleolithic, and probably even earlier. Aboriginal peoples, forced to rely on nature for food, medicines, and palliatives of all sorts, have likewise actively sought and used psychoactive plants for various magical, religious, medicinal, or recreational purposes. Perhaps nowhere in the world has the knowledge and use of endemic hallucinogenic plants developed to the extent that it has in the upper Amazon Basin of South America. Of the hundred or so species of plants employed as hallucinogens throughout the world, fully 60% of them are native to the New World; this fact is significant in itself and has been repeatedly remarked upon in the ethnobotanical literature [4,5]. Even more curious is that of the ten or so New World hallucinogens that have major ethnobotanical significance, fully two-thirds have their geographic and ethnological centers of distribution in the Northwest Amazon--the notable exceptions being the peyote cactus (Lophophora williamsii) of the American Southwest and the Psilocybe mushrooms of Oaxaca, Mexico. This oddly asymmetrical distribution of the New World hallucinogens may be partly due to ecological factors and partly to ethnological ones. Ecological

factors may contribute in that few other places on earth can boast of the sheer species diversity which abounds in the Amazon Basin, hence it is not surprising that a typical cross-section of representative species has a greater proportion of plants containing psychoactive constituents. Ethnologic factors may play a role in that no other indigenous peoples in the world depend as totally on the vegetal environment as do those inhabiting the Amazon Basin, nor does any other group exploit the vegetal environment as thoroughly as do the Amazonian tribes. Given this juxtaposition of species diversity and abundance, and intense exploitation of the vegetal environment, we would expect to find that this knowledge of the plant kingdom would extend as well (if not especially) to the locally available psychoactive plants.

Largely because of interdisciplinary ethnobotanical, ethnological, and phytochemical investigations carried out within the last two decades, the botanical sources and most of the active constituents of the major hallucinogenic plants used by Northwest Amazon tribes have now been identified [6]. Within the lowland areas of the upper Amazon Basin proper, i.e., within the region bounded on the West by the Andes, on the north by the Rio Orinoco, on the south by the Rio Ucayali drainage, and on the east by the conjunction of the borders of Peru, Colombia, and Brazil, the indigenous complex of hallucinogenic plants is totally dominated by two genera: Banisteriopsis in the Malpighiaceae and Virola in the Myristicaceae. The former is the basis for the hallucinogenic brew known variously as ayahuasca, caapi, or yage, while certain Virola spp. are most well known

as the source of potently hallucinogenic snuffs whose center of utilization extends from the Puinave Indians of the Rio Apaporis region of the Colombian Vaupes, north to the Waika Indian groups of the Orinoco headwaters in southern Venezuela [7]. One might suppose that since the main source plants and chemical constituents of the primary Northwest Amazon hallucinogens have been identified, there remains very little to interest the botanist or phytochemist. This assumption is unwarranted, however; a careful study of the literature pertaining to these indigenous drugs reveals that many of the most interesting problems, from the point of view of the biochemist, pharmacologist, and toxicologist, have been all but ignored. Some of the more interesting of these problems are the subject of the investigations reported in this thesis.

II. Botany and Chemistry of Malpighiaceous and Myristicaceous Hallucinogens

A. Botany and Chemistry of the Malpighiaceous Hallucinogens

The woody liana *Banisteriopsis caapi* (Spruce ex Griseb.)Morton (Malpighiaceae) forms the basis of the hallucinogenic beverage commonly known as *ayahuasca* (Quechua for "vine of the souls") although in different regions of the Amazon it is known by other vernacular names, including *yage*, *caapi*, *natema*, and *pinde* [8]. Although *B. caapi* is usually the species used, *B. inebrians* Morton, *B. quitensis* (Ndz.) Morton, and *Tetrapterys methystica* Schultes have all been reported as sources of the drink [8]. On occasion *ayahuasca* is prepared from

the boiled bark or stems of one of these species without the addition of any other botanical ingredients; more commonly, however, the leaves or bark of various admixture plants are added into the brew in order to strengthen or modify the effect [9]. The leaves of one Malpighiaceous species have been reported [10] as an admixture, Diplopterys cabrerana (Cuatrecasas) Gates. Formerly known as Banisteriopsis rusbyana, the name of this species has been changed in a recent taxonomic revision [11, Plowman, T., pers. Comm., 1980]. Although sundry species from numerous families are utilized as admixtures to ayahuasca (cf. Appendix I, also [9,12,13,14,15]), the admixtures used most commonly are Diplopterys cabrerana and Psychotria viridis R.&P. and Psychotria carthagenensis Jacq. (Rubiaceae). Admixtures in the Solanaceae are also common, including tobacco (Nicotiana spp.), Brugmansia spp., and Brunfelsia spp.

The most detailed chemical study to date of ayahuasca and its botanical ingredients is that of Rivier and Lindgren [15]. Using GC/MS analysis, these investigators found that the major active constituents of ayahuasca are the β-carboline alkaloids harmine, harmaline, and tetrahydroharmine, and N,N-dimethyltryptamine (cf. Fig 1 & 2, Chapter II). The β-carbolines are constituents of B. caapi [16] while DMT has been isolated as a constituent of D. cabrerana [17] and has also been reported [15] in both Psychotria carthagenensis and P. viridis. Hashimoto et al. [18,19] reported the isolation of 6 β-carboline bases, harmic amide, acetyl norharmine, ketotetrahydronorharmine, harmine N-oxide, harmic acid methyl ester, and harmalinic acid) from the leaves of B. caapi in addition to the three main

constituents. These workers also reported the isolation of the pyrrolidine bases shihunine and dihydroshihuhine [20] from leaves of B. caapi. These compounds are trace constituents in the plant, however, and their extremely low concentrations (0.007 - 0.0001 %) make it unlikely that they contribute significantly to the pharmacological activity of ayahuasca.

B. Botany and Chemistry of the Myristicaceous Hallucinogens

The genus Virola (Myristicaceae) includes some 45 to 60 species of tropical trees, native to the forests of Central and South America, and forming an especially abundant component of the Amazonian flora. At present about six Virola spp. , viz., V. calophylla, V. calophylloidea, V. elongata, V. cuspidata, Virola theiodora, and possibly V. peruviana, are known to be utilized in the preparation of hallucinogens. Species concepts within the genus are poorly elucidated, and this has led to considerable confusion in the ethnobotanical and phytochemical literature. For instance, the most recent systematic revision of the genus [21] states that Virola theiodora and V. cuspidata are equivalent to V. elongata, while V. calophylloidea is equated with V. calophylla. Thus, depending on which species concepts are accepted, one may say that as many as six or as few as three Virola spp. have been implicated as hallucinogens!

The phytochemical basis for the use of Virola spp. as hallucinogens is well established, since the bark, sap, leaves, and roots of a number of Virola species have been shown to contain substantial amounts of hallucinogenic tryptamines such as N,N-dimethyltryptamine, 5-methoxy-N,N-dimethyltryptamine, and

other tryptamine derivatives, including N-methyltryptamine, 5-methoxy-N-methyltryptamine, 5-methoxy-tryptamine, and tryptamine (cf. Fig. 1). β-carboline alkaloids (Fig. 2) have also been isolated from some Virola spp. Agurell et al. [22] reported the isolation of 6-MeO-2-methyl-tetrahydro-β-carboline from Virola theiodora and V. rufula, while a similar compound, 6-MeO-1,2-dimethyl-THβC, was isolated from Anadenanthera peregrina L. (Leguminoseae) which also contains tryptamine derivatives and is the source of a hallucinogenic snuff in the Caribbean and Brazil [2]. In a later publication [23] these workers detected both compounds in V. peruviana and an indeterminate Virola species; the related compound 2-methyl-THβC was detected in V. calophylla, V. elongata, and Virola theiodora. In all instances the β-carbolines were trace components compared to the tryptamines; V. cuspidata is the only species so far investigated in which the major alkaloids are β-carbolines. Cassady and co-workers [24] isolated 6-MeO-harman, 6-MeO-harmalan, and 6-MeO-tetrahydroharman from the leaves and stems of this species, but no tryptamines were detected. This rather distinctive biochemical character indicates that V. cuspidata is probably distinct from V. elongata and the other Virola spp. which have been claimed to be equivalent to V. elongata by Rodrigues [21].

III. Ethnopharmacology of Malpighiaceous and Myristicaceous Hallucinogens

Although the most important source-plants and the presumed psychoactive constituents of the Malpighiaceous and

Myristicaceous hallucinogens have been identified, the ethnopharmacology of both groups of hallucinogens is still incompletely understood.

A. Source Plants and Methods of Preparation

The hallucinogen ayahuasca is prepared by boiling the bark or crushed stems of B. caapi together with one or more of the sundry admixture plants mentioned above or listed in Appendix I. The exact mode of preparation varies from region to region; in some areas the stems and admixtures are boiled for many hours, and the brew is concentrated over a low fire to a fraction of its original volume; in other regions, the brew is boiled for a relatively short time (1-2 hours) and is not concentrated, while in still other cases, a cold-water infusion is prepared. Obviously the method of preparation would be expected to affect the concentration, proportions, and perhaps the kinds of active alkaloids present in the mixture. β-carbolines can be readily formed from tryptamines by aldehyde condensation followed by cyclization (Pictet-Spengler reaction) [25], and thus might well be formed as artifacts during the prolonged boiling and concentration of the brew. In any case the preparation as finally ingested would contain a relatively high proportion of harmine, harmaline, and tetrahydroharmine, and possibly other β-carbolines, and a low to moderate amount of DMT, depending on how much admixture was added.

The use of Myristicaceous species for hallucinogens centers around the ingestion of preparations derived from the "resin" (sap) of the tree, even though the leaves, bark, or roots of the

tree may also contain the active alkaloids. The mode of ingestion of the resin appears to be divided geographically and ethnologically into two major categories. In the region centered around the Rio Orinoco-Rio Vaupes draingage of Colombia, Venezuela, and Brazil, the indigenous people prepare a snuff from the dried, powdered resin of various Virola species. These tribes include the Puinave, Kubeo, Tukano, and various linguistic subgroups of the widely dispersed Waika (Yanomamo) tribe. The snuff may be prepared from the Virola resin alone but more often is mixed with the ashes or powdered leaves of other plants [26, see below]. The second mode of utilization of Virola resin as an hallucinogen is by oral ingestion, either of the resin directly or of pellets prepared from the resin and rolled in the ashes of other plants, whose identity may vary. The practice of oral ingestion of Virola resin has come to the attention of ethnobotanists only recently and seems to be geographically and ethnologically separated from the use of Virola resin as a snuff. The oral use of Virola is found primarily among the Bora, Muinane and Witoto tribes inhabiting the Caraparana-Igaraparana locality of the Colombian Putamayo. Field-work carried out by Schultes and Swain [7,27,28] near the village of El Encanto on the Rio Caraparana indicated that the best "kind" of oo-koo'-na (as the drug is called in Witoto) was derived from Virola theiodora. Subsequent field-work was carried out by Schultes, Swain, and Plowman [29] in the Bora and Witoto settlements of Tierra Firma, Brillo Nuevo, and Puco Urquillo on the Rio Ampiyacu near the Peruvian settlement of Pebas. The best sources indicated by the Bora for the preparation of the oral

drug (known in Bora as ku'-ru-ku) was V. elongata; V. pavonis, V. loretensis, and V. surinamensis were also indicated. The Witoto informants at Puco Urquillo indicated that V. elongata, V. surinamensis, and species in the related genus Iryanthera, viz., I. macrophylla, I. ulei, and I. tessmannii, were all suitable for the preparation of the drug. The genus Iryanthera had not been implicated previously as an hallucinogen; subsequent analysis of I. ulei detected a trace (.00013 mg/g) of 5-MeO-DMT in the bark. Both the Myristicaceous snuffs and the orally ingested Myristicaceous pastes would be expected to contain substantial concentrations of DMT and/or 5-MeO-DMT, as well as other tryptamines; lesser concentrations of β-carbolines might also be present, either because they are originally present in the resin of some Virola species, or as a result of formation from tryptamines during the cooking process. The alkaloid constituents of some Virola snuffs have been characterized [30] but the composition of the orally-ingested pastes has not been previously investigated.

B. Postulated Mechanism of Action

Although the source-plants and admixture plants utilized in the preparation of the Malpighiaceous hallucinogens are different from those used for the Myristicaceous snuffs and pastes, similar active alkaloids are responsible for the pharmacological activity of both of these Amazonian hallucinogens. The ayahuasca brews contain β-carbolines as the major alkaloids with DMT derived from the admixture plants present in lesser concentrations; the Myristicaceous drugs, on

the other hand, contain hallucinogenic tryptamine derivatives as the primary active constituents, but β-carbolines may be present as minor components. Although DMT and 5-MeO-DMT are extremely potent psychotomimetics, active in the 5 to 100 mg range, a peculiarity of their pharmacology is that they are not orally active [31] apparently because they are deaminated by peripheral monoamine oxidase (MAO), the mitochondrially localized enzyme that is responsible for the oxidative deamination of biogenic amines. Therefore these tryptamines either must be administered parenterally in order to manifest their activity, or they can be rendered orally active if taken together with a MAO inhibitor. On the other hand, the β-carbolines, although having limited activity as hallucinogens (some derivatives are active in the 500-1000 mg range; cf. Chapter II), are extremely potent short-acting inhibitors of monoamine oxidase, effective in vitro at concentrations on the order of 10^{-6} - 10^{-8} M [32,33,34]. The significance of this to the ethnopharmacology of these drugs may thus be understood: although different species and diverse admixtures are involved in the preparation of the hallucinogenic Banisteriopsis brews, the Myristicaceous snuffs used north of the Rio Negro and the orally-ingested Myristicaceous hallucinogens used in Peru and Colombia, in each case, it is the presence of methylated, psychotomimetic tryptamines that is the sine qua non for the hallucinogenic properties of these native preparations. The pharmacological rationale for the ingestion of Virola resin in the form of a snuff is now clear; the active tryptamines are not orally active, so this parenteral route of administration neatly sidesteps their inactivation by peripheral

MAO. Presumably the tryptamines alone are sufficient to account for the activity of the snuffs. In the case of the Malpighiaceous ayahuasca brews and the orally ingested Myristicaceous pastes, the MAO-inhibiting β-carbolines (or some other mechanism) is required in order to orally activate the tryptamines. The oral activation of the tryptamines by the β-carbolines has been proposed [9,10,13,27,28,29] as the underlying mechanism, but this has not been experimentally confirmed. Several questions need to be investigated experimentally before this postulated mechanism can be accepted. It must be shown that: 1.) The tryptamines in question are not orally active by themselves; enough data on the human pharmacology of these drugs has been accumulated in recent years (cf. Chapter II and [31]) that this point is fairly well established. 2.) The methylated tryptamines can be rendered orally active by MAOIs, specifically β-carbolines; tryptamines have been shown to be potentiated in vivo by some MAOIs, but this has not been specifically demonstrated for β-carbolines (cf. Chapter II and [35]). 3.) Hallucinogenic tryptamines are present in these native drug preparations in sufficient concentrations that the ingestion of an average dose would exceed the known threshold dose for these compounds, e.g., if DMT is the active tryptamine then at least 15 mg and preferably 60-75 mg would have to be ingested for the drug to manifest its hallucinogenic effects. Under conditions of peripheral MAO inhibition, substantially smaller doses of DMT may elicit hallucinogenic effects, since a greater proportion could escape degradative metabolism under these conditions. 4.) β-carbolines,

or some other type of MAOI, are present in the native preparation in sufficient concentration to effectively inhibit MAO and thus orally potentiate the tryptamines. Experimental investigations of some of these questions were among the primary objectives of the work described in this thesis.

C. Admixture Plants

Another aspect of these native hallucinogens which deserves further investigation is the influence of admixture plants as determinants of their pharmacological activity. On occasion the drugs may be prepared without the addition of any admixtures, but customarily, admixtures are employed. Particularly in the case of ayahuasca it seems that the utilization of admixture plants has reached a rather high degree of botanical and pharmacological sophistication. Besides the tryptamine-containing admixtures which are almost always included in ayahuasca, there is a virtual pharmacopoeia of admixtures which are occasionally used, depending on the magical, ritual, or medical purposes for which the drug is being made and consumed [9,10,13,14,15]. Many of these ayahuasca admixtures have not been botanically identified, much less chemically characterized; however the botanical identity of some admixtures has been established, and in many cases they are species which contain biodynamic constituents of potential medical value. The contribution that the admixtures may make to the pharmacological activity of ayahuasca is, at this time, a complete mystery, and an area well deserving of further research by ethnopharmacologists and phytochemists. The phytochemical

information that is available on approximately 70 of the species known to be used as admixtures has been summarized in Appendix I.

The admixture plants used in the preparation of the Myristicaceous pastes and snuffs have not received a great deal of attention in the ethnopharmacological literature. The two admixtures often used in the preparation of Virola snuff are Elizabetha princeps (Shomb. ex Bentham) Hooker (Leguminosae) and Justicia pectoralis Jacq. var. stenophylla Leonard (Acanthaceae) [26]. Ashes made from the bark of E. princeps are often added to the snuffs, and frequently the powdered leaves of Justicia pectoralis var. stenophylla are added to "make the snuff smell better." [26]. There are also reports [36, Prance, G. T., pers. comm., 1983] that occasionally a snuff is prepared from J. pectoralis by itself. Whether this snuff is psychoactive or whether this plant contains biodynamic constituents is not known as no phytochemical data is available on this species.

The only use of admixtures in the manufacture of the orally-ingested Myristicaceous pastes that has been noted [27,28,29] is that ocassionally the pellets of Virola resin are rolled in the "salts" of certain plants, the salts being the leachings of ashes made from the bark or leaves of various species. Schultes and Swain [28] have described the process of preparing this "salt". Among the plants used as the source of the salt are Eschweilera itayensis Kunth (Lecythidaceae) and Gustavia poeppigiana Berg ex. Martius (Lecythidaceae), Theobroma subinacum Mart. (Sterculiaceae) and several palm species including Geonoma juruana Dammer. Since only the ashes of these

plants are used in the preparation of the Virola pellets it is very unlikely that any biodynamic constituents are derived from these admixtures. One possibility, which seems to have been overlooked in the literature, is that the ashes are added to make the mixture alkaline and thus convert the active alkaloids to the free base form. This may facilitate absorption and uptake of the alkaloids. Ashes made from leaves of Cecropia spp. (Moraceae) are similarly added to the coca powder which is widely used by the same groups that use the oral Virola preparations. It is considered common knowledge that ashes must be added to the coca in order to make it strong. The addition of ashes to the Myristicaceous pastes may be an extension of this general rule, and may actually influence their biological activity.

IV. Ethnographic Aspects of Malpighiaceous and Myristicaceous Hallucinogens

The focus of the research reported in this thesis is on the botanical, chemical, and pharmacological aspects of the Malpighiaceous and Myristicaceous hallucinogens; however, a full understanding of these plant drugs must also consider the cultural context of their use. The effects of hallucinogens, unlike most other pharmacological agents, are influenced to a large degree by the expectations of the user. These expectations, and the nature of the individual's drug experience, will, in turn, partially be determined by psychological and neurophysiological variables, and partially by the prevailing cultural myths and beliefs surrounding the drug

in question. This is true in our own culture as well as in "traditional" non-Western cultures. Unlike our own culture, in most traditional societies in which hallucinogens are used, that use takes place in a magico-religious context. Use of the hallucinogen is surrounded by ritual and ceremony, the set and setting of the drug experience is carefully chosen and manipulated by the shaman or medicine man, and there is usually a specific purpose for consuming the drug--for divination, for instance, or to discover the cause of an illness, or to communicate with the spirit-world. In traditional cultures the boundaries between religion, magic, and medicine are not clearly delineated; the function of the shaman or traditional healer lies somewhere between the Western concepts of priest, doctor, and psychotherapist; illness may be precipitated by physical, psychological, or supernatural causes, or a combination of these, and all are amenable to treatment by the methods available to the shaman. In this sense the "holistic" therapies which have characterized recent trends in modern medicine are not that different from the therapeutic methods practiced by the traditional healer. Both proceed from the recognition that mind and body are an integrated unit and the most effective therapies are those that are directed at improving both physical and mental health. Thus it is not surprising that plant hallucinogens, which profoundly affect both the mind and the body, as well as affording access to and a certain degree of manipulation of (real or imagined) magical or supernatural dimensions, should occupy such a prominent position in the traditional healer's armamentarium.

It is not within the scope of this thesis to review the voluminous ethnographic and anthropological literature related to the use of plant hallucinogens in traditional healing; references [37,38] provide a general overview of this subject. Reichel-Dolmatoff [39] has provided a fascinating and detailed account of the central position of ayahuasca and the Myristicaceous snuffs in the religion and cosmology of the Tukano Indians, while Chagnon [40] has described the use of Virola snuff among the Yanomamo. Virtually nothing is known of the medical, magical, or religious use of the orally ingested Myristicaceous pastes beyond the fragmentary information recorded by Schultes [27] and the equally fragmentary observations made during the present investigation. Both are discussed more fully in Chapter III. Other observations related to the methods of preparation, use, and subjective effects of ayahuasca and the orally ingested pastes are also to be found in Chapter III.

Ethnographic aspects of the use of ayahuasca are more accessible to the outside observer than the Myristicaceous pastes chiefly because ayahuasca is used among urban mestizo populations residing on the outskirts of large riverine industrial and trade centers such as Iquitos and Pucallpa. Hence many of the cultural and linguistic barriers which are encountered in the ethnographic study of the Myristicaceous pastes are not a factor in the study of ayahuasca. Most members of the mestizo population in these urban or semi-urban settlements speak a dialectical form of Spanish (though not necessarily as the first language). Face-to-face interviews can

thus be conducted with informants, and this is generally a more fruitful procedure than interviews conducted through a Spanish-speaking interpreter. A more open attitude toward the outside investigator is often found among mestizos who use ayahuasca and this also facilitates gathering ethnographic information. Contemporary use of ayahuasca in Amazonian mestizo populations appears to be an amalgam of diverse tribal traditions. The large urban settlements have become melting pots; people of many different cultural backgrounds have migrated to these centers in search of employment in the lumber, petroleum, and similar resource-based industries, and have brought with them their own tribal traditions and belief systems (usually syncretically fused with Christianity due to prior contact with missionaries). The cultural background of these migrant laborers often extends to a knowledge of the medicinal plants valued in their own culture. Over the years this drug-plant lore derived from diverse sources has gradually diffused through the larger mestizo society and become melded into a coherent system of traditional medicine. This tradition, though it incorporates elements of its diverse tribal origins, is at the same time unique to the mestizo social class. This process of cultural amalgamation has occurred over the same period of time that most of the tribal societies in which reside the antecedents of mestizo folk medicine have disintegrated and/or disappeared. Hence mestizo folk-medicine as it is practiced today in the urban centers of the Amazon is a living system of traditional medicine based on the ethnomedicine of many cultures; in many cases it is the only place where such knowledge has been

preserved. Hence it is important, even urgent, that mestizo folk-medicine and the plants that form its basis be studied by investigators with scientific backgrounds in medicine, pharmacology, phytochemistry, and botany while the opportunity still exists. The ayahuasca admixtures listed in Appendix I provide an example. Some of the species known to be used as admixtures to ayahuasca contain highly biodynamic and potentially medically useful constituents; how many others may have value that have so far escaped investigation is not known. The insistence, nearly universal among ayahuasqueros, that ayahuasca teaches medicine, i.e., that one learns about the properties of plants and how to use them by taking them in conjunction with ayahuasca [14], cannot be dismissed out of hand. Further insights into the use of ayahuasca in contemporary mestizo folk medicine may be found in [14,38,41,42,43].

V. Scope and Objectives of the Present Investigation

It may be seen from the preceding discussion that several aspects of the chemistry, pharmacology, botany, and ethnography of the Malpighiaceous and Myristicaceous hallucinogens remain poorly understood. The objectives of the research described in this thesis were to investigate some of these unresolved problems. An interdisciplinary approach was adopted, in which ethnographic and ethnobotanical field investigations were combined with laboratory investigations of the chemistry and pharmacology of the Malpighiaceous and Myristicaceous hallucinogens and the plants used in their manufacture. The scientific rationale for specific aspects of the investigation

is given in the appropriate chapters; the over-all objectives of the research are enumerated below.

A. Field Work

This had several complementary objectives:

1. Collection of ethnobotanical and ethnographic information.

This included information related to the source-plants used in the manufacture of the drugs, the methods of preparation, the methods of ingestion, the purposes for which the drugs are used, and the occasions when they are consumed, and the cultural context of their use. Native vernacular names of the plants and information on their folk taxonomy was also collected.

2. Collection of plants and drug samples.

Collections of Malpighiaceous and Myristicaceous drug preparations were made and, when possible, the source plants and admixtures used in their preparation were also collected. Plant collections included both herbarium voucher specimens and samples for phytochemical analysis. As many collections of Malpighiaceous and Myristicaceous species as possible were made, even though they were not necesarily used to make drug preparations or indicated as being so usable. Special efforts were made to collect any admixture plants indicated by informants. Any other plants which were indicated by informants to have medicinal applications were also collected even though they were not constituents of hallucinogenic preparations.

3. Assessment of the biological activity of orally-active Malpighiaceous and Myristicaceous hallucinogens.

Although there is considerable discussion of the mechanism of action of the orally-ingested Myristicaceous and Malpighiaceous hallucinogens in the ethnobotanical literature, reports of their effects on outside observers are rare or non-existent. Thus it is only assumed that these drugs are orally-active as hallucinogens; substantiating evidence is required from an outside observer who is not immersed in the cultural belief system surrounding these drugs. Thus one of the objectives of the field-work was to determine whether these preparations are, in fact, orally effective. This was particularly important in the case of the orally-ingested Myristicaceous pastes, since their physiological and psychological effects have not previously been experienced by a non-Indian.

B. Laboratory Investigations

These were designed to complement the field investigations and to resolve several questions which could be approached experimentally:

1. Quantitative investigations of the active constituents of the Malpighiaceous and Myristicaceous hallucinogens.

Although previous investigators have determined that the active constituents of these drugs are in all likelyhood

psychotomimetic tryptamines and β-carbolines, quantitative data on the levels of alkaloids in these preparations is relatively sparse. It is not known, for instance, whether the amount of DMT in a "typical" dose of ayahuasca or Virola snuff exceeds the threshold dose required to elicit the psychotomimetic effects. Similarly, although it has been proposed that the DMT in ayahuasca and the orally ingested Myristicaceous pastes is rendered orally active by the MAO-inhibiting β-carbolines, it has not been shown that β-carbolines are present in sufficient concentrations to effectively inhibit MAO. Therefore quantitative methods were devised to determine the kinds and relative proportions of tryptamines and β-carbolines in the drug samples and in the plant samples.

2. Comparison of the alkaloid constituents of the drug preparations with the alkaloid constituents of the source-plants.

Quantitative and qualitative methods were applied in order to determine to what extent the alkaloid composition of the drug samples differed from that of the source-plants. Admixture plants were also screened for alkaloids and in some cases quantitatively analyzed.

3. Investigation of the effect of drug samples and their alkaloid constituents on MAO in vitro.

The mechanism of action postulated for the oral activity of these drugs is that the β-carbolines protect the psychoactive tryptamines from deamination by peripheral monoamine oxidase,

thus permitting their uptake into the CNS. Although reasonable, this mechanism had not been experimentally demonstrated. Therefore methods were developed for assessing the activity of various drug preparations and their constituents for activity as MAO inhibitors. The MAOI activity of synthetic tryptamine and β-carboline derivatives was also assessed in an effort to establish structure/activity correlations. Mixtures of synthetic compounds in concentrations approximating those found in the drug preparations were also assayed in order to determine whether the compounds might act synergistically in combination. The MAOI activity observed with the mixtures of synthetic standards was compared with that observed with the crude drug preparations in order to determine whether the activity was partly due to non-alkaloidal constituents. In vivo investigations of the MAOI activity of the drug preparations using animals were originally planned but were not carried out due to the unacceptable length of time required to develop and perfect the necessary techniques.

VI. Literature Cited

1. Schultes, R. E. (1970) The Botanical and Chemical Distribution of Hallucinogens. Annual Review of Plant Physiology 21:571-98

2. Schultes, R. E. & A. Hofmann (1980) The Botany and Chemistry of Hallucinogens. 2nd Edition. Charles C. Thomas, Publishers, Springfield, Ill.

3. Grinspoon, L. & L. B. Bakalar. (1979) Psychedelic Drugs Reconsidered. Basic Books, Inc. New York.

4. Schultes, R. E. (1963) The Botanical Sources of the New World Narcotics. The Psychedelic Review 1:145-66

5. Schultes, R. E. (1970) The New World Indians and Their Hallucinogenic Plants. Morris Arboretum Bulletin 21:3-14

6. Efron, D. H., B. Holmstedt, & N. S. Kline (eds.) (1967) Ethnopharmacologic Search for Psychoactive Drugs. U. S. Public Health Service Publication # 1645

7. R. E. Schultes (1979) Evolution of the Identification of the Myristicaceous Hallucinogens of South America. Journal of Ethnopharmacology 1:211-239 1979

8. Schultes, R. E. (1957) The Identity of the Malipighiaceous Narcotics of South America. Harvard Botanical Museum Leaflets 18:1-56

9. Pinkley, H. V. (1969) Plant Admixtures to Ayahuasca, the South American Hallucinogenic Drink. Lloydia 32:305-14

10. Der Marderosian, A. H., H. V. Pinkley, & M. F. Dobbins IV. (1968) Native Use and Occurrence of N,N-dimethyltryptamine in the Leaves of Banisteriopsis rusbyana. American Journal of Pharmacy 140:137-147

11. Gates, B. (1979) New Names in Banisteriopsis and Diplopterys (Malpighiaceae) of the Guayana Highland. Brittonia 31:108-9

12. Schultes, R. E., (1979) New Data on the Malpighiaceous Narcotics of South America. Harvard Botanical Museum Leaflets 23:137-47

13. Schultes, R. E. (1972) Ethnotoxicological Significance of Additives to New World Hallucinogens. Plant Science Bulletin 18: 34-41

14. Luna, L. E. (1983) The Concept of Plants as Teachers Among Four Mestizo Shamans of Iquitos, Northeast Peru. Paper presented at the Symposium on Shamanism, XIth International Congress of Anthropological and Ethnological Sciences, Phase 2. Vancouver, B. C., August 20-23 1983.

15. Rivier, L. & J. Lindgren (1972) Ayahuasca, the South American Hallucinogenic Drink: Ethnobotanical and Chemical Investigations. Economic Botany 29:101-129

16. Deulofeu, V. (1967) Chemical Compounds Isolated from Banisteriopsis and Related Species. pp. 393-402 in D. H. Efron, B. Holmstedt, & N. S. Kline (eds.) Ethnopharmacologic Search for Psychoactive Drugs. U. S. Public Health Service Publication #1645

17. Agurell, S., B. Holmstedt, & J. E. Lindgren (1968) Alkaloid Content of Banisteriopsis rusbyana. American Journal of Pharmacy 140:148-151

18. Hashimoto, Y. & K. Kawanishi (1975) New Organic Bases from Amazonian Banisteriopsis caapi. Phytochemistry 14:1633-35

19. Hashimoto, Y. & K. Kawanishi (1976) New Alkaloids from Banisteriopsis caapi. Phytochemistry 15:1559-60

20. Kawanishi, Y., Y. Uhara & Y. Hashimoto (1982) Shihunine and Dihydroshihunine from Banisteriopsis caapi. Journal of Natural Products 45:637-8

21. W. A. Rodrigues. (1980) Revisao Taxonomica das Especies de Virola Aublet (Myristicaceae) do Brasil. Acta Amazonica 1:1-123

22. Agurell, S., B. Holmstedt, J. E. Lindgren, & R. E. Schultes. (1968) Identification of Two New β-carboline Alkaloids in South American Hallucinogenic Plants. Biochemical Pharmacology 17:2487-88

23. B. Holmstedt, J. E. Lindgren, T. Plowman, L. Rivier, R. E. Schultes & O. Tovar. (1980) Indole Alkaloids in Amazonian Myristicaceae: Field and Laboratory Research. Harvard Botanical Museum Leaflets 28:215-234

24. J. M. Cassady, G. E. Blair, R. F. Raffauf & V. E. Tyler. (1971) The Isolation of 6-methoxyharmalan and 6-methoxyharman from Virola cuspidata. Lloydia 34:161-162

25. Allen, J. R. F., & B. Holmstedt (1980) The Simple β-carboline Alkaloids. Phytochemistry 19:1573-1582

26. Schultes, R. E. & B. Holmstedt (1968) The Vegetal Ingredients of the Myristicaceous Snuffs of the Northwest Amazon. Rhodora 70:113-160

27. Schultes, R. E. (1969) Virola as an Orally-administered Hallucinogen. Harvard Botanical Museum Leaflets 22:229-40

28. Schultes, R. E. & T. Swain (1976) Further Notes on Virola as an Oral Hallucinogen. Journal of Psychedelic Drugs 8:317-324

29. Schultes, R. E., T. Swain, & T. Plowman (1977) Virola as an Oral Hallucinogen Among the Boras of Peru. Harvard Botanical Museum Leaflets 25:259-272

30. Agurell, S., B. Holmstedt, & J. E. Lindgren (1969) Alkaloids in Certain Species of Virola and Other South American Plants of Ethnopharmacologic Interest. Acta Chemica Scandinavica 23:903-916

31. Shulgin, A. T. (1976) Psychotomimetic Agents. ch. 4 in Maxwell Gordon (ed.) Psychopharmacological Agents Vol. IV. Academic Press

32. Udenfriend, S., B. Witkop, B. G. Redfield, & H. Weissbach (1958) Studies with Reversible Inhibitors of Monoamine Oxidase: Harmaline and Related Compounds. Biochemical Pharmacology 1:160-165

33. McIsaac, W. M. & V. Estevez (1966) Structure-action Relationships of β-carbolines as Mono-amine Oxidase Inhibitors. Biochemical Pharmacology 26:1625-27

34. Buckholtz, N. S. & W. O. Boggan (1977) Monoamine Oxidase Inhibition in Brain and Liver Produced by β-carbolines: Structure-activity Relationships and Substrate Specificity. Biochemical Pharmacology 26:1991-96

35. Squires, R. F. (1978) Monoamine Oxidase Inhibitors: Animal Pharmacology. in L. L. Iverson, S. D. Iverson, & S. H. Snyder (eds.) Handbook of Psychopharmacology Vol. 14. Plenum Press, New York.

36. Chagnon, N., P. LeQuesne, & J. M. Cook (1971) Yanomamo Hallucinogens: Anthropological, Botanical, and Chemical Findings. Current Anthropology 12:72-74

37. Harner, M. (ed.) (1973) Hallucinogens and Shamanism. Oxford University Press, London.

38. Furst, P. T. (ed.) (1976) Hallucinogens and Culture. Chandler and Sharp, San Francisco.

39. Reichel-Dolmatoff, G. (1975) The Shaman and the Jaguar: A Study of Narcotic Drugs Among the Indians of Colombia. Temple University Press, Philadelphia.

40. Chagnon, N. (1968) Yanomamo: The Fierce People. Holt, Rinehart, and Winston, New York.

41. De Rios, M. Dobkin (1972) Visionary Vine: Psychedelic Healing in the Peruvian Amazon. Chandler, San Francisco.

42. De Rios, M. Dobkin (1971) Ayahuasca: The Healing Vine. International Journal of Social Psychiatry 17:256-69

43. De Rios, M. Dobkin (1970) Banisteriopsis in Witchcraft and Healing in Iquitos, Peru. Economic Botany 24:296-300

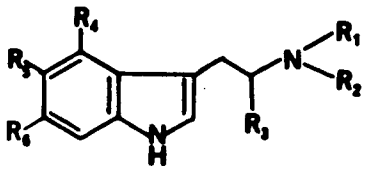
CHAPTER II: CHEMISTRY AND PHARMACOLOGY OF TRYPTAMINES AND β-CARBOLINES

I. Occurrence, Distribution, and Biosynthesis of Tryptamines and β-carbolines

The simple derivatives of tryptamine and β-carboline (Fig. 1 & 2) are among the most widely distributed alkaloids in the plant, animal, and fungal kingdoms [1,2]. As of 1977, some 19 simple derivatives of tryptamine had been identified in nature, distributed among 26 higher plant families and the Agaricales [1]; new species are continually being added to the list (cf., e.g., [3]) and undoubtedly the tabulation presented in [1] stands in need of revision. At the time of publication of [2] in 1980, 64 simple β-carboline derivatives distributed among 25 higher plant families and 3 species of fungi had been identified. Comparison of the species cited in [1] and [2] shows that in many instances, plants containing tryptamine derivatives also contain structurally related β-carbolines; this observation is not surprising since β-carbolines are biosynthesized from tryptophan and/or tryptamine via condensation with 1 or 2 carbon moieties [4,5] or via N-acetylation of tryptamine followed by cyclodehydration to 3,4-dihydro-β-carbolines [6]. The biosynthesis of tryptamine derivatives has been reviewed by Smith [7].

Both tryptamine derivatives and β-carbolines have been detected as endogenous metabolites in mammals, including man. Bufotenine and various related 5-hydroxy-indolethylamines are constituents of frog and toad venoms [8], being common in the genera Hyla, Leptodactylus, Rana, and Bufo. A wider spectrum of

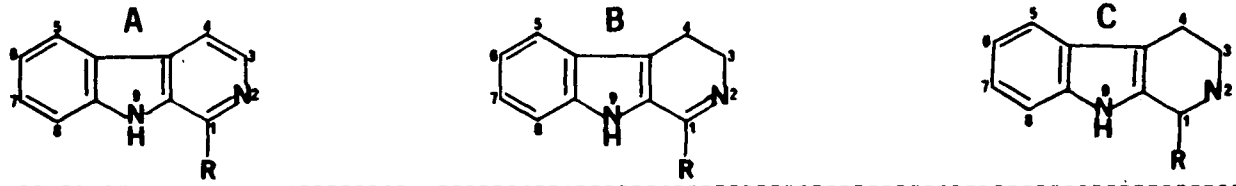
Figure 1 - Some Naturally-Occurring Tryptamine Derivatives*

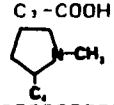


Name of Compound	Abbreviation	Substitution Pattern: R_1	R_2	R_3	R_4	R_5	R_6
tryptophan	-	H	H	COOH	H	H	H
5-hydroxy-tryptophan	-	H	H	COOH	H	OH	H
tryptamine	TA	H	H	H	H	H	H
5-hydroxy-tryptamine	5HT	H	H	H	H	OH	H
N-methyl-tryptamine	NMT	H	CH_3	H	H	H	H
5-methoxy-tryptamine	5-MeO-T	H	H	H	H	OCH_3	H
6-methoxy-tryptamine	6-MeO-T	H	H	H	H	H	OCH_3
5-methoxy-N-methyl-tryptamine	5-MeO-NMT	H	CH_3	H	H	OCH_3	H
5-methoxy-N-acetyl-tryptamine (melatonin)	-	H	$O{=}CCH_3$	H	H	OCH_3	H
N,N-dimethyl tryptamine	DMT	CH_3	CH_3	H	H	H	H
5-hydroxy-N,N-dimethyl-tryptamine (bufotenine)	5HO-DMT	CH_3	CH_3	H	H	OH	H
5-methoxy-N,N-dimethyl-tryptamine	5MeO-DMT	CH_3	CH_3	H	H	OCH_3	H
4-hydroxy-N,N-dimethyl-tryptamine (psilocin)	4HO-DMT	CH_3	CH_3	H	OH	H	H
4-phosphoryl-N,N-dimethyl-tryptamine (psilocybin)	-	CH_3	CH_3	H	OPO_3H	H	H
4-phosphoryl-N-methyl-tryptamine (baeocystine)	-	H	CH_3	H	OPO_3H	H	H
4-phosphoryl-tryptamine (norbaeocystine)	-	H	H	H	OPO_3H	H	H
N-methyl-tryptophan-methyl ester (abrine methyl ester)	-	H	CH_3	$COOCH_3$	H	H	H
N,N-dimethyl-tryptophan	-	CH_3	CH_3	COOH	H	H	H

*cf. Table 1 for structures of some psychoactive synthetic tryptamine derivatives.

Figure 2 - Some Naturally-Occurring β-carboline Derivatives



Trivial Name	Ring Type	R=	Additional Substitutents & Their Location	Abbreviation
norharman	A	H	-	-
norharmalan	B	H	-	-
tetrahydro-norharman	C	H	-	-
harman	A	CH_3	-	-
harmalan	B	CH_3	-	-
tetrahydro-harman	C	CH_3	-	-
harmine	A	CH_3	C_7-OCH_3	-
harmaline	B	CH_3	C_7-OCH_3	-
tetrahydro-harmine	C	CH_3	C_7-OCH_3	-
harmic amide	A	$CONH_2$	C_7-OCH_3	-
acetyl norharmine	A	C=OCH_3	C_7-OCH_3	-
harmine N-oxide	A	CH_3	C_7-OCH_3, N_2-->O	-
ketotetrahydro-norharmine	C	=O	C_7-OCH_3	-
harmic acid methyl ester	A	$COOCH_3$	C_7-OCH_3	-
harmalinic acid	B	COOH	C_7-OCH_3	-
harmol	A	CH_3	C_7-OH	-
harmalol	B	CH_3	C_7-OH	-
6-methoxy-harman	A	CH_3	C_6-OCH_3	-
6-methoxy-harmalan	B	CH_3	C_6-OCH_3	-
6-methoxy-tetrahydro-harman	C	CH_3	C_6-OCH_3	-
6-methoxy-2-methyl-tetrahydro-β-carboline	C	H	N_2-CH_3, C_6-OCH_3	6-MeO-MTHβC
6-methoxy-1,2-dimethyl-tetrahydro-β-carboline	C	CH_3	N_2-CH_3, C_6-OCH_3	6-MeO-DMTHβC
2-methyl-tetrahydro-β-carboline	C	H	N_2-CH_3	MTHβC
1,2-dimethyl-tetrahydro-β-carboline	C	CH_3	N_2-CH_3	DMTHβC
β-carboline-3-carboxylate	A	H	C_3-COOH	βCC
β-carboline-3-carboxylate ethyl ester	A	H	C_3-$COOCH_3$	βCCEE
tetrahydroharman-3-carboxylate	C	CH_3	C_3-COOH	-
brevicoline	A	CH_3		

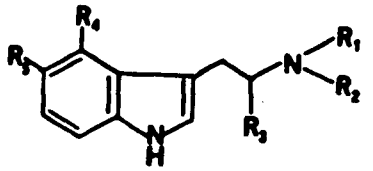
bufotenine derivatives is found in the last genus, including bufoviridine, the sulfate ester of bufotenine, dehydrobufotenine, and its sulfate ester, bufothionine. Although it is closely related to the hallucinogenic tryptamine derivatives, bufotenine itself is not hallucinogenic (see below, and [9]), acting as a pressor rather than a hallucinogen in man. The skin of Bufo alvarius contains 5-MeO-DMT at the rather staggering concentration of 50-160 mg/g of skin [8]; this is the only Bufo species known to contain a hallucinogenic tryptamine. Methyl transferases which catalyze the synthesis of tryptamines including DMT, 5-MeO-DMT, and bufotenine have been characterized in human lung, brain, blood, cerebrospinal fluid, liver, and heart, and also in rabbit lung, toad, mouse, steer, guinea pig, and baboon brains, as well as in other tissues in these species [10]. The in vivo formation of DMT from labelled precursors has been demonstrated in rabbit lung and in rat brain [10]. The state of knowledge of DMT biosynthesis in mammals as of 1981 has been summarized in [10]. Endogenous β-carboline derivatives have also been detected in human and rat tissues (cf. section III.A, below). Both tetrahydro-β-carbolines and the fully aromatic derivatives have been detected [11]. An aromatic β-carboline has been isolated from aging human lens protein [12] which is interesting in light of the recently discovered photocytotoxicity of aromatic β-carbolines [13]. Endogenous β-carboline derivatives and tetrahydroisoquinolines have been detected in human urine following ethanol loading. References [11,14] provide comprehensive recent reviews.

II. Pharmacology of Hallucinogenic Tryptamines

A. Structure/Activity Relationships

Most clinical and pharmacological studies with hallucinogenic tryptamines have focused on their *in vitro* metabolism and/or the possible endogenous synthesis of methylated tryptamine derivatives in pathological states such as schizophrenia. There is a paucity of scientific literature on the systematic investigation of structural influences on the hallucinogenic activity of tryptamine derivatives in humans. Investigations of structure/activity relationships of hallucinogens are complicated by the fact that no adequate animal model exists for this purpose; the drug discrimination test developed by Appel and co-workers [15] represents a step in the right direction, however. As a result, much of the currently available information is derived from "underground" sources of dubious credibility. Shulgin [16,17] has summarized the available information in two recent reviews. The substitution sites on the tryptamine nucleus that are important determinants of hallucinogenic activity are the indole ring, the side-chain carbons, or the side-chain nitrogen (cf. Table I). The N-alkyl homologs of DMT in which the N,N-dimethyl substituents are replaced with more aliphatic moieties include N,N-diethyltryptamine (DET), N,N-dipropyltryptamine (DPT), N,N-diisopropyltryptamine (DIPT), N,N-diallyltryptamine (DAT), and N,N-dibutyltryptamine (DBT). All of these homologs are psychoactive in man except for N,N-dibutyltryptamine, and all are apparently orally active except for DMT itself, which is

Table I - Orally and Parenterally Active
Psychotropic Tryptamine Derivatives*



Name of Compound	Substitution Pattern					Dosage (mg)	Route: Oral/Parenteral
	R_1	R_2	R_3	R_4	R_5		
tryptamine	H	H	H	H	H	100†	par/oral?
DMT	CH_3	CH_3	H	H	H	60	par
DET	C_2H_5	C_2H_5	H	H	H	60	par/oral
DPT	n-prop	n-prop	H	H	H	60	par/oral
DAT	C_3H_5	C_3H_5	H	H	H	60	par/oral
DIPT	i-prop	i-prop	H	H	H	30	oral
5MeO-DIPT	i-prop	i-prop	H	H	OCH_3	12	oral
5MeO-DMT	CH_3	CH_3	H	H	OCH_3	6	par
psilocin	CH_3	CH_3	H	OH	H	12‡	oral
CZ-74	C_2H_5	C_2H_5	H	OH	H	15‡	oral
serotonin	H	H	H	H	OH	100#	oral
bufotenine	CH_3	CH_3	H	H	OH	16þ	par
IT-290	H	H	CH_3	H	H	30	oral
4-hydroxy-α-methyl-tryptamine	H	H	CH_3	OH	H	20#	oral
MP-809	H	H	CH_3	H	CH_3	60ξ	oral
5-fluoro-α-methyl-tryptamine	H	H	CH_3	H	F	25ø	oral
5-methoxy-α-methyl-tryptamine	H	H	CH_3	H	OCH_3	3	oral
4-hydroxy-diisopropyl-tryptamine	i-prop	i-prop	H	OH	H	12ø	oral
4-hydroxy-N-isopropyl,N-methyl-tryptamine	i-prop	CH_3	H	OH	H	6ø	oral
N-t-butyl-tryptamine	H	t-butyl	H	H	H	?ø	oral
3-[2-(2,5-dimethylpyrrolyl)ethyl]-indole (side-chain=1-ethyl(2,5-dimethyl)pyrrole)			H	H	H	?	?

* Data compiled from [9,16,17,18].
† Autonomic symptoms; little central activity.
‡ The phosphate esters are psilocybin and CEY-19, respectively; both are stoichiometrically equivalent to the 4-hydroxy isomers.
# Cardiovascular and autonomic symptoms; little central activity.
þ A pressor amine rather than a hallucinogen in man.
ξ An antidepressant rather than a hallucinogen in man.
ø Based on anonymous reports in the lay press. No clinical studies have been published.

orally inactive in doses exceeding 1000 mg (cf. Table I). Presumably the oral inactivity of DMT is due to its deamination by monoamine oxidase (cf. discussion below) and those derivatives having bulkier N-alkyl substituents are orally active due to steric hindrance of the enzyme. Potency of all of the N,N-dialkyl derivatives mentioned above is considerably enhanced by hydroxyl substitution at the indole 4-position; this substitution also confers oral activity on the parent compound, DMT (psilocin = 4-hydroxy-DMT). The mechanism underlying the oral activity of psilocin is unelucidated, but it probably is due to the formation of an intramolecular ionic bond between the anionic ring hydroxyl and the charged side-chain nitrogen; this configuration could form a "pseudo" C ring and thus protect the side-chain from deamination [9]. Interestingly the 5-hydroxyl isomer of psilocin, bufotenine, is inactive as an hallucinogen and acts primarily on the peripheral autonomic systems, causing severe cardiovascular stimulation, salivation, hypertension, lachrymation, and hyperventilation, but no central effects [17]. Apparently the 5-hydroxyl substitution does not permit the formation of the intramolecular zwitterionic bond and the anionic character of the oxygen substituent interferes with the uptake of the compound into the central nervous system. The 5-O-methyl analogue of bufotenine (5-MeO-DMT) is parenterally active as an hallucinogen in man but is not orally active. 5-MeO-DMT exhibits similar psychological and somatic symptoms in man as DMT, but is approximately an order of magnitude more potent on a milligram basis (cf. Table I). Both 5-MeO-DMT and bufotenine are closely related structurally to the CNS neurotransmitter

serotonin. The N,N-diisopropyl analogues of DMT and 5-MeO-DMT are both orally active as hallucinogens in man [18]. Little is known of the hallucinogenic activity of the N-monosubstituted, N-dealkyl, or N-cycloalkyl tryptamines; the mono-tert-butyl derivative is "reputed" to be orally active in the underground literature [17].

In addition to indole ring substituents and aliphatic N-alkyl substituents, the side chain α-carbon represents a third substitution site affecting hallucinogenic activity. Methyl substitution of the α-carbon confers oral psychotomimetic activity on the compounds α-methyltryptamine and 5-MeO-α-methyltryptamine [9,17]. The mechanism of oral activity in the case of these analogues is undoubtedly related to steric hindrance of enzymatic deamination by the α-substituent. α-methyltryptamine and α-ethyltryptamine have been shown to act as competitive inhibitors of MAO [19]. No information is available on the activity of α-substituted N,N-dialkyltryptamines.

B. Metabolism of Hallucinogenic Tryptamines

The synthetic and degradative metabolism of DMT in mammals has been recently reviewed [10]. Indole N- and O-methyl transferases which catalyze the synthesis of DMT, 5-MeO-DMT, and bufotenine have been characterized in human lung, brain, blood, and cerebrospinal fluid [20]. Tryptamine, 5-hydroxytryptamine, and N-methyltryptamine have been identified as substrates for indole-N-methyltransferases but there is considerable variation in substrate specificity in different organisms and tissues. Two INMTs have been characterized in the Australian grass Phalaris

tuberosa which have different affinities for the primary amine (tryptamine) and the secondary amine (NMT), indicating that both are required in the biosynthesis of the tertiary amine [21]. Presence of two or more types of INMT in mammals has not been proven but would explain the varying substrate affinities in different tissues of the same species. S-adenosyl methionine (SAM) functions as the methyl donors in this transmethylation reaction; however, both SAM and 5-methyltetrahydrofolic acid (MTHF) have been found to participate in the synthesis of 2-methyl-tetrahydro-β-carboline (MTHβC) and tetrahydro-β-carboline (THβC) when incubated in vitro with NMT and tryptamine, respectively [22]. The tetrahydro-β-carboline formation probably occurs via the enzymatic formation of HCHO from the methyl donors followed by non-enzymatic condensation with the indole substrates via a Pictet-Spengler reaction. Both DMT and SAH, the de-methyl derivative of SAM, are potent inhibitors of the INMT activity [22]. Possible mechanisms involved in the regulation of the INMT activity have been reviewed by Barker, et al. [10].

Degradative metabolism of DMT has also been recently investigated [22] and is reviewed in [10]. A quantitative study of DMT metabolism in rat whole brain homogenates using deuterated DMT [22] found IAA, NMT, MTHβC, and DMT-N-oxide as metabolites. IAA was the major metabolite when DMT was incubated at 6×10^{-8} M, but at 2×10^{-5} M, DMT-NO was the major metabolite. Incubation of 6×10^{-8} M DMT in homogenates obtained from rats pretreated with the MAO inhibitor iproniazid resulted in the inhibition of IAA formation by 83%; however NMT and DMT-NO formation was inhibited by 90%, and no MTHβC was formed.

Based on these observations, the authors [22] speculated that a high proportion of IAA probably arose as a secondary metabolite resulting from the oxidative deamination of NMT. DMT itself is a poor substrate for MAO [23,24]; the relative rate of oxidation of NMT is some 9 times faster than DMT and 280 times faster than DMT-NO. The tetrahydro-β-carbolines detected as trace metabolites may be formed from the nonenzymatic condensation of tryptamine and/or NMT with the HCHO formed as an intermediate in the N-demethylation of DMT. Barker et al. [22] have made the interesting observation that direct C-hydroxylation of tertiary amines and tertiary amine N-oxide rearrangement results in the formation of identical intermediates (carbinolamines,iminium ions) which can undergo intramolecular cyclization to tetrahydro-β-carbolines. Earlier studies of DMT metabolism in rat liver microsomes obtained from animals pretreated with iproniazid detected 6-hydroxy-DMT and 6-hydroxy-DMT-NO in addition to tryptamine, NMT, and DMT-NO, giving rise to speculation that 6-hydroxylation might be an important metabolic pathway for DMT [25]. More recent studies have shown that, while 6-hydroxylation is characteristic of DMT metabolism in peripheral tissues, it apparently does not occur in brain [22].

Other investigators have focused on the metabolism of 4-hydroxy-DMT (psilocin) [26,27]. These studies indicate that oxidative deamination of the side-chain may be a relatively minor route of degradation for this compound. A metabolic study of the fate of ^{14}C-psilocin in the rat [26] indicated that unchanged psilocin and 4-hydroxy-IAA accounted for only 40% of total urinary metabolites, the remainder being present as

extremely hydrophilic metabolites, which could not be positively identified as glucuronides. Horita and Weber [27] studied the incubation of psilocybin in rat kidney homogenates. They found that psilocybin was readily dephosphorylated to psilocin by alkaline phosphatase; psilocin was then rapidly metabolized to a blue-colored product which they speculated was the o-quinone of psilocin. The formation of the blue product was unaffected by the presence of MAO inhibitors, but could be inhibited by KCN. Subsequently, psilocin and other hydroxy-indoles including serotonin and bufotenine were shown to be oxidized to colored products in the presence of mammalian cytochrome oxidase [28]. The significance of these metabolic investigations of DMT, psilocin, and related tryptamines lies in the recognition that oxidative deamination by MAO is not necessarily the only, or even the major, pathway available for the degradative metabolism of these compounds.

C. Hallucinogenic Tryptamines and MAO Inhibition

The activity of some tryptamine derivatives as MAO inhibitors has been investigated [23,24,29]. Unlike the β-carbolines, however, extensive studies of the structure/activity relationships of tryptamines with respect to MAOI activity have not been carried out. Lessin and co-workers [29] examined the SAR of a series of substituted tryptamines and β-carbolines on activity as MAOI and inhibitors of 5HT uptake. Among the tryptamines the most potent analog was 6-MeO-α,α-dimethyltryptamine which had 1.5% of the inhibitory potency of harmaline. The activity of N,N-dimethyltryptamine, 2-methyl-DMT

and 5-benzyloxy-DMT as MAOI in the guinea pig liver was investigated [23] at various inhibitor and substrate concentrations. In all cases DMT had significantly more activity than the other derivatives. The activity of DMT as MAOI was also influenced by the substrate; greatest inhibition was observed with 5HT as substrate, intermediate inhibition with tyramine as substrate, while the lowest activity was found with tryptamine as substrate. These results are consistent with the postulate that DMT may be a specific inhibitor of MAO-A (cf. Chapter VI). Ho et al. [24] investigated the MAOI activity of a series of 5-substituted gramines, *a*-methyltryptamines, and dimethyltryptamines using tryptamine as substrate. The order of MAOI activity of the tryptamine derivatives tested was DMT>5-methyl-DMT>5-MeO-DMT>5-hydroxyl-DMT. The DMT derivatives were generally more potent than the gramine derivatives except for 5-bromogramine which gave comparable inhibition at 72% of the concentration of DMT.

The potentiation of the behavioural and pharmacological effects of tryptamine derivatives by MAO inhibitors has been investigated, although the specific question of the oral potentiation of DMT and other parenterally-active derivatives has apparently not been investigated. The effect of DMT in human volunteers was assessed before and 3 days after treatment with the MAOI iproniazid [30]. Patients receiving DMT at a reduced dose following the iproniazid treatment experienced none of the visual hallucinations or disturbances of time and space perception which typify the symptoms of the drug; they reported only a feeling of "strangeness". Patients receiving a dose

equivalent to that given prior to iproniazid had a two-phase response. The first stage was similar to the usual DMT psychosis, but less pronounced; illusions and hallucinations were present, but less colorful, and only manifested with the eyes closed; the second phase was characterized by a persistent feeling of "strangeness" to which the patients often reacted negatively, or indifferently. Based on these trials the authors [30] speculated that the reduced effects may have been due to the higher 5HT concentration in the brain due to MAO inhibition, thus mitigating the 5HT blocking effects of DMT. This observation was also supported by the observation that prior administration of 1-methyl-d-lysergic acid butanolamide, a powerful serotonin antagonist, greatly exacerbated the psychotomimetic effects of DMT [31]. Moore and co-workers [32] studied the effects of iproniazid, chlopromazine, and methiothepin on DMT-induced changes in body temperature, pupillary dilatation, blood pressure and EEG in rabbits. Chlorpromazine attentuated or blocked the effects of DMT on these parameters but iproniazid prolonged the elevated rectal temperatures and mydriasis induced by DMT. Arterial blood pressure and EEG were not markedly altered by pre-treatment with iproniazid. Wang-Lu and Domino [33] investigated the effect of the MAOI iproniazid and tranylcypromine on DMT half-life in rat liver and brain and found a greatly increased half-life in both tissues after treatment. Tranylcypromine prolonged DMT half-life more than iproniazid. Interestingly, treatment with a larger dose of DMT in the absence of MAOI prolonged the half-life in brain but not in liver. The authors speculated that this may be

due to the fact that DMT itself is a weak MAOI and that its clearance from brain is mainly dependent on MAO. Liver may have other enzymes capable of metabolizing DMT even though MAO is inhibited. Half-life in brain and liver was similar in the presence of MAOI indicating that similar enzymes participate in the metabolism of DMT following MAO inhibition. Shah and Hedden [34] studied behavioural effects and metabolism of DMT in mice pretreated with SKF-525A, iproniazid, and chlorpromazine and found that iproniazid prolonged the behavioural effects of DMT and also significantly elevated DMT levels in plasma, brain, and liver with respect to controls. SKF-525A, an inhibitor of a wide variety of hepatic microsomal enzymes, did not prolong the behavioural effects of DMT or result in increased tissue or plasma levels, thus providing further evidence that MAO is the primary enzyme involved in the metabolism of DMT in vivo. It also indicated that the central effects of DMT are due to the parent compound rather than the 6-hydroxy-metabolite detected in some studies [25]. 6-hydroxy-derivatives of DMT and related compounds are inactive as hallucinogens [16]. Although the available literature indicates that MAOI do have significant influences on both the metabolism and behavioural effects of DMT, apparently the specific interactions of DMT with β-carbolines have not been investigated. This apparent oversight is especially remarkable in view of the close structural relationships of tryptamine derivatives and β-carbolines [1,2], the probable metabolic interconversion of DMT, other tryptamines, and β-carbolines [22,57], the involvement of both classes of compounds in important neuro-regulatory functions

such as MAO activity and amine uptake [11,14,29] and the probable role of tryptamine/β-carboline combinations in the mechanism of oral activity of the hallucinogens ayahuasca and the orally-ingested Virola pastes (cf. Chapter I). The available evidence strongly suggests that tryptamines and β-carbolines are closely related, not only structurally but also pharmacologically, yet very little is known about their interactions in mammalian systems.

III. Biochemistry and Pharmacology of β-carboline Derivatives

β-carbolines have been known to science since the isolation of harmaline from Peganum harmala by Goebel in 1847, followed a few years later by the isolation of harmine from the same species by J. Fritsche [35]. The structure elucidation of both compounds was accomplished by Manske in 1927, and their total synthesis was published by Spath and Lederer in 1930 [35]. In spite of their long history, many aspects of the biochemistry and pharmacology of β-carboline derivatives remain poorly understood. Since the discovery in 1959 that harmaline and related derivatives are competitive reversible inhibitors of monoamine oxidase [36], there has been a resurgence of interest in β-carbolines. The present section focuses primarily on the psychopharmacology of β-carbolines; other aspects of their pharmacology have been discussed in [37] and will only be mentioned here.

A. Psychopharmacology of β-carboline Derivatives

1. β-carbolines as Monoamine Oxidase Inhibitors

The activity of β-carboline derivatives as competitive reversible inhibitors of MAO was first demonstrated by Udenfriend and co-workers [36]. Subsequently, structure/activity relationships were investigated by McIsaac & Estevez [38], Ho et al. [39], and Buckholtz & Boggan [40,41]. Direct comparison of the results of these studies are complicated by the use of different animals and tissues as the source of MAO, and also by the use of different substrates. Other investigations [42,43,44,45] have provided evidence that at least two species of MAO exist; these forms of MAO, designated MAO-A and MAO-B, have different substrate specificities, are differentially sensitive to various MAO inhibitors, and have different kinetic properties. Fuller [42] demonstrated that harmaline (and presumably other β-carbolines) is a selective inhibitor of MAO-A. Some SAR studies of β-carboline MAOI activity have used tyramine as substrate [38], others have used tryptamine [39,40,41] and still others have used tryptamine, 5HT, and β-phenylethylamine as substrate [41]. 5HT is a specific substrate of MAO-A while tryptamine and tyramine are substrates of both MAO A and B [46]; β-phenylethylamine is probably metabolized mainly by MAO-B <u>in vivo</u> [46]. In view of the different substrates and enzyme species used in different studies it is not surprising that there are considerable differences in the I_{50} values and other structure/activity parameters. McIsaac and Estevez [38] using tyramine as substrate

found that fully aromatic β-carbolines were most active as MAOI while tetrahydro-β-carbolines had the least activity; dihydro-derivatives were intermediate in potency, a result that agrees with other studies. Little difference in potency was found between 6- or 7- methoxylated, or unsubstituted β-carbolines but hydroxyl substitution reduced the activity. Buckholtz and Boggan [41] using tryptamine as substrate reported results in general agreement with those of [38] except that 7-methoxy-β-carbolines were more potent than 6-methoxy- or unsubstituted derivatives; methyl substitution at C_1 decreased activity for liver MAO but increased it for brain MAO. Other differences in potency between liver and brain MAO were also noted for various derivatives. Generally most β-carbolines gave lower I_{50} values with 5HT as substrate than with tryptamine as substrate. See Chapter IV for a comparison of these results with a study carried out in the present work. Ho and co-workers [39] studied the influence of various substituents on C_1,N_2, and N_9 of the β-carboline nucleus on MAOI activity. Methyl, ethyl, or carboxyl substitution of C_1 resulted in progressive decrease in activity, the carboxyl substituted compounds being some eighteen times less active than the unsubstituted derivatives. Ethyl or N-propyl substitution of N_2 did not significantly reduce activity, although N_2-acetyl substitution essentially abolished the activity. Methyl substitution of the indolic nitrogen (N_9) significantly enhanced activity of the tetrahydro-β-carbolines but only slightly enhanced the aromatic derivatives. Buckholtz and Boggan [40] studied tetrahydro-β-carbolines in mouse brain and liver and found that they were more potent inhibitors in brain than in

liver. 6-MeO-THβC was without inhibitory activity in liver but had about the same activity in brain as THβC.

2. β-carbolines as Hallucinogens

The little that is known about the hallucinogenic activity of β-carbolines stems primarily from the studies of Pennes and Hoch [47] and Naranjo [48]. No systematic human studies of the hallucinogenic activity of β-carboline derivatives have been conducted in over 25 years; many of the results reported by Naranjo [48] were obtained from a single human trial. What does seem clear, however, is that the hallucinogenic action of β-carbolines only manifests at threshold dosage levels which are several orders of magnitude greater than the doses required to manifest their activity as MAOI or as competitive inhibitors of 5HT and epinephrine uptake [cf. 40,41]. It is unlikely, therefore, that these activities can be invoked as the mechanism responsible for the hallucinogenic action of some β-carbolines. Hoch [47] reported that harmine was orally inactive at doses in excess of one gram, but observed threshold hallucinogenic effects at i.v. doses between 200-250 mg. Slotkin [49] observed comparable effects at 50 mg i.v. Naranjo [48] reported that harmaline was orally active at 4 mg/kg; tetrahydroharmine was apparently less active, however the equation 300 mg tetrahydroharmine=100 mg harmaline resulted from a single trail. Naranjo [50] reported that 6-MeO-harmalan, the 6-substituted analog of harmaline, gave threshold oral activity at 1.5 mg/kg; comparable levels of 6-MeO-tetrahydroharmine gave "milder effects". 6-MeO-harman, the analog of harmine, has apparently

not been investigated.

3. Endogenous Synthesis of β-carbolines in Mammals

The hypothesis that endogenously synthesized hallucinogens might play a role in the etiology of schizophrenia or other mental disorders has fallen in and out of favor among researchers ever since Osmund and Smythies first proposed the idea in 1952 [51]. The issue has still not been resolved, even though unequivocal evidence [cf. 20] has been obtained indicating that hallucinogens can arise in mammalian systems under certain conditions. McIsaac [52] was the first to suggest that an endogenous β-carboline, formed from the cyclodehydration of the pineal hormone melatonin (5-methoxy-N-acetyltryptamine), could be an etiological factor in mental illness. Subsequently McIsaac [53] demonstrated that 1-methyl-6-MeO-THβC could be formed from 6-MeO-tryptamine and acetaldehyde under mild physiological conditions *in vitro* and also *in vivo* in rats pretreated with iproniazid and disulfiram (Antabuse). The hypothesis appeared to be confirmed when Farrel and McIsaac [54] claimed to have isolated a pineal hormone, adrenoglomerulotropin, which was identical to 1-methyl-6-MeO THβC. This initial tentative identification could not be confirmed and eventually the claim was withdrawn [55]. Although McIsaac's original hypothesis that the pineal gland is a likely site of endogenous β-carboline formation has been confirmed only for birds [56], the evidence that β-carbolines are synthesized in a variety of tissues is now overwhelming. β-carbolines have been unequivocally identified in human plasma and platelets, and

in the rat whole brain, forebrain, arcuate nucleus, and adrenal gland. Most of the endogenous β-carbolines so far characterized are 6-methoxy-, 6-hydroxy-, or unsubstituted tetrahydro-β-carbolines, however the fully aromatic derivative harman has been found in the rat arcuate nucleus [11]. It has been shown [57,58] that in some instances endogenous β-carbolines are formed as a result of condensation of indolamines with formaldehyde released non-enzymatically from 5-methyl-tetrahydrofolic acid (MTHF). Recent interest has centered on the possible involvement of endogenous β-carbolines, tetrahydroisoquinolines, and other amine/aldehyde condensation products in the etiology of alcoholism [59,60]. 2-methyl-THβC and harman were identified in human urine after ethanol loading [61]. Presumably these constituents are formed via the condensation of biogenic amines such as serotonin or dopamine with acetaldehyde, the primary metabolite of ethanol. See references [11,14] for comprehensive recent reviews of the biochemistry, pharmacology, and pathology of endogenous mammalian alkaloids.

B. Other Neurological and Biological Activities of β-carbolines

β-carbolines exhibit a wide spectrum of neurophysiological and biological activities in addition to those discussed above. It is not within the scope of this review to discuss each of them in detail; they are mentioned here for the sake of completeness. The appropriate references should be consulted for

further information. A number of β-carbolines have been shown to inhibit the uptake of 5HT, dopamine, nor-epinephrine, and epinephrine into synaptosomal suspensions [37,40]. Other β-carboline derivatives are inhibitors of membrane ATPases in human erythrocytes, rat brain, and squid retinal axon [37,64]. Interference with synthesis of biogenic amines by some β-carbolines has also been reported [37]. β-carboline-3-carboxylate ethyl ester (BCCE) and other β-carboline derivatives have been implicated [62,63] as possible endogenous ligands for the benzodiazepine (Valium) receptors, although other compounds, including purines and kynuramines, have also been proposed [cf. 63 for a comprehensive review]. Harmaline and related derivatives exert a potent "vasopressin"-like effect on Na and water transport in isolated toad skin, stimulating hydrosmotic flow across the membrane [64,65]. Failure of harmaline to elicit any effect in preparations pretreated with vasopressin and/or nor-epinephrine suggested a competitive mechanism of action. Harmine, harmaline and related compounds have tremorogenic effects, cardiovascular effects, and also influence homeothermic mechanisms, causing hypothermia in some animals (rats, mice) and hyperthermia in others (rabbits) [37].

β-carbolines also have biological activities other than their effects on neurophysiological systems. For instance Hopp and co-workers [66] found that harmine exhibited significant anti-trypanosomal activity against Trypanosoma lewisi. The mutagenic or co-mutagenic effect of certain β-carbolines has been noted [67] and the mechanism responsible may be related to the interaction of β-carbolines with nucleic acids [68,69]. More

recently, the ultra-violet-mediated photocytotoxic and photogenotoxic activity of some β-carboline derivatives has been described [13,70].

IV. Literature Cited

1. Smith, T. A. (1977) Tryptamine and Related Compounds in Plants. Phytochemistry 16:171-75

2. Allen, J. R. F., & B. Holmstedt (1980) The Simple β-carboline Alkaloids. Phytochemistry 19:1573-1582. 1980

3. Skaltsounis, A. L., F. Tillequin, & M. Koch. (1983) Plantes de Nouvelle-Caledonie LXXXIII. Alcaloides des Tiges Feuillees de Melicope leptococca. Journal of Natural Products 46:732-35

4. O'Donovan, D.G., & M. F. Kenneally (1967) The Biosynthesis of Eleagnine. Journal of the Chemical Society Sect. C:1109-10

5. O'Donovan, D. G. (1976) Proceedings of the Royal Irish Academy Section B. 76:187

6. Slator, M., & I. J. McFarlane. (1968) The Biosynthesis and Metabolism of Harman in Passiflora edulis. Phytochemistry 7:605-11

7. Smith, T. A. (1977) Recent Advances in the Biochemistry of Plant Amines. Chapter 2 in Progress in Phytochemistry V. 4. L. Reinhold, J. B. Harbourne, and T. Swain (eds.). Academic Press.

8. Daly & B. Witkop. (1971) Chemistry and Pharmacology of Frog Venoms. Chapter 39 in Venomous Animals and Their Venoms V. II, W. Bücherl & E. E. E. Buckley (eds.). Academic Press

9. Kantor, R. E., S. D. Dudlettes, & A. T. Shulgin. (1980) 5-Methoxy-α-Methyl-tryptamine (α,O-dimethylserotonin), a Hallucinogenic Homolog of Serotonin. Biological Psychiatry 15:349-52

10. Barker, S. A., J. A. Monti, & S. T. Christian. (1981) N,N-dimethyltryptamine: An Endogenous Hallucinogen. International Review of Neurobiology 22:83-110

11. Melchior, C., and M. A. Collins. (1982) The Route and Significance of Endogenous Synthesis of Alkaloids in Mammals. CRC Critical Reviews in Toxicology 9:313-356

12. Dillon, J., A. Spector, & K. Nakanishi. (1976) Identification of β-carbolines Isolated from Fluorescent Human Lens Proteins. Nature 259:422-3

13. McKenna, D. & G. H. N. Towers. (1981) Ultra-violet Mediated Cytotoxic Activity of β-carboline Alkaloids. Phytochemistry 20:1001-1004

14. Bloom, F., J. Barchas, M. Sandler, & E. Usdin (eds.) (1982) β-carbolines and Tetrahydroisoquinolines. proceedings of a symposium held at the Salk Institute, La Jolla, Ca, Dec. 12-13, 1981. Alan R. Liss, publisher, New York

15. Appel, J. B., F. J. White, & A. M. Holohean (1982) Analyzing Mechanism(s) of Hallucinogenic Drug Action with Drug Discrimination Procedures. Neuroscience and Biobehavioural Reviews 6:529-36

16. Shulgin, A. T. (1976) Psychotomimetic Agents. chapter 4 in Maxwell Gordon (ed.) Psychopharmacological Agents V. IV. Academic Press

17. Shulgin, A. T. (1982) Chemistry of Psychotomimetics. Chapter 1 in F. Hoffmeister & G. Stille (eds.) Handbook of Experimental Pharmacology Vol. 55 Pt. III: Psychotropic Agents Pt. III: Alcohol & Psychotomimetics, Psychotropic Effects of Central-Acting Drugs. Springer-Verlag

18. Shulgin, A. T. & M. F. Carter (1980) N,N-diisopropyltryptamine (DIPT) and 5-Methoxy-N,N-diisopropyltryptamine (5-MeO-DIPT). Two Orally Active Tryptamine Analogs with CNS Activity. Communications in Psychopharmacology 4:363-69

19. Greig, M. K., R. A. Walk, & A. J. Gibbon (1959) The Effect of Three Tryptamine Derivatives on Serotonin Metabolism in vitro and in vivo. British Journal of Pharmacology 127:110-115

20. Rosengarten, H. & A. J. Friedhoff (1976) A Review of Recent Studies of the Biosynthesis and Excretion of Hallucinogens Formed by Methylation of Neurotransmitters or Related Substances. Schizophrenia Bulletin 2:90-105

21. Mack, J. P. G., & M. Slaytor (1979) Indolethylamine N-methyl Transferases of Phalaris tuberosa: Purification and Properties. Phytochemistry 18:1921-5

22. Barker, S. A., J. A. Monti, and S. T. Christian (1980) Metabolism of the Hallucinogen N,N-dimethyltryptamine in Rat Brain Homogenates. Biochemical Pharmacology 29:1049-57

23. R. B. Barlow. (1961) Effects on Amine Oxidase of Substances Which Antagonize 5-hydroxytryptamine More Than Tryptamine on the Rat Fundus Strip. British Journal of Pharmacology 16:153-162.

24. B. T. Ho, W. M. McIsaac, R. An, R. T. Harris, K. E. Walker, & P. M. Kralik. (1970) Biological Activities of Some 5-Substituted N,N-dimethyltryptamines, α-methyltryptamines, and Gramines. Psychopharmacologia 16:385-394.

25. Szara, S. & J. Axelrod. (1959) Hydroxylation and N-demethylation of N,N-dimethyltryptamine. Experientia 15:216-17

26. Kalberer, F., W. Kreis & J. Rutschmann (1962) Fate of Psilocin in the Rat. Biochemical Pharmacology 11:261-69

27. Horita, A. and L. J. Weber (1961) The Enzymic Dephosphorylation and Oxidation of Psilocybin and Psilocin by Mammalian Tissue Homogenates. Biochemical Pharmacology 7:47-54

28. Weber, L. J. & A. Horita (1963) Oxidation of 4- and 5-hydroxyindole Derivatives by Mammalian Cytochrome Oxidase. Life Sciences 1: 44-49

29. Lessin, A. W., R. F. Long, & M. W. Parkes (1967) The Central Stimulant Properties of some Substituted Indolylalkylamines and β-carbolines and Their Activities as Inhibitors of Monoamine Oxidase and the Uptake of 5-hydroxytryptamine. British Journal of Pharmacology and Chemotherapy 29:70-79

30. Sai-Halasz, A. (1963) The Effect of MAO Inhibition on the Experimental Psychosis Induced by Dimethyltryptamine. Psychopharmacologia 4:385-88

31. Sai-Halasz, A. (1962) The Effect of Antiserotonin on the Experimental Psychosis Induced by Dimethyltryptamine. Experientia 18:137-138

32. Moore, R. H. S. K. Demetriou & E. F. Domino (1975) Effects of Iproniazid, Chloropromazine and Methiothepin on DMT-induced Changes in Body Temperature, Pupillary Dilatation, Blood Pressure and EEG in the Rabbit. Archives Internationels de pharmacodynamie et de therapie 213:64-72

33. Wang Lu, L. J., & E. F. Domino (1976) Effects of Iproniazid and Tranylcypromine on the Half-Life of N,N-dimethyltryptamine in Rat Brain and Liver. Biochemical Pharmacology 25:1521-27

34. Shah, N. S., & M. P. Hedden (1977) Behavioural Effects and Metabolic Fate of N,N-dimethyltryptamine in Mice Pretreated with β-diethylaminoethyl-diphenylpropylacetate (SKF-525-A), Iproniazid, and Chlorpromazine. Pharmacology, Biochemistry and Behaviour 8:351-56

35. Holmstedt, B. (1982) β-carbolines and Tetrahydroisoquinolines: Historical and Ethnopharmacological Background. in F. Bloom, et al., (eds.) op. cit.

36. Udenfriend, S., B. Witkop, B. G. Redfield, & H. Weissbach (1958) Studies with Reversible Inhibitors of Monoamine Oxidase: Harmaline and Related Compounds. Biochemical Pharmacology 1:160-165

37. Ho, B. T. (1977) Pharmacological and Biochemical Studies with β-carboline Analogs. chapter VI in W. B. Essman & L. Vazelli, (eds.) Current Developments in Psychopharmacology V. 4. Spectrum Publications, Inc.

38. McIsaac, W. M. & V. Estevez (1966) Structure-action Relationships of β-carbolines as Mono-amine Oxidase Inhibitors. Biochemical Pharmacology 26:1625-27

39. Ho, B. T., W. M. McIsaac, K. E. Walker, & V. Estevez (1968) Inhibition of Monoamine Oxidase: Influence of Methyl Substitution on the Inhibitory Activity of β-carbolines. Journal of Pharmaceutical Sciences 57:269-74

40. Buckholtz, N. S., and W. O. Boggan (1976) Effects of Tetrahydro-β-carbolines on Monoamine Oxidase and Serotonin Uptake in Mouse Brain. Biochemical Pharmacology 25:2319-2321

41. Buckholtz, N. S. & W. O. Boggan (1977) Monoamine Oxidase Inhibition in Brain and Liver Produced by β-carbolines: Structure-activity Relationships and Substrate Specificity. Biochemical Pharmacology 26:1991-96

42. Fuller, R. W., B. J. Warren, & B. B. Molloy (1970) Selective Inhibition of Monoamine Oxidase in Rat Brain Mitochondria. Biochemical Pharmacology 19:2934-2936

43. Donnelly, C. H., E. Richelson & D. L. Murphy (1976) Properties of Monoamine Oxidase in Mouse Neuroblastoma NIE-115 Cells. Biochemical Pharmacology 25:1629-1643

44. Donnelly, C. H., & D. L. murphy (1977) Substrate- and Inhibitor-related Characteristics of Human Platelet Monoamine Oxidase. Biochemical Pharmacology 26:853-858

45. Houslay, M. D. & K. F. Tipton (1974) A Kinetic Evaluation of Monoamine Oxidase Activity in Rat Liver Mitochondrial Outer Membranes. Biochemical Journal 139:645-652

46. Neff, N. H. and H.-Y. T. Yang (1974) Another Look at the Monoamine Oxidases and the Monoamine Oxidase Inhibitor Drugs. Life Sciences 14:2061-2074

47. Pennes, H. H. & P. H. Hoch (1957) Psychotomimetics, Clinical and Theoretical Considerations: Harmine, WIN-299 and Nalline. American Journal of Psychiatry 113:887-92

48. Naranjo, C. (1967) Psychotropic Properties of the Harmala Alkaloids. pp. 385-391 in D. H. Efron, B. Holmstedt, & N. S. Kline (eds.) Ethnopharmacologic Search for Psychoactive Drugs. U. S. Public Health Service Publication #1645

49. Slotkin, T. A., V. DiStefano & W. Y. W. Yu (1970) Blood Levels and Urinary Excretion of Harmine and Its Metabolites in Man and Rats. The Journal of Pharmacology and Experimental Therapeutics 173:26-30

50. Naranjo, C. (1969) Psychotherapeutic Possibilities of New Fantasy-enhancing Drugs. Clinical Toxicology 2:209-224

51. Osmund, H., and J. R. Smythies (1952) Schizophrenia: A New Approach. Journal of Mental Sciences 98:309-315

52. McIsaac, W. M. (1961) A Biochemical Concept of Mental Disease. Postgraduate Medicine 30:111-118

53. McIsaac, W. M. (1961) Formation of 1-methyl-6-methoxy-1,2,3,4-tetra-hydro-β-carboline under Physiological Conditions. Biochimica et Biophysica Acta 52:604-606

54. Farrell, G. & W. M. McIsaac (1961) Adrenoglomerulotropin. Archives of Biochemistry & Biophysics 94:543-44

55. Farrell, G., W. M. McIsaac, & A. N. Taylor (1964) Neurohumoral Factors from the Brainstem Epiphysis. pp. 141-47 in L. Martini & A. Pecile (eds.) Hormonal Steriods - Biochemistry, Pharmacology, and Therapeutics V. I. Academic Press.

56. Kari, I. (1981) 6-methoxy-1,2,3,4-tetrahydro-β-carboline in Pineal Gland of Chicken and Cock. FEBS Letters 127:277-280

57. Hsu, L. L., & A. J. Mandell (1975) Enzymatic Formation of Tetrahydro-β-carbolines from Tryptamine and 5-Methyltetrahydrofolic Acid in Rat Brain Fractions: Regional and Subcellular Distribution. Journal of Neurochemistry 24:631-36

58. Rommelspacher, H., H. Caper & S. Strauss (1976) On the Mode of Formation of Tetrahydro-β-carbolines. Life Sciences 18:81-88

59. Rahwan, R. G. (1975) Toxic Effects of Ethanol - Possible Role of Acetaldehyde, Tetrahydroisoquinolines, & Tetrahydro-β-carbolines. Toxicology and Applied Pharmacology 34:3-27

60. Davis, V. E. and M. A. Walsh (1970) Alcohol, Amines, and Alkaloids: A Possible Biochemical Basis for Alcohol Addiction. Science 167:1005-7

61. Rommelspacher, H., S. Strauss & J. Lindemann (1980) Excretion of Tetrahydroharmane and Harmane into the Urine of Man and Rat After a Load with Ethanol. FEBS Letters 109:209-12

62. Mennini, T., S. Cotecchia, S. Garattini (1982) Does Ethyl-β-carboline-3-carboxylate Interact with Mouse Brain Benzodiazepine Receptors in Vivo? Journal of Pharmacy and Pharmacology 34:394-5

63. Lapin, I. P. (1983) Structure/Activity Relationships in Kynurenine, Diazepam and Some Putative Endogenous Ligands of the Benzodiazepine Receptors. Neurosciences and Biobehavioural Reviews 7:107-118

64. Canessa, M., E. Jaimovich, & M. de la Fuente (1972) The Effect of Harmane Derivatives on the Na-dependent Phosphorylation from the Membrane ATPase System. p. 28ff Vth International Congress of Nephrology. Mexico City

65. de Sousa, R. C., & A. Grosso (1978) Vasopressin-like Effects of a Hallucinogenic Drug - Harmaline - on Sodium and Water Transport. Journal of Membrane Biology 40:77-94

66. Hopp, K. H., L. V. Cunningham, M. C. Bromel, L. J. Schermeister and S. K. W. Kahlil (1976) In Vitro Antitrypanosomal Activity of Certain Alkaloids Against Trypanosoma lewisi. Lloydia 39:375-77

67. Umezawa, K., A. Shirai, T. Matsushima, & T. Sugimura (1978) Comutagenic Effect of Norharman and Harman with 2-Acetylaminofluorene Derivatives. Proceedings of the National Academy of Sciences 75:928-30

68. Remson, J. F. & P. A. Cerutti (1979) Inhibition of DNA Repair and DNA Synthesis by Harman in Human Alveolar Tumor Cells. Biochemical and Biophysical Research Communications 86:124-129

69. Hayashi, K., M. Nagao, & T. Sugimura (1977) Interactions of Harman and Norharman with DNA. Nucleic Acids Research 4:3679-85

70. Towers, G. H. N., & Z. Abramowsky. (1983) UV-mediated Genotoxicity of Furanoquinolines and of Certain Tryptophan-Derived Alkaloids. Journal of Natural Products 46:576-81

PART II: ETHNOBIOLOGICAL INVESTIGATIONS OF MALPIGHIACEOUS AND MYRISTICACEOUS HALLUCINOGENS

CHAPTER III: ETHNOBOTANICAL COLLECTIONS AND ETHNOGRAPHIC OBSERVATIONS

I. Ethnobotanical Collections

A. Collection and Identification of Herbarium Voucher Specimens

During the field-work conducted in connection with this research, herbarium voucher specimens were collected for all plant species indicated by informants to have medicinal properties or other ethnobotanically significant uses. Particular efforts were made to secure voucher specimens for all source-plants and admixture plants used in the preparation of the Malpighiaceous and Myristicaceous drug samples which have been analyzed in this study. These efforts were not always totally successful, i.e. in some instances it was possible to collect only limited amounts of voucher material (as, for example, when collecting from the cultivated plot of an ayahuasquero), or the actual source-plants used in the manufacture of a particular sample were not available. In these instances plants of the same species from the same general locality were collected for comparative purposes. Collection numbers cited throughout refer to the personal collection numbers of D. McKenna and are prefaced with "DMK-". Exceptions are Plowman 6040 (Diplopterys cabrerana), Plowman 6041 (B. caapi var. "cielo") and Plowman 12,218 (Virola spp.). These samples were kindly supplied by Dr. Timothy Plowman of the Field Museum of Natural History in Chicago. Yanomamo snuff samples were a gift to the author by Dr. Ernesto Migliazza, formerly of the

Department of Anthropology, University of Maryland, College Park; no accompanying voucher specimens are available for the snuff samples. Authenticated herbarium vouchers for all "DMK-" collection numbers have been deposited in the Herbarium of the Department of Botany, University of British Columbia. Duplicate vouchers for most collections are also on deposit at the Field Museum; other herbaria in North and South America have duplicates of some but not all collections. All voucher collections of ethnobotanical significance which were made in connection with this work are listed in Appendix II.

B. Collection of Plant Material and Drug Samples for Phytochemical Analysis

Bark and leaf samples used in the phytochemical analyses consisted either of air-dried plant material or of material preserved in 100% methanol at the time of collection. Most of the drug samples were also preserved in methanol at the time of collection; exceptions were two ayahuasca samples, the orally-ingested paste sample from La Chorrera, Colombia (collected in 1971--no voucher available) and the Yanomamo snuff samples. Details of the preparation of the plant materials and drug samples for phytochemical analysis are cited in the "Materials and Methods" sections of the appropriate chapters.

II. Ethnographic Observations

The ethnographic aspects of the Malpighiaceous and

Myristicaceous hallucinogens have been the subject of study by anthropologists, ethnobotanists, scholars and explorers since before the turn of the century. In some respects the ethnography of these native hallucinogens is much more thoroughly documented than the botanical, phytochemical, or pharmacological facets, as these have only begun to receive clarification within the last thirty years. The relevant ethnobotanical literature on the subject was reviewed in chapter I; this chapter documents personal observations made during the course of fieldwork.

A. Ethnographic Aspects of *ayahuasca*

During the fieldwork carried out for this study, various *ayahuasqueros* (persons who specialize in the preparation and use of *ayahuasca* in folk medicine) were contacted in the vicinities of Iquitos, Pucallpa, and Tarapoto. An effort was made to interview and to collect *ayahuasca* samples from as many different practitioners as possible. This was done in an attempt to learn a.) whether there were significant differences in the alkaloid composition of *ayahuasca* samples prepared by different practitioners, either in terms of types of alkaloids found, or in concentration. b.) whether ethnographic elements of *ayahuasca* use are different from one region to another, or from one practitioner to another. In all, *ayahuasca* samples prepared by six different practitioners were collected, and in some cases, samples of different batches prepared by the same person were collected. The phytochemical results are discussed in Chapter IV. In some instances, contact with the *ayahuasquero* was superficial, and consisted either of attending the weekly

ayahuasca session as a paying participant or of visiting his home to purchase samples of ayahuasca;* in other instances, repeated visits were made and it was possible to conduct more detailed interviews. Of the several ayahuasqueros contacted, I became best acquainted with Don Fidel Mosambite, who lived in the small village of Jose Olaya, not far from Pucallpa. Most of the remarks which follow have been derived from our conversations. Don Fidel was quite willing to talk about the use of ayahuasca and other medicinal plants. He had some understanding of Western medicine and science and seemed impressed by the fact that we were botanicos. Don Fidel seemed to look upon Western medicine as complementary to his own ethnomedical tradition; he was quite aware that some diseases are caused by microbes or dietary deficiencies and could be treated with antibiotics or vitamins. He would often send his patients to seek treatment from Western medical practitioners, if antibiotics were called for, and occasionally he would supply them with vitamins himself. In most instances, however, he felt that the causes of illness were supernatural in origin or were amenable to treatment with any of several hundred medicinal plants with which he was familiar. In these cases he would undertake the treatment himself. Ayahuasca was usually incorporated into the treatment, although it was not always given to the patient; at times only Don Fidel consumed ayahuasca, in order to diagnose the illness, and at other times the patient would receive it as well. In other cases, the

---

*Although the masculine pronoun is used in this description, ayahuasqueros can be (and frequently are) women.

treatment involved medicinal plants other than ayahuasca and neither Don Fidel or the patient would take ayahuasca. In all cases that I observed, however, the administration of the botanical remedy, which might take the form of a tea, potion, poultice, or tincture, was accompanied by magical songs, chants, and whistling. The patient, the remedy, and the general vicinity were liberally censed with tobacco smoke which Don Fidel burned in a special pipe which he had made himself. Some ayahuasqueros make passes over the patient's body with a rattle made of dried palm fronds in order, presumably, to drive out evil spirits or purge the patient of malevolent influences. I never saw Don Fidel use such a rattle, however, he would often massage the affected part of the patient's body, and accompany this with a vigorous and noisy sucking of the affected area. This is a common element of shamanic practice everywhere, the purpose of the sucking being to draw out a magical object which has been placed in the body of the patient by a sorcerer or malevolent shaman. Luna [1] has described in detail the magical symbols, objects, and powers which are intrinsic to the shamanic practices of the mestizo ayahuasqueros. Although Luna's field investigations were conducted among ayahuasqueros in Iquitos, Don Fidel's magical cosmology contained essentially the same elements, even though he lived several hundred miles to the south. Evidence based on two samples is hardly conclusive, but indicates that the magical cosmology shared among ayahuasqueros is remarkably consistent from region to region and between individuals; this may mean that it is derived from some much older, geographically and culturally restricted, tribal

tradition that has gradually been assimilated into mestizo folk medicine.

Although I visited the house of Don Fidel frequently during my field studies in the Pucallpa area, I was unable to witness the actual preparation of ayahuasca. The ayahuasca which Don Fidel used was usually prepared by his uncle, Juan Salas Coumari, a much older man of considerable repute as an ayahuasquero, who lived twenty km from Pucallpa. On one occasion I visited the house of Don Juan accompanied by Don Fidel and was able to see the ayahuasca, already cut up and set to boil, along with the leaves of the "chacruna" admixture (Psychotria viridis), in a large aluminum pot set over the fire in Don Juan's cooking shed. Preparation of ayahuasca is a multistage process, involving prolonged boiling of the plant material with several changes of fresh water, followed finally by combining the fractions and concentrating them over a low fire to a fraction of the original volume. Luna [2] has provided excellent photographic documentation of the steps in the preparation of ayahuasca. The length of boiling, the amount of plant material initially extracted, and the degree of final concentration, must all vary significantly, since considerable variation in alkaloid content is found in brews prepared by different ayahuasqueros (cf. Chapter IV).

An ethnobotanically interesting aspect of folk taxonomy related to the identification of the "proper" Psychotria spp. for use as admixtures came to light during the course of fieldwork. Some Psychotria species, including P. viridis, possess tiny spine-like extensions of the mid-rib on the abaxial

surface of the leaf. These appear to be slightly swollen glandular structures which may be equivalent to the "domatia" found in some Psychotria spp. (Gentry, A., pers. comm., 1981). A domatium is a part of a leaf, petiole, stem or other plant part that is inhabited by ants, mites, or other insects. The domatia-like structures of the Psychotria viridis specimens I examined appeared to be much too small to accomodate ants of any visible size, but may be inhabited by mites (Prance, G. T., pers. comm., 1983). These tiny domatia-like structures were pointed out by the ayahuasqueros I interviewed as the key feature used to identify the chacrunas suitable for use as admixtures. "Chacruna" is the vernacular term for Psychotria spp. All of the ayahuasqueros save one insisted that these structures--termed by them espinas (spines)--had to be present; plants lacking espinas were regarded as false chacrunas and were considered to have no value as admixtures. Indeed, all of the P. viridis collections which were analyzed (cf. Table VI) possessed these structures, and all contained DMT; the single specimen which lacked these structures also contained no tryptamines or other alkaloids. This specimen (DMCK #109) may correspond to Psychotria carthagenensis Jacq., although the collection is sterile and the identification therefore tentative; further doubt is cast on the identification by the fact that Rivier & Lindgren [3] reported DMT in Psychotria carthagenensis, while none was detected in DMCK #109.

I attended the weekly ayahuasca sessions held at Don Fidel's house on several occasions. They were always held at night, and would commence soon after dark. Various people from

the neighborhood attended, trickling by ones and twos into Don Fidel's dooryard, murmuring greetings to their friends as they took up positions in his "living room"--actually a thatched veranda open on one side to the cool evening air. Everyone sat around, smoking and talking quietly among themselves while they waited for the stragglers to arrive. Some patients desiring treatment from Don Fidel might come from considerable distances to attend the sessions; people were often late. Those attending appeared to be a typical cross-section of local mestizo society; some were regular participants who showed up every week; others attended only a single session for some specific treatment. Some of those attending were patients seeking treatment, while one or two men were apparently apprentices of Don Fidel's, there to learn the ayahuasca songs and the plant medicines; still other participants used ayahuasca "recreationally", or were merely curious about it. Not everyone who attended would necessarily take ayahuasca; some received some other form of treatment from Don Fidel, others merely watched. Usually everyone had arrived by about 9:30. Don Fidel would uncork the wine bottle of ayahuasca, which by this time had been appropriately blessed with songs and whistling and thoroughly fumigated with tobacco, and pour about 70 ml of the dark brown, intensely bitter liquid into a small gourd cup which he passed to each of the participants in turn. Everyone drained the cup at one gulp, grimacing as they handed it back so Don Fidel could pour the next libation. Don Fidel took the last draught himself, often taking two cupfuls to everyone else's one. After the ayahuasca had been consumed, conversation fell to a minimum. Everyone sat

quietly, waiting for the initial effects of the drug. Presently Don Fidel would extinguish the oil lamp, plunging the room into blackness. Not long after, the first of the many ayahuasca songs and chants would begin, and continue off and on throughout the night. A few of these were in Spanish, most were in Quechua, and some were a combination of the two languages. Their tenor and tempo was low and stately, reminding me of some medieval Russian Orthodox liturgical chants which I had heard years before. The visions and hallucinations began at about the same time as the singing, and seemed to be keyed into and sustained by the singing. The visions faded or became less interesting whenever the chanting and singing stopped; then a new song would trigger a renewed exfoliation of hypnagogic imagery. The nature of the visions is difficult to characterize. They did not have the intense, hard-edge, geometrical quality typical of "psychedelic" imagery; rather their colors were muted, rich in soft browns, greens, and reds, the sorts of colors one might encounter on the forest floor, or in ancient tapestries. Jungle plants with enormous leaves, twining lianas, and underwater scenes of large bony-plated fishes were common motifs. The drug did not always elicit profound mental changes in me; only on two occasions, when I persuaded Don Fidel to double my usual allotment, did I experience more than the very mildest effects. How much of the content of the visions, and the degree of response to the drug, were functions of my own physical and psychological state, is hard to determine. I suspect that one's diet, as well as body weight, may influence the amount of ayahuasca required to achieve threshold effects. Certainly the ayahuasqueros

themselves all insist that diet is a major factor [1]. Since I was heavier than most participants, and unaccustomed to the typical mestizo diet of yuco, plantains, and smoked fish, my absorption and metabolism of the drug may have been different and thus I required a higher dose. I never experienced the nausea or vomiting that usually forms an intrinsic part of the ayahuasca experience and which gives it a reputation as an excellent purge. Most of the other participants excused themselves to go have their purge in the backyard during the sessions; some went several times. Everyone kept an outward demeanor of calm during the sessions, and I did not observe any negative or fearful reactions. Like the host in Catholicism, ayahuasca is the central mystery but the experience is given coherence and meaning by the social and psychological set and setting. This was very carefully orchestrated and controlled by Don Fidel through his use of songs, chants, tobacco-blowing, and other ritual manipulations. By this means, he was able to insure that everyone had a positive experience and came away from the session with a sense of personal and spiritual renewal. The sessions would continue, punctuated by chants and singing, almost until dawn. No one ever took more than the initial dose of ayahuasca. Occasionally Don Fidel and one of his assistants would take time to minister to one of the patients (if any were present for treatment). This ministration invariably involved more singing, tobacco-blowing, and usually noisy and vigorous sucking, designed to draw out the magical poisonous object discussed above and in [1]. Eventually, after every patient had been attended to and the effects of the drug had begun to wane,

the session would draw to a close, and the participants would file out in the cool predawn hours to return to their own homes and daily lives.

B. Ethnographic Aspects of Orally Active Myristicaceous Pastes

Although mestizo folk medicine has changed as diverse tribal elements have been syncretically assimilated into it, nevertheless it remains a vital and living tradition. Ayahuasca is an intrinsic part of this tradition and there is little danger that its use will disappear in the foreseeable future. The situation is much different with respect to the orally-ingested Myristicaceous pastes. Knowledge of the preparation and uses of the orally active Virola pastes represents a body of tribal lore which is rapidly disappearing as the fragile tribal cultures become increasingly fragmented under the impact of Western culture. Unlike ayahuasca, whose contemporary use among mestizos is an amalgam of many traditions, the orally-ingested Myristicaceous drugs were never used outside the Bora, Witoto, and possibly the Muinane tribes [4,5,6]; even within these groups their use was a jealously guarded secret known only to the medicine men (Calle, H., pers. comm., 1971). The most detailed knowledge of the Myristicaceous drug is the possession of the older men.* It is they who know which of the many Virola

*Unlike ayahuasca, in which both men and women may participate, the use of the orally-active Myristicaceous drug is apparently an activity from which women are excluded.

species are suitable for the preparation of the drug; it is they who know the right admixture plants, and the proper way to extract and concentrate the tryptamine-laden Virola resin. Most of these men have long since died, and with them have died the ethnomedical traditions of their people. In the meantime, outside influences brought by the white men--alcoholism, Christianity, foreign diseases for which the indigenous people have little resistance, cultural and geographical dislocation occasioned by the ruthless exploitation of these tribes in the rubber industry in the early part of this century--all of these circumstances have had a shattering impact on these once proud and self-sufficient jungle peoples. Their ethnomedical knowledge has been swept away by the onslaught of "progress," along with their religion, cosmology, magic, art, poetry and music. They are a people waiting to die; they are regarded as little more than an embarassing reminder of the brutal past and an impediment to the future. As a result, the Bora and Witoto have become the victims of a not-so-benign neglect. The sooner contemporary historical, social, and economic forces have completed the decimation of these people, the sooner the way will be clear to implement the grand plan of Amazonian "development". This attitude, which unfortunately prevails among many Latin Americans today, is typical of that which has too often characterized the attitudes of Western European man toward aboriginal peoples in other times and places. Although the preceding remarks reflect my own opinions, formed as a result of necessarily incomplete personal observations, others [7,8] have documented the atrocities committed in the name of the rubber

industry and the current less than optimistic outlook for the continued survival of the indigenous Amazonian peoples.

The orally-ingested Myristicaceous pastes, like so much of Bora and Witoto culture, are only a fading memory. They are remembered, by some of the middle-aged and older men, as something that was once used by their fathers and grandfathers. The purposes for which the drug was used seem to have been largely forgotten, the identification of the proper source-plants has become tentative, the method of preparation is now uncertain, a subject of debate among themselves. This is essentially the situation I encountered when our party arrived at the little Witoto/Bora village of Puco Urquillo an the Rio Ampiyacu, just a short ride by motorboat from the Peruvian river town of Pebas, at the confluence of the Ampiyacu and the Amazon. My observations are not unique; others [5,6] have remarked on the fairly loose grasp informants in this region had on the botanical and ethnopharmaceutical fine points associated with the preparation of the Myristicaceous pastes. The North American scientist comes in his motorboat, bringing money and sugar and salt, shotgun shells and cigarette lighters and malaria pills, and many other coveted items. He asks the informants to teach him the secrets of their fathers and their fathers' fathers, assuring them that these matters are of interest to his white-coated colleagues in their shining hospitals in America, Canada, Europe. Well, it seems a small enough favor to ask; they are impressed by the friendly visitors, and are eager to acquire the attractive items they have brought to trade; so, they are happy to help out, they are anxious to show their goodwill. Perhaps if

they co-operate they will receive a few extra shot gun shells, or a pretty piece of cloth for their wife or sister. So they set out, with the best will in the world, to lead us to the plants, to show us how the paste is made, to take us through the procedure step by step, as best they can reconstruct it according to the formula of the long dead medicine men. What does it matter if a few details have been forgotten, if one or two of the plants which were formerly used, which grew, perhaps, in their original home but not in this new village spawned by the migration, what does it matter if these minor details have been lost or forgotten or deliberately omitted? The American scientist will go away satisfied. How is he to know if the drug has been prepared in exactly the manner prescribed by the ancient medicine men? How, for that matter, are the Bora and Witoto themselves to know?

I encountered three informants who were willing to provide samples of the oral Virola pastes during our two-week stay in the Rio Ampiyacu region. Two of these informants, Jorge Churay and Marcos Vega Flores, were Bora Indians; Jorge Churay lived in Puco Urquillo while Marcos Flores lived in Brillo Nuevo, a small village about half a day by motorboat upriver from Puco Urquillo, on the banks of the Yaguasyacu, a tributary of the Ampiyacu. The population of Brillo Nuevo was Bora while that of Puco Urquillo was roughly half Bora and half Witoto; each group inhabited a different part of the village separated by about half a kilometer. The Bora and Witoto in Puco Urquillo did not get along very well; there was an uneasy truce between them that reminded me of the situation that might exist between

Protestants and Catholics sharing the same Boston ghetto. The third informant, Alfredo Moreno, in addition to being the local shaman, was the headman of the Witoto part of Puco Urquillo. Don Alfredo was by far the oldest of these informants, and also was the most secretive about the method of making the paste. He agreed to provide me with a sample of "oo'-koey" as he called it, but would not allow me to accompany him to find the tree or to observe or photograph the procedure followed in its manufacture. Only after he had delivered his first sample, several days after we had struck our bargain, did he take me to visit the cumala that he had cut down (cumala is a generic vernacular term for Myristicaceous species in Peru) so that I could collect bark and voucher specimens. The first sample, derived from V. sebifera, (DMK-40) turned out to have high levels of tryptamines (cf. Chapter V) and definitely exhibited some oral activity in a subsequent bioassay (see below). Before departing for Brillo Nuevo on the second leg of our fieldwork, I arranged with Don Alfredo to purchase three more paste samples; I was to pick them up on our way back downriver in about ten days time. I paid for the samples in advance, the enormous sum of nearly thirty dollars, which for Don Alfredo was probably a small fortune. It was not enough, apparently, to make an honest man out of him. The samples were waiting for us on our way back through, each small banana-leaf packet of paste accompanied by its own bundle of Virola leaves collected as vouchers (DMK-67,68, and 69); however, analyses (Chapter V) showed very much lower levels of tryptamines in these pastes than in the first sample. Also, the similarities of the voucher specimens and the

almost identical chemical profiles of these samples led me to conclude that I had been given three samples which had all been derived from the same tree. This was contrary to our agreement but of course did not become clear until months later when the samples were analyzed in Canada. The task of locating three different suitable specimens to prepare three different paste samples is considerable, and I do not fault Don Alfredo for taking the easy way out; after all, he was just trying to make some easy money. I do not know to this day whether the exceptionally low tryptamine levels found in these three paste samples were a result of deliberate deception on the part of Don Alfredo, or whether he just happened to select a specimen that was poor in the active compounds. Subsequent analyses (Chapter V) have shown that there is considerable variation from plant to plant. Despite the fact that the last three samples received from Don Alfredo were disappointing (the question of their oral activity remains unresolved, since these three samples were immediately preserved in methanol and were not bioassayed) I still feel that Don Alfredo's knowledge of the pastes was the most extensive and the closest to the "real" tradition. This feeling is partly based on his first sample, which had high levels of tryptamines and did exhibit oral activity, and partly on the fact that he pointed out several other "suitable" Virola specimens which proved to contain substantial amounts of the right kinds of tryptamines; he also seemed to have a more extensive knowledge of admixtures than the other informants I contacted. In fact, Don Alfredo utilized two unusual admixtures in making the paste which have not been reported in the

literature. One of these was a fern, Anemia sp., (DMK-39), the leaves of which are made into a tea; the water from this infusion is then used to cook the Virola resin. The other admixture was a green crustose lichen of indeterminant identity, which was growing on the bark of a small tree, Rinora racemosa (Mart. & Zucc.)Kuntze (DMK-38) (Violaceae); scrapings of this lichen were added to the Virola resin while it was simmering.

Jorge Churay and his brother-in-law Rey were our Bora informants in Puco Urquillo. They freely admitted that they had never used the pastes, but claimed that their fathers had done so and that they had watched its manufacture many times. They agreed to take us to collect bark samples to make the "ku'-ru-ku", the Bora name for the Myristicaceous pastes, and also to point out some of the different kinds of cumalas which are recognized by the Bora. The Bora distinguish 15 kinds of cumala and have different vernacular designations for each. Not all of them would be considered different by a taxonomist. (cf. Appendix II). One morning we set out, in the company of Jorge Churay, Rey, and two other Bora to find a suitable cumala to make "ku'-ru-ku". The Boras pointed out several cumalas to us along the way, but these were passed over as "not strong". Finally we came to a large tree, about 70 feet high with a DBH of about 25 cm (DMK-34, V. pavonis). They removed the bark in long strips from the lower part of the trunk. The inner cambial layer exuded a copious resin with a very aromatic spicy odor; this resin was clear but immediately oxidized to a reddish-orange color. After we had brought the bark back to the main Bora malocca in Puco Urquillo, the Bora proceeded to chip away

the outer layers of the bark using a machete. The inner phloem layer with the cambium was then pounded into long fibrous strips with an ax-head. These were stuffed into a large aluminum pot and tamped down into the bottom of the vessel. A minimal amount of water--barely enough to cover the strips of bark-- was added and then the mixture was boiled over a low fire for about 3 hours. After cooling, the bark strips were removed and thoroughly rung out to remove excess water; the resulting reddish-brown liquid was concentrated further to a thick paste; this was mixed with an approximately equivalent amount of sifted ashes of Cecropia sp. Ashes of the same species are also added to the powdered coca which most of the Bora men chew frequently. No other admixtures were added to this paste sample. The completed "ku'-ru-ku" was a dark reddish-brown, flecked with black, and was essentially odorless and tasteless; it had lost most of the spicy, sharp odor which had characterized the "raw" Virola resin.

The following evening I bioassayed this sample in an attempt to determine whether it was in fact an orally active hallucinogen. It produced no effect at all, despite the fact that I had fasted previously and over the course of the evening ingested several times the recommended dose. Subsequent analysis revealed that this particular paste sample as well as the source-plant from which it was derived (DMK-34) did not contain alkaloids of any sort (Chapter V). A second sample made by Jorge Churay (for which vouchers are unavailable) was also completely without activity. I think that their failure to select a suitable species on two occasions is an indication of just how

much the tribal knowledge of this oral hallucinogen has deteriorated over the last few decades. Schultes [5,6] has also pointed out that sometimes species indicated by native informants as suitable for making the pastes were found not to contain any active tryptamines. Although some informants remember how the pastes were once prepared, since they themselves no longer utilize the drug, their botanical grasp of just which species are suitable has become somewhat loose.

Another paste sample was made by Marcos ("el nino") Vega Flores, a Bora from the village of Brillo Nuevo, upriver from Puco Urquillo. Don Marcos, although not a particularly old man (I placed him at about 45 years old) was highly regarded in Brillo Nuevo as an expert on drugs, poisons, and medicines of all sorts. He had learned most of it from his father, Eugenio Flores, now in his mid-70s, from whom he had inherited the office of village medicine man. Don Marcos prepared the paste quite differently than the Bora at Puco Urquillo had done. For one thing, he took a lesser amount of bark from the tree, and was careful to take it from one side only. He explained that this was so that the tree would not die. The tree was a flowering specimen of V. elongata (DMK-59). Once back at his hut, instead of chipping off the outer bark with a machete, he carefully removed only the innermost cambial layer. This he stripped off in long flexible fibrous strips, only a few millimeters thick. The remainder of the bark was discarded. He beat the cambial strips with a wooden mallet, then dipped them into a vessel of water and rung the juice into a small enamel bowl. He repeated this wringing two or three times for each

strip, then discarded it. The bowl containing the cambial wringings was simmered until the liquid was concentrated to a thick paste about the color and consistency of chocolate syrup. This was then mixed with ashes made from the dried fruit husk of Theobroma bicolor (DMK-64) until it was the consistency of thick dough, and could be rolled between the fingers into a little ball. Prior to addition of the ashes, I dipped my finger into the syrupy paste and placed a dab on the tip of my tongue, as Don Marcos indicated I should do. I immediately felt a sharp, burning sensation, which turned my tongue numb for a few minutes; at such a low dose, however, I did not expect any hallucinogenic activity, nor was there any.

Four of the seven paste samples which were collected in the Rio Ampiyacu area were tested for oral activity, either by myself or by other persons in our party; in addition, the La Chorrera "oo'-koey", collected at La Chorrera, Colombia, in 1971, was also tested. The two paste samples made by Jorge Churay at Puco Urquillo were completely inactive as hallucinogens and appeared to be physiologically inert, even though an amount several times that supposedly required was ingested. The first sample made by Alfredo Moreno at Puco Urquillo (made from V. sebifera, DMK-40) appeared to exhibit some degree of oral activity at the dosage assayed. No physiological symptoms were noted. The activity detected consisted of the elicitation of hypnagogic imagery with the eyes closed. The subject who assayed the sample felt that oral activity was definitely present, and that a larger dose might have triggered the full spectrum of hallucinogenic effects. On

analysis, this sample proved to contain substantial concentrations of DMT and 5-MeO-DMT, although no β-carbolines were detected. The fourth sample assayed was derived from V. elongata (DMK-59) and was made by Marcos Flores at Brillo Nuevo. This sample proved to be the most physiologically active of any of the four assayed. Oral ingestion of approximately a gram and a half of the paste elicited a rapid and profound response, characterized by considerable physiological distress rather than by the perceptual and psychological disturbances usually typical of hallucinogens. The initial effects, which manifested within ten minutes following ingestion, consisted of a strong burning sensation in the mouth and throat, quickly developing into a feeling of numbness in the lips, tongue, and throat. Swallowing became difficult and breathing was labored. The numbness gradually spread over the rest of my body, and my limbs felt a tingling sensation in their extremities. My body felt heavy and inert, and I became chilled. My mind was racing and felt dissociated from my body. I became somewhat alarmed at the rapidity of onset of the symptoms and felt physically most uncomfortable. I focused on my own breathing, which was irregular and shallow, and concentrated on trying to maintain a steadier, deeper rhythm of breathing. I found myself reflecting on the use of Virola as an arrow poison, and felt that I now understood how it might be effective in this way; I felt incapable of any physical exertion, and thought that any further stress placed upon my respiratory system might be dangerous if not lethal. There were no hypnagogic images, perceptual changes, or hallucinations, but I had the feeling that they might have

manifested had I taken a slightly higher dose. Acuity of hearing proved to be greatly heightened, and I was extremely sensitive to the constant background hum of insect noises from the forest. The symptoms of physical distress persisted for about one half-hour to 45 minutes, than gradually faded. Breathing became easier, and I became drowsy and fell into a state of "twilight" sleep which may have lasted ten or fifteen minutes. Gradually most of the symptoms disappeared, but I continued to feel chilled for most of the night. The over-all effect of the paste sample was more akin to the actions of a pressor amine or general anaesthetic than a hallucinogen, but the bioassy left no doubt that the sample did definitely possess oral activity. It was not particularly pleasant or enjoyable, however. On later analysis, this sample was found to contain a high concentration of 5-MeO-DMT and a trace of NMT; several biologically active lignans were isolated from DMK-59 by Don MacRae (MacRae, unpublished data, 1982), and these may well have contributed to the pharmacological activity. No β-carbolines were detected in this paste sample. The fifth paste sample which has been subjected to human bioassay is the La Chorrera "oo'-koey", collected in 1971 in Colombia. No hallucinogenic or other physiological activity was ever detected with this sample in several trials, even though excessive doses were ingested on some occasions. Interestingly, this sample contained substantial amounts of tryptamines and detectable amounts of tetrahydro-β-carbolines (cf. Chapter V).

Although the limited numbers of human bioassays reported here are not conclusive, they do indicate that the oral activity

of these Myristicaceous pastes may be determined by variables other than the fortuitous combination of tryptamine derivatives and β-carbolines. The paste sample showing the greatest activity contained only a single alkaloid, 5-MeO-DMT, which is supposedly not orally active; on the other hand, the La Chorrera sample, which did contain the active tryptamines and β-carbolines in combination was completely without oral physiological activity. Samples lacking alkaloids also had no activity, and this result is at least consistent with expectations. The factors determining presence or absence of oral activity in those samples which do contain alkaloids still remain unelucidated. The results of these human trials, however, considered together with those obtained from the in vitro MAOI assays of the paste samples (cf. Chapter VI) indicate that the oral activity is not due simply (or solely) to the oral potentiation of the tryptamines by the β-carbolines. In fact, the alkaloidal profiles of the Myristicaceous paste samples has been shown to be highly variable, reflecting the chemical variability in the source-plants used to prepare the drug (cf. Chapter V). This, in turn, would probably result in considerable variation in pharmacological activity from sample to sample. In fact, the difficulties encountered in exercising some degree of pharmaceutical quality-control over the composition of different batches of the drug may explain some of the ethnopharmacological puzzles about these pastes, e.g., the fact that they are made and used secretly by the medicine men, the fact that they have never been used outside the Bora, Witoto, and Muinane groups although Virola snuffs are widely used in the Amazon Basin; and

the fact that their use even in these tribes has diminished or disappeared as contacts with outside cultures have increased.

III. Literature Cited

1. Luna, L. E. (1983) The Concept of Plants as Teachers Among Four Mestizo Shamans of Iquitos, Northeast Peru. Paper presented at the Symposium on Shamanism, XIth International Congress of Anthropological and Ethnological Sciences, Phase 2. Vancouver, B. C., August 20-23 1983.

2. Luna, L. E. (1983) Don Emilio y sus Doctoricitos. Film presented at XIth International Congress of Anthropological and Ethnological Sciences, Phase 2. Vancouver, B. C., August 20-23 1983.

3. Rivier, L. & J. Lindgren (1972) Ayahuasca, the South American Hallucinogenic Drink: Ethnobotanical and Chemical Investigations. Economic Botany 29:101-129

4. Schultes, R. E. (1969) Virola as an Orally-administered Hallucinogen. Harvard Botanical Museum Leaflets 22:229-40

5. Schultes, R. E., T. Swain, & T. Plowman (1977) Virola as an Oral Hallucinogen Among the Boras of Peru. Harvard Botanical Museum Leaflets 25:259-272

6. Schultes, R. E. & T. Swain (1976) Further Notes on Virola as an Oral Hallucinogen. Journal of Psychedelic Drugs 8:317-324

7. Collier, Richard (1968) The River that God Forgot: The Story of the Amazon Rubber Boom. E. P. Dutton & Co., New York.

8. Shoumatoff, Alex (1978) The Rivers Amazon. Sierra Club Books, San Francisco.

PART III: PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATIONS

CHAPTER IV: ALKALOID CONSTITUENTS OF *AYAHUASCA* BREWS, SOURCE-PLANTS, AND ADMIXTURE PLANTS

I. Introduction

The hallucinogenic beverage *ayahuasca* (Quechua for "vine of the souls") is widely used for medicinal, ritual, and recreational purposes by the aboriginal and mestizo populations inhabiting the Amazon Basin. While *ayahuasca* is the most common term for the drug in the Peruvian Amazon, in different regions it is known by various vernacular names, including *yage*, *caapi*, *natema*, and *pinde* [1]. The bitter, coffee-colored beverage is prepared by boiling the bark of the jungle liana *Banisteriopsis caapi* (Spruce ex Griseb.) Morton (Malpighiaceae) together with the leaves of various admixture plants, the addition of which is believed to intensify or modify the effect [2]. Many of the admixture plants used remain uncharacterized either botanically or chemically, however those used most commonly are *Psychotria viridis* Ruiz & Pavon, *Psychotria carthagenensis* Jacq., and *Diplopterys cabrerana* (Cuatrecasas)Gates; the latter is also a liana in the Malpighiaceae formerly known as *Banisteriopsis rusbyana* [3, Plowman, T., pers. comm., 1980].

The most complete study to date of the chemistry of the *ayahuasca* beverage, and of the source-plants and admixture plants used in its manufacture, is that of Rivier and Lindgren [4]; these investigators studied *ayahuasca* samples and vouchered botanical material collected among the Sharanahua and Culina tribes of the upper Rio Purús in Peru. Using GC/MS as the primary analytical method, Rivier and Lindgren found that the β-carboline alkaloids harmine, harmaline, and tetrahydroharmine,

and DMT were the major active constituents of ayahuasca (cf. Fig. 1 & 2). The β-carbolines are constituents of Banisteriopsis caapi [5] while N,N-dimethyltryptamine has been reported in the two Psychotria spp. [4] as well as in D. cabrerana [6]

Hashimoto et al. [7,8] have reported the isolation of 6 β-carboline bases from the leaves of Banisteriopsis caapi in addition to the three main constituents (viz.: harmic amide, acetyl norharmine, ketotetrahydronorharmine, harmine N-oxide, harmic acid methyl ester, and harmalinic acid). However the extremely low concentrations of these compounds in the plant (.007-.0001 %) make it unlikely that they contribute significantly to the pharmacological activity of ayahuasca.

Although the active alkaloids of ayahuasca are now known, certain aspects of the pharmacology of the drug remain to be clarified. DMT is known to be a potent hallucinogen but is also known to be inactive when ingested orally [9], probably due to deamination by intestinal and hepatic monoamine oxidase (MAO). The β-carbolines present in B. caapi are known to be highly active reversible inhibitors of MAO [10,11,12] and are probably also hallucinogenic. The psychotomimetic activity of the β-carbolines is not well understood because there are relatively few clinical investigations of the effects of β-carbolines on human subjects. Naranjo [13] reported harmaline to be hallucinogenic at oral doses of 4 mg/kg but could not report similarly unequivocal results for harmine or tetrahydroharmine. Pennes & Hoch [14] reported harmine was orally inactive at levels approaching 12 mg/kg. It has been suggested [2,6,9,15,16] that the hallucinogenic properties of the crude ayahuasca brew

result from a synergistic interaction among the various constituents; specifically, that it results from an oral activation of the DMT through the inhibition of MAO by the β-carbolines. This mechanism would render the DMT orally active by blocking its degradation by visceral MAO. Although this mechanism is reasonable and has long been accepted in the ethnopharmacological literature, the effect of ayahuasca on monoamine oxidase has not been experimentally determined. This chapter presents the results of phytochemical investigations of the alkaloid constituents of ayahuasca and the source-plants used in its manufacture. An evaluation of the effect of ayahuasca on MAO in vitro is reported in Chapter VI.

II. Materials and Methods

A. Field Collection of Drug Samples and Plant Materials

Collection numbers cited throughout this paper refer to the personal collection numbers of D. McKenna, with the exception of Plowman 6040 (Diplopterys cabrerana) and Plowman 6041 (B. caapi var. "cielo"). Plant material for Plowman 6040 and 6041 was kindly supplied by Dr. Timothy Plowman of the Field Museum in Chicago. Authenticated herbarium vouchers for all collection numbers cited have been deposited in the Herbarium of the Dept. of Botany, University of British Columbia. Duplicate vouchers of most collections are also on deposit at the Chicago Field Museum.

During ethnobotanical fieldwork in the spring of 1981, eight samples of ayahuasca preparations were obtained from

ayahuasqueros living on the outskirts of the Peruvian towns of Iquitos, Pucallpa, and Tarapoto. These samples were qualitatively analyzed using 2-dimensional TLC and quantified using HPLC. Identification of alkaloids was based on comparison with authentic standards. Ayahuasca samples used for analysis are identified in this chapter by the name of the ayahuasquero from whom they were obtained, and also by a number. In some cases more than one sample was obtained from the same person. See Chapter III for details of methods used in the collection of drug samples and source plants for phytochemical analysis; information on herbarium voucher collections is tabulated in Appendix II.

B. Two-dimensional Thin-layer Chromatography

The fluorescence characteristics, Ehrlich's color reactions, and Rf values in two solvent systems were determined for the tryptamine and β-carboline standards used in this study (Table II). Two-dimensional TLC of the tryptamine and β-carboline standards is shown graphically in Fig. 3; Fig. 4 illustrates the Rf values of selected mixtures of tryptamine standards following 1-dimensional development in solvent 1 and solvent 2.

Two-dimensional thin-layer chromatography was carried out using 10 x 10 cm Polygram Silica Gel G UV^{254} precoated plates (Brinkmann Instruments). The origin was marked with pencil in the lower left hand corner of the plate, 1.0 cm from the bottom and left-hand edge. Five μl aliquots of the material to be analyzed (consisting either of the crude ayahuasca samples or in

TABLE II: HRF* VALUES OF TRYPTAMINE AND β-CARBOLINE STANDARDS†

Compound Name	Solvent 1‡	Solvent 2#	UV fluorescence	Ehrlich's reactionþ
β-carbolines:				
norharman	62.7	84.8	blue	-
harman	67.9	84.8	dark blue	-
harmalan	49.0	67.7	grey-green	ntξ
tetrahydro-harman	30.0	40.3	nf - UVAΦ	nt
tetrahydro-3-carboxy-harman	0.0	55.9	nf - UVA	nt
harmine	58.2	82.9	dark blue	-
harmaline	33.2	45.7	aqua	-
tetrahydro-harmine	24.0	35.7	nf - UVA	lt. blueψ
harmol	35.7	81.3	dark blue	-
harmalol	17.3	35.7	aqua	-
6-MeO-harman	66.0	82.7	lt. blue	-
6-MeO-harmalan	44.3	56.8	tan	-
6-MeO-tetrahydro-harman	26.0	34.1	nf - UVA	nt
brevicoline	56.5	79.5	dark blue	nt
mean % standard deviationω =	6.24	5.5		
Tryptamines:				
l-tryptophan	0.0	51.5	nf - UVA	blue-gray
5-hydroxy-l-tryptophan	0.0	46.2	"	blue-gray
tryptophol	75.1	85.6	"	grey
tryptamine	35.0	29.5	"	blue-gray
5-hydroxy-tryptamine	13.5	18.9	"	gray
5-MeO-tryptamine	29.4	25.0	"	lt. blue
6-MeO-tryptamine	29.3	24.0	"	dk. blue
gramine	28.8	25.9	"	lt. violet
N-methyl-tryptamine	10.7	17.8	"	blue-gray
DMT	41.3	41.9	"	blue-gray
5-hydroxy-DMT	16.4	28.7	"	lt. blue
5-MeO-DMT	35.5	37.1	"	lt blue
psilocin	42.9	41.6	"	dk. violet
psilocybin	0.0	3.2	"	lt. violet
melatonin	46.3	83.7	"	lt. blue
5-MeO-diisopropyl-tryptamine	93.7	54.1	"	lt. blue
mean % standard deviationω =	1.7	8.0		

$$* \text{ hRf} = \frac{\text{distance migrated by compound}}{\text{distance migrated by solvent}} \times 100$$

† All standards used in TLC and in the MAO assays reported in Chapter VI were purchased from Sigma Chemical Co., St. Louis, Mo, or Aldrich Chemical Co., Milwaukee, Wis., with the following exceptions: Psilocybin and psilocin standards (Sandoz: lot numbers 8001 & 5001, respectively) were gifts of the Chief of Scientific Services, Health and Welfare Canada, Ottawa, Ontario; 5-MeO-diisopropyltryptamine and 3-[2-(2,5 dimethyl)pyrrolylethyl]-indole were gifts of Dr. B. Abeysekara, Radiopharmaceuticals, Inc., Vancouver, B. C.; 6-MeO-MTHβB was a gift of Dr. Bo Holmstedt, Karolinska Institute, Stockholm; brevicoline was a gift of Dr. E. Leet, Dept. Of Chemistry, University of Minnesota, Minneapolis, Minn.; tetrahydroharmine was synthesized from 6-MeO-tryptamine (Sigma) and acetaldehyde, according to the method of Akabori and Saito [29].

‡ Solvent 1: ether/2-butanone/conc. NH_4OH 5:4:1 (upper phase)

Solvent 2: n-propanol/1.5% NH_4OH 9:2

þ reaction to Ehrlich's reagent (cf. [20])

ξ nt= not tested

Φ nf - UVA = not fluorescent; visible as UV-absorbing spot under short wave UV

ψ Slow color-reaction visible after 24 hours

$$\omega \text{ mean \% standard deviation} = \frac{\text{mean standard deviation}}{\text{mean hRf}} \times 100$$

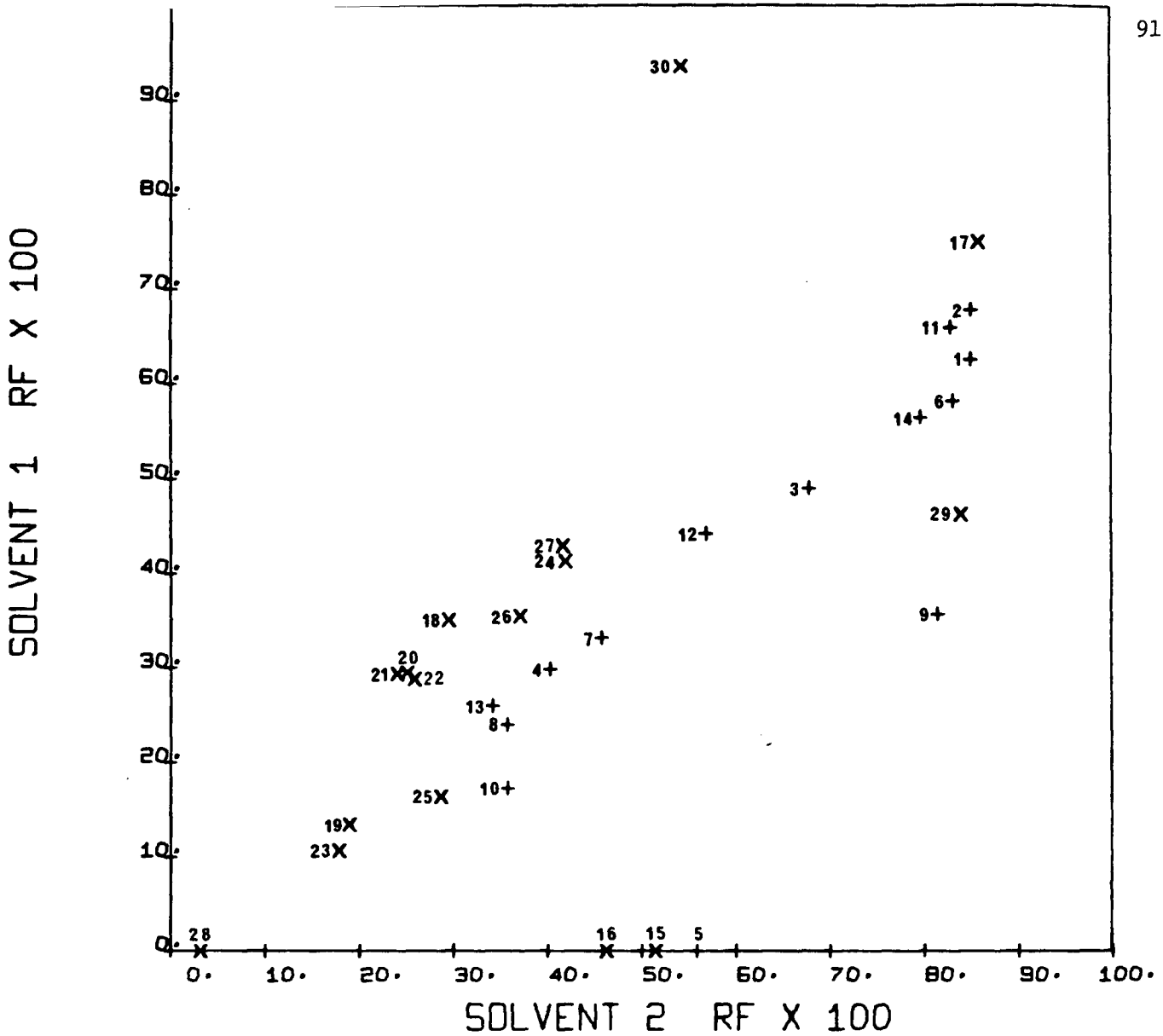


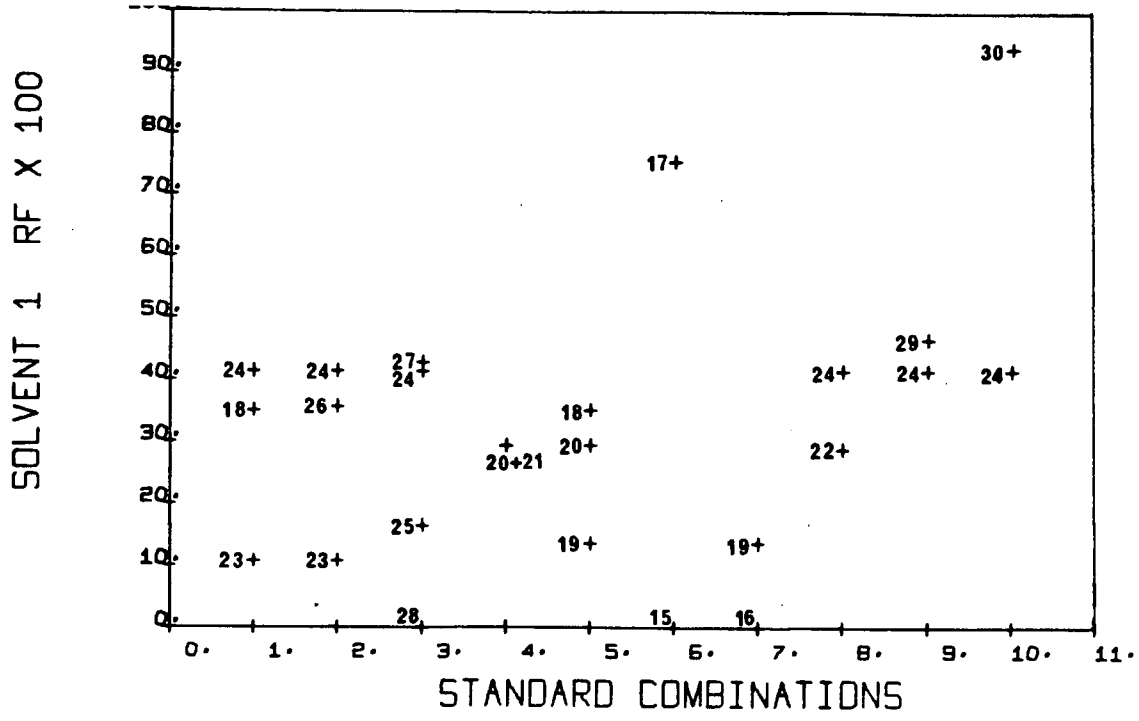
Figure 3 - Positions of Tryptamine and β-carboline Standards in Two Dimensional Thin Layer Chromatography

+ = β-carbolines

1. Norharman
2. Harman
3. Harmalan
4. Tetrahydroharman
5. Tetrahydroharman-3-carboxylate
6. Harmine
7. Harmaline
8. Tetrahydroharmine
9. Harmol
10. Harmalol
11. 6-MeO-harman
12. 6-MeO-harmalan
13. 6-MeO-tetrahydroharman
14. Brevicoline

X = Tryptamines

15. L-tryptophan
16. 5-hydroxy-l-tryptophan
17. Tryptophol
18. Tryptamine
19. 5-hydroxy-tryptamine
20. 5-MeO-tryptamine
21. 6-MeO-tryptamine
22. Gramine
23. N-methyl-tryptamine
24. N,N-dimethyltryptamine
25. 5-hydroxy-N,N-dimethyltryptamine
26. 5-MeO-N,N-dimethyltryptamine
27. Psilocin
28. Psilocybin
29. Melatonin
30. 5-MeO-diisopropyltryptamine



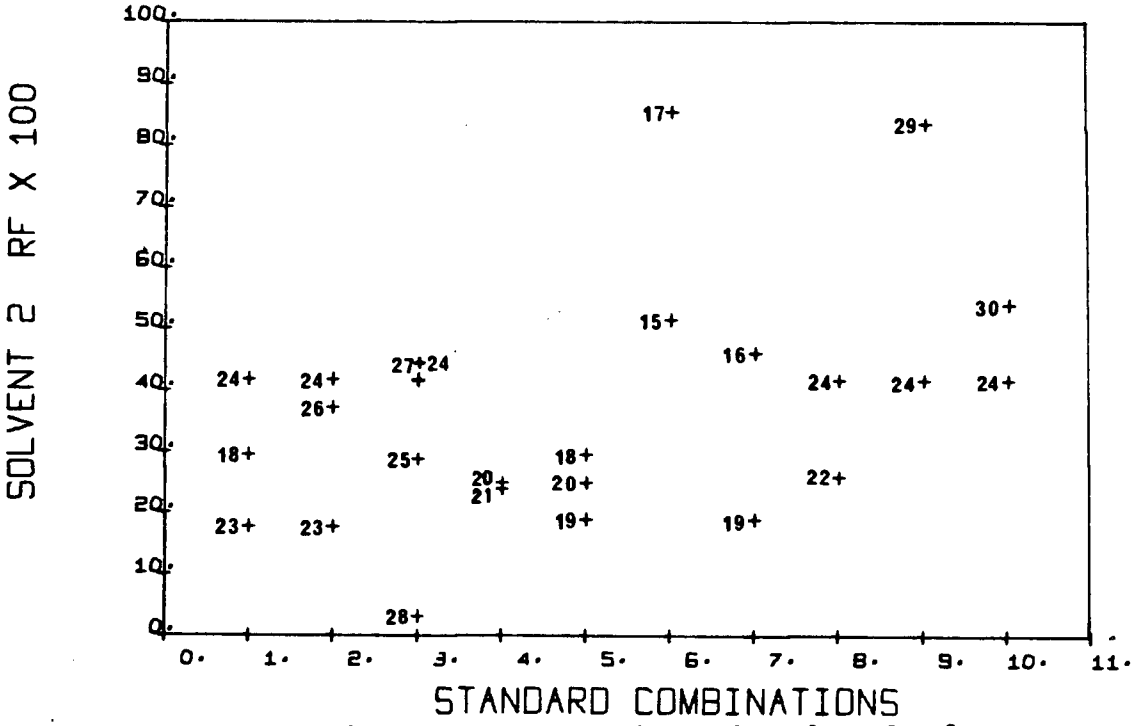


Figure 4 - One Dimensional TLC of Mixtures of Tryptamine Standards

Composition of standard mixtures:*
1. Tryptamine + NMT + DMT
2. DMT + 5-MeO-DMT + NMT
3. DMT + 5-HO-DMT + psilocin + psilocybin
4. 5-MeO-tryptamine + 6-MeO-tryptamine
5. Tryptamine + 5-MeO-tryptamine + 5HT
6. L-tryptophan + tryptophol
7. 5-hydroxy-l-tryptophan + 5HT
8. DMT + gramine
9 DMT + melatonin
10. DMT + 5-MeO-diisopropyltryptamine
* Numbering of individual constituents is according to key in Fig. 3.

the case of the admixture plants of a methanol solution of the purified alkaloid fraction) were applied to the origin using a Microcap applicator. The applied sample was dried under a gentle stream of air, and then developed in the first direction using ether/2-butanone/conc. NH_4OH 5:4:1 (Solvent 1). Solvent 1 was freshly prepared in a separatory funnel and the upper phase was collected for TLC. Following development in Solvent 1, the plates were removed and allowed to air dry in a fume hood. Development in the second dimension was commenced when the plates were completely free of Solvent 1, indicated by the absence of any solvent odor. Plates were then rotated 90° to the left with respect to their position in Solvent 1, and developed in Solvent 2, consisting of n-propanol/1.5% NH_4OH 9:2; this solvent was stable for 2-3 days at room temperature if kept sealed in a ground-glass stoppered flask. Development in both Solvent 1 & 2 was carried out at ambient temperature in an unlined 10 x 30 x 26 cm glass chromatographic tank containing 50 ml ± 5 ml of solvent. Following development in Solvent 2, plates were removed and air-dried in a fume hood for 30-60 min or overnight on the laboratory bench. Plates were examined under short- and long-wave UV light to visualize the alkaloids. DMT and tetrahydro-β-carbolines are visible as dark spots under short-wave UV while the aromatic and dihydro-β-carbolines give characteristic strong fluorescent colors under long-wave UV. Duplicate plates were sprayed with Ehrlich's reagent [17] which gives blue to violet colors with DMT and other tryptamine derivatives following exposure to HCl vapors. Aromatic and dihydro-β-carbolines do not react with Ehrlich's reagent however

tetrahydroharmine gives a characteristic robin's egg blue color which develops over 24 hr. This slow reaction can be used to distinguish tetrahydroharmine from its more aromatic analogues and also from the tryptamines which give darker blue reactions that appear within 30 min of exposure to HCl vapors. A TLC plate containing aliquots of known β-carboline and tryptamine standards was developed simultaneously with the sample plates; constituents in the samples corresponding to known standards could thus be readily identified by comparison of the sample plates with the "standard" plate (cf. "ayahuasca analogue", Fig. 6).

C. High Pressure Liquid Chromatography (HPLC)

1. Analytical conditions

A Varian model 5000 HPLC interfaced with a Spectra-physics model SP4100 computing integrator was used for the quantitative analysis of the ayahuasca samples, the Banisteriopsis caapi cultivars, and the DMT-containing admixture plants (Fig. 5). Constituents were detected by UV absorption at 260 nm with a Varian model 634 variable wavelength UV/visible spectrophotometer. Column consisted of a Varian Micropak MCH-10 reverse phase column, 30 cm x 4 mm i.d. Solvents were methanol/water containing 0.05% triethylamine. A gradient elution program was used for the analysis, from 60-90 % methanol at a rate of 1%/min. Solvent flow rate was 2 ml/min. Samples were applied to the column via a Rheodyne model 7125 syringe loading sample injector fitted with a 20 μl sample loop. Samples

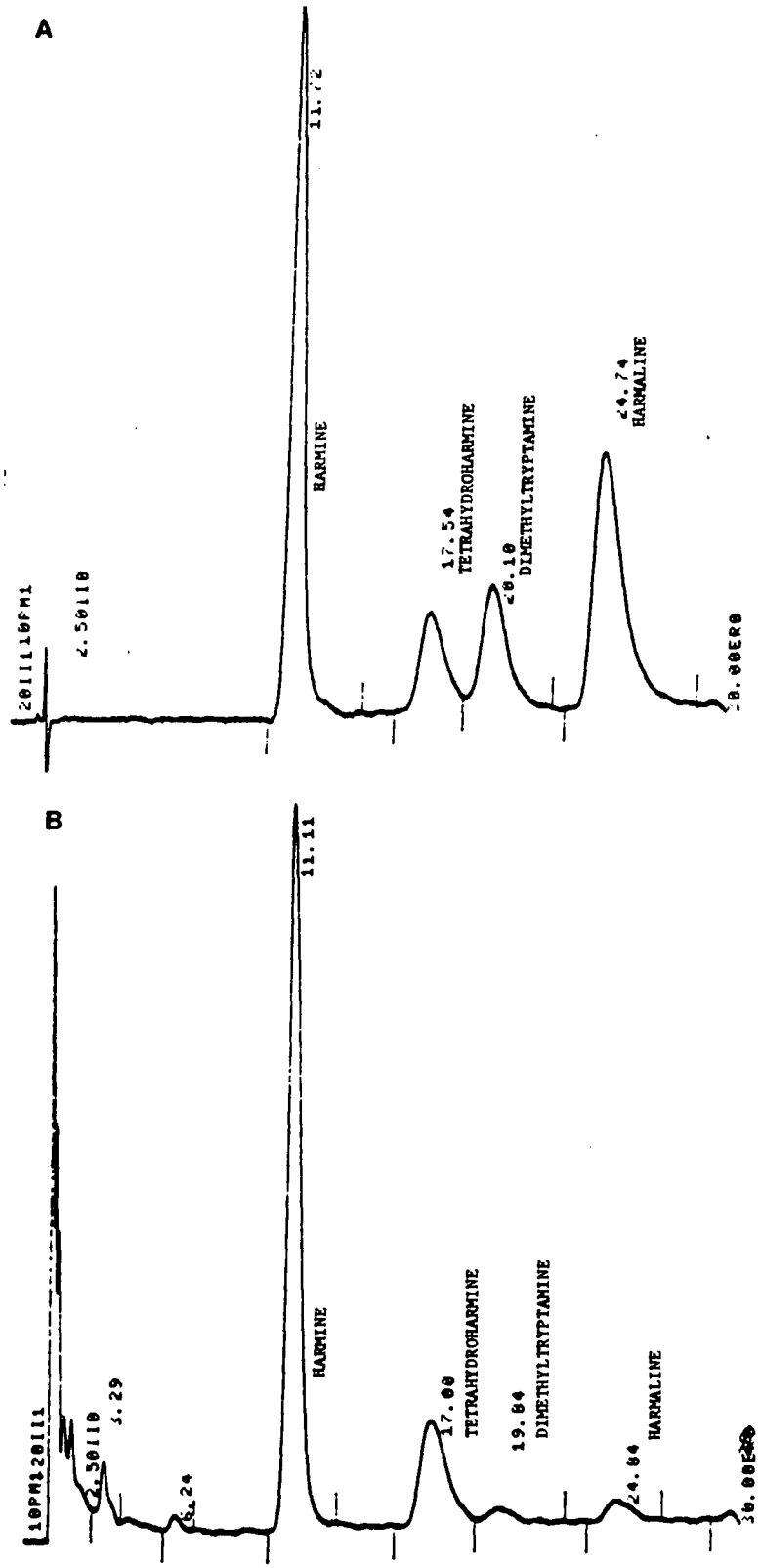


Figure 5 - HPLC Elution Profile of Peruvian Ayahuasca
A.) Alkaloid standards. B.) Ayahuasca sample.

Analytical Conditions:
Solvent: methanol/H_2O containing 0.05% triethylamine
Column: Varian Micropak MCH-10, 30 cm x 4 mm i. d.
Program: 60 - 90 % methanol, 1%/min; flow rate = 2 ml/min
Detection: 260 nm, Varian Model 634 UV/visible spectrophotometer

were loaded onto the sample injector using a 25 µl Hamilton #702 microliter syringe fitted with a #22 gauge 90° beveled needle.

2. Quantitative methods

Quantitative analyses were carried out using the external standard program supplied with the SP4100 computing integrator. In this method, the column is calibrated with a mixture containing known concentrations of standard compounds. The integrator program calculates the peak area of each standard in the calibration mixture and normalizes the response factor. Calibration at two or more concentrations enables the integrator to generate the coefficients of a linear equation relating sample concentration to peak area. This calibration data is stored in the memory circuits of the integrator and is used to calculate the concentration of components of interest in subsequent sample runs by comparison of the peak areas of sample components with the peak areas of standards in the calibration mix.

Stock solutions of harmine, harmaline, tetrahydroharmine and DMT standards were made up to a precise concentration of 1 mg/ml. Equal aliquots from the stock solutions were combined, giving a calibration mixture of 0.25 mg/ml of each component; 1:1 dilution of a portion of this mixture with methanol gave a second calibration mixture in which the concentration of each component was 0.125 mg/ml. The SP4100 integrator was calibrated by making single 20 µl injections of the calibration mixture at each concentration level. The integrator was recalibrated following every five sample injections.

3. Sample preparation

a. Ayahuasca samples. The ayahuasca samples which had not been diluted with methanol on collection were diluted for analysis so that the alkaloids present were within the concentration range of the calibration standards. A 1.5 ml aliquot of the crude preparation was diluted to 15 ml with chilled methanol and a white, flocculent, proteinaceous precipitate which separated from solution was removed by filtration. This diluted preparation, after filtration through a Pasteur pipette plugged with glass wool, was injected directly into the HPLC. Two replicates of each ayahuasca sample were prepared, and each replicate sample was injected twice during separate runs following calibration of the integrator using the standard mixtures. Values reported in Table III are the means of these four replicate injections. Values given are 10x the actual value measured since the samples measured were 1/10 the concentration of the undiluted brew.

The ayahuasca samples which had been diluted with methanol in the field were quantified for alkaloids in terms of mg/g dry weight of the lyophilized sample. Fifteen ml of the methanol-diluted sample was evaporated on a steam bath, frozen, then lyophilized. A portion of the freeze-dried residue was ground to a fine powder and 100 mg was transferred to a 100 ml round bottom flask. Ten ml 100% methanol was added and the solution was extracted over a steam bath for 5 min. The methanol was removed with a Pasteur pipette and filtered through glass wool. The extraction was repeated using a second ten ml aliquot of methanol. The filtered extracts were combined, and the final

volume was adjusted to 10 ml. Twenty μl aliquots of this solution were injected onto the HPLC. As with the previous samples, two replicates of each sample were prepared, and each replicate was injected twice. Figures given in Table IV are the average of these four replicate injections.

b. Banisteriopsis caapi cultivars. Quantitation of the alkaloid content of the Banisteriopsis caapi cultivars was carried out on stem samples which had been dried under low heat (<60°) in a plant dryer. The air-dried stems were ground to powder in a Wiley mill and 5 g was extracted with 2x 100 ml methanol for 24 hrs on a rotary shaker. The combined extracts were concentrated under vacuum, filtered through glass wool and the volume adjusted to 25 ml with methanol. Ten μl aliquots were injected directly onto the HPLC. Four replicate injections of each sample were made, and figures given in Table V reflect the mean of these four replicate injections.

c. DMT-containing admixture plants. All of the Psychotria viridis samples were analyzed using methanol-preserved leaf material collected in the field; the Diplopterys cabrerana sample consisted of freeze-dried leaves derived from a greenhouse propagated clone of Plowman 6040. The methanol was decanted from the methanol-preserved material and the remaining solid matter was frozen, then lyophilized. The freeze-dried leaf material was powdered and extracted overnight on a rotary shaker with methanol (10-20 ml/g d. wt.). The D. cabrerana leaves were extracted directly with methanol and otherwise treated in the same manner as the Psychotria samples. The methanol extracts were filtered, combined with the original methanol used to

preserve the samples, and concentrated in a rotary evaporator to a known volume. The crude methanol extracts were sealed and stored at 4° C. For purposes of alkaloid quantitation, an aliquot of the methanolic extract equivalent to 2.0 g dry wt of the freeze-dried leaf material was transferred to a 50 ml round-bottom flask and evaporated to dryness on a rotary evaporator. The residue was shaken with 5 ml of 1 N HCl and filtered. The acidic filtrate was washed with 1 x 5 ml CH_2Cl_2, and the organic layer discarded. The aqueous layer was basified to pH 8-9 with saturated $NaHCO_3$ and extracted with 3 x 5 ml CH_2Cl_2. It was then further basified to pH 11-12 with 2 N NaOH and extracted with 2 x 5 ml CH_2Cl_2. The organic layers were combined, dried over anhydrous Na_2SO_4, evaporated to dryness, redissolved in methanol, and filtered through glass wool. Twenty μl aliquots of this purified alkaloid fraction were injected into the HPLC. Concentration of the DMT present was determined by comparison with a standard curve constructed by injecting known amounts of DMT standard. Figures given in Table VI are means ± s.e. of 2 to 5 replicate injections.

D. GC/MS

DMT-containing admixtures were screened by GC/MS to confirm that the major indole base detected was N,N-dimethyltryptamine. The instrument used was a Finnigan model 1020 automated GC/MS interfaced with a Perkin-Elmer Sigma 3B gas chromatograph. The chromatograph was equipped with a 30 m x .25mm SE-54 fused silica capillary column (J&W Scientific). The chromatograph was temperature-programmed from an initial temperature of 180° to a

final temperature of 250°. The ramp rate was 3°/min initiated 3 min after injection of the sample. Injector block temperature was 250°, detector temperature 260° C. The carrier gas was helium. One μl aliquots of DMT standard, or of the purified alkaloid extracts of the leaf samples were injected. Eluted compounds were detected as peaks in the reconstructed ion chromatogram (RIC) generated by the mass spectrometer data system. Under these conditions, DMT had a retention time of 9.5-10 min, base peak 58, M^+ 188. The indole base present in the Diplopterys cabrerana and Psychotria leaf extracts had a mass spectrum and retention time identical with the standard.

E. Alkaloid Tests & TLC of Uncommon Admixture Plants

The uncommon admixture plants were screened for alkaloids following the method of Farnsworth & Euler [18]. Material used in the analysis was preserved in methanol, and this was worked up in a manner identical to that described above for the Psychotria samples. Aliquots of the acidic filtrate were tested with 1-2 drops of either Meyer's, Valser's, or Dragendorff's reagent. Appearance of either a marked turbidity or a heavy precipitate on addition of the reagent was interpreted as a positive alkaloid reaction; slight turbidity indicated possible traces of alkaloid. Composition of the reagents used is given in Martello & Farnsworth [19]. After testing with the precipitation reagents, the acidic aqueous solutions were basified to pH 8-9 with saturated $NaHCO_3$ and and extracted with 3 x 5 ml CH_2Cl_2; it was then basified to pH 11-12 and extracted with 2 x 5ml CH_2Cl_2. The combined organic layers were evaporated to dryness under

vacuum, and the residue taken up in 2.0 ml methanol. Five μl aliquots of this final methanol fraction were applied to precoated Polygram silica gel plates UV^{254}, and the plate was developed in one direction in butanol/acetic acid/water 4:1:1. Following development, the plates were air-dried, examined under short- and long-wave UV light, and sprayed with Dragendorff's modified reagent [20].

III. Results and Discussion

A. Ayahuasca Brews

Comparison of the TLC profiles of the eight samples (Fig. 6) shows that the major constituents vary little from sample to sample; different batches made by the same ayahuasquero (cf. Don Fidel # 1 & 2, and Don Juan, # 1 & 2) are generally similar, and there is also little variation in the constituents of brews made by different ayahuasqueros. Harmine, harmol, harmaline, and tetrahydroharmine were found to be the major β-carbolines present in all of the samples, while harmalol was not detected in any samples save one (Don Milton # 1). Dimethyltryptamine was found in all samples except that from Tarapoto. No other significant Ehrlich-positive spots were detected. Known constituents were identified by comparison with a mixture of authentic standards (ayahuasca "analogue", upper left in Fig. 6) Traces of other fluorescent compounds were also detected in most samples; it is assumed that these represent β-carbolines of undetermined structure. Absence of DMT in the sample from Tarapoto is significant, since this is the only sample in which

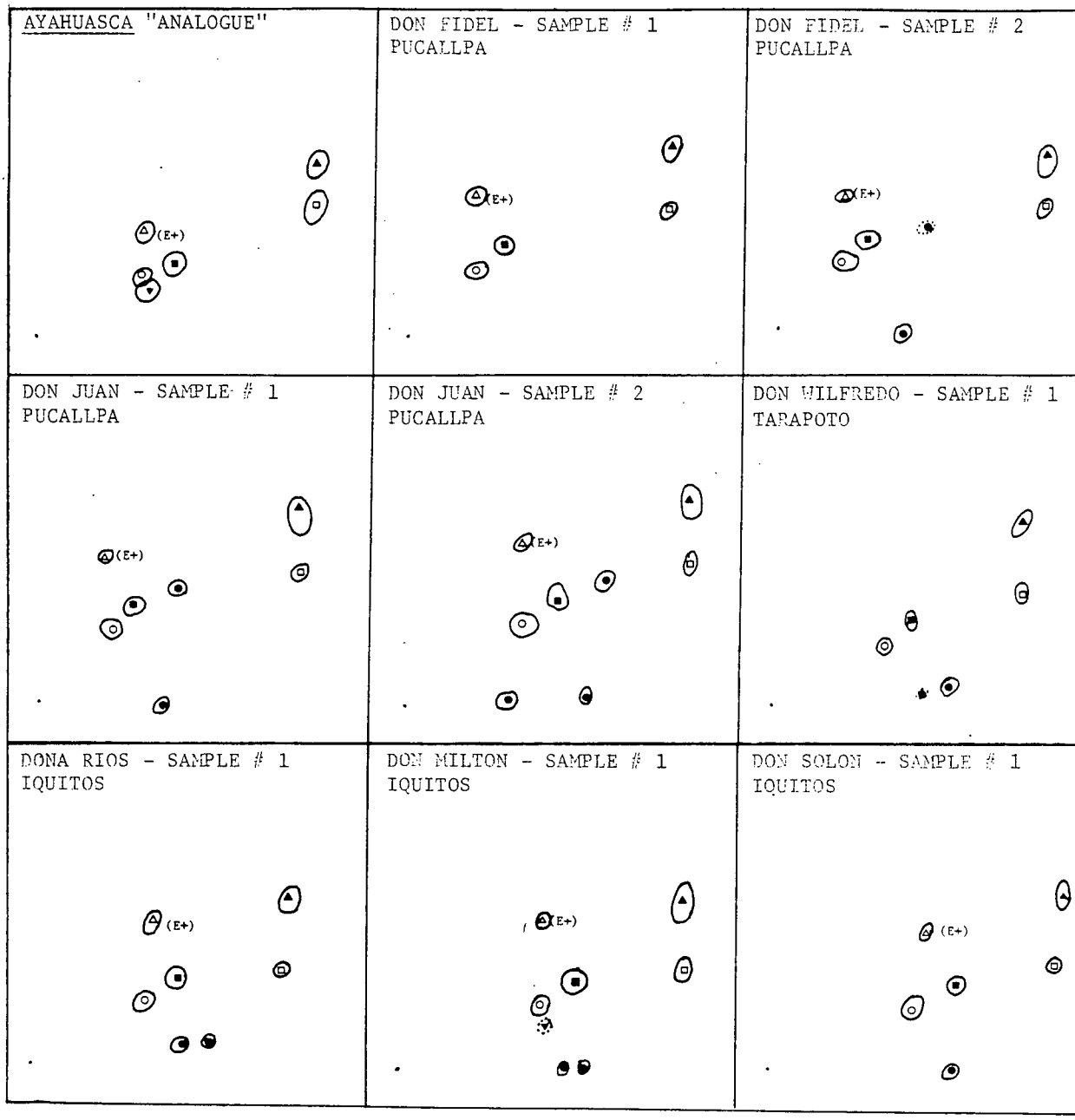


Figure 6 - TLC Analysis of Peruvian Ayahuasca Samples

△ Dimethyltryptamine(DMT)
▲ Harmine
■ Harmaline
□ Harmol
○ Tetrahydroharmine
▼ Harmalol
● Unknown

Solvents:
I. ether/2-butanone/
28% NH_4OH 5:4:1 (upper phase)
II. n-propanol/1.5% NH_4OH 9:2

Plates:
Polygram silica gel GF^{254}
(Brinkmann Instruments)

(E+) = positive to Ehrlich's Reagent

Psychotria carthagenensis was employed as the admixture rather than the more commonly used P. viridis.

The quantitative HPLC analysis of five undiluted samples prepared by two ayahuasqueros in Pucallpa shows little variation from batch to batch, either in total alkaloid content or in the proportions of constituents (Table III). The methanol-diluted samples were lyophilized and their alkaloid contents compared on a dry weight basis (Table IV). These samples showed considerable differences in alkaloid content (expressed as mg alkaloid/g dry wt). The sample from Pucallpa had the highest total alkaloid content (75.7 mg/g d wt.) of which 76% was harmine, 10.6% was tetrahydroharmine, and 7.6% was DMT. The samples from Iquitos and Tarapoto generally had lower total alkaloid levels, and also differed in the proportions of different constituents. The reason for the difference in relative proportion of harmine and tetrahydroharmine in the Pucallpa samples and those from other regions of Peru could be related to the type of B. caapi cultivar employed to prepare the drink, or to the method of preparation. Environmental variables, such as the type of soil in which the cultivar is grown, or different conditions of exposure to sunlight, may also be involved. Table III shows, however, that all of the ayahuasca samples from Pucallpa consistently show approximately the same relative proportions of harmine to tetrahydroharmine to harmaline. This may indicate that all of these samples were prepared either from the same B. caapi cultivar or clones of the same cultivar. This would not be surprising since Don Juan is the uncle of Don Fidel and they often collaborate in the preparation of their brews.

TABLE III
HPLC QUANTITATION OF UNDILUTED AYAHUASCA SAMPLES

Name of Sample	Alkaloid Concentration (mg/ml)* harmol	harmine	%	THH	%	harmaline	%	DMT	%	total
Don Fidel#1	tr	3.85 (.01)	66	1.06 (.05)	18	0.3 (.1)	5	0.61 (.01)	10	5.85 (.19)
Don Fidel#2	tr	4.64 (.3)	62	1.77 (.1)	24	0.45 (.1)	6	0.6 (.1)	8	7.48 (.16)
Don Fidel#3	tr	3.4 (.2)	53	1.94 (.06)	30	0.34 (.2)	5	0.7 (.2)	11	6.38 (.1)
Don Juan#1	tr	5.3 (.36)	66	1.73 (.1)	21	0.51 (.16)	6	0.51 (.16)	6	8.05 (.36)
Don Juan#2	tr	5.51 (.26)	67	1.67 (.07)	20	0.41 (.14)	5	0.6 (.2)	7	8.19 (.28)
Average Alkaloid content†		4.67 (.2)	65	1.60 (.08)	22	0.41 (.06)	6	0.6 (.06)	8	7.28 (.23)

* figures given are mg alkaloid/ml of undiluted sample, ± s. e., shown in parentheses; percentages are % total alkaloid.
† average based on n=18 replicate injections.

TABLE IV
HPLC QUANTITATION OF LYOPHILIZED AYAHUASCA SAMPLES

Sample & Origin	Alkaloid Concentration (mg/g d.wt.)*								
	harmine	%	THH	%	harmaline	%	DMT	%	total
Don Fidel#1 Pucallpa	57.6 (.33)	76	8.0 (.53)	11	4.2 (.22)	5	5.8 (.5)	8	75.6 (3.3)
Don Solon Iquitos	28.3 (1.3)	42	25.5 (1.4)	38	5.8 (.9)	9	7.2 (1.7)	11	66.8 (3.4)
Don Wilfredo Tarapoto	14.4 (.44)	50	10.5 (1.2)	36	4.2 (.3)	14	n.d.†	-	29.1 (1
Don Milton Iquitos	10.2 (.32)	33	10.2 (1.7)	33	5.2 (1.7)	16	5.7 (1.6)	18	31.3 (5.6)
Dona Rios Iquitos	8.6 (.3)	27	9.6 (1.8)	31	6.3 (1.8)	20	7.0 (2.1)	22	31.5 (5.6)
Average Alkaloid Content‡	23.8 (4.2)	51	11.1 (1.9)	24	5.1 (.5)	11	6.4 (.74)	14	46.9 (4.9)

* figures given are mg alkaloid/g d. wt. of lyophilized sample; standard errors (n=4) are given in parentheses. Percentages given are % total alkaloid.
† n. d. = not detected
‡ based on n=16 replicate injections

Summarizing the results of their quantitative studies, Rivier and Lindgren [4] state that a typical 200 ml dose of ayahuasca contains a total of 65 mg alkaloid, of which 30 mg is harmine, 10 mg is tetrahydroharmine, and 25 mg, DMT. This is some ten to thirty times less than the dosage at which the β-carbolines are hallucinogenically active in the pure form, (cf. Chapter II) although it is well within the range at which they are effective as MAO inhibitors. Twenty five mg is just above the threshold dose for DMT when this compound is injected intramuscularly [21] but it is possible that the threshold may be lower under conditions of MAO inhibition. Commenting on their findings, Rivier & Lindgren [4] conclude: "In view of these results, new pharmacological experiments for a better understanding of the hallucinogenic action of ayahuasca seem necessary."

The alkaloid levels found in the five samples from Pucallpa (Table III) exceed the levels reported by Rivier and Lindgren [4] in samples collected on the upper Rio Purús by at least an order of magnitude. Thus, (based on the average of the five samples) a 100 ml dose of the Pucallpa ayahuasca (cf. Table III) contains 728 mg total alkaloid, of which 467 mg is harmine, 160 mg is tetrahydroharmine, 41 mg is harmaline, and 60 mg is DMT. This is well above the threshold dose for DMT but is still considerably below the hallucinogenic dose level for the β-carbolines. In practice the typical dose ingested in the Pucallpa ceremonies rarely exceeds 75 ml and is usually closer to 55-60 ml. The relatively large differences in the alkaloid content of the upper Purús ayahuasca analyzed by Rivier and

Lindgren and the Pucallpa ayahuasca analyzed in the present study may be readily explained by the differences in the method of preparation in the two regions. In the upper Purús method, stems of Banisteriopsis caapi totaling about 900 cm in length and 1-4 cm diameter are cut into short sections, crushed, and packed in a 15 liter metal vessel together with alternating layers of leaves of Psychotria spp. Ten liters of water are added and the mixture is boiled for one hour, strained, and cooled. The mixture is then consumed without further processing. The method employed in Pucallpa starts out similarly but the mixture is boiled for a much longer time, approximately 10-15 hours. The water may be drained off and replaced with fresh water several times during this boiling process. The separate batches are combined, allowed to cool, and filtered through a strainer or cheesecloth. The plant material is removed from the cooking pot and discarded, and then the strained ayahuasca is poured back into the pot and simmered over a low fire until it has been concentrated to about half its original volume. Five or six liters of ayahuasca are obtained from this process; these may be kept for up to six months without refrigeration in wine or beer bottles stoppered with corks.

B. Alkaloid Content of Banisteriopsis caapi Cultivars

All of the ayahuasqueros that we interviewed during our field studies in Peru recognized several different "kinds" of ayahuasca which were claimed to vary in their psychological effect. The differentiation of these varieties of ayahuasca was based in part on the types of admixture plants which were added,

and in part on the type of B. caapi which was utilized. Several types of Banisteriopsis caapi were generally recognized by these practitioners and were distinguished by different adjectives, e.g., "cielo" ayahuasca, "lucero" ayahuasca, "rumi" ayahuasca. Some claimed to distinguish as many as ten kinds of Banisteriopsis vine (the term ayahuasca is indiscriminately applied either to the B. caapi vine or to the beverage made from it) but most were familiar with only two or three kinds. Presumably these "kinds" of B. caapi are referable to different cultivars, races, or chemical or morphological varieties of Banisteriopsis caapi. There were no outstanding morphological differences between the three or four kinds of B. caapi which we collected, and the relevant voucher specimens have all been determined as Banisteriopsis caapi by taxonomic specialists in the Malpighiaceae (W. R. Anderson & B. Gates, University of Michigan). HPLC analysis of the alkaloid levels in the dried stems of three of the recognized varieties plus one specimen (DMK#125) for which the vernacular name is unknown has shown that there is considerable variation between samples (Table V). The lowest level was found in DMK#126 which contained 1.7 mg/g total alkaloid, while DMK#125 contained the highest level, 13.6 mg/g. There appears to be no consistent correlation of alkaloid content with particular recognized cultivars, however. The variation observed probably has more to do with the age of the plant, and the soil, light, water, and other environmental conditions affecting the growth of the particular specimen. The amounts of alkaloids are in the same general range as those detected in the B. caapi samples analyzed by Rivier & Lindgren

TABLE V

HPLC QUANTITATION OF BANISTERIOPSIS CAAPI CULTIVARS

Collection #, Name, & Origin	DMK #110 "cielo" Tarapoto		DMK #124 "Pucahuasca" Tarapoto		DMK #125 - Iquitos		DMK #126 "cielo" Iquitos		DMK #128 "rumi" Iquitos		Plowman 6041 "cielo" Tarapoto-1976 (UBC-1982)	
Alkaloids Detected (mg/g d wt)												
Harmine	5.3	72%	5.9	47%	6.35	47%	0.57	34%	4.4	51%	1.0	35%
THH	0.95	13%	3.3	26%	1.95	14%	0.25	15%	1.45	17%	1.3	47%
harmaline	1.1	15%	3.2	26%	3.8	28%	0.75	44%	2.07	24%	.5	18%
harmol	0.05	.7%	0.06	.5%	1.2	9%	0.1	6%	0.65	8%	.01	.3%
harmalol	n.d.*	-	trace	-	.35	2%	n.d.	-	n.d.	-	n.d.	-
total alkaloids	7.4		12.5		13.6		1.7		8.6		2.8	

* n.d. = not detected

[4]. Further clarification of this question of possible chemical or morphological differences between recognized types of B. caapi cultivars would require a systematic sampling of as many different individuals of each type as possible; climatic, edaphic, and other environmental factors should also be considered.

In most of the B. caapi cultivars examined, harmaline constituted a greater proportion of the total alkaloid content than in the ayahuasca brews. In the B. caapi cultivars, harmaline consistently represented 25-50% of the total alkaloids, while in the ayahuasca samples, it was approximately 5-15% of the total alkaloids in most samples. This indicates that the process of boiling and concentration of the ayahuasca brews may result in the oxidation of a significant fraction of the harmaline to the more aromatic derivative, harmine.

C. Alkaloid Content of Ayahuasca Admixture Plants

1. DMT-containing Admixtures

In Peru,the admixture plant employed most frequently in the preparation of ayahuasca appears to be Psychotria viridis R. & P. We encountered only one ayahuasquero during our fieldwork who preferred to use another species of Psychotria, tentatively identified as Psychotria carthagenensis Jacq. Interestingly, no alkaloids of any kind were detected in this collection (DMK#109, Tarapoto) however, all of the P. viridis collections contained N,N-dimethyltryptamine as the single major base. Identity of the compound was confirmed by GC/MS and comparison of its HPLC

TABLE VI
DMT CONTAINING ADMIXTURE PLANTS: ANALYSIS BY TLC, HPLC, & GC/MS

Collection #, Name, & Origin	DMK#21 Psychotria viridis "chacruna" Iquitos	DMK#108 Psychotria viridis "suija" Tarapoto	DMK#109 Psychotria carthagenensis "yage-chacruna" Tarapoto	DMK#139 Psychotria viridis "chacruna" Pucallpa	Plowman 6040 Diplopterys cabrerana "chagro-panga" Tarapoto	DMT Standard
TLC:						
solvent 1 hRf*	42	42	n.d.‡	38	42	41.3
solvent 2 hRf†	23	24	-	27	25	25
reaction to Ehrlich's reagent	+(blue)	+(blue)	-	+(blue)	+(violet)	+(blue)
HPLC:						
Ret. time (min)	18.4(.1)þ	18.3(.03)	-	18.7(.05)	18.4(.15)	19.1
mg/g d wt.	1.58(.3)	1.02(.04)	-	1.2(.17)	1.74(.4)	
GC/MS: trace constituents	n.d.	n.d.	n.d.	2-Me-THβC	5-HO-DMT	M⁺=188 m/z 58=100%

* Solvent 1: ether/2-butanone/conc NH_4OH 5:4:1 (upper phase);
hRf= Rf x 100
† Solvent 2: n-propanol/1.5% NH_4OH 9:2
‡ n.d.= not detected
þ Figures in parentheses are standard errors

retention time, TLC hRf and Ehrlich's color reaction with that of an authentic standard (Table VI). The P. viridis samples analyzed contained fairly substantial amounts of DMT, between 1 and 1.6 mg/g dry wt. in the leaves. No alkaloid was detected in fruits or stems of P. viridis. No other alkaloids were detected in any of the Psychotria samples with the exception of DMK #139, in which a trace constituent with a mass spectrum corresponding to that reported [4] for 2-methyl-tetrahydro-β-carboline was detected. A single sample of Diplopterys cabrerana (Plowman 6040), the Malpighiaceous admixture, was available for analysis and this also contained N,N-dimethyltryptamine together with a trace amount of 5-hydroxy-DMT. The alkaloid extract of dried leaves of the original (1976) collection of Plowman 6040 had an ion chromatogram that was essentially identical to leaf extracts of greenhouse propagated clones of this specimen. Plowman 6040 contained slightly higher levels of DMT (1.74, mg/g d wt.) than the P. viridis samples, but otherwise was indistinguishable in terms of alkaloid content. Although Plowman 6040 was collected in Tarapoto where it was being utilized as an ayahuasca admixture, this use of Diplopterys cabrerana in Peru is uncommon; this species is the usual admixture in Southern Colombia and Ecuador [22] and in fact Plowman 6040 was originally brought to Tarapoto as a live cutting from the Rio Pastaza in Ecuador (Plowman, T., pers. comm., 1980). Psychotria viridis, or less frequently, Psychotria carthagenensis are the admixtures of choice in Peru and few of my informants in Peru were familiar with D. cabrerana under its common names, chagropanga or oco-yage.

2. Uncommon Admixture Plants

Ayahuasca is usually prepared using one of the DMT-containing admixture plants mentioned above, either Diplopterys cabrerana or a Psychotria sp.; less commonly, however, other admixtures are utilized, either in conjunction with the tryptamine-containing admixtures, or in place of them. Many of these admixtures have been identified in the ethnobotanical literature [2,4,22,23,] although little is known of their chemical or biodynamic properties. (cf. Appendix I). This would appear to be a promising area for further research. Three collections were made of plants which were stated by informants to be used as admixtures to ayahuasca (Table VII). One of these, Teliostachya lanceolata, has been discussed by Schultes [23] as an admixture, but the other two, Abuta grandifolia (Menispermaceae) and Cornutia odorata (Verbenaceae) have not previously been reported as admixtures. Plant material from these collections, preserved in methanol, were screened for alkaloids using alkaloid precipitation tests and TLC (Table VII). Insufficient material was available to permit further chemical characterization. The only collection giving an unambiguously positive test was Abuta grandifolia (DMK #74). This species has recently been reported [24] to contain palmatine, a typical quaternary base of the bis-benzylisoquinoline family which characterizes the Menispermaceae. Although palmatine is one of the commonest alkaloids in nature, investigations of its pharmacology are surprisingly sparse. One study [25] found that palmatine inhibited the effect of epinephrine on blood pressure of

TABLE VII
TEST FOR ALKALOIDS IN UNCOMMON ADMIXTURE PLANTS

Coll.#	DMK#74	DMK#22	DMK#119	DMK#1
Genus:	Abuta	Teliostachya	Cornutia	Justicia
species:	grandifolia	lanceolata	odorata	pectoralis
family:	Menispermaceae	Acanthaceae	Verbenaceae	Acanthaceae
Part ext'd(g):	bark(2)	leaves(2.7)	leaves(5.7)	leaves(1.2)
Reaction to:				
Meyer's rgt.	++	-	±	-
Valser's rgt.	++	-	±	-
Dragendorff's	++	-	-	-
TLC:				
Dragendorff's*	++	-	-	-
Fluorescent Spots detected:				
Long wave UV	+	-	-	+
Short wave UV	+	-	-	+

* modified for TLC according to Stahl [21]

rabbits, on the isolated rat seminal vesicle and on the toad hind-leg; its derivative dl-tetrahydropalmatine inhibited the effect of 5-hydroxytryptamine on isolated rat uteri, colon, and stomach. Palmatine also exhibited anticholinesterase activity. Both alkaloids had ACTH and bactericidal activity. Some of these properties may be antagonistic to the effects of the β-carbolines while others may be synergistic. For instance harmaline causes an increase in 5HT concentration in the whole brain, while harmine causes a significant decrease in acetylcholine in brain; on the other hand, harmine strongly inhibits the ATP-Mg^{++} dependent uptake of norepinephrine into isolated adrenal medullary vesicles. [26]. Whether the overall effect of palmitine is agonistic or antagonistic to the action of the β-carbolines, there seems little doubt that addition of the bark of Abuta grandifolia to ayahuasca could modify its effect. Further investigations of the pharmacology of this and many other admixture plants are needed in order to clarify their contribution to the effects of ayahuasca.

Justicia pectoralis (Acanthaceae) is also included in Table VII. Justicia pectoralis var. stenophylla is not used as an ayahuasca admixture but has been reported as an admixture to the Virola snuffs, (cf. Chapter VI, and [27]) and these authors have suggested that it may be used by itself as an hallucinogenic snuff. No tryptamines or alkaloids of any other type were detected in our collection of this species. GC/MS analysis of extracts of Justicia pectoralis indicate that it contains the coumarin derivative umbelliferone and the quaternary nitrogen derivative betaine (cf. Chapter VI, & [28]).

IV. Summary

The alkaloidal constituents of a number of ayahuasca brews, cultivars of B. caapi and a variety of admixture plants were qualitatively and quantitatively investigated using 2-dimensional thin-layer chromatography (TLC) and high-pressure liquid chromatography (HPLC) as the analytical methods. Admixture samples were also analyzed using gas chromatography/mass spectrometry (GC/MS). Some admixture plants were screened for alkaloids using precipitation tests and TLC. The levels of β-carbolines found in most ayahuasca samples were insufficient to account for the hallucinogenic properties of ayahuasca at the doses typically used, however the concentration of DMT would be well above the threshold level in most samples; apparently DMT is responsible for the hallucinogenic action of ayahuasca, assuming that it can be orally-activated by the blockade of visceral MAO. Different batches of ayahuasca prepared by the same person were remarkably consistent, both in terms of amount of total alkaloids and proportions of individual constituents. Considerable variation was found in samples prepared by different practitioners. Variation in alkaloid content of B. caapi cultivars was also found but may be due to environmental factors rather than actual genetic differences between clones. Substantial concentrations of DMT were found in several collections of Psychotria viridis, and in one collection of Diplopterys cabrerana, but was not detected in Psychotria carthagenensis. DMT was the single major base detected in these admixtures; only traces of other alkaloids were present. Of

several uncommon admixture plants which were screened for alkaloids, only Abuta grandifolia gave an unambiguously positive reaction.

V. Literature Cited

1. Schultes, R. E. (1957) The Identity of the Malipighiaceous Narcotics of South America. Harvard Botanical Museum Leaflets 18:1-56

2. Schultes, R. E. (1972) Ethnotoxicological Significance of Additives to New World Hallucinogens. Plant Science Bulletin 18:34-41

3. Gates, B. (1979) New Names in Banisteriopsis and Diplopterys (Malpighiaceae) of the Guayana Highland. Brittonia 31:108-9

4. Rivier, L. & J. Lindgren (1972) Ayahuasca, the South American Hallucinogenic Drink: Ethnobotanical and Chemical Investigations. Economic Botany 29:101-129

5. Deulofeu, V. (1967) Chemical Compounds Isolated from Banisteriopsis and Related Species pp. 393-402 in D. H. Efron, B. Holmstedt, & N. S. Kline (eds.) Ethnopharmacologic Search for Psychoactive Drugs U. S. Public Health Service Publication #1645

6. Agurell, S., B. Holmstedt, & J. E. Lindgren (1968) Alkaloid Content of Banisteriopsis rusbyana. American Journal of Pharmacy 140:148-151

7. Hashimoto, Y. & K. Kawanishi (1975) New Organic Bases from Amazonian Banisteriopsis caapi. Phytochemistry 14:1633-35

8. Hashimoto, Y. & K. Kawanishi (1976) New Alkaloids from Banisteriopsis caapi. Phytochemistry 15:1559-60

9. Shulgin, A. T. (1976) Psychotomimetic Agents. Ch. 4 in Maxwell Gordon (ed.) Psychopharmacological Agents Vol. IV. Academic Press

10. Udenfriend, S., B. Witkop, B. G. Redfield, & H. Weissbach (1958) Studies with Reversible Inhibitors of Monoamine Oxidase: Harmaline and Related Compounds. Biochemical Pharmacology 1:160-165

11. Buckholtz, N. S. & W. O. Boggan (1977) Monoamine Oxidase Inhibition in Brain and Liver Produced by β-carbolines: Structure-activity Relationships and Substrate Specificity Biochemical Pharmacology 26:1991-96

12. McIsaac, W. M. & V. Estevez (1966) Structure-action Relationships of β-carbolines as Mono-amine Oxidase Inhibitors Biochemical Pharmacology 26:1625-27

13. Naranjo, C. (1967) Psychotropic Properties of the Harmala Alkaloids pp. 385-391 in D. H. Efron, et al., (eds.) op. cit.

14. Pennes, H. H. & P. H. Hoch (1957) Psychotomimetics, Clinical and Theoretical Considerations: Harmine, WIN-299 and Nalline. American Journal of Psychiatry 113:887-92

15. Der Marderosian, A. H., H. V. Pinkley, & M. F. Dobbins IV. (1968) Native Use and Occurrence of N,N-dimethyltryptamine in the Leaves of Banisteriopsis rusbyana. American Journal of Pharmacy 140:137-147

16. Agurell, S., B. Holmstedt, J. E. Lindgren, & R. E. Schultes. (1968) Identification of Two New β-carboline Alkaloids in South American Hallucinogenic Plants. Biochemical Pharmacology 17:2487-88

17. Repke, D. B., D. T. Leslie & G. Guzman (1977) Baeocystin in Psilocybe, Conocybe, and Panaeolus. Lloydia 40:566-78

18. Farnsworth, N. R. & K. L. Euler (1962) An Alkaloid-screening Procedure Utilizing Thin-layer Chromatography Lloydia 25: 186-195

19. Martello, R. & N. R. Farnsworth (1962) Observations on the Sensitivity of Several Common Alkaloid Precipitating Reagents. Lloydia 25:176-185

20. Stahl, E., (1969) Handbook of Thin-layer Chromatography 2nd. ed. Springer-Verlag, New York.

21. Szara, S. (1957) The Comparison of the Psychotic Effect of Tryptamine Derivatives with the Effects of Mescaline and LSD-25 in Self-experiments. pp. 460-467 in S. Garattini & V. Ghetti (eds.) psychotropic drugs. Elsevier, amsterdam.

22. Pinkley, H. V. (1969) Plant Admixtures to Ayahuasca, the South American Hallucinogenic Drink. Lloydia 32:305

23. Schultes, R. E., (1979) New Data on the Malpighiaceous Narcotics of South America. Harvard Botanical Museum Leaflets 23:137ff

24. Setor de Fitoquimica, INPA, Manaus, Amazonas, Brazil. (1971) Chemical Composition of Amazonian Plants. Acta Amazonica 1:83-86

25. Ch'en, Mu-Ch'un & Chen-Yu Ch'i (1965) Comparative Pharmacology of Palmatine and dl-tetrahydropalmatine. Yal Hseuh Hseuh Pao 12:185-92. Cited in Chemical Abstracts 63:3492g (1965)

26. Ho, B. T. (1977) Pharmacological and Biochemical Studies with β-carboline Analogs. Current Developments in Psychopharmacology Vol. 4:153-177

27. Chagnon, N., P. LeQuesne, & J. M. Cook (1971) Yanomamo Hallucinogens: Anthropological, Botanical, and Chemical Findings. Current Anthropology 1: 2-74

28. MacRae, D. & G. H. N. Towers (1984) Justicia pectoralis - A Study of the Basis for its Use as a Virola Snuff Admixture. Journal of Ethnopharmacology. Submitted.

29. Akabori, S. & K. Saito (1930) Synthetische Versuche in der Indol-Gruppe, VIII.: Synthese von Harman und Harmin. Chemische Berichte 63:2245-2248

CHAPTER V: ALKALOID CONSTITUENTS OF ORALLY-ACTIVE MYRISTICACEOUS HALLUCINOGENS

I. Introduction

Various species of the genus Virola (Myristicaceae) have been employed by Amazon Indian tribes as the basis of hallucinogenic preparations [1]. The drug obtained from the Virola trees is in all cases derived from the "resin", the colorless to reddish exudate of the cambial layer of the bark. Most commonly, the resin is evaporated to dryness over a low fire and ground into a powder, which is ingested in the form of a snuff. The exact method of preparation varies from region to region and tribe to tribe; in some instances the snuff is prepared without the addition of other ingredients, while in others, the powdered leaves or ashes of other plants are added [2]. Although the use of Virola resin as a snuff has the widest geographical and ethnological distribution in the Amazon Basin, a few tribes utilize orally-ingested Virola preparations [1], and there are also reports [3] that the bark of certain Virola spp. may be smoked for hallucinogenic effects. Extensive chemical investigations have been carried out on many Virola spp. and related genera [4] and the active hallucinogenic constituents of the Virola snuffs have been determined to be indole bases related to tryptamine, e.g., N,N-dimethyltryptamine, 5-methoxy-N,N-dimethyltryptamine, and related derivatives [5,6]. Trace amounts of β-carbolines have been reported from some species [6], and in one species, not known to be used as an hallucinogen, β-carbolines are the major

alkaloids present [7]. The alkaloidal constituents of the orally-ingested Myristicaceous preparations have not previously been investigated. A more detailed discussion of the ethnobotanical, chemical, and pharmacological aspects of the Myristicaceous hallucinogens may be found in Chapter I.

In the present study a number of Myristicaceous bark and leaf samples collected in the Rio Ampiyacu region of Peru were screened for alkaloids using alkaloid precipitation tests, TLC, GC, HPLC, and GC/MS. In addition, the alkaloid constituents of several native drug samples were investigated; these consisted of six samples of orally-ingested Virola paste collected in the Rio Ampiyacu region in spring, 1981; one orally-ingested Virola paste sample collected at La Chorrera, Colombia, in 1971; and four samples of Yanomama snuff collected in Venezuela in 1972.

II. Materials and Methods

A. Detection of Alkaloids in Myristicaceous Samples

Collection numbers of Myristicaceous bark and leaf samples cited in this work refer to the personal collection numbers of D. McKenna (cf. Appendix II for a complete list of herbarium voucher collections relevant to this work). Herbarium vouchers are available for all of the orally-ingested pastes collected on the Rio Ampiyacu, however no vouchers are available for the La Chorrera paste sample or for the Yanomama snuff samples. All of the Myristicaceous bark and leaf samples were preserved in 100% methanol at the time of collection. The methanol was decanted from the preserved material and the remainder was frozen, then

lyophilized. The freeze-dried material was powdered and extracted overnight on a rotary shaker (10-20 ml/g dry wt). The extracts were filtered, combined with the original methanol used to preserve the sample, and concentrated under vacuum to a known volume. The crude extracts were sealed and stored at 4° C. For alkaloid screening, aliquots of the extracts equivalent to 3 g dry wt were evaporated to dryness in a 50 ml round bottom flask. The residue was shaken with 5 ml 1 N HCl, and filtered. A small aliquot of the acidic filtrate (ca. 0.5 ml) was removed and used for the alkaloid precipitation tests. The remainder was washed with 1 x 5 ml CH_2Cl_2 and the organic layer discarded. The aqueous layer was basified to pH 8-9 with saturated $NaHCO_3$ and extracted with 15 ml portions of CH_2Cl_2; it was further basified to pH 10-12 with 2 N NaOH and extracted until acidified aliquots of the aqueous fraction no longer gave a positive reaction to Meyer's reagent. The organic fractions were combined, dried over anhydrous Na_2SO_4, evaporated to dryness and redissolved in 3 ml methanol. The orally-ingested paste samples collected on the Rio Ampiyacu were also preserved in methanol and were treated in the same manner as the bark and leaf samples. The La Chorrera paste sample was frozen, lyophilized, and then extracted with methanol (20 ml/g dry wt). The Yanomama snuff samples were dried, powdered plant materials; these were extracted directly with methanol and prepared in the same manner as the other samples.

Alkaloid precipitation tests were carried out on small aliquots of the acidic filtrate during the alkaloid isolation procedure. Tests were made with Valser's, Meyer's, and Dragendorff's reagent [8]; all of these reagents form

precipitates or turbid solutions in the presence of alkaloids, while slight turbidity indicates possible traces of alkaloid. Valser's reagent is approximately an order of magnitude more sensitive than the other two. TLC of the samples was conducted with the purified base extracts which were redissolved in methanol. Three μl aliquots were applied to Polygram Silica Gel UV^{254} precoated TLC plates (Brinkmann Intruments) at a point 1.0 cm from the bottom edge. Following application the samples were dried under a stream of air, and developed to a distance of 9 cm in ether/2-butanone/conc. NH_4OH 5:4:1. The solvent was freshly prepared in a separatory funnel and the upper phase collected for TLC. Development was carried out at ambient temperature in an unlined 10x23x26 cm glass chromatographic tank containing 50 ± 5 ml of solvent. Following development, plates were air-dried in a fume hood for 30-60 min, then examined under long- and short-wave UV light. Tryptamine bases and tetrahydro-β-carbolines appear as dark UV-absorbing spots under short-wave UV while aromatic and dihydro-β-carbolines have characteristic fluorescences under long-wave UV. Following examination under UV, plates were sprayed with Ehrlich's reagent [9] which gives blue to violet colors with tryptamine bases upon exposure to HCl vapors. Tryptamines detected in the samples could be tentatively identified from TLC based on comparison of the Rfs and color reactions with authentic standards (cf. Table II and Figs. 3 and 4, Chapter IV for complete TLC data of the tryptamine and β-carboline standards). For some samples, identities of constituents detected using TLC were confirmed by GC/MS. Conditions for the GC/MS analysis were identical to those

described in Chapter IV. For some samples, GC was used to confirm the identities of the tryptamine bases detected, based on comparison with the retention times of authentic standards. Conditions of the GC analyses are described below. Following TLC those samples which were positive for alkaloids were evaporated to dryness under nitrogen and stored at -20° C.

B. Quantitative Analysis of Tryptamine Standards

Those Myristicaceous samples which were alkaloid-positive were quantified using gas chromatography (GC). The instrument used was a Sigma 3B Gas Chromatograph (Perkin Elmer) equipped with a hydrogen flame ionization detector. The column was a 15 M x 0.25mm SE-30 fused silica capillary column (J & W Scientific). Carrier gas was helium; inlet pressure for the carrier gas was 18 psi; inlet pressure for both the hydrogen and air was 30 psi; split ratio was 0.67. Attenuation of the GC detector was 1x4 mv and the attenuation of the chart recorder was 1x5 mv. The chromatograph was temperature programmed, from an initial temperature of 120° C to a final temperature of 200° C. Initial temperature was held for 3 min following injection and then increased at a rate of 10°/min.

Retention times, peak heights, and peak height ratios of tryptamine derivatives were determined using standards (Table VIII). Retention times were calculated as the mean ± standard error of a minimum of 10 injections. Mixtures of tryptamine standards were used to construct calibration curves used in the quantitative analyses of the Myristicaceous samples. Concentration of the tryptamine calibration mixtures ranged from

TABLE VIII - TRYPTAMINE STANDARDS: GC ANALYTICAL DATA

tryptamine standard	retention time (min)	concentration (mg/ml) .0625	.125	.25	.5	1.0
tryptamine	6.6±.15					
peak height (cm)		n.d.*	n.d.	1.6	7.5	-
phr†		-	-	.6	2.8	-
5-MeO-T	9.3±.15					
peak height		n.d.	n.d	n.d	2.1	9.3
phr		-	-	-	.8	3.5
NMT	7.1±.21					
peak height		n.d	.42	1.7	3.2	-
phr		-	.11	.43	.82	-
DMT	7.3±.13					
peak height		.94	2.9	6.7	17.7	-
phr		.24	.74	1.7	4.5	-
5-MeO-DMT	9.7±.13					
peak height		.65	1.9	4.3	10.3	-
phr		.16	.48	1.1	2.6	-

* n.d. = not detected
† phr = peak height ratio.

$$\text{phr} = \frac{\text{height of standard peak at concentration X}}{\text{height of gramine peak at 0.25 mg/ml}}$$

value of denominator was 3.91 for n=42 injections.

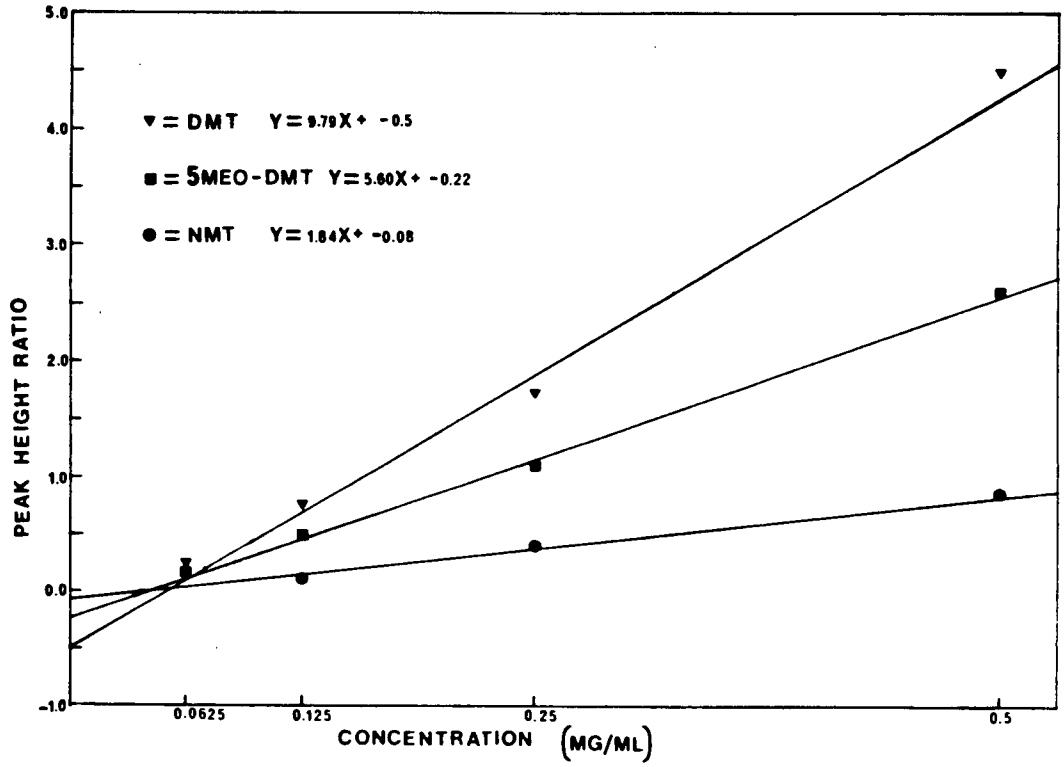


Figure 7 - GC Quantitation of Tryptamine Standards

0.0625 to 0.5 mg/ml for DMT, 5-MeO-DMT, and NMT, and from 0.25 to 1.0 mg/ml for tryptamine and 5-methoxy-tryptamine. Standards were dissolved in methanol/pyridine 9:1 and aliquots of 1 μl were injected. Tryptamine and 5-MeO-tryptamine, being polar and readily ionized compounds, could not be reliably detected if less than 250 ng was injected; well below 50 ng of the less polar methylated tryptamines could easily be detected, however. Ten replicate injections of each calibration mixture were made at each concentration level. Gramine was included as an internal standard in all of the calibration mixtures, at a constant concentration of 0.25 mg/ml. The mean peak height ratio for each standard at each concentration, with respect to the internal standard, was determined (cf. Table VIII). A linear relationship was observed over the concentration ranges stated, when the peak height ratio was plotted against concentration (Fig. 7).

C. Preparation of Myristicaceous Samples for GC Quantitation

The base fractions of the Myristicaceous bark and leaf samples had been previously evaporated to dryness and stored at -20° C. Each sample represented the alkaloids extracted from a known amount of dry weight of plant material, usually 3.0 g. One ml of methanol/pyridine 9:1 was added to the samples, which were heated briefly over a steam bath and filtered through a cotton plugged Pasteur pipette. The filtrate was collected in a small graduated cylinder, the pipette was washed with additional methanol and the sample was adjusted to a final volume of 1.5 ml. An aliquot equivalent to 1.0 g dry wt was removed and

adjusted to 0.5 ml with additional solvent; this sample was further diluted to 1.0 ml by adding 0.5 ml of solvent containing gramine at a concentration of 0.5 mg/ml. Thus the final concentration of the sample was 1.0 g dry wt/ml, and the final concentration of the gramine internal standard was 0.25 mg/ml. One μl aliquots were injected into the GC. Five replicate injections of each sample were run. Concentrations of tryptamines in the samples were calculated based on their peak height ratios with respect to the internal standard, using the calibration curves constructed using tryptamine standards. This gave the concentration of tryptamines in the samples in terms of mg/ml, which was equivalent to mg/g dry wt, since the sample consisted of 1.0 g dry wt/ml.

The Myristicaceous paste and snuff samples had not been evaporated to dryness and therefore the alkaloid fractions from known dry weights of plant material were dissolved in approximately known volumes of methanol. These samples were heated briefly over a steam bath, filtered through a cotton-plugged Pasteur pipette and brought to a known volume with additional methanol. Thus the concentration of the sample could be expressed in terms of g dry wt/ml, e.g., "2.0 g in 4 ml". At this point 1 μl of sample was injected into the GC to estimate the dilution required to bring the sample within the range of the calibration curves. An aliquot representing an appropriate portion of the total dry wt of the sample was diluted with an equal aliquot of methanol/pyridine 9:1 containing gramine (0.5 mg/ml) to give a final concentration of gramine of 0.25 mg/ml. Peak height ratios with respect to the internal standard were

calculated, and the concentrations of tryptamines in the samples were estimated as described above. Five replicate injections (1 μl) were made for each sample; for the paste samples, duplicate samples were prepared as described and 5 replicate injections of each were made.

III. Results and Discussion

The results are presented in Tables IX, X, XI, and Figs. 8, 9, 10 and 11.

A. Detection and Identification of Alkaloids in Myristicaceous Samples

1. Bark and leaf samples

The base composition of the Myristicaceous bark and leaf samples varies considerably (Table IX); the variation observed extends both to different parts of the same plant, and to different collections of the same species. These observations are consistent with a similar recent survey of Myristicaceous species [6] whose authors also remarked upon the variation found in the base composition of different plants and also in different parts of plants. In the present study, alkaloids were detected in 13 of the 27 collections. Among those which were negative for alkaloids were several collections of V. pavonis, and 6 Iryanthera spp. Of the Iryanthera species examined in the present survey, only one (I. ulei) was included in the survey of Holmstedt et al. [6]. These investigators detected a trace (0.013%) of 5-MeO-DMT in I. ulei. The V. pavonis samples, of

which 4 collections were examined, were all negative for alkaloids with the exception of DMK-30 which contained a low level (0.07 mg/g dry wt) of DMT in the leaves and shoot tips. This collection has been tentatively determined (W. Rodrigues 1983) as V. pavonis on the basis of sterile material, however the presence of DMT tends to cast doubt on this determination. An indeterminate Virola species from Brazil (Plowman 12,218) the bark of which reportedly is smoked by witch doctors as an additive to tobacco (Plowman, T., pers. comm., 1983) was also negative for alkaloids. Six collections of V. elongata were analyzed and all were positive for alkaloids. Five of these were found to contain tryptamine bases, viz., DMT, 5-MeO-DMT, NMT; all three compounds were detected in some collections while others contained only one or two out of the three. All samples in which DMT was detected also contained lesser amounts of N-methyltryptamine, an observation which probably reflects the position of this compound as the penultimate step in the biosynthesis of DMT. No similar association of either DMT or NMT with 5-MeO-DMT was found. DMK-35, 36, 37 were all collected at the same site, and all had a similar base composition; although the voucher material for these collections is sterile, they probably represent V. elongata. DMK-45, determined as V. elongata, possessed a completely anomalous alkaloid profile; only traces of Ehrlich-positive compounds could be detected by TLC. The major bases are apparently fluorescent compounds similar to β-carbolines. The fluorescent constituents detected did not match the Rfs and mass spectra of the available β-carboline standards, however. The limited amount of plant

TABLE IX - DETECTION OF ALKALOIDS IN MYRISTICACEOUS BARK AND LEAF SAMPLES

name & Collection #	alkaloid reagent tests:* D	M	V	TLC:† UVfluor.	UVabs.	Ehrlich's reaction:‡	compounds detectedþ
DMK-30 Virola pavonis							
leaves (3.0 g d wt.)	++	+/-	++	-	+	+(2)	DMT,NMT
twigs (3.0 g)	++	+	++	-	+	+(2)	DMT,NMT
DMK-32 V. pavonis							
leaves (3.0 g)	-	-	-	-	-	-	-
twigs (3.0 g)	-	-	-	-	-	-	-
DMK-34 V. pavonis							
leaves (3.0 g)	+/-	+/-	+/-	-	+	-	-
twigs (3.0 g)	-	-	-	-	+	-	-
bark (3.0 g)	+/-	+/-	+/-	+	-	-	-
DMK-35 Virola sp.							
leaves (3.0g)	++	-	++	-	+	+(2)	DMT,NMT
DMK-36 Virola sp.							
leaves (3.0 g)	+/-	-	-	-	+	+(2)	DMT,NMT
DMK-37 Virola sp.							
leaves (3.0 g)	++	-	++	-	+	+(2)	DMT,NMT
DMK-40 V. sebifera							
leaves (3.0 g)	+	-	+	+	-	+(1)	NMT
bark (3.0 g)	++	+	++	-	+	+(3)	DMT,NMT,5MeO-DMT
DMK-41 V. elongata							
bark (3.0 g)	++	+	++	-	+	+(2)	NMT,DMT
DMK-44 Iryanthera longiflora							
bark (3.0 g)	-	-	-	-	+	-	-
DMK-45 V. elongata							
bark (3.0 g)	++	-	++	++	+	+/-(1)	-
DMK-46 V. callophylla							
bark (6.8 g)	+	+/-	+	-	+	+(3)	NMT,5MeO-DMT,TA
DMK-47 Iryanthera macrophylla							
bark (3.0 g)	-	-	-	-	+	-	-
DMK-48 I. ulei							
bark (3.0 g)	-	-	-	-	+	-	-

Table IX (cont'd)

name & Collection #	alkaloid reagent tests:* D	M	V	TLC:† UVfluor.	UVabs.	Ehrlich's reaction:‡	compounds detected♭
DMK-49 I. crassifolia bark (2.3 g)	-	-	-	-	-	-	-
DMK-50 I. juruensis bark (3.0 g)	-	-	-	-	+	-	-
DMK-51 I. paraensis bark (3.0 g)	-	-	-	-	-	-	-
DMK-52 Virola multinervia bark (3.0 g)	-	-	-	-	-	-	-
DMK-56 V.callophylla seeds & fruits (3.0 g)	++	+	++	-	+	+(2)	DMT,NMT
DMK-59 V. elongata bark (3.0 g)	++	+	++	-	+	+(2)	NMT,5-MeO-DMT
leaves (3.0 g)	+	-	+	+	-	+(1)	NMT,DMT
DMK-63 V. pavonis bark (3.0 g)	-	-	-	-	+	-	-
DMK-67 V. elongata leaves (3.0 g)	++	+	++	+	+	+(1)	5MeO-DMT
bark (3.0 g)	+/-	-	+/-	-	-	-	-
DMK-68 V. elongata leaves (3.0 g)	++	+	++	+	+	+(1)	5MeO-DMT
bark (3.0 g)	+/-	-	+/-	-	-	-	-
DMK-69 V. elongata leaves (3.0 g)	++	+	++	+	+	+(1)	5-MeO-DMT
bark (3.0 g)	+/-	-	+/-	-	-	-	-
DMK-75 V. loretensis leaves (3.0 g)	-	-	-	-	-	-	-
DMK-78 Osteophloem platyspermum leaves (3.0 g)	++	+	++	-	+	+(1)	N-methyltryptophan methyl ester

Table IX (cont'd)

name & Collection #	alkaloid reagent tests:* D	M	V	TLC:† UVfluor.	UVabs.	Ehrlich's reaction:‡	compounds detectedþ
DMK-82 V. albidiflora bark (3.0 g)	-	-	-	-	-	-	-
Plowman 12218 Virola sp. bark (2.0 g)	-	-	-	-	+	-	-

* reaction to alkaloid precipitation reagents. D=Dragendorff's reagent; M=Meyer's reagent; V=Valser's reagent. Composition of reagents is according to Farnsworth [8].
† Results of TLC of basic fractions: presence/absence of UV-fluorescent or UV-absorbing spots is indicated.
‡ Presence/absence of Ehrlich-positive spots (diagnostic for tryptamines) on TLC plate is indicated. Numbers in parentheses indicate # of Ehrlich-positive spots detected.
þ Indicates major compounds identified in sample, using a combination of methods including TLC, GC, HPLC, and in some cases GC/MS.

TABLE X - DETECTION OF ALKALOIDS IN MYRISTICACEOUS PASTE AND SNUFF SAMPLES

name & Collection # of source plant	amt. extracted (g d wt.)	alkaloid reagent test:* D	M	V	TLC:† UV fluor.	UVabs.	Ehrlich's reaction:‡	compounds detected♭
A. Orally Active Paste Samples:#								
1. DMK-40 V. sebifera Alfredo Moreno #1 - Puco Urquillo	2.0	+++	++	+++	-	+	+(2)	NMT, DMT, 5-MeO-DMT 5-MeO-NMT
2. DMK-67 V. elongata Alfredo Moreno #2 - Puco Urquillo	2.0	+++	++	+++	-	+	+(1)	NMT, DMT, MTHβC (GC/MS)
3. DMK-68 V. elongata Alfredo Moreno #3 - Puco Urquillo	2.0	+++	++	+++	-	+	+(1)	NMT
4. DMK-69 V. elongata Alfredo Moreno #4 - Puco Urquillo	2.0	+++	++	+++	-	+	+(1)	NMT
5. DMK-34 V. pavonis Jorge Churay - Puco Urquillo	2.0	-	-	-	-	-	-	-
6. DMK-59 V. elongata Marcos Flores- Brillo Nuevo	5.4	+++	++	+++	-	+	+(2)	5MeO-DMT, 5MeO-NMT
7. no voucher "oo'-koey" La Chorrera, Colombia (1971)	2.0	+++	++	+++	-	+	+(2)	DMT, NMT, MTHβC, DMTHβC (GC/MS)
B. Yanomamo snuffs (Venezuela, 1972):ϕ								
1. no voucher "buhenak + mashahara" = Justicia pectoralis + unknown species?	1.7	++	-	++	+	+	-	coumarin, umbelliferone (TLC,GC/MS)
2. no voucher "mashahari" = Justicia pectoralis	3.0	++	++	++	+	+	+(3)	DMT, 5-MeO-DMT, NMT, umbelliferone, coumarin (TLC,GC/MS)
3. no voucher "caraknak" = Virola sp. carbonized resin?	3.0	++	++	++	+	+	+(2)	DMT,5MeO-DMT
4. no voucher "yakuana-sagona" = Virola sp. or complete snuff	4.0	+++	+++	+++	+	+	+(2)	NMT, 5MeO-DMT

* reaction to alkaloid precipitation reagents. D=Dragendorff's reagent; M=Meyer's reagent; V=Valser's reagent. Composition of reagents according to Farnsworth [8].
† Results of TLC of basic fractions. UVfluor. Indicates presence/absence of fluorescent spots; UVabs. Indicates presence/absence of UV-absorbant spots.
‡ Presence/absence of Ehrlich-positive spots (diagnostic for tryptamines) on TLC. Numbers in parentheses indicate # of Ehrlich-positive spots detected.
b Indicates major compounds identified in sample, using combination of methods including TLC, GC, HPLC, & GC/MS.
First line indicates collection # and identity of source-plant, where known; second line indicates name of person manufacturing sample and village of origin
Φ No vouchers available for Yanomama snuff samples. Second line lists Yanomama name of sample and probable botanical identity based on names cited in Schultes [2].

material precluded further investigation. DMK-45 may represent a genetic variant of V. elongata in which tryptamine biosynthesis has been re-directed to the synthesis of β-carbolines, or it may represent a misidentification of the collection. Cassady et al. [7] reported the isolation of 6-MeO-tetrahydroharman, 6-MeO-harmalan, and 6-MeO-harman as the major bases of Virola cuspidata; these investigators did not detect tryptamines in this species. The most recent taxonomic monograph of the genus Virola [10] does not recognize V. cuspidata as a legitimate species, considering it equivalent to V. elongata.

This is not the only instance in which confusion has arisen in the phytochemical and ethnobotanical literature as a result of the ill-defined species concepts in the genus Virola. Numerous publications [1,2,3,5,6,11,12] refer to the use of Virola theiodora in the preparation of hallucinogenic snuffs and orally-ingested pastes; yet this species is not recognized by Rodrigues [10] and is treated as equivalent to V. elongata or V. calophylla. For instance the species list cited in [6] contains 2 entries for V. cuspidata, 5 entries for V. elongata, and 2 entries for Virola theiodora; if Rodrigues' revision of the genus is followed, all of these are properly designated by the binomial V. elongata. Inasmuch as the majority of the Myristicaceous voucher collections examined in the present study were determined by Dr. Rodrigues, the species concepts established in [10] have been followed.

Table IX shows several other differences from the survey reported in [6] which deserve comment. Holmstedt et al., examined three collections of Virola multinervia, 2 of which

contained DMT in the bark while the third was alkaloid negative. A single specimen of this species was examined in the present survey and was alkaloid negative. In [6], no alkaloids were detected in the leaves of V. sebifera and this was the only tissue examined; in our survey, this species contained substantial amounts (cf. Table XI) of DMT, 5-MeO-DMT, and NMT in the bark and traces of NMT in the leaves. Our collection of this species (DMK-40) was the source-plant for one of the orally-ingested paste samples obtained at Puco Urquillo. Corothie and Nakano [13] reported the isolation of DMT as the sole base from the bark of V. sebifera. Holmstedt et al., [6] examined 3 collections of Osteophloem platyspermum (incorrectly cited in Table I of [6] as Osteophloem platyphyllum). DMT, 5-MeO-DMT, and 5-hydroxy-DMT were detected in one of the samples, while the others were alkaloid-negative. O. platyspermum was represented in the present survey by a single sample of leaves (DMK-78) which contained a single Ehrlich positive base not corresponding to any of the available tryptamine standards. This compound gave Rf=0.6 in the TLC solvent used (cf. Materials and Methods) and a GC retention time of 10.1 min under the conditions stated. The compound was isolated by collecting the peaks from HPLC runs. UV spectral analysis was typical of unsubstituted indoles (Fig. 8) with absorption maxima at 289, 279, and 273 nm and minima at 286. The mass spectrum (Fig. 9) matched that of a reference spectrum of N-methyl-L-tryptophan methyl ester; the base peak was 130 mass units and the parent ion was m/z 232. The compound N,N-dimethyl-L-tryptophan has an identical molecular weight and base peak, however N-methyl-L-tryptophan methyl ester can be

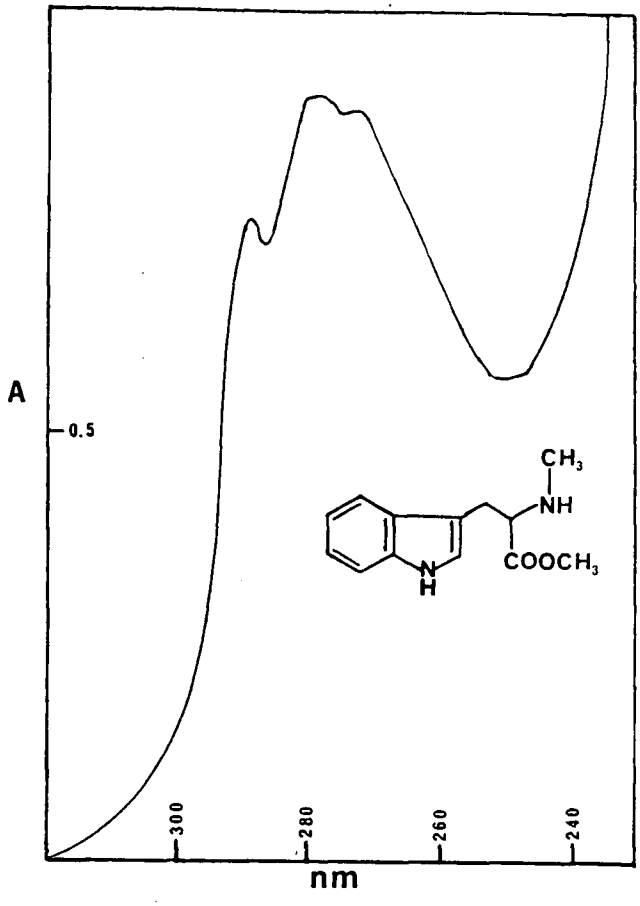


Figure 8 - UV Spectrum of N-methyl-tryptophan methyl ester from leaves of Osteophloem platyspermum

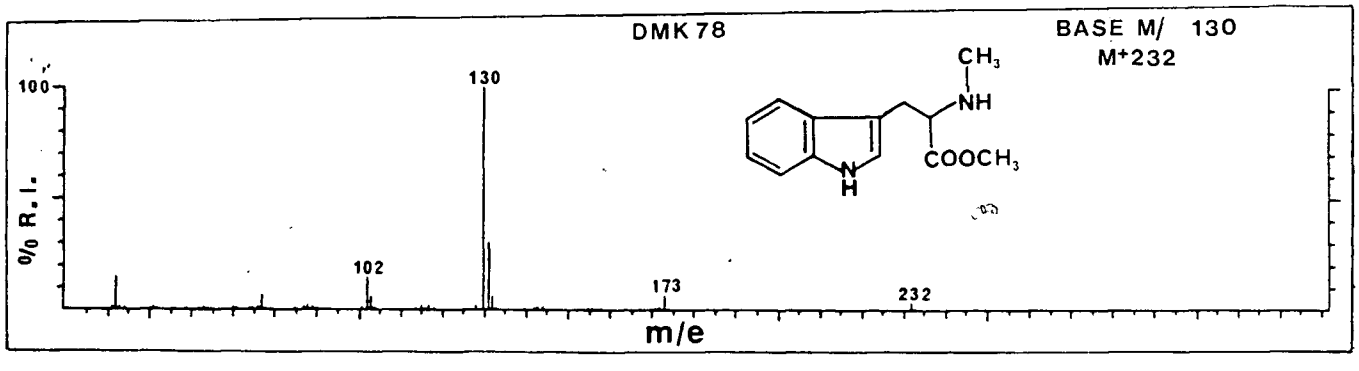


Figure 9 - Mass Spectrum of N-methyl-tryptophan methyl ester from leaves of Osteophloem platyspermum

distinguished from it by the presence of a peak at 173 mass units which represents the loss of the methyl carboxylate fragment (COOMe) from the parent ion (M-59). This peak is absent from the mass spectrum of N,N-dimethyl-L-tryptophan. The compound detected in O. platyspermum clearly shows an M-59 peak at m/z 173, thus providing strong evidence that it is in fact N-methyl-l-tryptophan methyl ester. This compound has not been reported in the Myristicaceae although it has been found in several species in the Leguminosae [14,15] and in Sida cordifolia (Malvaceae) [16]. The mass spectral data for our sample is consistent in all respects with that reported in [16]. Identification of an N-methylated tryptophan in a Myristicaceous genus is interesting from a biosynthetic standpoint as it indicates that the pathway to tryptamines in this family may involve methylation of the side chain nitrogen prior to decarboxylation.

2. Snuff and paste samples

The alkaloid constituents of a number of samples of Myristicaceous drug preparations were also investigated (Table X). These samples included one sample of orally-ingested paste collected at La Chorrera, Colombia, in 1971, and 6 paste samples collected in 1981 at the Witoto/Bora villages of Puco Urquillo and Brillo Nuevo on the Rio Ampiyacu in Peru. Four samples of Virola snuff from Venezeula were also screened.

All but one of the Myristicaceous paste samples contained substantial concentrations of alkaloid (Table X and XI). In most cases the base composition of the pastes reflected the base composition of the source plants used to prepare them; the

concentrations of tryptamines in the pastes were usually 1 to 2 orders of magnitude greater than the concentration in the crude plant material. β-carbolines were found in only two of the samples and in both instances were present in such low concentrations that mass spectral analysis was the only method capable of detecting them. The second sample prepared by Alfredo Moreno (DMK-67, V. elongata) contained low concentrations of DMT, NMT and a trace constituent with a mass spectrum closely matching that published [5] for 2-methyl-tetrahydro-β-carboline. In this sample the β-carboline component was so close to the limit of detection that a poor spectrum was obtained. However the sample from La Chorrera, for which no voucher is available, contained high levels of NMT and DMT, and traces of two β-carbolines; one of these could be unequivocally established as 2-methyl-THβC based on its identity with the published mass spectrum [5] (M^+=186, m/z 143=100%, additional peaks at m/z 130, 115, 89, 77) while the other, eluting immediately after 2-Me-THβC, had M^+=200, m/z 185=100%, and strong peaks at m/z 157, 144, 129, 118, 92, 77 (Fig. 10). The fragmentation pattern is similar to 2-Me-THβC except that all major peaks are 14 to 15 mass units higher. Like 2-Me-THβC, this compound also shows a prominent peak at M-43, corresponding to the loss of the CH_3-$N{=}CH_2$ fragment, although the base peak at m/z 185 (M-15) probably represents the facile loss of a methyl from C_1 and the formation of a stabilized intermediate with positive charge on the piperidine N. The prominent peak at m/z 157 is produced by loss of the piperidine N and the adjacent carbon 3, a typical fragmentation pattern for tetrahydro-β-carbolines [17]. A

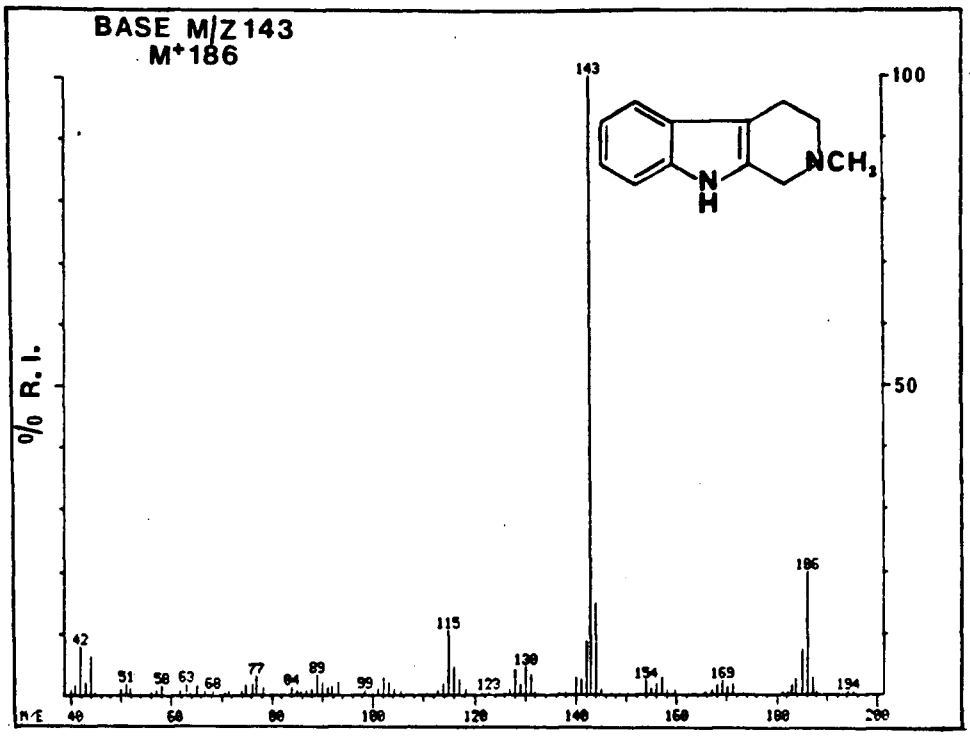


Figure 10 - Mass Spectrum of MTHβC from La Chorrera Oo'-koey

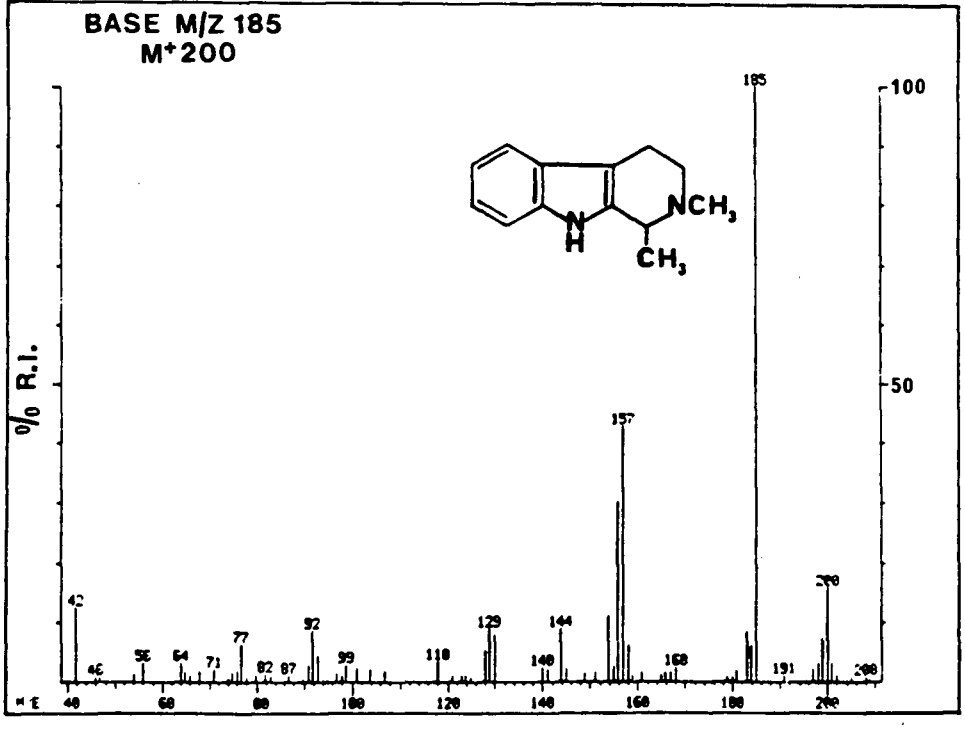


Figure 11 - Mass Spectrum of DMTHβC from La Chorrera Oo'-koey

reasonable structure can be postulated for this compound, viz., 1,2-dimethyl tetrahydro-β-carboline. A similar compound, 6-MeO-1,2-dimethyl-THβC, was isolated by Augurell and coworkers [18] from the seeds of Anadenanthera peregrina (Leguminoseae).

The failure to detect β-carbolines in all of the paste samples and the extremely low levels found in two samples raises some interesting questions with regard to the postulated mechanism of oral activity of these pastes. The hallucinogenic activity of the Myristicaceous pastes almost certainly results from the high concentrations of psychotomimetic tryptamines which they contain, yet these compounds are not orally active by themselves, requiring an MAO inhibitor to protect them from intestinal and hepatic degradation. The β-carbolines are effective MAO inhibitors and theoretically could orally potentiate the tryptamines in the pastes, provided they were present in sufficient concentration to effectively inhibit MAO. This mechanism has been postulated [11,12,6] to account for the oral activity of the Myristicaceous pastes as it probably also does for ayahuasca (cf. Chapter IV also [18,19,20]). The aromatic β-carbolines found in high concentration in ayahuasca (cf. Chapter IV) are considerably more effective as MAO inhibitors than the tetrahydro-β-carbolines detected in the Virola pastes (cf. Chapter VI, also [21]). Since not all of the paste samples contain β-carbolines, and those which do contain trace amounts of the less effective tetrahydro-β-carbolines, it seems extremely unlikely that these constituents could have any pharmacological significance in terms of orally potentiating the tryptamines in the pastes. In fact, the question of whether the

Myristicaceous pastes are orally effective as hallucinogens remains open (cf. Chapter III). In any case, it appears that their oral activity, if present, must depend on some mechanism other than β-carboline mediated inhibition of MAO. The low levels of β-carbolines detected in the pastes, and the failure to detect similar compounds in the source-plants used for the drugs, also leaves open the possibility that they may be formed as artifacts either during the cooking and concentration of the Virola resin or during the work-up of the alkaloid extract. This possibility has also been raised by Holmstedt et al. [6]. Tetrahydro-β-carbolines of the type found in the paste samples can be readily formed from tryptamine derivatives such as NMT by aldehyde condensation followed by cyclization [22].

Four non-Myristicaceous collections utilized as admixtures to the orally-ingested pastes were screened for alkaloids using precipitation tests. All were alkaloid-negative. The use of these species (viz.: Rinora racemosa (Mart. & Zucc.)Kuntze, (DMK-38), Anemia sp. (DMK-39), Theobroma subinacum Mart. (DMK-43), T. bicolor H. & B. (DMK-65), and Philodendron nervosum (Schult. & Schult.)Kunth (DMK-64)), as admixtures to the Virola pastes is discussed more fully in Chapter III.

In addition to the orally-ingested Myristicaceous samples, four Myristicaceous snuff samples collected among the Yanomama Indians of Venezeula were analyzed. These samples represent various components used in the manufacture of the snuffs, but are not accompanied by voucher specimens. They were collected by Dr. Ernesto Migliazza, an ethnolinguist who conducted field research on Yanomama language and grammar from 1970-72. They

were given to the author in 1974 by Dr. Migliazza, who at that time was on the faculty of the Department of Anthropology at the University of Maryland, College Park. Dr. Migliazza collected the samples in connection with his liguistic studies; although no vouchers were collected, the correct native name of each of the samples was noted by Dr. Migliazza. By comparing these names with those reported by Schultes [2], I was able to arrive at the probable botanical identity of each sample. Subsequent chemical analyses tended to confirm these tentative identifications. Two of the samples are aromatic powdered leaves of an herbaceous plant which is almost certainly Justicia pectoralis Jacq. Var. stenophylla Leonard. One of these samples is labelled "mashahari" while the other is labelled "buhenak + mashahara". Schultes [2] states that the Karauetari Indians, a Yanomama subgroup on the Rio Cauaburi in Brazil, apply the name mashahari to Justicia pectoralis var. stenophylla, while Indians of the Rio Tototobi, Brazil, have two names for Justicia pectoralis: masha-hara-hanak (hanak means "leaf") and boo-hanak ; this latter term is sufficiently close to "buhenak" that the terms are probably synonymous. Another possibility is that the Yanomama may recognize more than one kind or variety of Justicia pectoralis and apply different terms to each type. Chemical analyses of the samples lends support to this latter supposition. The sample labelled "mashahara + buhenak" contained no alkaloids (cf. Table X) but did contain the benzopyran derivatives coumarin and umbelliferone. These compounds were found in the Peruvian collections of Justicia pectoralis made by the author (DMK-1, [23]); their identity has been established by

comparison of their mass spectra and TLC Rf values with authentic standards. Based on this evidence, it seems reasonable to speculate that the sample labelled "buhenak + mashahara" consists of powdered Justicia pectoralis leaves without any admixtures. The situation is less clear with respect to the sample labelled "mashahari". Although the label indicates that only one ingredient (Justicia pectoralis) is present, chemical analysis (Table X & XI) has detected DMT, 5-MeO-DMT, and NMT in this sample in addition to the coumarin derivatives characteristic of Justicia pectoralis. Several explanations may account for this difference but unfortunately the issue cannot be decided in the absence of botanical voucher specimens. One explanation is that the labels have been exchanged on the two samples, and the sample labelled "mashahari" is really "buhenak + mashahara"; if this is the case, then the ingredient "buhenak" may not represent Justicia pectoralis, but instead may be a term applied to another ingredient, probably a Virola species, which does contain tryptamines and is used in the manufacture of Yanomama snuff. An alternative explanation is that this sample does in fact represent Justicia pectoralis or a related species, which synthesizes tryptamines as well as coumarins. There exist reports [24, Prance, G. T., pers. comm., 1983] that occasionally the Yanomama do prepare an intoxicating snuff using Justicia pectoralis as the sole ingredient; these reports need to be corroborated by further ethnobotanical and chemical investigations. A third sample consists of black, partially charred and carbonized plant material, labelled with the Yanomama term "caraknak". This term does not correspond to any

of those reported by Schultes [2], but may consist of partially carbonized Virola resin. Schultes, describing the preparation of the snuff on the Rio Tototobi [2], mentions that a portion of the resin becomes carbonized in the process of boiling the resin; this carbonized material is powdered separately from the rest of the resin to form a blackish brown powder. The remainder of the uncarbonized resin is ground into a light coffee-colored powder, which is then mixed with the carbonized material to produce the final snuff. Based on this description, it seems probable that the partly charred material labeled "caraknak" consists of carbonized Virola resin. The low levels of DMT and 5-MeO-DMT which this sample contained (cf. Table XI) support this identification. The fourth sample is labelled "yakuana-sagona" and consists of dark reddish brown powdered bark material. Both its appearance and its high alkaloid content (cf. Table XI) make it virtually certain that this sample consists either of powdered, concentrated Virola resin or of the finished snuff containing Virola resin plus other admixtures. According to Schultes [2], the term applied to the snuff on the Rio Tototobi is nyakwana, which is reasonably close to yakuana. Determining the exact equivalence of these native terms is complicated by the fact that the Yanomama linguistic family includes at least four major languages, each with numerous dialects; many of these dialects are mutually unintelligible [25]. In view of this it is remarkable that the names recorded by Migliazza for the various snuff components can be matched so closely with those recorded by Schultes.

B. Quantitative Analyses of Myristicaceous Samples

The quantitative data for the tryptamines found in the various Myristicaceous drug samples (Table XI) show that the Virola snuff ("yakuana-sagona") and the various paste samples contain similar amounts of alkaloids on a dry wt basis. The snuff sample contained 19.7 mg/g 5-MeO-DMT, a figure of the same order of magnitude as that reported by Holmstedt et al. [5] who found 7.15 mg/g in a sample of "epena" snuff from the Rio Cauaburi. A sample of nyakwana snuff from the Rio Tototobi was reported by Holmstedt et al. [5] to contain more than 110 mg/g DMT and 5-MeO-DMT, or more than 11% alkaloids. In light of the other quantitative work reported in this and other studies [5,6], this figure is open to question. It is possible that an error was made either in the measurement of the alkaloid content of the sample, or in the calculation of the experimental data. The other components of the Yanomama snuffs which were found to contain tryptamines ("mashahari" and "caraknak") contained quite low levels compared to the "yakuana" sample. Tryptamine concentrations found in the orally-ingested Myristicaceous drugs were generally of the same order of magnitude as those in the snuff. For instance, the first batch of paste (designated oo'-koey in Witoto) obtained from Alfredo Moreno at Puco Urquillo, contained 18.8 mg/g alkaloid, 70% of which was 5-MeO-DMT. The second, third, and fourth batches obtained from Alfredo Moreno contained anomalously low levels of alkaloid. These batches were derived from DMK-67,68, and 69, respectively, all of which have been determined as V. elongata. Comparison of the voucher specimens, and the qualitative and quantitative similarities of

TABLE XI - QUANTITATIVE ANALYSIS OF TRYPTAMINES IN MYRISTICACEOUS SAMPLES

Sample name & origin	Species & Collection #	alkaloids detected	mg/g d wt.	%
A. Orally active Myristicaceous pastes:				
"oo'-koey" La Chorrera, Colombia	no voucher	DMT	1.58	14
		NMT	9.43	86
		Total:	11.01	
"oo'-koey" Puco Urquillo Alfredo Moreno #1	DMK-40 V. sebifera	DMT	3.8	20
		5-MeO-DMT	13.2	70
		NMT	1.78	10
		Total:	18.78	
"oo'-koey" Puco Urquillo Alfredo Moreno #2	DMK-67 V. elongata	DMT	0.065	7
		NMT	0.86	93
		Total:	0.92	
"oo'-koey" Puco Urquillo Alfredo Moreno #3	DMK-68 V. elongata	DMT	0.25	10
		NMT	2.2	90
		Total:	2.45	
"oo'-koey" Puco Urquillo Alfredo Moreno #4	DMK-69 V. elongata	DMT	0.164	10
		NMT	1.43	90
		Total:	1.59	
"ku'-ru-ku" Brillo Nuevo Marcos Flores #1	DMK-59 V. elongata	5-MeO-DMT	15.72	100
B. Yanomama snuffs & snuff admixtures:				
"mashahari" = Venezuela	Justicia pectoralis (no voucher)	DMT	0.09	15
		5-MeO-DMT	0.52	85
		Total:	0.61	
"caraknak" = Venezuela	Virola sp. carbonized resin (no voucher)	DMT	0.064	17
		5-MeO-DMT	0.32	83
		Total:	0.384	
"yakuana-sagona" = Venezuela	Virola sp. (snuff) (no voucher)	5-MeO-DMT	19.7	100

Table XI (cont'd)

Sample name & origin	Species & Collection #	alkaloids detected	mg/g d wt.	%
C. Virola bark & leaf samples:				
Virola sp.	DMK-30			
	leaves	DMT	0.08	100
		NMT	trace	
	twigs	DMT	0.085	100
		NMT	trace	
Virola sp.	DMK-35			
	leaves	DMT	0.097	100
		NMT	trace	
Virola sp.	DMK-36			
	leaves	DMT	0.106	100
		NMT	trace	
Virola sp.	DMK-37			
	leaves	DMT	0.097	100
		NMT	trace	
Virola sebifera	DMK-40			
	leaves	NMT	trace	
	bark	DMT	0.078	30
		5-MeO-DMT	0.181	70
		NMT	trace	
		Total:	0.259	
Virola elongata	DMK-41			
	bark	DMT	0.063	38
		NMT	0.102	62
		Total:	0.165	
Virola callophylla	DMK-46			
	bark	DMT	0.56	100
Virola callophylla	DMK-56			
	seeds & fruit	DMT	0.185	100
		NMT	trace	
Virola elongata	DMK-59			
	bark	5-MeO-DMT	0.23	100
		NMT	trace	
	leaves	DMT	0.17	100
		NMT	trace	

the alkaloid profiles of the bark, leaf and paste samples obtained from these three specimens (cf. Tables IX, X, XI) make it quite clear that these three paste samples were all derived from the same tree.

The variation seen in the levels of alkaloid in the orally ingested samples (Table XI) ranged from no alkaloid to substantial concentrations of alkaloid (e.g. Alfredo Moreno #1). This quantitative variation is accompanied by considerable variation in the kind and number of tryptamine bases present. For example the sample prepared from DMK-40 contained measurable levels of DMT, 5-MeO-DMT, and NMT, while that prepared from DMK-59 contained only 5-MeO-DMT in any significant quantity. Similar differences in composition were found in the snuff samples. Generally the base composition of the pastes reflected that of the source-plants, at least in those instances where the source-plants were available for examination (Table IX & X). The alkaloid concentrations in the prepared pastes were usually 60 - 200 times greater than in the source plants. Therefore it seems that the technology of preparing the drug, which basically involves extracting, cooking, and concentrating the resin, does not result in a drastic change in the base composition compared to the source-plants; however minor constituents, such as the tetrahydro-β-carbolines, may be artifacts of the cooking process. The fact that these compounds have also been isolated from "raw" untreated Virola bark argues against this, however.

Disregarding for the moment the question of whether the orally-ingested Myristicaceous drugs require a MAOI in order to potentiate their activity, we can use the quantitative data from

Table XI to estimate the amount of drug which would be required to elicit psychotomimetic effects typical of tryptamines. Szara [26] reported in self-experiments that the lower threshold dose for DMT administered i.m. was 0.2mg/kg, i.e., 15 mg for a 75 kg adult, with optimal effects reached at 0.7 - 1.0 mg/kg, i.e,, between 50 and 75 mg. Thus from Table XI it is clear that for samples with the highest alkaloid content a threshold dose would require the ingestion of somewhat less than 1 gram of paste or snuff, while an "optimal" dose would require the ingestion of 4 - 5 g dry wt of plant material. However if 5-MeO-DMT instead of DMT were the major constituent then approximately one-tenth as much plant material would be an adequate threshold dose (cf. Table I). The ingestion of several grams of the orally-ingested pastes would not be a problem but the absorption of 4 grams of Virola snuff through the mucous lining of the nose and throat might present some mechanical difficulties. The most active pastes and snuffs may be those that contain a relatively greater proportion of 5-MeO-DMT; certainly this was a characteristic of the two paste samples which showed the greatest activity in the bioassays (cf. Chapter III and Table XI). In fact, the actual pharmacological activity of these preparations is probably determined by more than a simple dose-response relationship; the presence or absence of MAO inhibitors, the route of ingestion, the diet, body weight, and general health of the person ingesting the drug, the frequency of use and whether or not tolerance is developed, are all factors which could affect the physiological action of these drugs. The variability in response which was observed in self-experiments with some samples

examined in the present study (cf. Chapter III) also tends to indicate that the activity/inactivity of these Myristicaceous preparations is a function of numerous variables, not all of them botanical or chemical in nature.

IV. Summary

Alkaloid constituents in Myristicaceous bark and leaf samples and in purportedly hallucinogenic preparations derived from Myristicaceous sources were qualitatively and quantitatively analyzed using TLC, GC, alkaloid precipitation tests and GC/MS. Sixteen of the twenty eight bark and leaf samples analyzed contained detectable amounts of alkaloids. The major bases were DMT and/or 5-MeO-DMT; much smaller amounts of tryptamine and/or NMT were also usually present. Detectable levels of β-carbolines were not found in the bark or leaf samples. Considerable variation in alkaloid profiles was found, extending to different collections of the same species. Fourteen of the eighteen *Virola* samples contained alkaloids; none of the six *Iryanthera* species had detectable alkaloids. A single sample of *Osteophloem platyspermum* contained an indolic base, identified as N-methyl-tryptophan methyl ester on the basis of UV and mass spectral characteristics. Seven samples of an orally-ingested drug made from *Virola* spp. were analyzed. All except one contained substantial amounts of tryptamines, but the types and proportions of tryptamines present varied greatly between samples. Samples of *Virola* snuff including various admixtures were analyzed and all components but one contained

detectable levels of tryptamines. The drug samples having the highest concentrations of alkaloids contained 15-20 mg/g dry wt while the Myristicaceous bark and leaf samples had much lower concentrations ranging from 0.05 to 0.26 mg/g dry wt. Only two of the Myristicaceous drug samples contained detectable amounts of β-carbolines which had mass spectra corresponding to MTHβC and DMTHβC. The β-carbolines were trace constituents, however. These results indicate that the inactivation of peripheral MAO by β-carbolines is unlikely to account for the oral activity of the Myristicaceous pastes, since the amounts detected are probably not pharmacologically significant.

V. Literature Cited

1. R. E. Schultes. (1979) Evolution of the Identification of the Myristicaceous Hallucinogens of South America. Journal of Ethnopharmacology 1:211-239

2. R. E. Schultes, & B. Holmstedt (1968) The Vegetal Ingredients of the Myristicaceous Snuffs of the Northwest Amazon. Rhodora 70:113-160

3. R. E. Schultes & A. Hofmann. (1980) The Botany and Chemistry of Hallucinogens (2nd Ed.) Charles C. Thomas, Publishers. Springfield, Ill

4. O. R. Gottlieb. (1979) Chemical Studies on Medicinal Myristicaceae from Amazonia. Journal of Ethnopharmacology 1:309-323

5. S. Agurell, B. Holmstedt, & J. E. Lindgren. (1969) Alkaloids in Certain Species of Virola and Other South American Plants of Ethnopharmacologic Interest. Acta Chemica Scandinavica 23:903-916

6. B. Holmstedt, J. E. Lindgren, T. Plowman, L. Rivier, R. E. Schultes & O. Tovar. (1980) Indole Alkaloids in Amazonian Myristicaceae: Field and Laboratory Research. Harvard Botanical Museum Leaflets 28:215-234

7. J. M. Cassady, G. E. Blair, R. F. Raffauf & V. E. Tyler. (1971) The Isolation of 6-methoxyharmalan and 6-methoxyharman from Virola cuspidata. Lloydia 34:161-162

8. R. Martello & N. Farnsworth. (1962) Observations on the Sensitivity of Several Common Alkaloid Precipitating Reagents. Lloydia 25:176-185

9. D. B. Repke, D. T. Leslie & G. Guzman. (1977) Baeocystin in Psilocybe, Conocybe, and Panaeolus. Lloydia 40:566-78

10. W. A. Rodrigues. (1980) Revisao Taxonomica das Especies de Virola Aublet (Myristicaceae) do Brasil. Acta Amazonica 1:1-123

11. R. E. Schultes (1969) Virola as an Orally-administered Hallucinogen. Harvard Botanical Museum Leaflets 22:229-40.

12. R. E. Schultes & T. Swain. (1976) Further Notes on Virola as an Oral Hallucinogen. Journal of Psychedelic Drugs 8:317-324.

13. E. Corothie & T. Nakano. (1969) Constituents of the Bark of Virola sebifera. Planta Medica 17:184-88

14. S. R. Johns, J. A. Lamberton, & A. A. Sioumis. (1971) The Major Alkaloid of Aotus subglauca (Leguminoseae), S-(+)-N - methyltryptophan methyl ester. Australian Journal of Chemistry 24:439-40

15. N. Mandava, J. D. Anderson & S. R. Dutky. (1974) Indole Plant-growth Inhibitor from Abrus precatorius Seeds. Phytochemistry 13:2853-2856

16. S. Ghosal, R. Ballav, P. S. Chauhan & R. Mehta. (1975) Alkaloids of Sida cordifolia. Phytochemistry 14:830-832

17. R. T. Coutis, R. A. Locock & G. W. A. Slywka. (1970) Mass Spectra of Selected β-carbolines [β-9H-pyrido(3,4-b)indoles]. Organic Mass Spectrometry 3:879-889

18. S. Agurell, B. Holmstedt, J. E. Lindgren, & R. E. Schultes. (1968) Identification of Two New β-carboline Alkaloids in South American Hallucinogenic Plants. Biochemical Pharmacology 17:2487-88

19. Schultes, R. E. (1972) Ethnotoxicological Significance of Additives to New World Hallucinogens. Plant Science Bulletin 18: 34-41

20. Der Marderosian, A. H., H. V. Pinkley, & M. F. Dobbins IV. 91968) Native Use and Occurrence of N,N-dimethyltryptamine in the Leaves of Banisteriopsis rusbyana. American Journal of Pharmacy 140:137-147

21. N. S. Buckholtz & W. O. Boggan. (1976) Effects of Tetrahydro-β-carbolines on Monoamine Oxidase and Serotonin Uptake in Mouse Brain. Biochemical Pharmacology 25:2319-2321 1976

22. R. G. Taborsky & W. M. McIsaac. (1964) The Synthesis and Preliminary Pharmacology of Some 9H-Pyrido[3,4-b]indoles (β-carbolines) and Tryptamines Related to Serotonin and Melatonin. Journal of Medicinal Chemistry 7:135-141

23. D. MacRae & G. H. N. Towers (1984) Justicia pectoralis, a Study of the Basis for its Use as a Virola Snuff Admixture. Journal of Ethnopharmacology. Submitted.

24. Chagnon, N., P. LeQuesne, & J. M. Cook (1971) Yanomamo Hallucinogens: Anthropological, Botanical, and Chemical Findings. Current Anthropology 12:72-74

25. E. C. Migliazza. (1972) Yanomama Grammar and Intelligibility. PhD. Thesis, Indiana University.

26. S. Szara (1957) The Comparison of the Psychotic Effect of Tryptamine Derivatives with the Effects of Mescaline and LSD-25 in Self-experiments. pp. 460-467 in S. Garattini & V. Ghetti (eds.) Psychotropic Drugs Elsevier, Amsterdam.

CHAPTER VI: ORALLY-ACTIVE MALPIGHIACEOUS AND MYRISTICACEOUS HALLUCINOGENS AND THEIR CONSTITUENTS AS MONOAMINE OXIDASE INHIBITORS

I. Introduction

The mitochondrially-localized enzyme monoamine oxidase (MAO) plays an important role in mammalian metabolism. MAO catalyzes the oxidative deamination of biogenic monoamines including tyramine, tryptamine, serotonin, norepinephrine, dopamine, and other monoamines, according to the general reaction equation [1]:

$RCH_2NH_2 + O_2 + H_2O$ --MAO--> $RCHO + NH_3 + H_2O_2$

The enzyme is widely distributed throughout various tissues in vertebrates and invertebrates, including the liver, brain, small intestine, blood plasma and platelets, heart, and lungs [2]. The most significant metabolic function of MAO is related to its ability to inactivate endogenously produced monoamines such as serotonin or dopamine, which function as CNS neurotransmitter substances [3]. Considerable experimental evidence has been accumulated [2] which indicates that the visceral MAO system functions as a detoxification mechanism serving to protect the nervous and cardiovascular systems from toxic biogenic amines ingested in the diet and formed as a result of aromatic amino acid decarboxylation. Evidence based on substrate specificity and selective sensitivity to certain MAO inhibitors indicates that MAO consists of two species, designated MAO-A and MAO-B [4,5,6,7]. Houslay & Tipton [7] carried out a kinetic evaluation of the two species using mixed substrate assays and concluded

that the substrates for species A activity were substituted β-phenylethylamines and β-phenylethanolamine derivatives having a free p-hydroxyl group; and 5-hydroxytryptamine. Substrates for species B activity included substituted benzylamine and β-phenylethylamine derivatives, but not substituted β-phenylethanolamines. Tryptamine, 5-methoxytryptamine, dopamine, and the m-O-methyl derivatives of dopamine are substrates for both MAO-A and MAO-B. Neff et al., [8] point out that substances which interact with MAO-A such as serotonin and norepinephrine have more polar aromatic rings than substances which interact with MAO-B (e.g., β-phenylethylamine, benzylamine). In general adding a polar hydroxyl group to β-phenylethylamines (e.g. tyramine) or removing one from serotonin to form tryptamine results in a common substrate. The specific metabolism of DMT has not been investigated, but if it conforms to the above rule it would be a substrate for both MAO-A and MAO-B since it lacks aromatic substituents. Further evidence for the existence of two species of MAO comes from experiments using selective MAO inhibitors [5].

Most of the MAO inhibitors which have been the focus of biochemical and clinical investigations belong to one of four classes, viz., hydrazine derivatives, phenylcyclopropylamine derivatives, N-benzyl-N-methyl propargylamine, or 2-methyl-3-piperidinopyrazine [9]. These are synthetic compounds which are not known to have corresponding analogues in nature. The major exception is the β-carboline derivatives, a group of tricyclic indole alkaloids which are widespread in nature [10] (cf. Fig. 2). These compounds are biosynthetically derived from tryptamine

and its derivatives and can be readily synthesized from tryptamine derivatives [11]. Udenfriend et al., [12] were the first to demonstrate that harmaline and related compounds are extremely potent reversible inhibitors of monoamine oxidase. These investigators showed that the fully aromatic β-carbolines were the most effective inhibitors, and that activity decreased with increasing saturation of the piperidine ring; tetrahydro-β-carbolines still showed significant activity, however. Subsequently, experiments by Fuller et al.[4] showed that harmaline selectively inhibited oxidation of serotonin, indicating that it was a specific inhibitor of MAO-A.

The capacity of β-carbolines to inhibit MAO has been suggested to be the mechanism responsible for the oral activity of ayahuasca [13,14,15,16] and possibly also for the activity of other orally ingested drugs derived from Virola spp. [17,18,19].

Both ayahuasca and the orally-ingested hallucinogenic pastes derived from Virola spp. contain psychotomimetic tryptamines such as N,N-dimethyltryptamine (DMT), 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) and related tryptamine derivatives, as well as β-carboline derivatives (cf. Chapters IV & V). Ayahuasca contains substantial amounts of the 7-substituted β-carbolines harmine, harmaline, and tetrahydroharmine as well as DMT derived from the admixture plant Psychotria viridis, commonly used in the preparation of ayahuasca (cf. Chapter IV). The orally-ingested Virola pastes, which are prepared from the cambial sap of various Virola species, contain psychoactive tryptamines as the major alkaloids; in addition some samples contain trace amounts of

tetrahydro-β-carbolines (cf. Chapter V and [16,19]). The tryptamines DMT and 5-MeO-DMT are potent hallucinogens, but are not orally active, presumably due to deamination by peripheral MAO; in the presence of β-carbolines, however, the compounds may be protected from deamination and thus rendered orally active. Although this mechanism has been proposed to underly the oral activity of ayahuasca and the Virola paste preparations, the inhibition of MAO by these drugs or their alkaloid fractions has not previously been investigated.

In the present study, the activity of various β-carboline and tryptamine derivatives as specific inhibitors of MAO-A was investigated. The structure/activity relationships of a selected sample of derivatives from each class were determined. Activity measured with individual compounds was compared with the activity of mixtures of compounds which approximated the composition of the alkaloid fractions of ayahuasca and the orally-ingested Virola pastes. The mixtures of standards were assayed in order to determine whether combinations of alkaloids exhibit synergistic activity as MAOI. The MAOI activity of two ayahuasca samples and the basic fractions of three Virola paste samples were investigated. The activity of one Virola paste sample lacking alkaloids and a crude lignan fraction isolated from V. elongata was also investigated [26].

II. Materials and Methods

A. Preparation of rat-liver cytosol

Rat-liver, obtained from mature female Wistar rats, was

used as the source of monoamine oxidase for the assays. The rats were sacrificed by stunning followed by cervical dislocation. The abdominal cavity was opened by removal of the belly skin and peritoneum and the liver perfused with cold 0.1 M sodium phosphate buffer, pH 7.2 [20] by injection into the hepatic vein with a beveled #22 stainless steel syringe. The perfused liver was removed from the abdominal cavity and immediately placed in cold buffer. Portions of liver were transferred to a glass homogenizer and homogenized in cold buffer to a smooth paste (± 1 g liver/3 ml buffer). The homogenate was centrifuged at 3000 x g for 20 min to remove nuclear fragments. The supernatant was collected and diluted 1:1 with buffer. The diluted cytosol fraction was sonicated for 10 sec. This diluted whole cytosol fraction was kept on ice for the duration of the experiment, and was used without further centrifugation.

B. Preparation of reaction mixture and addition of labelled substrate

The assay was conducted at 37.5° C in 13 x 100 mm disposable test tubes. Volume of the reaction mixture was 0.5 ml. Composition of the reaction mixture in controls lacking added inhibitor consisted of 375 µl buffer, 100 µl whole cytosol, and 25 µl labelled substrate. The substrate consisted of 5-hydroxytryptamine (side-chain-2-^{14}C)creatinine sulfate (Amersham). The labelled substrate was diluted with cold carrier 5HT to a specific activity of 0.835 mCi/mmole and a concentration of 2 x 10^{-3} M. At this dilution, addition of a 25 µl aliquot to the reaction mixture results in a final volume of

500 µl and a 5HT concentration of 0.1 mM, containing a total of 0.04 µCi of labelled 5HT. All components of the reaction mixture except the labelled substrate were added to the reaction tubes which were maintained at 0° C in an ice bath. Inactivated control blanks, identical in composition to the active controls, were prepared by immersing the tubes in boiling water for 10 min. Prior to initiation of the assay, the reaction tubes were immersed in a 37.5° C water bath and equilibrated for 5 min. The assay was initiated by adding a 25 µl aliquot of the diluted substrate to the tubes, mixing for 1-2 sec on a vortex mixer, and replacing in the water bath. Following addition of the substrate the assay tubes were incubated for 30 min at 37.5° C. After 30 min the reaction was terminated by adding 1.0 ml of 1.0 M citrate. This was followed by the addition of 0.5 ml saturated NaCl, and 2.0 ml ethyl acetate to each tube. Tubes were then vortexed for 5 sec each and centrifuged at 2000 rpm for 10 min to separate the organic and aqueous layers. In this method, [21] the labelled reaction products are soluble in the organic (upper) phase while the protonated substrate remains in the lower aqueous phase. Following centrifugation, a 1.0 ml aliquot of the upper phase was transferred to a scintillation vial containing 9.0 ml Aquasol-2 (New England Nuclear). Scintillation vials were counted for 10 min in a Searle Isocap 300 Liquid Scintillation Counter.

C. Assays using tryptamine and β-carboline standards

Stock solutions of various β-carboline and tryptamine derivatives, dissolved in 0.1 N HCl, were prepared so that

addition of a 50 µl aliquot to a reaction mixture would result in an inhibitor concentration of 10^{-3} M and a total volume of 500 ul. The tryptamine derivatives 5-Methoxy-diisopropyl tryptamine and 3-[2-(2,5-dimethyl)pyrrolylethyl]-indole were poorly soluble in 0.1 N HCl so these compounds were dissolved in 10% Tween-80. Stock solutions for lower concentrations were prepared by making serial 1:10 dilutions, over a concentration range from 10^{-3} to 10^{-10} M. Assay mixtures containing inhibitor consisted of 325 µl buffer, 100 µl whole cytosol, 50 µl inhibitor solution of the appropriate concentration, and 25 µl substrate. The inhibitor solution was added just before the assay tubes were transferred to the warm water bath, so that the incubation time of the cytosol plus inhibitor at 37.5°C was 5 min. Boiled blanks containing denatured enzyme were included in each run. In addition controls containing active enzyme but no inhibitor were also assayed simultaneously (inhibitor was replaced either with buffer, 10% Tween-80, or with 50 µl 0.1 N HCl). There was no appreciable difference between controls in which buffer replaced the inhibitor and those in which 0.1 N HCl or 10% Tween-80 replaced the inhibitor. The controls lacking inhibitor represented 0% MAO inhibition; and the % inhibition of tubes containing varied concentrations of inhibitor was calculated relative to these controls, by dividing the amount of labelled reaction products recovered (measured as cpm) by the amount recovered from the control tubes. The background count represented by the activity detected in the boiled blanks (usually 200-300 cpm) was subtracted from both control counts and inhibitor counts.

D. Preparation and quantitation of ayahuasca samples for MAO assay

The ayahuasca samples used in the assay were Don Fidel #1 and Don Juan #2. Five ml aliquots of each were diluted to 50 ml with chilled methanol and the white nonalkaloidal precipitate removed by filtration. The filtered solution was then evaporated to dryness under reduced pressure and resolubilized in 5 ml 0.01 N HCl to reconstitute the original volume. A 1:1 dilution of this reconstituted solution was used to quantify the alkaloids present in the mixture using the HPLC method described in Chapter IV. This quantification showed the reconstituted samples to contain 3.5 mg/ml and 4.8 mg/ml total alkaloid, respectively. Serial 1:10 dilutions of the reconstituted samples, ranging from 0 to 10^{-7}, were made using 0.01 N HCl. Fifty μl aliquots of the appropriately diluted ayahuasca solutions were added to the reaction mixture in the same manner as the inhibitors, and % inhibition was calculated relative to the controls.

Inhibitor assays were also conducted using mixtures of standards which approximated the composition of ayahuasca. Mixtures having the following compositions were assayed:

a. Equimolar mixture of harmine, harmaline, and tetrahydroharmine; total alkaloid concentration of undiluted stock solution: 2.115 mg/ml (10^{-3} M, based on average MW).

b. Equimolar mixture of harmine, harmaline, tetrahydroharmine, and DMT; total alkaloid concentration of undiluted solution: 2.16 mg/ml (10^{-3} M, based on average MW).

c. "ayahuasca analogue #1" - 69% harmine, 4.6% harmaline, and 26% tetrahydroharmine; overall concentration equivalent to the

equimolar mixture.

d. "ayahuasca analogue #2" - 65% harmine, 6% harmaline, 22% tetrahydroharmine, and 7% DMT; overall concentration equivalent to the equimolar mixture.

E. Preparation and quantitation of Myristicaceous paste samples for MAO assay

The Myristicaceous paste samples used for the assay were Alfredo Moreno sample #1, prepared from Virola sebifera, (DMK-40), Don Marcos sample #1, (V. elongata, DMK-59) and the "oo'-koey" from La Chorrera, Colombia (no voucher). Aliquots of the methanol extracts of each paste sample equivalent to 0.2-0.4 g dry wt of the original sample were evaporated to dryness and resolubilized in 2 ml methanol. One ml was removed, diluted 1:1 with methanol, and filtered through a cotton-plugged Pasteur pipette. Twenty µl aliquots of this diluted sample were injected onto the HPLC in order to quantitatively determine the alkaloid content of the sample. The quantitative methods and analytical conditions were identical to those used in the quantitation of the ayahuasca samples (cf Chapter IV). The molar concentration of tryptamines in each sample was calculated from the quantitative data (Table XII). An "analogue" of each sample was prepared using tryptamine standards. Each paste "analogue" consisted of a mixture of tryptamine standards in the same proportions and concentrations as were measured in the paste samples. Each paste sample and its analogue were simultaneously assayed in the monoamine oxidase system. If the MAOI activity of the paste sample and the analogue were equivalent, this would

Table XII: Alkaloid Concentration in Myristicaceous Paste Samples Assayed for MAOI Activity*

Sample Name	Alkaloids Detected	Alkaloid Concentration (mg/ml)	Molar Concentration (M)
La Chorrera	NMT	1.38	7.9×10^{-4}
"oo'-koey"	DMT	0.1	0.53×10^{-4}
(no voucher)	Total	1.48	8.43×10^{-4}
Don Marcos #1 (DMK-59)	5-MeO-DMT	2.03	9.3×10^{-4}
Alfredo Moreno #1	5-MeO-DMT	1.19	5.48×10^{-4}
(DMK-40)	DMT	0.3	1.54×10^{-4}
	Total	1.49	7.02×10^{-4}

* HPLC quantitation carried out on samples resolubilized in 10% Tween-80. Cf. Materials and Methods.

indicate that enzyme inhibition by the pastes was attributable solely to the tryptamines, which are competitive substrates of 5HT for MAO-A. If the MAOI activity of the paste exceeded the activity of the analogue, however, this would indicate that some constituents in addition to the tryptamines were contributing to the over-all inhibitory activity. Following HPLC quantitation, the paste samples were diluted with an equivalent amount of 10% Tween-80 and the methanol was removed under nitrogen. If necessary additional Tween-80 was added to restore the sample to its original volume. The paste "analogues" were prepared from stock solutions of tryptamine standards dissolved in 10% Tween-80. The alkaloid-free paste sample (derived from DMK-34, V. pavonis) and the lignan fraction from V. elongata (DMK-59) were also prepared in 10% Tween-80. Concentrations of the undiluted stock solutions of each was 3.0 g dry wt/ml and 10 mg/ml, respectively.

III. Results and Discussion

A. β-carbolines and Tryptamines as MAOI: Structure/Activity Relationships

1. β-carbolines

In order to assess the influence of structural variation of the β-carboline skeleton (cf. Fig. 2) on the MAO inhibitory activity of these compounds, a selected group of standards (Table XIII) were assayed in vitro using the rat-liver cytosol fraction as the source of enzyme and ^{14}C-labelled 5-

hydroxytryptamine creatinine sulfate as substrate. Table XIII lists I_{50} values for the present experiment and also includes the values reported by McIsaac & Estevez [22] and Buckholtz, et al., [23] for comparison. The I_{50} value corresponds to the molar concentration of inhibitor at which enzyme activity is 50% inhibited with respect to controls lacking inhibitor. McIsaac & Estevez [22] used a calf-liver mitochondrial fraction and ^{14}C tyramine as substrate, while Buckholtz et al. [23] utilized mouse whole brain homogenate and ^{14}C-tryptamine as substrate. Since the degree of MAO inhibition measurable in vitro is partially determined by the tissue source and preparation of the enzyme, and partially by the particular substrate used, the I_{50} values reported in Table XIII for the present study are not directly comparable to those reported in [22] and [23]. In general, however, the conclusions suggested by the present study regarding the structure/activity relationships of β-carbolines as monoamine oxidase inhibitors are in agreement with the previous studies. Thus, for example, the fully aromatic and dihydro-β-carbolines are significantly more potent inhibitors than analogues in which the piperidine ring is fully saturated (cf. harmine, harmaline, and tetrahydroharmine). Inhibitory potency is roughly equivalent between the 7-methoxylated fully aromatic and dihydro derivatives harmine and harmaline, but of the 6-methoxylated analogues the fully aromatic compound is more potent. The 7-substituted β-carbolines were generally more potent inhibitors than the corresponding 6-substituted analogues in this study and that of Buckholtz et al. [23] but McIsaac & Estevez [22] found 6-methoxy-harman was equipotent with harmine

TABLE XIII
STRUCTURE/ACTIVITY RELATIONSHIPS OF β-CARBOLINES AS MAO INHIBITORS

Inhibitor	I_{50}*	I_{50} McIsaac et al.†	I_{50} Buckholtz et al.‡
Harmine	1.26×10^{-8}	1.5×10^{-8}	8.0×10^{-8}
Harmaline	1.58×10^{-8}	1.0×10^{-6}	6.0×10^{-8}
THH	1.77×10^{-6}	not tested	1.4×10^{-5}
6MeO-harmalan	1.20×10^{-6}	4.5×10^{-7}	1.8×10^{-5}
6MeO-harman	7.08×10^{-7}	1.5×10^{-8}	3.1×10^{-5}
Harmol	5.00×10^{-7}	2.7×10^{-8}	5.8×10^{-6}
Harman	4.47×10^{-7}	5.0×10^{-9}	3.3×10^{-6}
Norharman	3.55×10^{-6}	7.5×10^{-10}	2.0×10^{-5}
6-MeO-MTHβC	3.98×10^{-7}	not tested	not tested

* I_{50} = molar concentration of inhibitor equal to 50% inhibition of activity with respect to controls (present experiment). Values given are means of at least three separate determinations.
† Results reported by McIsaac et al. [22] using calf-liver mitochondria & tyramine as substrate.
‡ Results reported by Buckholtz et al. [23] using mouse whole brain homogenate and tryptamine as substrate.

TABLE XIV - MAOI ACTIVITY OF AYAHUASCA SAMPLES AND MIXTURES OF β-CARBOLINES

Inhibitor	I_{50}	% inhibition at 10^{-3} M
Harmine + THH + Harmaline- equimolar mix #1	3.16×10^{-7}	94
Harmine + THH + Harmaline - equimolar mix #2	2.51×10^{-8}	99.6
Harmine + THH + DMT + Harmaline - equimolar mix #3	7.1×10^{-8}	99.2
ayahuasca "analogue" #1*	3.98×10^{-7}	99.3
ayahuasca "analogue" #2†	2.82×10^{-8}	99.3
ayahuasca samples:‡		
Don Fidel #1	1.58×10^{-5}	-
Don Juan #2	$<1.0 \times 10^{-7}$	-

* Mixture of β-carbolines approximating proportions found in ayahuasca, viz., 69% harmine, 26% THH, and 4.6% harmaline.
† Mixture of β-carbolines + DMT approximating proportions found in ayahuasca, viz., 65% harmine, 6% harmaline, 22% THH, and 7% DMT.
‡ Figures given for ayahuasca represent dilution factor with respect to undiluted samples.

while 6-methoxy-harmalan was a more active inhibitor than harmaline. Hydroxyl substitution on the aromatic ring results in a less active compound than the corresponding methoxy-substituted compound (cf. harmine and harmol). In the present study harmane, lacking substituents on the aromatic ring, was less active than the 7-methoxylated analogue but approximately equipotent to the 6-methoxylated fully aromatic compounds, results which agree with those of Buckholtz, et al. [23]. McIsaac et al., [22] reported harmane to be somewhat more active than either harmine or 6-methoxy-harman. Lack of a methyl group at C_1 (cf harmine and norharmane) resulted in reduced activity in these assays and in those of Buckholtz, et al. [23] but McIsaac and Estevez [22] reported somewhat greater activity for norharmane than for harmane. I found 6-methoxy-2-methyl-tetrahydro-β-carboline, a compound reported from Virola spp., [16] to be in the same general range of potency as harman and harmol. Thus 6-MeO-MTHβC exhibited the greatest inhibitory activity of any of the 6-methoxylated β-carbolines tested, and was approximately an order of magnitude more active than THH. The greater activity of this compound with respect to THH and the other 6-methoxylated analogues may be due primarily to the methylation of the piperidine nitrogen. 6-MeO-MTHβC was not investigated by either McIsaac & Estevez [22] or Buckholtz, et al.[23]; the former authors, however, reported that acetylation of the piperidine nitrogen resulted in a compound lacking inhibitory activity. This substitution would completely abolish the basicity of the piperidine nitrogen, which is probably a structural requirement for MAOI activity.

2. Tryptamines

Although the structure/activity relationships of the β-carboline derivatives as MAO inhibitors has been systematically investigated [22,23] similar investigations of the MAOI activity of tryptamine derivatives have not been conducted. Barlow [24] investigated the effects of DMT and several related methylated tryptamines as inhibitors of amine oxidase in guinea-pig liver homogenates and found a greater degree of inhibition when tyramine and 5HT were used as substrates than when tryptamine was the substrate. In a more recent study [25] MAO inhibition by 5-substituted dimethyltryptamines, α-methyltryptamines, and gramines was examined. These investigators could find no correlation of inhibitory activity with the steric and electronic characteristics of the 5-position substituents. The DMT derivatives were more active than the gramine derivatives as MAOI and both were generally less active than the α-methyltryptamines. Of the 4 DMT derivatives tested, greatest inhibitory activity was shown by DMT, a 1 mM concentration giving 50% inhibition; 5-MeO-DMT was 3.4 times less active on a molar basis. The present study was undertaken to assess the influence of various indole ring substituents and modifications of the alkyl side-chain on the action of tryptamines as MAOI. Presumably the activity exhibited by the various derivatives is also a reflection of their activity as competitive substrates of MAO-A.

Comparison of the I_{50} values for the tryptamine derivatives (Table XV) with the I_{50} values of the β-carbolines (Table XIII) indicates that most of the tryptamines assayed are one to two

TABLE XV - MAOI ACTIVITY OF TRYPTAMINE DERIVATIVES AND MYRISTICACEOUS PASTES

Compound or sample Assayed	I_{50}*	% Inhibition at 1×10^{-3} M
Tryptamine derivatives:		
5-hydroxy-tryptophan	6.31×10^{-4}	56
Tryptamine	2.82×10^{-5}	89
5-MeO-tryptamine	3.98×10^{-5}	89
5-MeO-N-acetyl-tryptamine (melatonin)	3.98×10^{-4}	70
N-methyl-tryptamine	2.24×10^{-5}	90
N,N-dimethyltryptamine	1.58×10^{-6}	99
5-MeO-N,N-dimethyltryptamine	4.47×10^{-5}	98
4-hydroxy-N,N-dimethyl-tryptamine (psilocin)	2.82×10^{-5}	96
4-phosphoryl-N,N-dimethyl-tryptamine (psilocybin)	$>1.00 \times 10^{-3}$	35
5-MeO-N,N-diisopropyl-tryptamine	$>1.00 \times 10^{-3}$	40
3-[2(2,5dimethyl)-pyrrolyl-ethyl]-indole)	2.51×10^{-5}	91

Table XV (cont'd)

Compound or sample Assayed	I_{50}*	% Inhibition at highest concentration
Myristicaceous pastes and paste analogues:		
La Chorrera "oo'-koey"	1.51×10^{-5}	96 (8.43×10^{-4} M)†
La Chorrera analogue	1.90×10^{-5}	94 (8.43×10^{-4} M)
Alfredo Moreno sample #1	8.91×10^{-6}	96 (7.02×10^{-4} M)
A. Moreno analogue	6.30×10^{-6}	97 (7.02×10^{-4} M)
Marcos Flores sample #1	2.95×10^{-5}	92 (9.30×10^{-4} M)
Marcos Flores analogue	2.34×10^{-5}	92 (9.30×10^{-4} M)
Non-alkaloidal samples:		
Jorge Churay sample #1 (DMK-34, V. Pavonis)	1200 μg d wt/ml‡	88 (15×10^{3} μg d wt/ml)
Crude lignan fraction (DMK-59, V. Elongata)	20 μg d wt/ml	64 (50 μg d wt/ml)

* Molar concentration at 50% inhibition of enzyme activity
† Figures given are % inhibition at highest molar concentration of tryptamines in sample or analogues.
‡ Figures given are concentrations expressed as μg dry weight/ml.

orders of magnitude less effective than the β-carbolines as inhibitors of MAO. The major exception is N,N-dimethyltryptamine; with the lowest I_{50} value of all tryptamine derivatives tested, this compound has an I_{50} comparable to tetrahydroharmine and 6-MeO-harmalan (Table XIII). All other tryptamine derivatives are one to several orders of magnitude less effective than DMT as MAOI, and this reduction in activity may be related to the relative size and polarity of ring and side-chain substituents and also to the basicity of the side-chain N. Thus 5-MeO-DMT is some one and one-half orders of magnitude less active than DMT as MAOI, and the corresponding 5-MeO-diisopropyl analogue is essentially without significant MAOI activity at the highest concentration used. The influence of bulky ring substituents is well illustrated by comparison of psilocin and psilocybin; the 4-hydroxy substituted compound psilocin shows reduced but still significant MAOI activity compared to DMT, but substitution with the large phosphoryl ester (psilocybin) essentially abolishes the activity. The effect of various alkyl side-chain substituents appears to be more complex than simply relative size; for instance 3-[2-(2,5-dimethyl)-pyrrolylethyl]-indole exhibits approximately the same inhibitory activity as tryptamine, even though it has by far the largest alkyl-N substituent; in this case the lipophilic nature of the N-substituent may permit a considerable degree of interaction with lipophilic residues of the enzyme despite its size. A certain degree of basicity of the side chain nitrogen appears to be a requirement for MAOI activity, presumably because the protonated amine interacts with an anionic moiety at

the active site of the enzyme. Substitutions which abolish this basic character, e.g. the zwitterionic 5-hydroxy-tryptophan, and the N-acetylated compound melatonin, appear also to abolish the MAOI activity. Presence of non-polar substituents on the basic nitrogen may enhance interactions with a secondary lipophilic site; this would explain why DMT is a more active MAOI than tryptamine or NMT. In the case of the diisopropyl and higher homologs, however, activity may be diminished due to steric factors even though the substituents are non-polar.

B. Activity of ayahuasca and ayahuasca analogues as MAOI

Once the relative inhibitory activity of a representative sample of β-carbolines had been evaluated in our assay system, the next step was to evaluate the activity of appropriately diluted ayahuasca samples. The activity exhibited by the ayahuasca samples was compared to that shown by mixtures of harmine, harmaline, and tetrahydroharmine, which are known to be the major β-carbolines found in ayahuasca. Four types of standard mixtures were compared. One was an equimolar mixture of harmine, harmaline, and tetrahydroharmine while the second contained the 3 β-carbolines + DMT at an equimolar concentration; DMT was included in the mixture in order to determine whether it interfered with or enhanced the MAO inhibition by the β-carbolines. The third and fourth mixtures contained approximately the same molar concentration of alkaloids as the equimolar mixtures, but the proportions reflected the proportions found in the ayahuasca brews; DMT was omitted from analogue mixture #3, but was included in analogue

mixture #4 (cf. Materials and Methods).

Two of the ayahuasca samples from Pucallpa, Don Juan sample #2 and Don Fidel sample #1, were analyzed using quantitative HPLC in order to determine the concentration of alkaloids in the undiluted samples. Total alkaloid concentration was determined to be 4.8 mg/ml and 3.5 mg/ml, respectively. The samples were then subjected to serial 1:10 dilutions so that the most dilute solution was 1 x 10^{-7} as concentrated as the undiluted sample. 50 µl aliquots of the appropriate dilutions from each sample were assayed for MAO inhibitory activity using the in vitro rat-liver cytosol system (Fig. 12, Table XIV). Fig. 12 shows clearly that both ayahuasca samples are extremely effective MAO inhibitors; Don Fidel's #1 still showed >40% inhibition of the enzyme at 10^{-5} full strength, while Don Juan's #2 exceeded 50% inhibition even at one ten-millionth (10^{-7}) the concentration of the undiluted brew. These in vitro results indicate that ayahuasca is active as an MAO inhibitor even when diluted by many orders of magnitude. These observations constitute the first empirical demonstration of the effect of ayahuasca on MAO and provide evidence for the hypothesis that the hallucinogenic properties of ayahuasca are due to its inactivation of visceral MAO and consequent oral potentiation of the DMT in the preparation. It should be emphasized, however, that the present in vitro study represents only the first step toward understanding the pharmacology of ayahuasca. Further investigations are required, particularly in vivo studies of the action of ayahuasca in both animals and humans, in order to fully elucidate the pharmacology of this Amazonian drug. Another

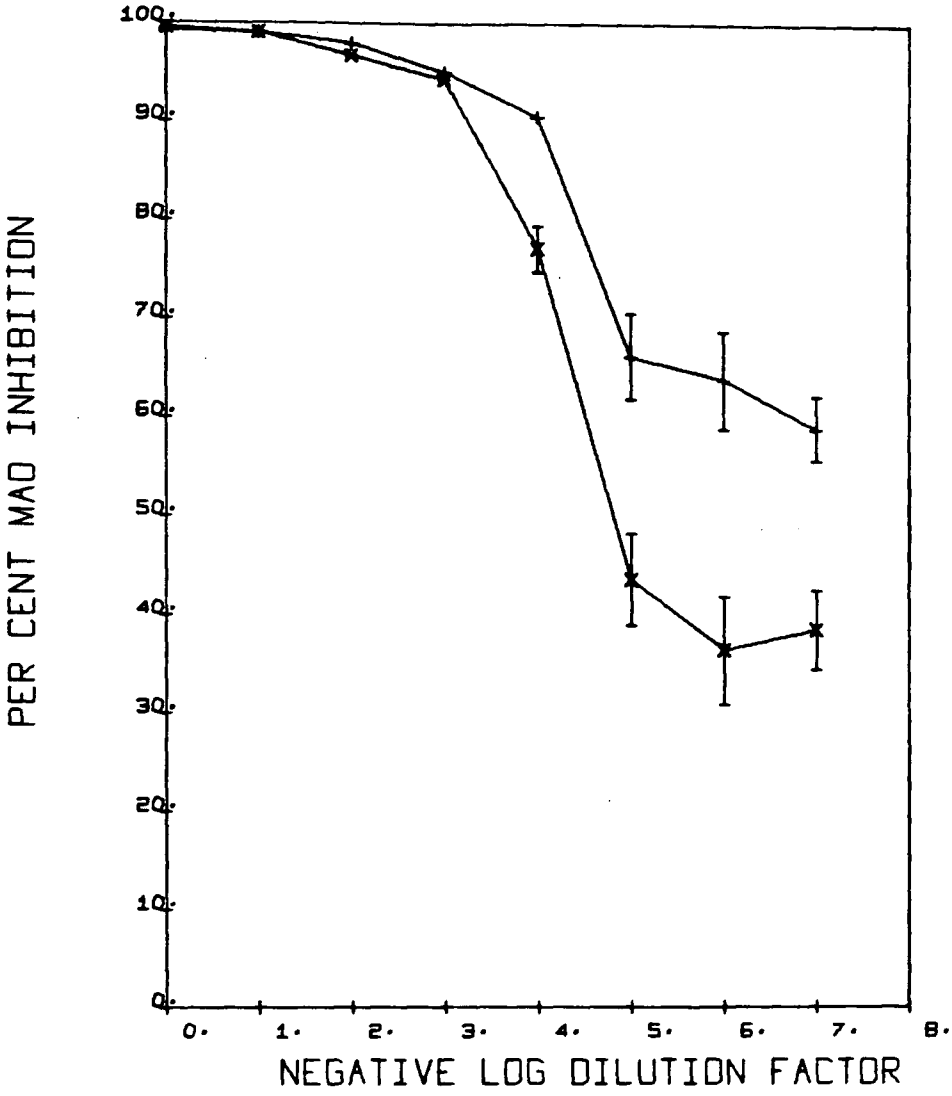


Figure 12 - MAOI Activity of Ayahuasca

\+ = Don Juan Sample #2

x = Don Fidel Sample #1

point worth mentioning is the fact that on occasion ayahuasca is prepared from B. caapi alone without the addition of any admixture plants; in these instances, DMT would be absent from the preparation. This would alter its pharmacology and presumably the hallucinogenic effects, if present, would be due to the β-carbolines alone. In this case concentrations of β-carbolines considerably greater than those measured in our samples would be required if a nonsynergistic mechanism is assumed.

It is informative to compare the % MAO inhibition produced by the mixtures of β-carboline standards with the inhibition elicited by single components of the mixture (Table XIII & XIV, Figs. 13 & 14). In the first assay, an equimolar mixture of harmine, harmaline, and tetrahydroharmine was assayed in parallel with "ayahuasca analogue #1" - having the same overall concentration as the equimolar mixture but with the individual components present in approximately the proportions found in ayahuasca, viz.: 69% harmine, 4.6% harmaline, and 26% tetrahydroharmine. The equimolar mixture inhibited 50% of the enzyme activity at a concentration of 3.16×10^{-7} M. This value is intermediate between the I_{50} value of the most active constituent of the mixture (harmaline, $I_{50} = 1.58 \times 10^{-8}$ M) and the least active (tetrahydroharmine, $I_{50} = 1.77 \times 10^{-6}$ M) indicating that these compounds act additively rather than synergistically with respect to their inhibition of MAO. A synergistic interaction would result in I_{50} values considerably lower than the I_{50} value of any one constituent by itself; such a result is not observed, indicating that the inhibitory

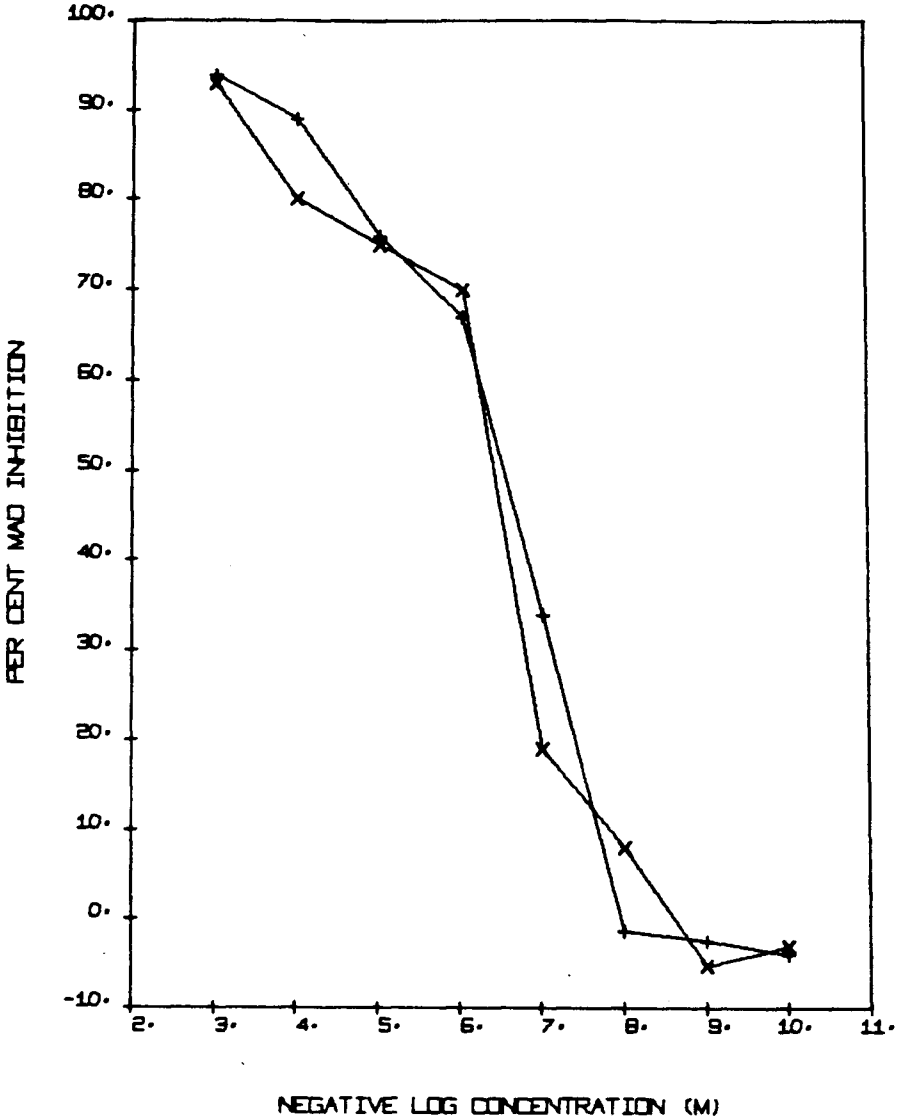


Figure 13 - MAOI Activity of
β-carboline Mixtures

\+ = harmine + harmaline + THH: Equimolar Mixture

x = "Ayahuasca analogue #1" - 69% harmine,
4.6% harmaline, 26% THH

activity of the three compounds collectively is not greater than the activity of the most effective compound of the group. The I_{50} value of "analogue #1" was nearly identical with the equimolar mixture (3.98×10^{-7} M and 3.16×10^{-7} M, respectively) indicating that the combination of harmine and tetrahydroharmine alone can account for most of the inhibitory activity exhibited by ayahuasca. Although harmaline is equivalent to or slightly stronger than harmine, it is essentially a trace component in ayahuasca and probably does not contribute significantly to the monoamine oxidase inhibition which this drug elicits.

In the second assay (Fig. 14) an equimolar mixture of harmine, harmaline, and tetrahydroharmine was assayed in parallel with an equimolar mixture of the three β-carbolines plus DMT. This assay was carried out in order to determine whether the presence of significant concentrations of DMT would affect the activity of the β-carbolines. The I_{50} values measured (Table XIV) were fairly close, however it appears that in this in vitro system, the presence of DMT in the equimolar mixture may slightly mitigate the effectiveness of the β-carbolines as MAOI. It must be remembered, however, that in the equimolar mixture containing DMT the total concentration of β-carbolines is less than in the mixture lacking DMT; therefore it is reasonable to expect the mixture with DMT to have a slightly higher I_{50} value. "Analogue #2" - containing 65% harmine, 6% harmaline, 22% tetrahydroharmine, and 7% DMT - was also assayed and found to have an I_{50} value of 2.82×10^{-8} M, quite close to the value measured with the equimolar mixture containing β-

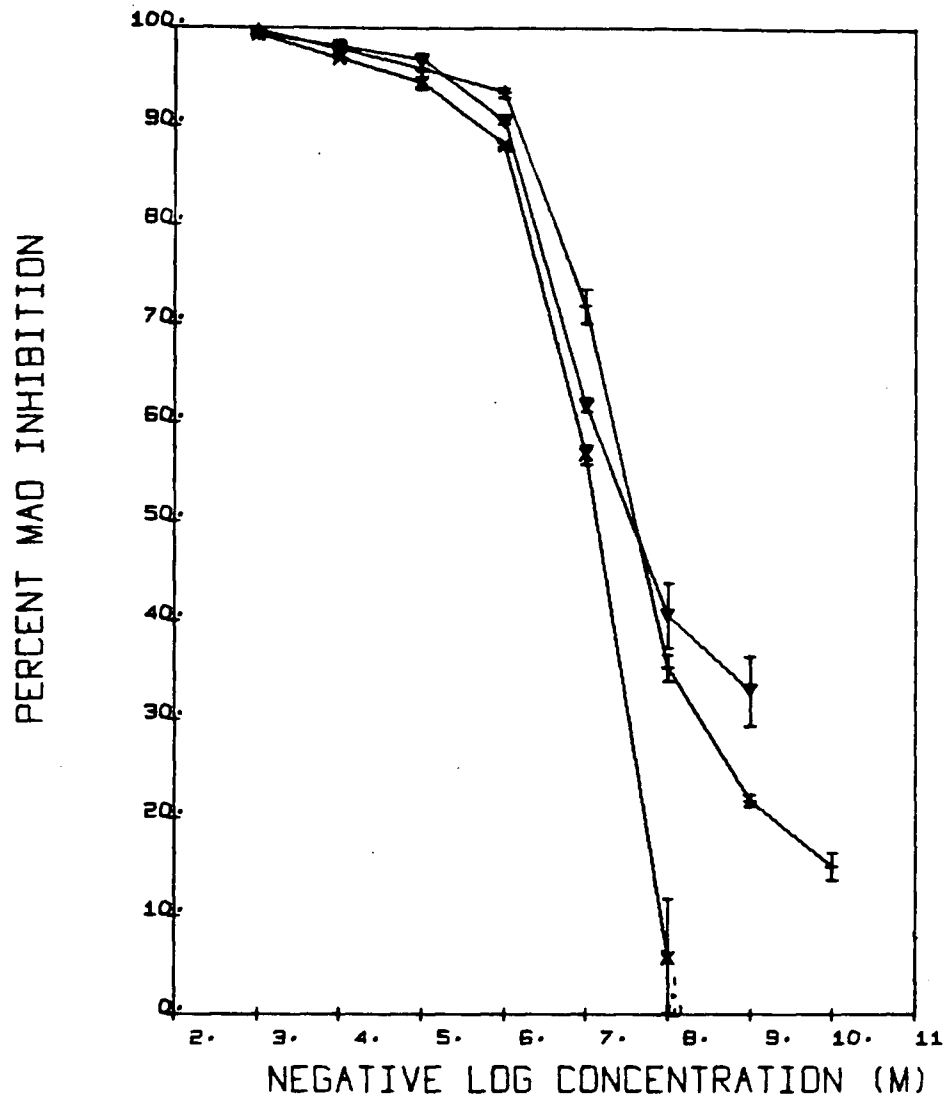


Figure 14 - MAOI Activity of
β-carboline/DMT Mixtures

+ = harmine + harmaline + THH: Equimolar Mixture

x = harmine+ harmaline + THH + DMT: Equimolar Mixture

= "Ayahuasca analogue #2" - 65% harmine, 6% harmaline, 22% THH, 7% DMT

carbolines alone. All of the I_{50} values measured in this second group of assays were somewhat lower than those measured in the first group; the discrepancy is probably related to batch to batch variability in the rat-liver enzyme preparations.

C. Activity of Myristicaceous pastes and paste "analogues" as MAOI

The three Myristicaceous paste samples which quantitative GC analysis had previously shown to have the highest concentrations of tryptamines (Table XI) were assayed for MAOI activity in the in vitro rat-liver enzyme system. The concentrations and proportions of tryptamines in the paste samples were determined by HPLC after the samples had been prepared for use in the assay. Paste "analogues", consisting of mixtures of tryptamine standards in the same concentrations and proportions as in the pastes themselves were run simultaneously with each paste sample (cf. Materials & Methods). The results of these assays (Table XV, Fig. 15-17,) show that the MAOI activity exhibited by the orally-ingested pastes is paralleled almost exactly by the activity of the paste analogues, containing only tryptamines. In all cases, the I_{50} of the paste sample is quite close to that of the analogue mixture indicating that the MAOI activity of the pastes is due solely to the presence of the tryptamines; it seems unlikely, therefore, that the presence of trace amounts of β-carbolines in addition to the tryptamines (as in, e.g., the La Chorrera sample) has any significance in terms of enhancing the MAOI action of the pastes or of orally potentiating the tryptamines present. The relative MAOI activity

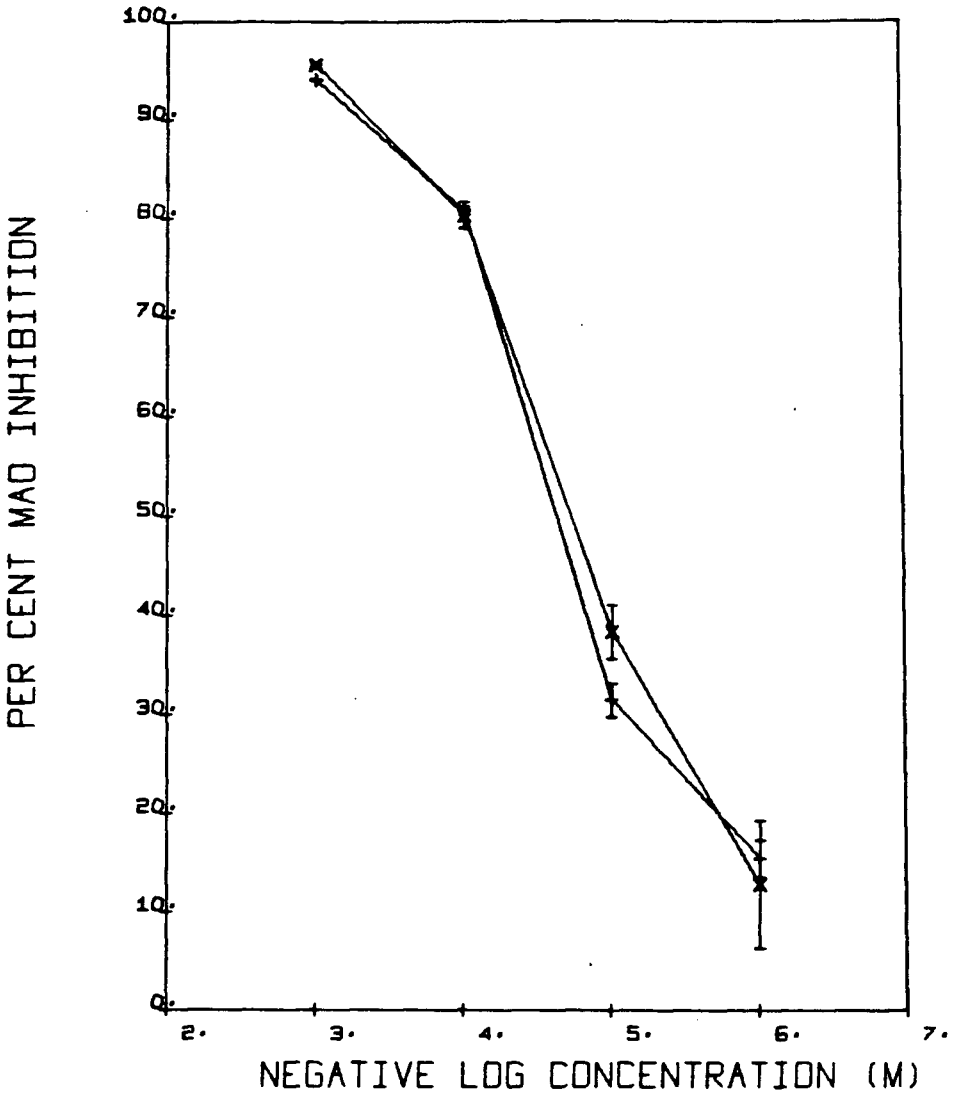


Figure 15 - MAOI Activity of Myristicaceous Pastes: La Chorrera Oo'-koey

\+ = La Chorrera Oo'-koey (no voucher)

x = La Chorrera "analogue" (cf.Tables XII & XV)

of the pastes appears to be a function of the types and amount of tryptamines present, e.g., the La Chorrera sample had an I_{50} only slightly lower than the Don Marcos sample; the former sample contained NMT + DMT, both of which are more active MAOI than 5-MeO-DMT which is the single major tryptamine in the Don Marcos sample. Alfredo Moreno's sample #1 had the lowest I_{50} value of the three, which is consistent with expectations since this sample contained proportionately the highest concentration of DMT, the most effective MAOI of all the tryptamine derivatives tested. Although in vivo experiments would be required to confirm these results, the in vitro evidence indicates that these pastes do not exhibit MAOI activity beyond what is due to the tryptamine bases alone. Therefore if these pastes are orally effective as hallucinogens, it appears that some mechanism other than MAO inhibition must be sought to explain their oral activity.

The MAOI activity of the paste samples may also be partially due to the presence of non-nitrogenous MAOI in addition to the tryptamines and traces of β-carbolines. In order to investigate this possibility, a paste sample (derived from DMK-34, V. pavonis) which was alkaloid-free (cf. Table X, Chapter VI) was assayed in the system; a crude lignan fraction obtained from V. elongata (DMK-59) was also assayed. Some degree of inhibition was observed with both samples (Fig. 18) but was significant only at the highest concentrations. For the paste sample, this was 15000 μg dry wt/ml of crude extract, and for the lignan sample, the highest concentration was 50 μg dry wt/ml of the crude lignan fraction. By contrast, the tryptamine-

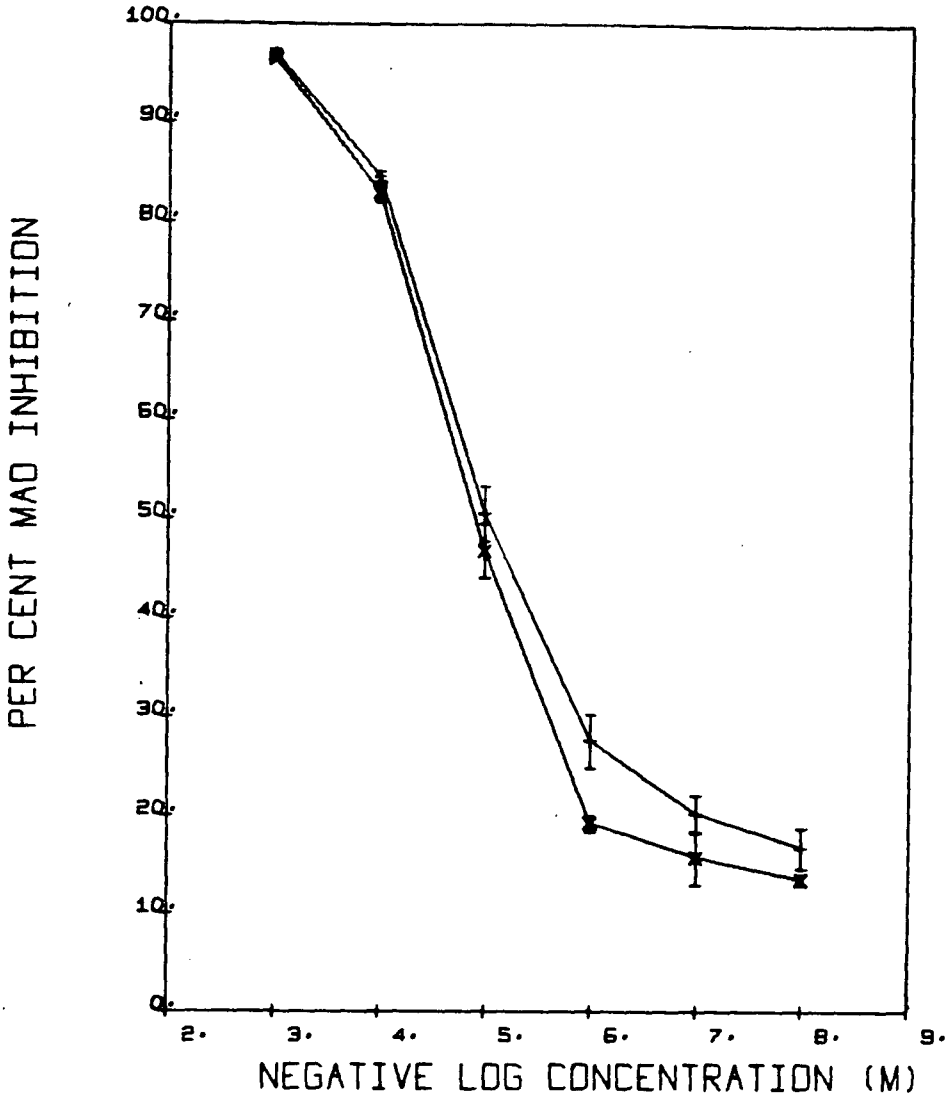


Figure 16 - MAOI Activity of Myristicaceous Pastes: Alfredo Moreno Oo'-koey Sample #1

x = Alfredo Moreno Oo'-koey Sample #1 (DMK-40, V. sebifera)

+ = Alfredo Moreno Sample #1 "analogue" (cf. Tables XII & XV)

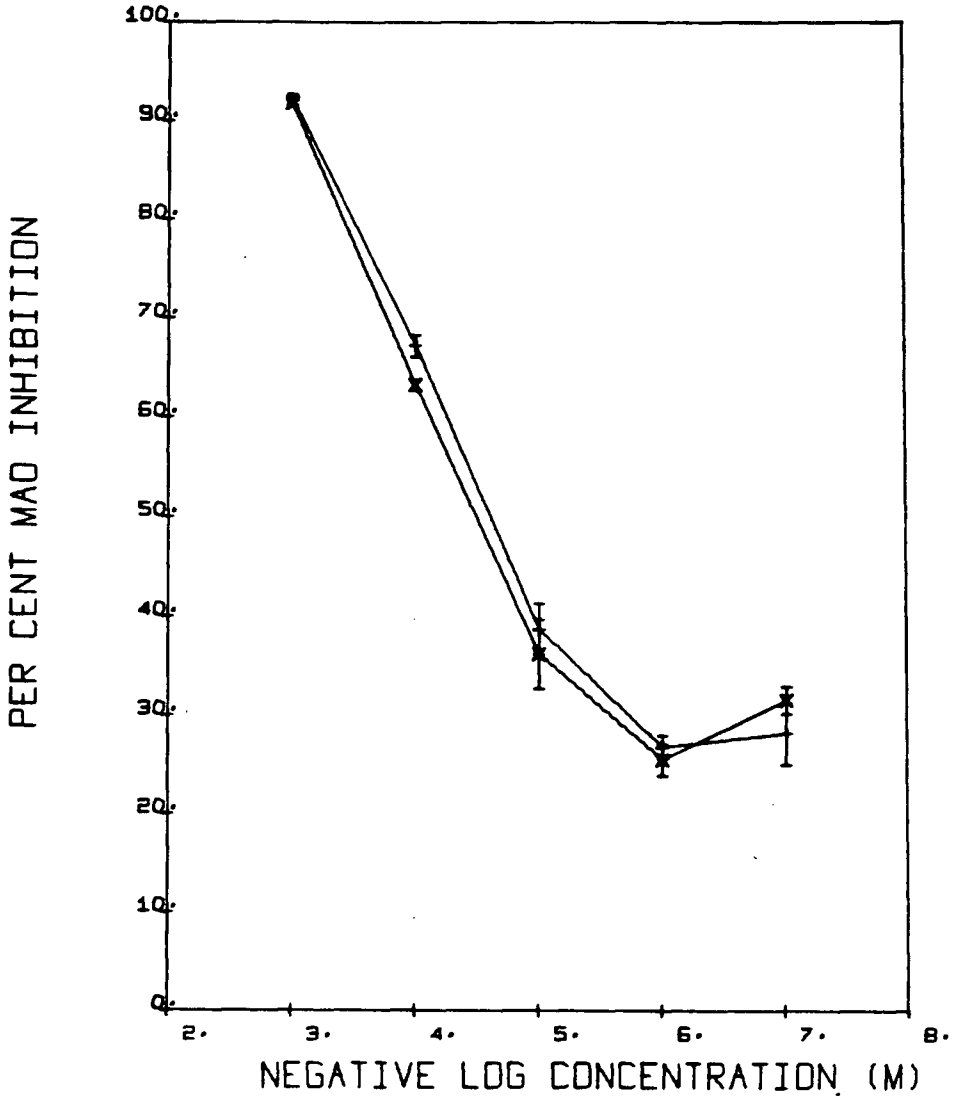


Figure 17 - MAOI Activity of Myristicaceous Pastes: Marcos Flores Ku'-ru-ku Sample #1

x = Marcos Flores Ku'-ru-ku Sample#1 (DMK-59, V. elongata)

+ = Marcos Flores Sample #1 "analogue" (cf. Tables XII & XV)

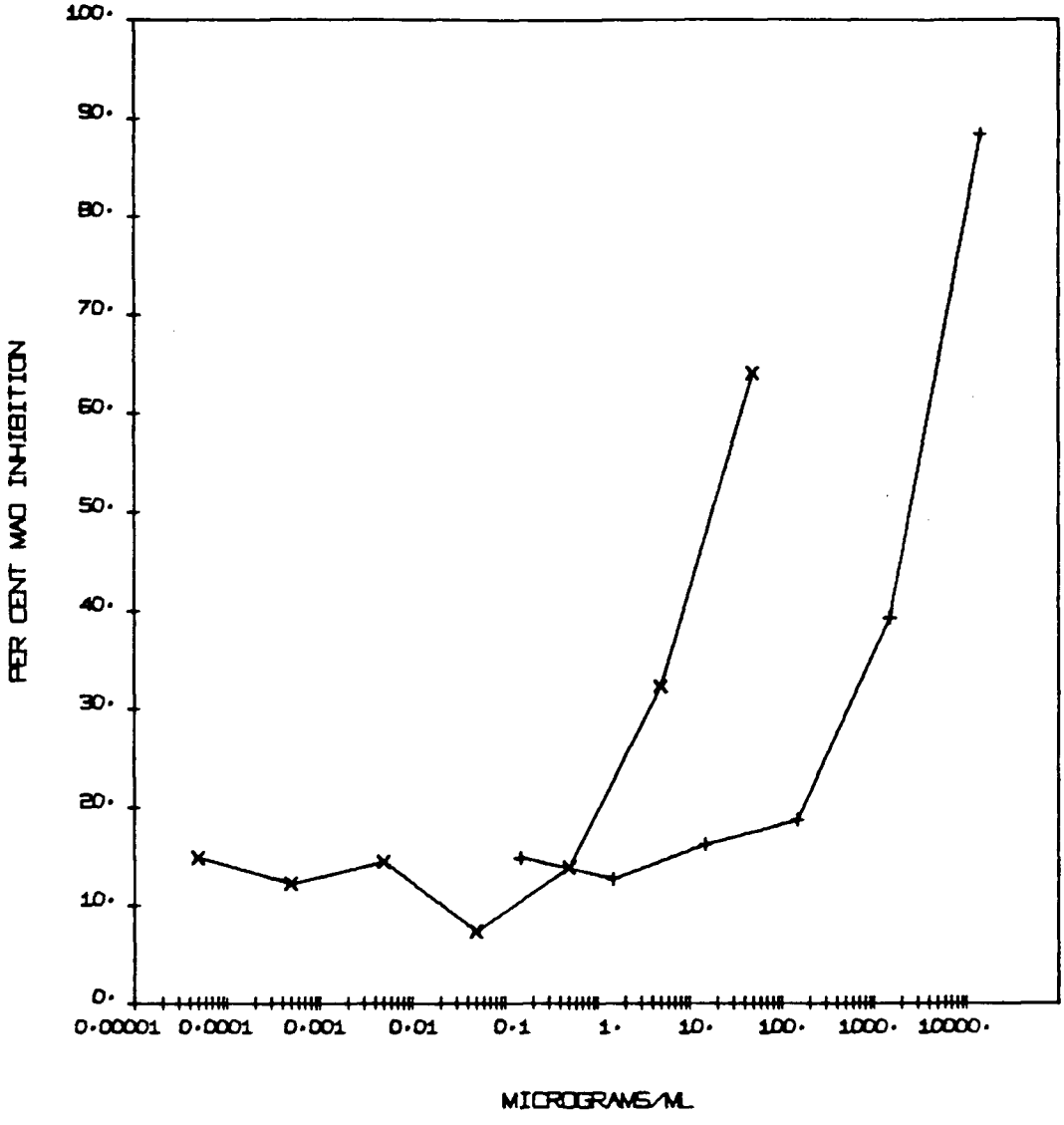


Figure 18 - MAOI Activity of Non-alkaloid Constituents of Myristicaceous Pastes

\+ = non-alkaloidal paste sample (DMK-34, V. pavonis)

x = lignan fraction (DMK-59, V. elongata)

containing paste samples showed comparable amounts of inhibition at dry wt concentrations on the order of 3 to 4 μg dry wt/ml of crude extract. The inhibition shown by the alkaloid-free samples therefore appears to be fairly nonspecific and probably is due to a general denaturing of the proteins in the enzyme preparation due to the high phenolic content of the samples. The possibility that the Myristicaceous pastes may contain some highly specific non-nitrogenous MAO inhibitor seems somewhat remote.

IV. Summary

The activity of a number of tryptamine and β-carboline derivatives as MAOI was investigated using an *in vitro* enzyme assay and 5-hydroxy-^{14}C-tryptamine as substrate. Activity was measured using single compounds and mixtures of compounds and the results were compared to the activity of samples of *ayahuasca* and samples of orally-ingested Myristicaceous pastes. The MAOI activity of β-carboline derivatives was found to be several orders of magnitude greater than the activity measured with tryptamine derivatives. Mixtures of β-carbolines were not significantly more effective as MAOI than the single most active compound in the mixture, indicating an additive rather than a synergistic mechanism of action. Some structural correlations for MAOI activity were found for both the tryptamines and β-carbolines. Samples of *ayahuasca* were found to be highly active as MAOI even when diluted by several orders of magnitude, and the activity observed was similar to that measured for comparable concentrations and proportions of β-carboline

mixtures. Based on this evidence it appears likely that ayahuasca could function effectively as MAOI in vivo and thus orally potentiate the DMT which is probably responsible for the hallucinogenic action of the drug. A lesser degree of inhibition was measured when samples of orally-ingested Myristicaceous pastes were assayed. The inhibition elicited by the paste samples were closely matched by mixtures of tryptamine standards having comparable proportions and concentrations. These observations indicate that the MAOI activity of the pastes is due mainly to the high concentrations of tryptamines; the traces of β-carbolines or non-nitrogenous inhibitors present probably do not contribute significantly to the total inhibition. Thus it appears unlikely that the oral activity of the Myristicaceous pastes is due to the potentiation of the tryptamines via inhibition of MAO by β-carbolines; some mechanism other than MAO inhibition must be invoked to account for the oral hallucinogenic activity of the Myristicaceous pastes if they are, in fact, orally active.

V. Literature Cited

1. Yasunobu, K. T., H. Ishizaki, & N. Minamiura (1976) The Molecular, Mechanistic and Immunological Properties of Amine Oxidases. Molecular and Cellular Biochemistry 13:3-29

2. Marley, E., & B. Blackwell (1970) Interactions of Monoamine Oxidase Inhibitors, Amines, & Foodstuffs. Advances in Pharmacology & Chemotherapy 8:185-239

3. Iverson, L. L., (1979) The Chemistry of the Brain. Scientific american Sept. 1979:134-149

4. Fuller, R. W., B. J. Warren, & B. B. Molloy (1970) Selective Inhibition of Monoamine Oxidase in Rat BraiM mitochondria. Biochemical Pharmacology 19:2934-2936

5. Donnelly, C. H., E. Richelson & D. L. Murphy (1976) Properties of Monoamine Oxidase in Mouse Neuroblastoma NIE-115 Cells. Biochemical Pharmacology 25:1629-1643

6. Donnelly, C. H., & D. L. Murphy (1977) Substrate- and Inhibitor-related Characteristics of Human Platelet Monoamine Oxidase. Biochemical Pharmacology 26:853-858

7. Houslay, M. D. & K. F. Tipton (1974) A Kinetic Evaluation of Monoamine Oxidase Activity in Rat Liver Mitochondrial Outer Membranes. Biochemical Journal 139:645-652

8. Neff, N. H. And H.-Y. T. Yang (1974) Another Look at the Monoamine Oxidases and the Monoamine Oxidase Inhibitor Drugs. Life Sciences 14:2061-2074

9. Pletscher, A. (1966) Monoamine Oxidase Inhibitors. Pharmacological Reviews 18:121-129

10. Allen, J. R. F., & B. Holmstedt (1980) The Simple β-carboline Alkaloids. Phytochemistry 19:1573-1582

11. R. G. Taborsky & W. M. McIsaac. The Synthesis and Preliminary Pharmacology of Some 9H-Pyrido[3,4-b]indoles (β-carbolines) Related to Serotonin and Melatonin. Journal of Medicinal Chemistry 7:135-141 1964

12. Udenfriend, S., B. Witkop, B. G. Redfield, & H. Weissbach (1958) Studies With Reversible Inhibitors of Monoamine Oxidase: Harmaline and Related Compounds. Biochemical Pharmacology 1:160-165

13. Schultes, R. E. (1972) Ethnotoxicological Significance of Additives to New World Hallucinogens. Plant Science Bulletin 18: 34-41

14. Shulgin, A. T. (1976) Psychotomimetic Agents. Ch. 4 in Maxwell Gordon (ed.) Psychopharmacological Agents Vol. IV. Academic Press.

15. Der Marderosian, A. H., H. V. Pinkley, & M. F. Dobbins IV. (1968) Native Use and Occurrence of N,N-dimethyltryptamine in the Leaves of Banisteriopsis rusbyana. American Journal of Pharmacy 140:137-147

16. Agurell, S., B. Holmstedt, J. E. Lindgren, & R. E. Schultes. (1968) Identification of Two New β-carboline Alkaloids in South American Hallucinogenic Plants. Biochemical Pharmacology 17:2487-88

17. Schultes, R. E. (1969) Virola as an Orally-administered Hallucinogen. Harvard Botanical Museum Leaflets 22:229-40

18. Schultes, R. E. & T. Swain (1976) Further Notes on Virola as an Oral Hallucinogen. Journal of Psychedelic Drugs 8:317-324

19. Schultes, R. E., T. Swain, & T. Plowman (1977) Virola as an Oral Hallucinogen Among the Boras of Peru. Harvard Botanical Museum Leaflets 25:259-272

20. Dawson, R. M. C., D. C. Elliot, W. H. Elliot, K. M. Jones (eds.) (1969) Data for Biochemical Research, 2nd ed. Oxford University Press, London; p. 484

21. Tipton, K. F. & M. B. H. Youdim (1976) Assay of Monoamine Oxidase. In Monoamine Oxidase And Its Inhibition. Ciba Foundation Symposium 39 (N.S.). Elsevier, Amsterdam.

22. McIsaac, W. M. & V. Estevez (1966) Structure-action Relationships of β-carbolines as Mono-amine Oxidase Inhibitors. Biochemical Pharmacology 26:1625-27

23. Buckholtz, N. S. & W. O. Boggan (1977) Monoamine Oxidase Inhibition in Brain and Liver Produced by β-carbolines: Structure-activity Relationships and Substrate Specificity. Biochemical Pharmacology 26:1991-96

24. R. B. Barlow. (1961) Effects on Amine Oxidase of Substances Which Antagonize 5-hydroxytryptamine More than Tryptamine on the Rat Fundus Strip. British Journal of Pharmacology 16:153-162.

25. B. T. Ho, W. M. McIsaac, R. An, R. T. Harris, K. E. Walker, & P. M. Kralik (1970) Biological Activities of Some 5-Substituted N,N-dimethyltryptamines, α- methyltryptamines, and Gramines. Psychopharmacologia 16:385-394

26. D. MacRae. (1984) Ethnobiological and Chemical Investigations of Selected Amazonian Plants. PhD thesis, University of British Columbia, Vancouver, B. C.

CHAPTER VII: COMPARATIVE ETHNOPHARMACOLOGY OF MALPIGHIACEOUS AND MYRISTICACEOUS HALLUCINOGENS: SUMMARY AND CONCLUSION

I. Introduction

This thesis presents the results of ethnographic, ethnobotanical, phytochemical, and pharmacological investigations of two Amazonian hallucinogens. The hallucinogenic drink ayahuasca is prepared from the liana Banisteriopsis caapi (Malpighiaceae) and sundry admixture plants, notably Diplopterys cabrerana and various Psychotria spp. The cambial resin of certain members of the genus Virola (Myristicaceae) is the source of hallucinogenic snuffs and orally-ingested hallucinogenic pastes used by some tribes. Although derived from entirely different botanical sources, similar alkaloids--tryptamines and β-carbolines--are the active constituents of both preparations. In the case of ayahuasca and the orally-ingested Virola pastes, it has been suggested that monoamine oxidase inhibition--due to the β-carbolines--protects the psychotomimetic tryptamines from oxidative deamination by peripheral MAO and thus permits their oral activity. Experimental investigations of this postulated mechanism of oral activity--involving in vitro evaluations of the drugs and their constituents as MAOI--were one of the primary objectives of this thesis. Other objectives were the collection of ethnobotanical and ethnographic data on the use of these drugs among Indian and mestizo populations, the collection of voucher specimens and material for phytochemical analysis, and the analysis of alkaloidal constituents in source-plants, admixture plants, and drug samples. Most of these objectives have been met, and it is

now appropriate to summarize and compare the results obtained for ayahuasca with those found for the Myristicaceous hallucinogens.

II. Ayahuasca

The contemporary use of ayahuasca in South America occurs primarily within the context of mestizo folk medicine, which is comprised of an amalgam of many tribal traditions. Although most of these tribes have long since fragmented or disappeared, much of their ethnomedical lore has survived and been adopted among mestizos. Ayahuasca is among these, and it occupies a central and important position in mestizo folk medicine. Not only is it employed as a general cure-all for the treatment of many disorders ranging from mental illness to parasites, it is also the ayahuasquero's or healer's passport to supernatural dimensions where the skills intrinsic to his profession can be acquired. It enables the healer to learn the medicinal uses of plants and the songs and chants used in the healing ceremonies; with it he is able to diagnose diseases, divine the supernatural causes of illness, predict the future and see and communicate across distances. Whether or not there is a rational basis for any of these practices, an impressive pharmacopoeia of plants--many of which do contain highly biodynamic constituents--are (or can be) used in conjunction with ayahuasca. The phytochemical investigations reported here have found that most mestizo ayahuasca brews contain substantial amounts of β-carbolines and N,N-dimethyltryptamine. The major β-carbolines are harmine and tetrahydroharmine, and lesser amounts of harmaline; only traces

of other β-carbolines were detected. The amounts of β-carbolines in these samples were several orders of magnitude greater than those reported in an earlier study of ayahuasca prepared by tribes inhabiting the upper Rio Purús in southwestern Peru [1]. A "typical" 100 ml dose of mestizo ayahuasca contains between 500 and 800 mg β-carbolines and 40-80 mg DMT; this is well above the threshold dose for DMT and within the range at which the β-carbolines are effective MAOI; it is still well below the threshold level for hallucinogenic activity of the β-carbolines, however. Therefore these data indicate that the hallucinogenic activity of ayahuasca is probably due to DMT, which is orally activated by some mechanism, presumably the MAO inhibition induced by the high concentration of β-carbolines. This hypothesis is supported by the finding that ayahuasca is a very effective inhibitor of MAO in vitro even when diluted by many orders of magnitude. A number of ayahuasca samples, brewed by different practitioners in different parts of Peru, were analyzed. The same alkaloids were consistently found; the samples differed mainly in the concentration and proportions of various components. Concentration differences are expected since this is dependent on the amount of plant material extracted and other variables in the preparation procedure. Proportional differences may be attributable to the use of different B. caapi cultivars. It is remarkable that, faced with so many variables, ayahuasqueros all across Peru manage to manufacture a drug having a high degree of pharmacological consistency from batch to batch; except for concentration and proportional differences all of the ayahuasca samples analyzed in this study could have

been taken from the same pot. All of the DMT-containing admixture plants which were analyzed were similar; DMT was the single major base in all of them; only traces of other alkaloids were detected. The concentration of DMT was between 1-2 mg/g dry wt. in all samples. Similar consistency was not found in the several B. caapi cultivars which were analyzed. More or less the same constituents were present but concentrations ranged from 1.7 mg/g d wt (total alkaloids) to 13.6 mg/g dry wt. These differences probably are due to environmental factors rather than to genetically based biochemical differences between cultivars.

III. Myristicaceous Hallucinogens

Unlike ayahuasca, the use of hallucinogenic preparations derived from Virola spp. or other Myristicaceous genera is a practice confined to a few indigenous Amazonian tribes and has never become integrated into mestizo folk medicine. As a result the use of these Myristicaceous drugs has diminished or in some cases disappeared as the tribal societies have fragmented due to outside influences. This is particularly true of the orally-ingested Myristicaceous pastes. Use of Virola spp. as an oral hallucinogen is more ethnologically restricted than its use as a snuff; it has been reported among the Bora, Witoto, Muinane, and possibly the Maku and apparently does not occur outside these groups. Even within these tribes, the source-plants, methods of preparation, and modes of usage of the oral Virola drug are the specialized knowledge of the medicine men and are not known to most members of the tribe. Another complicating factor is that

the Bora and Witoto populations inhabiting the Rio Ampiyacu region, where the fieldwork for this study was carried out, are not indigenous to that area but migrated there from north of the Rio Putumayo in the early decades of this century, as a result of the dislocations produced by the rubber industry. This has not contributed to the preservation of their ethnomedical traditions nor any other tribal institutions; the result is that local knowledge of the oral Myristicaceous drugs has become, in some cases, rather inexact. The work reported here has shown that there is a high degree of variability in the type and amount of alkaloid constituents, not only among different Virola species, but even among different collections of the same species. The composition of the orally-ingested paste samples which were analyzed in this study is similarly variable, both qualitatively and quantitatively, and presumably this is a reflection of the chemical variability of the source-plants. Thus, while all of the ayahuasca samples had essentially the same constituents (with DMT being present or absent depending on which Psychotria sp. was used as admixture), not one of the oral Virola pastes had the same base composition as any other one. Large differences in the concentration of alkaloids in various samples were also found. Either this high degree of chemical variability has always been a feature of these orally-ingested pastes, or the criteria which were formerly used to select the "best" (i.e., strongest) Virola species to prepare the paste (e.g., taste, smell, and/or visual appearance of the resin) have been largely forgotten and the selection has now become a fairly haphazard process. If the former possibility is true, this may

explain why the paste was restricted to the medicine men and why they usually consumed it alone: if only one out of every three or four samples actually is orally active, this information was probably kept from the people at large, in order to preserve their faith in the efficacy of the medicine man and his medicines.

Another objective of these investigations was to examine the mechanism for the (presumed) oral activity of these Myristicaceous pastes; is it due, like ayahuasca, to the oral activation of the tryptamine constituents through the inhibition of visceral MAO by β-carbolines? In the process of answering some of these questions this investigation has created new and even more puzzling ones. In the first instance β-carbolines were not consistently found as constituents of all of the paste samples; even in the two samples containing β-carbolines, only traces were detected. Those β-carbolines which were found belong to the tetrahydro-β-carboline type, which are poor MAO inhibitors compared to the dihydro- and fully aromatic β-carbolines. A further point is that presence or absence of β-carbolines in the paste samples apparently had little or nothing to do with their oral activity or inactivity as determined in self-experiments; the La Chorrera oo'-koey, which contained the highest levels of β-carbolines, was completely inactive in repeated self-experiments while Marcos Flores' ku'-ru-ku, which contained only high concentrations of 5-MeO-DMT in the base fraction, was highly orally active although the activity was not typical of hallucinogens. Although the Myristicaceous paste samples did exhibit some degree of MAO inhibition, this was

shown to be primarily if not entirely due to the tryptamine constituents; non-alkaloidal constituents from the samples showed only a slight degree of non-specific inhibition. Most of the synthetic tryptamine derivatives which were assayed as MAOI did exhibit some activity but the I_{50} values were usually several orders of magnitude lower than those for the β-carboline derivatives. Interestingly, DMT showed the most MAOI activity of all the tryptamine derivatives tested; its I_{50} was comparable to THH, one of the least active of the β-carbolines. All of this evidence considered together suggests that the Myristicaceous pastes, when they <u>are</u> orally active, must owe their activity to some mechanism <u>other</u> than MAO inhibition, whether due to β-carbolines or other constituents.

What alternative mechanism might account for the oral activity of some samples? There are basically two possibilities worth consideration; both would require further experimental investigations to confirm. The first is that the oral activity of the Myristicaceous pastes is <u>not</u> due to the tryptamines at all, but rather to some other biologically active constituents, such as lignans. Certainly this would help to explain why the oral activity observed in self-experiments was atypical for hallucinogens. It is equally possible, however, that tryptamines taken orally differ significantly in their effects from tryptamines administered parenterally. That <u>Virola</u> resin (including the sample assayed) does contain biologically active lignans has been established; but whether these lignans are capable of eliciting the spectrum of biological responses observed in the bioassay, is not known. The second possibility,

which also calls for much further experimental investigation, is that the oral inactivation of DMT, 5-MeO-DMT, and related derivatives is not due to oxidative deamination by peripheral MAO; alternative metabolic pathways may be relatively more significant in the degradation of these compounds in the periphery. Both in vitro and in vivo studies of DMT metabolism [2,3] (cf. Chapter II) suggest that 6-hydroxylation and/or N-oxidation of DMT occurs more readily in peripheral tissues than deamination by MAO. 6-hydroxylation has been shown to occur in peripheral tissues but apparently not in brain [2,3]; significantly, 6-hydroxy derivatives of DMT and related compounds are inactive as hallucinogens [4]. Little is known of the hallucinogenic action of DMT-NO, but if it follows the general pattern for tertiary amine N-oxides, [5] it would either be completely inactive, or ten to one hundred times less active than DMT. Other studies of in vivo DMT metabolism in the presence of MAO inhibitors and microsomal mixed function oxidase (MFO) inhibitors [6] have found that while the MAOI iproniazid prolongs plasma and tissue half-life of DMT, the MFO inhibitor SKF-525A does not; the authors interpret these results as support for the hypothesis that DMT is metabolized mainly by MAO in vivo. A problem with most in vivo studies of the type reported in [6], is that the DMT is administered to the animal i.p. rather than orally; thus the compound reaches the circulation directly and avoids "intestinal/hepatic-portal shunt" metabolism. It is possible that the metabolism of DMT via the intestinal/hepatic-portal shunt may differ in important respects from its metabolism when introduced directly into the

bloodstream or body cavity. In the hepatic shunt 6-hydroxylation, and/or N-oxidation, may be relatively more important than MAO as a catabolic route for the compound. In any event, *in vivo* metabolic studies involving i.p. or other parenteral routes of administration actually shed little light on DMT metabolism following oral administration. The fact is that our understanding of the peripheral metabolism of DMT and related compounds is far from complete; all that is known for certain is that more than type of oxidative reaction is involved. MAO may be partially responsible for the degradation of tryptamines in the periphery, but microsomal MFOs, which are involved in both the 6-hydroxyl and N-oxide pathways, are possibly even more important. Interestingly, if orally-administered DMT *is* a substrate for microsomal oxidases, then a mechanism can be proposed to explain the oral activity of some *Virola* pastes, even though they lack β-carbolines and are not significantly effective as MAOI. This alternative mechanism postulates that some of the non-alkaloid constituents in the pastes may possess anti-oxidant activity and/or exhibit activity as specific inhibitors of microsomal MFOs. In the former case, presence of high concentrations of non-specific anti-oxidants could scavange a high proportion of the molecular oxygen in the vicinity, thus making less of it available as co-substrate for the microsomal enzymes catalyzing DMT N-oxidation and 6-hydroxylation. In the latter case, constituents in the *Virola* resin may specifically inhibit hepatic microsomal MFO and thus block the oxidation(s) of DMT caused by these enzymes. In either case the compound could be protected from oxidative

transformation in the intestinal/hepatic shunt and thus be taken up into the CNS in the form of the unchanged tertiary amine. A number of lignans have been characterized which exhibit protective activity against hepatotoxins [7]; inhibition of hepatic MFO has been proposed as the probable mechanism. All of the active compounds possessed a methylenedioxyphenyl moiety, but analogs lacking this configuration did not have hepatoprotective properties. The methylenedioxyphenyl group has been implicated in other studies as the main pharmacophore responsible for mixed function oxidase inhibition [8]. The Myristicaceous genera Virola and Iryanthera are both rich in constituents incorporating the methylenedioxyphenyl group, including fatty acid derivatives, neolignans, flavans, and diarylpropanoids [9]. Several novel lignans having this substitution were characterized in the bark of DMK-59 (V. elongata) which was the source-plant for Marcos Flores' paste sample; this sample showed the greatest degree of oral activity in self-experiments. A number of other phenolic compounds, including flavans, flavanoids, isoflavonoids, diarylpropanoids, and neolignans, have been isolated from a number of Virola and Iryanthera spp.; some of these compounds could act as antioxidants and could contribute to the inactivation of MFOs via this nonspecific mechanism. In any case the peripheral metabolism of DMT and related compounds, following oral administration, may be significantly altered in the presence of antioxidants and/or specific MFO inhibitors; under these conditions the compounds might well reach the CNS in the form of the unchanged tertiary amine. Further in vivo and in

vitro experiments would be required to confirm or disconfirm this alternative mechanism of oral activity. In view of the phytochemical and pharmacological data accumulated in the present study, it appears that this alternative hypothesis is at least as probable, if not more probable, than MAO inhibition as the mechanism responsible for the oral activity of the Myristicaceous pastes.

IV. Conclusion

Phytochemical and pharmacological information collected in the course of this study has been insufficient to definitely establish the mechanism of oral activity in these two Amazonian hallucinogens; however it has provided phytochemical data and *in vitro* pharmacological evidence which indicates that in the case of *ayahuasca* the original hypothesis proposed to explain the oral activity cannot be disproved. Certainly *ayahuasca* contains high enough concentrations of β-carbolines to effectively inhibit MAO and by this mechanism the active hallucinogenic constituent, DMT, may be protected from peripheral degradative metabolism. The alternative mechanism proposed in the above discussion may also be implicated in the pharmacology of *ayahuasca*, but at least it is not *necessary* to invoke this mechanism for *ayahuasca*. In the case of the orally-ingested Myristicaceous preparations, however, the data reported here indicate that MAO inhibition--whether due to β-carbolines or some other constituents--is almost certainly *not* the mechanism responsible for their oral activity. The pastes do not contain more than traces of β-carbolines, they show poor activity as

MAOI, and their oral activity, when present, is not correlated with the presence of β-carbolines. Some alternative mechanism must therefore be invoked to explain the oral activity of these Myristicaceous pastes. Two such alternatives have been discussed above; one is that the oral activity is due to biologically active constituents other than tryptamines. The other and perhaps more attractive possibility is that the active tryptamines are protected from peripheral degradation by constituents which inhibit hepatic mixed-function oxidases, the enzymes responsible for 6-hydroxylation and N-oxidation of tryptamine derivatives. MFO inhibitors require the presence of a methylenedioxyphenyl configuration as the active pharmacophore, and Virola spp. are excellent sources of compounds possessing this moiety. Unfortunately neither alternative mechanism can be proven or disproven until more has been learned about the in vivo metabolism of DMT and related compounds. An obvious place to start would be to study the metabolism of orally-administered DMT in the presence of known MFO inhibitors.

V. Literature Cited

1. Rivier, L. & J. lindgren (1972) Ayahuasca, the South American Hallucinogenic Drink: Ethnobotanical and Chemical Investigations. Economic Botany 29:101-129

2. Barker, S. A., J. A. Monti, and S. T. Christian (1980) Metabolism of the Hallucinogen N,N-dimethyltryptamine in Rat Brain Homogenates. Biochemical Pharmacology 29:1049-57

3. Szara, S. & J. Axelrod. (1959) Hydroxylation and N-demethylation of N,N-dimethyltryptamine. Experientia 15:216-17

4. Shulgin, A. T. (1976) Psychotomimetic Agents. chapter 4 in Maxwell Gordon (ed.) Psychopharmacological Agents V. IV. Academic Press

5. Bickel, M. H. (1969) The Pharmacology and Biochemistry of N-oxides. Pharmacological Reviews 21:325-358

6. Shah, N. S., & M. P. Hedden (1977) Behavioural Effects and Metabolic Fate of N,N-dimethyltryptamine in Mice Pretreated with β-diethylaminoethyl- Diphenylpropylacetate (SKF-525-A), Iproniazid, and Chlorpromazine. Pharmacology, Biochemistry and Behaviour 8:351-56

7. MacRae, W. D., & G. H. N. Towers (1984) Biological Activities of Lignans. Phytochemistry. In press.

8. Brattsten, L. B. (1977) Biochemical Defense Mechanisms in Herbivores Against Plant Allelochemicals. Chapter 5 in G. A. Rosenthal, and D. H. Janzen (eds.) Herbivores: Their Interactions with Secondary Metabolites. Academic Press.

9. Gottlieb, O. R. (1979) Chemical Studies on Medicinal Myristicaceae from Amazonia. Journal of Ethnopharmacology 1:309-323

10. D. MacRae. (1984) Ethnobiological and Chemical Investigations of Selected Amazonian Plants. PhD thesis, University of British Columbia, Vancouver, B. C.

APPENDIX I

TABLE XVI - BIOLOGICALLY ACTIVE CONSTITUENTS IN AYAHUASCA ADMIXTURES

The following list of plant species has been compiled from various sources and is intended as a summary of the current state of phytochemical knowledge of those genera and species which are known to be utilized as admixtures to ayahuasca. The information on the use of these species as ayahuasca admixtures is compiled primarily from references 1,2,3,4,5, and 86; information on the vernacular names of the plants used is compiled from the references cited above and also from 85. The phytochemical information is derived primarily from a computer search of the Biological Abstracts Data Base and the American Chemical Society Data Base, covering the years 1970-present. The references cited in this appendix are not intended to be exhaustive but rather are intended as indicators of the existence or nonexistence of information regarding biodynamic constituents in the species listed. In the case of certain genera, especially Tabernaemontana, Tabebuia, Maytenus, Alchornea, Ocimum, Erythrina, Ficus, and Uncaria, the number of available references runs well into the thousands; in these instances only a few key references have been cited. In most instances the references cited do not refer specifically to the species used as an ayahuasca admixture, but to some closely related species in the same genus. Often information is not available on the constituents of the particular species used in conjunction with ayahuasca, but is available for other members of the genus.

Family: Genus & Species	Vernacular Name	Biodynamic Constituents	References
Acanthaceae: Teliostachya lanceolata Nees var. Crispa Nees in Mart.	"toe negro"	none reported	1,3
Amaranthaceae: Iresine sp. P. Br.	-	hydroxy-cinnamic acid amides	1,44
Alternanthera lehmanii Ilieron	"picurullana-quina"	none reported	2,4,85
Apocynaceae: Malouetia tamaquarina (Aubl.)A. DC.	"cuchura-caspi"	steroid alkaloids, conopharyngine	2,26,62
Tabernaemontana sp. L.	"uchu-sanango"	bisindole alkaloids, terpenoids, cornaridine	1,2,8,9,31,76,84
Himatanthus succuba (Spruce)Woods	"bellaco-caspi"	flavonoids, fulvoplumieron	1,32,43
Araceae: Montrichardia arborescens Schott.	"raya balsa"	none reported	1
Bignoniaceae: Mansoa alliaceae (Lem.)A. Gentry	"ajo sacha"	none reported	1
Tabebuia heteropoda (DC)Sandwith.	"tahuari"	dibenzoxanthenes, napthoquinones, lapachol	1,22,53,73
Tynnanthus panurensis (Bur.)Sandwith.	"clavohuasca"	none reported	1

Table XVI (cont'd)

Family: Genus & Species	Vernacular Name	Biodynamic Constituents	References
Bombacaceae:			
Ceiba pentandra L.	"lupuna"	none reported	1
Cavanillesia hylogeiton Ulb.	"puca lupuna"	?	1,37
Cactaceae:			
Opuntia sp. Mill	"tchai"	N-methyl-tyramine, mescaline	5,40,42
Epiphyllum sp. Haw.	"pokere"	none reported	5
Caryocaraceae:			
Anthodiscus pilosus Ducke	"tahuari"	none reported	1
Celastraceae:			
Maytenus ebenifolia Reiss	"chuchuhuasi"	sesquiterpene alkaloids, nicotinoyl alkaloids, triterpenes, maytensine, tingonane, ansa macrolides, etc.	1,20,41,49 52,78,
Cyclanthaceae:			
Carludovica divergens Drude	"tamshi"	none reported	1
Cyperaceae:			
Cyperus sp. L.	"piri-piri"	quinones, essential oils saponins, sesquiterpenes	1,7,57 64
Cyperus digitatus Roxb.	"chicorro"	none reported	86
Euphorbiaceae:			
Alchornea castenifolia (Willd.) Juss.	"hiporuru"	alchornine, imadazole alkaloids, corynanthe-type alkaloids, antifeedants, etc.	1, 63, 70 79,84,
Hura crepitans L.	"catahua"	tiglione diterpenes, piscicidal compounds, lectins	1,47,54,65
Guttiferae:			
Clusia insignis Mart.	"renaco"	clusianone, xanthochymol, triterpenoids	1,25,36,66
Labiateae:			
Ocimum micranthum Willd.	"pichana, abaca"	neolignans, sesquiterpenes, antihelmentics	2, 19, 30, 50
Lecythidaceae:			
Couroupita guianensis Aubl.	"ayahuma"	indole alkaloids	1,12,60

Table XVI (cont'd)

Family: Genus & Species	Vernacular Name	Biodynamic Constituents	References
Leguminosae:			
Calliandra angustifolia Spruce	"bobinsana"	imino acids	1,33,38
Cedrilinga catenaeformis Ducke	"huairacaspi"	none reported	1
Pithecellobium laetum (Poepp. & Endl.) Benth.	"remo caspi"	phytomitogens, lupeol, spinasterol	1,13,69
Sclerobium setiferum Ducke	"palisangre" = "palisanto"?	none reported	1,85
Erythrina poeppigiana (Walp.) O. F. Cook	"amaciza" = "amasisa"?	Erythrina alkaloids, etc.	1,85,34,77
Voucapoua americana Aubl.	"huacapu"	none reported	1
Lomariopsidaceae:			
Lomariopsis japurensis (Mart.)J. Sm.	"shoka"	none reported	5
Loranthaceae:			
Phrygilanthus eugenoides var. robustus Glaz.	"miya"	none reported	5
Phtirusa pyrifolia H. B. K. Eichler	"suelda con suelda"	none reported	1
Marantaceae:			
Calathea veitchiana Veitch. Ex Hooker	"pulma"	tryptophan	2,75
Menispermaceae:			
Abuta grandifolia (Mart.) Sandwith.	"abuta", "caimitillo", "sanango"	tropolone isoquinolines, oxo-aporphines, palmatine	1,86,15,58,59
Moraceae:			
Coussapoa tessmannii Mildbr.	"renaco"	none reported	1
Ficus ruiziana Standl.	"renaco"	furocoumarins, triterpenes, biphenylhexa-	1,28,29,71
Ficus insipida Willd.	"oje"	hydroindolizines, phenanthroxindolizines	
Pourouma Aubl. sp. aff. foleata McBr.	"chullachaqui caspi"	none reported	1

Table XVI (cont'd)

Family: Genus & Species	Vernacular Name	Biodynamic Constituents	References
Myristicaceae: Virola sp. Aubl.	"cumala"	diaryl propanoids, 2-methyl ketones tryptamines, β-carbolines, neolignans	1,6,24,61 74
Virola surinamensis Warb.	"caupuri"	neolignans	1,6
Nymphiaceae: Cabomba aquatica Aubl.	"murere" = "mureru"?	none reported	1,85
Polygonaceae: Triplaris surinamensis Mart. var. chamissoana Meisn.	"tangarana"	none reported	1
Pontederiaceae: Pontederia cordata L.	"amaron borrachero"	none reported	2,3
Phytolaccaceae: Petiveria alliaceae L.	"mucura"	oligo sulfides, triterpenes, trithiolanes	1,16,18,39
Rubiaceae: Calycophyllum spruceanum (Benth.)Hooker	"capirona negro"	none reported	1
Guettarda ferox Standl.	"garabata"	canthemine, hetero-yohimbine alkaloids	1,10,48
Uncaria guianensis (Aubl.)Gmel	"garabata"	spiro-oxindoles,bis-indoles, hetero-yohimbines	1,27,67, 76,82,84
Schizaeceae: Lygodium venustum Sw.	"tachai del monte"	antifertility agents	5,55
Scrophulariaceae: Scoparia dulcis L.	"nucnu pichana"	triterpenes, 6-MeO-benzoxazolilinone	1,21,56,72

Table XVI (cont'd)

Family: Genus & Species	Vernacular Name	Biodynamic Constituents	References
Solanaceae: Datura suaveolens (Willd.) Brechtold & Presl.	"toe"	tropane alkaloids	1,2,3,4
Brunfelsia chiricsanango Plowman	"chiricsanango"	scopaletin, CNS depressants, antiinflammatory compounds	1,2,11,87,88
Iochroma fuchsoides Meers in Hooker	"borrachero"	none reported	2,3,4
Juanulloa ochracea Cuatr.	"ayahuasca"	none reported	3
Verbenaceae: Cornutia odorata (P.& E.) Peopp.	"shinguarana"	none reported	85,86
Vitex triflora Vahl.	"tahuari"	diterpenes lactones, iridoid glycosides, flavonoid glycosides	1,17,23,51,68

References

1. Luis E. Luna. The Concept of Plants as Teachers Among Four Mestizo Shamans of Iquitos, Northeast Peru. Paper presented at the Symposium on Shamanism, XIth International Congress of Anthropological and Ethnological Sciences, Phase 2. Vancouver, B. C., August 20-23, 1983.

2. R. E. Schultes. Ethnotoxicological Significance of Additives to New World Hallucinogens. Plant Science Bulletin 18: 34-41. 1972.

3. R. E. Schultes. New Data on the Malpighiaceous Narcotics of South America Harvard Botanical Museum Leaflets, 23:137ff. 1979.

4. H. V. Pinkley. Plant Admixtures to Ayahuasca, the South American Hallucinogenic Drink. Lloydia 32:305. 1969.

5. L. Rivier & J. Lindgren. Ayahuasca, the South American Hallucinogenic Drink: Ethnobotanical and Chemical Investigations. Economic Botany 29:101-129. 1972.

6. L. E. S. Barata, P. M. Baker, O. R. Gottlieb, & E. A. Ruveda. Neolignans of Virola Surinamensis. Phytochemistry 17:783-86. 1978.

7. R. D. Allan, R. J. Wells, R. L. Correll, & J. K, MacLeod. The Presence of Quinones in the Genus Cyperus as an Aid to Classification. Phytochemistry 17:263-266. 1978.

8. D. G. Kingston. Plant Anti-cancer Agents Part 6: Isolation of Voacangine, Voacamine and Epi-voacorine from Tabernaemontana arborea Sap. Journal of Pharmaceutical Sciences 67:271-72. 1978.

9. D. G. Kingston, B. B. Gerhart, F. Ionescu, M. M. Mangino, & S. M. Sami. Plant Anti-cancer Agents Part 5: New bis-Indole Alkaloids from Tabernaemontana johnstonii Stem Bark. Journal of Pharmaceutical Sciences 67:249-51. 1978.

10. H. P. Husson, C. Kan-Fan, T. Sevenet, & J. P. Vidal. Structure of Cathenamine, Key Intermediate in the Biosynthesis of Indole Alkaloids. Tetrahedron Letters 22:1889-92. 1977.

11. M. Chaubal, & R. P. Iyer. Carbon-13 NMR Spectra of Scopoletin. Lloydia 40:618. 1977.

12. J. Bergman, B. Egestad, & J-O. Lindstrom. The Structure of Some Indolic Constituents in Couroupita guainensis. Tetrahedron Letters 30:2625-26. 1977.

13. M. Yadav, V. K. C. Ganaswaran. Phyto-Mitogens of Tropical Legumes Part I: Isolation from Parkia speciosa and Pithecellobium jiringa. Malaysian Journal of Science 4:25-26. 1976.

14. C. K. Kokate & K. C. Varma. Pharmacological Study on Essential Oil of Cyperus scariosus Part I: Effect on Central Nervous System pp. 189-193 in L. D. Kapoor & R. Krishnan (eds.) Advances in Essential Oil Industry. Today & Tomorrow's Printers & Publishers, New Delhi. 1977.

15. J. V. Silverton, C. Kabuto, K. T. Buck, M. P. Cava. Structure of Imerubrine, a Novel Condensed Tropolone Isoquinoline Alkaloid. Journal of the American Chemical Society 99:6708-12. 1977.

16. C. Von Szczepanski, J. Heindl, G-A. Hoyer, & E. Schroeder. Biologically Active Compounds from Plants Part 2: Synthesis and Anti-microbial Activity of Some Dissymmetric Oligo Sulfides. European Journal of Medicinal Chemistry 12:279-84. 1977.

17. R. U. Kodanda, R. E. Venkata, R. D. Venkata. Phenolic Constituents of the Bark of Vitex negundo. Indian Journal of Pharmacy 39:41. 1977.

18. F. P. Segelman, & A. B. Segelman. Constituents of Petiveria alliaceae (Phytolaccaceae) Part 1: Isolation of Isoarborinol, Isoarborinol Acetate and Isoarborinol Cinnamate from the Leaves. Lloydia 38:537. 1975.

19. R. G. Roy, N. M. Madesayaa, R. B. Ghosh, D. V. Gopalkrishnan, N. N. Murthy, T. J. Doraira, & N. L. Sitaraman. Study on Inhalation Therapy by an Indigenous Compound on Plasmodium vivax and Plasmodium falciparum Infections: A Preliminary Communication. Indian Journal of Medicinal Research 10:1451-55. 1976.

20. S. M. Kupchan, & R. M. Smith. Maytoline, Maytine, and Matolidine, Novel Nicotinoyl Sesquiterpene Alkaloids from Maytenus serrata. Journal of Organic Chemistry 42:115-118. 1977.

21. C-M. Chen & C-T. Chen. 6-Methoxy Benoxazolinone and Triterpenoids from Roots of Scoparia dulcis. Phytochemistry 15:1997-99. 1976.

22. R. Y. Wong, K. J. Palmer, G. D. Manners, & L. Jurd. The Structure of Guayacanin with Acetone of Crystallization, A Naturally Occurring Dibenzoxanthene from Tabebuia guayacan. Acta Crystollographica Sec. B 8:2396-2400. 1976.

23. H. Taguchi. Studies on the Constituents of Vitex cannibolia. Chemical & Pharmaceutical Bulletin 7:1668-70. 1976.

24. R. Baruffaldi, E. Fedeli, & N. Cortesi. Study of the Fat of Virola surinamensis Part 1: Acidic and Glyceride Composition and Chemical Nature of Some of the Unsaponifiable Components. Revista de Farmacia e Bioquimica da Universidade de Sao Paulo 13:91-102. 1975.

25. L. E. McCandlish, J. C. Hanson, & G. H. Stout. The Structures of 2 Derivatives of Bi-cyclo-3,3,1-nonane-2,4,9-tirone, a Natural Product Clusianone and Trimethylated Catechinic Acid. Acta Crystallographica, Sec. B: Structural Crystallography and Crystal Chemistry 32:1793-1801. 1976.

26. J. D. Medina & R. Bracho. Constituents of the Bark of Malouetia glandulifera. Planta Medica 29:367-69. 1976.

27. Y. Ban, M. Seto, & T. Oishi. The Synthesis of 3 Spiro Oxindole Derivatives Part 7: Total Synthesis of Alkaloids Racemic Rhynchophylline and Racemic Isorhynchophylline. Chemical & Pharmaceutical Bulletin 11:2605-13 1975.

28. Y. I. Eidler, G. L. Genkina, T. T. Shakirov. Quantitative Determination of Furocoumarins in Ficus carica Leaves. Khimiya Prirodnykh Soedinenii 3:349-51. 1975.

29. M. H. A. Elgamal, B. A. H. El-tawil, & M. B. E. Fayez. The Triterpenoid Constituents of the Leaves of Ficus nitida. Naturwissenschaften 62:486. 1975.

30. S. J. Terhune, J. W. Hoff, & R. W. Lawrence. Bicyclosesquiphellandrene and 1-epi Bicyclosesquiphellandrene 2 New Dienes Based on the Cadalene Skeleton. Phytochemistry 13:1183-85. 1974.

31. B. Talapatra, A. Patra, & S. K. Talapatra. Terpenoids and Alkaloids of the Leaves of Tabernaemontana coronaria. Phytochemistry 14: 1652-53. 1975.

32. R. R. Paris, & S. Duret. The Flavonoids of Various Apocynaceae. Plantes Medicinales et Phytotherapie 8:318-25. 1974.

33. G. A. Dardenne. New Free Amino Acids from Leguminosae. Phytochemistry 14:860. 1975.

34. K. Ito, M. Haruna, & H. Furuka. Studies on the Erythrina Alkaloids Part 10: Alkaloids of Several Erythrina sp. Plants from Singapore Malaysia. Yakugaku Zasshi 3:358-62. 1975.

35. S. R. Hemingway, & J. D. Phillipson. Alkaloids from South American Species of Uncaria (Rubiaceae). Journal of Pharmacy and Pharmacology 26(suppl.):113p. 1974.

36. D. L. Dreyer. Xanthochymol from Clusia rosea (Guttiferae). Phytochemistry 13:2883-84. 1974.

37. J. E. Lopez Guillen & I. K. De Cornelio. Medicinal Plants of Peru part 5. Biota 10:76-104. 1974.

38. J. E. Lopez Guillen & I. K. De Cornelio. Medicinal Plants of Peru part 4. Biota 10:28-56. 1974.

39. E. K. Adesogan. Trithiolaniacin, a Novel Trithiolane from Petiveria alliacea. Journal of the Chemical Society, Chemical Communications 21:906-7. 1974.

40. R. L. Vander Veen & L. G. West. N-methyl Tyramine from Opuntia clavata. Phytochemistry 13:866-67. 1974.

41. M. Leboeuf. Maytansine and Maytansinoids. Plantes Medicinales et Phytotherapie 12:53-70. 1978.

42. J. Pardanani, B. N. Meyer, & J. L. McLaughlin. Mescaline and Related Compounds from Opuntia spinosior. Lloydia 41:286-88. 1978.

43. G. P. Perdue & R. N. Blomster. South American Plants Part 3: Isolation of Fulvoplumierin from Himatanthus sucuuba (Apocynaceae). Journal of Pharmaceutical Sciences 67:1322-23. 1978.

44. J. Martin-Tanguy, F. Cabanne, E. Perdrizei, & C. Martin. The Distribution of Hydroxy-cinnamic Acid Amides in Flowering Plants. Phytochemistry 11:1927-8. 1978.

45. P. K. Mehrotra & V. P. Kamboj. Hormonal Profile of Coronaridine Hydrochloride, an Antifertility Agent of Plant Origin. Planta Medica 33:345-49. 1978.

46. H. Wagner & J. Burghart. Spermidine Alkaloids and Triterpenes from Maytenus heterophylla ssp. heterophylla and Pleurostyla africana: Chemical Constituents of the Celastraceae Part 4. Planta Medica 32A:9-14. 1977.

47. F. J. Evans & C. J. Soper. The Tigliane Daphnane and Ingenane Diterpenes: Their Chemistry, Distribution, and Biological Activities; A Review. Lloydia 4:193-233. 1978.

48. C. Kan-Fan & H. P. Husson. Stereochemical Control in the Biomimetic Conversion of Heteroyohimbine Alkaloid Precursors: Isolation of a Novel Key Intermediate. Journal of the Chemical Society, Chemical Communications 14:618-19. 1978.

49. H. Nozaki, H. Suzuki, K-H. Lee, & A. T. Mcphail. Structure and Stereochemistry of Maytenfolic Acid and Maytenfoliol, 2 New Anti- Leukemic Triterpenes from Maytenus Diversifolia: X-Ray Crystal Structures. Journal of the Chemical Society, Chemical Communications 0:1048-51. 1982.

50. D. G. Desai, N. S. Ambade, & R. R. Mane. Synthesis of Ocimum a New Neolignan from Ocimum americanum. Indian Journal of Chemistry Section B, Organic Chemistry 5:491-92. 1982.

51. C. K. Sehgal, S. C. Taneja, K. L. Dhar, & C. K. Atal. 2' p Hydroxybenzoyl Musaenosidic Acid, a New Iridoid Glycoside from Vitex negundo. Phytochemistry 2:363-66. 1982.

52. J. G. Gonzales, G. Delle Monache, F. Delle Manache, G. B. Marini-Bettolo. Chuchuhuasha, a Drug Used in Folk Medicine in the Amazonian and Andean Areas of South America: A Chemical Study of Maytenus Laevis. Journal of Ethnopharmacology 5:73-78. 1982.

53. D. G. Kingston & M. M. Rao. Isolation, Structure Elucidation and Synthesis of 2 New Cytotoxic Napthoquinones from Tabebuia cassinoides. Planta Medica 3:230-31. 1980.

54. M. Pere, D. Pere, & P. Rouge. Isolation and Studies of the Physicochemical and Biological Properties of Lectins from Hura crepitans. Planta Medica 41:344-50. 1981.

55. B. B. Gaitonde & R. T. Mahajan. Antifertility Activity of Lygodium flexuosum. Indian Journal of Medical Research 72:597-604. 1980.

56. S. B. Mahato, M. C. Das, & N. P. Sahu. The Terpenoids of Scoparia dulcis. Phytochemistry 20:171-73. 1981.

57. P. N. Singh & S. B. Singh. A New Saponin from Mature Tubers of Cyperus rotundus. Phytochemistry 19:2056. 1980.

58. J. W. Skiles, J. M. Saa, & M. P. Cava. Splendidine, a New Oxo Aporphine Alkaloid from Abuta rufescens. Canadian Journal of Chemistry 57:1642-46. 1979.

59. Setor de Fitoquimica, INPA, Manaus,Amazonas, Brazil. Chemical Composition of Amazonian Plants. Acta Amazonica 1:83-86. 1971.

60. A. K. Sen 6 S. B. Mahato. Couroupitine a New Alkaloid from Couroupita guianensis. Tetrahedron Letters 7:609-10. 1974.

61. O. R. Gottlieb, & A. A. Loureiro. Distribution of Diaryl Propanoids in Amazonian Virola Species. Phytochemistry 12:1830. 1973.

62. F. Khuong-Huu, & M-J. Magdelaine. Steriod Alkaloids from Malouetia brachyloba and from Malouetia heudelotii. Phytochemistry 7:1813-16. 1973.

63. F. Khuong-Huu & J. P. Le Forestier. Alchorneine, Isoalchorneine and Alchorneinone, Products Isolated from Alchornea floribunda. Tetrahedron 28:5207-20. 1972.

64. H. Hikino, & K. Aota. Structure and Absolute Configuration of Alpha Rotunol and Beta Rotunol, Sesquiterpenoids of Cyperus rotundus M. Tetrahedron 27:4831-36. 1971.

65. K. Sakata, & K. Kawazu. Studies on a Piscicidal Constituent of Hura crepitans D. Part 1: Isolation and Characterization of Hura Toxin and its Piscicidal Activity. Agricultural and Biological Chemistry 35:1084-91. 1971.

66. S. B. Mathur Triterpenoid Constituents of Clusia rosea D. Phytochemistry 11:1513-14. 1972.

67. M. Lavault, C. Moretti, & J. Bruneton. Alkaloids of Uncaria guianensis. Planta Medica 47:244-5. 1983.

68. A. H. Jackson, & A. S. Chawla. Studies of Erythrina Alkaloids. Part IV. GC/MS Investigations of Alkaloids in the Leaves of E. poeppigiana, E. macrophylla, E. berteroana, and E. salviflora. Allertonia 3:39-45. 1982.

69. S. P. Gunasekera, G. A. Cordell, & N. R. Farnsworth. Constituents of Pithecellobium multiflorum. Journal of Natural Products 45:651. 1982.

70. B. L. H. Hankinson. Investigation of Constituents and Antifeedant Activity of Alchornea triplinervia. Dissertation Abstracts International Sec. B # DA8217228. 1982

71. S. R. Venkatachalam, & N. B. Mulchandani. Isolation of Phenanthroindolizidine Alkaloids and a Novel Biphenylhexahydroindolizine Alkaloid from Ficus hispida. Naturwissenschaften 69:287-8. 1982.

72. J. Li, Y. Li, R. Nie, & J. Zhou. Coixol and Betulinic Acid of Scoparia dulcis L. Yun-nan Chih Wu Yen Chiu 3:475-7. 1981.

73. L. Prakash & R. Singh. Chemical Examination of the Leaves and Stem heartwood of Tabebuia pentaphylla (Linn) Hemsl. (Bignoniaceae). Journal of the Indian Chemical Society 58:1122-23. 1981.

74. O. R. Gottlieb. Chemical Studies on Medicinal Myristicaceae from Amazonia. Journal of Ethnopharmacology 1:309-323. 1979.

75. W. E. Splittstoesser & F. W. Martin. The Tryptophan Content of Tropical Roots and Tubers. Hortscience 10:23-24. 1975.

76. G. A. Cordell & J. E. Saxton. Bisindole Alkaloids. Chapter 1 in R. H. F. Manske & R. G. A. Rodrigo, (eds.) The Alkaloids Vol XX.Academic Press. 1981.

77. S. F. Dyke & S. N. Quessy. Erythrina and Related Alkaloids. Chapter 1 in Manske & Rodrigo, op. cit., Vol XVIII. 1981.

78. D. M. Smith. The Celastraceae Alkaloids. Chapter 4 in R. H. F. Manske (ed.) The Alkaloids Vol. XVI. 1977.

79. D. S. Seigler. Plant Systematics and Alkaloids. Chapter 1 in Manske, op. cit., Vol XVI. 1977.

80. R. L. Clarke. The Tropane Alkaloids. Chapter 2 in Manske, op. cit., Vol XVI. 1977.

81. J. E. Saxton. Alkaloids of Mitragyna and Related Species. Chapter 3 in Manske, op. cit., Vol. XIV. 1973.

82. J. S. Bindra. Oxindole Alkaloids. Chapter 2 in Manske, op. cit., Vol XIV. 1973.

83. M. Shamma & R. L. Castenson. The Oxoaporphine Alkaloids. Chapter 6 in Manske, op. cit., Vol. XIV. 1973.

84. V. Snieckus. Distribution of Indole Alkaloids in Plants. Chapter 1 in Manske, op. cit., Vol XI. 1968.

85. J. Soukup. Vocabulario de los Nombres Vulgares de la Flora Peruana Colegio Salesiana, Lima. 1970.

86. D. McKenna, G. H. N. Towers, & F. S. Abbott. Monoamine Oxidase Inhibitors in South American Hallucinogenic Plants: Tryptamine and β-carboline Constituents of Ayahuasca. Journal of Ethnopharmacology. 10:195-223. 1984

87. Plowman, T. Brunfelsia in Ethnomedicine. Harvard Botanical Museum Leaflets 25:289-320. 1977.

88. Iyer, R. P., J. K. Brown, M. G. Chaubal & M. H. Malone. Brunfelsia hopeana I. Hippocratic Screening and Anti-inflammatory Evaluation. Journal of Natural Products 40:356-60. 1c77.

APPENDIX II

The following list of herbarium voucher specimens includes the collection data on all of the specimens discussed in the text for which herbarium vouchers are available. In addition Appendix II includes any other plant collection which was stated by informants to have a medicinal or ethnobotanically sigificant use. Appendix II also includes all collections from the Myristicaceae and Malpighiaceae, even though these may not have been utilized in the preparation of drug samples analyzed in this work or may not be used in this manner. Plant collections which were not known to have ethnobotanical or medicinal significance have been omitted from this collection list. In instances where multiple collections were made of the same species, the taxonomic authority is given in the first citation; the authority is omitted in subsequent citations.

TABLE XVII - LIST OF HERBARIUM VOUCHER COLLECTIONS

Collection # (DMK-)	Binomial & authority	Vernacular Name	Determined by:*	UBC Herbarium Access # V172-	Duplicate Vouchers:	Remarks:
DMK-1*†	Justicia pectoralis Jacq.	masha-hiri	T. Plowman(F)	175	F,USM,MO‡	additive to Virola snuff used to treat cold sores
DMK-3	Banisteriopsis caapi (Spr.ex Griseb) Morton	ayahuasca	T. Plowman(F)	177	F,USM	source of ayahuasca
DMK-16*	Sanchezia tigrina Leonard	-	P. Matekaitis(F)	189	F,USM,ECON UNAP	leaves smoked or brewed as hallucinogen
DMK-20*	Psychotria viridis R. & P.	chacruna	T. Plowman(F)	193	UBC only	admixture to ayahuasca orig. stock for DMK-21
DMK-21*	Psychotria viridis	chacruna	T. Plowman(F)	194	F,USM,ECON, MICH,RVH,UNAP	see above
DMK-22*	Teliostachya lanceolata Nees	toe negro	T. Plowman(F)	195	F,USM,ECON, UNAP,MO,RVH	ayahuasca admixture
DMK-23*	Virola pavonis (A. DC.)A. C. Smith	cumala	W. Rodrigues(INPA)	196	F,USM,ECON, UNAP,INPA,RVH	
DMK-30*	Virola pavonis	cumala	W. Rodrigues(INPA)	201	F,ECON,INPA UNAP,RVH	alkaloid +
DMK-31*	Palicourea sp.	-	J. Ruiz(UNAP)	203	F,USM,MO, UNAP,RVH	alkaloid ++
DMK-32*	Virola pavonis	cumala	W. Rodrigues(INPA)	204	F,USM,ECON, INPA,UNAP,RVH	alkaloid -
DMK-34*	Virola pavonis	cumala; ku'-ru-ku	W. Rodrigues(INPA)	205	F,USM,ECON, UNAP,MICH, INPA,MO,RVH	alkaloid -;source of Bora paste at P.U.
DMK-35*	Virola sp.	cumala	P. Matakaitis(F)	206	UBC only	alkaloid +
DMK-36*	Virola sp.	cumala	P. Matakaitis(F)	207	UBC only	alkaloid +
DMK-37*	Virola sp.	cumala	P. Matakaitis(F)	208	UBC only	alkaloid +
DMK-38*	Rinora racemosa (Mart.&Zucc.)Kuntze	-	A. Gentry(MO)	209	F,USM,ECON, UNAP,MO,RVH	alk. -; lichen on bark added to Virola paste
DMK-39*	Anemia sp.	-	R. G. Stolze(F)	210	F,USM,ECON, UNAP,RVH	alk.-;tea fr. leaves used to cook Virola paste
DMK-40*	Virola sebifera Aubl.	cumala roja; oo'-koey; piri;	W. Rodrigues(INPA)	211	F,USM,MICH, INPA,MO,RVH ECON,UNAP	alk.++;source of D.A. Moreno sample #1

Table XVII (Cont'd)

Collection # (DMK-)	Binomial & authority	Vernacular Name	Determined by:*	UBC Herbarium Access # V172-	Duplicate Vouchers:	Remarks:
DMK-41*	Virola elongata (Benth.)Warb.	cumala;oo'-koo-na;	W. Rodrigues(INPA)	212	F,USM,ECON, UNAP, INPA, RVH	alkaloid ++ suitable for paste
DMK-42	Pityrogramma calomelos(L.)Link	-	R.G. Stolze(F)	213	F,USM,UNAP RVH	juice of croziers used for cataracts
DMK-43*	Theobroma subinacum Mart.	macambo del monte; Bora:a'-he	P. Matakaitis(F)	214	F,USM,ECON, UNAP,RVH	ashes of fruits added to Virola paste
DMK-44*	Iryanthera longiflora Ducke	Bora: ehu-ghwa-o-oo-e	W. Rodrigues(INPA)	215	F,USM ECON,UNAP INPA	alkaloid -
DMK-45*	Virola elongata	Bora:ugr-pah-laye	W. Rodrigues(INPA)	216	INPA	alkaloid +
DMK-46*	Virola calophylla Warb.	Bora:kuhr'-re-ko	W. Rodrigues(INPA)	217	F,USM,ECON UNAP	alkaloid +;suitable for making paste
DMK-47*	Iryanthera macrophylla (Bth.)Warb.	Bora:hachiurne-e	W. Rodrigues(INPA)	219	F,USM,ECON, UNAP, INPA,RVH	alkaloid -
DMK-48*	Iryanthera ulei Warb.	Bora:hachi-e	W. Rodrigues(INPA)	218	USM,INPA, RVH	alkaloid -
DMK-49*	Iryanthera crassifolia A. C. Smith	Bora:chi-chi-ch	W. Rodrigues(INPA)	220	F,USM,ECON INPA, UNAP,RVH	alkaloid -
DMK-50*	Iryanthera juruensis Warb.	Bora:piji-hah'-eh	W. Rodrigues(INPA)	221	F,USM,ECON, INPA,UNAP,RVH	alkaloid -
DMK-51*	Iryanthera paraensis Warb.	Bora:ti-ti-mueh	W. Rodrigues(INPA)	222	F,USM,ECON, INPA,UNAP,RVH	alkaloid -
DMK-52*	Virola multinervia Ducke	Bora:kat'-so-eh	W. Rodrigues(INPA)	223	F,USM,ECON, INPA,UNAP RVH	alkaloid -
DMK-53	Virola calophylla	Witoto:oo-koo'-na	W. Rodrigues(INPA)	224	F,USM,ECON, UNAP,INPA	suitable for paste
DMK-54	Virola sp.	cumala	P. Matakaitis(F)	225	USM,ECON,UNAP,RVH	
DMK-56*	Virola calophylla	Witoto:ti-ti-mueh	W. Rodrigues(INPA)	226	F,USM,ECON INPA,UNAP, RVH	alkaloid + (seeds & fruits)
DMK-57	Banisteriopsis muricata(Cav.)Cuatr.	sacha-ayahuasca	B. Gates (MICH)	227	F,USM,ECON, UNAP,MICH, RVH	used to make ayahuasca

Table XVII (Cont'd)

Collection # (DMK-)	Binomial & authority	Vernacular Name	Determined by:*	UBC Herbarium Access # V172-	Duplicate Vouchers:	Remarks:
DMK-59*	Virola elongata	cumala	W. Rodrigues(INPA)	229	F,USM,ECON, UNAP,MICH, INPA,MO,RVH	source of Don Marcos' ku'-ru-ku; alkaloid ++
DMK-63*	Virola pavonis	cumala blanca; Bora:ku'-ru-ku	W. Rodrigues(INPA)	233	F,USM,ECON, UNAP,MICH	alk. -; said suitable for paste
DMK-64*	Philodendron nervosum (Schult.& Schult.)Kunth	-	T. Plowman(F)	234	F,USM,ECON, UNAP,MICH, MO,RVH	admixture to Virola paste; alkaloid -
DMK-65	Theobroma bicolor H.&B.	macambo; Bora:a'-hep	T. Plowman(F)	235	F,USM,ECON, UNAP,MO,RVH	admixture to Virola paste; alkaloid -
DMK-66*	Calliandra angustifolia Spruce	-	C. Niezgoda(F)	236	F,USM,UNAP, RVH	tea used as sedative; admix. to ayahuasca
DMK-67*	Virola elongata	cumala; Witoto:oo'-koo-na	W. Rodrigues(INPA)	237	F,USM,UNAP, INPA,RVH	alk.+;source of D.A. Moreno sample #2
DMK-68*	Virola elongata	cumala; Witoto:oo'-koo-na	W. Rodrigues(INPA)	238	F,USM,UNAP, INPA,RVH, ECON	alk.+;source of D.A. Moreno sample #3
DMK-69*	Virola elongata	cumala; Witoto:oo'-koo'-na	W. Rodrigues(INPA)	239	F,USM,UNAP, INPA,ECON, RVH	alk.+;source of D.A. Moreno sample #4
DMK-70	Virola elongata	cumala	W. Rodrigues(INPA)	240	F,USM,ECON, INPA,RVH	source of seeds
DMK-71	Virola elongata	cumala	W. Rodrigues(INPA)	241	F,USM,ECON, MO,INPA,UNAP,RVH	source of seeds
DMK-73	Banisteriopsis martiniana (Adr.Juss.)Cuatr. var. subenervia Cuatr.	-	B. Gates(MICH)	243	F,USM,MO, ECON,MICH, UNAP,RVH	
DMK-74*	Abuta grandifolia (Mart.)Sandwith	abuta,caimitillo, sanango	T. Plowman(F)	244	F,USM,ECON, UNAP,RVH	admixture to ayahuasca; alkaloid ++
DMK-75*	Virola loretensis A.C. Smith	cumala	W. Rodrigues(INPA)	245	F,USM,ECON, UNAP,MICH, INPA,MO,RVH	alkaloid -
DMK-78*	Osteophloem platyspermum (A.DC)Warb.	-	W. Rodrigues(INPA)	248	F,USM,ECON, INPA,UNAP, RVH	alk. +; N-Me-tryptophan-methyl-ester in lvs.
DMK-82*	Virola albidiflora Ducke	cumala de los brujos	W. Rodrigues(INPA)	251	F,USM,UNAP, INPA,RVH	alkaloid -

Table XVII (Cont'd)

Collection # (DMK-)	Binomial & authority	Vernacular Name	Determined by:*	UBC Herbarium Access # V172-	Duplicate Vouchers:	Remarks:
DMK-83*	Mascagnia sinemariensis (Aubl.)Griseb.	-	W. Anderson(MICH)	252	F,USM,UNAP, MICH,MO,RVH	
DMK-84*	Byrsonima poeppigiana Adr. Juss.	-	W. Anderson(MICH)	253	F,USM,UNAP, MICH,MO,RVH	
DMK-86	Burdachia prismatocarpa Adr. Juss. var. loretoensis Anderson	-	W. Anderson(MICH)	255	F,USM,UNAP, MICH,MO,RVH	
DMK-87	Mascagnia benthamiana (Griseb.)Anderson	-	W. Anderson(MICH)	256	F,USM,UNAP, MICH,MO,RVH	
DMK-88	Mascagnia benthamiana	-	W. Anderson(MICH)	257	F,USM,UNAP, MICH,MO,RVH	
DMK-89	Mascagnia benthamiana	-	W. Anderson(MICH)	258	F,USM,UNAP, MICH,MO,RVH	
DMK-90	Heteropterys orinocensis (H.B.K.)Adr. Juss.	-	W. Anderson(MICH)	259	F,UNAP,MICH, RVH	
DMK-91	Stigmaphyllon hypoleucum Miq	-	W. Anderson(MICH)	260	F,USM,UNAP, MICH,MO,RVH	
DMK-92	Byrsonima arthropoda Adr. Juss.	-	W. Anderson(MICH)	261	F,USM,UNAP, MICH,RVH	
DMK-93	Heteropterys orinocensis	-	W. Anderson(MICH)	262	F,UNAP,MICH, USM,MO,RVH	
DMK-94	Heteropterys orinocensis	-	W. Anderson(MICH)	263	F,UNAP,MICH, RVH	
DMK-95	Heteropterys orinocensis	-	W. Anderson(MICH)	264	UNAP,MICH,RVH	
DMK-96	Heteropterys orinocensis	-	W. Anderson(MICH)	265	UNAP,MICH,RVH, F,USM,MO	
DMK-97	Brunfelsia grandiflora D. Don ssp. schultesii Plowman	chiricsanango	T. Plowman(F)	266	F,UNAP,RVH	febrifuge;admix. to ayahuasca
DMK-103	Byrsonima chrysophylla H.B.K.	-	W. Anderson(MICH)	272	F,USM,UNAP, MICH,RVH	
DMK-104	Hyptis suaveolens (L.)Poit.	-	C.Niezgoda(F)	273	F,USM,MO, UNAP,RVH	used for rheumatism

Table XVII (Cont'd)

Collection # (DMK-)	Binomial & authority	Vernacular Name	Determined by:*	UBC Herbarium Access # V172-	Duplicate Vouchers:	Remarks:
DMK-107*	Davilla nitida (Vahl.)Kub.	pucahuasca	P. Matakaitis(F)	276	UBC only	
DMK-108*	Psychotria viridis	yage-semiruca; chacruna;suija	T. Plowman(F)	277	F,USM,ECON, UNAP,MICH, MO;RVH	alk.+;admix. To ayahuasca
DMK-109*	Psychotria carthagenensis Jacq.	yage;yage-chacruna	P. Matakaitis(F)	278	F,UNAP	alk. -; admix. to ayahuasca
DMK-110*	Banisteriopsis caapi	cielo ayahuasca	T. Plowman(F)	279	F,UNAP	ayahausca cultivar
DMK-111	Banisteriopsis caapi	lucero ayahuasca	T. Plowman(F)	280	F,UNAP	ayahuasca cultivar
DMK-112	Banisteriopsis caapi	lucero ayahuasca	T. Plowman(F)	281	F,UNAP	ayahuasca cultivar
DMK-113	Senna occidentalis (L.)Link	retama	R. Barnaby(NY)	282	UBC only	medicinal; use uncertain
DMK-115	Petiveria alliaceae L.	mukura	P. Matakaitis(F)	284	F,USM,UNAP, MO,RVH	used in the bath; admix. to ayahuasca
DMK-116	Piper aduncum L.	cordoncillo	W. Burger(F)	285	F,UNAP,RVH	tea of leaves used to treat asthma & heal bones
DMK-117	Ocimum micranthum Willd.	albaca	T. Plowman(F)	286	UNAP	for the stomach; ayahuasca admixture
DMK-118	Hippeastrum puniceum (Lam.)Kuntze	samangi	T. Plowman(F)	287	UNAP	for dropsy, & sore arches
DMK-119	Cornutia odorata (P.& E.)Poepp.	shinguarana	T. Plowman(F)	288	UBC only	admixture to ayahuasca alkaloid -
DMK-120	Sambucus mexicana Presl. ssp.bipinnata(S.&C.)Schwer.	salco	T. Plowman(F)	289	UNAP,RVH	treatment of epilepsy, cough, bronchitus
DMK-121	Chenopodium ambrosioides L.	buseta	T. Plowman(F)	290	F,USM,MO, RVH	used in bath
DMK-122	Maranta arundinaceae L.	shimpanpana	T. Plowman(F)	291	UNAP,RVH	used in bath

Table XVII (Cont'd)

Collection # (DMK-)	Binomial & authority	Vernacular Name	Determined by:*	UBC Herbarium Access # V172-	Duplicate Vouchers:	Remarks:
DMK-123	Alpinia speciosa Schum	miski-panga	P. J. M. Maas(F)	292	F,USM,ECON, MO,UNAP,RVH	use uncertain
DMK-124*	Banisteriopsis caapi	pucahuasca	B. Gates (MICH)	293	UNAP,MICH, RVH	ayahuasca cultivar
DMK-125*	Banisteriopsis caapi	ayahuasca	B. Gates (MICH)	294	UNAP,MICH, RVH	ayahuasca cultivar
DMK-126*	Banisteriopsis caapi	cielo ayahuasca	B. Gates (MICH)	295	UNAP,MICH, RVH	ayahuasca cultivar
DMK-127*	Banisteriopsis caapi	cielo ayahuasca	B. Gates (MICH)	296	UNAP,MICH, RVH	ayahuasca cultivar
DMK-128*	Banisteriopsis caapi	rumi ayahuasca	T. Plowman (F)	297	UBC only	ayahuasca cultivar
DMK-129	Psychotria viridis	chacruna -'la hembra' form	P. Matakaitis(F)	298	UBC only	admix. to ayahuasca; cultivar
DMK-130	Psychotria viridis	chacruna - 'la hembra' form	P. Matakaitis(F)	299	UBC only	admix. to ayahuasca; cultivar
DMK-131	Psychotria viridis	chacruna - - 'el macho' form	P. Matakaitis(F)	300	UBC only	admix. to ayahuasca; cultivar
DMK-132	Psychotria viridis	chacruna	P. Matakaitis(F)	301	UBC only	admix. to ayahuasca; cultivar
DMK-134	Brunfelsia grandiflora D. Don	chiricsanango - 'grande' form	T. Plowman(F)	302	UBC only	admix. to ayahuasca; cultivar
DMK-135	Brunfelsia grandiflora ssp. schultesii Plowman	chiricsanango -'pequeno' form	T. Plowman(F)	303	UBC only	admix. to ayahuasca; cultivar
DMK-136	Iryanthera lancifolia Ducke	cumala	W. Rodrigues(INPA)	304	INPA	source of large seeds
DMK-137	Iryanthera juruensis	cumala	W. Rodrigues(INPA)	305	INPA	source of small seeds

Table XVII (Cont'd)

Collection # (DMK-)	Binomial & authority	Vernacular Name	Determined by:*	UBC Herbarium Access # V172-	Duplicate Vouchers:	Remarks:
DMK-138*	Spilanthus alba L'Her	-	P. Matakaitis(F)	306	F,USM,MO	leaves chewed for toothache
DMK-139*	Psychotria viridis	chacruna	P. Matakaitis(F)	307	UBC only	admix. to ayahuasca
DMK-142	Tripogandra glandulosa (Seub.)Rohw. var.(vel. sp. aff.)	-	B. Faden(US)	308	F,USM,MO	use uncertain; sold in market places in Peru
DMK-143*	Cyperus digitatus Roxb.	chicorro; chicorro-piri-piri	D. Adams(F)	309	USM	tubers used as ayahuasca admix.; or smoked as hallucinogen
DMK-144	Eleutherine bulbosa (Mill)Urb.	-	T. Plowman(F)	310	UBC only	bulb made into tea to aid in childbirth

* = Name of taxonomist making determination. Herbarium codes are shown in parentheses
† - * indicates additional material was collected for phytochemical analysis
‡ - Herbaria where duplicate vouchers are on deposit. Herbarium codes are according to Index Herbariorum I (1974):
F = Field Museum of Natural History, Chicago, Ill.
UBC = Herbarium of University of British Columbia
ECON = Oakes Ames Economic Herbarium, Harvard Botanical Museum, Harvard University
USM = Herbario San Marcos, Museo de Historia Natural, Lima, Peru
MICH = Herbarium of the University of Michigan, Ann Arbor, Mich.
INPA = Instituto Nacional de Pesquisas do Amazonia, Manaus, Brazil
MO = Missouri Botanical Garden, St. Louis, Mo.
US = U.S. National Herbarium, Smithsonian Institution, Washington, D. C.
UNAP = Herbarium of the Universidad Nacional de Amazonense Peruana, Iquitos, Peru (not listed in Index Herbariorum)
RVH = Herbarium of R.V. Heraclitus, Institute of Ecotechics,Aix-en-Provence, France (not listed in Index Herbariorum)