The University of British Columbia

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FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

of

JOSEPH E. GERVAY

M.Sc., The University of British Columbia

1963

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IN ROOM 261, CHEMISTRY BUILDING

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STUDIES IN NATURAL PRODUCTS

ABSTRACT

Part I. THE BIOSYNTHESIS OF ERYTHRINA ALKALOIDS

Hypotheses for the biogenesis of Erythrina alkaloids are discussed. Di-(β-3, 4-dihydroxyphenyl)-ethylamine, the theoretical precursor predicted by the biogenetic theory, was prepared and ring closure to the erythrinane ring system by oxidative coupling was attempted under various conditions. Consequently, the biogenesis of the Erythrina alkaloids was re-examined and a new proposal is advanced for the biosynthesis of these alkaloids. Synthetic routes to a hypothetical precursor, proposed here for the first time as a potential intermediate, are described.

The biogenetic-type synthesis of the spiro-amine ring system present in the Erythrina alkaloids was achieved by oxidative coupling of the blocked diphenolic precursor, as predicted by the proposed biosynthetic scheme. Oxidation of di-(β-3-hydroxy-4-methoxyphenyl)-ethylamine by alkaline potassium ferricyanide afforded 3, 15-dimethoxy-16-hydroxy-2-oxoerythrina-1 (6), 3-diene in 15% yield. Reduction of the latter by sodium borohydride gave 3, 15-dimethoxy-2, 16-dihydroxyerythrina-1 (6), 3-diene. Acetylation of the dienone yielded 3, 15-dimethoxy-16-acetoxy-2-oxoerythrina-1(6), 3-diene. The total biogenetic-type synthesis of erysodine is therefore but two steps from completion.

The results as a whole confirm the hypothesis that Erythrina alkaloids are produced in Nature by oxidative coupling of diphenols. They also demonstrate the directing role of the protective groups in the phenolic precursor. The evidence allows a biosynthetic pathway for the aromatic Erythrina alkaloids to be considered, and the mechanism for the ring closure process is discussed.

The isotopically labelled precursor 3-hydroxy-4-methoxy-N-(3-hydroxy-4-methoxyphen (1-^{14}C) ethyl) -

phenethylamine was prepared to test the biosynthetic hypothesis in the plant. Feeding experiments are in progress.

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The biogenesis of cholesterol and methods for functionalising inert methyl groups are reviewed, and a new theoretical approach to removal of the 14 - methyl group from lanosterol is described. The removal of this methyl group in vitro could not be achieved, but a series of interesting compounds was obtained. Evidence for the structures of these compounds is presented.

Thus, photosensitized Oxygenation of dihydrolanosteryl acetate in the presence of para-nitrobenzenesulphonyl chloride yielded 3β-acetoxylanosta-7,9(11)-diene, 3β-acetoxylanost-8-ene-7-one and 3β-acetoxylanost-8-ene-7α- hydroperoxide. In addition a compound having an ambiguous structure and designated as IP1 was obtained. The dibromo-derivative of the latter is 3β-acetoxy-7α, 11α-dibromolanostane-8α,9α-epoxide, the structure of which was determined by X-ray crystallographic study. A working structure for compound IP1 based on the physical and chemical evidence is discussed.

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PUBLICATION

A. Rosenthal and J. Gervay: "Direct High Pressure Carbonylation of Aromatic Nitriles with Dicobalt Octacarbonyl" Chemistry and Industry, 1623 (1963).

A. Rosenthal and J. Gervay: "Direct Conversion of Aromatic Nitriles into Phthalimidines and Ureas Using Dicobalt Octacarbonyl"
Canadian Journal of Chemistry, 42 1490 (1964).

STUDIES IN NATURAL PRODUCTS

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Part II. AN ATTEMPTED IN VITRO DEMETHYLATION OF LANOSTEROL

by

JOSEPH EDMUND GERVAY
B.Sc. Honours, University of Montreal, Loyola College, 1961
M.Sc., University of British Columbia, 1963

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

in the Department
of
Chemistry

We accept this thesis as conforming to the
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THE UNIVERSITY OF BRITISH COLUMBIA
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ABSTRACT

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ACKNOWLEDGMENTS

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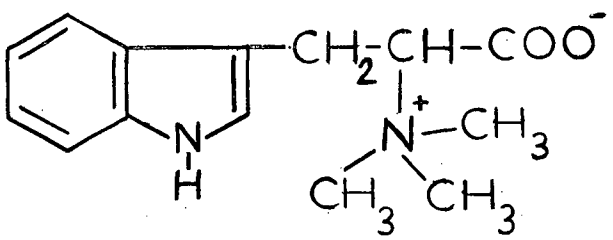
PART I

THE BIOSYNTHESIS OF ERYTHRINA ALKALOIDS

INTRODUCTION

The Erythrina Alkaloids

The alkaloids found in numerous species of the genus Erythrina have attracted general interest both because of their physiological activity, and because they contain a type of structure not previously encountered among the alkaloids. The occurrence of hypaphorine (1), an indole alkaloid,[1] in the species of Erythrina has long been known[2] and the presence of other uncharacterized amorphous alkaloids has been reported repeatedly.[3,4,5,6]



(1)

An intensive search for alkaloids in plants belonging to this genus was started some twenty five years ago following the discovery of the curare-like action of extracts of various species of Erythrina, an action definitely not attributable to hypaphorine.[6,7,8,9,10] The first systematic examination of some fifty-one species of Erythrina showed that all contained alkaloids with paralyzing activity, the potency varying widely.[11,12,13] Physiologically, the Erythrina alkaloids are curarizing agents of high potency, although their activity presents several unusual features. Unlike other known curarizing agents, both synthetic and naturally-occurring, the Erythrina alkaloids are unique in that they are tertiary bases with rela-

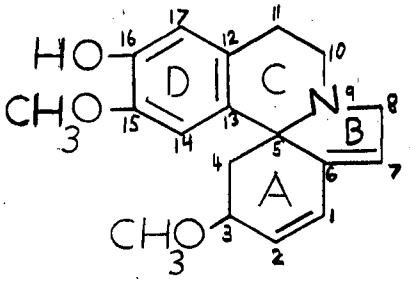
tively high activity. Furthermore, quaternization greatly diminishes the curarizing potency; this also is the only class of compounds in which this is true.[7,14] They are effective when administered orally and have been used clinically with some success.

The pioneering work on the isolation and characterization of alkaloids from various species of Erythrina was done by Folkers and his associates.[11, 12,13] The elucidation of their constitution has been achieved mainly by Prelog, who first suggested the nature of the ring system in the bases, and by Boekelheide.[15,16,17]

By 1960 the isolation and characterization of the members of this family of alkaloids was essentially complete, and excellent reviews of the history and chemistry of the Erythrina alkaloids have been presented in authoritative manner.[1,14]

Besides hypaphorine, which occurs in a number of Erythrina species, the bases found in these plants fall into two groups. The bases of the first group or "free" alkaloids, are named from the prefix "erythr-", and these alkaloids are isolated directly from extracts of the plant without the necessity of previous hydrolysis. The second group or "combined" alkaloids occur in the plant in combination with some other moiety, usually sulphoacetic acid[18,19] or glucose[20] and on hydrolysis yield the "liberated" alkaloids. The stem "eryso-" is used to indicate the "liberated" alkaloids which do not apparently occur as such in the plant but are liberated in the course of isolation by the hydrolytic action of dilute mineral acids. The quantities of liberated alkaloids obtained from the plants generally predominate and often greatly exceed those of the free alkaloidal fractions.

The known naturally-occurring Erythrina alkaloids can be classified depending whether or not the alkaloids, as originally isolated, contain an aromatic ring. There are seven members of the aromatic group, including the erysodine (2) and erythraline (3) types; whereas the second group in which an aromatic ring is not present has only two members, α-erythroidine (4) and β-erythroidine (5). The two groups differ only in that the erythroidines have a lactone ring where the benzenoid ring occurs in the aromatic Erythrina alkaloids. The principal aromatic alkaloids differ only in the nature of the oxygen function attached to the benzenoid ring or in their degree of unsaturation. The numbering of the Erythrina alkaloids follows that devised earlier as a common numbering for all the Erythrina alkaloids.[21]



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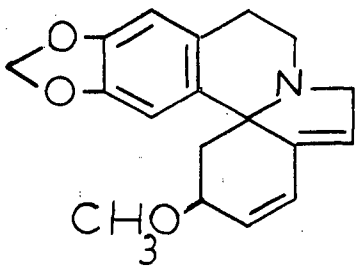

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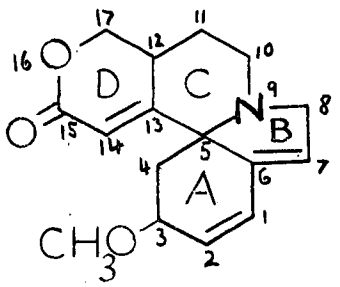

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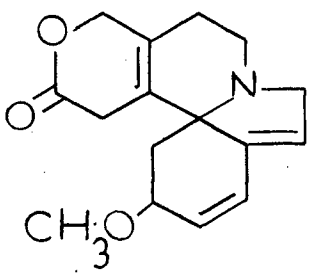
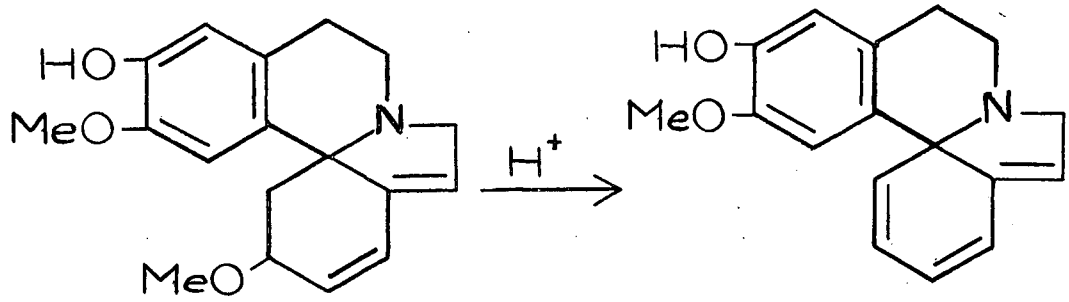


(5)

One interesting feature of structures (2) - (5) is the heteroannular diene system present in rings A and B. All the Erythrina alkaloids readily undergo catalytic reduction to the corresponding di- and tetrahydro derivates.[15,17] Furthermore, when the original alkaloids are treated with acid under mild conditions, loss of the aliphatic methoxyl group occurs as methanol and the diene system becomes lengthened to a conjugated triene system,[15,17,22,23] as shown for the desmethoxy derivative (6) of erysodine (2).

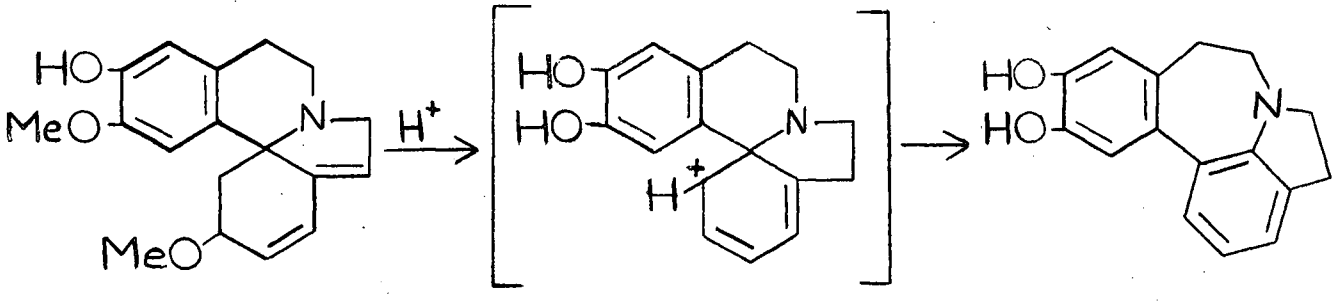


(2) (6)

When the original alkaloids or their desmethoxy derivatives are treated with acid under more severe conditions, i.e. boiling hydrobromic acid or polyphosphoric acid at 125°, a rearrangement with aromatization of ring A occurs.[23,15,17,24] This reaction, known as the "apo-rearrangement", leads to a dihydroindole derivative as shown (8). By analogy with other known carbonium ion rearrangements, it is probable that an intermediate of the type shown by (7) is involved.

In the case of aromatic Erythrina alkaloids, the "apo-rearrangement" is accompanied by cleavage of the aromatic ether linkages in ring D so that the product in each case is apoerysopine (8). Also, since the "apo-

rearrangement" results in destruction of both asymmetric atoms (C-3 and C-5) apoerysopine and apo-β-erythroidine are optically inactive. The conversion of the dihydroindole derivative to a true indole structure has been accomplished in both the aromatic series and with β-erythroidine.[25,26,27,28]



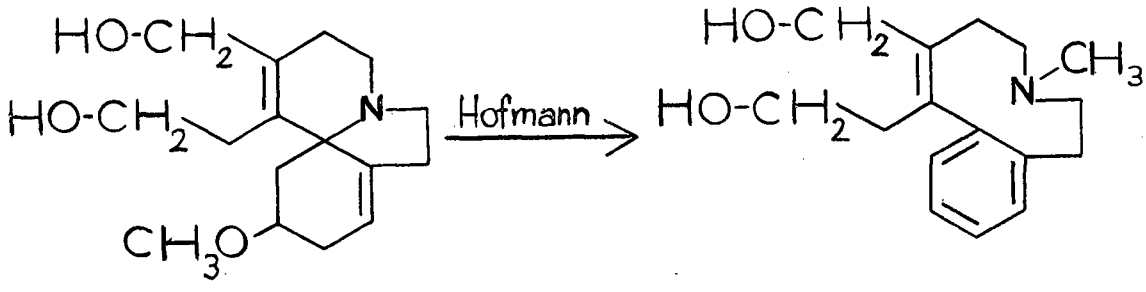
(2) (7) (8)

The Hofmann exhaustive methylation procedure has been used extensively in degrading the Erythrina alkaloids.[14] With less highly hydrogenated derivatives the Hofmann reaction is accompanied by aromatization of ring A and frequently elimination of functional groups may occur as well. This was first observed by Boekelheide and Agnello[29] and later this aromatization was studied extensively in the case of dihydro-β-erythroidinol (9).[17,30] The diol (9), formed by the lithium aluminium hydride reduction of dihydro-β-erythroidine, was subjected to the exhaustive methylation procedure. In this case aromatization of ring A was accompanied by loss of methanol but the diol function remained intact as shown by (10).

A similar aromatization of ring A was demonstrated by Prelog with the von Braun degradation of aromatic Erythrina alkaloids.[31]

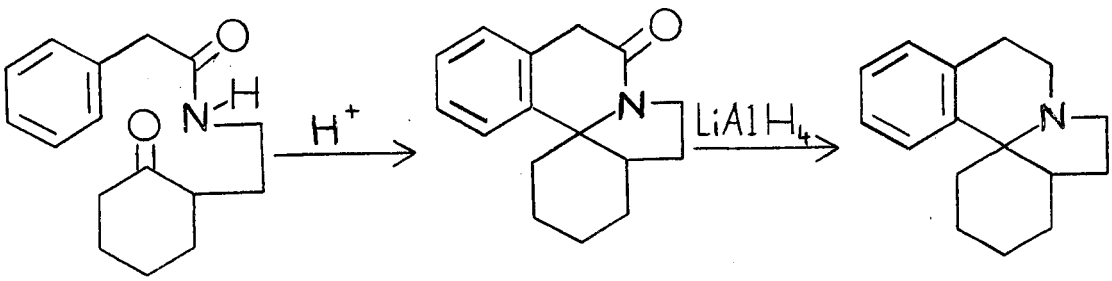
From the degradative evidence it can be seen that the Erythrina alkaloids give two important series of products in which ring A has become aromatized. As shown, the rupture of the carbon to nitrogen bond at

C-5—N-9 gives rise to the ortho disubstituted benzenoid system of structure (10), whereas, the migration of carbon atom 13 from C-5 to C-4 gives rise to the indoline-type structure (8). To explain the origin of these products, the spiro amine structure (2) (5) proposed for these alkaloids appeared to be not only a reasonable one to correlate and explain the degradative evidence, but a necessary requirement.



(9) (10)

A number of methods have been explored for the synthesis of molecules of this type to obtain valuable evidence regarding the proposed structures, since the spiro amine system was first deduced as being common to all of the Erythrina alkaloids.[15,17,21] To obtain a correlation between synthetic and natural material, it was necessary to accomplish the synthesis of a suitable derivative of the Erythrina alkaloids in which the spiro system remains intact. Most of the preliminary work on the synthesis of compounds structurally related to the Erythrina alkaloids is due to Wiesner and his collaborators.[32] The first successful synthesis of a compound containing the desired spiro amine system was that of Belleau.[33] His elegant synthesis is shown below for the aromatic derivative (11) to which the trivial name erythrinane has been given.



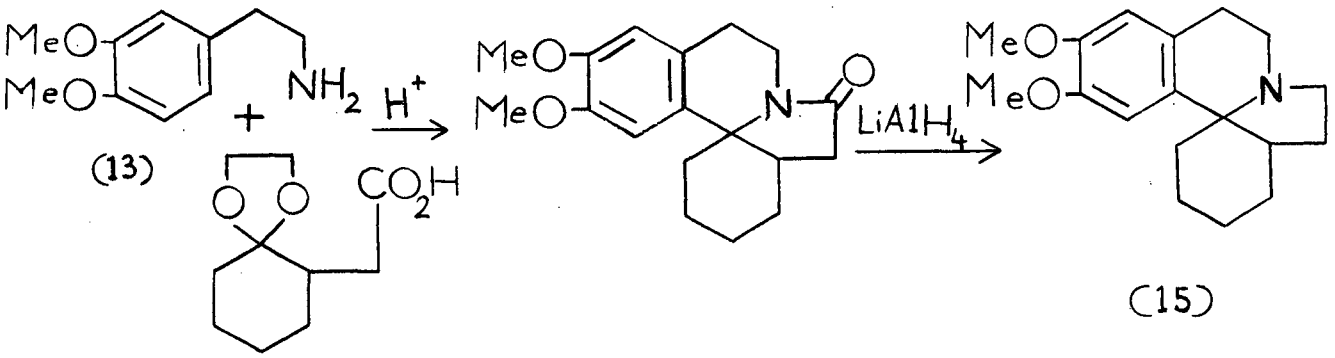
(11)

Belleau[34] repeated the synthesis using the corresponding dimethoxy derivative and obtained racemic 15,16-dimethoxyerythrinane (15). The infrared spectrum of the picrate of this racemic mixture proved to be superimposable on the spectrum of the picrate of natural 15,16-dimethoxy-erythrinane obtained from degradations.[15]

Mondon[35] has demonstrated that cyclization to form the spiro amine system occurs even more readily when the lactam carbonyl is placed in the five membered ring instead of the six. When the ketal acid (12) is warmed with 3,4-dimethoxyphenethylamine (13) in the presence of acid, the condensation-cyclization reaction occurs in excellent yield to give the amide (14). This on reduction with lithium aluminium hydride, gives the same racemate of 15,16-dimethoxyerythrinane (15) obtained previously by Belleau.

The resolution of 15,16-dimethoxyerythrinane (15) was accomplished independently by Belleau[36] and Boekelheide.[37] The levorotatory enantiomorph was shown to be identical with the natural material. Similarly, the identity of synthetic and natural derivatives established the presence of the spiro amine system in β-erythroidine (5).[38] α-Erythroidine differs from

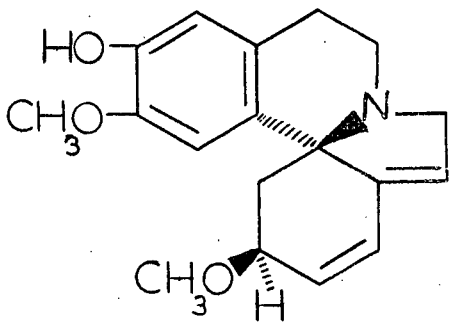
β-erythroidine in having the aliphatic double bond linking the 13-14 rather than the 12-13 positions, but, since α-erythroidine has been converted to β-erythroidine the synthesis conclusively established the spiro amine structure postulated for α-erythroidine and its derivatives.[39] Thus, the fact that the synthetic and natural isomers have the same mode of ring fusion is beyond question.



In the past few years important advances have been made in elaborating the chemistry of the erythrina alkaloids. Other routes leading to the spiro amine system have been devised.[38,46,47,48,75] Mondon[40,41,42,43,44,49] has extended his previous synthetic studies and he also reported[45] a new rearrangement of the erythrinane ring skeleton.

The important question of stereochemistry of these alkaloids have been settled quite recently. The aromatic Erythrina alkaloids with the exception of erythratine, have been interrelated and shown to have the same configuration of the spiro carbon atom, C-5.[14] From an X-ray crystallographic study of erythraline hydrobromide[50] and from chemical studies of erysodine and the proof of its structure through synthesis,[47] the relative configurations at C-3 and C-5 are known for the aromatic erythrina alkaloids.

The methoxyl and spiro amine groups have a "cis" relationship as shown (16).



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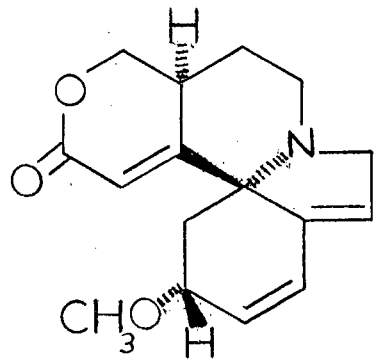


(17)

The conversion of α-erythroidine to β-erythroidine established that both have the same configuration at C-3 and C-5.[39] Degradations[51,52] and X-ray study[53] allowed the complete assignment of relative and absolute configurations of the erythroidines as shown for (3R,5S,12S)-α-erythroidine (17).

Boekelheide[54] has shown, by the method of molecular rotation differences and by optical rotatory dispersion measurements, that the aromatic erythrina alkaloids and the erythroidines have the same configuration at the spiro carbon atom C-5. In view of this evidence, and of the determination of absolute configuration of erythroidines, the absolute configuration of the aromatic series can be given (16).[51]

The Biosynthesis of Alkaloids.

The structures of some 1700 naturally occurring alkaloids are known at present, and the processes by which alkaloids are synthesized in plants

have long been the subject of study and speculation among organic chemists and biochemists.[55,56,57,58] A proper understanding of the pathways demands a knowledge of the substances which are involved as intermediates and also of the mechanisms by which the various transformations are carried out. In the last ten years, this complex of problems, which previously was only the object of speculations, has been attacked successfully on a broad front.[59, 60,61,62,63] The biogenetic studies with alkaloidal compounds are now at the very interesting stage of development where hypothesis and experiment can be combined, and at the moment the main interest lies in the clarification of the major formal relationships between alkaloids and their precursors. The difficult problems of the details of the reaction mechanisms now seem to rest in many cases on a secure theoretical basis.[64]

No simple synthesis has yet been devised for the alkaloids, and the multistep syntheses described in the last half century seem unsatisfactory when compared to the paths used by nature. This problem has intrigued many workers, and as early as 1917 Sir Robert Robinson[65] had devised and executed the famous sythesis of tropinone, patterned along lines considered also to represent reasonable biosynthetic routes. He put forward many important ideas on alkaloid biosynthesis,[58] and his ideas have been invaluable in guiding experimental work on living plants.

The term "biogenetic-type"[59] has been selected to describe an organic synthesis designed to follow, in at least major aspects, biosynthetic pathways proved or presumed, to be used in the natural construction of the end products. It implies that the relationship of the laboratory synthesis to the biosynthesis is not necessarily very close and that the in vitro route may be based on an in vivo scheme which is reasonable yet only

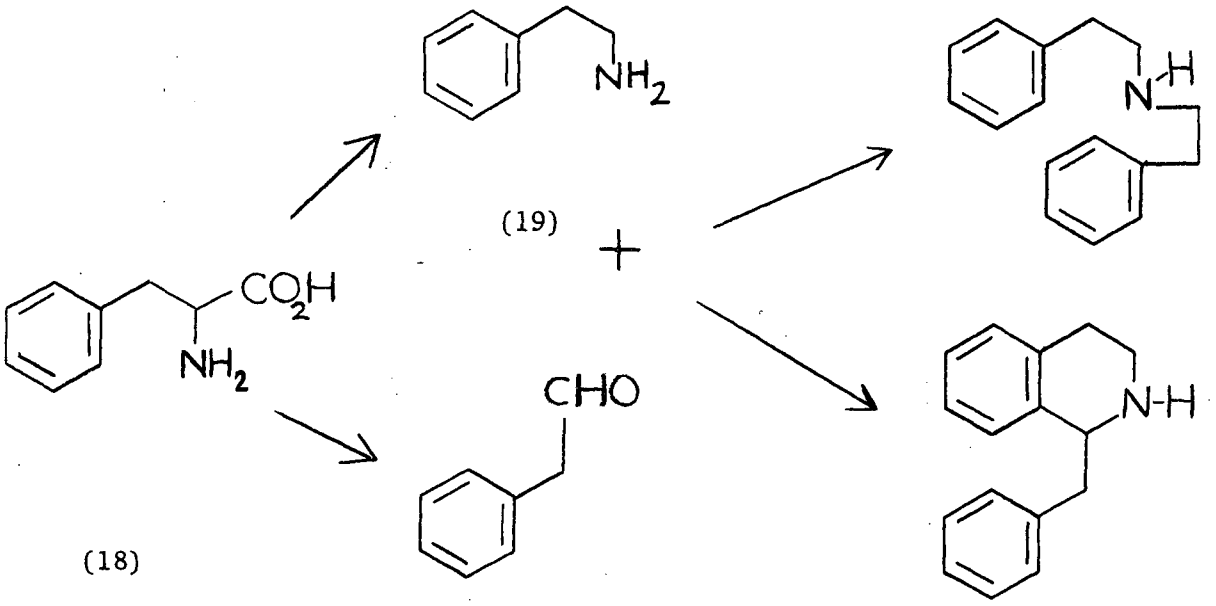
speculative, or for which only meager evidence may be available. The term is meant to refer to presumed intermediates and biosynthetic paths, and little emphasis is placed on reagents and conditions. In the laboratory duplication of the key biological step, any conditions or reagents may be used which are necessary for the completion of the reaction. The striking success of certain "biogenetic-type" syntheses may depend upon utilization of reaction types which parallel enzyme-promoted processes, and in lieu of the enzyme system, the organic chemist may need to resort to reagents and conditions not available to the living system, in order to follow the overall biosynthetic route. The key intermediate may possess the exact structure proposed in the biosynthetic scheme or it may be a simple modification, to direct the intermediate along desired channels and preclude other reaction courses. Biogenetic-type synetheses often are neater, shorter, and more efficient than normal routes in which no attention is paid to natural processes. Sometimes, it is found that the only satisfactory route to some natural product is the biogenetic type. However, the success of a "biogenetic-type" synthesis by itself does not constitute evidence for the operation of a particular chemical step in nature, and the temptation is great in many cases to draw such a conclusion.

Real progress in the study of alkaloid biosynthesis began when organic compounds labelled with carbon-14 and with other isotopes became readily available in the early nineteen-fifties. Since then, many groups of workers have tackled problems in this area and the information is accumulating rapidly.[63,64] Isotopes have proved particularly important in clarifying the mutual relationships among the various alkaloids found in any given plant at the same time. Many biosynthetic processes of fundamental importance can be studied by tracer methods. Examples of

such processes are condensation, ring closure, methylation and demethylation, several different oxidation reactions, dehydrogenation, and so on. By means of this new experimental technique, it was possible to establish the biogenetic relationships between certain "precursors" and the alkaloids within relatively short periods.[60]

There have been a considerable number of postulated biogenetic routes, and looking back at these speculations one can see two main thought processes, which are often closely allied. One approach has been the comparative anatomy method, involving inspection of the formulae of alkaloids to seek common structural units within a group of alkaloids, and suggesting possible relationship of these units to simpler natural products. Such deductions are particularly useful for correlating different groups of alkaloids and for predicting the structures of new alkaloids. The simple units from which the alkaloids may arise are the amino acids resulting from the decomposition of proteins. The recognition of the extremely close relationship between alkaloids, simple plant bases, and basic amino acids led to important information concerning the synthesis of alkaloids. The structural examination based hypothesis can be supported by tools such as tracers or the isolation of enzymes which catalyse the successive steps of the biosynthesis, since both direct and indirect approaches to biogenesis complement each other. The other approach has been to correlate alkaloidal structures on the basis of a unifying reaction mechanism. The amino acid phenylalanine (18) can, by decarboxylation, give the amine (19) and, by oxidation, the aldehyde (20). In this connection Robinson[58] recognized that if condensation of β-substituted ethylamines (19) with aldehydes (20) could occur in plants, then one could account for a wide variety of alkaloidal structures, as this reaction is particularly important for the

synthesis of N-heterocyclic systems.

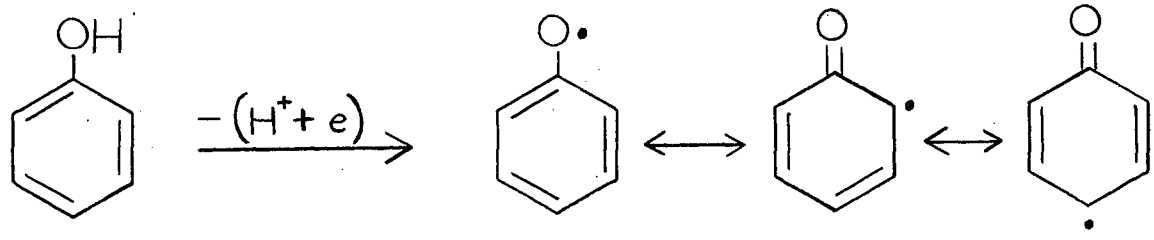


Other synthetic reactions which can be considered to be most important as keys to the synthesis of alkaloids are: the aldol condensation between aldehydes and β-keto acids and the similar condensation of carbinolamines, -C(OH)-N- with the active methylene groups ($-CH_2-C=O$) of ketones or β-keto acids.[66,61]

The biosynthetic significance of phenol oxidations has long been recognized, and the role of oxidative condensations in the biosynthesis of alkaloids have been discussed in great detail in excellent reviews.[64,67,68,69] Of the known alkaloids more than 10% can be derived, in principle, by coupling of appropriate phenolic precursors. The interpretative mechanistic approach to biosynthesis, of correlating the structures of natural products in terms of oxidative coupling of phenolic precursors in biogenetic pathways, is now classified as Biogenetic Analysis.[64] It

includes in its operation those feeding experiments with radiochemically labelled substrates which can be used to evaluate such an analysis.

The oxidation of phenols or of phenol anions by one-electron transfer oxidizing agents affords mesomeric phenol radicals as shown (21).



(21)

These are stable radicals, relative to alkyl radicals, because of the spread of the odd electron by resonance over the ortho and para positions of the aromatic ring. The free radicals have been detected by magnetic susceptibility and electron spin resonance measurements,[64] and detailed studies have shown that the free electron density is greater at the para- than at the ortho-position. The experiments are in full accord with the view that the first step in the oxidation of a monohydric phenol, by a one electron transfer oxidant is the generation of the phenoxyl radical. Once phenol radicals have been generated they may be converted to stable molecular products depending on the substitution pattern by several processes. Reduction gives back the parent phenol, coupling with reactive molecules, for example oxygen and halogens afford non-radical products, self coupling furnishes dimers. Dimers can be formed by carbon-carbon, carbon-oxygen or oxygen-oxygen coupling. Simple dimerization of the radicals gives rise to diphenyls or diphenyl ethers by ortho- or para-coupling. (See Figure 1).

The carbon-carbon coupling is the most important and it can be ortho-ortho, ortho-para or para-para.

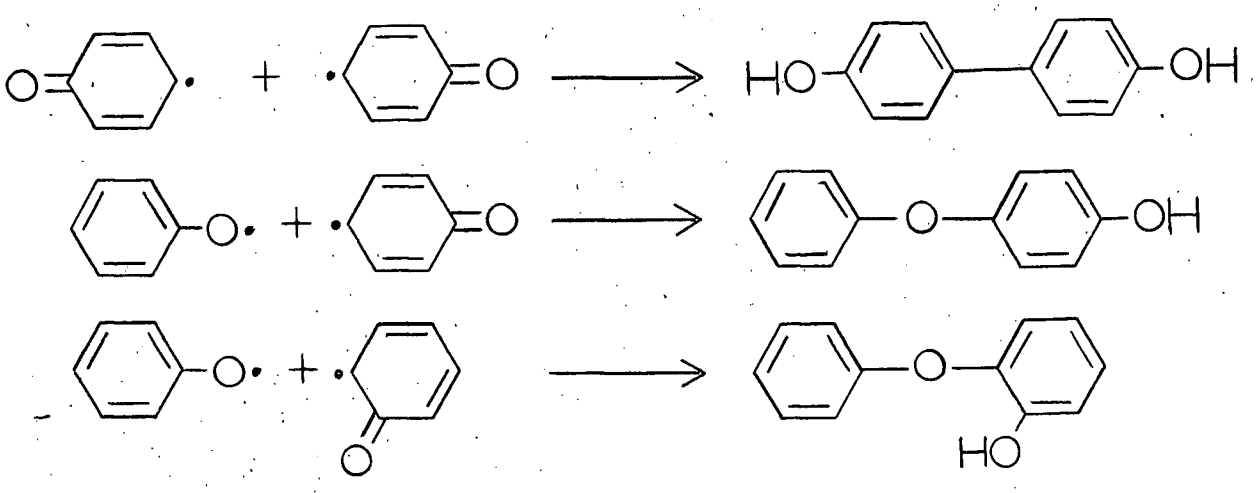


Figure 1. Simple dimerization of phenol radicals.

If oxidative dimerization of the molecule is assumed, one must distinguish, in principle, between the radical coupling process (homolytic coupling) and the substitution of a phenol radical (radical insertion) into a molecule of phenol followed by further oxidation. Both mechanisms predictortho-para type substitution. Although radical substitution into a phenol anion cannot be disregarded, the intervention of radical insertion processes in such phenol oxidations seems unlikely, and the radical coupling is favoured and accepted without further qualification.[68] Evidence is also lacking for the intrusion of cationic species (2-electron oxidation) in these oxidations.[64]

Phenols in which one or preferably two reactive positions are blocked by suitable substituents, e.g. methoxyl, methyl or acetyl groups, give good yields of diphenyl derivatives on oxidation. It is clear that 2,4,6-tri-substituted phenols not having α-CH in the substituents produce stable

phenol radicals, which are readily detected not only by their chemical reactivity but also by their paramagnetism. This is because phenol coupling is prevented by steric reasons, and the absence of α-hydrogen in the substituents prohibits the formation of a methylenequinone.[67] Amino phenols and amines also couple via mesomeric radicals.[64]

Successful coupling reactions illustrating the formation of C-C, C-O and C-N bonds have been carried out in the laboratory, however, the selection of reagent and the experimental conditions for a given substrate are largely empirical. A great variety of electron acceptors have been employed to effect the coupling of phenols e.g. ferricyanides, ferric chloride, hydrogen peroxide, manganese dioxide, quinones or enzyme preparations. The yields of the isolable products of the reaction vary within wide limits and are greatly dependent upon the structure of the phenol, the stability or solubility of the reaction products and the reaction conditions (pH). As a rule large amounts of amorphous by-products, generally ill defined, are simultaneously formed due to hydroxylations, ring fission or polymerization. In the living cell phenol oxidations occur in a highly organized surrounding and it is probable that the reacting phenol molecules become suitably oriented so as to make a directed coupling possible, thus minimizing the formation of by-products. This is very difficult to imitate in the laboratory, and it is customary to work in high dilution to prevent polymerization.

If one assumes that radicals such as (21) disappear by coupling in pairs to furnish molecular products, then certain restrictions are imposed on the mode of coupling. If such radicals are also involved in biogenetic pathways, then by blocking reactive positions interesting restrictions are imposed on the precursors and products in the biogenetic sequence. It was

first recognized by Barton and Cohen,[67] that completely rigorous application of the principle of ortho- and para-C-C and C-O coupling accounts for the structural features found in many classes of alkaloids. They also postulated that the coupling step is the result of phenol oxidation. Numerous papers have appeared since concerning the use of oxidative procedures on phenolic and amino compounds and a variety of biosynthetic successes have been obtained.[63,68,64]

Amongst the many types of compounds which can, at least formally, be derived by the coupling of phenoxide radicals the alkaloid galanthamine (25) present in the Amaryllidaceae provides an outstanding example. According to biogenetic theory the three main classes of Amaryllidaceae alkaloids are all derived from a precursor (22) now known to be the alkaloid norbelladine, having two phenolic rings with the phenolic hydroxyl groups in such positions as to direct ortho- and/or para-coupling between the rings.[67] The validity of this scheme has been demonstrated by independent researches in three laboratories.[63,68]

Galanthamine was regarded as biosynthesised from norbelladine ON-dimethyl ether (24) by oxidation to the dienone (26), ring closure to the enone (27), and reduction of the latter to the allylic alcohol galanthamine (25) (see Figure 2). At the time when this scheme was put forward the alkaloid narwedine (27) had not been characterized, and the formula for galanthamine was uncertain. The correct formula (25) was, in fact, chosen on the basis of the proposed biogenesis. Biogenetic analysis has shown that not only was the correct formula (25) chosen for galanthamine but it was proved that labelled norbelladine ON-dimethyl ether (24) and norbelladine (22) was incorporated into galanthamine.[71] In earlier

experiments, incorporation of [2-^{14}C] tyrosine (23) into galanthamine (25) was observed. The constitution of galanthamine has been placed beyond question by a total synthesis from norbelladine ON-dimethyl ether (24) based on the biogenetic scheme.[70] An important result which bears on the phenol coupling theory for these alkaloids is the evidence obtained for the presence of phenolic norbelladine derivatives in Amaryllidaceae plants.[63]

The aporphine alkaloid isothebaine (29) has an unusual oxygenation pattern. It was suggested[67] that bases of this type are biosynthesized from the 1-benzyltetrahydroisoquinoline (28) by phenol oxidation to give the dienone (30) followed by reduction to the dienol (31) (see Figure 3). Migration of a bond by dienol benzene rearrangement leads directly to isothebaine (29).[63] The first synthesis of isothebaine by this sequence has been achieved,[72] and tracer studies carried out with oriental poppies showed that orientaline (28) is incorporated by plants into isothebaine.[73] The influence of selective protection of phenolic function is nicely illustrated in this case, and it seems probable that methylation controls the direction of oxidative coupling in the biosynthesis of alkaloids.

An extensive investigation[74] of benzyltetrahydroisoquinolines has shown, that, provided they contain quaternary nitrogen, simple phenolic bases can undergo oxidative condensation under conditions similar to those of biogenesis to form alkaloids of the isoquinoline series with good yields. By means of such oxidative condensation, more than sixty alkaloids of various structural types have become more easily obtainable. Of the numerous possible condensations of the intermediate mesomeric radicals only those which lead to naturally occuring alkaloids give good yields and few by-products. These results suggest that oxidative condensations of

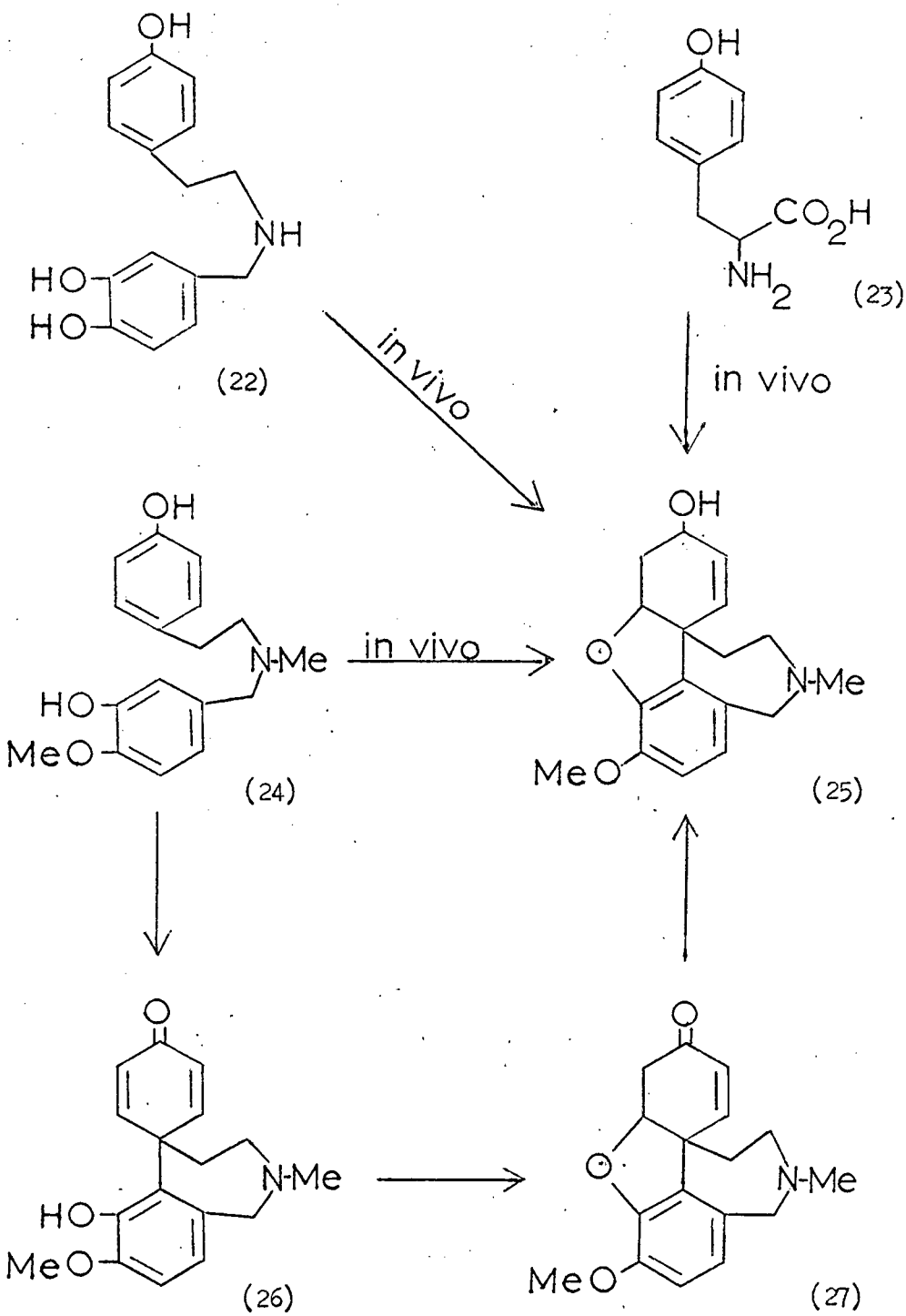


Figure 2. The Biosynthesis of Galanthamine (25).

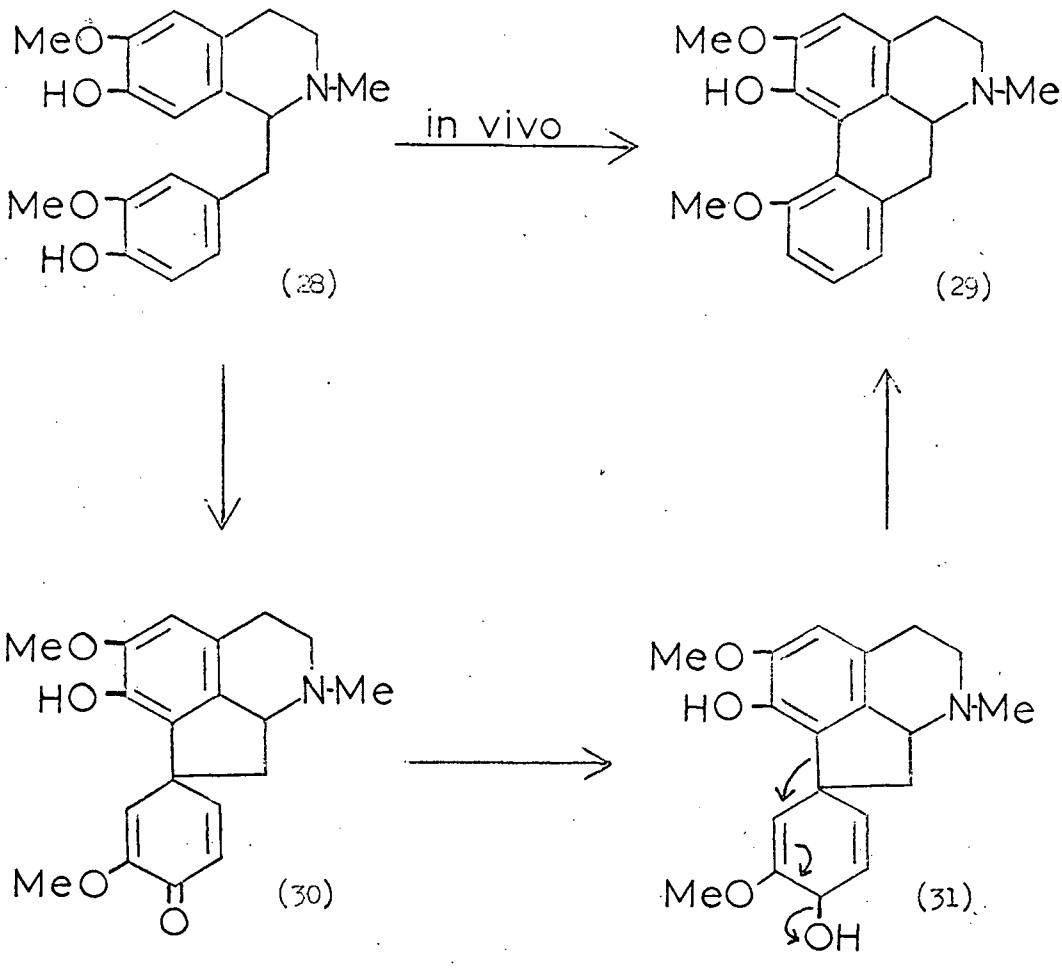


Figure 3. The Biosynthesis of Isothebaine (29).

quaternary bases may also be involved in the biosynthesis of alkaloids in the plant cell.

There have been a number of speculations on the biogenesis of the Erythrina alkaloids,[14] however, no experimental evidence has been reported so far with which to evaluate these proposals.

The greater the importance of the complex natural products which can be isolated and structurally identified in modern times, the more important does it become to learn to synthesize them as simply and as rapidly as in the cell. It is hoped that, by imitating such biosynthetic methods the increasing demand for physiologically active biological products can be met more efficiently than by the time-consuming extractions from the plant cell.

DISCUSSION

The object of this investigation was to obtain fundamental information concerning the biosynthesis of Erythrina alkaloids, and to achieve a model synthesis of the characteristic spiro amine structure along the lines of a proposed biogenetic scheme. The problem arose as a direct consequence of much speculation[14,58,67,76,77,78] concerning the formation of these alkaloids in the plants, since the biogenesis of the Erythrina alkaloids had not been previously explored.

The biogenesis of Erythrina alkaloids cannot readily be related to any of the schemes proposed for other alkaloids. It appeared that a new approach is required to explain the formation of the spiro amine system, and it might be expected to arise through a new type of variation in biogenesis. A study of the molecular structures of the Erythrina alkaloids, leads to certain firm convictions as to the sort of precursors and processes involved in their biosynthesis. The modes of possible biogenesis are set out as connected series of reactions, however, a definite order for the various stages is not assumed.

According to biogenetic theory[58] the spiro amine system is derived from a precursor (33), having two phenolic rings with phenolic hydroxyl groups at 3,4-positions, synthesised in Nature from two C_6-C_2 units (Ar-C-C). The condensation presumably proceeds by a mechanism whereby one amino acid becomes reactive by decarboxylation to an amine and the other by oxidative deamination to an aldehyde or its equivalent. One satisfactory scheme[14] (see Figure 4) envisions the building blocks to be two molecules of 3,4-dihydroxyphenylalanine (32), their union to give the precursor (33) and its subsequent oxidation to the orthoquinone (35). It

is not known at what stage decarboxylation occurs. The ring closure to the dihydroindole (34) and subsequently to the intermediate (37) is a plausible one. Similar ring closures occur when 3,4-dihydroxyphenethylamines and and 3,4-dihydroxyphenylalanine itself is subjected to mild oxidation.[79,80] Methylation to give erysopine (38) may occur at some later stage. The outstanding feature of this scheme is that it represents an overlap of the two great biogenetic pathways for forming indole and isoquinoline alkaloids, and it explains the formation of the spiro amine system in a very simple way. According to this scheme erysopine (38) appears to be the key intermediate for the elaboration of the various aromatic alkaloids as well as for the erythroidines (4) (5). The non-aromatic alkaloids of this family may be derived by Woodward fission[17] of the dihydroxybenzenoid ring of erysopine (38), followed by appropriate modifications of the side-chains and ultimate lactonization (5). Since these further alterations do not affect the stereochemistry at C-3 and C-5 it is possible that erysopine (38) is formed first in the plant and then converted enzymatically to other alkaloids, including β-erythroidine (5), the extent to which this could occur would depend on the enzyme systems possessed by a particular plant species. It seems to be quite possible that the starting material for the biogenesis of the Erythrina alkaloids is 3,4-dihydroxyphenylalanine (32), since an appropriate union of two molecules of this amino acid can lead directly to a structure (38) representative of the "aromatic" alkaloids.

The role that the coupling of phenoxide radicals can play in explaining the biosynthesis of natural products is by now well appreciated.[64] The formation of all Erythrina alkaloids can be accommodated by this elegant and simple biogenetic hypothesis,[67] that the carbon skeletons

are produced by oxidative phenolic coupling of a precursor of the type (33). The aromatic Erythrina alkaloids are plausibly derived by oxidative coupling of this intermediate (see Figure 5). Oxidation of the base (33), by some one-electron transfer system to generate radicals which, by coupling, would yield the diphenoquinone (39). Addition of the amino group to the quinonoid system in (39) leads to the dienone (37) and then subsequent unexceptional steps yield the aromatic Erythrina alkaloids (40) (38). A variation in this scheme is the carbon-nitrogen coupling of the precursor (33) to give the hydroindole (34), further para-para carbon-carbon coupling will furnish the same dienone (37) which was obtained from the other route. The non-aromatic members of this family can be formulated by oxidative fission of the catechol ring as suggested above. Such processes as dehydration, hydrogenation and dehydrogenation may occur at any stage after the spiro amine system was formed. O-methylation can occur at some previous or subsequent stage, as it is not known yet whether the methylation pattern is built in from the basic precursors or at the diphenol (33) level. However, since this hypothetical precursor possesses the specific hydroxylation pattern from which all the known Erythrina alkaloids can be derived, it is strongly believed that methylation occurs at this stage in the biosynthesis. This hypothesis is firmly supported by the now well known "directing" role of the protective groups in both the synthesis and biosynthesis of alkaloids.[64, 70, 73, 97] The difference in the methylation pattern of the phenolic rings demonstrates that biosynthesis is directed to different final skeletons, at least in part, by O-methylation.

With the biogenetic theory[58,67] as a background in mind the programme of research was initiated, first to examine the in vitro synthesis of the erythrinane ring system, followed by tracer experiments to determine in vivo

in the plant how near to the truth the assumptions may be. Consequently, in order to prepare the base precursor (33) predicted by the biogenetic theory, it was first necessary to develop a synthetic pathway to this proposed key intermediate for the in vitro synthesis (see Figure 6).

3,4-Dimethoxyphenylacetic acid (42) was treated with thionyl chloride and the resultant acid chloride was condensed with 3,4-dimethoxyphenethylamine (13) to give homoveratroyl-homoveratrylamine (43). The amide was reduced with lithium aluminium hydride in ether to di-(β-3, 4-dimethoxyphenyl)-ethylamine (45). This diamine was also prepared on a larger scale in one step by the catalytic reduction of 3,4-dimethoxyphenylacetonitrile (44).[81] Demethylation of this product in refluxing concentrated hydrobromic acid then yielded di-(β-3,4-dihydroxyphenyl)-ethylamine hydrobromide (33) identified by chemical analysis. Its infrared 3400 cm^{-1} (O-H) and ultraviolet spectrum λ_{max} 284 mμ (ε 7770) were in agreement with the assigned structure. This hypothetical precursor (33) of the Erythrina alkaloids was then oxidized with alkaline potassium ferricyanide under various conditions. In 24 oxidation experiments it was not possible to isolate any products or to obtain reproducible results. The major product in all cases was an intractable chloroform-insoluble polymer. Even in very dilute solution extensive polymerization was observed. One crude product of minute amount had absorptions at 3450 (O-H) and 1720 cm^{-1} (C=O) in the infrared. The ultraviolet spectrum showed λ_{max} at 253 mμ and a shoulder at 284 mμ. Similar results were obtained when ferric chloride was used as oxidizing agent. It was clear from these experiments that polymerization of the tetrahydroxy-precursor cannot be avoided under laboratory conditions and the proposed dienone (37), if formed at all, could not be present in

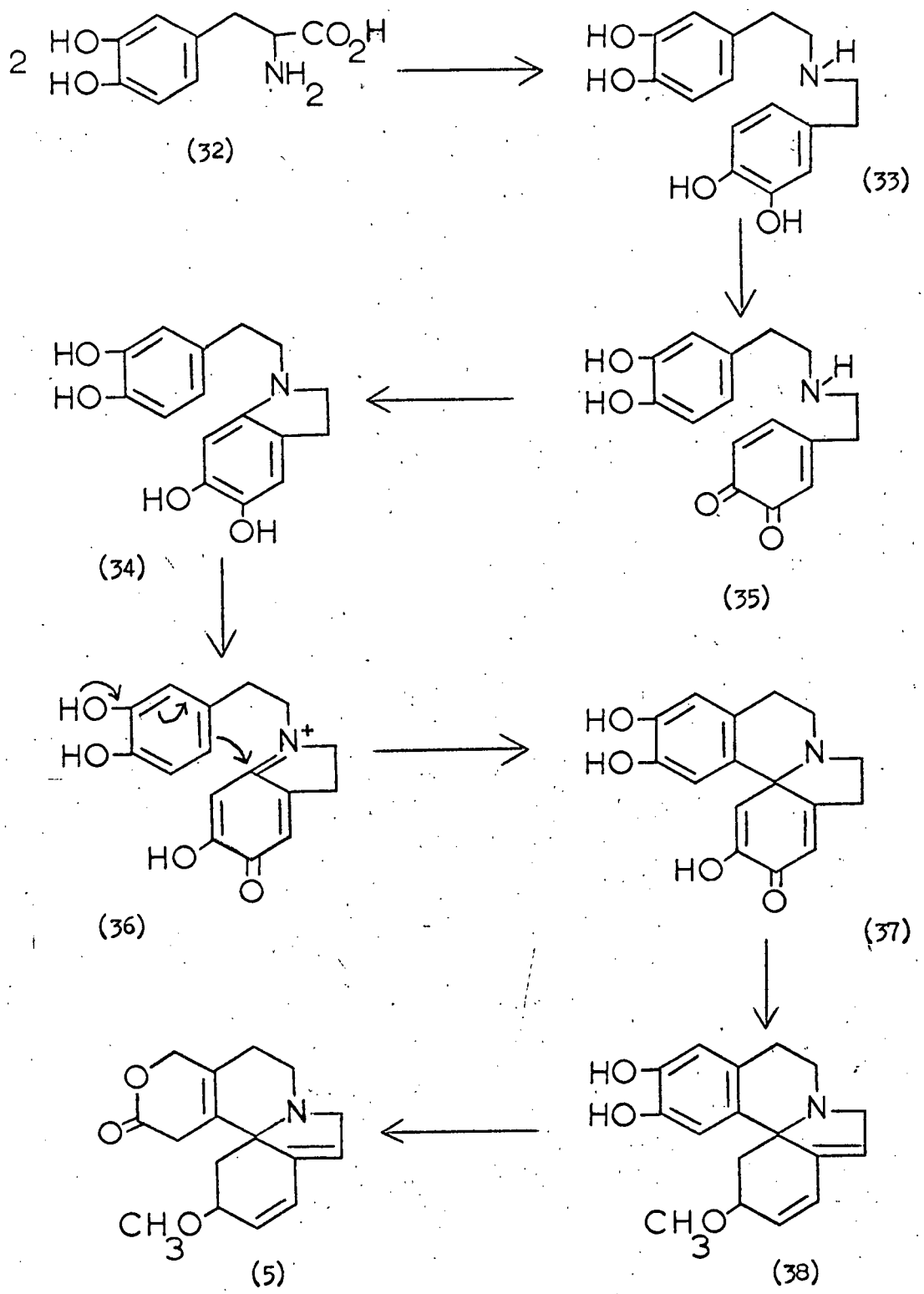


Figure 4. Hypothetical Biogenesis of the Erythrina alkaloids.

yields much greater than 1%. Many combinations are possible in the condensation, since each phenolic hydroxyl group may produce two different radical positions in this manner.

In view of the above results the biogenesis of the Erythrina alkaloids was re-examined, and a new hypothesis was put forward. It was reasonable to suggest that blocking by methylation of the 4-hydroxyl group in both 3,4-dihydroxybenzenoid rings of the diphenol (33) should reduce the number of reactive sites (both ortho and/or para) liable to oxidative coupling, and direct the reaction along the proposed route to the dienone (37) which is, of course, the desired initial product of phenol radical coupling. It seemed quite probable that oxidation of the modified diphenol (58), proposed here for the first time as a potential precursor of the spiro amine ring system, would prove more successful. In this hypothetical precursor (58), where the subsequent coupling reaction is directed only by the free phenolic groups, the phenoxide radicals have a better opportunity to couple intramolecularly than before, for the very same reasons which were discussed earlier in other similar ortho-methoxyphenol systems. To test this new proposal in vitro, the chemical synthesis started from isovanillin (46), and the accomplished plan for this new synthetic approach is outlined in Figures 7,8, and 9.

Isovanillin (46) was benzylated with benzyl chloride, and its condensation with hippuric acid gave the oxazolone (49), the alkaline hydrolysis of which yielded a mixture of 3-benzyloxy-4-methoxyphenylpyruvic (52) and benzoic acid. These acids were separated after treatment of the mixture with alkaline hydrogen peroxide, resulting in 3-benzyloxy-4-methoxyphenylacetic acid (55) separated by column chromatography.[85] This acid was prepared in much better yields by the alkaline hydrolysis of 3-benzyloxy-4-

methoxyphenylacetonitrile (54).

A considerable quantity of 3-benzyloxy-4-methoxy-β-nitrostyrene (50) was desired for the preparation of 3-benzyloxy-4-methoxyphenethylamine (53). Unsuccessful attempts were made to repeat an earlier procedure[82] for the condensation of O-benzylisovanillin (47) with nitromethane using methylamine as the condensing agent. The experiment was repeated six times but the product described was never obtained. Instead an unknown brown amorphous substance, melting above 200° was obtained and this compound might be the polymer of the desired product. The nitrostyrene (50) was finally prepared by the method of Lange and Hambourger[38] using aqueous sodium hydroxide instead of methylamine and carrying out the condensation reaction at 10°. The nitrostyrene was then reduced with lithium aluminium hydride[84] in tetrahydrofuran to yield 3-benzyloxy-4-methoxyphenethylamine (53). This amine was also prepared by the reduction of 3-benzyloxy-4-methoxyphenylacetonitrile (54) with lithium aluminium hydride in ether.

The phenylacetonitrile (54), which turned out to be an important intermediate in the laboratory synthesis of the desired phenolic diamine precursor (58), was prepared as follows. O-benzylisovanillin (47) from isovanillin (46) was reduced with sodium borohydride to the corresponding alcohol (48) which, with thionyl chloride yielded 3-benzyloxy-4-methoxybenzyl chloride (51). This underwent exchange with potassium cyanide in dimethyl sulphoxide to yield 3-benzyloxy-4-methoxyphenylacetonitrile (54).[86]

The acid chloride of 3-benzyloxy-4-methoxyphenylacetic acid (55), freshly prepared, was treated at once with 3-benzyloxy-4-methoxyphenethylamine (53), yielding 3-benzyloxy-N-(3-benzyloxy-4-methoxyphenethyl)-4-methoxyphenylacetamide (56). The amide was best reduced by borane in tetrahydrofuran to give di-(β-3-benzyloxy-4-methoxyphenyl)-ethylamine (57) in good yields, identified as the hydrochloride. This new compound analysed

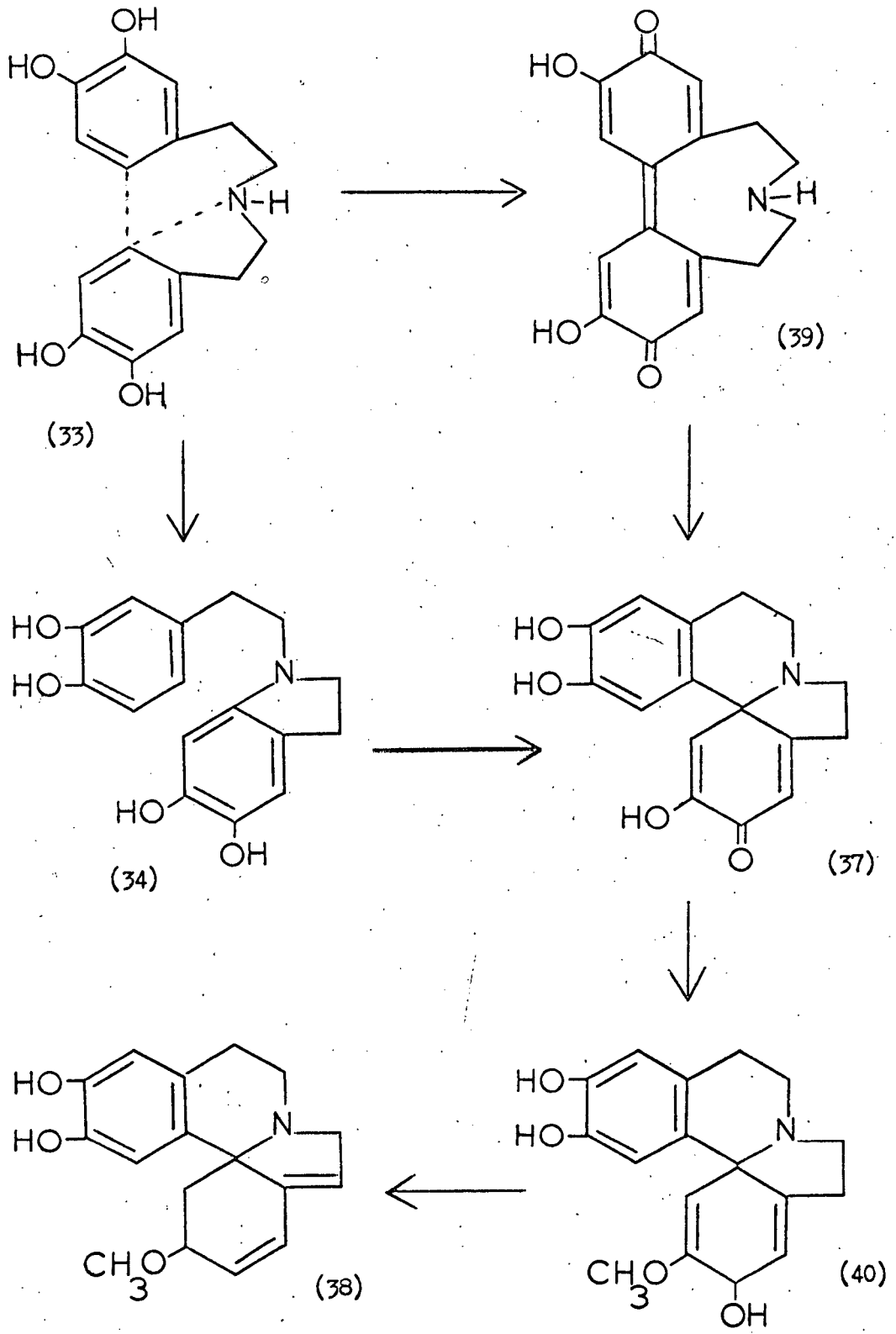


Figure 5. Hypothetical biogenesis of the Erythrina alkaloids via oxidative coupling of phenols.

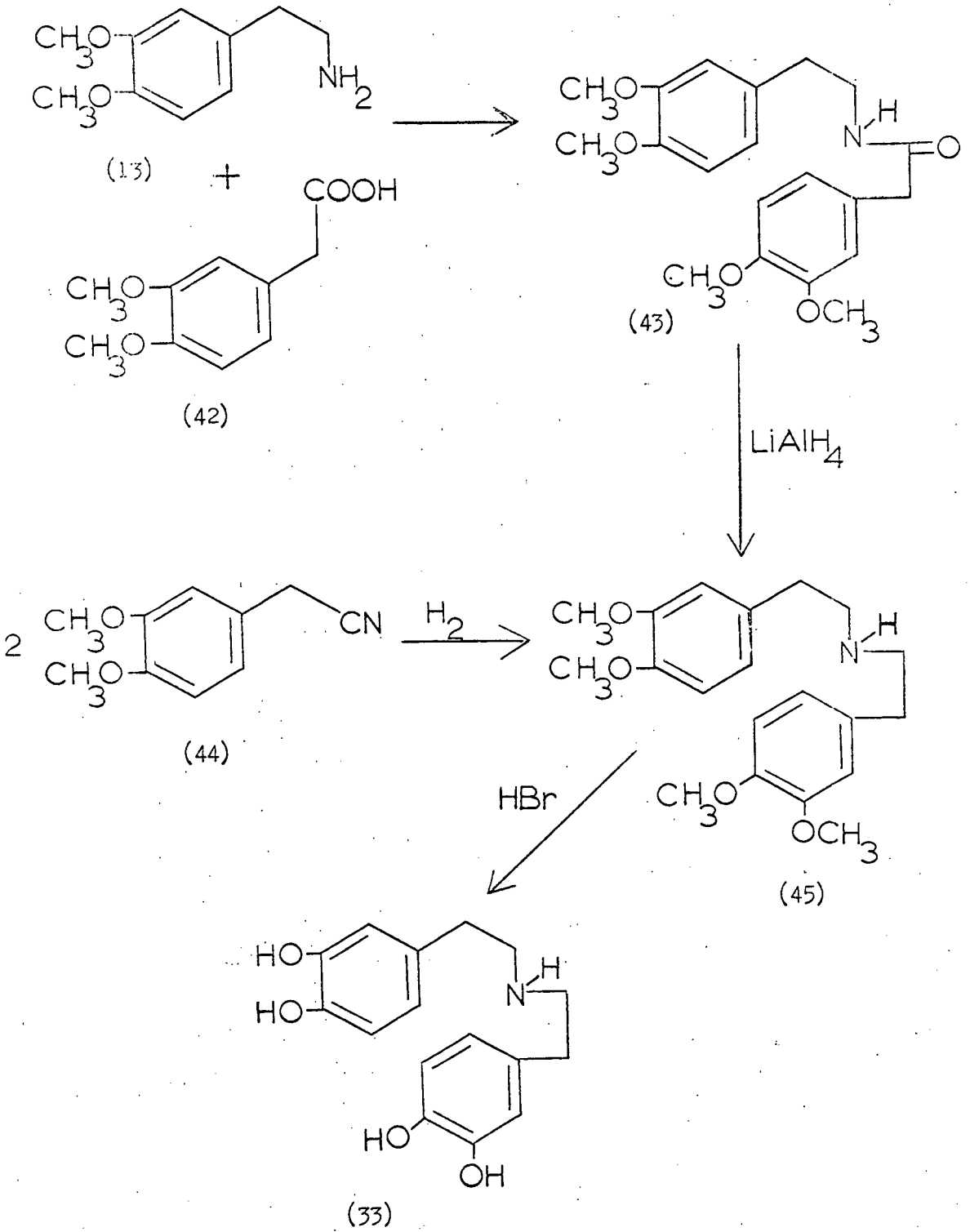


Figure 6. Reaction sequence, leading to di-(β -3,4-dihydroxyphenyl)-ethylamine (33).

correctly for $C_{32}H_{36}O_4NCl$. Its n.m.r. spectrum in deuterochloroform showed the expected aromatic resonance as a multiplet at τ 2.65 and a singlet at τ 3.24, a singlet for the methylene (-O-CH_2-) at τ 4.94 and for the methyls (-O-CH_3) at τ 6.2, and a broad singlet centered at τ 6.88 for the protons of the phenethylamine side chain.

The benzyl-groups of the amine (57) were removed by hydrogenolysis in methanol containing concentrated hydrochloric acid over 10% palladised charcoal; to give the desired di-(β-3-hydroxy-4-methoxyphenyl)-ethylamine (58). The fact that the right precursor was at hand was shown by its subsequent synthesis in one step from 3-benzyloxy-4-methoxyphenylacetonitrile (54) by catalytic reduction. Microelemental analysis was in agreement with the proposed structure, and its infrared spectrum in Nujol showed a strong absorption at 3550 cm^{-1} (O-H) characteristic of phenols substituted by ether groups in the ortho position. n.m.r. spectrum in deuterochloroform showed the aromatic proton resonance as a multiplet centered at τ 3.36, a singlet at τ 4.47 for the phenolic protons which disappeared on addition of D_2O (deuterium exchange), a singlet at τ 6.2 for the methyls (-O-CH_3), and a broad triplet centered at τ 7.25 for the protons of the phenethylamine side chain.

The previous oxidative condensations had given unsatisfactory results chiefly because of side reactions. Thus, to achieve a biogenetic-type synthesis by oxidative condensation, it appeared necessary to suppress unwanted phenol oxidation. This new approach has been very rewarding and with the modified diphenolic precursor (58), where the arrangement of the hydroxy groups is such as to promote and direct the condensation in the phenolic moiety, a simple synthesis of the spiro amine system was achieved in very reasonable yield (see Figure 9).

The exploratory experiments investigating the new potentialities of such phenol-coupling showed (U.V., I.R.) that these oxidations actually

proceed much better than had been expected. First, at the most two equivalents of oxidizing agent were used and potassium ferricyanide appeared to be a convenient reagent. The oxidation proceeded best when a very dilute solution of the diphenol was added slowly to a fourfold excess (by equivalent) of the ferricyanide containing potassium hydrogen carbonate (pH 8). By working up the product as detailed in the experimental section, crystalline 3,15-dimethoxy-16-hydroxy-2-oxoerythrina-1(6),3-diene (59) was isolated in 9% yield m.p. 224-227°. In more elaborate preparative experiments with ferricyanide as oxidant the yield of dienone (59) actually isolated was increased to 15%. There is little doubt that further improvement in yield could be achieved by increasing the dilution of the reactants still more. Elemental analysis and spectral data confirmed beyond doubt that the long sought dienone (59) had been obtained. T.L.C. of the dienone (59) gave one spot on silica gel. The ultraviolet spectrum in ethanol showed λ_{max} at 238 (ε 19370) and 283 (ε 4150) mμ. The infrared spectrum in Nujol had absorptions at 3500 (O-H), 1690 (C=O) unsaturated six membered ring ketone), 1665 (vinyl ether), 1630 (C=C), 1595 (aromatic) and 915, 845 (substituted diene) cm^{-1}. The n.m.r. spectrum in deuterochlorofrom (see Figure 10) showed the lone aromatic protons as singlets at τ 3.36 and τ 3.62, the olefinic protons as a triplet centered at τ 3.72 (H_1)(J_{17}= 1.5 c.p.s) in complete agreement with the magnitude of long-range allylic spin-spin coupling[87] and at τ 3.99 (H_4). The phenolic proton appeared at τ 3.79 and exchanged on addition of D_2O, the expected methyl resonances as singlets at τ 6.28 and at τ 6.38, and broad multiplets in the region τ 6.5-7.8 corresponding to the 8 aliphatic protons of ring B and C of the erythrinane ring system. The mass spectrum showed the molecular ion at m/e 313, and other significant peaks at m/e 312 (M-1), 298 (M-15)(M-CH_3),

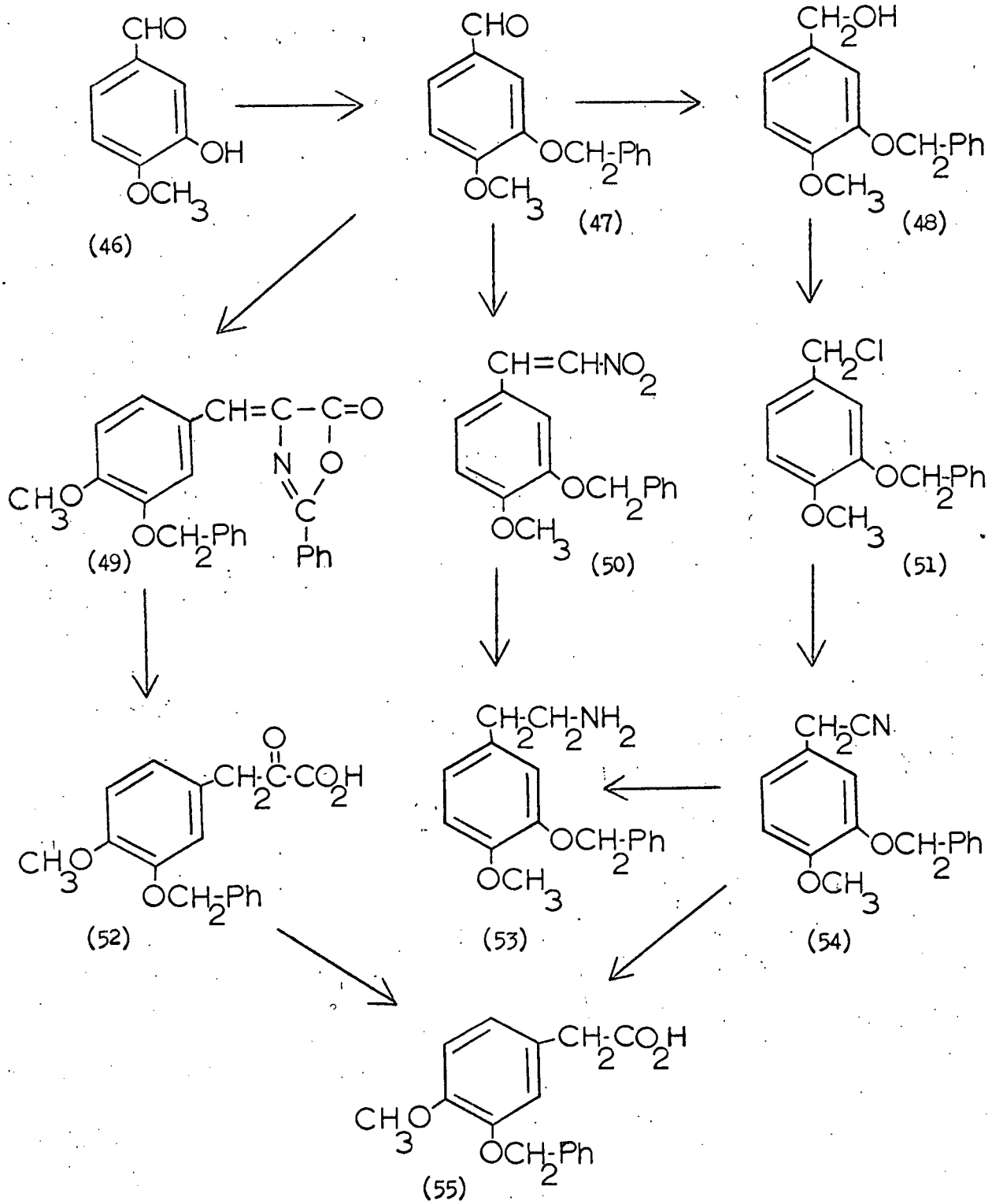


Figure 7. Reaction scheme for the preparation of 3-benzyloxy-4-methoxyphenethylamine (53) and 3-benzyloxy-4-methoxyphenylacetic acid (55).

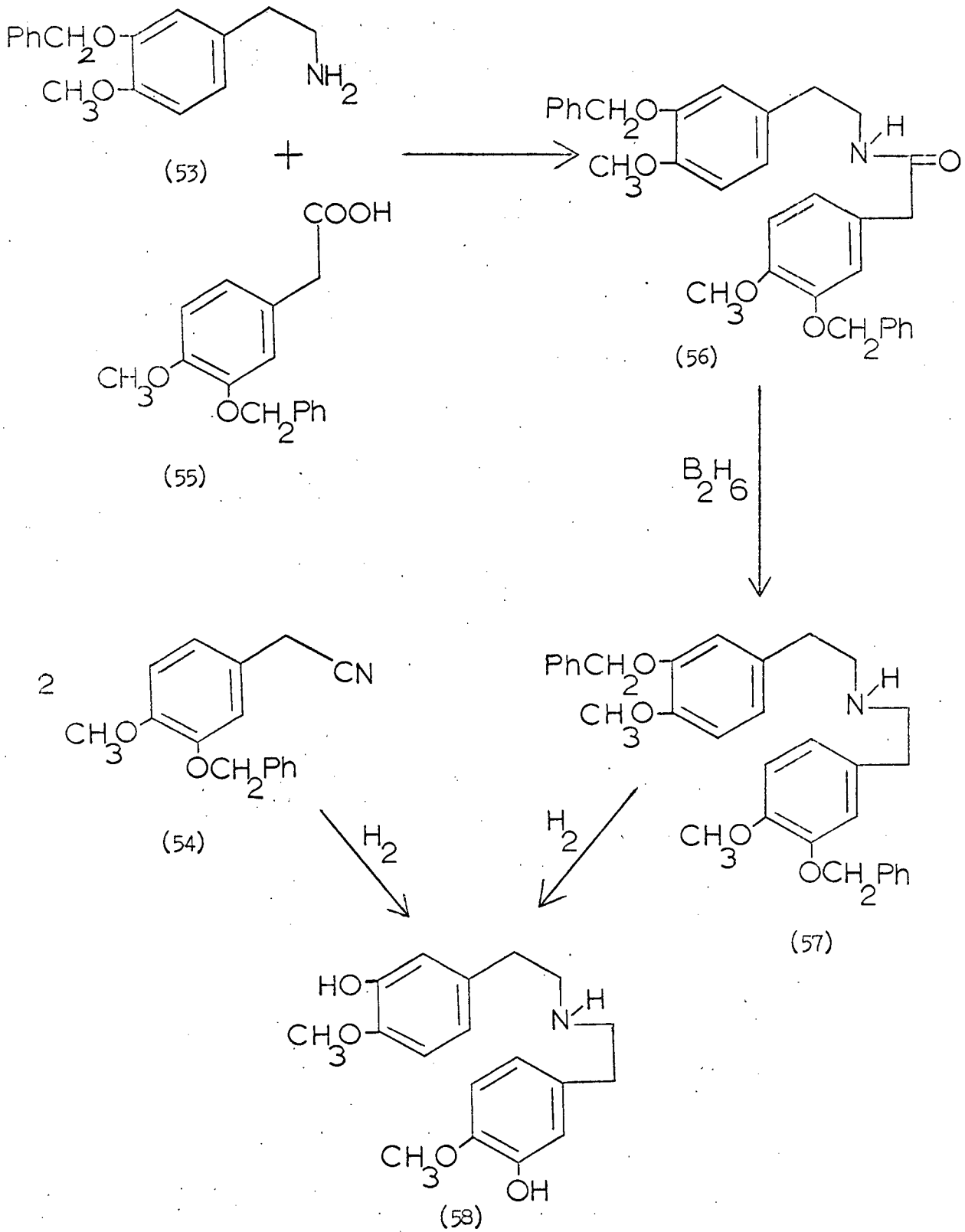


Figure 8. Reaction sequence, leading to di-(β-3-hydroxy-4-methoxyphenyl)-ethylamine (58).

285 (M-28)(M-CO)(M-C_2H_4), 283 (M-30)(M-C_2H_6), 282 (M-31)(M-1-C_2H_6).

Additional evidence was gained by acetylation of the dienone (59) with acetic anhydride and dry pyridine at room temperature to give 3,15-dimethoxy-16-acetoxy-2-oxoerythrina-1(6), 3-diene (60). T.L.C. of this compound showed one spot on silica gel. Ultraviolet spectrum in ethanol showed λ_{max} at 214, 235 and 285 mμ. Infrared spectrum in Nujol had absorptions at 1780 (phenol acetate), 1690 (unsaturated six membered ring ketone), 1665 (vinyl ether), 1630 (C=C) and 1205 (phenol acetate) cm^{-1}. This also established that a tertiary nitrogen is present in the molecule, since a secondary nitrogen would acetylate readily.

Reduction of the dienone (59) by sodium borohydride in ethanol at room temperature yielded 3,15-dimethoxy-2,16-dihydroxyerythrina-1(6),3-diene (61). This compound was purified by chromatography on an alumina column, m.p. 166-169°. T.L.C. on silica gel showed one spot. Infrared spectrum in Nujol had absorptions at 3510, 3450 (O-H), 1655 (vinyl ether), 1610 (C=C) and 1110, 1260 (secondary alcohol) cm^{-1}. Ultra-violet spectrum in ethanol showed λ_{max} at 212, 240 (shoulder) and 287 mμ. The mass spectrum showed the molecular ion at m/e 315, and other significant peaks at m/e 316 (M+1), 300 (M-15)(M-CH_3), 297 (M-18)(M-H_2O), 287 (M-28)(M-C_2H_4) and 285 (M-30) (M-C_2H_6). The n.m.r. spectrum in deuterochloroform showed the two lone aromatic protons as singlets at τ 3.43 and at τ 3.68, a multiplet at τ 4.24 and a singlet at τ 4.95 for the two olefinic protons, a broad singlet at τ 4.72 which exchanged on addition of D_2O and was assigned to a hydroxyl proton, a multiplet centered at τ 5.16 for the proton on the carbon atom bearing the hydroxyl group in ring A, the methyl resonances (-O-CH_3) at τ6.28 and at τ 6.47 and a complex pattern of lines between τ 6-8 corres-

ponding to the aliphatic protons in rings B and C of the erythrinane ring system.

Considering the mechanism of the cyclization reaction via oxidative coupling, there are two routes leading to the spiro amine ring system. One has as its first step the para-para coupling of the generated phenolic radicals to give a diphenoquinone type intermediate (39) (see Figure 5). The fourth ring may then be formed by the addition of the amino group to the quinonoid system to yield the dienone (37). There are two objections to this scheme. The major inherent objection to this route is the steric difficulty of attaching the nitrogen atom to the diphenoquinone system. A careful examination of the molecular model showed clearly that it is almost impossible to utilize the proposed diphenoquinone intermediate (39). The nitrogen atom is too far out and above the ring system and the site of its proposed attack, so that bond formation would be sterically difficult. The second objection is that it has been reported that diphenoquinone does not undergo addition reactions with amines.[88,89] The second route (see Figure 11) can be visualized by the coupling of the phenolic radical with the unpaired electron on the nitrogen to give the phenethyl-indole intermediate (65). This can either, by para-para phenolic coupling (66), or by an indoline type of ring closure (64), furnish (67) which, on aromatization of ring D will give rise to the dienone (59). This second mechanism is the favoured one and it is proposed for the cyclization, strongly supported by analogous products obtained enzymatically[80] and by ferricyanide oxidation of N-substituted 3,4-dihydroxyphenethylamines.[90] The exact analogy for our case is, of course, oxidation of the methylester of 3-hydroxy-4-methoxyphenylalanine to the corresponding indole by means of Fremy-salt.[90]

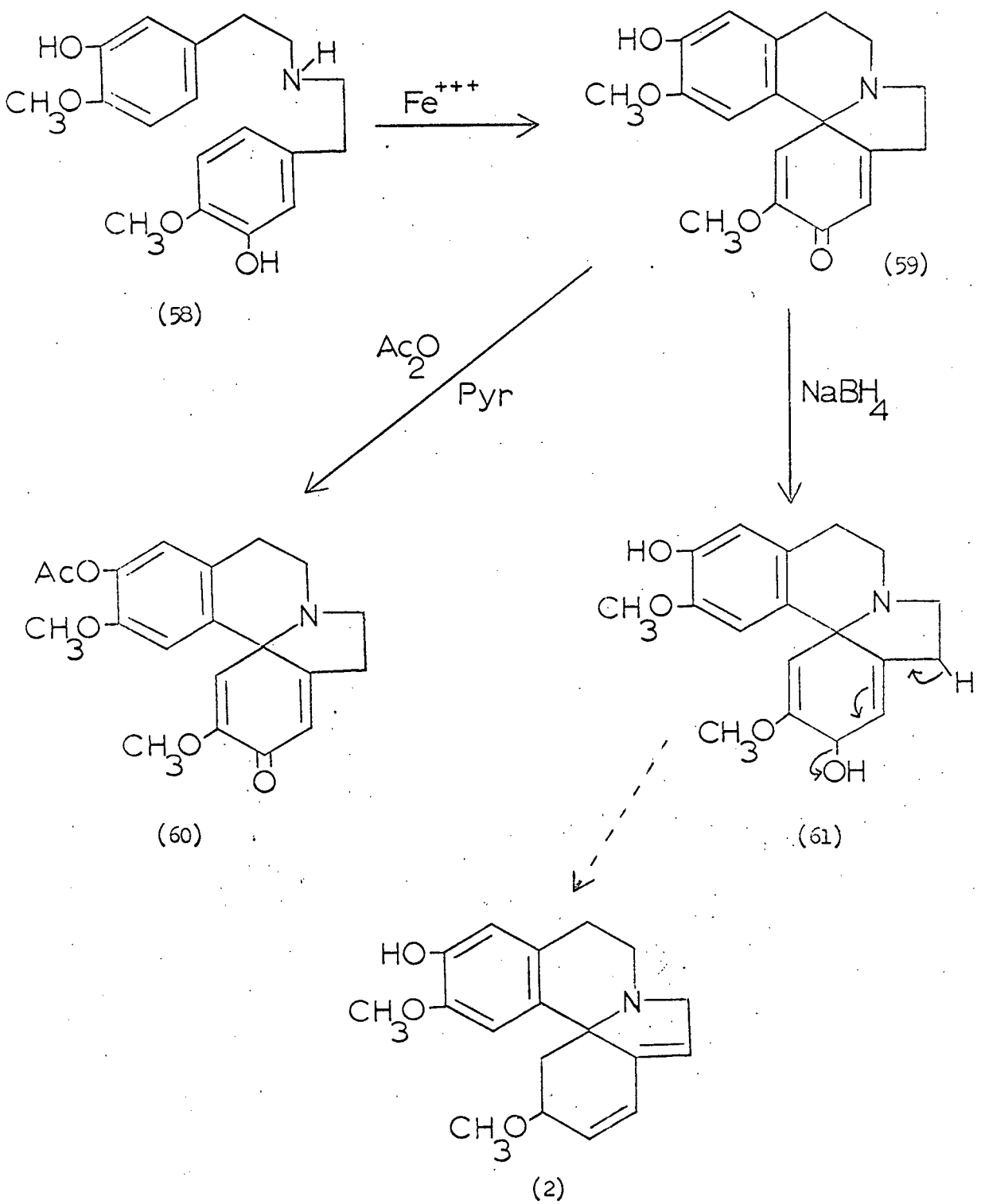


Figure 9. Biogenetic-type synthesis of the erythrinane spiro-amine ring system.

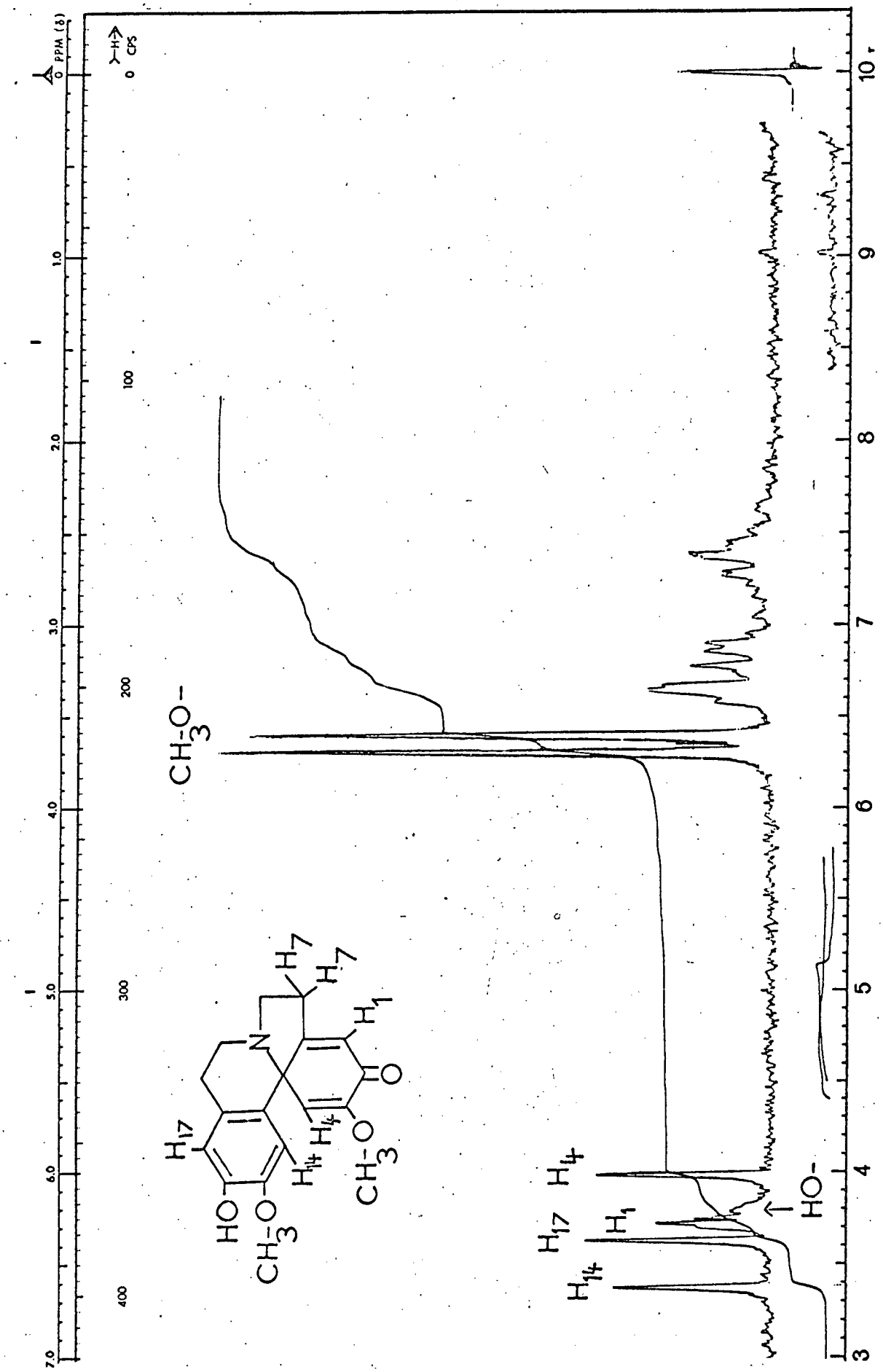


Figure 10. n.m.r. spectrum of 3,15-dimethoxy-16-hydroxy-2-oxoerythrina-1(6),3-diene (59).

This first laboratory realization of such cyclization, to provide the spiro amine skeleton present in the Erythrina alkaloids by radical coupling, has numerous interesting facets both from a biogenetic and a synthetic standpoint. It certainly renders strong support for the attractive proposal that in Nature these alkaloids are formed by oxidative coupling, which previously has received no experimental verification. The results of this investigation are consistent with the ideas presented earlier, but are not decisive. However, they are sufficient to indicate in broad outline the biosynthetic pathway to the spiro amine system. The importance of choosing the correct protection pattern for the phenolic groups and also the proper state of the nitrogenous function is well illustrated by the synthesis. The most important conclusion to be drawn is that it seems very probable that methylation precedes and controls the direction of oxidative coupling in the biosynthesis of Erythrina alkaloids. The tetrahydroxy-diphenol (33) may well be the true precursor of these alkaloids in the plants, and the introduction of allylic hydroxyl or methoxyl at a later stage represents a minor divergence from our in vitro scheme. Such a precursor would have the conceptual advantage of a single oxygenation pattern in the precursor for all the Erythrina alkaloids. The structural aspects of the theory are thus consistent with the results. Proof that the mechanism of coupling really involves two phenolate radicals is difficult to secure by direct experiment, but so far as circumstantial evidence will go the theory is supported. Therefore, the case is a very strong one in favour of the biosynthesis of Erythrina alkaloids by coupling of the diphenol (58).

Although the result of the feeding experiments are not yet available, it seems quite certain that the reaction carried out in the laboratory also

takes place in vivo. The evidence shows that such couplings under laboratory conditions are facile, and the formation of the dienone (59) from the phenolic rings could be the key step in the biosynthesis of Erythrina alkaloids. The intermediates, therefore, must occupy attention. Isolation of intermediates from plants is quite difficult since they are present only in minute amounts. However, since it was possible to prepare the dienone (59), based on our present biosynthetic knowledge it is possible and quite safe to predict that this hypothetical precursor is present in the Erythrina species. The validity of this proposal is supported by the examples of other similar alkaloidal structures occuring in plants, and known to originate from phenolic precursors.[98,99,100] The hypothesis that methylenedioxy-groups in alkaloids could be derived biogenetically by cyclization of O-methoxyphenols is by now well established.[71] Therefore it seems reasonable to assume that the methylenedioxy-group present in erythraline (3) is formed by a radical cyclization mechanism from the dienone (59). There are five examples of derivation of this group from the O-methoxyphenol system in different alkaloids, and the generality of this step is in little doubt.[96]

From the synthetic standpoint, the dienol (61) is a potential intermediate for a total biogenetic-type synthesis of erysodine (2) in view of its methylation pattern (see Figure 9). Elimination of the alcohol function in (61) with the possibility of introducing the C-6 C-7 double bond and reduction at C-3 C-4 will lead directly to erysodine (2). Experiments in this direction are under way in our laboratory.

A final decision on the biosynthetic pathway can only be made by radiochemical labelling experiments. Therefore an extensive feeding program was initiated in our laboratory. First, in order to obtain an in

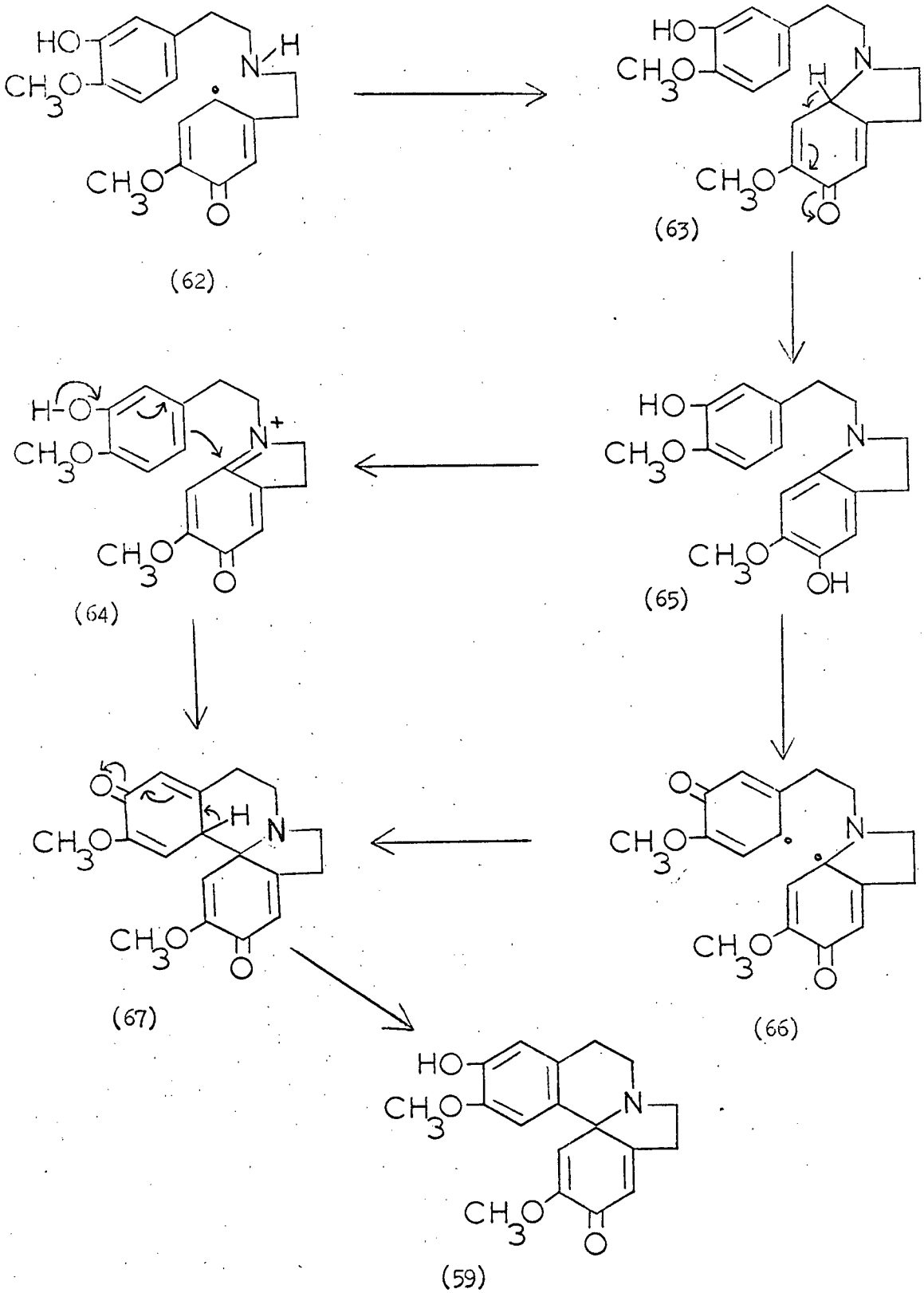


Figure 11. Proposed mechanistic scheme for the formation of the spiro-amine ring system via oxidative coupling.

vivo confirmation for our in vitro laboratory synthesis the diphenol (58) labelled with ^{14}C was prepared according to the scheme shown in Figure 12. 3-Benzyloxy-4-methoxybenzyl chloride (51) was reacted with potassium cyanide containing potassium [^{14}C] cyanide in dimethyl sulphoxide to yield 3-benzyloxy-4-methoxyphenyl[1-^{14}C]acetonitrile (68). The nitrile was hydrolysed to 3-benzyloxy-4-methoxyphenyl[1-^{14}C]acetic acid (70) which on condensation with 3-benzyloxy-4-methoxyphenethylamine (53) gave 3-benzyloxy-N-(3-benzyloxy-4-methoxyphenethyl)-4-methoxyphenyl[carbonyl-^{14}C]acetamide (69). The amide was reduced by borane in tetrahydrofuran (71) and debenzylated by hydrogenolysis to give the desired 3-hydroxy-4-methoxy-N-(3-hydroxy-4-methoxyphen[1-^{14}C]ethyl)—phenethylamine (72) shown in radio-inactive runs to be identical with an authentic sample. This labelled substance, which is assumed to be a precursor of the aromatic Erythrina alkaloids and introduced into the plant's biosynthetic system, is expected to produce on isolation radiochemically labelled erysodine (2), erysopine (38) and erythraline (3). Feeding experiments are in progress, the results of which will be of major interest, and they are expected to provide good support for the suggested mode of biosynthesis of the Erythrina alkaloids.

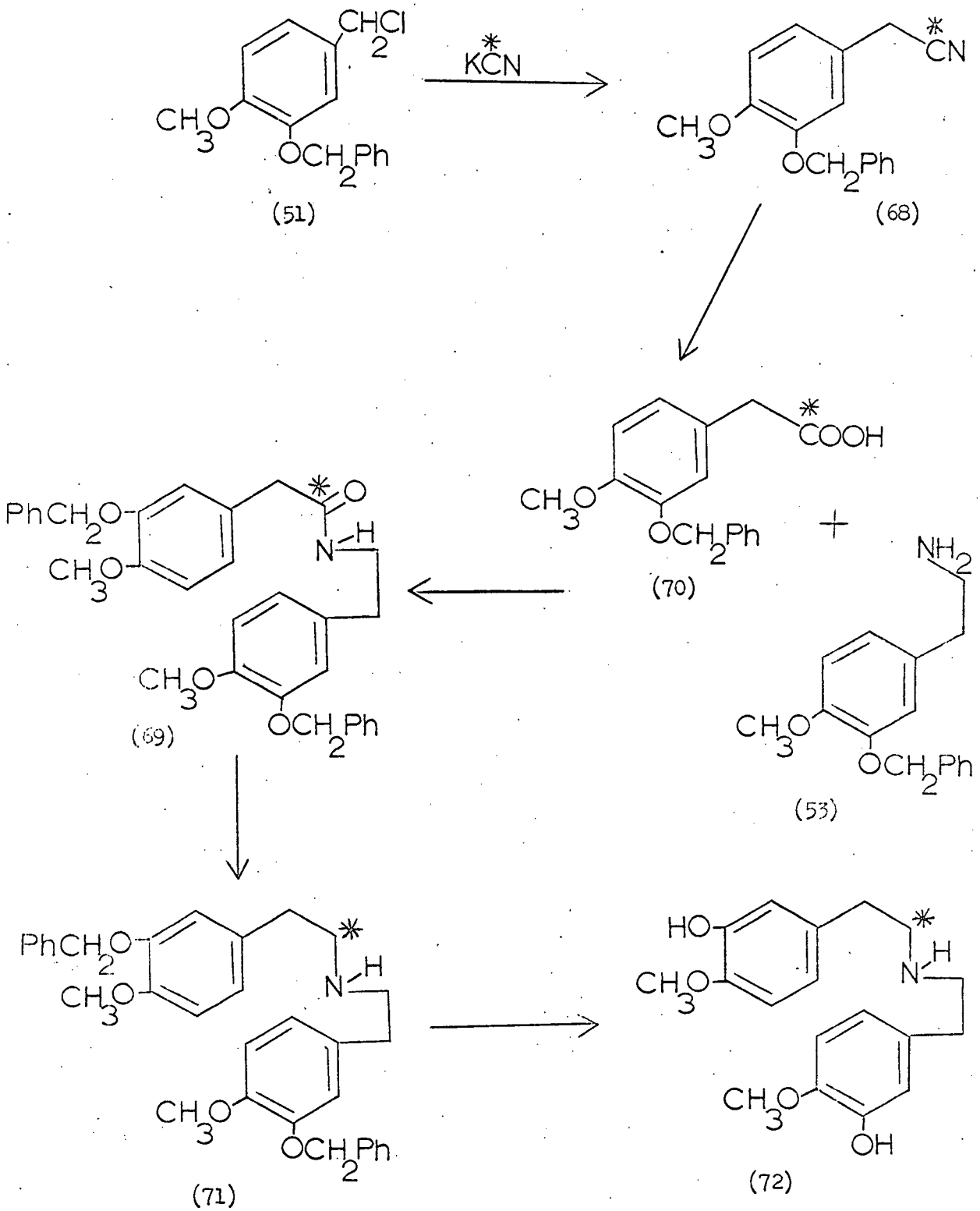


Figure 12. Reaction scheme for the preparation of 3-hydroxy-4-methoxy-N-(3-hydroxy-4-methoxyphen[1-^{14}C]ethyl)-phenethylamine (72). The labelled carbon atom [^{14}C] is marked with asterisk.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Ultraviolet (U.V.) spectra were measured on a Cary 14 spectrophotometer and infrared spectra (I.R.) were taken on a Perkin-Elmer model 137B spectrophotometer. Nuclear magnetic resonance (n.m.r.) spectra were recorded at 60 Mc/s on a Varian A60 instrument. The line positions or centers of multiplets are given in the Tiers τ scale with reference to tetramethylsilane as the internal standard, with the types of protons and integrated areas being indicated in parentheses. Silica gel G and alumina G (according to Stahl) plates were used for thin layer chromatography (T.L.C.) and were developed as given below. The alumina used for column chromatography was Shawinigan reagent, neutralized with ethyl acetate, dried and deactivated with 60% of water. Every molecular weight quoted was determined mass spectrometrically. The mass spectrum was determined on a A.E.I. MS9 double focusing mass spectrometer.

Elemental microanalyses were performed by Mrs. C. Jenkins of this Department, and by Dr. A. Bernhardt and his associates of the Max Planck Institute, Mulheim, Ruhr, West Germany. The n.m.r. and mass spectrometric determinations were done by Mrs. A. Brewster and Mr. F. G. Bloss of this Department respectively.

The radioactivity was determined with a Nuclear Chicago Model D47 gas flow detector operated as a Geiger counter and mounted in a Model M-5 Semiautomatic Sample Changer, all in conjunction with a Model 181B Decade Scalar. The activities were measured by depositing samples of 0.1 to 0.5 mg as thin films on standard 1.125 inch diameter aluminum planchets. The total activities of synthetic precursors are given in millicuries (mc), a

counter efficiency of 39.1% being assumed.

Homoveratroyl-homoveratrylamine (43)

3,4-Dimethoxyphenylacetic acid (1 g) L. Light & Co., England) and thionyl chloride (15 ml) were heated on a water-bath for 1 hour (45°), and the excess of thionyl chloride was evaporated under reduced pressure. A solution of the residue in anhydrous ether (50 ml) was added to 3,4-dimethoxyphenethylamine (1.5 g) (Eastman Kodak) in ether (20 ml). The amide which precipitated immediately was filtered off and the crude product recrystallized from ethanol (1.5 g). m.p. 124° (literature m.p. 124°).[92] Anal. Found: C, 66.59%; H, 6.5%; N, 3.79%. Calc. for $C_{20}H_{25}NO_5$: C, 66.9%; H, 6.97% N, 3.9%. Infrared spectrum in chloroform: 3450 (s) (N-H), 3050 (s), 2990 (s), 1680 (s), 1610 (s), 1530 (s), 1480 (s), 1430 (s), 1270 (s) (broad), 1160 (s), 1150 (s), 1035 (s), 965 (w), 865 (s) and 815 (s) cm^{-1}. n.m.r. signals in deuterochloroform, given in τ units: multiplet centered at 3.29 (aromatic H, area 6 H), multiplet centered at 6.15 (methyl H of $-O-CH_3$, area 12 H), multiplet centered at 6.53 (methylene H of $-CH_2-\overset{O}{\overset{\|}{C}}-$ and methylene H of $-N-CH_2-$, area 4H), triplet centred at 7.32 (β-methylene H of homoveratrylamine side chain, area 2H).

Di-(β-3,4-dimethoxyphenyl)-ethylamine (45)

Homoveratroyl-homoveratrylamine (500 mg) was extracted (Soxhlet) into a refluxing suspension of lithium aluminium hydride (600 mg) in dry ether (180 ml) (48 hours). The excess of reagent was decomposed with ethyl acetate (10 ml) and water was added (50 ml). The ether layer was separated

and the pasty aqueous layer further extracted with ether. Evaporation of the dried (anhydrous magnesium sulphate) ethereal solutions gave the oily amine (400 mg). The hydrochloride, prepared in anhydrous ether, was recrystallized from ethanol. m.p. 197° (literature m.p. 195-196°).[81] The amine was also prepared on a larger scale by a previously described method[81] as follows. A solution of 3,4-dimethoxyphenylacetonitrile (25 g) (K & K) in ethanol (500 ml) was hydrogenated over activated Raney-Nickel catalyst at 1000 p.s.i. at 100° for 6 hours. After filtration the solvent was removed under reduced pressure and then the amine was distilled off at 118-120°/1.5 mm. The residual oil was dissolved in dry ether (50 ml) and dry hydrogen chloride passed in. The precipitated hydrochloride was collected, recrystallized from ethanol, giving di-(β-3,4-dimethoxyphenyl)-ethylamine hydrochloride (7 g) identical with the product from the amide route (mixed m.p., I.R., n.m.r.). Anal. Found: C, 62.98%; H, 7.23%; N, 3.75%. Calc. for $C_{20}H_{28}NO_4Cl$: C, 62.8%, H, 7.32%; N, 3.67%. Infrared Spectrum in Nujol: 2925 (s), 2500 (w), 1600 (m), 1525 (s), 1460 (s), 1380 (s), 1340 (m), 1240 (s), 1260 (s) 1165 (s), 1030 (s), 860 (s), 815 (s), 773 (w) and 725 (w) cm^{-1}. n.m.r spectrum in deuterochloroform, given in τ units: doublet centered at 3.25 (aromatic H, area 6 H), singlet centered at 6.19 (methyl H of $-O-CH_3$, area 12 H), broad singlet centered at 6.75 (H of phenethylamine side chain, area 8 H).

Di-(β-3,4-dihydroxyphenyl)-ethylamine Hydrobromide (33)

Di-(β-3,4-dimethoxyphenyl)-ethylamine hydrochloride (1.3 g) was refluxed for 3 hours with hydrobromic acid (48%) (50 ml). The resulting solution was evaporated to dryness under reduced pressure on the steam-bath, and the

residual solid recrystallized from ethanol (charcoal) giving di-(β-3,4-dihydroxyphenyl)-ethylamine hydrobromide (1 g). It was necessary to dry the crystals at 100° under vacuum for 5 hours. m.p. 151°. Anal. Found: C, 49.8%; H, 5.29%; N, 3.21%. Calc. for $C_{16}H_{20}NO_4BrH_2O$: C, 49.6%; H, 5.15%; N, 3.61%. Infrared spectrum in Nujol: 3400 (s) (O-H), 2995 (s), 2750 (w), 1625 (m), 1545 (m), 1470 (s), 1380 (s), 1340 (w), 1295 (s), 1270 (m), 1195 (m), 1155 (w), 1120 (m), 1070 (m), 1050 (m), 1039 (w), 950 (w), 888 (w), 825 (m), 810 (w), 783 (s), and 725 (w) $cm.^{-1}$

Ultraviolet spectrum in ethanol: λ_{max} 284 mμ (ε 7770).

Oxidation of Di-(β-3,4-dihydroxyphenyl)-ethylamine Hydrobromide with Potassium Ferricyanide and Ferric Chloride

In one typical experiment 356 mg (1.08 mMole) of potassium ferricyanide in 900 ml of distilled water containing 20 g of sodium bicarbonate was added through a fine capillary to a stirred solution of di-(β-3,4-dihydroxyphenyl)-ethylamine hydrobromide (100 mg, 0.27 mMole) in 340 ml of distilled water under nitrogen atmosphere during 6 hours. The reaction mixture was stirred for another 3 hours and filtered to remove the large amount of polymer which separated. The solution was then extracted with ethylacetate and chloroform (4x200 ml respectively). The combined and dried (anhydrous sodium sulphate) extracts were evaporated under reduced pressure to give a gummy material (18 mg). The infrared spectrum (NaCl) of the crude product has absorptions at 3450 (OH) and 1720 cm^{-1} (C=O). Ultraviolet spectrum in ethanol: $\lambda_{max.}$ 253 mμ and a shoulder at 284 mμ. Similar results were obtained when ferric chloride was used as oxidizing agent. It was not possible to isolate any products or to obtain reproducible results in these

oxidation experiments.

O-Benzylisovanillin (47)

A mixture of isovanillin (30 g) (K & K), benzyl chloride (37.8 g), finely powdered anhydrous potassium carbonate (15 g) and potassium iodide (7.5 g), and absolute methanol (100 ml) was refluxed for 15 hours. After filtration from inorganic materials and concentration under reduce pressure, the product was steam distilled yielding a gum-like residue which solidified on standing at 0°. The residue was dissolved in ether, washed first with dilute sodium hydroxide solution then with water and dried (anhydrous magnesium sulphate). The brownish oil, obtained by evaporation of the ether solution under vacuum, was recrystallized twice from benzene-petroleum ether (30-60°) to give colorless needles (32 g), m.p. 62° (literature m.p. 62°).[93] n.m.r signals; given in τ units, spectrum obtained in deuterochloroform: singlet centered at 0.25 (aldehyde H, area = 1 H), multiplet centered at 2.7 (aromatic H, area = 8 H), singlet centered at 6.12 (methyl H of $-O-CH_3$, area = 3 H) and a single centered at 4.91 (methylene of $-O-CH_2-C_6H_5$, area = 2 H).

3-Benzyloxy-4-methoxybenzyl Alcohol (48)

A stirred solution of O-Benzylisovanillin (25 g), in methanol (260 ml) was treated portionwise with sodium borohydride (3.5 g) over $1^1/_2$ hours. The solution was warmed at 40° for 1 hour, acidified with concentrated hydrochloric acid, and then basified with 2 N. sodium hydroxide. The organic solvent was evaporated under reduced pressure and the residue, an aqueous suspension, extracted thoroughly with chloroform. After drying

(anhydrous sodium sulphate) the chloroform solution was evaporated under vacuum, and the product recrystallized from ether and light petroleum-ether (40-60°) to yield the alcohol (24 g), m.p. 72° (literature m.p. 72-73°).[86]

3-Benzyloxy-4-methoxybenzyl chloride (51)

Thionyl chloride (38 ml) was added dropwise during 30 minutes to a rapidly stirred suspension of 3-benzyloxy-4-methoxybenzyl alcohol (21 g) in ether (150 ml). After a further 30 minutes, the clear solution was evaporated under reduced pressure. The crude product was dissolved in petroleum-ether (80-110°), purified (charcoal), and filtered by gravity. On evaporation of the organic solvent 24 g of the required chloride was obtained, which was recrystallized from petroleum-ether (80-110°) and ether. m.p. 72° (literature m.p. 72-73°).[86]

3-Benzyloxy-4-methoxyphenylacetonitrile (54)

Potassium cyanide (15 g) was stirred for 15 minutes with dimethylsulphoxide (500 ml), and then 3-benzyloxy-4-methoxybenzyl chloride (40 g) was added to the solution. After stirring the solution for 6 hours at room temperature, 200 ml of water was added and the aqueous solution extracted six times with ether-petroleum-ether (80-110°) (1:1 by volume), washing each time with water (100 ml). Evaporation of the combined, dried (anhydrous magnesium sulphate) extracts gave 28 gm of the nitrile. It was recrystallized from chloroform-petroleum ether (30-60°). m.p. 78° (literature m.p. 79.5-80.5°).[86] Anal. Found: C, 75.31%; H, 5.33%; N, 5.18%. Calc. for $C_{16}H_{15}NO_2$: 75.8%; H, 5.93%; N, 5.51%. The infrared spectrum in

chloroform had the characteristic nitrile absorption band at 2280 cm^{-1} (C≡N stretching).

3-Benzyloxy-4-methoxy-β-nitrostyrene (50)

12 g of O-benzylisovanillin was dissolved in 400 ml of 95% ethanol at room temperature and the solution then cooled to 5-10°, after which 6 g of nitromethane was added. Then a solution of 5 g of sodium hydroxide dissolved in the minimum amount of water in 100 ml of ethanol, cooled to 5-10°, was added from a dropping funnel at a rate of 5 ml per minute. The solution of the nitromethane and O-benzylisovanillin in alcohol was vigorously stirred and kept below 15° during the addition of the alcoholic sodium hydroxide. As the reaction proceeded, the insoluble sodium salt of the condensation product precipitated. After all of the alkali had been added and with the temperature kept below 15°, ice water was slowly added until the precipitate dissolved. The clear cold solution was added in a fine stream through a funnel to a stirred solution of 60 ml of concentrated hydrochloric acid in 90 ml of water. No attempt was made to control the temperature during the addition. A fine, yellow precipitate was immediately formed and after standing for $^1/_2$ hour was filtered with suction and then washed with ethanol. 8 g of nitrostyrene was obtained, m.p. 127°. (literature m.p. 127-128°).[82] The product thus formed was quite pure and was, without further purification by recrystallization, used in the next step.

3-Benzyloxy-4-methoxyphenethylamine (53)

A solution of 3-benzyloxy-4-methoxy-β-nitrostyrene (10.5 g) in anhydrous tetrahydrofuran (200 ml) was added to a suspension of lithium

aluminium hydride (10 g) in the same solvent (200 ml). A vigorous reaction was observed. After the mixture had been heated under reflux for four days, it was treated with ice cold water (25 ml), stirred for 2 hours and filtered, the filter-pad being washed twice with ether. The combined organic solutions were evaporated, and the residue was dissolved in 10% hydrochloric acid. The acidic solution was extracted with ether, basified and extracted three times with chloroform (400 ml). Evaporation of the dried (anhydrous sodium sulphate) chloroform extracts left a residue (5 g), which was dissolved in anhydrous ether and treated with dry hydrogen chloride in the same solvent. Recrystallization of the bulky precipitate from ethanol-ether yielded the required amine hydrochloride (5 g) m.p. 163-165° (literature m.p. 162-166°).[82]

This amine was also prepared by another method, as follows. A solution of 3-benzyloxy-4-methoxyphenylacetonitrile (4 g) in anhydrous ether (150 ml) was added to a stirred solution of lithium aluminium hydride (5 g) in ether (100 ml). After the mixture had been heated under reflux for 5 hours, it was cooled, treated with ice water, stirred for 1 hour and filtered. The ethereal solution was evaporated under reduced pressure, and the residue was dissolved in 10% hydrochloric acid. The acidic solution was extracted with ether, basified and extracted three times with chloroform. Evaporation of the dry (anhydrous sodium sulphate) chloroform extracts yielded the amine (1.8 g). The hydrochloride was recrystallized from ethanol-ether to give crystals, identical with the product from the other route. n.m.r. signals: given in τ units, spectrum obtained in deuterochloroform: multiplet centered at 2.67 (aromatic H of $O\text{-}CH_2\text{-}C_6H_5$, area 5 H), multiplet centered at 3.2 (aromatic H of trisubstituted benzene, area 3 H), singlet centered at 4.94 (methylene of $-O\text{-}CH_2\text{-}C_6H_5$, area 2 H), singlet centered at

6.22 (methyl H of $-O-CH_3$, area 3 H), multiplet centered at 6.95 (H of phenylethylamine side chain, area 4H). Infrared spectrum in Nujol: 2990 (s), 1620 (m), 1530 (s), 1460 (s), 1375 (s), 1275 (s), 1238 (s), 1150 (s), 1030 (s), 940 (m), 860 (m), 812 (s), 750 (m) 735 (s) 706 (m) and 695 (m) cm^{-1}.

4-(3-Benzyloxy-4-methoxybenzylidene)-2-phenyloxazolone (49)

The oxazolone was obtained by heating O-benzylisovanillin (20 g), hippuric acid (14.5 g), anhydrous sodium acetate (A.R., 8.5 g) and acetic anhydride (50 ml) at 100° for 2 hours. The crude product was mixed with alcohol, and the yellow solid filtered by suction and washed with much boiling water (1 liter). The oxazolone crystallized from acetic acid in yellow prismatic needles (16 g). m.p. 154° (literature m.p. 155°).[82] The substance is sparingly soluble in hot alcohol.

3-Benzyloxy-4-methoxyphenylacetic Acid (55)

A mixture of the oxazolone (35 g) and 10% sodium hydroxide (200 ml) was refluxed under nitrogen until evolution of ammonia ceased (8 hours), then saturated with carbon dioxide (pH 8-8.5) (4 hours), and cooled to 5°. Then 6% aqueous hydrogen peroxide was added (25 ml) at such a rate that the temperature of the reaction mixture did not rise 5° (1 hour). After storage at 0° for 24 hours the mixture was acidified with concentrated hydrochloric acid, and the precipitated gummy acids were exhaustively extracted with chloroform. The extracts, when washed, dried (anhydrous sodium sulphate), and evaporated under reduced pressure, afforded an oil (34 g) which was chromatographed on silica gel (B.D.H.) (600 g). The size

of the column was 27 x 5 cm diameter. The material was added to the top of the column by dissolving it in the minimum amount of benzene. On elution the following fractions were obtained consecutively:

(A) 3000 ml of benzene removed no material

(B) 1000 ml of benzene-ether (10%) yielded nothing.

(C) 1000 ml of benzene-ether (10%) gave a small amount of solid material

(D) 2000 ml of benzene-ether (10%) eluted benzoic acid, which gave a melting point of 122° undepressed on admixture with an authentic sample of benzoic acid.

(E) 1000 ml of benzene-ether (10%) eluted oils in two fractions.

(F) 2500 ml of benzene-ether (10%) yielded 5 g of 3-benzyloxy-4-methoxyphenylacetic acid.

The last fractions eluted from the column were oils, and because of the large amount of solvent involved, further development of the chromatographic column was abandoned. The acid obtained was recrystallized twice from benzene. m.p. 122-124°. (literature m.p. 125°),[82]

The required acid was prepared more readily and in better yields, as follows. A solution of 3-benzyloxy-4-methoxyphenylacetonitrile (15 g) in ethylene glycol (300 ml) and water (80 ml) was heated under reflux for 13 hours with potassium hydroxide (8 g). After dilution of the cooled solution with water (200 ml), it was extracted twice with ether, then acidified with concentrated hydrochloric acid and extracted again with ether. The combined second set of extracts gave 3-benzyloxy-4-methoxyphenylacetic acid (11 g) which was recrystallized from benzene to give crystals identical with the product from the other route. Anal. Found: C, 71.06%; H, 5.96%. Calc. for $C_{16}H_{16}O_4$: C, 70.70%; H, 5.83%.

Infrared spectrum of the crystals showed the following major absorptions in Nujol: 2990 (s), 1720 (s), 1605 (w), 1530 (m), 1460 (s), 1380 (s), 1260 (m), 1220 (m), 1160 (m), 1140 (m), 1010 (m), 860 (w), 815 (w), 780 (w), 745 (m), and 695 (w) cm^{-1}. The n.m.r. signals: given in τ units, spectrum obtained in deuterochloroform: singlet centered at -1.4 (H of COOH), multiplet centered at 2.65 (aromatic H of $O\text{-}CH_2C_6H_5$, area 5 H), multiplet centered at 3.19 (aromatic H of trisubstituted benzene, area 3 H), singlet centered at 4.9 (methylene H of $O\text{-}CH_2\text{-}C_6H_5$, area 2 H), singlet centered at 6.2 (methyl H of $O\text{-}CH_3$, area 3 H), singlet centered at 6.5 (methylene H of $-CH_2\text{-}COOH$, area 2 H).

3-Benzyloxy-N-(3-benzyloxy-4-methoxyphenethyl)-4-methoxyphenylacetamide (56)

3-Benzyloxy-4-methoxyphenylacetic acid (600 mg) in dry chloroform (50 ml) was added portionwise to 3.5 ml of thionyl chloride, and the reaction mixture was then allowed to stand on a water-bath (45°) for one hour. The solvent and the excess of thionyl chloride were evaporated under diminished pressure. The residual acid chloride solidified and was used immediately.

The acid chloride (from 600 mg of acid) in dry tetrahydrofuran (40 ml) was added dropwise during 1 hour to a stirred solution of 3-benzyloxy-4-methoxyphenethylamine hydrochloride (800 mg) in tetrahydrofuran (50 ml) and aqueous sodium hydroxide (0.25 g in 1 ml). After an additional $^1/_2$ hour the tetrahydrofuran was removed under reduced pressure, The residue was taken up in chloroform, washed successively with dilute hydrochloric acid, aqueous sodium bicarbonate and water, dried (anhydrous magnesium

sulphate), and evaporated under vacuum to give an oil which solidified on standing at room temperature. Recrystallization from ethyl acetate gave the amide (900 mg) m.p. 116° (literature m.p. 118°.[82] Anal. Found: N, 2.73%, Calc. for $C_{32}H_{33}NO_5$: N, 2.74%. Infrared spectrum in Nujol: 3350 (m) (N-H), 2950 (s), 1650 (s), 1600 (m), 1525 (s), 1460 (s), 1380 (s), 1260 (s), 1240 (s), 1160 (w), 1140 (s), 1080 (w), 1020 (s), 940 (w), 855 (w), 813 (m), 780 (w), 742 (m), 725 (m), and 700 (s) cm^{-1}. n.m.r. signals; given in τ units, spectrum obtained in deuterochloroform: multiplet centered at 2.65 (aromatic H of $-O-CH_2-C_6H_5$, area 10 H), singlet centered at 3.23 (aromatic H of trisubstituted benzene, area 6 H), singlet centered at 4.95 (methylene H of $-O-CH_2-C_6H_5$, area 4 H), singlet centered at 6.2 (methyl H of $-O-CH_3$, are 6 H), multiplet centered at 6.5 (methylene H of $CH_2-C=O$ and methylene H of $-N-CH_2-$, area 4 H), triplet centered at 7.25 (β methylene H of phenethylamine side chain, area 2 H).

Di-(β-3-benzyloxy-4-methoxyphenyl)-ethylamine (57)

To a solution of 3-benzyloxy-N-(3-benzyloxy-4-methoxyphenethyl)-4-methoxyphenylacetamide (258 mg) in dry tetrahydrofuran (50 ml) in a 100 ml flask (nitrogen atmosphere) was added 10 ml of 1 M borane in tetrahydrofuran over 20 minutes.[94] The temperature was maintained at approximately 0° during the addition. After the reaction mixture had been heated under reflux for 8 hours, it was cooled to room temperature and 2 ml of dilute hydrochloric acid was added. The tetrahydrofuran was removed by distillation. Sodium hydroxide pellets were added to saturate the aqueous phase and the latter was extracted three times with a total of 100 ml of ether. The combined ether extracts were dried (anhydrous sodium

sulphate) and evaporated. The residual amine was converted into its hydrochloride (250 mg) with dry ethereal hydrogen chloride, m.p. 130°. The final product was obtained as the crystalline amine hydrochloride by recrystallization from ethanol-ether and drying at 100° under vacuum for five hours. m.p. 164-167°. Anal. Found: C, 72.13%; H, 6.83%; N, 2.47%, Calc. for $C_{32}H_{36}O_4NCl$: C, 72.1%; H, 6.76%; N, 2.63%. Infrared spectrum in Nujol: 2950 (s), 2480 (w), 1600 (w), 1530 (s), 1460 (s), 1380 (s), 1285 (s), 1240 (s), 1150 (s), 1085 (w), 1030 (s), 990 (m), 880 (w), 858 (w), 814 (m), 775 (w), 752 (m), 735 (s) and 700 (m) cm^{-1}. n.m.r. signals; given in τ units, spectrum obtained in deuterochloroform: mulitplet centered at 2.65 (aromatic H of $-O-CH_2-C_6H_5$, area 10 H), singlet centered at 3.24 (aromatic H of trisubstituted benzene, area 6 H), singlet centered at 4.94 (methylene H of $-O-CH_2-C_6H_5$, area 4 H), singlet centered at 6.2 (methyl H of $-O-CH_3$, area 6 H), singlet (broad) centered at 6.88 (H of phenylethylamine side chain, area 8 H).

Di-(β-3-hydroxy-4-methoxyphenyl)-ethylamine (58)

The corresponding dibenzyl ether, Di-(β-3-benzyloxy-4-methoxyphenyl)-ethylamine hydrochloride (56 mg) was hydrogenolysed in methanol (10 ml) containing concentrated hydrochloric acid (0.1 ml) and 10% palladised charcoal (25 mg), the hydrogen uptake being complete in 30 minutes. Filtration of the solution and evaporation of the solvent gave di-(β-3-hydroxy-4-methoxyphenyl)ethylamine hydrochloride. It was recrystallized from ethanol. m.p. 230°.

The amine was also prepared on a larger scale by another method, as follows. A solution of 3-benzyloxy-4-methoxyphenylacetonitrile (3 g) in

methanol (250 ml) was placed into a high pressure bomb, with 1.5 g of freshly prepared active Raney-Nickel catalyst.[95] The hydrogen gas was passed into the bomb (1100 p.s.i.) and the mixture heated at 90° with stirrring for 8 hours. The bomb was allowed to cool before it was opened and then the contents were removed. The reaction product was then filtered and the ammoniacal methanol solution evaporated. The residue was dissolved in absolute ethanol and dry hydrochloric acid was added to the ethanolic solution in the same solvent. The amine hydrochloride (1g) crystallized overnight at 0° and after recrystallization from absolute ethanol it was found to be identical (mixed m.p., I.R., n.m.r.) with that obtained from the amide route above. Anal. Found: C, 61.08%; H, 6.7%; N, 3.49%. Calc. for $C_{18}H_{24}O_4NCl$: C, 61.3%; H, 6.8%; N, 3.96%. Infrared spectrum in Nujol: 3550 (m), 2950 (s), 2500 (w), 1600 (m) 1460 (s),, 1380 (s), 1340 (m), 1300 (m), 1265 (w), 1230 (s), 1205 (w), 1158 (m), 1132 (m), 1025 (s), 955 (w), 870 (s), 812 (s), 768 (w), 755 (w) and 725 (w) cm^{-1}. n.m.r. signals; given in τ units, spectrum obtained in deuterochloroform: multiplet centered at 3.36 (aromatic H, area 6 H), singlet centered at 4.47 (phenolic H, area 2 H), singlet centered at 6.2 (methyl H of $-O-CH_3$, area 6 H), triplet (broad) centered at 7.25 (H of phenylethylamine side chain, area 8 H).

3,15-Dimethoxy-16-hydroxy-2-oxoerythrina-1(6),3-diene (59)

A solution of di-(β-3-hydroxy-4-methoxyphenyl)-ethylamine hydrochloride (150 mg, 0.424 mMole) in distilled water (900 ml) was added through a fine capillary to a vigorously stirred solution of potassium ferricyanide (570 mg, 1.73 mMole) in 100 ml of distilled water and 240 ml of 1 N. sodium bicarbonate, under nitrogen atmosphere, during 6 hours. The reaction

mixture was stirred for another three hours, then extracted with chloroform (6 x 200 ml). The combined extracts, after drying (anhydrous sodium sulphate), on evaporation of the solvent under reduced pressure yielded a brown gum (110 mg). The experiment was repeated ten times and the combined gummy residue (1.140 g) was chromatographed on alumina (30 g) (Shawinigan reagent, neutralized with ethyl acetate, dried at 100° and then deactivated with 6% of water). The material was introduced in 20 ml of chloroform into the column (size 13 x 2 cm diameter), and on elution the following fractions were obtained consecutively:

(A) 50 ml of chloroform removed 61 mg of colourless oil.

(B) 90 ml of chloroform gave 250 mg of crystalline solid.

(C) 525 ml of chloroform yielded 165 mg of oil.

(D) 250 ml of chloroform-ethanol (4:1) eluted 145 mg of oil material.

The total recovery of organic material was 621 mg.

The crystals obtained from fraction (B) were recrystallized from ethanol to give an analytical sample. m.p. 224-229° with decomposition. One spot in T.L.C. on silica gel (Stahl G) in n-butanol-water-acetic acid (12:5:2) (R_f=0.29). This compound was identified as 3,15-dimethoxy-16-hydroxy-2-oxoerythrina-1(6),3-diene (225 mg, 15%). Molecular weight 313 (determined by mass spectrometry). Anal. Found: C, 69.21%; H, 6.19%, N, 4.45%. Calc. for $C_{18}H_{19}NO_4$: C, 69.02%; H, 6.06%; N, 4.46%.

Ultraviolet spectrum in ethanol: λ_{max} 238 mμ (ε 19370) and 283 mμ (ε 4150). Infrared spectrum in Nujol: 3500 (m), 1690 (s), 1665 (s), 1630 (s), 1595 (m), 1505 (s), 1460 (s), 1380 (s), 1320 (w), 1265 (m), 1200 (s), 1175 (s), 1130 (w), 1100 (w), 1070 (w), 1030 (m), 975 (w), 915 (w), 885 (m),

845 (m), 812 (w), 782 (m) and 723 (w) cm^{-1}. n.m.r. signals; given in τ units, spectrum obtained in deuterochloroform: singlet centered at 3.36 (aromatic H, area 1H), singlet centered at 3.62 (aromatic H, area 1 H), triplet centered at 3.72 (J = 1.5 c.p.s) (vinyl H, area 1 H), broad singlet centered at 3.79 which disappeared on addition of D_2O (hydroxyl H, area 1 H), singlet centered at 3.99 (vinyl H, area 1 H), singlets centered at 6.28 and 6.38 (methyl H of $-O-CH_3$, area 3 H respectively) and broad multiplets centered at 6.75 and 7.38 (aliphatic protons of ring B and C of erythrinane skeleton, area 4 H respectively).

Mass spectrum showed significant peaks at m/e = 313 (M^+), 312 (M-1), 298 (M-15), 285 (M-28), 283 (M-30), 282 (M-31), 269, 254, 241, 238, 226, 210, 198, 176, and 170.

3,15-Dimethyoxy-16-acetoxy-2-oxoerythrina-1(6),3-diene (60)

6 mg of 3,15-dimethoxy-16-hydroxy-2-oxoerythrina-1(6),3-diene was acetylated, with 3 ml of acetic anhydride (reagent) and 3 ml of dry pyridine (reagent) at room temperature overnight. The excess of reagent and the pyridine was removed under reduced pressure on the steam bath. The residue was dissolved in 10 ml of chloroform, washed twice with water and dried (anhydrous sodium sulphate). Evaporation of the chloroform solution under reduced pressure gave 7 mg of an oily material which solidified on standing at room temperature. Infrared spectrum in Nujol: 1780 (s), 1690 (s), 1630 (s), 1600 (shoulder), 1510 (s), 1465 (s), 1380 (s), 1260 (s), 1205 (s), 1180 (s), 1100 (m), 1080 (m), 980 (w) and 900 (w) cm^{-1}. Ultraviolet spectrum in ethanol: λ_{max} 214, 234, 285 mμ. T.L.C. gave one spot on silica gel. R_f= 0.18 in n-butanol-water-acetic acid

(12:5:2).

3,15-Dimethoxy-2,16-dihydroxyerythrina-1(6),3-diene (61)

To a solution of 3,15-dimethoxy-16-hydroxy-2-exoerythrina-1(6),3-diene (115 mg) in ethanol (20 ml) was added sodium borohydride (70 mg), and the reaction mixture stirred for 6 hours at room temperature. The ethanolic solution was then concentrated under reduced pressure and 40 ml of water added. The aqueous solution was extracted three times with chloroform, and the combined extracts (150 ml) after drying (anhydrous sodium sulphate), on evaporation yielded 102 mg of oily material which was chromatographed on alumina (3 g). The size of the column was 3.5 x 2 cm diameter. The material was introduced into the column in 4 ml of chloroform, and on elution the following fractions were obtained consecutively: 180 ml of chloroform eluted a yellow oil in 3 fractions. The required alcohol was then eluted with 50 ml of chloroform (65 mg). Recrystallization from ether-ethanol gave a m.p. 166-169°. T.L.C. gave one spot. R_f= 0.19 in n-butanol-water-acetic acid (12:5:2). Infrared spectrum in Nujol: 3510 (s), 3450 (s), 1655 (s), 1610 (s), 1505 (s), 1450 (s), 1375 (s), 1260 (s), 1220 (s), 1110 (s), 1085 (s), 1050 (s), 1020 (s), 985 (m), 920 (m), 870 (m), 840 (w), 806 (s) and 770 (m) cm^{-1}. Ultraviolet spectrum in ethanol: λ_{max} 212, 240 (shoulder), 287 mμ. Mass spectrum showed significant peaks at m/e = 315 (M^+), 316 (M + 1), 300 (M-15), 297 (M-18), 287 (M-28), 285 (M-30), 279, 260, 259, 251, 242, 212, 199, 167, 149, 147, 129, 113 and 112. The n.m.r. spectrum in deuterochloroform showed the expected methyl resonances ($-OCH_3$) at τ 6.28 and τ 6.47, two singlets at τ 3.43 and τ 3.68 for the aromatic protons, a multiplet centered at τ 4.24 and a singlet at τ 4.95

for the two olefinic protons, a multiplet centered at τ 5.16 for the lone proton on the carbon bearing the hydroxyl group in ring A of the erythrinane ring system, a broad singlet at τ 4.72 which disappeared on addition of D_2O and was assigned to one hydroxyl proton, and multiplets τ 6-8 corresponding to the aliphatic protons in rings B and C.

3-Benzyloxy-4-methoxyphenyl[1-^{14}C]acetic Acid (70)

Potassium cyanide (39.4 mg) was stirred for 10 minutes with dimethyl sulphoxide (3 ml) and potassium[^{14}C]cyanide (6.5 mg, 1.0 mc) was added and washed in with dimethyl sulphoxide (4 ml). After 15 minutes, 3-benzyloxy-4-methoxybenzyl chloride (185.5 mg) was added, and the solution was stirred at room temperature for 6 hours. It was shaken with water (50 ml) and ether-petroleum ether (80-110°) (50 ml, 1:1 by volume) and the aqueous layer was further extracted twice with the same solvent, washing each time with water (10 ml). Evaporation of the combined, dried (anhydrous sodium sulphate) extracts left 3-benzyloxy-4-methoxyphenyl[1-^{14}C]acetonitrile (151 mg), shown in radio-inactive runs to be identical with authentic material (mixed m.p.). All the active sample was dissolved in ethylene glycol (3 ml) and water (1 ml) and heated with potassium hydroxide (0.5 g) under reflux for 12 hours. The cooled solution was partitioned between water and ether and the aqueous phase, after acidification, was extracted four times with ether and dried to afford 3-benzyloxy-4-methoxyphenyl[1-^{14}C]acetic acid (137 mg; 0.8 mc), m.p. 123°.

3-Hydroxy-4-methoxy-N-(3-hydroxy-4-methoxyphen[1-^{14}C]ethyl)-phenethylamine (72).

3-Benzyloxy-4-methoxyphenyl[1-^{14}C]acetic acid (137 mg) was warmed on a steam bath for 30 minutes with thionyl chloride (3 ml) and the excess of reagent was evaporated under reduced pressure. A solution of the residue in anhydrous ether (10 ml) was added dropwise to a stirred solution of 3-benzyloxy-4-methoxyphenethylamine (417 mg) in anhydrous ether 10 ml at 0°. The mixture was shaken with 2N hydrochloric acid (20 ml) and ethyl acetate (60 ml), and the aqueous layer was extracted twice with ethyl acetate. After the combined extracts had been shaken with an excess of aqueous potassium carbonate and water, they were dried and evaporated to yield 3-benzyloxy-N-(3-benzyloxy-4-methoxyphenethyl)-4-methoxyphenyl-[carbonyl-^{14}C]acetamide as a solid. Recrystallization from ethyl acetate gave the amide (150 mg), m.p. 116°, suitable for reduction. To a solution of the foregoing amide in dry tetrahydrofuran (50 ml) in a 100 ml flask (nitrogen atmosphere) was added 10 ml of 1M borane in tetrahydrofuran over 20 minutes. The temperature was maintained at approximately 0° during the addition. After the reaction mixture had been heated under reflux for 6 hours, it was cooled to room temperature and 2 ml of dilute hydrochloric acid was added. The tetrahydrofuran was removed by distillation at atmospheric pressure (steam bath) as hydrogen was evolved from the hydrolysis of excess reagent. Sodium hydroxide pelletts were added to saturate the aqueous phase and the latter was extracted three times with a total of 100 ml of ether. The combined ether extracts were dried (anhydrous sodium sulphate) and evaporated. The residual amine was debenzylated by hydrogenolysis in methanol (10 ml) containing concentrated hydrochloric acid

(0.1 ml) and 10% palladised charcoal (25 mg). The hydrogen uptake was complete in 30 minutes. Filtration of the solution and evaporation of the solvent gave 3-hydroxy-4-methoxy-N-(3-hydroxy-4-methoxyphen[1-^{14}C]ethyl-phenethylamine hydrochloride which was recrystallized from ethanol m.p. 230° (60 mg; 0.08 mc). It was shown in radio-inactive runs to be identical with an authentic sample (mixed m.p.).

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PART II

AN ATTEMPTED IN VITRO DEMETHYLATION OF LANOSTEROL

INTRODUCTION

The investigation of the biogenesis of cholesterol (18) is the most exhaustive and thorough of all the work which has been done on the biosynthesis of steroids, and the topic has been well reviewed.[1,2,3,4] The knowledge of this centrally important precursor of other steroids is vital and its biogenesis illustrates a "general" mechanism for sterol formation.

The first work which demonstrated the chemical nature of this biosynthesis began over 20 years ago when it was found[5] that steroidal substances are constructed from numerous small molecules, and that acetate is the source of the carbon atoms of sterols in yeast[6] and in animal tissue.[7,8,9] Bloch's[10] pioneering work showed that acetic acid molecules are incorporated into the structure of cholesterol according to a definite pattern. This work took on even greater interest with the realization that the biogenesis of cholesterol and steroids is a small part of a vast biosynthetic panorama that now includes all terpenes and derived substances.[4] Subsequent work,[11,12,13,14,15,16] which stands as a brilliant achievement, has located the origin of every carbon atom of cholesterol in either the carboxyl or methyl carbon of acetate, by suitable degradations of the sterol and measurement of the relative isotope incorporation into both nuclear and side-chain moieties. Thus, all of the carbon atoms in cholesterol can be derived from acetate. Besides acetate, many other low molecular weight compounds were tested for the possibility for their incorporation into cholesterol.[17] It appeared that the efficiency of the many suggested precursors was proportional to their ability to yield the two carbon fragment, acetate.

The observations[18,19,20] that hydroxymethylglutaryl CoA (3) is

synthesized from acetoacetyl CoA (2) and acetyl CoA (1) and could be incorporated into cholesterol (18),[21] raised the possibility that hydroxymethylglutarate (3) might be a key intermediate.

The search for intermediates in the transformation of acetate into cholesterol met with little success until 1956 when the Merck group isolated mevalonic acid (4).[22,23,24] The obvious similarity to hydroxymethylglutarate (3), which is transformed into cholesterol only poorly,[21] prompted investigation[25] of the possible role of mevalonic acid (4) in cholesterol biosynthesis. The results indicated that this compound was capable of being incorporated into cholesterol in very high yield. Subsequent study showed[26] that a carboxyl group is lost as carbon dioxide early in the sequence of reactions and also conversion to squalene (11) with high efficiency was observed under anaerobic conditions.[27,28] This indicated that decarboxylation occurred, apparently at the six carbon atom level, to give a five carbon atom active intermediate. Ferguson[29] has established that hydroxymethylglutaryl CoA (3) can be converted to mevalonic acid (4) in yeast and Knauss[30] has recently demonstrated the formation of mevalonic acid (4) from acetate (1) in liver tissue, so an excellent case for their intermediacy has now been made.

Tchen[31] working with mevalonic acid (4) in yeast preparations found an enzyme which transforms the acid to 5-phosphomevalonic acid (5). In addition he found that ATP was necessary to transform the 5-phosphomevalonate (5) to squalene (11). Lynen[32] and Bloch[33] have described the further reactions which convert 5-phosphomevalonate (5) to 5-pyrophosphomevalonate (6) and isopentenyl pyrophosphate (7), the isoprenoid intermediate that evaded isolation for a long time. The condensation of dimethylallyl pyrophosphate (8), an isomerization product of isopentenyl pyrophosphate

(7), and isopentenyl pyrophosphate (7) has been reported by Lynen[34] to result in geranyl pyrophosphate (9). This compound can then condense with an additional molecule of isopentenyl pyrophosphate (7) to yield farnesyl pyrophosphate (10). The reductive dimerization of two molecules of farnesyl pyrophosphate (10), tail to tail, to yield squalene (11) has been demonstrated in liver and yeast.[32, 35]

The incorporation of acetate into terpenes and their derivatives, such as steroids, differs at an early stage from that for the biosynthesis of acetogenins.[4] The acetogenins include compounds biogenetically derivable by the acetate hypothesis and exclude the terpenes, which, although ultimately derived from acetate, are themselves a homogeneous family arising from linear combination of isoprenoid units. Whereas the acetogenins are formed by a linear linking of acetate units, the terpenes are generated by conversion of acetate to a branched-chain intermediate, isopentenyl pyrophosphate (7), the biological isoprene unit. The series of reactions as now postulated for the biogenesis of squalene (11) are given in Figures 1 and 2 ($P = PO_3^{-2}$). The intermediates in this biosynthetic sequence prior to mevalonate (4) are capable of interconversion to many other substances.[4] However, the formation of mevalonate (4) is an irreversible process, and mevalonate once formed has essentially only one biochemical role, the production of isoprenoid substances. Its discovery was, therefore, one of the important break-throughs in terpene biosynthesis.

As early as 1926 it was suggested[35] simply on the bases of structural similarities, that squalene (11) is biogenetically related to the steroids. The early experiments[36] did indicate some involment of squalene (11) in cholesterol (18) biosynthesis, however , it was not until 1953 that Bloch[37]

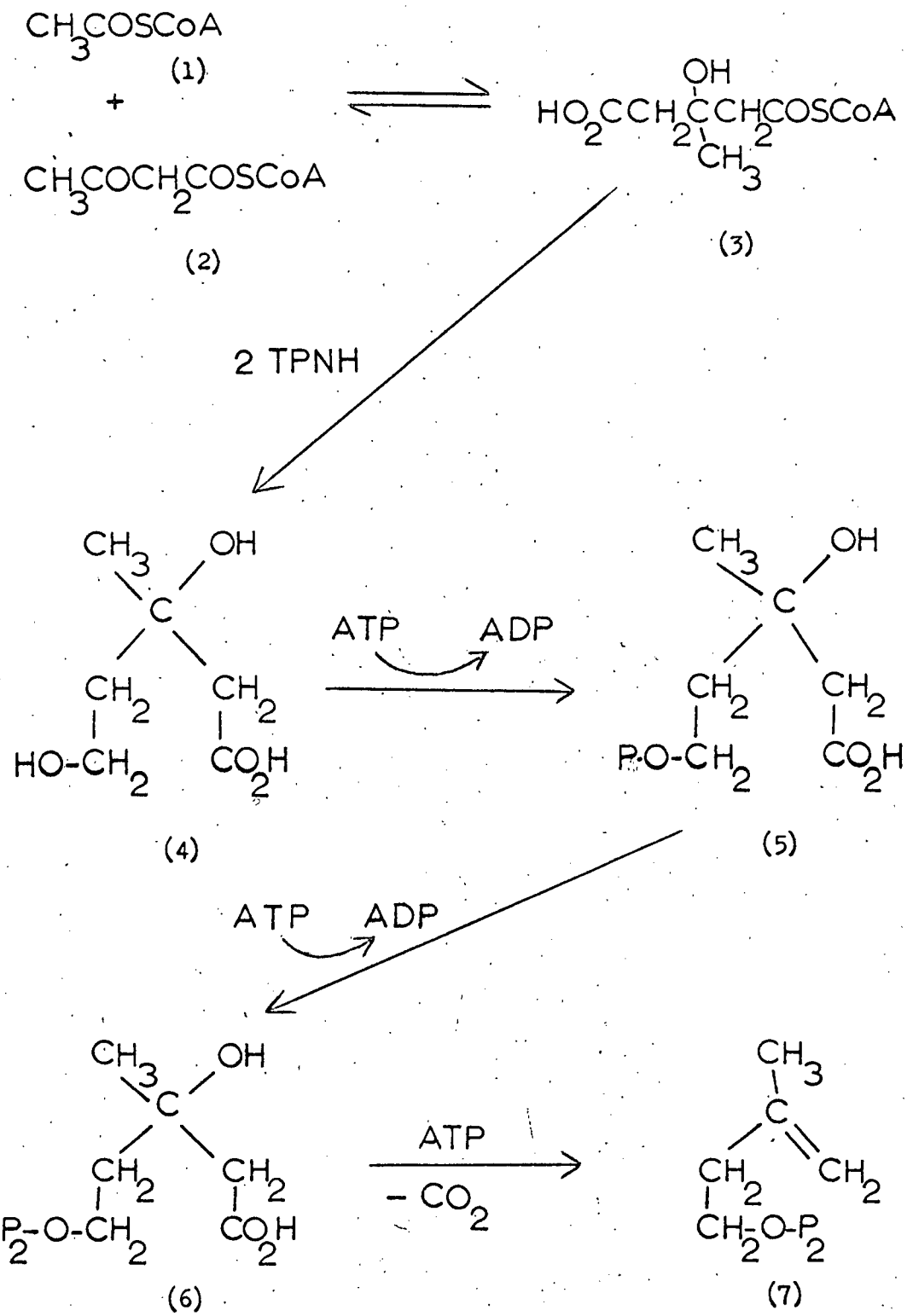


Figure 1. The Biosynthesis of Isopentenyl Pyrophosphate (7).

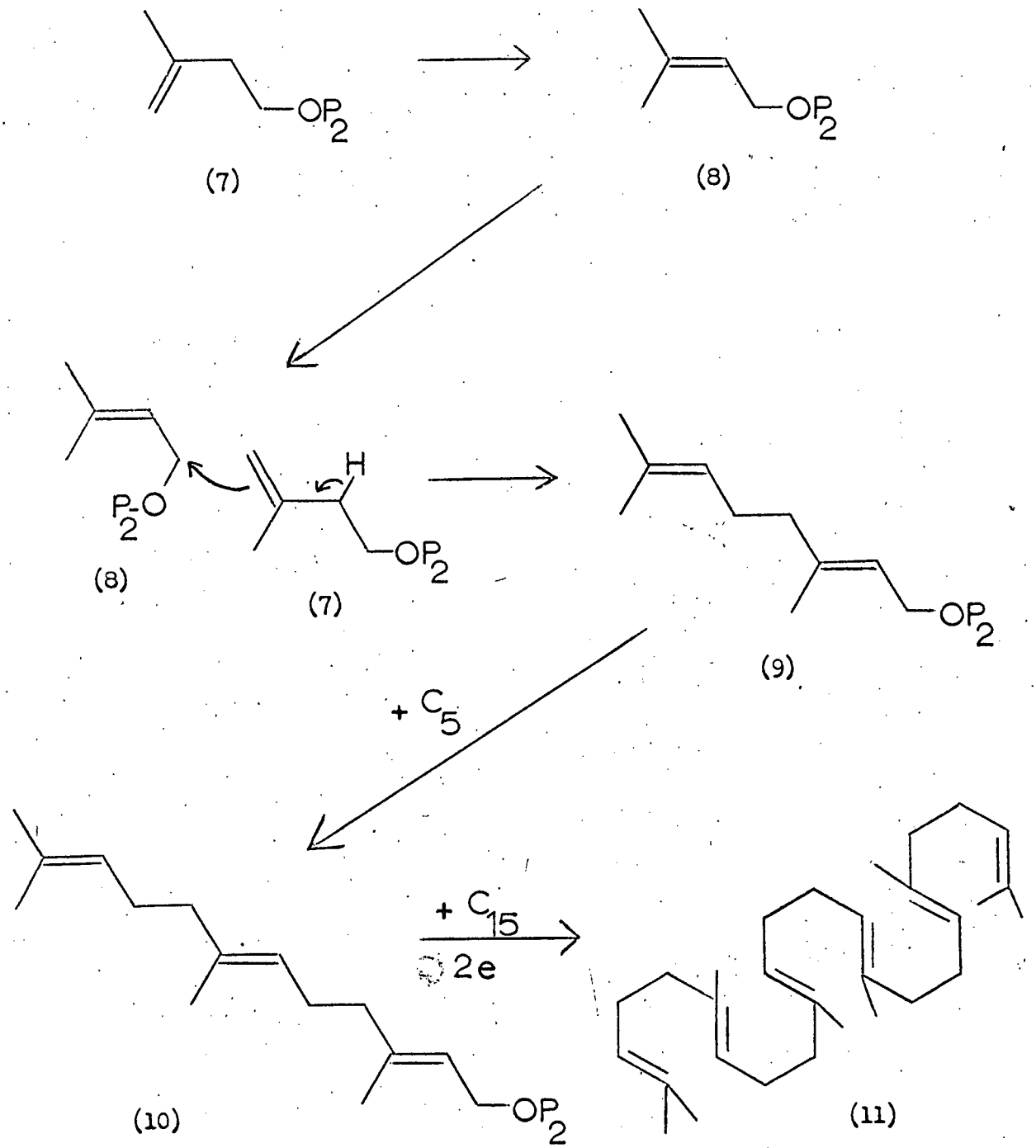


Figure 2. Polymerization of Isopentenyl Pyrophosphate (7).

and his collaborators reinvestigated the matter using isotopes and were able to demonstrate that squalene (11) is indeed converted into cholesterol (18). The cyclization of squalene (11) to lanosterol (13) (see Figure 3) is of particular interest because of the intermediacy of the latter in the pathway of steroid biogenesis. The role of squalene as an obligatory precursor of cholesterol, and the mechanism of its conversion to lanosterol proposed on purely structural and theoretical grounds, now has firm experimental support. Woodward and Bloch[38] in their suggested mechanism implicated lanosterol (13) as an intermediate in cholesterol biosynthesis, and their proposal is consistent with all of the isotope distribution data and enzymoligical evidence that has been obtained so far.[4] The conversions of squalene (11) to lanosterol[39] (13) and of lanosterol (13) to cholesterol[40] (18) have been demonstrated. Very interestingly, the formation of squalene from its precursors does not require oxygen,[41] the cyclization of squalene to lanosterol and cholesterol does require oxygen.[42] Squalene (11) cyclises concertedly by a process initiated by atmospheric oxygen probably as the $HO^{\oplus}$ cation on C-3[42,43] to give the hypothetical intermediate (12), which can undergo two 1,2-methyl shifts[44,45] to yield lanosterol (13).

With the attainment of lanosterol (13), the biosynthesis of the structural feature most characteristic of the steroids, the cyclopentanoperhydrophenanthrene skeleton, has been achieved. For the final transformation of lanosterol (13) to cholesterol (18) there remains only the removal of three methyl groups and appropriate alteration of the two olefinic centers in the lanosterol (13). There are undoubtedly many enzymatic reactions involved in these transformations, but to date only some of them have been elucidated in detail. One of the two pathways by which the changes in lanosterol (13) are effected has as its terminal step the saturation of the

sidechain double bond (see Figure 3). The alternate route[4] involves earlier reduction of the side-chain double bond. There is of course the distinct possibility of intermediates crossing from one of these pathways to the other.

Some steps in the conversion of lanosterol (13) to cholesterol (18) have recently been established. The conversion of desmosterol (17) to cholesterol (18) has been shown by Stokes.[46] Bloch[47] and Schwenk[48] have demonstrated the conversion of biosynthetically labelled zymosterol (16) to cholesterol (18). The three methyl groups at C-4 and C-14 of lanosterol (13) are lost as carbon dioxide in a sequence which requires oxygen and is belived to involve oxidation of the methyl groups to carboxyl groups[49, 50, 51] in the final formation of cholesterol (18). Another intermediate has been isolated in the sequence between lanosterol (13) and cholesterol (18), and by a series of ingenious biochemical[49] and chemical[51] methods it has been shown to possess the structure of 14-desmethyllanosterol (14). It gives rise in turn to cholesterol, thus, it is established that the 14α is the first of the three extra methyl groups to be lost in lanosterol (13).

The exact order of events resulting in loss of the C-4 methyl groups is not known with certainty, but there is some argument for the existence of a hypothetical C-4-monomethyl intermediate compound (15), based upon the recently identified 4-monomethyl steroids in Nature.[4] The scheme for the conversion of squalene (11) to cholesterol (18), as we now understand it, is shown in Figure 3.

It must be emphasized that the above pathways were the result of an intracellular "in vivo" process. This process made extensive use of enzymes to synthesize the desired product. There is an increasing amount of interest in the mechanism of these "in vivo" reactions and, by utilizing the same

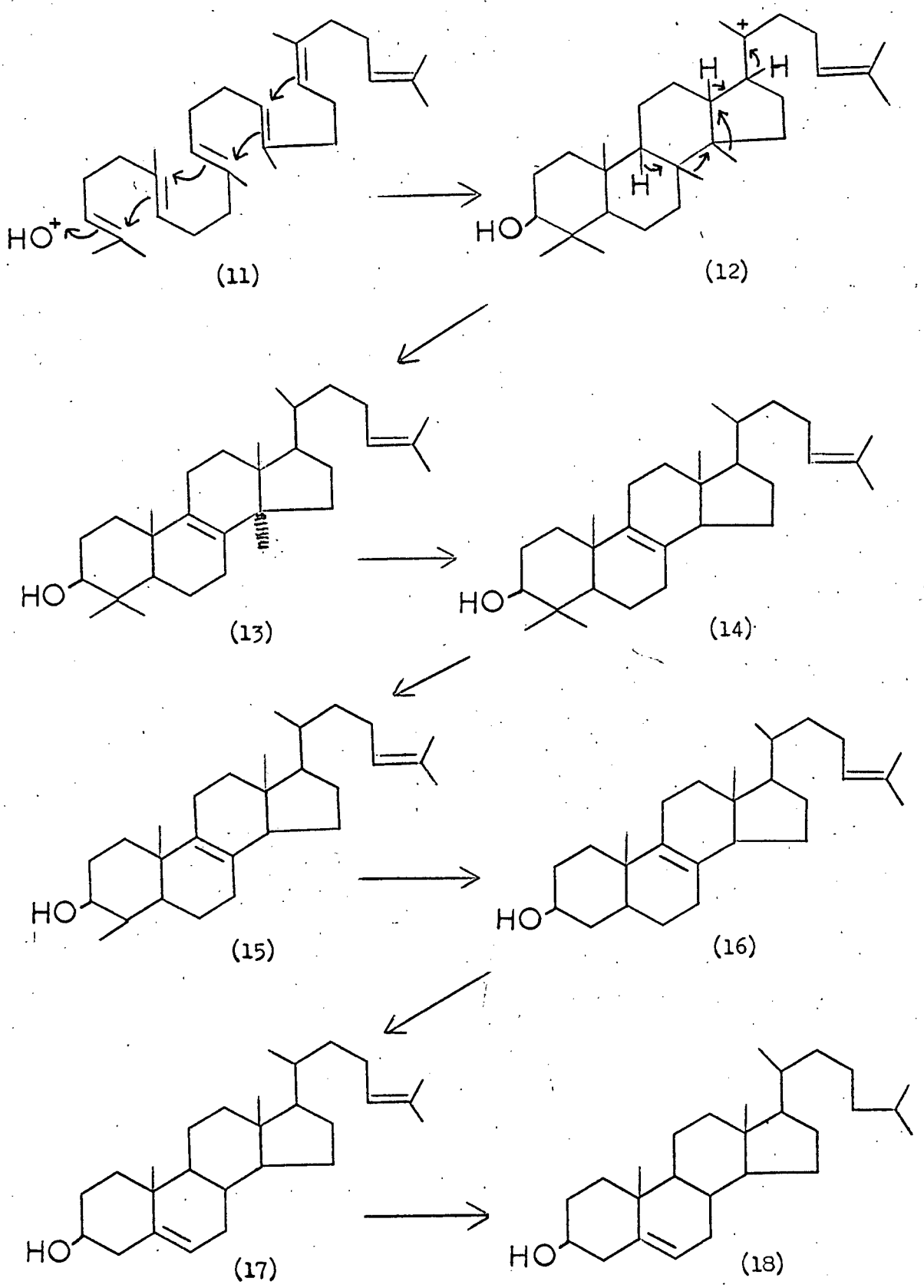


Figure 3. Scheme for the formation of Cholesterol (18) from Squalene (11).

intermediates as the cell, organic chemists now attempt to reproduce these reactions under laboratory conditions 'in vitro' without enzymes. This is possible since the enzyme lowers the activation energy of the reaction by acting as a catalyst. In vitro, these reactions which occur "in vivo" should thus only require stronger conditions. A successful reaction of this type is, of course, a "biogenetic type" synthesis.[52]

The loss of the methyl groups in the lanosterol-cholesterol interconversion has been of considerable interest. Since C-18 and C-19 hydroxylated sterols have been found in nature[53,54,55] and C-19 hydroxysteroids are considered to be likely precursors of ring A nor-steroids,[56] it is reasonable to assume that the first step in the demethylation may be hydroxylation of the methyl groups. The hydroxylation of saturated carbon atoms that are unactivated in the classical sense is of great importance. Investigation of biological hydroxylation of steroids has indicated that the hydroxylation occurs by replacement of hydrogen without inversion of configuration,[57,58,59] i.e., the hydroxyl group occupies the same steric location as the hydrogen which it replaces. This stereochemical result is finding increasing analogy in studies of electrophilic substitution reactions at saturated carbon. Corey[60] has suggested electrophilic oxygen as the active agent, comparing the enzyme-oxygen complex to a peroxide or peracid.

Microrganisms which effect steroid hydroxylation at a specific saturated carbon atom often produce 1,2-epoxides from steroids in which that carbon is part of an olefinic linkage, and it has been pointed out[61] that the same enzyme may be involved in both processes. As a result the enzymatic reagent has been compared to a peroxide or peracid and has been con-

sidered as an electrophilic, non-radical species. Furthermore, it has been established that the hydroxylation process does not involve hydration of an olefinic intermediate, but direct incorporation of molecular oxygen into the reagent and hence into the steroid and that metal ions and TPNH are involved,[62] providing additional evidence for a peroxidic intermediate.

Another reaction of importance is the introduction, removal, and rearrangement of double bonds. The first may involve prior hydroxylation and subsequent loss of water. Rearrangement of double bonds may in some cases occur as a result of series of oxidations and reductions, as in the case of the movement of C-8 C-9 unsaturation of zymosterol (16) to C-5 C-6 in cholesterol (18).

In vitro functionalisation of non-active methyl groups, by intramolecular attack by groups near these groups in space, has been achieved by a variety of methods. The problem posed an interesting challenge in synthetic chemistry, magnified by the widespread occurrence of such transformations in nature under the influence of enzymes. The first reported functionalisation of inactive methyl groups was the use of the Loeffler-Freytag reaction[63,64,70] to prepare conessine derivatives as shown (19) - (21) (see Figure 4). The mechanism is of the free radical chain type,[65] and radical abstraction by nitrogen is favoured by the juxtaposition of the two groups involved. The radical decomposition of the 20-N-chloroamino steroid (19) in acid solution led to a ring closure between C-18 and nitrogen. A similar radical abstraction reaction involving oxygen rather than nitrogen as the abstracting radical, was the photolysis of C-20 ketosteroids which gave rise to cyclobutanol products.[66]

The pyrolysis of 21-diazo-5α-pregnan-20-one (22) resulted in the for-

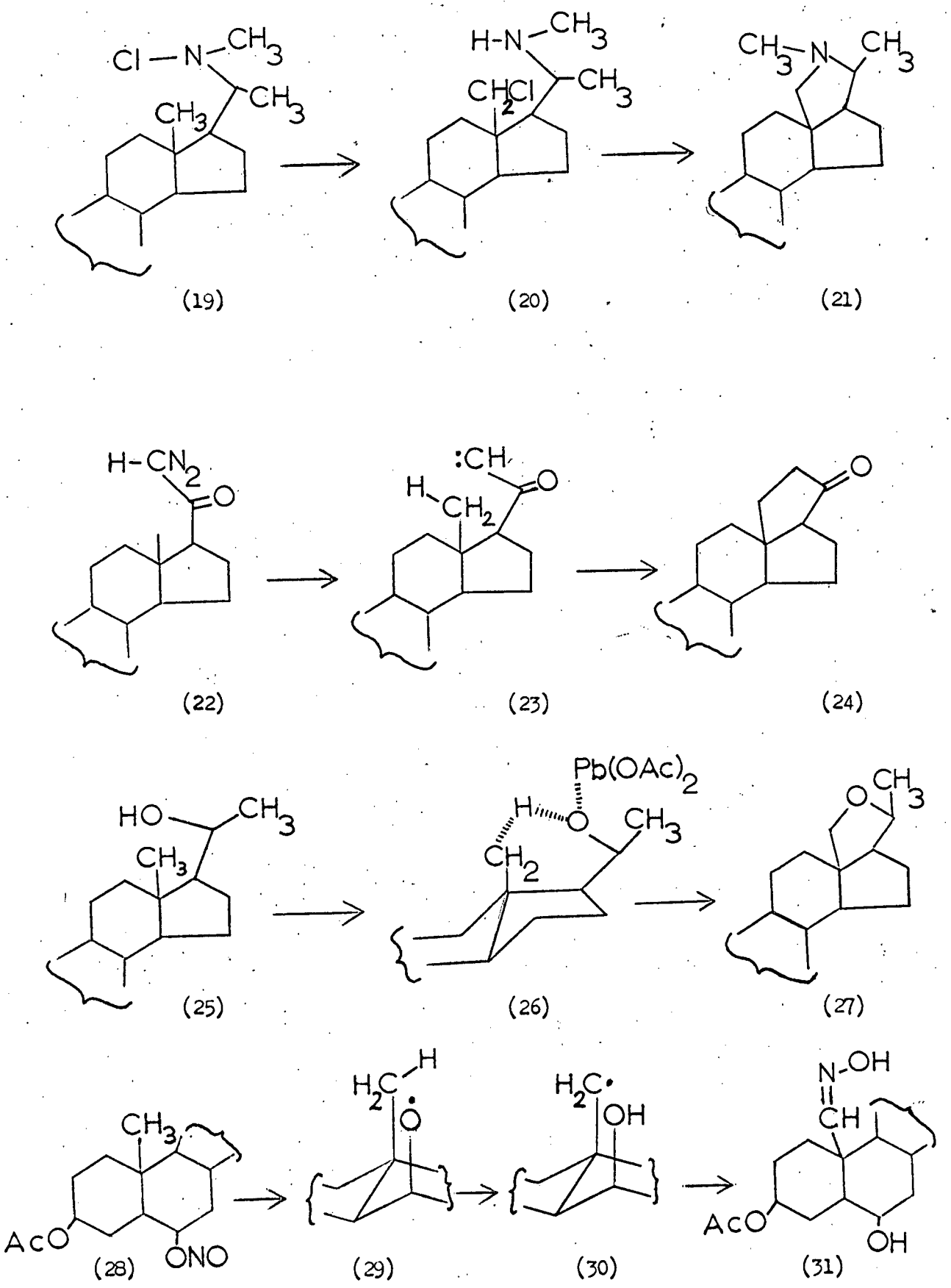


Figure 4. In vitro functionalisation of non-active methyl groups.

mation of a carbon-carbon bond between the C-18 methyl group and C-21 (24) by carbene (23) insertion as shown. Photolysis of azides will cause nitrene insertion in a similar manner, and use has been made of this in Barton's conessine synthesis[68] and Edward's diterpene alkaloid synthesis.[69]

The action of lead tetraacetate on alcohols leads directly to high yields of tetrahydrofuran derivatives, without the formation of a free carbonium ion, when all the partcipating centers are fixed in the arrangement which favours hydrogen abstraction.[70] The treatment of steroidal alcohols such as (25) with lead tetraacetate will give rise to cyclic ethers (27), and the reaction is thought to involve the transition state (26). The reaction has been used to functionalise the C-18 methyl group in an aldosterone synthesis.[71,72]

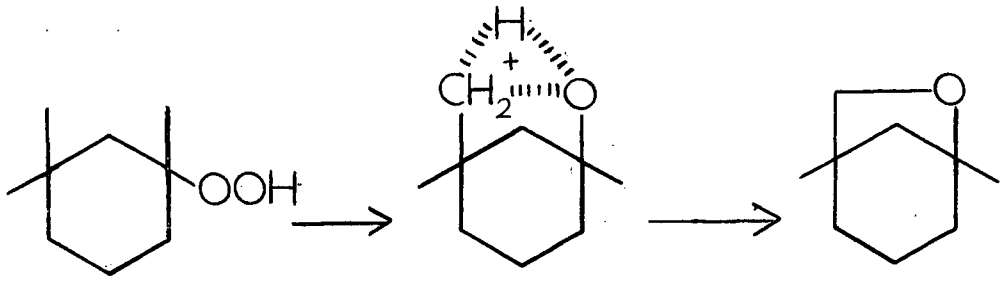
Hypochlorites can be photolysed to give functionalisation of suitably placed inert methyl groups,[70,73] and lead tetraacetate treatment of hypohalites gives similar results.[74]

The photolysis of suitably constituted organic nitrites provokes an intramolecular exchange of the NO of the nitrite residue with a hydrogen atom attached to a carbon in the γ-position. The C-nitroso compounds thus formed can be isolated as the oximes. This reaction has been used to functionalise both C-18 and C-19 methyl groups in steroids by a mechanism as shown (28) - (31).[75,76] The whole process is believed to take place within a solvent cage. The reaction has been used in a partial synthesis of aldosterone.[77]

The methods of functionalising methyl groups by intramolecular abstraction of hydrogen, corresponds to the transfer of a hydrogen atom to an attacking free radical in the same molecule, and hence to a hydrogen shift.

The most frequently observed intramolecular hydrogen transfers are 1,5 shifts. The most favourable transition state for the hydrogen transfer is that of a 6-membered ring in the chair form and the steric requirements for these reactions are more important than the energetic ones. Hydrogen shifts corresponding to 1,2- and 1,3-hydride shifts observed with carbonium and oxonium ions[78] do not occur with oxy-radicals.

The methods of functionalising methyl groups, summarised above, have all involved some sort of free radical reaction, and are thus not good analogies for Corey's[60] model for biological hydroxylation which is presumed to involve attack of cationic oxygen. A closer analogy to this model, of non radical displacement of alkyl hydrogen by oxygen which can be regarded as especially similar to steroid hydroxylation, is the reaction of decalin with ozone. Both cis- and trans-decalin with ozone give cis- and trans-9-hydroxydecalin, respectively, a stereospecific substitution proceeding with retention of configuration[79] and involving electrophilic oxygen. Another close chemical analog for this type of biological reactions of steroids is the formation of a cyclic ether (34) from a hydroperoxide tosylate (32), which involves attack on a carbon unactivated in any classical fashion.[80,85]

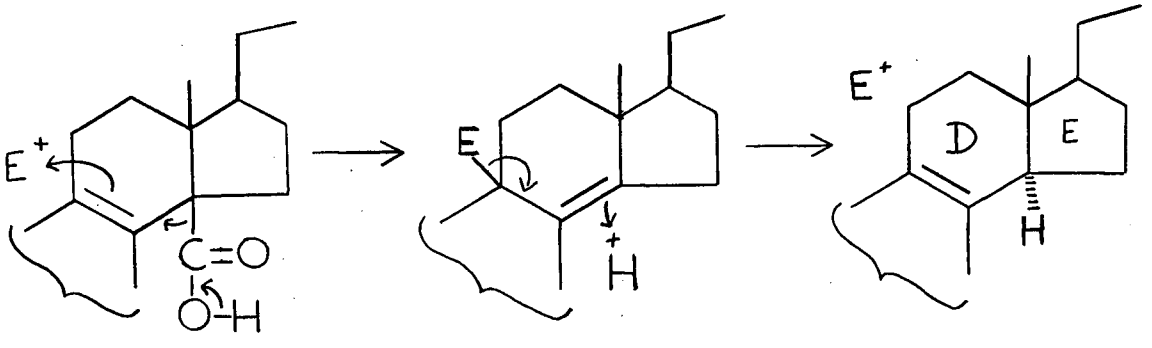


(32) (33) (34)

Thus 1,3,3-trimethylcyclohexane hydroperoxide (32) reacted with para-nitrobenzenesulphonyl chloride in cold pyridine and methylene chloride. Since O-O cleavage of the para-nitrobenzensulphonyl derivative of the hydroperoxide is probably heterolytic under the conditions used the change from hydroperoxide to bicyclic ether (34) is considered proceeding via the cationic transition state such (33). The mechanism is an intermediate between the extremes for attack of cationic oxygen, exclusively on hydrogen or exclusively on carbon. The bicyclic ether has not been detected under different reaction conditions which are known to proceed via a radical mechanism. One important feature of the reaction in this case is its steric facilitation, owing to the proximity of the methyl group and hydroperoxide function. This reaction is interesting as a chemical counterpart of enzymatic hydroxylation at a saturated carbon which also appears to involve electrophilic oxygen, and which proceeds by frontal displacement as implied by the intermediate (33).

DISCUSSION

The first stage in the conversion of lanosterol to cholesterol, "in vivo", is the removal of the 14α-methyl group. There are several possibilities for the removal of the extra methyl carbons: direct loss as a methyl group; preliminary oxidation to a hydroxy-methylene group and loss as formaldehyde; further oxidation to a formyl group and loss as formic acid; or, finally, complete oxidation and loss as CO_2. From the evidence presented[50] the last of these possibilities seems actually to occur. The location of the 8,9-double bond in lanosterol (13) is attractive as an activating feature for the decarboxylation of the C-14 carboxylic acid (35). Such an activated decarboxylation usually results in the migration of the double bond, which would in this case form the 8(14)-unsaturation (36). It is possible that this substance is indeed an intermediate but that it rapidly rearranges to the thermodynamically more stable isomer (37) or that, in the reaction, C-9 acquires an electrophile from an enzyme surface that initiates the loss of CO_2 as shown.



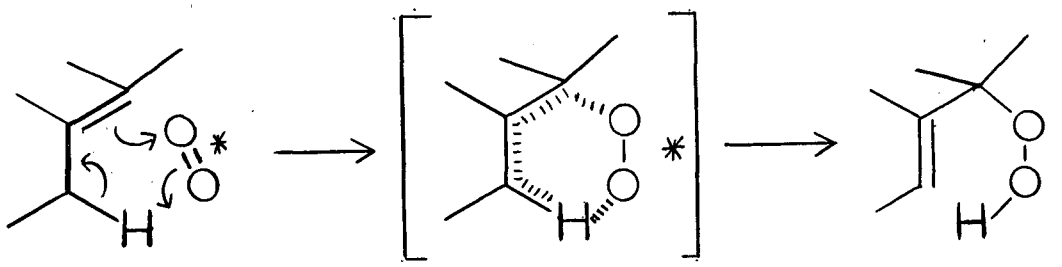
(35) (36) (37)

The enzyme-steroid complex is then cleaved by attack of a proton at C-14 producing the isolated intermediate (37). Such a mechanism will lead to a product with the correct stereochemistry about the D/E ring juncture.

To achieve "in vitro" oxidation of the 14α-methyl, a suitable group would have to be introduced in a 1,3-diaxial relationship to it. The 8(9)-double bond makes the 9α and 7α positions particularly labile, and these are, therefore, the best positions for substitution with a view to removal of the 14α-methyl group as they are both in a 1,3-diaxial relationship to it. Since molecular oxygen is involved in biological hydroxylation[81] a reaction involving molecular oxygen would be a closer "in vitro" analogy.

Autoxidation of olefins is known to give allylic hydroperoxidation,[78, 82] and lanosterol would be expected to oxidize in the C-7 and C-11 positions. Autoxidation of lanosterol is known, however, to give the 7β and 11β hydroperoxides,[83] and this would be useless as a means of obtaining the 7α-hydroperoxide. Photosensitized oxygenation of olefinic double bonds gives allylic hydroperoxides in which the double bond has rearranged.[84] The initially formed hydroperoxide usually survives the reaction conditions and can be isolated and reduced to the allylic alcohol by any of a number of ways. When applied to olefins having nearby functional groups, this oxygenation reaction has considerable potential as a synthetic tool, and also yields information on various factors that might influence sensitized photochemical processes.[84,87] The proposed mechanism[84] of this reaction involves excitation of the sensitizing dye on absorption of light energy, and the formation of a labile photosensitizer-oxygen complex, which in turn oxidizes the substrate. The findings[86] suggest a cyclic mechanism for the olefin-oxygen combination, after the system has been suitably energized.

The sensitizer-oxygen complex breaks down to yield excited oxygen (O_2*) which will react with the double bond (38) to give the rearranged allylic hydroperoxide (40)via the six membered transition state (39).



(38) (39) (40)

Several detailed variants for such a cyclic process can be envisaged according to whether it is concerted or not, and depending on the nature of the bonds in the transition states or intermediates, and the extent (if any) of participation by the sensitizer when the oxygen attacks.

This photosensitized oxygenation reaction is also of special interest as a possible pathway for biological oxidations, and various steroidal olefins have been hydroperoxidized in this manner.[84,86,87] The attack has usually been from the less hindered α-face of the molecule and the bonds formed and broken (C-O and C-H respectively) are cis to each other, with no implication about the timing of the events or the extent of participation by the sensitizer. The result with steroids and simpler olefins indicated that the reaction is subject to steric hindrance.[87] In dihydrolanosterol (41) the C-8 position is more hindered than the C-9 position due to the proximity of the 14α-methyl group and thus 9α-hydroperoxylanost-7-ene-3β-ol (42) would be the expected product of photosensitized oxidation.

When dihydrolanosterol was oxygenated in the presence of light and haematoporphyrin, and the resultant product was reduced and acetylated, 3β-acetoxylanosta-7,9(11)-diene (48) was obtained as the only solid product.[88] This result might indicate that 9-hydroperoxidation had in fact occurred since the 9α-alcohol would be readily dehydrated under acetylation conditions. However, the photo-oxygenation of dihydrolanosteryl acetate (47) gave the same result, and attempts to isolate the 9α-hydroperoxide were unsuccessful.

Dihydrolanosterol (41) could be used to functionalize the C-14 methyl group in situ under the influence of a powerful leaving group such as the para-nitrobenzenesulphonyl group (see Figure 5). The C-9 C-14 methyl ether (43) would be the expected product by analogy to the Corey reaction (see Introduction) and this ether could be oxidized to the unsaturated lactone (44) which would open to the acid (45) from which the C-14 group would be readily lost by decarboxylation. In this sequence, the C-14 methyl group would be removed, and the double bond would be shifted to the 7 position. Both of these are steps in the in vivo conversion of lanosterol to cholesterol, and an in vitro realization of these steps via a series of oxidation reactions, would establish a mile-stone in an area traditionally considered to be a sector of biochemistry, namely, the field of enzyme mechanism. Previous attempts[88] of C-14 methyl activation by photosensitized oxygenation were unsuccessful, as the expected C-9 C-14 cyclic ether could not be isolated or detected in the reaction product. When in the hope of functionalizing the C-14 methyl group via the C-7 C-14 methyl ether 3β-acetoxylanost-8-ene-7α-hydroperoxide (50) was treated with para-nitrobenzensulphonyl chloride in pyridine, no reaction occurred, the only compound isolated was the starting material.

The lability or intractability of any 9α-hydroperoxide formed in the

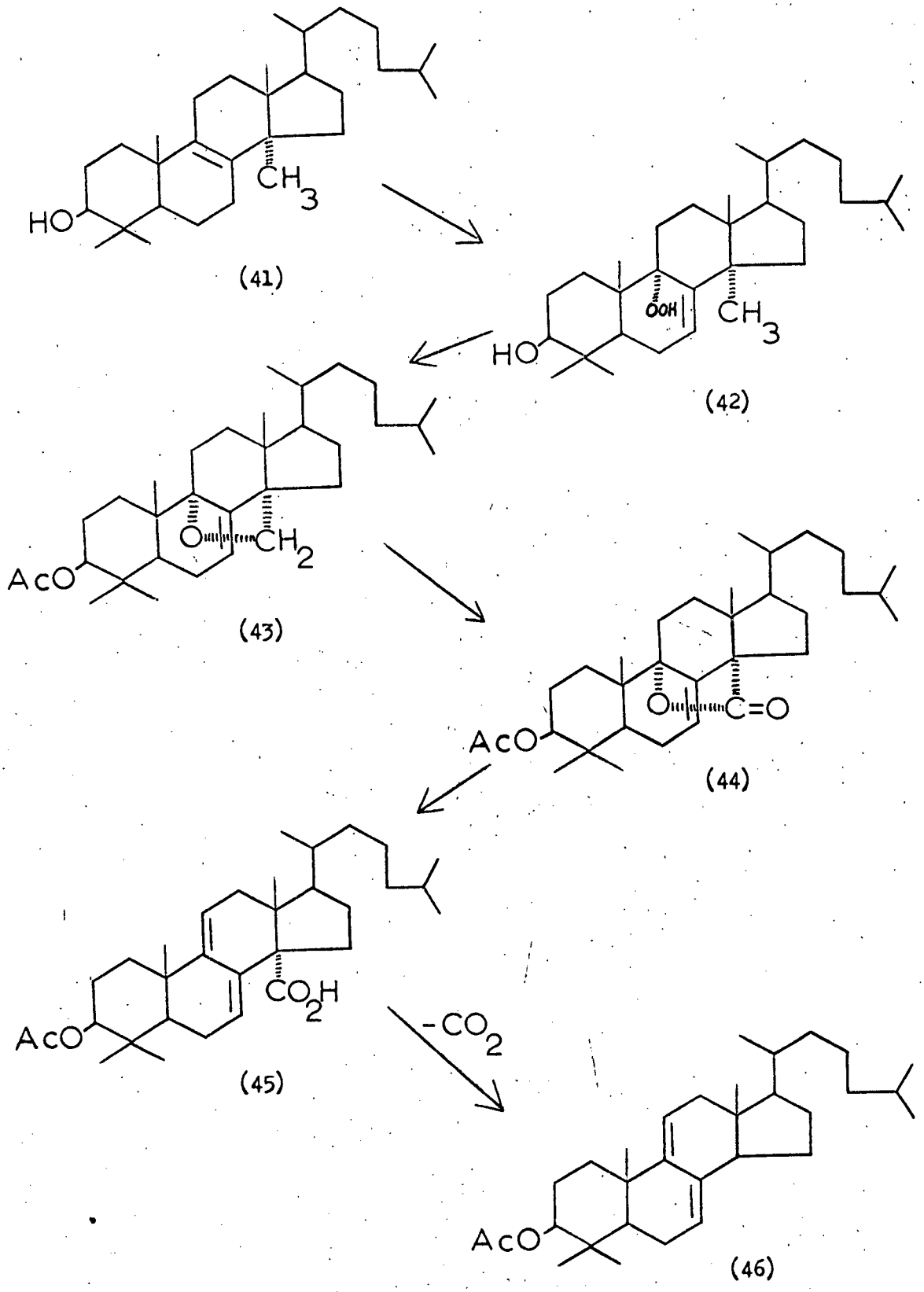


Figure 5. Proposed scheme for the removal of the 14α-methyl group in dihydrolanosterol (41).

photo-oxygenation reaction might be overcome by carrying out the reaction in the presence of para-nitrobenzensulphonyl chloride so that the peroxide might react with the 14α-methyl group. Therefore the in situ photo-oxygenation reaction was repeated. Dihydrolanosteryl acetate (47) (see Figure 6) was oxygenated in pyridine in the presence of light, haematoporphyrin, and para-nitrobenzensulphonyl chloride. The reaction product was worked up the usual way, and a semi-solid was obtained which was chromatographed on an alumina colum.

On developing the chromatographic column, first, petroleum ether eluted unchanged para-nitrobenzensulphonyl chloride followed by 3β-acetoxylanosta-7,9(11)-diene (48).

Next petroleum ether eluted a compound which was designated as IP1 m.p. 141-144°. Its infrared spectrum had peaks at 1730, 1240 (acetate) and 823 cm^{-1}, the ultraviolet absorption was at 209 mμ (ε 8960). This compound was found to be identical to that obtained, and not identified, in a previous study[88] (Infrared, Ultraviolet spectrum and mixed m.p.).

In subsequent fractions on elution with petroleum ether and petroleum ether-benzene, 3β-acetoxylanost-8-ene-7-one (49) and 3β-acetoxylanost-8-ene-7α-hydroperoxide (50) were obtained respectively. Since allylic hydroperoxidation[88] had yielded the 7α-hydroperoxide, it was possible that the latter compound had been formed by rearrangement of the first formed 9α-hydroperoxide. The rearrangement of tertiary to secondary hydroperoxides had been reported,[89,90] and the mechanism requires retention of configuration.

The compound IP1 analyzed correctly for the required cyclic ether (43), and the mass spectroscopic molecular weight of 484 is in agreement with the correct analysis for $C_{32}H_{52}O_3$. Since it had no carbonyl or hydroxyl

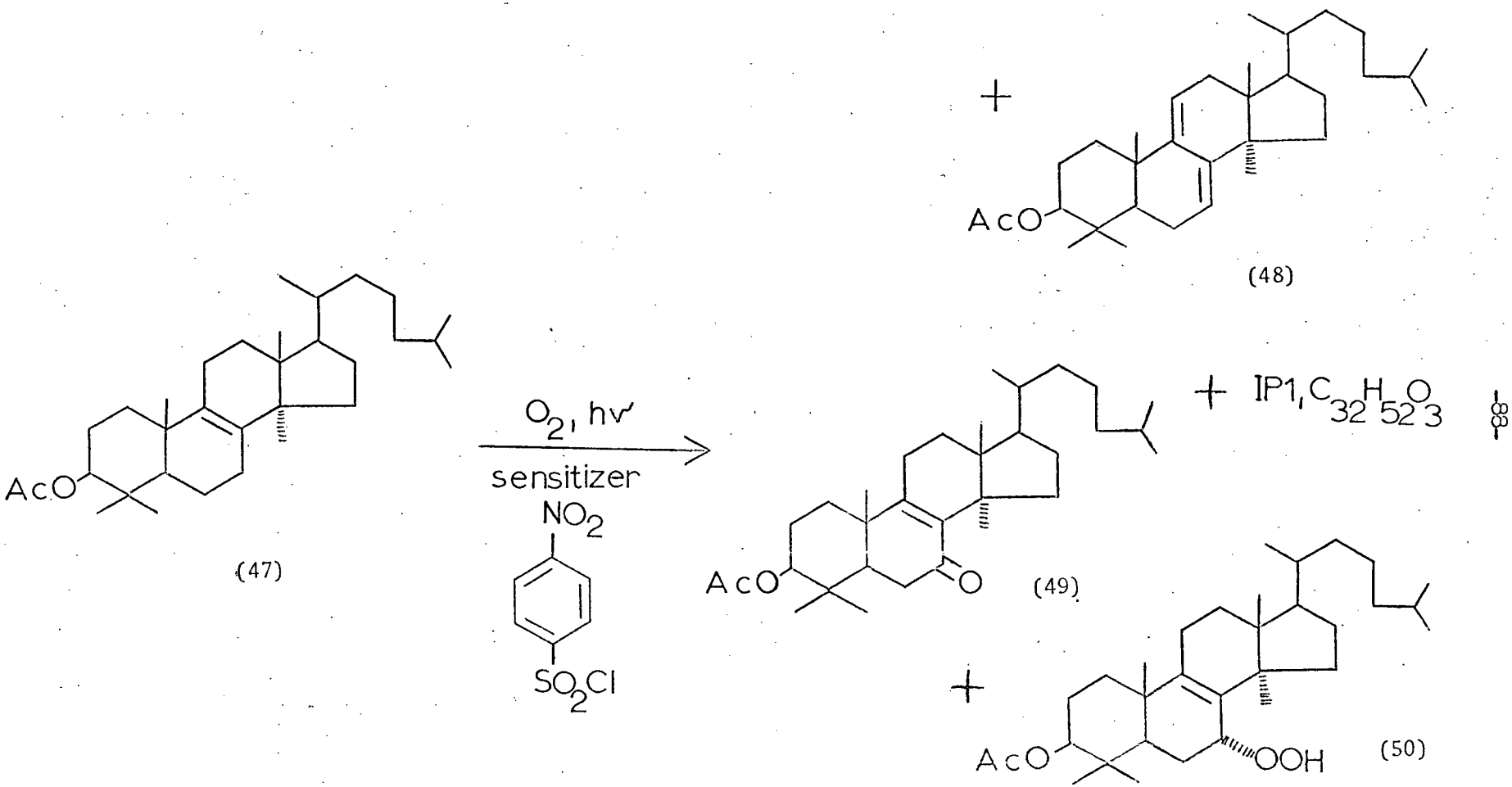


Figure 6. Photosensitized oxygenation of dihydrolanosteryl acetate (47), in the presence of para-nitrobenzenesulphonyl chloride.

functions, the compound was presumably an ether. An earlier study[88] showed that treatment of this compound with chromium trioxide in acetic acid gave 3β-acetoxylanost—8-ene-7-one (49), and reduction with potassium iodide in acetic acid gave 3β-acetoxylanosta-7,9(11)-diene (48) (see Figure 7). Oxidation under conditions which had yielded lactones from steroidal cyclic ethers failed to give products with lactone absorption in the infrared. On treatment with boron trifluoride etherate compound IP1 gave two products. The first one of these was an enone $C_{32}H_{52}O_3$, supported by Infrared and Ultraviolet spectral data. The n.m.r. showed no olefinic proton, so the enone double bond was tetrasubstituted. The second product from the boron trifluoride treatment of the ether IP1 was a ketol, substantiated by its infrared spectrum. The Ultraviolet spectrum was transparent. The alcoholic grouping was considered to be secondary in view of a proton at τ 6.8 in the n.m.r spectrum. This proton was coupled with two protons on an adjacent carbon atom and was considered to be axial since the quartet had similar spin-spin coupling to the 3α axial proton at τ 5.51. Since this proton is axial, the alcoholic group must be equatorial.

At this point none of the various hypothetical structures which are possible has fitted all of the physical and chemical data available for compound IP1. Therefore, it was decided to tackle the problem by X-ray methods. Since the compound is presumably an ether but not the expected cyclic ether (43), it was hoped that it might be a suitable intermediate which can be functionalized in subsequent steps for the removal of the 14α methyl group. The compound IP1 was readily brominated by pyridine perbromide to give the dibromo-derivative which analyzed correctly for $C_{32}H_{52}O_3Br_2$. Its Ultraviolet spectrum was transparent, and the Infrared spectrum showed absorptions at 1745 and 1240 cm^{-1} (acetate).

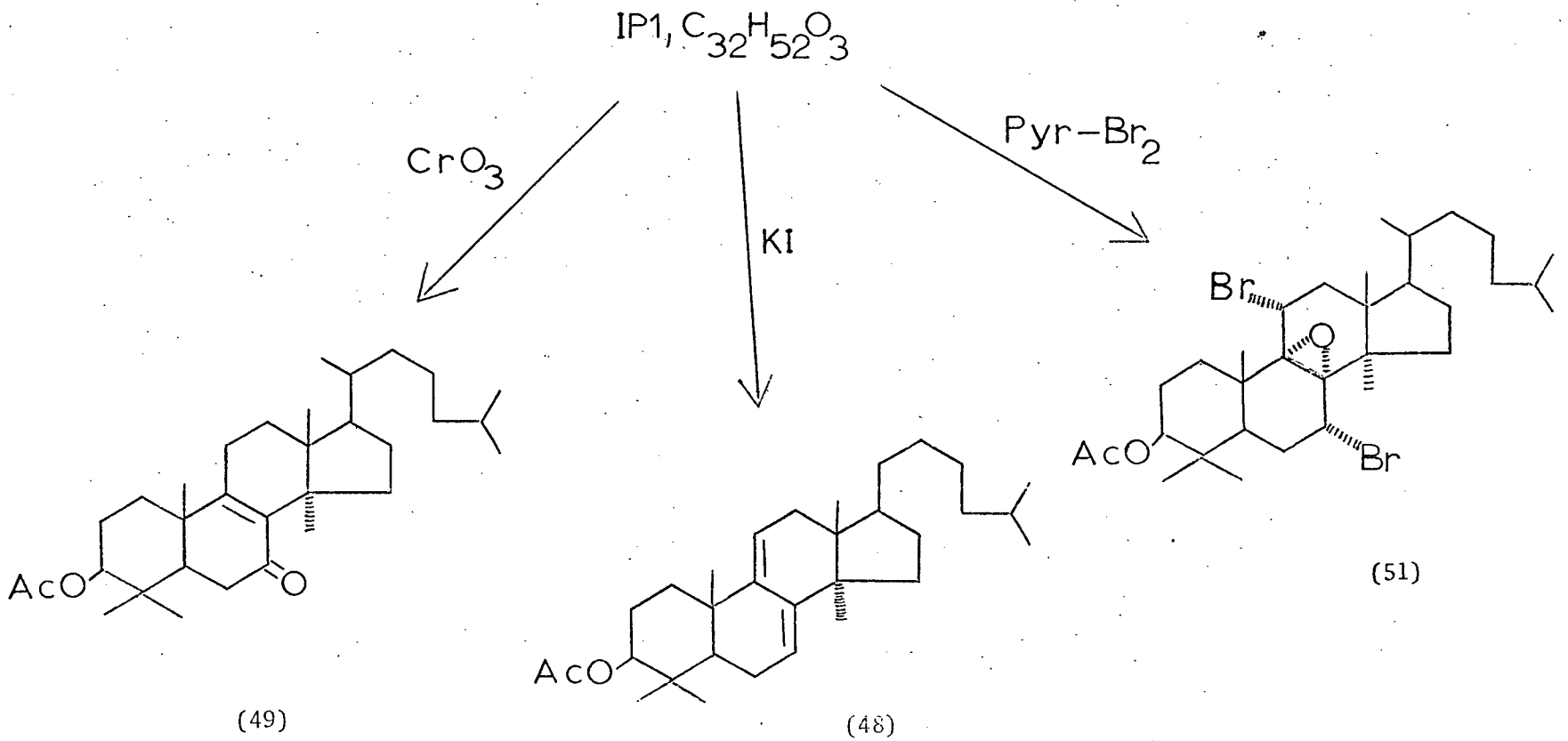


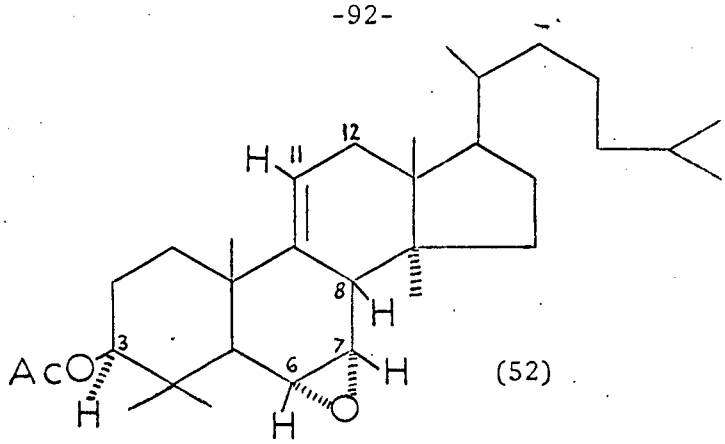
Figure 7. Chart showing the reactions of compound IP1 with chromium trioxide, potassium iodide and pyridine perbromide.

The X-ray crystallographic study was carried out in this Department.[91] The crystals of the dibromo-derivative are orthorhombic, $\underline{a}$ = 26.03_5, $\underline{b}$ = 9.88_5, $\underline{c}$ = 12.26_8 A, $\underline{Z}$ = 4 space group $\underline{P2_12_12_1}$. The intensities of about 2400 reflections were measured on a G.E. spectrogoniometer with scintillation counter, using Cu$\underline{K}\alpha$ radiation. The two bromine positions were determined by Patterson methods and all carbon atoms were located on three successive three-dimensional electron-density distributions. Positional and isotropic temperature parameters were refined by four cycles of least squares; a further six cycles of anisotropic least squares completed the refinement giving a final $\underline{R}$ value of 13.3%. The absolute configuration was determined by the anomalous dispersion method.

The dibromo-derivative of compound IP1 is 3β-acetoxy-7α,11α-dibromolanostane-8α,9α-epoxide (51). Steroid ring A is in the normal chair form and ring D has a half-chair conformation. The epoxide prevents rings B and C from adopting the chair form. The bond lengths and valency angles are normal, and the intermolecular separations correspond to Van der Waals interactions.

In summary, the oxidation product IP1 is an ether and we feel strongly that it is a disubstituted 1,2-epoxide in view of the protons in the 7τ region of the n.m.r. spectrum. The formation of a ketone-secondary alcohol with boron trifluoride etherate would also indicate that the ether termini are very probably secondary, and the structure (52) can be assigned tentatively which would fit all the physical at hand.

The n.m.r. spectrum in deuterochloroform could not be evaluated with certainty. However, in benzene solution, where a solvent shift was observed,



(52)

a better resolution was obtained and assignments were readily made as follows: The olefinic proton at C-11 was assigned to a quartet at τ5.15 (area = 1 H). This proton is coupled with the two proton on C-12. The 3α-axial proton on the same carbon as the acetate function occurred at τ5.48 with $J_{axial\text{-}axial}$= 8 c.p.s. and $J_{axial\text{-}equatorial}$= 3 c.p.s. as expected (area = 1 H). The pair of doublets at τ6.95 and at τ7.15 were assigned to the two epoxide protons (area = 1 H and J = 3.5 c.p.s., respectively). A doublet at τ7.55 (J = 5 c.p.s., area = 1 H) was assigned to the proton at C-8, and the abnormal downfield shift may be attributed to the deshielding by the double bond. A complex pattern of lines occurred between τ7.6-9.5 where individual assignments are not possible.

Finally, it is clear from the above that a most unusual rearrangement, not encountered so far, must occur in the bromination of compound IP1. It is difficult to conceive of a disubstituted 1,2-epoxide in the lanosterol skeleton from which the dibromide (51) can be derived, and which also fits all the chemical and physical data available.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Ultraviolet spectra were measured on a Cary 14 spectrophotomer and infrared spectra were taken on a Perkin Elmer Model 137B spectrophotometer. Nuclear magnetic resonance (n.m.r.) spectra were recorded at 60 Mc/s on a Varian A60 instrument. The line positions or centers of multiplets are given in the Tiers τ scale with reference to tetramethylsilane as the internal standard. Alumina G (according to Stahl) plates were used for thin layer chromatography (T.L.C.). The alumina used for column chromatography was Woelm neutral reagent, deactivated with 6% of water. The mass spectrum was determined on a A.E.I. MS9 Double Focusing Mass Spectrometer.

Elemental microanalyses were performed by Mrs. C. Jenkins of this Department, and by Dr. A. Bernhardt and his associates of the Max Planck Institute, Mulheim, Ruhr, West-Germany. The n.m.r. and mass spectrometric determinations were done by Mrs. A. Brewster and Mr. G. Bloss of this Department respectively.

The X-ray crystallographic study was carried out by Dr. J. Trotter and by Mr. J. K. Fawcett in this Department.

Lanosteryl Acetate

30 g of Lanosterol (K & K) was acetylated, with 50 ml of acetic anhydride (reagent) and 80 ml of dry pyridine (reagent) at room temperature overnight. The excess of reagent was removed under reduced pressure on the steam bath and the residue was dissolved in ether, washed with dilute hydrochloric acid and then several times with water and dried (anhydrous

magnesium sulphate). Evaporation of the ethereal solution under reduced pressure gave 28.5 g of lanosteryl acetate which was recrystallized from ethanol. m.p. 123-124°. Infrared spectrum in Nujol: 1739 (OAc) and 1240 (OAc) cm^{-1}.

Dihydrolanosteryl Acetate (47)

Lanosteryl acetate (25 g) was hydrogenated in ethyl acetate (200 ml) containing 1 g of platinum oxide, under hydrogen atmosphere with stirring for 24 hours. After removal of the catalyst by filtration the dihydrolanosteryl acetate was recrystallized to constant melting point from ethyl acetate. m.p. 119-120°. (literature m.p. 120-121°).[83]

Anal. Found: C, 81.5%; H, 11.3%. Calc. for $C_{32}H_{54}O_2$: C, 81.7%, H, 11.5%. T.L.C. one spot. R_f = .87 in benzene-chlorofrom (1:1).

Infrared spectrum in Nujol: 1750 (s), 1242 (s), 1035 (s), 1010 (m), 905 (w) and 870 (w) cm^{-1}.

Oxygenation of Dihydrolanosteryl Acetate in the presence of Para-nitrobenzene Sulphonyl Chloride

10 g of dihydrolanosteryl acetate (3-β-acetoxylanost-8-ene), 4.9 g of para-nitrobenzene sulphonyl chloride and 150 mg of haematoprophyrin were dissolved in 260 ml of dry pyridine (reagent) contained in a 500 ml. round-bottom flask. The solution was irradiated with three G. E. 20 watt 250 volt fluorescent tubes for 8 days while oxygen was bubbled through the solution. The pyridine was removed under reduced pressure and the solid residue was dissolved in ether. The ether solution was subsequently

extracted with water, dilute sulphuric acid and water. The ethereal solution was dried (anhydrous magnesium sulphate), and the solvent evaporated under reduced pressure to give 16 g of a semi-solid which was chromatographed on alumina (450 g). T.L.C. on the crude product showed 7 spots in benzene-chloroform (1:1), and the product liberated iodine from sodium iodide solution.

The organic material was added to the top of the column (22 x 2.5 cm diameter) by dissolving it in a mixture of petroleum ether (80-110°)-benzene (17:2). On elution the following fractions were then obtained consecutively:

Elution with 80 ml of petroleum ether (40-60°) gave unchanged para-nitrobenzene-sulphonyl chloride.

Elution with 250 ml of petroleum ether (40-60°) gave 3β-acetoxylanosta-7,9(11)-diene which was crystallized from acetone as needles (658 mg). m.p. 161-164°. Ultraviolet spectrum in ethanol: λ_{max} 237, 246 and 255 m . Infrared spectrum in Nujol: 1745 (s), 1240 (s), 1030 (m), 980 (m), 905 (w), 875 (w) and 820 (w) cm^{-1}. This compound gave no depression in melting point by admixing it, and had an identical infrared spectrum with an authentic sample.[88]

Elution with 500 ml of petroleum ether (40-60°) gave a solid which crystallized from acetone as needles (95 mg). m.p. 141-144°. This compound was designated IP1, and was found to be identical to that obtained earlier by another group of workers[88] (mixed m.p., Infrared and Ultraviolet Spectrum. $[\alpha]_D$ = +161 in ethanol. Ultraviolet spectrum in ethanol: λ_{max} 209 (ϵ 8960). Infrared spectrum in Nujol: 1730 (s), 1240 (s), 1095 (w), 1070 (m), 1050 (w) 1030 (m), 1010 (w), 975 (w), 960 (w) and 823 (m) cm^{-1}. T.L.C. showed one spot. R_f= 0.61 in benzene-chloroform (1:1). Mass spectrum showed significant peaks at m/e = 484 (M^+), 483 (M-1), 469 (M-15), 441 (M-43), 424 (M-60),

371, 329 (M-155), 311, 289, 247, 231, 208, 207, 193 and 161. n.m.r. signals; given in τ units, spectrum obtained in benzene: multiplet centered at 5.18, quartet centered at 5.4 correspondign to the 3α-axial proton on the same carbon as the acetate function, doublet centered at 6.96 (J = 3.5 c.p.s.), doublet centered at 7.15 (J = 3.5 c.p.s.) doublet centered at 7.52 (J = 5 c.p.s.), and a complex pattern of lines between 7.8-9.5.

Elution with 1000 ml of petroleum ether (40-60°) yielded nothing.

Elution with 500 ml of petroleum ether (80-110°) gave traces of crystals.

Elution with 1750 ml of petroleum ether (80-110°) gave 3β-acetoxylanost-8-ene-7-one which crystallized from petroleum ether (40-60°) as needles (1.32 g). m.p. 147-150°. Ultraviolet spectrum in ethanol: λ_{max} 254 mμ. Infrared spectrum in Nujol: 1740 (s), 1650 (s), 1580 (s), 1240 (s), 1080 (w), 1030 (m), 1010 (m), 975 (m) and 900 (w) cm^{-1} T.L.C. showed one spot. R_f= 0.64 in benzene-chloroform (1:1). This compound was found to be identical to that of an authentic sample (mixed m.p., infrared spectrum).[88]

Elution with 1500 ml of petroleum ether (80-110°) yielded 300 mg of oil.

Elution with 300 ml of petroleum ether (80-110°) gave nothing.

Elution with petroleum ether (80-110°)-benzene (2:1) gave 3β-acetoxylanost-8-ene-7α-hydroperoxide which crystallized from ethyl acetate as needles (310 mg). m.p. 174-175°. Infrared spectrum in Nujol: 3400 (s), 1730 (s), 1275 (s), 1040 (m) 1020 (m), 980 (m), 930 (w) and 860 (w) cm-1. T. L.C. showed one spot. R_f = 0.37 in benzene-chloroform (1:1). The compound had only end absorption in the ultraviolet, and it gave no depression

on a mixed melting point determination with an authentic sample.[88] At this point the development of the chromatographic column was discontinued.

Bromination of Compound IP1

The compound IP1 (45 mg) was dissolved in dioxan (8 ml) with pyridine perbromide (45 mg) and the solution was left 4 hours at room temperature. The excess of bromine was removed by the addition of sodium thiosulphate solution. The aqueous suspension was extracted three times with ether and the combined ethereal extracts on evaporation, after drying (anhydrous magnesium sulphate), gave a solid. The product was crystallized from acetone to give sturdy needles and dried. m.p. 199-200°. Anal. Found: C, 59.93%; H, 8.2%. Calc. for $C_{32}H_{52}O_3Br_2$: C, 59.7%; H, 8.03%. Infrared spectrum in Nujol: 1745 (s), 1240 (s), 1030 (m), and 905 (w) cm^{-1}. The ultraviolet spectrum was transparent. The dibromide of compound IP1 was identified by X-ray crystallographic analysis as 3-β-acetoxy-7α,11α-dibromo-lanostane-8α,9α-epoxide.

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