C.1

ANALYTICAL AND BIOLOGICAL STUDIES OF SESQUITERPENE LACTONES

by

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

Department of Botany

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

January 1981

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Date February 4, 1981

ABSTRACT

Three related analytical and biological studies of sesquiterpene lactones mainly from Compositae were conducted. In the first one I examined various thin-layer chromatography (TLC) techniques for identification of sesquiterpene lactones and polyacetylenes. Vanillin and p-dimethylaminobenzaldehyde were found to be most useful of several benzaldehyde and benzoic acid derivatives for the detection of these compounds. Colors produced with these two reagents and with 78 sesquiterpene lactones and 25 polyacetylenes were of all shades of the spectrum and were specific for individual compounds. Therefore, these reagents can be used as an aid in the identification of these compounds by TLC. These spray reagents are the most sensitive of all reagents which have so far been used for the detection of sesquiterpene lactones or polyacetylenes using TLC.

The vanillin spray reagent was used in the second study for the detection and preliminary identification of major sesquiterpene lactones in crude extracts of 29 samples of Parthenium hysterophorus representing specimens from various areas of its world distribution. Some sesquiterpene lactones detected by this technique were subsequently isolated and also identified by NMR analyses. Three sesquiterpene lactones, coronopilin, hysterin, and dihydroisoparthenin, were shown for the first time to occur in this species.

The chemical analyses of 10 selected compounds from 29 samples indicated the existence of 11 chemical types of P. hysterophorus. The most widespread is the North American type which is also present in Belize, Australia, and India. However, the South American samples differ greatly in their sesquiterpene lactone composition from the North American plants. In addition, the comparison of samples from various South American populations showed a high degree of diversity in chemistry. These results suggest the possibility of the existence of either several forms, or subspecies, or perhaps even several species of this plant.

In the third study I examined the relationship between various biological activities of sesquiterpene lactones and their chemical structures. Antimicrobial screening tests were performed with 57 sesquiterpene lactones against 6 bacteria and 45 sesquiterpene lactones against 3 fungi. The presence of certain structural moieties in the compounds seems to be necessary for antimicrobial activities. However, different functional groups appear to be required for the different skeletal classes of sesquiterpene lactones and for different types of microorganisms. In addition, some other functional groups appear to enhance activity whereas others reduce activity of sesquiterpene lactones. Finally, the configuration of some functional groups alone may significantly influence the activity of certain sesquiterpene lactones. These results indicate that there is no simple general relationship between the antimicrobial activity of sesquiterpene lactones and their chemical structure.

Four structurally similar sesquiterpene lactones were tested for their effects on survival of flour beetles. Three compounds containing the exocyclic methylene group on the lactone ring (coronopilin, helenalin, and parthenin) significantly reduced survival of beetles, whereas tenulin, lacking this moiety, had no effects. Therefore, the exocyclic methylene is most likely responsible for the detrimental properties of these sesquiterpene lactones on survival of the beetles.

Many sesquiterpene lactones are potential allergens. In the last study cross-reactivity to 6 structurally similar sesquiterpene lactones of parthenin sensitized guinea pigs was examined. The presence of the exomethylene on the lactone ring was found to be necessary for cross-reactivity. However, compounds which were progressively structurally more different than parthenin, gave weaker allergenic responses.

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ACKNOWLEDGEMENTS

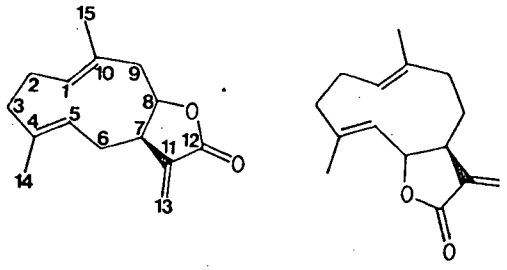
I would like to thank my supervisor, Professor G. H. N. Towers, for invaluable discussions and advice throughout this study as well as for helpful comments on this thesis. I also thank members of my research committee, Dr. A. D. M. Glass, Dr. D. G. Kilburn, Dr. J. C. Mitchell, and Dr. I. E. P. Taylor, for helpful suggestions on this manuscript. I am grateful to many individuals listed in the Sections I and II who kindly provided samples of sesquiterpene lactones, polyacetylenes, and Parthenium hysterophorus. The study would not have been possible without their help. I thank Z. Abramowski, F. Balza, Dr. I. Panfil, and Dr. C.-K. Wat for helpful discussions. F. Balza also assisted with the hydrogenation of parthenin, performed at the Department of Chemistry, U.B.C., and with the identification of hysterin. I thank M. M. Tracey of the Department of Chemistry, U.B.C., for measurements of many NMR spectra. I am grateful to Dr. R. H. Elliott who kindly provided the flour beetles for my experiements and R. A. Norton for a slide of P. hysterophorus. Finally, I would like to express my special thanks to my husband, Dr. J. Picman, for his encouragement and invaluable help in all aspects of my work.

PREFACE

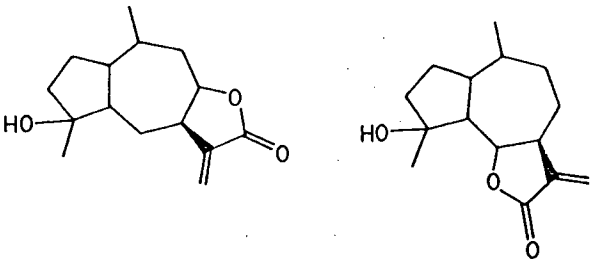
1. Chemistry of sesquiterpene lactones

Sesquiterpene lactones are a group of terpenoids derived from isoprenoid units attached together via head-to-tail condensation and subsequent cyclization followed by oxidative modifications (Geissman 1973, Herz 1973). Most of the known naturally occurring sesquiterpene lactones are classified according to their carbocyclic skeletons into four basic classes: germacranolides (with a ten-membered ring), eudesmanolides (6/6 bicyclic compounds), guaianolides and pseudoguaianolides (5/7 bicyclic compounds) (Geissman and Crout 1969, Yoshioka et al. 1973; Fig. 1). An important common feature of sesquiterpene lactones is the presence of a γ-lactone ring with an α-methylene in many of them. The lactone ring is β-oriented in all lactones of known stereochemistry. It is cis or trans fused to the C6,C7 or the C8,C7 position. Basic skeletal modifications involve the incorporation of an epoxide ring, hydroxyls or esterified hydroxyls, and/or tiglic or angelic acid at a variety of positions (Yoshioka et al. 1973). A few sesquiterpene lactones occur in glycosidic form (e.g. Herz et al. 1970, 1978) and some contain halogens (Siuda and DeBernardis 1973).

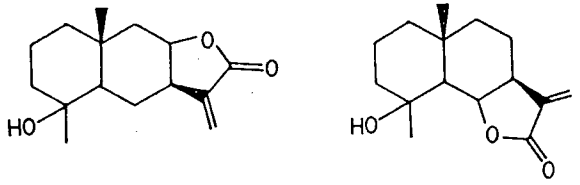
Fig. 1. Basic skeletal classes of sesquiterpene lactones.



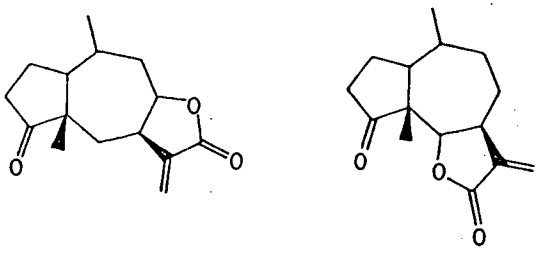
Germacranolides



Guaianolides



Eudesmanolides



Pseudoguaianolides

2. Distribution of sesquiterpene lactones

Approximately 900 sesquiterpene lactones are known at present (Mabry and Gill 1979) but the number of these compounds is rapidly increasing. A great majority of sesquiterpene lactones occur in members of the Compositae, and these compounds together with polyacetylenes characterize this large family of plants (Mabry and Bohlmann 1977). Differences in skeletal types and quantities of sesquiterpene lactones in different genera and species of this family have been utilized in chemotaxonomic studies (e.g. Mabry 1970, Herz 1973, Herout 1973, Geissman and Irwin 1973, Kelsey and Shafizadeh 1979).

Generally, an individual plant will produce sesquiterpene lactones of one skeletal type mostly with oxidative and esterification variations on that skeleton. However, different species of genera with a wide geographical range or sometimes even different populations of a single species may form different skeletal types, as is known, for example, for different species of the genus Ambrosia (Mabry 1970).

In many composites sesquiterpene lactones are located in trichomes which cover aerial parts of the plants (Rodriguez et al. 1976a, Picman 1977, Picman et al. 1979, Picman and Towers, unpublished results). In Parthenium hysterophorus sesquiterpene lactones are found also in pollen (Picman et al. 1980), achenes, and seedlings (in the first true leaf which bears trichomes). Younger seedlings, with cotyledons only, lack trichomes and do not contain detectable amounts of sesquiterpene lactones (Picman 1977, Picman et al. 1979). The highest concentration of

sesquiterpene lactones (up to 8% of the dry weight of plants) is usually found in the flowering heads and leaves. Stems have small quantities of these compounds (Rodriguez et al. 1976a), presumably because of a relatively smaller surface area covered with trichomes in stems than in leaves (Picman 1977). Roots usually have no or very small quantities of sesquiterpene lactones (Rodriguez et al. 1976a,b, Picman 1977, Picman et al. 1980), however, the isolation of two sesquiterpene lactones from the root bark of Liriodendron tulipifera (Magnoliaceae) has been reported (Doskotch and El-Feraly 1969).

Although most of all known sesquiterpene lactones have been isolated from species of the Compositae, these compounds occur sporadically also in other families of plants. In Angiosperms, they have been reported from Umbelliferae, Magnoliaceae, Lauraceaeae, Winteraceae, Illiciaceae, Aristolochiaceae, Menispermaceae, Cortiariaceae, and Acanthaceae (Yoshioka et al. 1973, and references therein). A few sesquiterpene lactones have also been isolated from liverworts (Hepaticeae), such as Frullania dilatata and F. tamarisci (Knoche et al. 1969), Diplophyllum albicans (Benesova et al. 1975, Asakawa et al. 1979), and Porella species (Asakawa et al. 1976). The fungus, Aspergillus terreus (Ranieri and Calton 1978), and some mushrooms, for example Lactarius vellereus, L. pergamenus (Magnusson and Thoren 1973), and L. blennius (Vidari et al. 1976), have been found to contain this type of compounds.

Sesquiterpene lactones along with many other groups of compounds such as alkaloids, phenolic compounds, and glycosides

are products of secondary metabolism whose functions in the primary metabolism of plants are not known (Czapek 1925, in Mothes 1976). It has been suggested (Fraenkel 1959) that these compounds evolved in plants as a means of defense against herbivorous predators such as insects. They may also play a role in reducing competition with other plants (Dalvi et al. 1971) and in the defense of plants against various pathogenic organisms (Wallace and Mansell 1976).

This thesis reports studies on certain biological and chemical aspects of sesquiterpene lactones. In the first section, I describe a new thin layer chromatography (TLC) visualization technique for identification of sesquiterpene lactones. In the section II, I report the use of this new TLC technique in a chemotaxonomic study of major sesquiterpene lactones present in various populations of P. hysterophorus. The purpose of this study was to establish the relationships between various populations of this common weed throughout its recent range of distribution. Finally, in the Section III, I report various biological activities of sesquiterpene lactones in relation to their chemical structures. This study was designed to elucidate the role of various functional groups in determining the activity of sesquiterpene lactones against various types of organisms.

S E C T I O N I

Visualization reagents for sesquiterpene lactones and polyacetylenes on thin layer chromatograms

Introduction

The Compositae family characteristically contains two biologically active types of secondary metabolites, namely sesquiterpene lactones and polyacetylenes (Heywood et al. 1977). In spite of an increasing interest in the cytotoxic, antibiotic, phototoxic, and antineoplastic activities of these compounds (Rodriguez et al. 1976b, Towers and Wat 1978) satisfactory reagents for their visualization on thin layer chromatograms have not been reported. The techniques generally used for the detection of sesquiterpene lactones by thin layer chromatography (TLC) or paper chromatography (PC) are UV light (Ivie et al. 1975a,b), exposure to iodine vapours (Mabry 1970, Rodriguez 1975a), spraying with a solution of $KMnO_4$ (Rodriguez 1975a), or with concentrated H_2SO_4 (Geissman and Griffin 1971), followed by heating (Hall et al. 1977). These methods are generally unspecific giving the same color reaction with almost all unsaturated compounds. Acid catalyzed color reactions of certain sesquiterpene lactones have been described (Geissman and Griffin 1971, Griffin et al. 1971), but the technique is restricted to those lactones which form color complexes in acidic solution. Polyacetylenic compounds are often identified by their highly characteristic UV spectra (Bohlman et al. 1973) but, with the

exception of thiophenes (Curtis and Phillips 1962), no simple reliable method for the visualization of these compounds by TLC or PC has been described.

In this section I describe a new technique for the separation, visualization, and identification of sesquiterpene lactones and polyacetylenes. This technique is based on the use of several acidic reagents containing either vanillin or related benzaldehyde or benzoic acid derivatives which are highly sensitive for these compounds and which give characteristic colors with individual sesquiterpene lactones or polyacetylenes after TLC on silica gel.

Spray reagents containing vanillin in mineral acids are commonly used in the detection of steroids (Stahl 1969, Bajaj and Ahuja 1979), higher alcohols, phenols, essential oils (Stahl 1969), and cannabinoids (Tewari and Sharma 1979). An acidic solution of p-dimethylaminobenzaldehyde is a known spray reagent for azulenes, indole derivatives, urea and ureides, and nitro compounds (Merck). Other structurally similar benzaldehydes have also been reported for use as spray reagents although mostly for steroid and alkaloid detection (Merck).

Experimental

(1) Chemicals

Sesquiterpene lactones melcanthin-B, confertiflorin, cinerenin, melampodin-A and -B, enhydrin, and melampodinin were kindly provided by Dr.N. H. Fisher (Louisiana State University, Baton Rouge, La., U.S.A.), helenalin, hymenin, hysterin, conchosin-A, cumanin, eupatoriopicrin, and santamarine by Dr. A. Romo de Vivar (Universidad Nacional Autonoma de Mexico, Mexico), ambrosin, axivalin, ivaxillarin, damsin, tenulin, iso-tenulin, tetraneurin-A, -B, -D, and -E, conchosin-B, and hymenin by Dr. E. Rodriguez (University of California, Irvine, Calif., U.S.A.), glaucolide-A, -B, -D, -E, -F, and -G and marginatin by Drs. T. J. Mabry and M. Betkouski (University of Texas, Austin, Tx., U.S.A.), parthenolide -9-α-hydroxyl and elephantopin by Dr. J. M. Cassady (Purdue University, West Lafayette, Ind., U.S.A.), quadrone by Dr. R. L. Ranieri (W. R. Grace & Co., Columbia, Md., U.S.A.), frullania lactone by Dr. G. Ourisson (University of Strasbourg, France). Alantolactone and iso-alantolactone (as a mixture "Helenin") were purchased from Sigma, Chem. Co. and α-santonin from Fluka, Switzerland. All other sesquiterpene lactones were provided by the late Professor T. A. Geissman (University of California, Los Angeles, Calif., U.S.A.), were previously isolated in our laboratory from various plant sources, or were prepared as described in the Section II. Polyacetylenes number 24 and 25 (Table 2) were isolated by K.

Downum and number 20 (Table 2) by R. A. Norton in our laboratory from various plant sources. Methyl 2-thienyl ketone was purchased from Eastman Kodak Co. and thiophene from ICN Pharmaceuticals, Inc. All other polyacetylenes were kindly provided by Dr. J. Lam (University of Aarhus, Denmark). All sesquiterpene lactones and polyacetylenes were used without further purification. Other chemicals which were used as standards or in spray solutions were obtained from common commercial sources and were used without further purification.

(2) Spray reagents

One of the following chemicals, vanillin, p-dimethylaminobenzaldehyde, p-hydroxybenzaldehyde, salicyl aldehyde, m-anisaldehyde, cinnamaldehyde, p-hydroxybenzoic acid, or vanillic acid (0.5g) was dissolved in a solution consisting of 9 ml of ethanol (95%), 0.5 ml of concentrated sulphuric acid, and 3 drops of acetic acid. This reagent should be freshly prepared before use.

(3) TLC plates

Silica gel plates, without gypsum and with fluorescent indicator (Polygram, Brinkmann Instruments, Inc.), were spotted with 5-10 µg of each compound. Sesquiterpene lactones were dissolved in chloroform or acetone, polyacetylenes in ethanol, other compounds (Table 4) in various solvents. The plates were developed in a standard chamber (without chamber saturation) with a solvent system of chloroform - acetone (6 : 1). After

chromatography the plates were air dried, sprayed with the reagent solution and directly placed on a hot plate (TekPro HeatStir 36, Scientific Products) and slowly heated (temperature on 70°C). When vanillin was used as a spray reagent the background become yellowish and with continued heating slightly violet. Colors formed by reaction with the compounds tested were recorded at 10 minutes after this treatment (Table 4) and also 24 hours later (Table 1). Some polyacetylenes changed colors after 48 hours (Table 2) where there was no change of color after 24 hours. The colors formed are unstable and change on standing. Some preservation was achieved by wrapping plates in plastic film (Saran Wrap) and storing plates in dark. The colors were recorded using the Handbook of Colour (Kornerup and Wauscher 1967).

(4) Reagent sensitivity test

Each of five sesquiterpene lactones (Table 3), in acetone solution, was applied in quantities ranging from 0.05 to 20.0 µg on TLC plates and developed as described above. Developed plates were visualized under UV light or exposed to iodine vapours or sprayed with a solution of either $KMnO_4$, vanillin or p-dimethylamonobenzaldehyde reagent followed by heating. The minimum quantities of sesquiterpene lactones forming visible spots on the chromatograms were recorded. In the same way the sensitivities of vanillin and p-dimethylaminobenzaldehyde were tested with two natural polyacetylenes, phenylheptatriyne (No. 20 in Table 2) and the thiophene derivative, α-terthienyl (No. 25 in Table 2). The sensitivity of isatin was tested on the

latter compound. The minimum quantities of both polyacetylenes (in ethanol) still showing distinct characteristic curves of their UV spectra were recorded (Unicam Sp. 800).

(5) Test of various reagents for their reactions with sesquiterpene lactones

Five to ten µg of each of 12 selected sesquiterpene lactones, tamaulipin-A, alantolactone, isoalantolactone, pulchellin-C, parthenin, coronopilin, helenalin, desacetoxymatricarin, cumambrin-B acetate, tenulin, iso-tenulin, and xanthinin, in chloroform or acetone solution were spotted on 13 silica gel plates. Each plate was directly (without developing) sprayed with one of the spray reagents listed above or 5% (w/v) aqueous solution of $KMnO_4$, concentated H_2SO_4, or 95% ethanol - hydrochloric acid (1 : 1) followed by heating to obtain the most intensive colors or was exposed to iodine vapours. Colors were recorded during first 10 minutes and after 24 hours.

Results and Discussion

Of the reagents tested for TLC visualization of sesquiterpene lactones in acidic solutions only benzaldehydes and vanillic acid produced distinct colors with individually tested compounds. In contrast to this other reagents (iodine vapours, $KMnO_4$ solution, and concentrated H_2SO_4) produced only greyish to brownish colors with sesquiterpene lactones. The ethanol-hydrochloric acid spray gave distinct colors with some sesquiterpene lactones but did not give any color with others. Sesquiterpene lactones representing all the basic skeletal classes (Fig.1) and a few modified sesquiterpene lactones (altogether 78; Table 1) and 25 polyacetylenes (Table 2) were tested with the vanillin and p-dimethylaminobenzaldehyde reagents. Vanillin was chosen because it produces a great range of colors and p-dimethylaminobenzaldehyde because it gives a different spectrum of colors when compared with vanillin. Vanillin was tested with a few representatives of other classes of compounds (Table 4). Monoterpenes, steroids, and carotenoids gave very bright colors, fatty acids gave greyish colors, and some aromatic acids did not give any color. Flavonoids generally produced yellow colors.

The smallest quantities of sesquiterpene lactones detectable by TLC by various reagents are shown in Table 3. The most sensitive were vanillin and p-dimethylaminobenzaldehyde which can be used to detect 0.05 µg of certain sesquiterpene

lactones. Dimethylaminobenzaldehyde, in some cases, was even more sensitive than vanillin. The smallest quantity of a sesquiterpene lactone which can be detected depends on the brightness and intensity of the colors developed on chromatograms. Thus tenulin (No. 63 in Appendix) which produces bright orange red colors with vanillin and p-dimethylaminobenzaldehyde is detectable on chromatograms at 0.05 µg while helenalin (No. 66 in Appendix) which gives a weaker reddish orange color with vanillin and a greyish yellow color with p-dimethylaminobenzaldehyde is only detectable at 0.5 and 0.1 µg. Parthenin (No. 47 in Appendix) which forms a bluish green color with vanillin can be detected at 0.5 µg but the golden yellow color with p-dimethylaminobenzaldehyde is visible at a concentration 10x lower. Alpha-terthienyl (No. 25 in Table 2) which gives a very bright color with vanillin, p-dimethylaminobenzaldehyde, and isatin has a lower limit of detection of 0.05 µg. Phenylheptatriyne (No. 20 in Table 2) (forming the least intensive color of all polyacetylenes I tested) can be detected on TLC plates with vanillin or p-dimethylaminobenzaldehyde reagent only at the level of 0.5 µg which, however, is still 1000x lower than the minimum amount needed for its detection by UV spectrophotometry.

Some of the reagents which I have tested, particularly acidic solutions of vanillin and p-dimethylaminobenzaldehyde, are the most suitable for the identification of sesquiterpene lactones and of polyacetylenic compounds. These reagents produce a variety of colors with structurally very different compounds. The differences in colors produced by various sesquiterpene

lactones and other compounds allows for the ready detection of impurities in crystalline preparations. The TLC method can be made highly specific by :(1) the use of several reagents that form different colors or shades with the same compound, (2) the use of standard compounds, and (3) evaluating Rf values.

The wide range of colors produced with all types of sesquiterpene lactones and polyacetylenes makes it difficult to assign the formation of a particular color to a chemical structure. This is supported by the fact that the structurally similar sesquiterpene lactones, such as damsin (No. 54 in Appendix), coronopilin (No. 53 in Appendix), and parthenin (No. 47 in Appendix), produce quite different colors with the vanillin reagent (Table 1) while diastereoisomers produce identical colors as is the case with parthenin and hymenin (numbers 47 and 51 in Appendix).

Hydroxybenzoic acid did not produce distinct colors with sesquiterpene lactones but p-hydroxybenzaldehyde formed a large variety of colors. Both vanillic acid and vanillin, on the other hand, gave a good range of colors. Although the most distinctive and variable colors were produced with p-hydroxybenzaldehyde and vanillin, certain sesquiterpene lactones might be more conveniently detected with a different spray reagent (e. g., cinnamaldehyde forms a very bright stable color with helenalin).

To conclude, I recommend this TLC technique as a simple, fast, sensitive, and specific method that can be used in a preliminary search for sesquiterpene lactones and polyacetylenes in crude (plant) extracts of the Compositae and also in the

identification of individual compounds. I used this technique in the study of chemistry of major sesquiterpene lactones of Parthenium hysterophorus which is described in the Section II.

Table 1. Color reactions of sesquiterpene lactones with vanillin or p-dimethylaminobenzaldehyde spray reagent.

Sesq. lactone		Reagent		Rf	
No. in Appendix	Name	vanillin	p-dimethylamino-benzaldehyde	($CHCl_3$: Me_2CO, 6:1)	Structure (reference)
Germacranolides:					
1	Parthenolide	dull blue dull violet	greyish ruby light brown	0.83	1
2	Pyrethrosin	dull green light brown	brownish red orange	0.80	1
3	Mikanolide, dihydro-	bluish grey grey	brownish orange brownish orange	0.52	1
4	Chamissonin, diacetyl	blue greyish yellow	brownish grey brownish grey	0.83	1
5	Tamaulipin-A	greyish violet greenish grey	dark brown dark brown	0.52	1
6	Tamaulipin-B	grey grey	brown light brown	0.57	1
7	Eupatoriopicrin	dull green greyish brown	dark brown dark brown	0.15	1
8	Melampodin-A	dull blue bluish grey	greyish brown olive brown	0.43	2
9	Melampodin-B	greyish green dull blue	brownish orange brownish orange	0.60	3
10	Enhydrin	violet pastel violet	light brown greyish green	0.77	2

Table 1. Continued.

11 Cinerenin	grey grey	brownish orange brownish orange	0.19	3
12 Melampodinin	greyish blue grey	greyish brown greyish brown	0.50	2
13 Melcanthin-B	dull blue reddish grey	greyish brown greyish brown	0.07	4
14 Glaucolide-A	olive grey greyish violet	purple → light brown brownish grey	0.75	5
15 Glaucolide-B	olive grey greyish violet	light brown brownish grey	0.68	5
16 Marginatin	dark ruby violet brown	olive brown olive brown	0.86	6
17 Glaucolide-D	dark violet dark violet	light brown light brown	0.70	7
18 Glaucolide-E	dark violet dark violet	light brown light brown	0.80	7
19 Glaucolide-F	olive grey greyish violet	purple → light brown brownish grey	0.74	8
20 Glaucolide-G	dark ruby violet brown	olive brown olive brown	0.87	8
21 Elephantopin	grey grey	yellowish grey greyish yellow	0.34	9
22 Parthenolide, 9-α-OH	dark blue dull violet	violet → light brown light brown	0.37	9

Table 1. Continued.

Guaianolides:				
23 Cumambrin-A	dark blue dull green	reddish brown light brown	0.59	1
24 Cumambrin-B	greyish turquoise dull green	light brown light brown	0.42	1
25 Cumambrin-B, dihydro-	dark blue dull green	reddish brown light brown	0.32	10
26 Cumambrin-B, tetrahydro-	dark blue dark purple	brownish red reddish brown	0.14	10
27 Cumambrin-B, acetate	greyish turquoise dull green	reddish brown light brown	0.61	10
28 Cumambrin-B, formyl	greyish turquoise dull green	light brown light brown	0.55	10
29 Matricarin	purplish red greyish red	greyish yellow greyish yellow	0.84	1
30 Matricarin, desacetoxy-	greyish ruby purplish pink	yellow yellow	0.83	1
31 Grossheimin	greyish yellow greyish yellow	greyish yellow greyish yellow	0.43	1
32 Ivalin, pseudo-	vivid violet → → dark blue olive	reddish brown reddish brown	0.43, 0.35*	11

Table 1. Continued.

Eudesmanolides:				
33 Alantolactone	dull blue grey	violet brown greyish Magenta	0.91	1
34 Alantolactone, tetrahydro-	dull blue blue	violet brown greyish Magenta	0.91	1
35 Alantolactone, iso-	dull blue bluish grey	violet brown greyish Magenta	0,91	1
36 Ivalin	purple greenish grey	dark brown purplish grey	0.51	1
37 Ivasperin	violet blue greenish grey	light brown light brown	0.15	1
38 Pinnatifidin	bluish red → red yellowish orange	vivid yellow vivid yellow	0.65	1
39 Pulchellin-C	brownish red greyish ruby	greyish orange greyish orange	0.14	1
40 α-Santonin	greyish brown → → dull green greyish orange	brownish orange brownish orange	0.81	1
41 Ludovicin-A	bluish grey brownish grey	dark brown brownish grey	0.58	1
42 Ludovicin-B	bluish grey brownish grey	dark brown brownish grey	0.41	1
43 Ludovicin-C	dark blue brownish grey	greyish ruby greyish ruby	0.89	1

Table 1. Continued.

44	Reynosin	dull blue violet grey	light brown light brown	0.58, 0.49*	1
45	Frullania lactone	dark green olive	greyish magenta purplish grey	0.91	1
46	Santamarine	dark blue dark violet	light brown light brown	0.63	1
	Pseudoguaianolides:				
47	Parthenin	bluish green greyish yellow	golden yellow golden yellow	0.44	1
48	Parthenin, dihydroiso-	vivid orange light yellow	light yellow greyish yellow	0.45	12
49	Parthenin, tetrahydro-	dull blue dull blue	orange → light brown brownish red	0.49	12
50	Parthenin, photolytic product	vivid violet yellowish grey	yellowish grey yellowish grey	0.76	13
51	Hymenin	bluish green greyish yellow	golden yellow golden yellow	0.44	1
52	Ambrosin	yellow → orange red greenish yellow	light orange light orange	0.67	1
53	Coronopilin	blue greyish magenta	yellow → orange red red	0.46	1
54	Damsin	vivid yellow yellowish white	golden yellow golden yellow	0.73	1
55	Hysterin	grey grey	greyish brown greyish brown	0.19	1
56	Tetraneurin-A	greyish green greyish green	brownish yellow brownish yellow	0.42	1

Table 1. Continued.

57 Tetraneurin-B	blue	brownish red	0.44	1
	bluish violet	brownish red		
58 Tetraneurin-D	dull green	brownish orange	0.09	1
	olive brown	light brown		
59 Tetraneurin-E	dull green	greyish orange	0.09	1
	olive brown	greyish orange		
60 Conchosin-A	lilac	pink→pastel blue	0.05	1
	greyish blue	pastel blue		
61 Conchosin-B	yellowish green	golden	0.42	1
	light yellow	golden		
62 Confertiflorin, desacetyl	greyish yellow	greyish yellow	0.25	1
	greyish yellow	greyish yellow		
63 Tenulin	orange red	reddish orange	0.44	1
	brownish orange	reddish orange		
64 Tenulin, iso-	greyish yellow	yellow→greyish orange	0.71	1
	greyish yellow	greyish orange		
65 Gaillardilin	brownish grey→ → dull green	greyish yellow	0.59	1
	dull green	greyish yellow		
66 Helenalin	yellow→reddish orange	greyish yellow	0.42	1
	greyish yellow	greyish yellow		
67 Flexuosin-B	violet red→ → greyish orange	yellow→reddish golden	0.53	1
	pale yellow	reddish golden		
68 Spathulin	black	golden brown	0.07	1
	brown	golden brown		
69 Balduilin	olive yellow	yellow	0.63	1
	greyish yellow	yellow		

Table 1. Continued.

70 Cumanin	brownish yellow→ →greyish brown greyish brown	brownish yellow→ →greyish brown light brown	0.21	1
Other sesquiterpene lactones:				
71 Xanthinin	brownish red ruby	vivid yellow orange yellow	0.82	1
72 Psilostachyin	dull green blue	grey grey	0.59	1
73 Psilostachyin-C	dull green greenish grey	blue→grey grey	0.50	1
74 Vernolepin	grey greyish brown	dark yellow dark yellow	0.28	1
75 Vernomenin	light brown grey	dark yellow dark yellow	0.29	1
76 Quadrone	deep yellow→ →light yellow light yellow	vivid yellow yellow	0.84	14
77 Axivallin	violet violet grey	bluish violet greyish violet	0.61	1
78 Ivaxillarin	orange red light orange	greyish violet yellow	0.43	1

→ =change during heating the plate

* =two spots of identical color and intensity

First color recorded after 10 min; second after 24 hours.

References:

1 Yoshioka et al. (1973)

2 Fisher et al. (1976)

3 Perry and Fisher (1975)

4 Fisher et al. (1978)

5 Padolina et al. (1974a)

6 Padolina et al. (1974b)

7 Betkouski et al. (1975)

8 M. Betkouski (pers. comm.)

9 J. M. Cassady (pers. comm.)

10 T. A. Geissman (pers. comm.)

11 Devon and Scott (1972)

12 Herz et al. (1962)

13 Kagan et al. (1971)

14 Ranieri and Calton (1978)

Table 2. Color reactions of polyacetylenic compounds with vanillin or p-dimethylaminobenzaldehyde spray reagents.

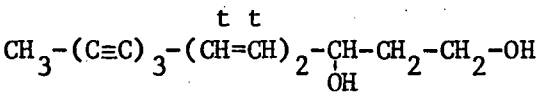
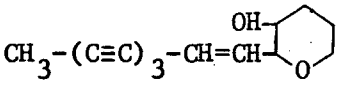
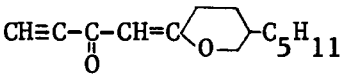
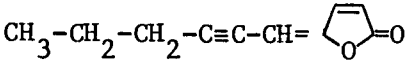
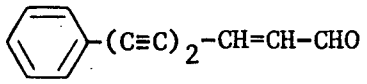
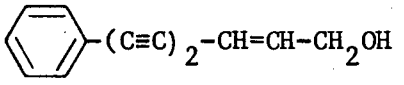
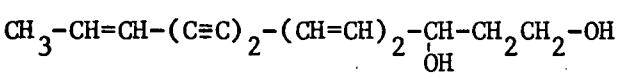
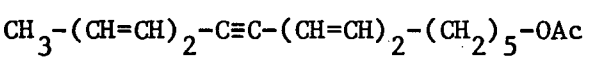
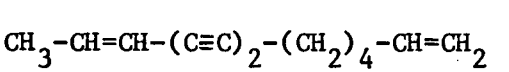
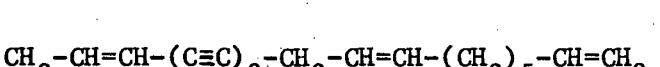
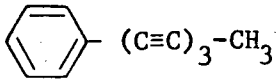
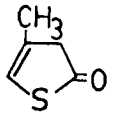
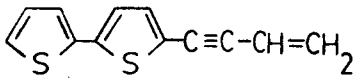
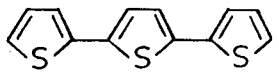
Compound	Reagent: vanillin	Reagent: p-dimethylamino-benzaldehyde	Rf ($CHCl_3$: Me_2CO, 6:1)
(1) 	dull blue	olive yellow	0.16
(2) 	greyish brown	brownish orange	0.71
(3) 	deep orange	reddish orange	0.87
(4) 	bluish violet blue*	greyish Magenta violet*	0.83
(5) 	dark yellow	olive yellow	0.91
(6) 	brownish beige	greyish yellow	0.63
(7) 	brownish beige	olive yellow	0.17
(8) 	olive brown	olive brown	0.92
(9) 	grey	yellowish brown	0.93
(10) 	reddish grey	light brown	0.93

Table 2. Continued.

Compound	Reagent		Rf ($CHCl_3$: Me_2CO, 6:1)
	vanillin	p-dimethylamino-benzaldehyde	
(11) $CH_3-CH=CH-(C\equiv C)_2-(CH=CH)_2-(CH_2)_3-OAc$	greyish brown	yellowish brown	0.91
(12) $CH_3-CH=CH-(C\equiv C)_2-CH=CH-\underset{OH}{CH}-\underset{OH}{CH}-CH=CH_2$	dull green	yellowish brown	0.36
(13) $CH_3-(C\equiv C)_3-(CH=CH)_2-\underset{OAc}{CH}-CH_2-\underset{OAc}{CH_2}$	olive brown and deep Magenta	olive and greyish ruby	0.89, 0.73**
(14) $CH_3-(C\equiv C)_2-CH\overset{c}{=}$ 	olive brown	olive brown	0.90
(15) $CH_3-(C\equiv C)_2-CH\overset{t}{=}$ 	deep violet violet*	greyish violet	0.79
(16) $CH_3-CH=CH-(C\equiv C)_3-CH=CH-\underset{OH}{CH}-CH_2OH$	greyish brown	olive greyish brown*	0.20
(17) $CH_3-CH=CH-(C\equiv C)_3-CH=CH-\underset{Cl}{CH}-CH_2OH$	olive	greyish brown	0.79
(18) $CH_3-(C\equiv C)_3-CH\overset{t}{=}CH-$ 	greenish grey	greenish grey	0.92

Table 2. Continued.

Compound	Reagent: vanillin	Reagent: p-dimethylamino-benzaldehyde	Rf ($CHCl_3$: Me_2CO, 6:1)
(19) $CH_2=CH-\underset{OH}{CH}-(C\equiv C)_2-\underset{OH}{CH}-CH=CH-C_7H_{15}$	greyish brown	dark brown olive brown*	0.58
(20) 	dark blue blue*	greyish yellow	0.95
(21) $CH_3-(CH_2)_2-(C\equiv C)_2-CH=CH-COOCH_3$	dark blue	grey	0.98
(22) 	olive→ →dull blue	dull blue	0.95
(23) 	vivid red	orange red	0.88
(24) 	greenish blue	violet grey	0.90
(25) 	bluish green	vivid blue	0.90

*=change of color after 48 hrs

→ =change during heating the plate

**=two spots of identical intensity

Table 3. Minimum quantities (μg) of selected sesquiterpene lactones and polyacetylenes detectable with various reagents.

Compound	UV light	iodine vapours	$KMnO_4$ (5%, H_2O)	vanillin	p-dimethylamino-benzaldehyde	isatin (0.4%, conc. H_2SO_4)	UV spectrophotometry
Parthenin	1.0	1.0	0.5	0.5	0.05	NT	NT
Coronopilin	5.0	1.0	0.5	0.05	0.05	NT	NT
Tenulin	1.0	1.0	0.5	0.05	0.05	NT	NT
Helenalin	1.0	1.0	0.5	0.5	0.1	NT	NT
Alantolactone	5.0	1.0	0.5	0.1	0.1	NT	NT
α-terthienyl	NT	NT	NT	0.05	0.05	0.05	2.5 mg/ml
Phenylhepta-triyne (PHT)	NT	NT	NT	0.5	0.5	NT	0.5 mg/ml

NT =not tested

Table 4. Color reactions of some naturally occurring compounds with vanillin spray reagent.

Compound	Color	Rf ($CHCl_3$:Me_2CO, 6:1)
Monoterpenes:		
d-limonene	dark blue	0.94
citronellol	dark blue	0.76
citronellal	dark blue	0.86
menthol acetate	dark blue	0.90
Steroids:		
cholesterol	purple	0.76
ergosterol	purple	0.76
Carotenoids:		
β-carotene	dark blue	0.93
Fatty acids:		
stearic	grey	0.79
oleic	greyish blue	0.79
Flavonoids:		
quercetin	yellow→ orange	0.18
kaemferol	yellow→ orange	0.37
myricetin-3-O-Arab	golden yellow	0.00
kaemferol-3-O-Glu	golden yellow	0.00
quercetin-3-O-Glu	golden yellow	0.00
Aromatic acids:		
anisic	-	
p-hydroxybenzoic	-	
vanillic	-	
caffeic	-	
ferulic	pastel violet	0.49
p-coumaric	pastel blue	0.47
p-aminobenzoic	vivid yellow	0.42

Table 4. Continued.

Compound	Color	Rf ($CHCl_3$:Me_2CO, 6:1)
Miscellaneous compounds:		
catechol	bluish red	0.46
resorcinol	vivid red	0.29
hydroquinone	deep magenta	0.29
coumarin	-	
hydroxycoumarin	-	

- =no color reaction

→ =change during heating the plate

S E C T I O N I I

A comparative study of the major sesquiterpene lactones and other selected compounds in various populations of PARTHENIUM HYSTEROPHORUS

Introduction

Parthenium hysterophorus L. is included in the tribe Heliantheae, subtribe Ambrosiinae of the Compositae (Asteraceae) family (Rollins 1950). This species is a representative of the section Argyrochaetae which is composed of nine herbaceous taxa (Rollins, op. cit.).

The plant (Fig. 2) is erect, branched, leafy, with a rigid herbaceous stem which persists usually for one growing season. The roots, however, can persist for at least three years and produce new shoots. Leaves are alternate, highly divided and covered on both sides with trichomes which are also present on the stems. The capitulum (flowering head) is heterogamous, numerous, and composed of five fertile pistillate ray florets, and about forty fertile staminate disk-florets. The mature fruits (achenes) with two disk-florets attached at the base and the subtending brack of female floret fall off together as a unit called an achene complex. The achenes alone are obovate, black, crowned by the persistent remnants of corolla, appendages and style (Rollins 1950). About 2000 mature achenes are produced per plant. Pollination is probably by wind, although insects might play a role. The chromosome number (2n=34) is probably the

same in all populations (Towers et al. 1977b).

Today P. hysterophorus is distributed throughout the tropics (Fig. 3), occurring primarily in areas disturbed by man (Towers et al. 1977b). According to Rollins (1950), the species is native to the region around the Gulf of Mexico, including the West Indies, and possibly also to central Argentina. Within the last hundred years this weed was introduced to Africa, Australia, and south-eastern Asia. In Australia it was first recorded in Queensland in 1955, then it was eradicated but accidentally re-introduced again in 1958 (Haseler 1976). In 1956 P. hysterophorus was also recorded in India (Rao 1956). Its spread in the following years resulted in serious agricultural problems in both countries and it has become a serious medical hazard in certain parts of India, being a source of allergic contact dermatitis (Towers et al. 1977b).

The detrimental properties of P. hysterophorus have been attributed mainly to the presence of a particular sesquiterpene lactone, parthenin (Fig. 4), whose content in this plant may be as high as 8% of the dry weight (Rodriguez et al. 1976a). Parthenin is not only the major allergen responsible for allergic contact dermatitis in India (Lonkar et al. 1976) but, in adition, it appears to be responsible for the allelopathic properties of P. hysterophorus (Kanchan 1975).

Parthenin was isolated from P. hysterophorus by Herz and Watanabe (1959) and shown to be a pseudoguaianolide (Herz et al. 1962). Hymenin (Fig. 4), the C-1 hydroxystereoisomer of parthenin, was first isolated and identified from Hymenoclea

salsola (Toribio and Geissman 1968) and later also from P. hysterophorus (Rodriguez 1975b). While parthenin was found to be the major constituent of P. hysterophorus from U.S.A., Mexico, West Indies, and India, hymenin was found in populations from southern Bolivia and central Argentina and also in one population in Texas (Rodriguez 1975b, Towers et al. 1977b). Ambrosin (No. 52 in Appendix) has also been reported to occur together with parthenin in plants from one population from Texas (Rodriguez et al. 1976a).

More recently Indian workers (Sohi et al. 1979) have identified tetraneurin-A (Fig. 4), known from P. alpinum var. tetraneuris (Ruesch and Mabry 1969), and a new sesquiterpene lactone which they named hysterophorin in extracts of P. hysterophorus. All the structures given by Sohi et al. (1979) are incorrect.

On the basis of differences in the occurence of parthenin and hymenin in various populations of P. hysterophorus Rodriguez (1975b) divided this species into the "parthenin race" and "hymenin race". In addition to differences in the chemistry of the sesquiterpene lactones, P. hysterophorus also displays a number of morphologically diverse forms. In South America, particularly, there is a great degree of variation in the size and other morphological characters of this species (Rollins 1950).

In this study I have examined the major sesquiterpene lactones and other selected constituents of P. hysterophorus from various localities using a sensitive visualization TLC

method described in the Section I. Using these results, I established:

(1) relationships between various populations from the Americas;

(2) the probable origin of *P. hysterophorus* which has been introduced into Australia and India.

Experimental

(1) Plant material

Plants were collected by various persons (see Table 5) in 1975-1980, air dried and kept as herbarium specimens or in paper envelopes. One sample (No. 15 in Table 5) consisted of achenes only. Vouchers have been deposited in the University of British Columbia Herbarium. When fresh plants were used in this study, they were grown from achenes collected in Belize, Australia, or Texas (Fig. 2). Achenes were left to germinate in wet soil in covered petri dishes. When cotyledons and the first leaves appeared seedlings were transfered to pots and placed in a greenhouse.

(2) Chemicals and instruments

Standards of sesquiterpene lactones were obtained as described in the Section I. The melting points were determined on a Thomas-Hoover capillary melting point apparatus. Nuclear magnetic resonance spectra were measured in $CDCl_3$ with TMS as an internal standard on Varian XL-100 (100 MHz) or Bruker Spectrospin (80 MHz) spectrometers in the Department of Chemistry, U.B.C. The Rayonet Photochemical Reactor used was provided by the Department of Chemistry, U.B.C. and the hydrogenation of parthenin was carried out in the Department of Chemistry, U.B.C.

(3) Thin layer chromatography

One-directional TLC with a solvent system chloroform-acetone (6:1) was carried out as described in the Section I. Two-directional TLC: plates 20x20 cm were developed firstly in chloroform-acetone (6:1), air dried, and then run in a second direction in a solvent system water-ethanol (5:1). All plates were visualized by the vanillin spray reagent as described in the Section I.

(4) Chromatographic identification of parthenin and hymenin

The separation of stereoisomers by TLC may sometimes be achieved by multiple development of plates in the same solvent system (Dr. I. Panfil, pers. communication). Two stereoisomers, parthenin and hymenin, in crude plant extracts were identified by TLC using this method. The TLC plates 20x20 cm were firstly developed in chloroform-acetone (6:1), air dried, and then developed in a second direction in heptane-ether-ethyl acetate (30:65:5). Chromatography in the second solvent was repeated ten times, with drying off the plates in between the runs. Parthenin moves ahead of hymenin under these conditions.

(5) Isolation and identification of sesquiterpene lactones from plant material

(a) Parthenin and coronopilin

Shoots of dried P. hysterophorus (Texas or Belize collection) were pulverized in a blender, covered with

chloroform and allowed to stand overnight. The chloroform extract was filtered and the filtrate evaporated to dryness on a rotatory evaporator *in vacuo*. The syrup was dissolved in 95% ethanol, 4% aqueous lead acetate solution was added to precipitate fatty acids, flavonoids, and phenolic acids, and the solution was filtered through celite (analytical filter aid). The filtrate was concentrated *in vacuo*, extracted with chloroform and the extract dried over anhydrous magnesium sulfate. The chloroform solution was filtered, evaporated to dryness, and the residue dissolved in benzene-acetone (2:1) and applied to a silica gel column packed in benzene. The column was eluted with benzene first, followed by increasing amounts of acetone (benzene:acetone 10:1, 10:5, 5:5, 5:10, 1:10, and acetone only). Fractions containing parthenin (checked by TLC with benzene-acetone, 1:4, and benzene-ethyl acetate, 7:3; iodine vapors) were combined and partly evaporated. After addition of isopropyl ether and cooling in a refrigerator white crystals appeared. However, using various solvent systems for TLC development and the vanillin spray reagent I found that the parthenin, isolated in this way, was a mixture of two compounds. Sixty mg of this mixture was chromatographed on a silica gel column and eluted with chloroform-acetone, 6:1. Early fractions gave a blue spot (Rf=0.46) on TLC plates. The combined fractions yielded white crystals, m.p. 177°C, NMR ($CDCl_3$ with TMS): δ 6.29 (*d*, H-13b), 5.61 (*d*, H-13a), 4.95 (*d*, H-6), 1.17 (*s*, C-10-Me), 1.10 (*s*, C-5-Me). The NMR and m.p. correspond to the reported values of coronopilin (see Fig. 4) (Yoshioka et al. 1973) and the Rf and color on TLC plates were identical with

those of an authentic sample of coronopilin (Prof. T. A. Geissman). Later fractions yielded pure parthenin (Fig. 4), giving a bluish-green color with vanillin (Rf=0.44, m.p. 167°C, NMR ($CDCl_3$ with TMS): δ 7.50 (d, H-2), 6.33 (d, H-13b), 6.25 (d, H-3), 5.62 (d, H-13a), 5.02 (d, H-6), 1.30 (s, C-5-Me), 1.14 (d, C-10-Me). The parthenin-coronopilin mixture before chromatography had m.p. 164°C and a NMR spectrum identical with that given for parthenin (Yoshioka et al. 1973).

(b) Hymenin

Dried shoots of Argentinian P. hysterophorus (No. 19 in Table 5) (only 8.5g were available) were cut with scissors and extracted with chloroform overnight. The extract was filtered, evaporated to a minimum volume and applied to a column (silica gel) packed with chloroform-acetone, 6:1. The column was eluted with the same solvent system and all fractions were checked by TLC. Fractions giving a bluish-green spot (Rf=0.44) were combined and partly evaporated. White crystals appeared after treatment with isopropyl ether. The NMR spectrum of this compound ($CDCl_3$ with TMS): δ 7.50 (d, H-2), 6.25 (d, H-13b), 6.18 (d, H-3), 5.55 (d, H-13a), 4.88 (d, H-6), 1.10 (d, C-10-Me), 1.05 (s, C-5-Me). This NMR spectrum corresponds to the reported values for hymenin (Fig. 4) (Yoshioka et al. 1973). The color and Rf on TLC plates were identical with those of an authentic sample of hymenin (Dr. A. Romo de Vivar).

(c) Hysterin

Dried leaves of an Argentinian sample (No. 20 in Table 5) (only 8g were available) were cut with scissors, extracted, and chromatographed as described above. Earlier fractions contained hymenin (TLC, NMR), later fractions gave a grey spot (Rf=0.19) after TLC and the NMR ($CDCl_3$ with TMS): δ 6.20 (d, H-13b), 5.45 (d, H-13a), 4.50 (d, H-6), 3.85 (s, C-10-CH O), 2.08 (s, acetyl-Me), 0.80 (s, C-5-Me). These NMR values correspond to those reported for hysterin (Fig. 4) (Yoshioka et al. 1973). The color and Rf on chromatograms were identical with those of an authentic sample of hysterin (Dr. A. Romo de Vivar).

(6) Preparation of dihydroisoparthenin and tetrahydroparthenin

The procedure of hydrogenation of parthenin described by Herz et al. (1962) was followed. A solution of 100 mg of parthenin in 10 ml of ethanol (95%) was hydrogenated at room temperature and atmospheric pressure with 10 mg of 10% palladium on charcoal (Nutritional Biochemicals, Corp.). After 5 hours of reaction the solution contained two new compounds but no parthenin (TLC, NMR). The solution was filtered and completely evaporated in vacuo. The residue was dissolved in acetone and crystals (needles) were formed after the addition of petroleum ether. Complete separation of these two compounds was achieved by using preparative TLC (silica gel) with the solvent system chloroform-acetone, 6:1. Purity of these compounds was checked by TLC and NMR. After vanillin spray and heating the plate, one of these compounds (Rf=0.45) gave a vivid orange spot fading

rapidly on cooling the plate giving light yellow color. The NMR spectrum ($CDCl_3$ with TMS): δ 5.42 (s broad, H-6), 0.83 (s, C-5-Me), 1.12 (d, C-10-Me) corresponds to the NMR values of dihydroisoparthenin (see Fig. 4) (Herz et al. 1962). The second compound (Rf=0.49) gave dull blue color with the same reagent and the NMR spectrum ($CDCl_3$ with TMS): δ 4.77 (d, H-6), 1.2 (s, C-5-Me) corresponding to that of tetrahydroparthenin (see Fig. 4) (Herz et al, 1962). The approximate ratio of dihydro- and tetrahydroparthenin formed from parthenin was 2:1 (NMR).

(7) Photolysis of parthenin and coronopilin

The procedures of Kagan et al. (1971) and Romo de Vivar et al. (1978) were followed. A solution of parthenin (100 mg) in ethyl acetate (100 ml) under a nitrogen atmosphere was distributed equally in four Pyrex tubes and these were placed in the Rayonet Photochemical Reactor (25°C, 350 nm) for 4 hours. Contents of all tubes were combined and evaporated on a rotatory evaporator, yielding honey-like material with some white crystals, presumably parthenin. The mixture was dissolved in ethyl acetate-hexane, 1:1, and applied to a column of silica gel packed and eluted with the same solvent system. The fractions giving a vivid violet spot (Rf=0.80), presumably of the photolytic product of parthenin, were pooled and evaporated on a rotatory evaporator. The compound was checked immediately by NMR ($CDCl_3$ with TMS): δ 6.34 (d, H-13b), 5.60 (d, H-13a), 4.48 (d, H-6), 3.60 (complex), 2.90-3.90 (complex), 1.80-2.40 (complex), 1.27 (s, C-5-Me), 1.03 (d, C-10-Me). These values correspond to the NMR values given for the photolytic product of parthenin

(Kagan et al. 1971).

To examine the rate of the photoreaction, one Pyrex tube with 10 ml of ethyl acetate with 10 mg of parthenin (under nitrogen) was placed in the Rayonet Photochemical Reactor and the solution checked by TLC at 20 minute intervals.

The formation of the photoproducts of parthenin and coronopilin in various solvents and in a crystalline form were also examined. Parthenin or coronopilin either in solutions (5 mg in 5 ml of benzene, ethyl acetate or water) or in a crystalline form in Pyrex tubes under nitrogen were placed horizontally under a UV lamp (Sylvania Blacklite; light output at 320-380 nm) for 24 hours. The solutions were then concentrated on a rotatory evaporator and checked by TLC for the presence of new compounds.

(8) Detection of sesquiterpene lactones in plant material

One gram of each P. hysterophorus sample (only parts of shoots were used) was cut with scissors into small pieces and covered with 30 ml of chloroform and occasionally shaken by hand during 2 days of extraction. Each extract was filtered through filter paper and the remaining plant material washed twice with 30 ml of chloroform. The combined filtrates were evaporated to dryness in vacuo on a rotatory evaporator and left in a desiccator in vacuo for one hour to remove remaining chloroform. Each sample was then dissolved in a minimum amount of $CDCl_3$ and used for NMR analysis (80 MHz). The NMR spectra (with the lower field peaks maximized and the methyl peaks minimized) of the

crude chloroform extracts (Fig. 5,6) were compared with lower field peaks of the NMR spectra of the authentic samples of the following sesquiterpene lactones:

Parthenin: H-2(d), H-3(d), H-13b(d), H-13a(d), H-6(d);

Coronopilin: δ 4.9 of H-6(d) was used for coronopilin identification in extracts containing parthenin (H-13a,b overlap with those of parthenin); coronopilin could not be identified in extracts containing hymenin because the doublets of H-6 overlap;

Hymenin: H-2(d), H-3(d), H-13b(d), H-13a(d), H-6(d);

Hysterin: H-6(d) only; peaks of H-13a,b overlap with those of hymenin;

Dihydroisoparthenin: H-6(s broad).

The crude chloroform extracts of all P. hysterophorus samples were chromatographed by one-directional and two-directional TLC (Fig. 7). All major spots were recorded 10 minutes and 24 hours later. The presence of the following sesquiterpene lactones was checked by comparison their position and color on TLC plates with authentic samples of parthenin or hymenin (both have identical color and position on chromatograms), coronopilin, hysterin, tetraneurin-A, tetrahydroparthenin, dihydroisoparthenin, the photolytic product of parthenin, and tetraneurin-E and -D.

Results and Discussion

Parthenin was identified by NMR in most of the samples (Table 5) as the major sesquiterpene lactone. Hymenin was present in all Argentinian samples and in two samples from Jamaica (No. 14 and 15; Table 5). It was identified by NMR and by two-directional TLC with multiple development.

Coronopilin (Fig. 4; identified by NMR, m. p., Rf and color on TLC plates) was isolated from extracts of dried *P. hysterophorus* collected in Belize and Texas. It was first isolated and its structure determined from *Ambrosia psilostachya* (Herz and Hognauer 1961) and later reported from numerous species of *Ambrosia* and *Parthenium* (Rodriguez 1975b), but not from *P. hysterophorus*. Coronopilin was not detected in this species earlier because it is not separated and distinguished from parthenin by the conventional methods of TLC analyses of sesquiterpene lactones (e.g. UV light, iodine vapors, or aqueous $KMnO_4$), and because its NMR spectrum overlaps to some extent with that of parthenin. Coronopilin was detected (TLC and NMR) in all samples of *P. hysterophorus* in which parthenin (Fig. 4) was found to be the major sesquiterpene lactone (Table 5). The ratio of parthenin to coronopilin was always approximately 10:1 (determined by NMR). Coronopilin was also detected by TLC in two Argentinian samples (No. 22, 23; see Table 5) containing hymenin (Fig. 4) as the major sesquiterpene lactone, and by TLC and NMR in the Bolivian sample, which contains neither parthenin nor hymenin (sample No. 18; Table 5).

Coronopilin from plants containing hymenin, might, in fact be the diastereoisomer of coronopilin, but because of a scarcity of Argentinian plant material this could not be verified by NMR. Therefore, both the Argentinian and one Bolivian samples were compared with the Belizian sample (containing parthenin) by two-directional TLC using multiple development of plates in solvents by which hymenin and parthenin were clearly separated. Coronopilin was present in all examined samples and there was no evidence for the presence of its diastereoisomer.

Hysterin (Fig. 4) was isolated and identified by NMR, Rf, and color on TLC plates from the Argentinian sample (No. 20; Table 5). This lactone was first isolated and its structure determined by Romo de Vivar et al. (1966) from plant material which was later established to be P. bipinnatifidum (Herz 1968, Rodriguez et al. 1971). Hysterin was detected by TLC and/or NMR in three Argentinian samples (No. 20, 21, 22; Table 5) and in one sample from Jamaica (No. 14; Table 5). In all these samples hymenin was the major sesquiterpene lactone (Table 5).

Tetraneurin-A (Fig. 4) was detected by TLC in all samples containing parthenin, with the exception of Brazilian plants. It was never found together with hymenin (Table 5).

Two derivatives of hydrogenated parthenin were checked for their presence in P. hysterophorus. Dihydroisoparthenin was detected by TLC and NMR only in one Argentinian sample (No. 23; Table 5), but tetrahydroparthenin was not detected in any sample.

Compound No. 1 (see Fig. 7) was present in crude extracts of both Brazilian and Bolivian samples and also in two samples from Argentina (No. 22, 23; Table 5).

Compound No. 6 (see Fig. 7) was typical of all Argentinian samples and occurred also in the samples from Jamaica (No. 14; Table 5) which was identical with Argentinian sample No. 20 (Table 5).

Compound No. 8 (Fig. 7) was detected only in two samples from Argentina (No. 21, 22; Table 5).

Compound No. 9 (see Fig. 7) had identical color and Rf on TLC as tetraneurin-D or -E. However, these two sesquiterpene lactones were not identified in crude extracts of P. hysterophorus samples by NMR. The compound number 9 was present in most samples examined (Table 5).

Compound No. 10 (see Fig. 7); three samples from Argentina (No. 9, 20, 22; Table 5) and one from Jamaica (No. 14; Table 5) contained a relatively large quantity of this compound, which on one-directional TLC completely covered hymenin and coronopilin spots, thereby preventing detection of these sesquiterpene lactones. This compound precipitates from crude chloroform extracts on standing for several days. It is not soluble in most solvents.

The photolytic product of hymenin, conferdiolide, has been found to occur naturally together with hymenin in P. confertum var. lyratum (Romo de Vivar et al. 1978). The fact that P. hysterophorus contains a large quantity of parthenin or hymenin,

and coronopilin suggests the possibility that this species might also contain the photolytic products of these sesquiterpene lactones. It was found that the photolytic product of parthenin (Fig. 4) was formed after 20 minutes of UV irradiation *in vitro* and its amount was increasing steadily for about three and half hours when the reaction mixture consisted of approximately 80% of this compound. There was no change in the amount of this product with further irradiation. The isolated product was unstable in crystalline form at room temperature (after 7 days several spots beside that of the main compound were detected). The photoreaction of parthenin occurred when parthenin was dissolved in ethyl acetate, benzene, or water, but parthenin in a crystalline form did not change after 24 hours of UV irradiation.

Coronopilin underwent the photolytic reaction only when dissolved in ethyl acetate or benzene but not in water or when it was in a crystalline form. When benzene was used, two new spots, beside coronopilin (Rf=0.46) were detected; one spot of identical Rf (0.80) and color (vivid violet) with the photoproduct of parthenin and a second, very intensive pink spot (Rf=0.63). Coronopilin in ethyl acetate formed the same products and, in addition, several new spots were detected. The compounds were not identified. The photolytic products of parthenin and coronopilin were not detected by TLC in crude extracts of any of the 29 dried samples of P. *hysterophorus* or in fresh leaves of plants grown from achenes from Belize and Australia.

Ambrosin (No. 52 in Appendix) was not detected in any plant

sample, in spite of the earlier report on its occurrence in trichomes of P. hysterophorus (Rodriguez et al. 1976).

Relationships between the chemistry of P. hysterophorus from various localities

The twenty nine samples of P. hysterophorus, examined in this study, could be divided into 11 chemical types, according to the presence or absence of compounds which were examined by the TLC method used and/or NMR of their crude extracts (Table 6). In North America only types I and II are present; these differ only with respect to the compound No. 9 (Tables 6 and 7); parthenin is the major sesquiterpene lactone. In Central America type I is present and parthenin is the major sesquiterpene lactone. The West Indies samples are of types II, VII, and XI; type II is the North American type with parthenin as the major sesquiterpene lactone, whereas types VII and XI contain hymenin (locality No. 14 is identical with the Argentinian sample No. 20; see Table 6). The South American samples can be distinguished as follows:

Brazil: the present types differ only in the compound No. 9 (Tables 6, 7); parthenin is the major sesquiterpene lactone.

Bolivia: the present type V is closest to type IV but differs by the absence of parthenin; coronopilin is the major sesquiterpene lactone.

Argentina: types VI-X are present (Table 6); types VI and VII different only with respect to the presence of

hysterin; hymenin is the major sesquiterpene lactone. Type VII is most similar to type VIII, from which it differs by the presence of the compound No. 8 and absence of the compound No. 10. Type IX differs greatly from all other types, with the greatest similarity to type VII and VIII from which it differs in three compounds (Tables 6, 7). Type X represents the most distinct population of P. hysterophorus, differing from all other types by at least four compounds (Tables 6, 7).

Only type I is present in samples from India and Australia; parthenin is the major sesquiterpene lactone.

All samples from North America, Belize, India, Australia, and one sample from Jamaica belong to type I and II, which are almost identical, differing only in one compound (Tables 6,7). These results suggest that the populations examined are closely related and may be of the same origin. Because samples of P. hysterophorus from India and Australia differ greatly from all South Ameriacan samples, it seems reasonable to conclude that these populations originated in North America. This is consistent with an earlier suggestion that P. hysterophorus was introduced along with grain imported from the U. S. A. to India (Vartak 1968) and Australia (Haseler 1976). Results of my chemical analyses exclude an alternative explanation that Indian populations of P. hysterophorus originate in Argentina (Lonkar and Jog 1972).

Compared to North American samples, South American populations of P. hysterophorus are extremely diverse. In fact, each sample examined represents a special chemical type (Table 6) differing in 1-8 compounds from all other South American samples (Table 7).

A high degree of diversity in chemistry and the earlier reported morphological differences between various South American populations of P. hysterophorus (Rollins 1950) suggest the existence of several different forms on this continent. These differences in South American populations could be a consequence of three situations.

First, hybridization with closely related species might promote a higher degree of diversity among P. hysterophorus populations in South America. This seems to be supported by observations of Rollins (1950) that many morphological characters of South American P. hysterophorus appear to derive from both, P. confertum and P. bipinnatifidum,. This view appears to be also supported by the fact that hymenin, which is present in P. confertum and hymenin and hysterin in P. bipinnatifidum (Rodriguez 1975b, Romo de Vivar et al. 1978) is also present in South American populations of P. hysterophorus (Table 5). On the other hand, the hypothesis invoking hybridization with P. bipinnatifidum and P. confertum, as a cause of a higher degree of diversity in South American populations of P. hysterophorus is unlikely because, according to Rollins (1950), P. bipinnatifidum and P. confertum are not known south of Mexico. If hybridization with these species was

an important source of new forms, then we would expect a higher degree of diversity among samples collected in North America. However, hybridization with other closely related species native to South America might be an important source of a greater degree of diversity among South American populations of P. hysterophorus. This possibility requires a detailed comparative study of morphology, anatomy, genetics, and chemistry of P. hysterophorus and other closely related species.

Secondly, it has been suggested that polyploidy has played a role in producing the diversity of forms of P. hysterophorus in central South America (Rollins 1950). The available data, however, show that chromosome number (2n=34) is uniform among various populations, including one from Cordoba, Argentina (Rollins 1950, Towers et al. 1977b).

Thirdly, different chemistry and differences in morphology among South American populations of P. hysterophorus may represent various adaptations of plants to local conditions, such as the degree of humidity, predator pressures, pathogens, competitive relationships with other plants, etc. This possibility requires the study of physiology and ecology of these probably locally adapted populations.

Assuming that P. hysterophorus originates in an area around the Gulf of Mexico (Rollins 1950), from my chemical analyses possible relationships between the examined populations of P. hysterophorus can be reconstructed. From Tables 6, 7 it can be seen that the two Brazilian samples are most similar to those from North America. These and the Bolivian sample, which is

chemically similar to the Brazilian populations (Tables 6, 7), thus represent forms of P. hysterophorus intermediate between North American types and the extremely divergent Argentinian types (Table 7). This view is supported also by the fact that the Argentinian populations, which are most specialized as indicated by the highest number of compounds examined (Table 6), exhibit a high degree of diversity among themselves (individual samples differ from one another in 1-5 compounds; Table 7).

The samples of P. hysterophorus from Jamaica indicate a close relationship with both North and South American types. One sample was identical with No. 20 from Argentina, whereas the second sample was identical with North American type II (Table 5). The third sample (type XI) differed from all other samples (Table 5) but because of insufficient information on three compounds, its relationships to other types could not be determined. The presence of hymenin in this sample, however, indicates the probable origin of this P. hysterophorus population in South America.

Conclusions

The use of vanillin as a TLC spray reagent for preliminary identification of major sesquiterpene lactones in *P. hysterophorus* resulted in finding three compounds, coronopilin, hysterin, and dihydroisoparthenin, which were shown for the first time to occur in this species. The presence of these sesquiterpene lactones was confirmed by NMR analyses.

The chemical analyses of selected compounds from various samples of *P. hysterophorus* indicated the existence of 11 chemical types of this species. However, it has to be considered that the number of samples was small and sampling did not cover the whole range of recently described *P. hysterophorus* distribution. Therefore, it is likely that the number of chemical types is an underestimation. A more extensive survey is necessary for a better understanding of the diversity and relationships between various populations, especially those in South America.

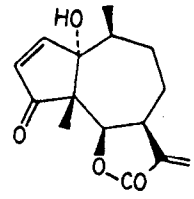
In any case, differences in the chemistry of various populations of *P. hysterophorus* shown by this study suggest the possibility of the existence of several forms, subspecies, or perhaps even species. This has already been indicated by Rollins (1950) on the basis of his study of various morphological features of *P. hysterophorus*. However, the implications of differences in chemistry between various populations of *P. hysterophorus* shown by this study will require a more detailed

taxonomic study.

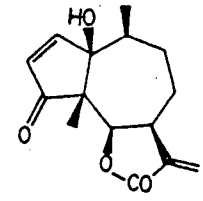
Fig. 2. Parthenium hysterophorus grown in a greenhouse (U.B.C.) from achenes collected in Austin, Texas.

Fig. 3. Distribution of P. hysterophorus (according to Rollins (1950) and Towers et al. (1977)) and localities from which plant samples for chemical analyses were obtained.

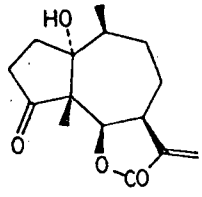
Fig. 4. Chemical structures of sesquiterpene lactones examined in this section.



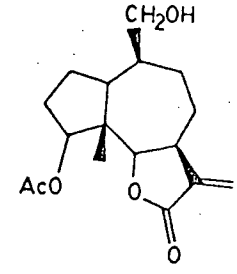
Parthenin



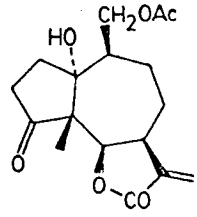
Hymenin



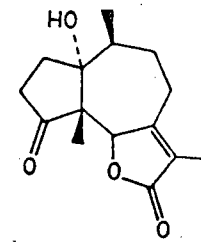
Coronopilin



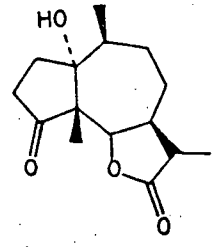
Hysterin



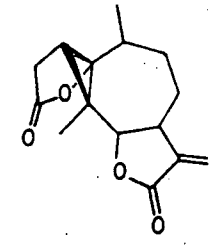
Tetraneurin-A



Dihydroisoparthenin



Tetrahydroparthenin



Parthenin, photolytic product

Fig. 5. NMR spectrum of crude chloroform extract of P. hysterophorus from Texas (locality 10). This represents the most widespread chemical type I containing parthenin and coronopilin as major sesquiterpene lactones.

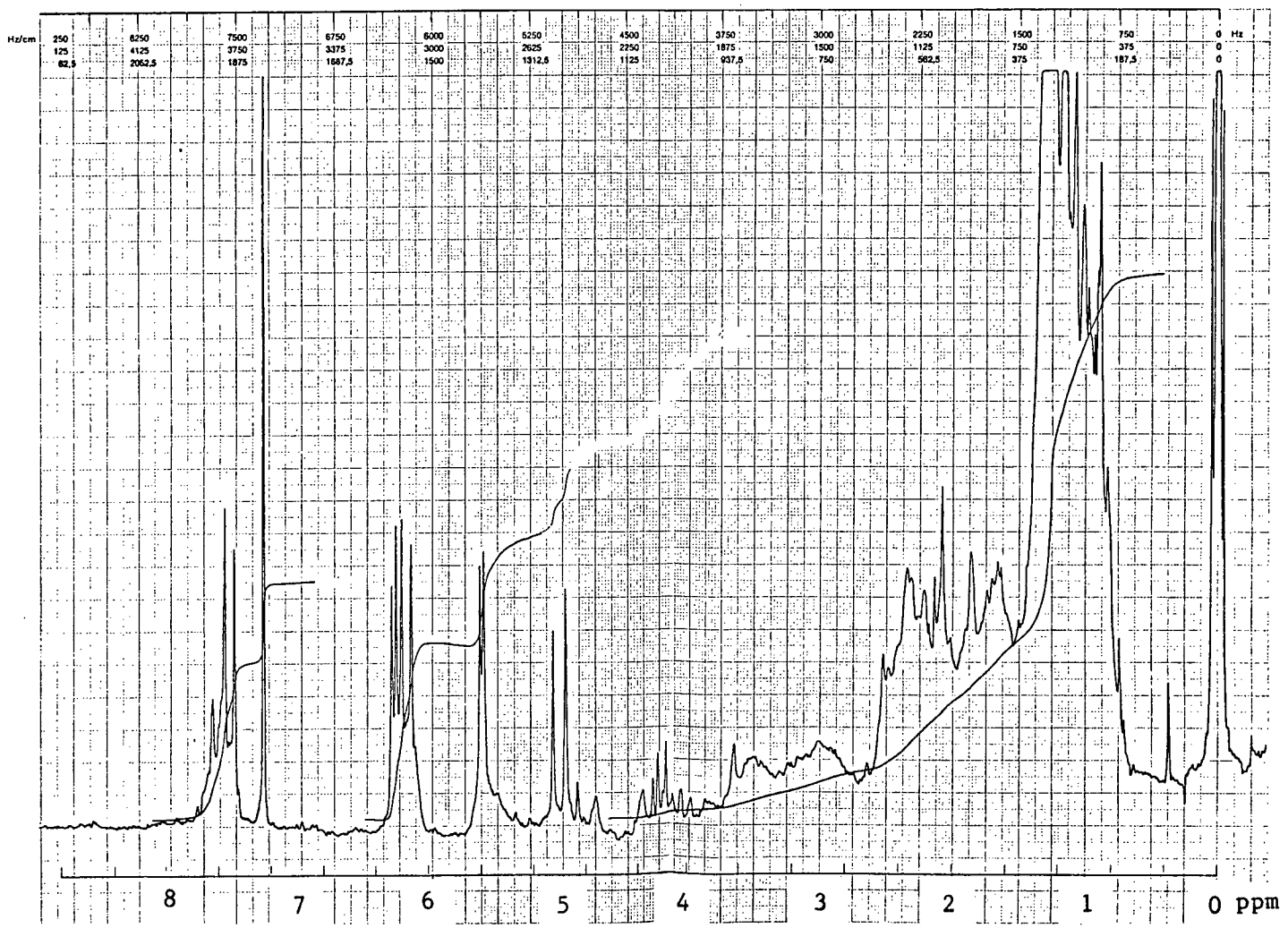


Fig. 6. NMR spectrum of crude chloroform extract of P. hysterophorus from Argentina (locality 20). This represents the chemical type VII containing hymenin and hysterin.

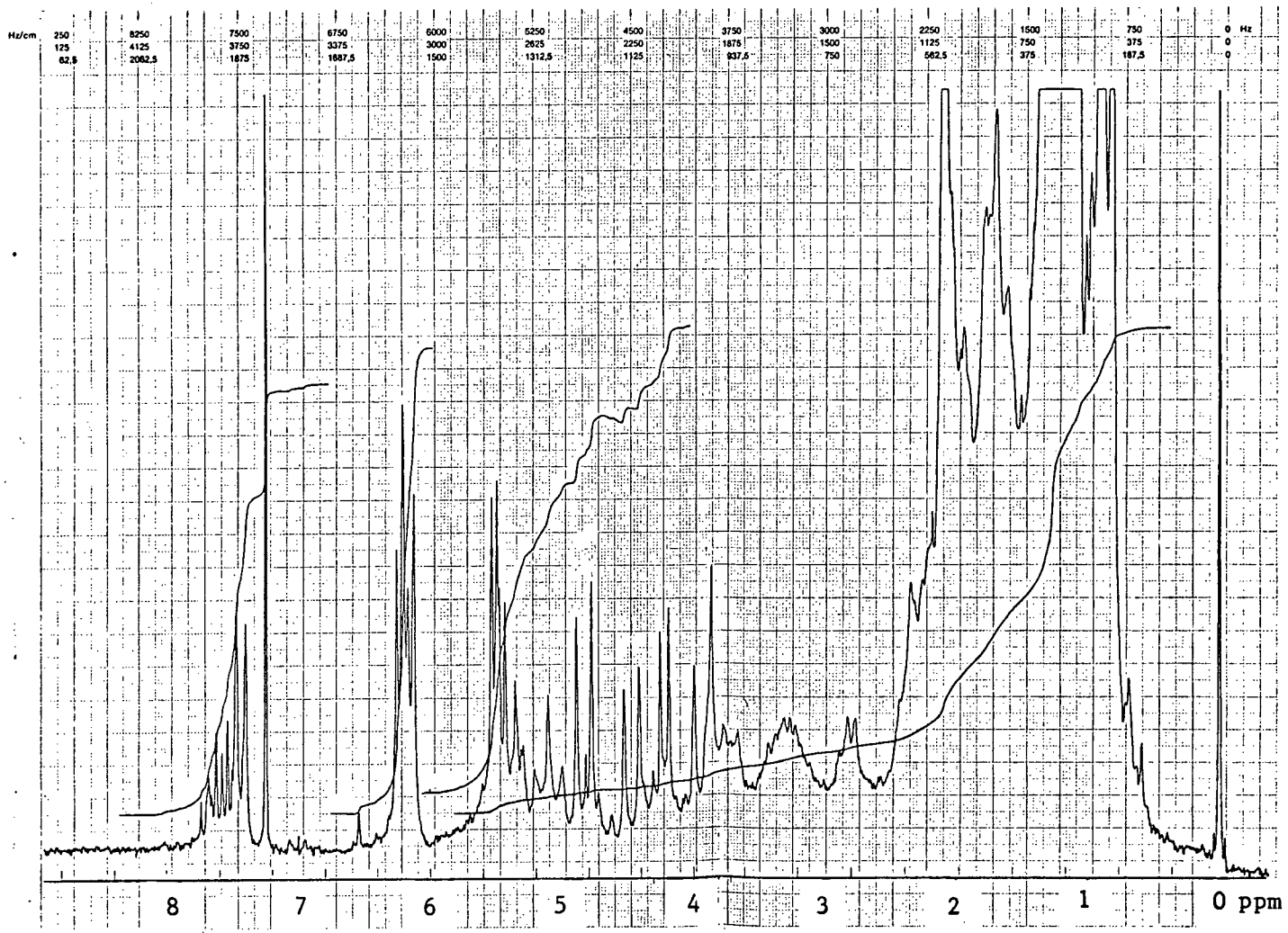
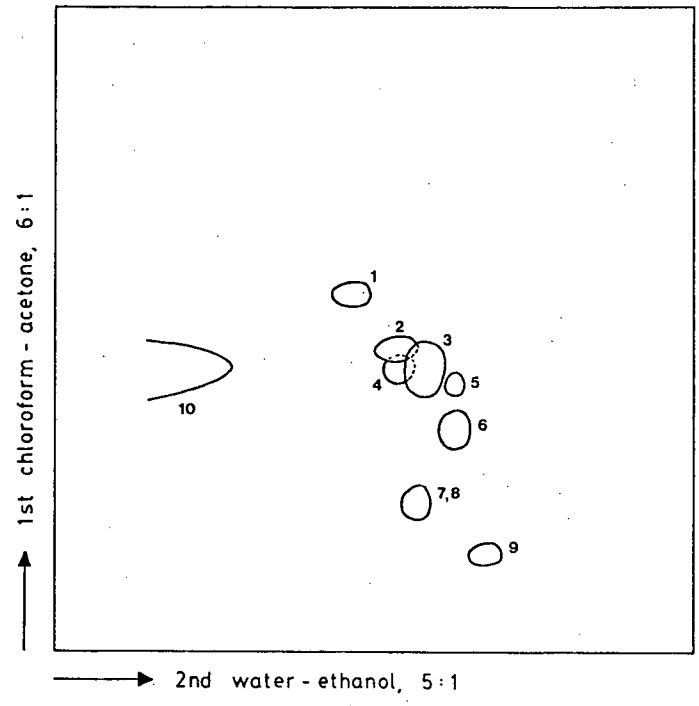


Fig. 7. Major spots detected by vanillin spray reagent on plates developed by two-directional TLC of crude chloroform extracts of P. hysterophorus.



Spot No.	Color after 10 min (after 24 hours)	Rf values	Compound
1	Bluish red (purple)	0.56; 0.46	unidentified
2	Blue (greyish magenta)	0.46; 0.54	coronopilin
3	Bluish green (greyish yellow)	0.44; 0.59	parthenin, hymenin
4	Vivid orange (light yellow)	0.45; 0.54	dihydroisoparthenin
5	Greyish green (greyish green)	0.42; 0.65	tetraneurin-A
6	Greenish blue (greenish blue)	0.32; 0.65	unidentified
7	Grey (grey)	0.19; 0.58	hysterin
8	Violet → greyish green (greyish green)	0.19; 0.58	unidentified
9	Dull green (olive brown)	0.09; 0.71	unidentified
10	Yellow (yellow)	0.44; 0.18	unidentified

Table 5. Summary of chemical analyses of P. hysterophorus from various localities by two-directional TLC and NMR (■ compound detected, □ compound not detected by TLC; ▲ compound detected, △ compound not detected by NMR). Numbers of individual localities correspond to those in Fig. 3.

No.	Locality; date of collection; collector	No.1 Unk.	No.2 Cor.	No.3 Par. or Hym.	Par.	Hym.	No.4 DHP	No.5 T.-A	No.6 Unk.	No.7 Hys.	No.8 Unk.	No.9 Unk.	No.10 Unk.
1	Florida: Hwy 90-Quincy; October 1975; G. H. N. Towers	□	■ ▲	■	▲	△	□ △	■	□	□ △	□	■	□
2	Florida: Hwy 90-Marianna; October 1975; G. H. N. Towers	□	■ ▲	■	▲	△	□ △	■	□	□ △	□	■	□
3	Alabama: Montgomery; October 1975; G. H. N. Towers	□	■ ▲	■	▲	△	□ △	■	□	□ △	□	□	□
4	Alabama: Selma; October 1975; G. H. N. Towers	□	■ ▲	■	▲	△	□ △	■	□	□ △	□	□	□
5	Alabama: Hwy 80W-Uniontown; October 1975; G. H. N. Towers	□	■ ▲	■	▲	△	□ △	■	□	□ △	□	□	□

Table 5. (continued)

6	Texas: Forney; October 1975; G. H. N. Towers	□	■	▲	■	▲	△	□	△	■	□	□	△	□	■	□
7	Texas: Dalas; October 1975; G. H. N. Towers	□	■	▲	■	▲	△	□	△	■	□	□	△	□	■	□
8	Texas: Hwy 84-Mexia; October 1975; G. H. N. Towers	□	■	▲	■	▲	△	□	△	■	□	□	△	□	■	□
9	Texas: Hwy 35-Waco; October 1975; G. H. N. Towers	□	■	▲	■	▲	△	□	△	■	□	□	△	□	□	□
10	Texas: Austin 1975 D. R. DiFeo	□	■	▲	■	▲	△	□	△	■	□	□	△	□	■	□
11	Mexico: Mexico City; November 1975; G. H. N. Towers	□	■	▲	■	▲	△	□	△	■	□	□	△	□	■	□
12	Belize: Indian Church; May 1979; T. Arnason	□	■	▲	■	▲	△	□	△	■	□	□	△	□	■	□

Table 5. (continued)

13	Jamaica: Montego Bay; December 1975; G. H. N. Towers	□	■ ▲	■	▲	△	□ △	■	□	□ △	□	□	□
14	Jamaica: Kingston; December 1975; G. H. N. Towers	□	□ △	■	△	▲	□ △	□	■	■ ▲	□	■	■
15	Jamaica: St. Mary; June 1980; K. Stuart	□	□	■	△	▲*	□	?	□	?	□	?	□
16	Brazil: Corumba 19°S 57°30'W 1979 I. Dale	■	■ ▲	■	▲	△	□ △	□	□	□ △	□	■	□
17	Brazil: Londrina 23°30'S 51°30'W; 1979; I. Dale	■	■ ▲	■	▲	△	□ △	□	□	□ △	□	□	□
18	Bolivia: Santa Cruz 17°47'S 63°10'W; 1979; I. Dale	■	■ ▲	□	△	△	□ △	□	□	□ △	□	□	□
19	Argentina: Jujuy, San Salvador de Jujuy; February 1976; G. H. N. Towers	□	□ △	■	△	▲	□ △	□	■	□ △	□	■	■

Table 5. (continued)

20	Argentina: Salta, Pampa Blanca 24°30'S 64°30'W; 1979; I. Dale	□	□ △	■	△	▲	□ △	□	■	■ ▲	□	■	■
21	Argentina: Tucuman, 26°30'S 65°W; 1979; I. Dale	□	□ △	■	△	▲	□ △	□	■	** ▲	■	■	□
22	Argentina: La Rioja 29°S 67°W; 1979; I. Dale	■	■ ***	■	△	▲	□ △	□	■	** ▲	■	■	■
23	Argentina: Cordoba, Arroyo la Pampa; February 1976; G. H. N. Towers	■	■ ***	■	△	▲	■ ▲	□	■	□ △	□	■	□
24	India: Bangalore; P. V. Subba Rao	□	■ ▲	■	▲	△	□ △	■	□	□ △	□	■	□
25	Australia: Queensland, Charter's Towers; March 1980; I. Dale	□	■ ▲	■	▲	△	□ △	■	□	□ △	□	■	□
26	Australia: Queensland, Collinsville; February 1980; I. Dale	□	■ ▲	■	▲	△	□ △	■	□	□ △	□	■	□

Table 5. (continued)

27	Australia: Queensland, Mt. Douglas; March 1980; I. Dale	□	■	▲	■	▲	△	□	△	■	□	□	△	□	■	□
28	Australia: Queensland, Elgen Downs; March 1980; I. Dale	□	■	▲	■	▲	△	□	△	■	□	□	△	□	■	□
29	Australia: Queensland, Clermont; 1979; I. Dale	□	■	▲	■	▲	△	□	△	■	□	□	△	□	■	□

? Analyses incomplete because of insufficient sample;

* Presence of hymenin was determined by two-directional TLC with multiple development (see Experimental for further explanation);

** Hysterin was undetectable by TLC because of being covered by compound No. 8;

*** Coronopilin was undetectable on NMR because its peaks overlapped with those of hymenin.

Note: Cor.=coronopilin; Par.=parthenin; Hym.=hymenin; DHP=dihydroisoparthenin; T.-A=tetraneurin-A; Hys.=hysterin; Unk.=unknown compound.

Table 6. Types of P. hysterophorus as determined by chemical analyses (NMR, TLC) of samples from various localities.

Chemical type	Compounds examined											Locality
	Par.	Hym.	Cor.	Hys.	T.-A	DHP	No.1	No.6	No.8	No.9	No.10	
I	+	-	+	-	+	-	-	-	-	+	-	Florida (1,2), Texas (6-8,10), Mexico (11), Belize (12), India (24), Australia (25-29)
II	+	-	+	-	+	-	-	-	-	-	-	Alabama (3-5), Texas (9), Jamaica (13)
III	+	-	+	-	-	-	+	-	-	+	-	Brazil (16)
IV	+	-	+	-	-	-	+	-	-	-	-	Brazil (17)
V	-	-	+	-	-	-	+	-	-	-	-	Bolivia (18)
VI	-	+	-	-	-	-	-	+	-	+	+	Argentina (19)
VII	-	+	-	+	-	-	-	+	-	+	+	Argentina (20), Jamaica (14)
VIII	-	+	-	+	-	-	-	+	+	+	-	Argentina (21)
IX	-	+	+	+	-	-	+	+	+	+	+	Argentina (22)
X	-	+	+	-	-	+	+	+	-	+	-	Argentina (23)
XI	-	+	-	?	?	-	-	-	-	?	-	Jamaica (15)

Note: Par.=parthenin; Hym.=hymenin; Cor.=coronopilin; Hys.=hysterin; T.-A=tetraneurin-A; DHP=dihydroisoparthenin; No.1, 6, 8, 9, 10=unknown compounds; + =compound present; - =compound absent.

Table 7. Degree of dissimilarity between individual chemical types of P. hysterophorus (type numbers correspond with those given in Table 6). Table gives total number of compounds in which individual types differ from all others. Type XI is not included because of insufficient data.

Chemical type	Chemical type									
	I	II	III	IV	V	VI	VII	VIII	IX	X
I	-	1	2	3	4	6	7	7	8	6
II	1	-	3	2	3	7	8	8	9	7
III	2	3	-	1	2	6	7	7	6	4
IV	3	2	1	-	1	7	8	8	7	5
V	4	3	2	1	-	6	7	7	6	4
VI	6	7	6	7	6	-	1	3	4	4
VII	7	8	7	8	7	1	-	2	3	5
VIII	7	8	7	8	7	3	2	-	3	5
IX	8	9	6	7	6	4	3	3	-	4
X	6	7	4	5	4	4	5	5	4	-

S E C T I O N I I I

Biological activities of sesquiterpene lactones

Introduction

Sesquiterpene lactones exhibit a variety of activities against different types of organisms. In the following part I will summarize the major findings on individual types of biological activities of these compounds.

1. Antimicrobial activity

Some sesquiterpene lactones have been reported to inhibit growth of bacteria and/or fungi (e.g. Olechmnowicz-Stepien and Stepien 1963, Vichkanova et al. 1971, Vanhaelen-Fastre 1968, 1972, Mathur et al. 1975, Char and Shankarabhat 1975, Norman et al. 1976, Lee et al. 1977b, Towers et al. 1977a).

It has been suggested (Lee et al. 1974, 1977b) that the unsubstituted cyclopentenone ring is a prerequisite for antimicrobial activity of sesquiterpene lactones, and that it is independent on the presence or absence of the α-methylene-γ-lactone moiety.

2. Allelopathic activity

Parthenium hysterophorus, a hazardous, widespread weed that has infested agricultural lands in many parts of India and Australia, causes a serious reduction of many economically important crop species. The growth and yield of several crop species were considerably affected when they were grown in soil containing dried root and leaf materials of this weed (Kanchan and Jaychandra 1976). These authors also observed that dried plant material or aqueous extracts from roots of P. hysterophorus caused a suppression in the growth and colonization of Rhizobia in leguminous plants. Sarma et al. (1976) found that the seed germination of three crop plants (Arachis hypogea, Crotalaria juncea, and Phaseolus mungo) was not affected by aqueous extracts of P. hysterophorus, however, the extracts had a profound inhibitory effect on radicle growth and dry weight of the plants examined.

Parthenin and water extracts of P. hysterophorus inhibited seed germination and growth of seedlings of Phaseolus vulgaris (Garciduenas et al. 1972), wheat, and ragi (Eleusine coracana) (Kanchan 1975). In the latter work almost all fractions of extracts showed inhibitory activity. On the basis of these and other data it has been suggested that P. hysterophorus contains a complex of inhibitors with parthenin and some phenolic acids as the prominent constituents (Kanchan 1975, Kanchan and Jayachandra 1976).

P. hysterophorus produces large quantities of pollen which, when carried away and deposited on floral parts of other plants,

inhibit the germination of pollen and the growth of pollen tube of other species. This has been observed under both experimental and natural conditions but the compounds responsible for this action have not been identified (Kanchan and Jayachandra 1976, 1980). The fact that sesquiterpene lactones have been found in pollen of P. hysterophorus (Picman et al. 1980) suggests that these compounds could be responsible for pollen allelopathy of this species.

Dalvi et al. (1971) demonstrated the phytotoxic action of alantolactone by its inhibitory effects on seed germination, seedling growth, and rate of respiration of Phaseolus mungo. The authors suggested that alantolactone inhibits amylases and proteases and enzymes involved in the synthesis of new proteins and nucleic acids.

Sesquiterpene lactones from species of Artemisia inhibited the growth but stimulated the respiration of Cucumis sativus (McCahon et al. 1973). Litter of Artemisia tridentata inhibited germination and growth of three species of grasses (Schlatterer and Tisdale 1969) possibly due to sesquiterpene lactones present in this species.

Sesquiterpene lactones from Helianthus tuberosus inhibited the elongation of Avena coleoptile sections and promoted adventitious root formation of Phaseolus mungo cuttings (Shibaoka et al. 1967 a,b). Only lactones with the exomethylene on the lactone ring exhibited this activity. Reduction of the lactones involving the methylene group led to inactive compounds. Also the adducts formed with cysteine (via the

exomethylene group) were inactive.

Some other sesquiterpene lactones have been reported to possess growth regulating activity (Gross 1975). A number of such compounds have been isolated in the search for antitumor agents of plant origin. For example, vernolepin from Vernonia hymenolepis inhibits the extension growth of wheat coleoptile sections (Sequiera et al. 1968).

3. Antihelminthic activity and chemoprophylaxis in schistosomiasis

It has been reported that alantolactone, which is a very strong sensitizor in human allergic contact dermatitis, has been used as a vermifuge (Dupuis et al. 1974). It possesses antihelminthic activity against Fasciola hepatica (Kim et al. 1961). Alpha-santonin and its derivatives are also well known as imporatant antihelminthic and ascaricidal agents (Haynes 1948). Eremanthin, costunolide, β-cyclocostunolide, and goyazenolide from the wood oils of the common brazilian trees (Eremanthus elaeagnus, E. goyazensis, Vanillosmopsis erythropappa, and Moguinia velutina) inhibit penetration of cercariae of the trematode Schistosoma mansoni into animal skin (Baker et al. 1972, Vichnewski et al. 1976). It has been suggested that the exocyclic methylene on the lactone ring of these sesquiterpene lactones is responsible for this activity by inhibiting either the penetration enzymes or enzymes within the cercaria.

4. Effects on insects

It has been established that the sesquiterpene lactone glaucolide-A from Vernonia species provides resistance to insect feeding and that the ingestion of this compound has a detrimental effect on the growth and development of Lepidopteran larvae (Burnett et al. 1974).

Another sesquiterpene lactone, alantolactone, is a feeding deterrent to Tribolium confusum and also affects the survival of beetles (Picman et al. 1978). Parthenin from P. hysterophorus inhibits heartbeat of grasshoppers most likely by blocking thiol containing compounds important for normal heart activity. This is indicated by the fact that the activity of parthenin-arrested hearts can be restored by thiol addition (Picman et al. 1981).

5. Effects on mammals

The sesquiterpene lactone, glaucolide-A, has been reported to act as a feeding deterrent to rabbits and deer (Burnett et al. 1977, Mabry and Gill 1979). In addition, there are several reports on the poisonous action of many species of the Compositae to livestock grazing on them (e.g. Sperry et al. 1964, Kingsbury 1964, Schmuz et al. 1968). Ivie et al. (1975a) showed that hymenovin, the major sesquiterpene lactone of Hymenoxys odorata, is responsible for toxicity of this plant to grazing sheep and goats. Also the extreme toxicity of Helenium microcephalum to cattle, sheep, and goats has been found to be caused by a high level of the sesquiterpene lactone, helenalin,

present in this species (Witzel et al. 1976; in Towers et al. 1977b). Vermeerin, a sesquiterpene dilactone of the physiologically active vermeeric acid, causes vomiting disease in sheep grazing on Geigeria species in South Africa (Anderson et al. 1967). This dilactone is also present in Hymenoxys richardsonii, an American plant poisonous to livestock (Herz et al. 1970). It has been known for many years that when plants, which contain sesquiterpene lactones, are consumed by dairy cattle, their milk tastes bitter (Herzer 1942). It has been established that tenulin from Helenium amarum is the active ingredient responsible for the bitter taste of the milk. Parthenium hysterophorus, when fed to cattle and buffaloes in India, was found to cause illness or death of the animals, most likely because of parthenin present in this species (Narasimhan et al. 1977). The extract of P. hysterophorus from which parthenin and other sesquiterepene lactones were removed could be used as a protein rich fodder (Savangihar and Joshi 1978), clearly showing that sesquiterpene lactones are responsible for the poisoning action of this plant.

Ivie et al. (1975a) suggested that the poisoning action of sesquiterpene lactones such as hymenovin on grazing mammals might be a consequence of: (1) their reaction with sulphydryl groups of key enzymes (see also Hanson et al. 1970); and (2) the effects of sesquiterpene lactones on the microbial composition of the rumen. In addition, Seth and Bhatia (1978) demonstrated that parthenin is a direct cardiac depressant in dogs and rats.

6. Allergic contact dermatitis

Information on allergic contact dermatitis caused by sesquiterpene lactones and photodermatitis caused by polyacetylenic compounds activated by UV light was reviewed by Towers (1979). Most of the sesquiterpene lactones causing allergic contact dermatitis have been reported from species of the Compositae but there are also examples from other families such as the Lauraceae and Magnoliaceae (Mitchell 1969, Mitchell et al. 1970, 1971a,b, 1972, Bleumink et al. 1976, Lonkar et al. 1974, Evans and Schmidt 1980). Cases of allergic contact dermatitis evoked by Frullania species (an epiphytic liverwort) in forest workers have also been reported (Mitchell et al. 1969). The major sensitizers from Frullania species were isolated and identified as sesquiterpene lactones (Knoche et al. 1969, Mitchell et al. 1970, Perold et al. 1972, Asakawa et al. 1976).

The recent findings of continuing research on dermatitis caused by P. hysterophorus were summarized by Towers et al. (1977b). Allergic contact dermatitis caused by this species was reported from the southern United States 50 years ago (French 1930) but mechanization of farming caused a decline in its incidence. On the contrary, in India, where the species was accidentally introduced in 1956, the frequency of cases of Parthenium dermatitis has been increasing since it was first recognized in 1965 (Lonkar and Jog 1972). Allergic contact dermatitis due to Parthenium develops from repeated contacts with this plant or possibly with its disseminated trichomes and

dried plant parts (Lonkar et al. 1974). Typical initial patients were farmers and weed pullers but more recently some persons living in cities, who were not directly exposed to the living plants, also developed this allergy. The weed has become an important dermatological and health problem (Towers et al. 1977b, and references therein). Parthenium dermatitis has been reported much more frequently in adult males than in females and no cases are known in children before puberty (Towers et al. 1977b). Sesquiterpene lactones, mainly parthenin, are considered to be the allergens of this plant species (Lonkar et al. 1976).

The genus Chrysanthemum (with its many species and varieties) is one of the most common causes of allergic contact dermatitis among florists and horticulturists. Sesquiterpene lactones present in these plants are responsible for the dermatitis (e.g. Hausen and Schulz 1973, 1975, 1976). Other common weeds, horticultural plants, or vegetables from the Compositae such as Ambrosia, Artemisia, Aster, Cichorium, Cosmos, Dahlia, Helianthus, Helenium, Matricaria, Solidago, and many others have been reported to cause allergic contact dermatitis (e.g. Mitchell 1969, Evans and Schmidt 1980).

It has been established that the exomethylene on the lactone ring is responsible for allergenicity of sesquiterpene lactones, however, this group alone is not always immunologically sufficient (Mitchell et al. 1970, 1971a,b; Mitchell and Dupuis 1971, Epstein et al. 1980). Since parthenin undergoes a reaction with cysteine via the exomethylene on the lactone ring as well as via the C2-C3 double bond (Picman et al.

1979) the presence of these two active sites in a molecule of parthenin could be responsible for its strong allergenicity.

7. Cytotoxic activity

Sesquiterpene lactones are of great interest in cancer research because many of these compounds exhibit antileukemic, cytotoxic, and/or tumor inhibitory activity.

The relationship between chemical structure of sesquiterpene lactones and their cytotoxic activity was investigated by many researchers. In their review of antineoplastic agents of plant origin, Hartwell and Abbott (1969) concluded that all known active sesquiterpene lactones possess an α,β-unsaturated lactone ring. Later it was established that the conjugated exomethylene group on the lactone is an essential requisite for cytotoxicity (Kupchan 1970, Kupchan et al. 1970, 1971). Changes such as saturation or additions to this methylene group resulted in the loss of cytotoxicity and tumor inhibition (e.g. Kupchan et al. 1971, Pettit and Cragg 1973, Howie et al. 1974). The presence of a conjugated ester, cyclopentenone, or exomethylene on the lactone in addition appear to enhance cytotoxicity. However, Lee et al. (1971) demonstrated that the most direct factor responsible for cytotoxicity among sesquiterpene lactones is the $O=C-C=CH_2$ system, whether it involves the lactone or cyclopentenone. Additional alkylating groups may enhance cytotoxicity significantly (Lee et al. 1973). In their further studies Lee et

al. (1972, 1974, 1977a) and Hall et al. (1977) concluded that the α-methylene-γ-lactone moiety is less important than the α,β-unsaturated ketone moiety with respect to cytotoxicity as well as antimicrobial activity (Lee et al. 1977b). However, bakkenolide-A, which is a β-methylene-γ-lactone that does not have the $O=C-C=CH_2$ system, is cytotoxic (Jamieson et al. 1976). This suggests that other structural features of sesquiterpene lactones also play an important role in cytotoxic activity of these compounds.

Schlewer et al. (1979) studied the cytotoxic activity of 20 synthetic methylene- -butyrolactones. Many of these compounds were cytotoxic, however, because chemically close compounds differed greatly in their activity, these authors concluded that there was no clear structure-activity relationship.

Chemical and biological studies support the view that sesquiterpene lactones inhibit tumor growth by selective alkylation of growth-regulatory biological macromolecules such as key enzymes which control cell division (Kupchan 1974). These tumor inhibitors may have a triple selectivity: (a) for thiols over other nucleophiles such as amines; (b) for particular sulphydryl enzymes; and (c) for particular sulphydryl groups within those enzymes (Kupchan 1974). This hypothesis is supported by the observed loss of activity of sulphydryl enzymes phosphofructokinase (Hanson et al. 1970) and glycogen synthase (Smith et al. 1972) after their reaction with some sesquiterpene lactones known to act as tumor inhibitors. In vivo studies (Lee et al. 1977a, Hall et al. 1977) showed that sesquiterpene

lactones inhibited nuclear DNA synthesis and DNA polymerase enzymatic activity in tumor cells and interfered with glycolytic and mitochondrial energy processes. These authors concluded that the inhibition of cellular enzyme activities and metabolism with these lactones is by their reaction with the available thiol groups of the enzymes in the tumor cells.

8. Mutagenic activity

Parthenin, a sesquiterpene lactone from P. hysterophorus, has been reported to have the ability to break human leucocyte chromosomes in vitro and to induce micronuclei formation in the polychromic erythrocytes of mice in vivo (Vaidya et al. 1978). The exact mechanism underlying the observed cytogenetic damage caused by parthenin is not known.

Bacillus thuringiensis which is extensively used as an insecticide can mutate to B. anthracis, a pathogenic microorganism for man and other animals. In their studies on effects of sesquiterpene lactones on B. thuringiensis Norman et al. (1976) found that hymenovin but not tenulin has this mutagenic effect.

9. Anti-inflammatory activity

Hall et al. (1979) tested some sesquiterpene lactones for anti-inflammatory activity in rodents. In the carageenan inflammation screening tests and in the tests for inhibition of

the writhing reflex, the exomethylene on the lactone ring of the sesquiterpene lactones was found to be required for potency. The sesquiterpene lactones were only marginally effective against induced pleurisy. In the anti-arthritic tests compounds with the methylene on the lactone ring, the unsubstituted cyclopentenone ring, and the epoxy cyclopentenone were significantly active at relatively low doses.

10. Analgesic activity

A crude extract of Helenium amarum was found to have analgesic activity and inhibited the writhing syndrome in mice previously induced by injection of acetic acid. Amarilin (a pseudoguaianolide) isolated from this plant extract was found to be responsible for the major part of the analgesic action. Also some other sesquiterpene lactones, helenalin, tenulin, isotenulin, and their derivatives, showed analgesic activity which was, however, weaker than that of amarilin (Lucas et al. 1964).

The above review of various biological activities of sesquiterpene lactones indicates that these naturally occurring compounds influence different physiological processes of many types of organisms. However, these earlier studies do not usually provide sufficient data for explaining the mode of action of sesquiterpene lactones and hence the relationship between chemical structures and various biological activities of these compounds. Therefore, in this study I examined activities

of selected sesquiterpene lactones against various types of organisms, in particular against bacteria (Chapter 1), fungi (Chapter 2), insects (Chapter 3), and mammals (Chapter 4). The main purpose of my study was: (1) to relate biological activities of sesquiterpene lactones to their chemical structure; and (2) to establish whether there is any clear pattern between chemical structures and activities of sesquiterpene lactones against various types of organisms.

CHAPTER 1

Antibacterial activity of sesquiterpene lactones

Introduction

Many plants exhibit antimicrobial activity caused by compounds of various structures (e.g. Vichkanova et al. 1971, Mitscher et al. 1972, Mitcher 1975, Towers et al. 1977a, Ieven et al. 1978). Sesquiterpene lactones have been reported to exhibit antibacterial activity, for example xanthatin from Xanthium pennsylvanicum (Little et al. 1950), cnicin from Cnicus benedictus (Vanhaelen-Fastre 1968, 1972), acroptilin from Acroptilon repens, mibulactone from Artemisia taurica, lactocin from Cichorium intybus, and saurin from Saussurea pulchella (Vichkanova et al. 1971), and mikanolide and dihydromikanolide from Mikania monaganensis (Mathur et al. 1975). Lee et al.(1977b) tested 36 natural sesquiterpene lactones or their derivatives against two Gram positive and three Gram negative bacteria and found 19 of them to be active. These studies showed that some sesquiterpene lactones inhibit growth of bacteria and that this activity is associated with the exocyclic methylene group on the lactone (Vanhaelen-Fastre 1972), or with an α,β-unsubstituted cyclopentenone ring (Lee et al. 1964, 1977b). Since only a small number of naturally occuring sesquiterpenes were examined, there is a need for a larger scale investigation.

Therefore, I screened 57 sesquiterpene lactones for their activity against 6 bacteria. The purpose of this investigation was: (1) to examine more thoroughly which sesquiterpene lactones exhibit antibacterial activities; (2) to compare activities of individual sesquiterpene lactones against various types of bacteria; (3) to evaluate antibacterial activities of sesquiterpene lactones in relation to their chemical structure.

Experimental

Cultures of Staphylococcus albus (U.B.C. #48), Bacillus subtilis (U.B.C. #221), Streptococcus faecalis (U.B.C. #197), Escherichia coli (U.B.C. #219), Proteus vulgaris (U.B.C. #15), Proteus mirabilis (U.B.C. #5) and Pseudomonas fluorescens (U.B.C. #9) were obtained from the Department of Microbiology, U.B.C. The sesquiterpene lactones were isolated, prepared, or obtained as described in the Sections I and II.

(1) Antimicrobial activity screening test

Each culture was spread over an agar plate containing the nutritional medium (Bacto Nutrient Broth, Difco, Lab., Michigan) using sterile cotton swabs. Crystals (approximately 1.5 mg) of the sesquiterpene lactones to be tested were placed directly on the plates prepared in this way. The plates, in duplicate, were incubated at 37°C in the dark and examined after 24 hours. Lactones which caused a clear area with complete bacterial growth inhibition were considered strongly active. Those in which some colonies were present in the otherwise clear area were considered weakly active, and those which did not inhibit growth of bacteria were considered to be inactive (Table 8).

Some sesquiterpene lactones obtained later during my study were tested on P. mirabilis instead of on P. vulgaris which was no longer available.

Results and Discussion

Results of the antibacterial screening tests (Table 8) show that the majority of sesquiterpene lactones tested are antimicrobial agents. Out of 309 tests performed with 57 sesquiterpene lactones against 6 bacteria (some lactones were tested against fewer bacteria) 88 (29%) were strongly positive and 48 (16%) were weakly positive. Out of 57 sesquiterpene lactones examined, 38 (67%) strongly inhibited the growth of at least one bacterium, 15 (26%) were only weakly active, and only 4 (7%) had no evident effect on their growth (Table 9).

While I examined approximately only 6% of all presently known sesquiterpene lactones, my studies show that when these compounds are categorized according to their skeletal classes, there are between 56 and 82% of strongly active compounds within individual classes (Table 9). Eudesmanolides appear to have the largest proportion of active compounds, whereas guaianolides seem to be the least frequently active against bacteria (Table 9).

(1) Activity against individual bacteria

Most of the sesquiterpene lactones examined were active against *B. subtilis* and *S. albus* (Table 10). However, the proportion of lactones displaying activity against individual bacterial species decreased in the following order: *P. vulgaris* (or *P. mirabilis*), *S. faecalis*, *E. coli*, and *P. fluorescens* (Table 10). Great differences in the proportion of compounds

inhibiting the growth of individual bacteria indicate that the antibacterial activity of sesquiterpene lactones must be partially determined by differences in biochemistry and physiology of various types of bacteria.

Generally more lactones inhibited Gram positive than Gram negative bacteria (Table 10). Approximately 64% of the sesquiterpene lactones tested inhibited the growth of Gram positive bacteria (this includes compounds exhibiting both strong and weak inhibition), whereas only 24% of the compounds were active against Gram negative bacteria. It is possible that the weaker antimicrobial activity of sesquiterpene lactones against Gram negative bacteria is a result of the more complex outer layers of Gram negative cells, which probably reduce their permeability to drugs (see Franklin and Snow 1975), such as sesquiterpene lactones. In addition, the presence of cysteine residues in the protein-lipopolysaccharide outermost layer, as known from E. coli (Franklin and Snow 1975), might prevent penetration of these compounds through the outer membrane by reacting with lactones.

The stronger antibacterial activity of sesquiterpene lactones against Gram positive than Gram negative bacteria is similar to that reported by Lee et al. (1977b) who examined activity of selected sesquiterpene lactones and their derivatives against 5 bacteria. They concluded, however, that the compounds they studied were active only against Gram positive bacteria, such as Staphylococcus aureus and B. subtilis , but were inactive against Gram negative species, such as E.

coli, Salmonella enteritidis, and Klebsiella pneumoniae. Using the screening test I found that sesquiterpene lactones inhibited growth of Gram negative bacteria strongly in 11% and weakly in 13% out of 150 tests (Table 10). The gram negative bacteria, P. vulgaris and P. mirabilis, were inhibited even more frequently than the Gram positive S. faecalis. These differences between the results of Lee et al. (1977b) and my study are likely a consequence of: (1) the use of a larger number of sesquiterpene lactones by me, (2) the fact that different sesquiterpene lactones were tested in these two studies, (3) the use of different microorganisms in the tests, and finally (4) the use of different techniques for testing the antimicrobial activity by Lee et al. (1977b) and in my study.

(2) Relationship between chemical structure and antibacterial activity

It has been suggested that the mechanism of antibacterial action of compounds with unsaturated ketone and lactone functions is by reaction of these groups with thiol groups of enzymes (Cavallito and Haskell 1945). More recently, in vitro reactions between the unsubstituted cyclopentenone and/or exocyclic methylene on the lactone ring of sesquiterpene lactones and thiol agents, such as cysteine, have been demonstrated (Kupchan et al. 1970, 1971, Lee et al. 1977a, Hall et al. 1977, Picman et al. 1979). This evidence suggests that the antibacterial activity of sesquiterpene lactones might be through their reaction with thiol containing compounds e.g.

enzymes.

Lee et al. (1977a) concluded that one of the structural requirements for cytotoxic antitumor as well as antimicrobial action of sesquiterpene lactones is the presence of the β-unsubstituted cyclopentenone. They also suggested that significant antimicrobial activity is independent of the presence or absence of an α-methylene-γ-lactone moiety. However, these conclusions were reached on the basis of tests of only 13 selected sesquiterpene lactones and some of their derivatives. Therefore, in the following section I examined the relationship between antibacterial activity and the presence of various functional groups of a larger sample of mainly naturally occuring sesquiterpene lactones. The exomethylene on the lactone represents one of the active sites of sesquiterpene lactones as demonstrated by the reaction of some of them with cysteine (e.g. Picman et al. 1979). To examine a possible role of this moiety in the antibacterial activity of sesquiterpene lactones, I divided all lactones into two groups based on the presence or absence of this moiety (Table 11). Approximately 50% of tests with sesquiterpene lactones possessing the exomethylene on the lactone were positive and 36% of tests including lactones lacking this moiety gave positive results. The fact that some lactones without the exomethylene were active (several of them strongly) suggests that this group is not necessary for antibacterial activity of sesquiterpene lactones. In addition, this moiety is not always sufficient for the activity because many sesquiterpene lactones possessing the C13-methylene gave negative results.

To eliminate possible differences in activity between major groups of sesquiterpene lactones due to their different basic skeletons, I evaluated the skeletal classes individually. Individual classes were grouped according to the presence or absence of the exocyclic methylene on the lactone ring; pseudoguaianolides were also divided according to the presence or absence of another possibly active site, the C2,C3 double bond. Furthermore, I also examined other functional groups for their possible importance in the antibacterial activity of sesquiterpene lactones. Since the largest number of tests were usually performed with S. albus whose growth was frequently inhibited by tested lactones (Table 10), I examined the relationship between the presence of functional groups in sesquiterpene lactones and their inhibition of growth of this species.

(a) Germacranolides

Out of 32 tests performed with a total of 12 germacranolides on three Gram positive bacteria, 19 tests included compounds possessing the C13-exomethylene on the lactone ring. Of these 84% were positive (mostly strongly; see Table 12). Of all 13 tests including lactones without the C13-methylene function, 77% were positive (Table 12). This clearly suggests that in germacranolides the methylene on the lactone is not solely responsible for antibacterial activity of these compounds, although this moiety might contribute to the observed activity in some cases.

Germacranolides were generally less active against Gram negative bacteria. Since this lack of activity was similar for compounds possessing or lacking the exomethylene (Table 12), these results support the conclusion reached above for Gram positive bacteria that this moiety does not play an important role in antimicrobial activity.

Table 13 gives information on other functional groups of germacranolides tested in relation to their antibacterial activity against S. albus. It appears that antibacterial activity of selected compounds or the lack of it cannot be simply explained either by the presence or absence of any given functional group. Some of these groups are present in both categories of lactones with and without activity. These individual groups either do not affect the activity of germacranolides or their activity might become expressed only in the presence (or absence) of some other group(s). On the other hand, functional groups present only in lactones with or without antibacterial activity might enhance or hinder their activity. For example, all glaucolides (A,D,F, and G) and marginatin, which have similar structures but lack the exomethylene on the lactone ring, are active (strongly and weakly) as well as inactive (Table 13). Marginatin and glaucolide-G which possess the double bonds between C1-C10 and C7-C11 and R2 on C8 are strongly active. Glaucolide-F, which does not possess the C1-C10 double bond but has the C7-C11 double bond as well as R2, is only weakly active. This indicates that the C7-C11 double bond is an important active site but that the C1-C10 double bond enhances the activity of marginatin and glaucolide-G.

Glaucolide-D, missing the C7-C11 double bond but possessing the C1-C10 double bond, is inactive. It is possible that: (a) the C1-C10 double bond itself is not an important active site of glaucolides, or (b) that the C1-C10 is an active site but its activity is inhibited in glaucolide-D by the acetyl group present on C2. This group, which is not present on other glaucolides or marginatin, might reduce the activity of sesquiterpene lactones (see also pseudoguaianolides, this chapter). Also the epoxy group which sometimes reduces or incresases the activity of sesquiterpene lactones (Lee et al. 1977b) in R4 on C8 might reduce the activity of glaucolide-D.

(b) Guaianolides

Out of 18 tests with guaianolides possessing the exomethylene 11 were positive (9 strongly) against three Gram positive bacteria (Table 14). However, of all tests performed with lactones lacking this group only 4 (out of 9) were positive (only weakly). These results indicate that the exocyclic methylene could play a role in determining the antibacterial activity of the selected guaianolides. The sample size is, however, too small for making a definite conclusion.

In contrast to Gram positive bacteria the exomethylene on the lactone does not seem to play any role in the activity against Gram negative bacteria where both groups of guaianolides with and without the C13-methylene showed very little activity (Table 14).

As can be seen from Table 15 there is no clear relationship

between the presence or absence of various functional groups on the skeleton of different guaianolides and the activity against S. albus. It is clear that certain potentially active groups (e.g. C3-C4 and C1-C10 double bonds in desacetoxymatricarin) are not by themselves sufficient for the inhibition of growth of this as well as other tested bacteria (Tables 8,15). But it is possible that (1) these groups might enhance or reduce the activity of some other group(s), or (2) the activity of such groups could become expressed only in the presence of another group(s). For example, cumambrin-A possessing a C3-C4 double bond is inactive whereas cumambrin-B acetate which lacks this double bond (this is the only difference between these two compounds) is active. Hence the C3-C4 double bond in this instance reduces activity of another functional group(s) present in both cumambrins. It is also possible that the activity of any given potentially active group might be influenced by its configuration on a sesquiterpene lactone molecule. Thus, in certain configurations, the activity of these presumably active functional groups might be reduced or even inhibited.

(c) Eudesmanolides

Out of 27 tests with eudesmanolides possessing the C13-methylene 67% were positive (mostly strongly) against Gram positive bacteria (Table 16). This suggests that this group is at least partially responsible for inhibiting properties of eudesmanolides against the bacteria examined. This is supported by a complete loss of activity of α-santonin which does not possess the exocyclic methylene. However, the presence of the

methylene-γ-lactone moiety is not sufficient for activity of these compounds since 9 out of 27 eudesmanolides possessing this group were inactive (Table 16).

Tests carried out on Gram negative bacteria were mostly negative for both groups of lactones with the exomethylene present or absent (Table 16).

Table 17 summarizes other functional groups present in various eudesmanolides tested against S. albus. All eudesmanolides strongly active against this bacterium possess the α-OH group (except pinnatifidin which possesses a ketone group instead) on C1 or C2. This suggests that this group might enhance the antibacterial activity of eudesmanolides. Thus alantolactone might be active as a result of the presence of the C13-exomethylene (and probably C5-C6 double bond) but it is weaker because it lacks the hydroxyl groups. Also the lack of the activity of α-santonin, compared with the strongly active, structurally similar ludovicin-C, might result not only from the absence of the exocyclic methylene but also from the lack of the hydroxyl groups on C1 and C2.

(d) Pseudoguaianolides

It has been previously suggested that pseudoguaianolides may possess two active sites (the exomethylene on the lactone ring and the C2,C3 double bond) which are responsible for their various biological activities (Lee et al.1974, 1977a,b, Hall et al. 1977, Picman et al. 1979). Table 18 summarizes results of antibacterial tests of selected pseudoguaianolides possessing

both, either, or neither of these functional groups. Compounds possessing the exocyclic methylene alone were strongly active against Gram positive bacteria in 32% of the tests (but none against S. faecalis). However, the fact that 45% of all tests were negative suggests that the presence of the α-methylene-γ-lactone moiety alone does not guarantee antibacterial activity of pseudoguaianolides possessing this group. This moiety apparently plays an even less important role in antimicrobial activity against Gram negative bacteria since 86% of the tests were negative (Table 18).

Out of 22 pseudoguaianiolides examined only two lactones (tenulin and isotenulin) lack the exocyclic methylene but possess the α,β-cyclopentenone ring. Both lactones were active against all three Gram positive bacteria. However, these lactones were inactive when tested against Gram negative bacteria, with the exception of tenulin which weakly inhibited growth of P. vulgaris (Tables 8, 18). This indicates that the presence of a C2-C3 double bond in pseudoguaianolides may be associated with the activity against Gram positive but not against Gram negative bacteria. However, 55% of tests carried out on Gram positive bacteria using lactones lacking this moiety (but possessing an exomethylene on the lactone) were positive. This fact indicates that the presence of the α,β-unsubstituted cyclopentenone is not always a requirement for the antibacterial activity of pseudoguaianolides.

Table 18 shows that when both active sites (the cyclopentenone ring and the exomethylene on the lactone ring)

are present, pseudoguaianolides exhibited a strong antibacterial activity against S. albus and B. subtilis. This indicates that the presence of both moieties enhances the activity of pseudoguiaianolides against these two bacteria. However, there was no clear relationship between the presence of the two moieties and the activity of those pseudoguaianolides against S. faecalis and three Gram negative bacteria (Table 18). These different results could possibly be explained by different chemistries of different bacteria involved.

The three pseudoguaianolides containing lactones lacking both moieties (the exocyclic methylene and the endocyclic C2,C3 double bond) were inactive against five bacteria tested (Table 18). However, two of these compounds (dihydroisoparthenin and tetrahydroparthenin) weakly inhibited growth of B. subtilis. These results are consistent with the view that the two moieties play the most important role but other functional groups might also influence the activity of some pseudoguaianolides against bacteria.

To establish whether the activity of the exocyclic methylene and β-unsubstituted cyclopentenone could be influenced by their interactions with other potentially active groups present in pseudoguaianolides, I examined the relationship between antimicrobial activity of 22 selected pseudoguaianolides against S. albus and the presence of various functional groups (Table 19). Two major conclusions can be made from the results. First, the exocyclic methylene group itself appears to be important but is not sufficient to completely inhibit the growth

of bacteria. Another active group must be present on a sesquiterpene lactone molecule to make the pseudoguaianolide an active compound. The group might for example be the C2,C3 double bond whose presence alone is sufficient for activity against Gram positive bacteria. This group augments the activity of the exomethylene on the lactone (or vice versa), leading to growth inhibition of at least two Gram positive bacteria (Table 18).

Secondly, as concluded earlier, the presence of a methylene function on C13 is usually associated with antimicrobial activity. However, there are also four compounds possessing this moiety which had no visible inhibitory effects on growth of S. albus (Table 19). Since the monoadduct of parthenin with L-cysteine via exomethylene on the lactone only slightly inhibited growth of S. albus, whereas parthenin had strong inhibitory effects (Picman 1977), the α-methylene-γ-lactone moiety must be partially responsible for the antimicrobial activity of parthenin and probably also of other pseudoguianolides. Therefore, the lack of activity of four pseudoguianolides possessing this moiety could most likely be explained by the presence of other group(s) which conteract the effects of the exomethylene group. This idea is supported by the fact that all four inactive pseudoguaianolides possessing the exomethylene on the lactone also have acetyl group(s) on C4 or C14 (Table 19). The suggestion that these acetyl groups might exert inhibitory effects on the activity of the C13-methylene is furthermore supported by evidence on activity of structurally similar lactones which differ only in the presence or absence of the acetyl groups. For example, coronopilin is strongly active but

tetraneurin-A, which differs from coronopilin only by the presence of a C15-acetyl group, is weakly active, and tetraneurin-B, which differs from coronopilin only by the presence of a C14-acetyl group, is completely inactive (Table 19). Hall et al. (1979) reached similar conclusions in their study on anti-inflammatory activity of sesquiterpene lactones. These authors concluded that the hydroxyl group may play a significant role in receptor binding since esterification (as $-OCOCH_2$) or elimination of the hydroxyl group drastically reduced the anti-inflammatory activity of the compounds studied.

However, in contrast to these suggestions, there are three pseudoguaianolides which exhibited strong antibacterial activity (Table 19), and which also possess a C13-methylene as well as an acetyl group(s). The activity of these compounds might be explained by the presence of additional active sites which further enhance the activity of the exocyclic methylene. Thus, conchosin-B is a ß-unsubstituted cyclopentenone, gaillardilin has an epoxy group, and spathulin has two hydroxyl groups.

In addition, the position of acetyls on the skeleton could play an important role and thereby influence the activity of pseudoguianolides. All inactive compounds have acetyl groups on C4 or C14. In contrast, the strongly active compounds have the acetyl group on C6, C9, or C15. (Table 19). The importance of the position of the acetyl group can further be seen from the fact that tetraneurin-A which has an acetyl on C15 is only weakly active, whereas tetraneurin-B which has acetyl on C14 (this is the only difference in chemical structure between the

two compounds) is inactive (Table 19).

Conclusions

There is no simple general relationship between the antibacterial activity of sesquiterpene lactones and their chemical structure. My results suggest that the antibacterial activity of these compounds could be determined by any combination of the following factors:

(1) The presence or absence of functional groups such as an exocyclic methylene on the lactone ring in some lactones, or an β-unsubstituted cyclopentenone ring in pseudoguianolides, or a C7-C11 double bond in germacranolides;

(2) The presence or absence of various additional groups which enhance (e.g. α-hydroxyl in eudesmanolides) or reduce (e.g. acetyl on C4 or C14 in paseudoguaianolides) the antibacterial activity of a given lactone;

(3) The position of individual additional groups, which increase or reduce the activity of sesquiterpene lactones, on a skeleton (e.g. the acetyl group on C4 and C14 apparently reduces the activity of some pseudoguiaianolides but in positions on C6, C9, and C15 it does not seem to play an important role);

(4) The configuration of functional groups on a skeleton (e.g. the α- or β- position of a hydroxyl function in eudesmanolides);

(5) The availability and accessibility of vital thiol compounds

present in bacteria and their chemical affinity for sesquiterpene lactones. For example, although parthenin *in vitro* forms a monoadduct with L-cysteine through the C13 exocyclic methylene (Picman et al. 1979), it forms a monoadduct *in vitro* with thiophenol through its unsubstituted cyclopentenone ring (Panfil and Towers; unpublished). Further, when L-cysteine is in excess, it forms two adducts with parthenin. In contrast, glutathione always forms with parthenin only a single adduct (Picman et al. 1979).

Table 8. Screening test for antimicrobial activity of selected sesquiterpene lactones against six bacteria.

Sesquiterpene lactone		Response to					
Number in Appendix	Name	*S. albus*	*B. subtilis*	*S. faecalis*	*E. coli*	*P. vulgaris*	*P. fluorescens*
	Germacranolides						
1	Parthenolide	+	NT	-	±	NT	NT
2	Pyrethrosin	+	+	+	-	-	±
4	Chamissonin, acetyl	+	+	±	-	-	-
5	Tamaulipin-A	±	-	-	-	-	-
7	Eupatoriopicrin	+	+	+	-	±	-
14	Glaucolide-A	±	±	-	-	-	NT
16	Marginatin	+	+	NT	-	±	NT
17	Glaucolide-D	-	-	±	-	-	-
19	Glaucolide-F	±	±	NT	-	NT	NT
20	Glaucolide-G	+	+	+	-	±	±
21	Elephantopin	-	+	+	-	-*	-
22	Parthenolide, 9-α-OH	+	+	+	+	+*	±

Table 8. Continued.

	Guaianolides						
23	Cumambrin-A	-	-	-	-	-*	-
24	Cumambrin-B	-	+	-	-	±*	-
25	Cumambrin-B, dihydro-	±	±	±	±	±	-
26	Cumambrin-B, tetrahydro-	±	-	-	-	-	-
27	Cumambrin-B, acetate	+	+	±	±	-	-
28	Cumambrin-B, formyl	+	+	-	-	-	-
30	Matricarin, desacetoxy-	-	-	-	-	-	-
31	Grossheimin	+	+	-	-	+*	-
32	Ivalin, pseudo-	+	+	±	±	±	+
	Eudesmanolides						
33	Alantolactone	±	+	-	-	-	-
35	Alantolactone, iso-	-	±	-	-	±	-
36	Ivalin	+	+	-	-	-	-

Table 8. Continued.

37	Ivasperin	+	+	-	-	+	-
38	Pinnatifidin	+	+	-	-	+	-
39	Pulchellin-C	+	±	±	±	-	-
40	α-Santonin	-	-	-	-	±	-
41	Ludovicin-A	+	NT	-	+	NT	NT
42	Ludovicin-B	+	NT	NT	-	NT	NT
43	Ludovicin-C	+	+	±	-	-	-
46	Santamarine	-	+	-	-	-*	-
	Pseudoguaianolides						
47	Parthenin	+	+	+	+	+	-
48	Parthenin, dihydroiso-	-	±	-	-	-	-
49	Parthenin, tetrahydro-	-	±	-	-	-	-
51	Hymenin	+	+	±	+	+	-
52	Ambrosin	+	+	NT	±	NT	NT
53	Coronopilin	+	+	-	+	+	-
54	Damsin	+	+	+	-	-	-

Table 8. Continued.

55	Hysterin	-	±	-	-	-	-
56	Tetraneurin-A	±	+	-	-	-*	-
57	Tetraneurin-B	-	+	NT	-	-*	NT
58	Tetraneurin-D	-	±	-	-	-	-
59	Tetraneurin-E	-	±	-	-	-*	-
60	Conchosin-A	±	-	-	-	±	-
61	Conchosin-B	+	NT	-	-	NT	-
63	Tenulin	+	+	+	-	±	-
64	Tenulin, iso-	±	+	±	-	-	-
65	Gaillardilin	+	NT	-	NT	NT	NT
66	Helenalin	+	+	-	+	+*	-
67	Flexuosin-B	-	-	-	-	-	-
68	Spathulin	+	+	-	-	-	-
69	Balduilin	+	+	±	-	-	-
70	Cumanin	±	+	-	-	±	-

Table 8. Continued.

	Other sesquiterpene lactones						
76	Quadrone	-	-	NT	NT	NT	NT
77	Axivalin	+	+	-	-	-*	-
78	Ivaxillarin	+	+	NT	+	NT	-

NT= not tested

*= tested on P. mirabilis

+= complete inhibition

±= some colonies present

-= no inhibition

Table 9. Summary of antimicrobial activity of sesquiterpene lactones from individual skeletal classes against six bacteria.

Class	No. (%) of sesq. lactones active against at least one bacterium		Number (%) inactive sesq. lact.	Total
	strongly	weakly		
Germacranolides	8	4	0	12
Guaianolides	5	2	2	9
Eudesmanolides	9	2	0	11
Pseudo-guaianolides	14	7	1	22
Other sesq. lactones	2	0	1	3
Total	38 (67)	15 (26)	4 (7)	57

Table 10. Summary of antimicrobial activity of selected sesquiterpene lactones against individual bacteria.

Bacterium	No. (%) pos. responses		Number (%) negative responses	Total No. tests
	Strong	Weak		
S. albus	31 (54)	10 (18)	16 (28)	57
B. subtilis	33 (64)	10 (19)	9 (17)	52
S. faecalis	8 (16)	10 (20)	32 (64)	50
Total Gram positive	72 (45)	30 (19)	57 (36)	159
E. coli	7 (13)	6 (11)	42 (76)	55
P. vulgaris/ *P. mirabilis*	8 (17)	11 (23)	29 (60)	48
P. fluo-rescens	1 (2)	3 (6)	43 (92)	47
Total Gram negative	16 (11)	20 (13)	114 (76)	150

Table 11. Summary of antibacterial activity of sesquiterpene lactones as related to the presence or absence of the exomethylene on the γ-lactone ring.

Bacterium	No. sesq. lact. with C13=CH_2	No. (%) responses			No. sesq. lact. without C13=CH_2	No. (%) responses		
		Positive				Positive		
		Strong	Weak	Negative		Strong	Weak	Negative
S. albus	42	28	5	9	15	3	5	7
B. subtilis	37	29	5	3	15	4	5	6
S. faecalis	38	6	7	25	12	2	3	7
E. coli	41	7	5	29	14	0	1	13
P. vulgaris/ *P. mirabilis*	35	8	6	21	13	0	5	8
P. fluorescens	36	1	2	33	11	0	1	10
Total	229 (100)	79 (35)	30 (13)	120 (52)	80 (100)	9 (11)	20 (25)	51 (64)

Table 12. Antimicrobial activity of germacranolides as related to the presence or absence of the methylene group on the γ-lactone ring.

Bacterium	No. sesq. lact. with $C13{=}CH_2$	No. (%) responses: Positive Strong	Positive Weak	Negative	No. sesq. lact. without $C13{=}CH_2$	No. (%) responses: Positive Strong	Positive Weak	Negative
S. albus	7	5	1	1	5	2	2	1
B. subtilis	6	5	0	1	5	2	2	1
S. faecalis	6	4	1	1	3	1	1	1
Total Gram positive	19 (100)	14 (74)	2 (10)	3 (16)	13 (100)	5 (39)	5 (39)	3 (23)
E. coli	7	1	1	5	5	0	0	5
P. vulgaris/ *P. mirabilis*	6	1	1	4	4	0	2	2
P. fluorescens	6	0	2	4	2	0	1	1
Total Gram negative	19 (100)	2 (11)	4 (21)	13 (68)	11 (100)	0	3 (27)	8 (73)

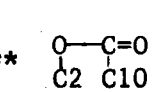
Table 13. Antimicrobial activity of germacranolides against S. albus as related to the presence or absence of various functional groups.

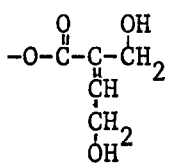
Sesquiterpene lactone		Presence or absence of functional groups																
No. in Appendix	Name	C1=C10	C1-C10 (O)	C1=O	C2-OAc	C3-OAc	C4-C5 (O)	C4=C5	C6-OAc	C6,C7 γ-lactone	C7,C8 γ-lactone	C7=C11	C8-R_1	C8-R_2	C8-R_3	C8-R_4	C13-OAc	C13=CH_2
Strongly active:																		
1	Parthenolide	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+
2	Pyrethrosin	-	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	+
4	Chamissonin, diacetate	+	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	+
7	Eupatoriopicrin	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-	-	+
16	Marginatin	+	-	-	-	-	+	-	-	+	-	+	-	+	-	-	+	-
20	Glaucolide-G	+	-	-	-	-	+	-	-	+	-	+	-	+	-	-	+	-
22	Parthenolide, 9-α-OH	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+
Weakly active:																		
5	Tamaulipin-A	+	-	-	*	-	-	+	-	+	-	-	-	-	-	-	-	+
14	Glaucolide-A	-	-	+	-	-	+	-	-	+	-	+	-	-	+	-	+	-

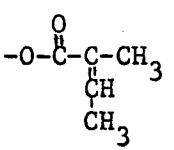
Table 13. Continued.

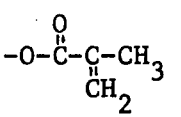
19	Glaucolide-F	-	-	+	-	-	+	-	-	+	-	+	-	+	-	-	+	-	
Inactive:																			
17	Glaucolide-D	+	-	-	+	-	+	-	-	+	-	-	-	-	-	+	+	-	
21	Elephantopin	+	-	-	**	-	+	-	-	+	-	-	-	-	+	-	-	+	

* OH

** 

R_1= 

R_2= 

R_3= 

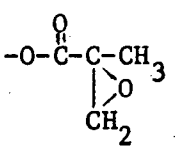
R_4= 

Table 14. Antimicrobial activity of guaianolides as related to the presence or absence of the methylene group on the γ-lactone ring.

Bacterium	No. sesq. lact. with $C13{=}CH_2$	No. (%) responses: Positive Strong	Positive Weak	Negative	No. sesq. lact. without $C13{=}CH_2$	No. (%) responses: Positive Strong	Positive Weak	Negative
S. albus	6	4	0	2	3	0	2	1
B. subtilis	6	5	0	1	3	0	1	2
S. faecalis	6	0	2	4	3	0	1	2
Total Gram positive	18 (100)	9 (50)	2 (11)	7 (39)	9 (100)	0	4 (44)	5 (56)
E. coli	6	0	2	4	3	0	1	2
P. vulgaris/ *P. mirabilis*	6	1	2	3	3	0	1	2
P. fluorescens	6	1	0	5	3	0	0	3
Total Gram negative	18 (100)	2 (11)	4 (22)	12 (67)	9 (100)	0	2 (22)	7 (78)

Table 15. Antimicrobial activity of guaianolides against S. albus as related to the presence or absence of various functional groups.

Sesquiterpene lactone		Presence or absence of functional groups											
No. in Appendix	Name	C1=C10	C2=O	C3=O	C3=C4	C6,C7 γ-lactone	C7,C8 γ-lactone	C8-OH	C8-OAc	C8-formyl	C10-OH	C10=CH_2	C13=CH_2
Strongly active:													
27	Cumambrin-B, acetate	-	-	-	-	+	-	-	+	-	+	-	+
28	Cumambrin-B, formyl	-	-	-	-	+	-	-	-	+	+	-	+
31	Grossheimin	-	-	+	-	+	-	+	-	-	-	+	+
32	Ivalin, pseudo	+	-	-	-	-	+	-	-	-	-	-	+
Weakly active:													
25	Cumambrin-B, dihydro	-	-	-	+	+	-	+	-	-	+	-	-
26	Cumambrin-B, tetrahydro	-	-	-	-	+	-	+	-	-	+	-	-

Table 15. Continued.

Inactive:													
23	Cumambrin-A	-	-	-	+	+	-	-	+	-	+	-	+
24	Cumambrin-B	-	-	-	+	+	-	+	-	-	+	-	+
30	Matricarin, desacetoxy	+	+	-	+	+	-	-	-	-	-	-	-

Table 16. Antimicrobial activity of eudesmanolides as related to the presence or absence of the methylene group on the γ-lactone ring.

Bacterium	No. sesq. lact. with C13=CH_2	No. (%) responses: Positive Strong	Positive Weak	Negative	No. sesq. lact. without C13=CH_2	No. (%) responses: Positive Strong	Positive Weak	Negative
S. albus	10	7	1	2	1	0	0	1
B. subtilis	8	6	2	0	1	0	0	1
S. faecalis	9	0	2	7	1	0	0	1
Total Gram positive	27 (100)	13 (48)	5 (19)	9 (33)	3 (100)	0	0	3 (100)
E. coli	10	1	1	8	1	0	0	1
P. vulgaris/ *P. mirabilis*	8	2	1	5	1	0	1	0
P. fluorescens	8	0	0	8	1	0	0	1
Total Gram negative	26 (100)	3 (12)	2 (8)	21 (81)	3 (100)	0	1 (33)	2 (67)

Table 17. Antimicrobial activity of eudesmanolides against S. albus as related to the presence or absence of various functional groups.

Sesquiterpene lactone		Presence or absence of functional groups												
No. in Appendix	Name	C1-α-OH	C1-β-OH	C1=C2	C2-α-OH	C3-α-OH	C3-β-OH	C3=C4	C4=CH_2	C4=C5	C5=C6	C6,C7 γ-lactone	C7,C8 γ-lactone	C13=CH_2
Strongly active:														
36	Ivalin	-	-	-	+	-	-	-	+	-	-	-	+	+
37	Ivasperin	-	+	-	+	-	-	-	+	-	-	-	+	+
38	Pinnatifidin	-	-	-	*	-	-	+	-	-	-	-	+	+
39	Pulchellin-C	-	-	-	+	-	+	-	+	-	-	-	+	+
41	Ludovicin-A	+	-	-	-	**	**	-	-	-	-	+	-	+
42	Ludovicin-B	+	-	-	-	+	-	-	+	-	-	+	-	+
43	Ludovicin-C	+	-	-	-	*	*	-	-	+	-	+	-	+
Weakly active:														
33	Alantolactone	-	-	-	-	-	-	-	-	-	+	-	+	+

Table 17. Continued.

Inactive:														
35	Alantolactone, iso-	-	-	-	-	-	-	-	+	-	-	-	+	+
40	α-santonin	-	-	+	-	*	*	-	-	+	-	+	-	-
46	Santamarine	-	+	-	-	-	-	+	-	-	-	+	-	+

* =O

** -CH-CH-
\O/

Table 18. Antimicrobial activity of pseudoguaianolides as related to the presence or absence of the methylene group on the γ-lactone and the unsubstituted cyclopentenone ring.

Bacterium	No. sesq. lact. with C13=CH_2 only	No. (%) responses: Positive Strong	Weak	Negative	No. sesq. lact. with C2=C3 only	No. (%) responses: Positive Strong	Weak	Negative
S. albus	11	4	3	4	2	1	1	0
B. subtilis	10	6	3	1	2	2	0	0
S. faecalis	10	0	1	9	2	1	1	0
Total Gram positive	31 (100)	10 (32)	7 (23)	14 (45)	6 (100)	4 (67)	2 (33)	0
E. coli	10	1	0	9	2	0	0	2
P. vulgaris/ P. mirabilis	10	1	2	7	2	0	1	1
P. fluorescens	10	0	0	10	2	0	0	2
Total Gram negative	30 (100)	2 (7)	2 (7)	26 (86)	6 (100)	0	1 (17)	5 (83)

Table 18. Continued.

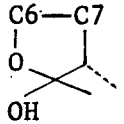
Bacterium	No. sesq. lact. with C13=CH_2 and C2=C3	No. (%) responses			No. sesq. lact. without C13=CH_2 and C2=C3	No. (%) responses		
		Positive				Positive		
		Strong	Weak	Negative		Strong	Weak	Negative
S. albus	6	6	0	0	3	0	0	3
B. subtilis	5	5	0	0	3	0	2	1
S. faecalis	5	1	2	2	3	0	0	3
Total Gram positive	16 (100)	12 (75)	2 (13)	2 (13)	9 (100)	0	2 (22)	7 (78)
E. coli	6	3	1	2	3	0	0	3
P. vulgaris/ P. mirabilis	4	3	0	1	3	0	0	3
P. fluorescens	5	0	0	5	3	0	0	3
Total Gram negative	15 (100)	6 (40)	1 (7)	8 (53)	9 (100)	0	0	9 (100)

Table 19. Antimicrobial activity of pseudoguaianolides against S. albus as related to the presence or absence of various functional groups.

Sesquiterpene lactone		Presence or absence of functional groups												
No. in Appendix	Name	C1-α-OH	C2-OH	C2=C3	C3-OH	C4-OH	C4=O	C6,C7 γ-lactone	C7,C8 γ-lactone	C6-OAc	C9-OAc	C13=CH_2	C14-OAc	C15-OAc
Strongly active:														
47	Parthenin	+	-	+	-	-	+	+	-	-	-	+	-	-
51	Hymenin	+(β)	-	+	-	-	+	+	-	-	-	+	-	-
52	Ambrosin	-	-	+	-	-	+	+	-	-	-	+	-	-
53	Coronopilin	+	-	-	-	-	+	+	-	-	-	+	-	-
54	Damsin	-	-	-	-	-	+	+	-	-	-	+	-	-
61	Conchosin-B	+	-	+	-	-	+	+	-	-	-	+	-	+
63	Tenulin	-	-	+	-	-	+	-	+	a	-	-	-	-
65	Gaillardilin	-	+(β)	-	b	-	-	-	+	+	-	+	-	-
66	Helenalin	-	-	+	-	-	+	-	+	c	-	+	-	-
68	Spathulin	-	+	-	-	+	-	-	+	+	+	+	-	-
69	Balduilin	-	-	+	-	-	+	-	+	+	-	+	-	-

Table 19. Continued.

Weakly active:														
56	Tetraneurin-A	+	-	-	-	-	+	+	-	-	-	+	-	+
60	Conchosin-A	+	d	-	-	-	+	+	-	-	-	+	-	-
64	Tenulin, iso-	-	-	+	-	-	+	-	+	+	-	-	-	-
70	Cumanin	-	-	-	+(β)	+(β)	-	-	+	-	-	+	-	-
Inactive:														
48	Parthenin, dihydroiso-	+	-	-	-	-	+	+	-	-	-	-	-	-
49	Parthenin, tetrahydro-	+	-	-	-	-	+	+	-	-	-	-	-	-
55	Hysterin	-	-	-	-	e	-	+	-	-	-	+	-	c
57	Tetraneurin-B	+	-	-	-	-	+	+	-	-	-	+	+	-
58	Tetraneurin-D	+	-	-	-	+(β)	-	+	-	-	-	+	+	-
59	Tetraneurin-E	+	-	-	-	e	-	+	-	-	-	+	-	c
67	Flexuosin-B	-	+(α)	-	-	-	+	-	+	f	-	-	-	-

a= 

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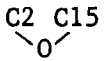
d= 

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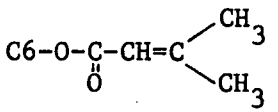
f= 

CHAPTER 2

Antifungal activity of sesquiterpene lactones

Introduction

Several studies of higher plants, their extracts, or chemicals isolated from them show that some species contain antifungal compounds (e.g. Vichkanova et al. 1971, Camm et al. 1975, Towers et al. 1977a, Ieven et al. 1978, Muir 1979, and references therein). Certain sesquiterpene lactones have been reported to possess antifungal properties. For example, xanthatin from Xanthium pennsylvanicum strongly inhibited the growth of Trichophyton mentagrophytes and Candida albicans and slightly inhibited growth of six other fungi (Little et al. 1950). Alantolactone and isoalantolactone have been reported to inhibit the growth of T. mentagrophytes, T. acuminatum, and Epidermophyton sp. (Olechnowicz-Stepien and Stepien 1963). The effect of parthenin on certain phases of fungal growth has been reported. This lactone inhibited sporangial germination and zoospore motility in Sclerospora graminicola but did not exhibit any activity in the conidial development of Aspergillus flavus at the same or greater concentrations (Char and Shankarabhat 1975). Mathur et al. (1975) found mikanolide and dihydromikanolide, from Mikania monagasensis, to be active against Candida albicans. A large scale study by Towers et al.

(1977a) showed that out of 65 sesquiterpene lactones tested against C. albicans only 3 compounds, mikanolide, glaucolide-B, and pseudoivalin were antibiotic. Lee et al. (1977b) who examined the activity of 36 sesquiterpene lactones and their derivatives against C. albicans also concluded that only 5 lactones inhibited growth of this yeast. These studies therefore suggest that sesquiterpene lactones rarely exhibit the activity against C. albicans.

In this study I tested 45 sesquiterpene lactones for their antifungal activities against 3 fungi and 7 lactones against two species of yeasts. The purpose of this study was to: (1) examine which sesquiterpene lactones exhibit antifungal activities, (2) compare the activities of individual sesquiterpene lactones against various types of fungi, and (3) to relate antifungal activities of sesquiterpene lactones to their chemical structure.

Experimental

Cultures of *Microsporum cookei* (U.B.C. #86), *Trichophyton mentagrophytes* (U.B.C. #132), *Fusarium* sp. (U.B.C. #77), *Saccharomyces cerevisiae* (U.B.C. #140), and *Candida albicans* (U.B.C. #54) were obtained from the Department of Microbiology, U.B.C. The sesquiterpene lactones were isolated, prepared, or obtained as described in the Section II.

(1) Antifungal activity screening test

Mycelia of *M. cookei*, *T. mentagrophytes*, and *Fusarium* sp. were scraped from the cultures, suspended in sterile water, and well mixed with cotton swabs to form a fine suspension. Each of the mycelial suspensions or cultures of yeast was evenly spread with sterile cotton swabs over agar plates containing the nutritional medium (Bacto Sabouraud Dextrose Agar, Difco Lab., Michigan). Crystals (approximately 1.5 mg) of the sesquiterpene lactones to be tested were placed directly on the agar plates. *M. cookei*, *T. mentagrophytes*, and *Fusarium* sp., in duplicate, were grown at room temperature in the dark and checked after 5 and 20 days of growth (Fig. 8). The plates with *C. albicans* and *S. cerevisiae*, in duplicate, were incubated at 37°C in the dark and examined 24 hours later. Lactones which caused a completely clear area of fungal growth inhibition around the site where they were placed were considered strongly active, whereas those inhibiting growth only within an area where crystals were placed were considered weakly active. Inactive compounds were those

which had no observable effects on the growth of fungi.

Results and Discussion

Results of the antifungal activity screening tests on M. cookei, T. mentagrophytes, and Fusarium sp. (Table 20) show that a majority of sesquiterpene lactones examined possess at least weak antifungal properties. Out of 130 tests with 45 sesquiterepene lactones 24 (19%) were strongly positive, 29 (22%) were weakly positive, and 77 (59%) were negative. Out of 45 sesquiterpene lactones examined 13 (29%) strongly inhibited growth of at least one of the three fungi, 15 (33%) were only weakly active. Only 17 (38%) sesquiterpene lactones did not have any apparent effect on the growth of the three fungi examined (Table 21).

Table 21 summarizes the antifungal activity of sesquiterpene lactones based on skeletal classes. Eudesmanolides have the highest proportion of strongly active lactones, whereas germacranolides have the highest proportion of inactive compounds. However, sample sizes within individual skeletal classes of sesquiterpene lactones are too small for making any definite conclusions on the importance of the basic skeletal structure in determining the antifungal activities of these compounds.

(1) Antifungal activity against individual fungi

Overall activity of sesquiterpene lactones examined was approximately the same for both dermatophytes, M. cookei, and T. mentagrophytes (Table 22); out of 45 sesquiterpene lactones

tested against these two species approximately one half inhibited growth (strongly or weakly) of these fungi. On the contrary, Fusarium sp. was inhibited (only weakly) in 13% out of 40 tests. These marked differences in the sensitivity of the skin fungi and Fusarium to sesquiterpene lactones suggests that these fungi must differ greatly in the their physiology and chemistry.

Four out of 14 tests of 7 sesquiterpene lactones against two yeasts (S. cerevisiae and C. albicans) were positive (Table 23). Pseudoivalin was active against both yeasts but parthenin and helenalin inhibited growth of S. cerevisiae only. This indicates that individual species of yeasts may greatly differ in their sensitivity to the same sesquiterpene lactones.

(2) Relationship between chemical structure and antifungal activity

To my knowledge, no study has yet been conducted that would examine the mechanism of antifungal activity of sesquiterpene lactones. In vitro reactions between the exocyclic methylene on the lactone ring or the unsubstituted cyclopentenone with various thiols have been demonstrated (e.g. Hall et al. 1977, Lee et al. 1977a, Picman et al. 1979). These results suggest that, in vivo, sesquiterpene lactones could react through these active sites with various vitally important thiols such as enzymes and consequently might have various detrimental effects on a given organism. In this way sesquiterpene lactones might

also interfere with various fungal physiolgical processes, consequently destroying the cells or slowing down their growth rate.

In the following part, I examine the relationship between the presence of various functional groups of sesquiterpene lactones and their antifungal activity. A study of adduct formation with thiols through the exomethylene on the lactone ring (e.g. Picman et al. 1979) suggests that this moiety presents a possible active site of sesquiterpene lactones which might be responsible for their various biological activities. Table 24 summarizes the antifungal activity of all selected sesquiterpene lactones according to the presence or absence of the methylene group on the lactone. About 50% of tests including sesquiterpene lactones possessing this group were positive and 50% were negative. Out of 39 tests with lactones lacking the C13-methylene only 6 were weakly positive, whereas the rest were negative. These results suggest that although the exocyclic methylene probably may play a role in the antifungal activity of sesquiterpene lactones, some other functionalities also contribute. Thus the presence or absence of the exomethylene on the lactone ring alone cannot fully explain the antifungal activity of sesquiterpene lactones.

To eliminate differences in activity of sesquiterpene lactones due to differences in their basic skeletal structure, I analyzed the individual skeletal classes separately. Firstly, I divided sesquiterpene lactones according to the presence or absence of the C13-methylene. Pseudoguaianolides were divided

also according to the presence or absence of the C2-C3 double bond which is another potentially active site. Furthermore, I also examined the role of other functional groups of sesquiterpene lactones which might influence the antifungal activity of these compounds. Since the strongest positive responses were obtained on T. mentagrophytes on which also all available sesquiterpene lactones were tested, I chose this fungus for a closer study of the relationship between the functional groups of sesquiterpene lactones and their ability to inhibit growth of fungi. In the following part I will discuss results obtained for individual classes of sesquiterpene lactones.

(a) Germacranolides

Out of 13 tests against three fungi, including germacranolides which possess the exocyclic methylene, 54% were either strongly or weakly positive (Table 25). On the other hand, all 15 tests including germacranolides without the exocyclic methylene were negative. Thus the presence of the exocyclic methylene is probably necessary but not always sufficient for antifungal activity of this group of lactones.

The presence or absence of various functional groups in germacranolides in relation to their activity against T. mentagrophytes is summarized in Table 26. Elephantopin is the only lactone possessing the exocyclic methylene on the lactone ring which did not inhibit growth of this fungus. The lack of

activity in this compound might be a result of the presence of two epoxy groups on a molecule (Table 26) which probably counteract the effects of the exocyclic methylene. The presence of at least one epoxy group is also common to all other inactive germacranolides but since all of these compounds also lack the exocyclic methylene, it is impossible to decide on the relative importance of these moieties. In addition, there are other functional groups which might play a role in determining antifungal activities of germacranolides. For example, the C4,C5 double bond is present in active germacranolides but is absent from all inactive compounds (Table 26). This functionality thus appears to enhance the antifungal activity of germacranolides. However, it will be necessary to examine a larger number of compounds more similar in structure before any conclusions can be drawn.

(b) Guaianolides

There is no clear relationship between the presence of the exomethylene on the lactone ring and the antifungal activity of guaianolides (Table 25). Because one compound without the exomethylene (desacetoxymatricarin) weakly inhibited growth of both dermatophytes, some other group(s) must be responsible for the antifungal activity of this and possibly other related compounds.

The activity of desacetoxymatricarin against T. mentagophytes might be explained by the presence of C1,C10

double bond which is also present in a strongly active pseudoivalin (Table 27). Data are insufficient to further examine the importance of other functional groups.

(c) Eudesmanolides

Out of 21 tests including 7 eudesmanolides possessing the exomethylene, 67% were positive while all tests including a lactone lacking this group (α-santonin) were negative (Table 25). Thus it appears that the presence of the C13-methylene may be associated with the antifungal activity of this class of lactones. However, since some lactones with this group did not exhibit any activity in the tests, the presence of this moiety is not always sufficient to cause the antifungal activity perhaps because some other functionality may negate it.

This possibility is supported by data on three structurally similar eudesmanolides. Ivasperin, which differs from the strongly active ivalin only by the presence of a hydroxyl group on C1, did not have any visible effects on growth of T. metagrophytes (Table 28). Similarly, pulchellin-C, which differs from ivalin only by the presence of a hydroxyl group on C3, was also inactive. Hence the lack of activity of ivasperin and pulchellin-C can be explained only by the additional hydroxyl group which must have counteracted the effects of the exocyclic methylene. Other functional groups (see Table 28) might also play a role, however, I do not have sufficient data on similar compounds to examine this possibility.

(d) Pseudoguaianolides

Table 29 summarizes results on activity of pseudoguaianolides in relation to the presence or absence of the exocyclic methylene and/or C2,C3 double bond. The exocyclic methylene alone does not appear to play an important role in activity of pseudoguaianolides against fungi because a majority of the compounds possessing this moiety did not exhibit activity against tested fungi (Table 29).

Also the C2,C3 double bond does not appear to play a role in antifungal activity because all 6 tests (including 2 lactones) gave negative results (Table 29). There thus appears to be no relationship between the presence or absence of both moieties and the activity of pseudoguaianolides against the fungi examined (Table 29). Therefore I conclude that either other functional groups are responsible for activity of these sesquiterpene lactones or these moieties are important but their effects are frequently counteracted by other functional groups.

To examine the two possible explanations, in Table 30, I summarized various functional groups of pseudoguaianolides in relation to their activity against T. mentagrophytes. Parthenin, which possesses a hydroxyl group on C1 in α-position, is strongly active but hymenin, which differes from parthenin only by β-position of this hydroxyl group is inactive. This indicates that the configuration of the hydroxyl on C1 alone is an important factor determining activity of these compounds. This hydroxyl group possibly influences the activity of pseudoguaianolides through its interactions with the

exomethylene and cyclopentenone ring. This view is supported by the fact that ambrosin which differs from parthenin and hymenin only by the absence of the hydroxyl group on C1 is strongly active (Table 30). This shows the importance of the exomethylene and/or the C2,C3 double bond. Damsin which also lacks this hydroxyl group and the C2,C3 double bond is still strongly active. This suggests that the C13-methylene alone might be responsible (if the hydroxyl group on C1 is absent) for strong antifungal activity. But the presence of the C1 hydroxyl in coronopilin, which is otherwise identical with damsin (Table 30), results in a complete loss of activity. The importance of the C2,C3 double bond is apparent from the inactivity of coronopilin which lacks this group but is otherwise identical to the strongly active parthenin (Table 30). Thus since the lack of the C2,C3 double bond results in a complete loss of activity, this moiety must also play an important role. These comparisons therefore suggest that: (1) both the exomethylene and the C2,C3 double bond are important constituents responsible for antifungal activities of pseudoguaianolides; and (2) the hydroxyl group on C1 (particularly in β-position) reduces the effects of the two moieties.

In addition, a comparison of other inactive pseudoguaianolides (shown in Table 30) shows that a common feature of all but one compound is the presence of either an ester function (usually acetyl) or other grouping (in tenulin), and in many cases also a hydroxyl group on either C1, C2, C3, C4, or C15. These groups might have reduced the activity of either the exomethylene, the C2,C3 double bond, or both

moieties. On the basis of these results I conclude that the exomethylene on the lactone ring and the C2,C3 double bond are probably the major constituents responsible for antifungal activity of pseudoguaianolides but that their effects are frequently counteracted by other functional groups.

Conclusions

The antifungal activity of sesquiterpene lactones appear to have a multiple causation, as with bacteria. The following structural features of sesquiterpene lactones may play a role either individually or in combination with others in producing the antifungal effects.

(1) The presence of the exomethylene the lactone ring appears to be necessary for antifungal activity in germacranolides and eudesmanolides. However, the presence of this group is not always sufficient for activity.

(2) The presence of the C1,C10 double bond in guaianolides or the C2,C3 double bond in pseudoguaianolides may be responsible for the activity of some compounds, or these groupings might enhance the activity of other functional group(s).

(3) Some other functional groups appear to influence the activity possibly through their interactions with the main active functionalities. For example, the C4,C5 double bond enhances the activity in germacranolides, whereas the hydroxyl groups in eudesmanolides and

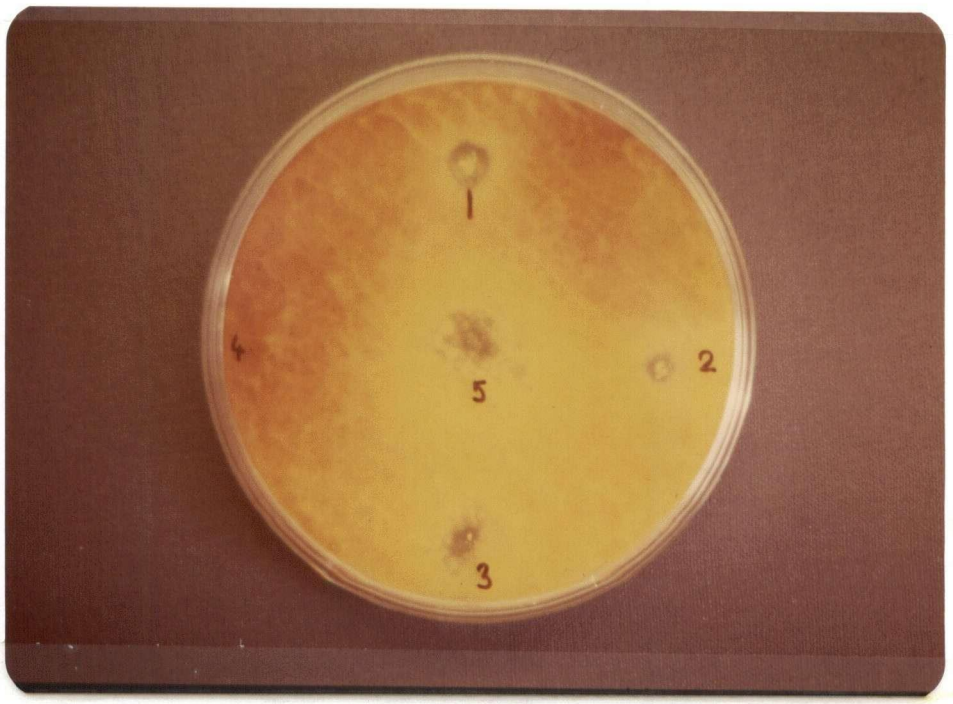
pseudoguaianolides, the epoxy group(s) in germacranolides, and the acetyl groups in pseudoguaianolides reduce the antifungal activities of some sesquiterpene lactones.

(4) The configuration of the hydroxyl group on C1 in some pseudoguaianolides influences their antifungal activity.

The activity of sesquiterpene lactones against fungi must also be determined by differences in the chemistry and physiology of individual types of fungi. The permeability of the various fungi examined here to the sesquiterpene lactones used could be an important factor which was not examined. Selective uptake of organic compounds by cells is a well-known phenomenon. In addition, the sesquiterpene lactones used in this study differ in their solubilities and this is undoubtedly also very important. These factors could explain different sensitivity of dermatophytes and other types of fungi tested in this study against the same sesquiterpene lactones.

Fig. 8. Antifungal activity of some sesquiterpene lactones (1- alantolactone, 2- isoalantolactone, 3- ivalin, 4- ivasperin, 5- pinnatifidin) against (a) Microsporum cookei and (b) Trichophyton mentagrophytes.

(a)



(b)

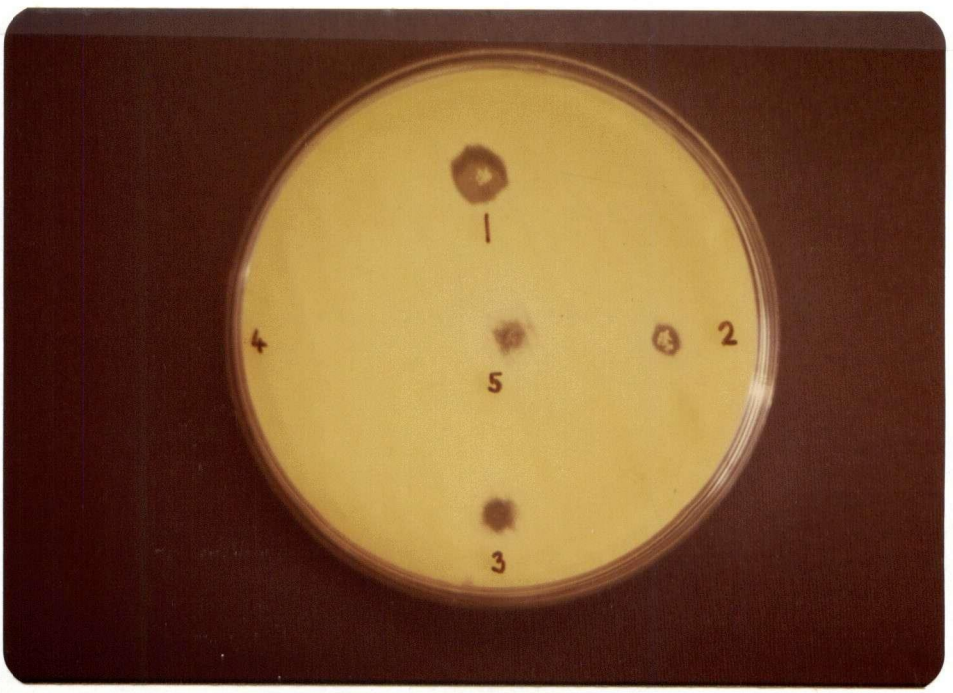


Table 20. Screening test for antimicrobial activity of selected sesquiterpene lactones against three fungi. The activity of individual sesquiterpene lactones was examined after 5 and 20 days of growth. If the activity after 20 days of growth differed from that after 5 days, the result is shown in parentheses.

Sesquiterpene lactone		Response to		
Number in Appendix	Name	M. cookei	T. mentagrophytes	Fusarium sp.
	Germacranolides			
2	Pyrethrosin	+	+	-
4	Chamissonin, diacetyl	±	±	-
7	Eupatoriopicrin	±	±	-
14	Glaucolide-A	-	-	-
16	Marginatin	-	-	-
17	Glaucolide-D	-	-	-
19	Glaucolide-F	-	-	-
20	Glaucolide-G	-	-	-
21	Elephantopin	-	-	NT
22	Parthenolide, 9-α-OH	-	+	NT
	Guaianolides			
23	Cumambrin-A	±	±	-
24	Cumambrin-B	±	±	-
26	Cumambrin-B, tetrahydro-	-	-	-
27	Cumambrin-B, acetate	±	±	-

Table 20. Continued.

30	Matricarin, desacetoxy-	±	±	-
31	Grossheimin	±	±	-
32	Ivalin, pseudo-	+	+	±
	Eudesmanolides			
33	Alantolactone	+	+	±
35	Alantolactone, iso-	+	+	±
36	Ivalin	+	+	-
37	Ivasperin	± (-)	-	-
38	Pinnatifidin	+	+	-
39	Pulchellin-C	± (-)	-	-
40	α-Santonin	-	-	-
46	Santamarine	+	+	-
	Pseudoguaianolides			
47	Parthenin	+	+	-
48	Parthenin, dihydroiso-	±	±	NT
49	Parthenin, tetrahydro-	±	±	NT
51	Hymenin	±	-	-
52	Ambrosin	+	+	±
53	Coronopilin	± (-)	-	-
54	Damsin	+	+	-
55	Hysterin	-	-	-

Table 20. Continued.

56	Tetraneurin-A	-	-	-
57	Tetraneurin-B	-	-	-
58	Tetraneurin-D	-	-	-
59	Tetraneurin-E	-	-	-
63	Tenulin	-	-	-
64	Tenulin, iso-	-	-	-
66	Helenalin	+	+	±
67	Flexuosin-B	-	-	-
69	Balduilin	-	-	-
70	Cumanin	±	-	-
	Other sesquiterpene lactones			
77	Axivalin	-	± (-)	-
78	Ivaxillarin	-	+ (±)	NT

NT= not tested

+= complete inhibition

±= weak inhibition

-= no inhibition

Table 21. Summary of antimicrobial activity of sesquiterpene lactones from individual skeletal classes against three fungi.

Class	No. (%) of sesq. lactones active against at least one fungus		Number (%) inactive sesq. lact.	Total
	strongly	weakly		
Germacranolides	2	2	6	10
Guaianolides	0	6	1	7
Eudesmanolides	5	2	1	8
Pseudo-guaianolides	4	5	9	18
Other sesq. lactones	1	1	0	2
Total	13 (29)	15 (33)	17 (38)	45

Table 22. Summary of antimicrobial activity of selected sesquiterpene lactones against individual fungi.

Fungus	No. (%) positive responses		Number (%) negative responses	Total No. tests
	Strong	Weak		
M. cookei	11 (24)	14 (31)	20 (45)	45
T. mentagrophytes	13 (29)	10 (22)	22 (49)	45
Fusarium sp.	0	5 (13)	35 (88)	40
Total	24 (19)	29 (22)	77 (59)	130

Table 23. Activity of selected sesquiterpene lactones against two species of yeasts.

Sesquiterpene lactone		Response to	
Number in Appendix	Name	*S. cerevisiae*	*C. albicans*
32	Ivalin, pseudo-	+	+
33	Alantolactone	-	-
35	Alantolactone, iso-	-	-
47	Parthenin	±	-
51	Hymenin	-	-
63	Tenulin	-	-
66	Helenalin	+	-

Table 24. Summary of antifungal activity of sesquiterpene lactones as related to the presence or absence of the exomethylene on the γ-lactone ring.

Fungus	No. sesq. lact. with C13=CH_2	No. (%) responses			No. sesq. lact. without C13=CH_2	No. (%) responses		
		Positive				Positive		
		Strong	Weak	Negative		Strong	Weak	Negative
M. cookei	32	11	11	10	13	0	3	10
T. mentagrophytes	32	13	7	12	13	0	3	10
Fusarium sp.	29	0	5	24	11	0	0	11
S. cerevisiae	6	2	1	3	1	0	0	1
C. albicans	6	1	0	5	1	0	0	1
Total	105 (100)	27 (26)	24 (23)	54 (51)	39 (100)	0	6 (15)	33 (85)

Table 25. Antifungal activity of germacranolides, guaianolides, and eudesmanolides as related to the presence or absence of the methylene group on the γ-lactone ring.

Fungus	No. sesq. lact. with C13=CH_2	No. (%) responses: Positive Strong	Positive Weak	Negative	No. sesq. lact. without C13=CH_2	No. (%) responses: Positive Strong	Positive Weak	Negative
Germacranolides:								
M. cookei	5	1	2	2	5	0	0	5
T. mentagrophytes	5	2	2	1	5	0	0	5
Fusarium sp.	3	0	0	3	5	0	0	5
Total	13 (100)	3 (23)	4 (31)	6 (46)	15 (100)	0	0	15 (100)
Guaianolides:								
M. cookei	5	1	4	0	2	0	1	1
T. mentagrophytes	5	1	4	0	2	0	1	1
Fusarium sp.	5	0	1	4	2	0	0	2
Total	15 (100)	2 (13)	9 (60)	4 (27)	6 (100)	0	2 (33)	4 (67)

Table 25. Continued.

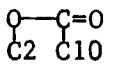
Eudesmanolides:								
M. cookei	7	5	2	0	1	0	0	1
T. mentagrophytes	7	5	0	2	1	0	0	1
Fusarium sp.	7	0	2	5	1	0	0	1
Total	21 (100)	10 (48)	4 (19)	7 (33)	3 (100)	0	0	3 (100)

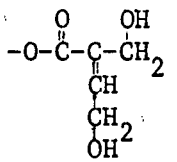
Table 26. Antimicrobial activity of germacranolides against T. mentagrophytes as related to the presence or absence of various functional groups.

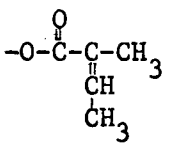
Sesquiterpene lactone		Presence or absence of functional groups																	
No. in Appendix	Name	C1=C10	C1-C10	C1=O	C2-OAc	C3-OAc	C4-C5	C4=C5	C6-OAc	C6,C7 γ-lactone	C7,C8 γ-lactone	C7=C11	C8-R_1	C8-R_2	C8-R_3	C8-R_4	C13-OAc	C13=CH_2
Strongly active:																		
2	Pyrethrosin	-	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	+
22	Parthenolide, 9-α-OH	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+
Weakly active:																		
4	Chamissonin, diacetate	+	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	+
7	Eupatoriopicrin	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-	-	+
Inactive:																		
14	Glaucolide-A	-	-	+	-	-	+	-	-	+	-	+	-	-	+	-	+	-
16	Marginatin	+	-	-	-	-	+	-	-	+	-	+	-	+	-	-	+	-
17	Glaucolide-D	+	-	-	+	-	+	-	-	+	-	-	-	-	-	+	+	-
19	Glaucolide-F	-	-	+	-	-	+	-	-	+	-	+	-	+	-	-	+	-

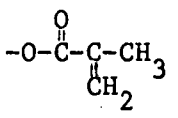
Table 26. Continued.

20	Glaucolide-G	+	-	-	-	-	+	-	-	+	-	+	-	+	-	-	+	-
21	Elephantopin	+	-	-	*	-	+	-	-	+	-	-	-	-	+	-	-	+

* 

R_1= 

R_2= 

R_3= 

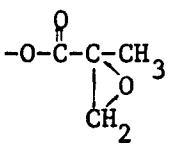
R_4= 

Table 27. Antimicrobial activity of guaianolides against T. mentagrophytes as related to the presence or absence of various functional groups.

Sesquiterpene lactone		Presence or absence of functional groups											
No. in Appendix	Name	C1=C10	C2=O	C3=O	C3=C4	C4-OH	C6,C7 γ-lactone	C7,C8 γ-lactone	C8-OH	C8-OAc	C10-OH	C10=CH_2	C13=CH_2
Strongly active:													
32	Ivalin, pseudo	+	-	-	-	+	-	+	-	-	-	-	+
Weakly active:													
23	Cumambrin-A	-	-	-	+	-	+	-	-	+	+	-	+
24	Cumambrin-B	-	-	-	+	-	+	-	+	-	+	-	+
27	Cumambrin-B, acetate	-	-	-	-	-	+	-	-	+	+	-	+
30	Matricarin, desacetoxy	+	+	-	+	-	+	-	-	-	-	-	-
31	Grossheimin	-	-	+	-	-	+	-	+	-	-	+	+
Inactive:													
26	Cumambrin-B, tetrahydro	-	-	-	-	-	+	-	+	-	+	-	-

Table 28. Antimicrobial activity of eudesmanolides against T. mentagrophytes as related to the presence or absence of various functional groups.

Sesquiterpene lactone		Presence or absence of functional groups											
No. in Appendix	Name	C1-OH	C1=C2	C2-OH	C3=O	C3-OH	C3=C4	C4=CH_2	C4=C5	C5=C6	C6,C7 γ-lactone	C7,C8 γ-lactone	C13=CH_2
Strongly active:													
33	Alantolactone	-	-	-	-	-	-	-	-	+	-	+	+
35	Alantolactone, iso-	-	-	-	-	-	-	+	-	-	-	+	+
36	Ivalin	-	-	+	-	-	-	+	-	-	-	+	+
38	Pinnatifidin	-	-	*	-	-	+	-	-	-	-	+	+
46	Santamarine	+	-	-	-	-	+	-	-	-	+	-	+
Inactive:													
37	Ivasperin	+	-	+	-	-	-	+	-	-	-	+	+
39	Pulchellin-C	-	-	+	-	+	-	+	-	-	-	+	+
40	α-Santonin	-	+	-	*	*	-	-	+	-	+	-	-

* =O

Table 29. Antifungal activity of pseudoguaianolides as related to the presence or absence of the methylene group on the γ-lactone and the unsubstituted cyclopentenone ring.

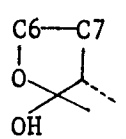
Fungus	No. sesq. lact. with C13=CH_2 only	No. (%) responses: Positive Strong	Positive Weak	Negative	No. sesq. lact. with C2=C3 only	No. (%) responses: Positive Strong	Positive Weak	Negative
M. cookei	8	1	2	5	2	0	0	2
T. mentagrophytes	8	1	0	7	2	0	0	2
Fusarium sp.	8	0	0	8	2	0	0	2
Total	24 (100)	2 (8)	2 (8)	20 (80)	6 (100)	0	0	6 (100)
	No. sesq. lact. with C13=CH_2 and C2=C3				No. sesq. lact. without C13=CH_2 and C2=C3			
M. cookei	5	3	1	1	3	0	2	1
T. mentagrophytes	5	3	0	2	3	0	2	1
Fusarium sp.	5	0	2	3	1	0	0	1
Total	15 (100)	6 (40)	3 (20)	6 (40)	7 (100)	0	4 (57)	3 (43)

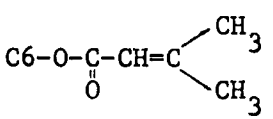
Table 30. Antimicrobial activity of pseudoguaianolides against T. mentagrophytes as related to the presence or absence of various functional groups.

Sesquiterpene lactone		Presence or absence of functional groups												
No. in Appendix	Name	C1-α-OH	C2-α-OH	C2=C3	C3-β-OH	C4-β-OH	C4=O	C6,C7 γ-lactone	C7,C8 γ-lactone	C6-OAc	C7=C11	C13=CH_2	C14-OAc	C15-OH
Strongly active:														
47	Parthenin	+	-	+	-	-	+	+	-	-	-	+	-	-
52	Ambrosin	-	-	+	-	-	+	+	-	-	-	+	-	-
54	Damsin	-	-	-	-	-	+	+	-	-	-	+	-	-
66	Helenalin	-	-	+	-	-	+	-	+	a	-	+	-	-
Weakly active:														
48	Parthenin, dihydroiso-	+	-	-	-	-	+	+	-	-	+	-	-	-
49	Parthenin, tetrahydro-	+	-	-	-	-	+	+	-	-	-	-	-	-

Table 30. Continued.

Inactive:														
51	Hymenin	+(β)	-	+	-	-	+	+	-	-	-	+	-	-
53	Coronopilin	+	-	-	-	-	+	+	-	-	-	+	-	-
55	Hysterin	-	-	-	-	b	-	+	-	-	-	+	-	+
56	Tetraneurin-A	+	-	-	-	-	+	+	-	-	-	+	-	b
57	Tetraneurin-B	+	-	-	-	-	+	+	-	-	-	+	+	-
58	Tetraneurin-D	+	-	-	-	+	-	+	-	-	-	+	+	-
59	Tetraneurin-E	+	-	-	-	b	-	+	-	-	-	+	-	+
63	Tenulin	-	-	+	-	-	+	-	+	c	-	-	-	-
64	Tenulin, iso-	-	-	+	-	-	+	-	+	+	-	-	-	-
67	Flexuosin-B	-	+	-	-	-	+	-	+	d	-	-	-	-
69	Balduilin	-	-	+	-	-	+	-	+	+	-	+	-	-
70	Cumanin	-	-	-	+	+	-	-	+	-	-	+	-	-

a= -OH a= -OAc c= 

d= 

CHAPTER 3

Effects of selected pseudoguaianolides on survival of the flour beetle, TRIBOLIUM CONFUSUM

Introduction

Secondary plant substances that do not have any apparent function in essential metabolic processes of plants are believed to play an important role in defense of plants against herbivores, particularly insects (Fraenkel 1959, Wallace and Mansell 1976). Sesquiterpene lactones have not been extensively examined for this protective function. To date only glaucolide-A (Burnett et al. 1974) and alantolactone (Picman et al. 1978) have been shown to be insect feeding deterrents; glaucolide-A influences the growth rate and survival of lepidopterous larvae (Burnett et al. 1974) and parthenin exhibits cardiac-inhibitory properties in grasshoppers (Picman et al. 1981).

In this study I examined the effects of several sesquiterpene lactones in relation to their chemical structure on survival of the confused flour beetle, Tribolium confusum (Coleoptera: Tenebrionidae). This species is frequently used to ascertain the toxic properties of chemicals on insects. All of the selected sesquiterpene lactones are pseudoguaianolides

differing mainly in the presence of two potentially active sites, α-methylene-γ-lactone and α,β-unsaturated ketone moiety.

Experimental

The beetles, obtained from Dr. R. H. Elliott, Plant Science Department, U.B.C., were maintained on wheat flour in a glass container covered with weekly moistened filter paper.

Coronopilin, helenalin, parthenin, and tenulin (Fig. 9) were isolated or obtained as described in the Section I.

(1) Feeding experiment

Different concentrations (0.2-10.0%) of sesquiterpene lactones were prepared in 95% ethanol. Approximately 0.3 ml of each ethanolic solution was added to 50mg samples of flour. After evaporation of the ethanol, the flour was transferred to petri dishes which were lined with filter paper. Five beetles were placed in each dish. A small strip of moistened filter paper was added weekly to maintain proper humidity. The beetles were checked every morning throughout a 60 day study period. The test was run in duplicates for all concentrations of parthenin and coronopilin. Because of the scarcity of tenulin only 0.2, 4, and 8% concentrations were duplicated, and for the same reason the test with helenalin was run only once. Two controls, one with flour only and the other one without any food, were also run in duplicate.

Data on survival rates of the beetles were analyzed by Duncan's Multiple Range Test. Reference to 'significance' in the text means a significant differences at the 0.05 level.

Results and Discussion

In a control trial, ten beetles which were offered flour without any lactone survived for an average of 58.9 ±3.5 (SD) days. The effects of food containing various sesquiterpene lactones are shown in Figures 10-13.

Helenalin (Fig. 9) significantly reduced survival of the beetles (Fig. 10). Its effects increased with increasing concentration of this compound up to 4%, but further increase in concentration had no additional significant efffects. The survival time of beetles offered food with 4% or higher concentrations of helenalin was similar to that of control beetles without food (Fig. 10).

Coronopilin (Fig. 9), in concentrations higher than 3%, also had a significant effect on survival of Tribolium. The strongest effect, comparable to that of the control without food, was recorded when food contained 6% or more coronopilin (Fig. 11).

Parthenin (Fig. 9) also significantly reduced survival of beetles, particularly in the two highest concentrations (Fig. 12). However, there was a great degree of variation in survival rates of individual beetles within individual trials (Fig. 13).

Tenulin (Fig. 9) which was offered in 6 different concentrations (0.2-8.0%) had no significant effects on survival of the beetles compared with that of control animals given food

without any sesquiterpene lactone (Fig. 13).

The three sesquiterpene lactones which reduced survival of flour beetles had no significant effects in lower concentrations; i.e. in 0.2-2.0% (parthenin and helenalin) or up to 3% (coronopilin). On the other hand, in higher concentrations (4-10%) all three lactones had a similar adverse effect on beetles' survival. Therefore, I conclude that parthenin, coronopilin, and helenalin had statistically similar effects on the survival of T. confusum.

Because in higher concentrations these lactones had similar effects on beetles' survival rates as starvation, it is difficult to establish whether the beetles died as a direct result of ingesting sesquiterpene lactones, or whether they refused to eat the food containing these compounds and consequently died of starvation. This problem is moreover complicated by the possibility that the ingestion of lactones produces the same effects as starvation. Sesquiterpene lactones could decrease the efficiency of digestion by their reaction with thiol-activated digestive enzymes. The presence of such proteolytic enzymes is known from insects (e. g. Houseman 1978). In addition, starvation-like effects might also be produced if a given sesquiterpene lactone had detrimental effects on microorganisms important for digestion. The available evidence is not sufficient to discriminate between these suggestions.

Establishing the biological significance of effects of selected sesquiterpene lactones on the survival of the flour beetles requires the evaluation of functional groups which are

responsible for biological activities of these compounds. The presence of two functional groups in the four lactones are summarized in Table 1. The three lactones which reduced survival of the beetles (parthenin, helenalin, and coronopilin) all possess the methylene on the lactone ring. A double bond between C2-C3, however, does not seem to be responsible for detrimental property because coronopilin which lacks this group had a similar effect to parthenin and helenalin which both possess it (Table 31). This suggests that the exomethylene on the lactone ring alone is responsible for reduced survival rates of the beetles. This view is further supported by results on tenulin which had no effect on beetles' survival. This lactone lacks the exocyclic methylene but possesses the double bond between C2-C3 (Table 31).

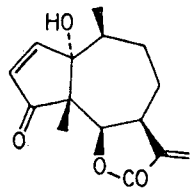
Other studies concerned with the effects of tenulin on mammals showed that this sesquiterpene lactone is relatively low in toxicity when compared to the highly toxic sesquiterpene lactone, hymenovin, containing the exocyclic methylene (Ivie et al. 1975a,b). These authors concluded that the exomethylene on the lactone ring accounts, at least in part, for the mode of action of the sesquiterpene lactones. They also suggested, however, that the toxicity of tenulin, which was observed only at extremely high concentrations of this compound, may be partly attributal to the α,β-unsaturated ketone moiety.

My results are consistent with the view that the α-methylene-γ-lactone moiety may be important for the various biological activities of sesquiterpene lactones (Rodriguez et

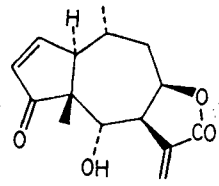
al. 1976b). However, these results seem to contrast the earlier findings that parthenin, helenalin, and coronopilin form different adducts with cysteine or glutathione (Picman et al. 1979). These studies suggested that lactones which have more than one active site (parthenin and helenalin; see Table 31) should exhibit a stronger activity. This was not the case, however, because coronopilin exhibited an activity similar to that of parthenin and helenalin. It is possible that the formation of the adduct through C2 is likely to occur only in case of thiols of lower molecular weight such as cysteine. This is supported by the fact that parthenin forms two adducts with cysteine but only one adduct with the tripeptide glutathione (Picman et al. 1979). If the thiol containing compounds inside the flour beetles are large molecules such as proteins, the sesquiterpene lactones may be bound only to the methylene function of the lactone. Then this would result in a similar effect of parthenin, helenalin, and coronopilin on survival rates of the beetles.

To conclude, this study suggests that the α-methylene-γ-lactone moiety is probably responsible for the detrimental properties of selected pseudoguaianolides on survival of flour beetles. The α,β-unsaturated ketone moiety does not seem to contribute to those properties.

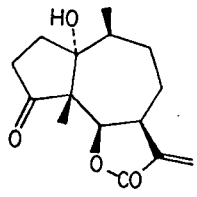
Fig. 9. Chemical structures of selected pseudoguaianolides tested for their effects on survival of flour beetles.



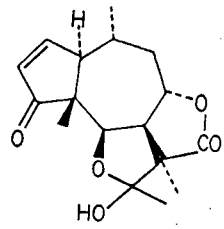
Parthenin



Helenalin



Coronopilin



Tenulin

Fig. 10. Effect of helenalin of various concentrations on survival of Tribolium confusum (mean ± SD).

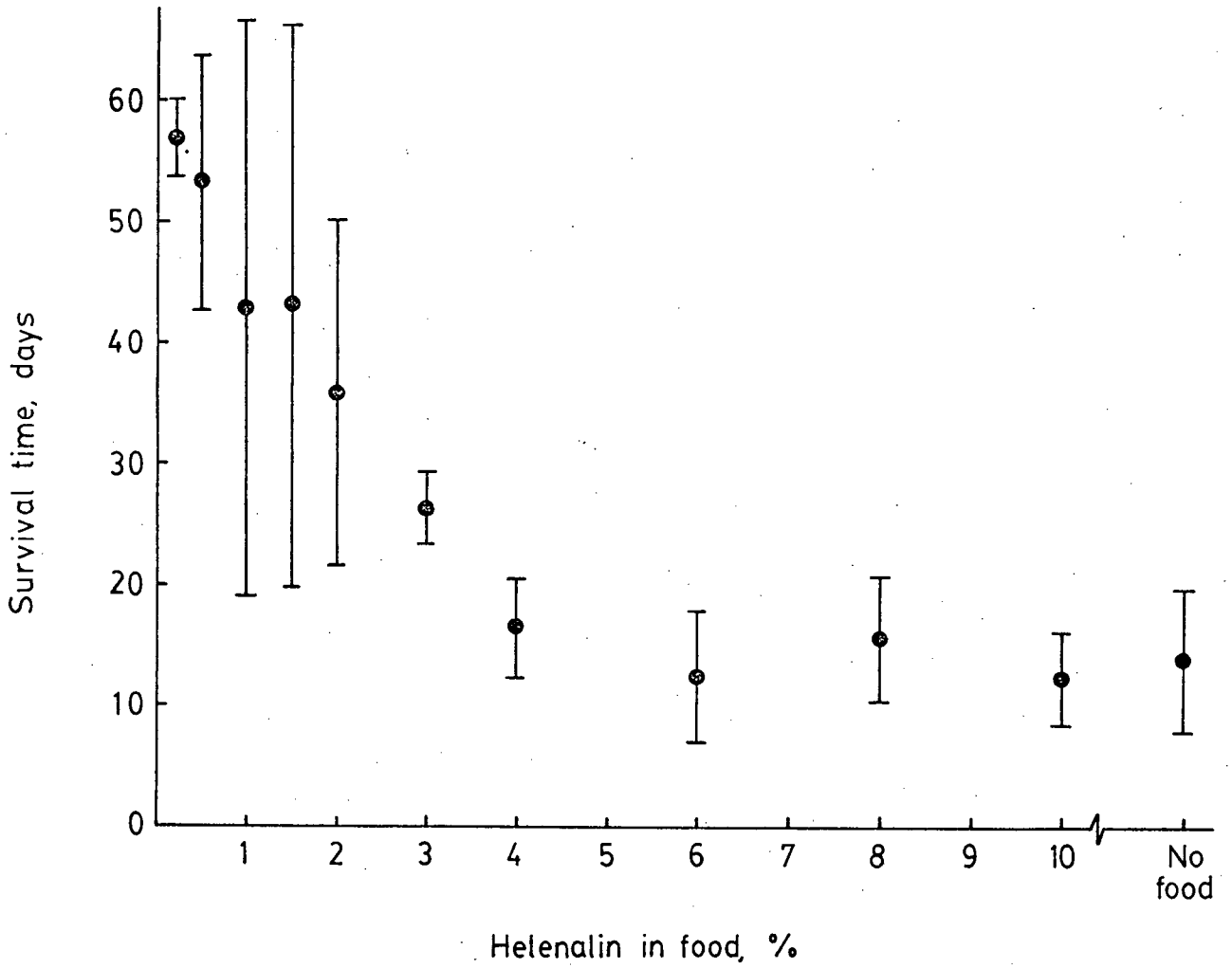


Fig. 11. Effect of coronopilin of various cancentrations on survival of Tribolium confusum (mean ± SD).

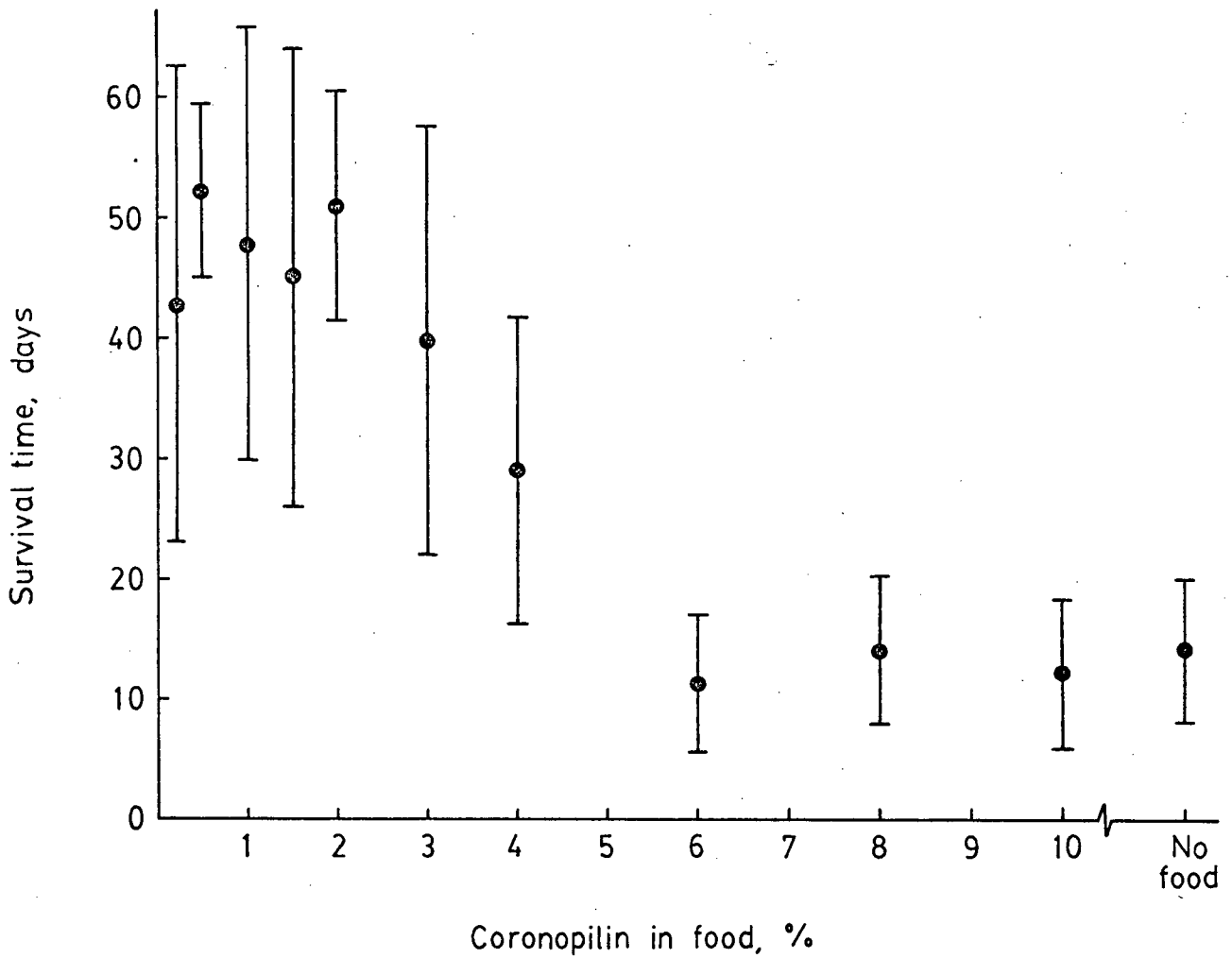


Fig. 12. Effect of parthenin of various concentrations on survival of Tribolium confusum (mean ± SD).

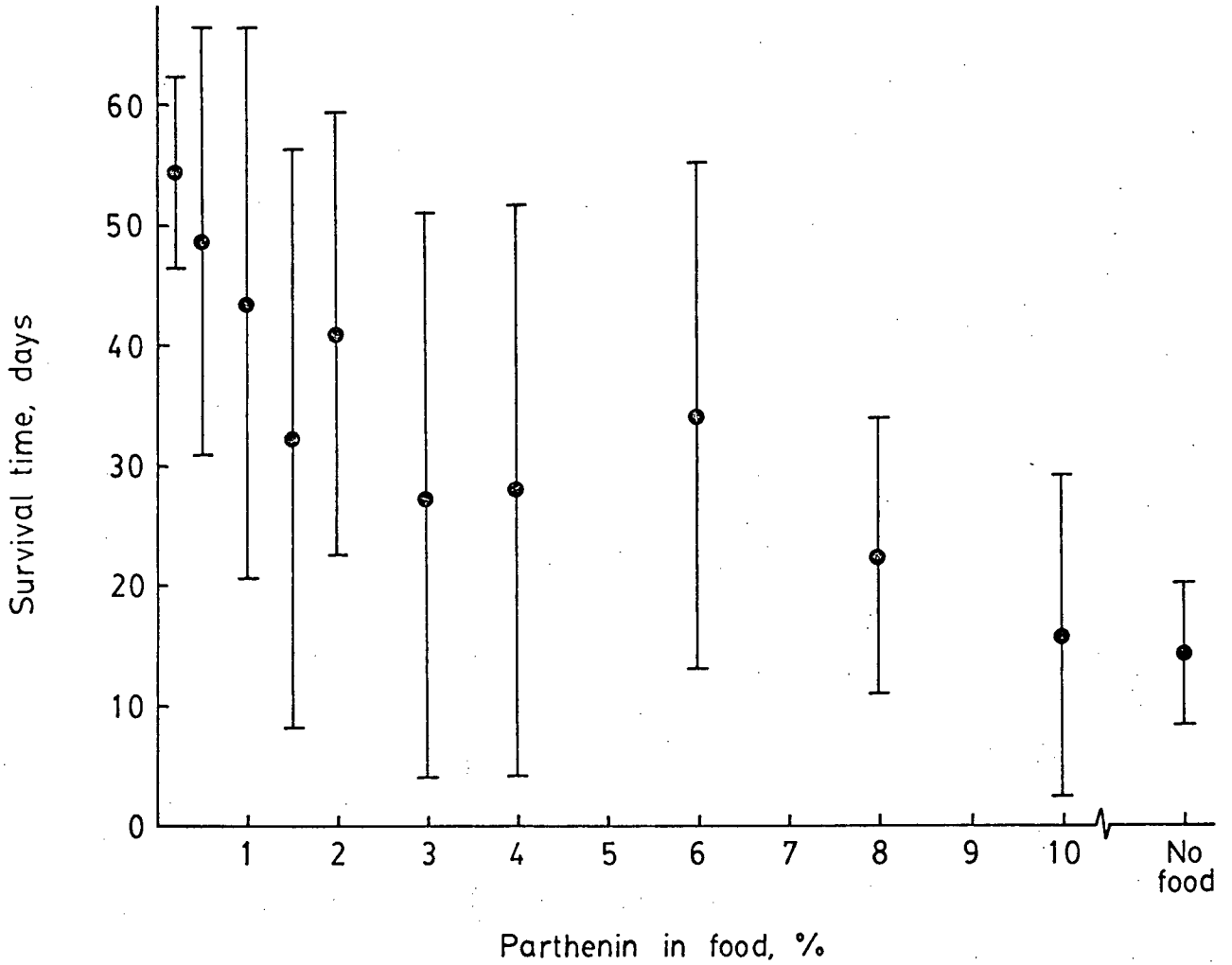


Fig. 13. Effect of tenulin of various concentrations on survival of Tribolium confusum (mean ± SD).

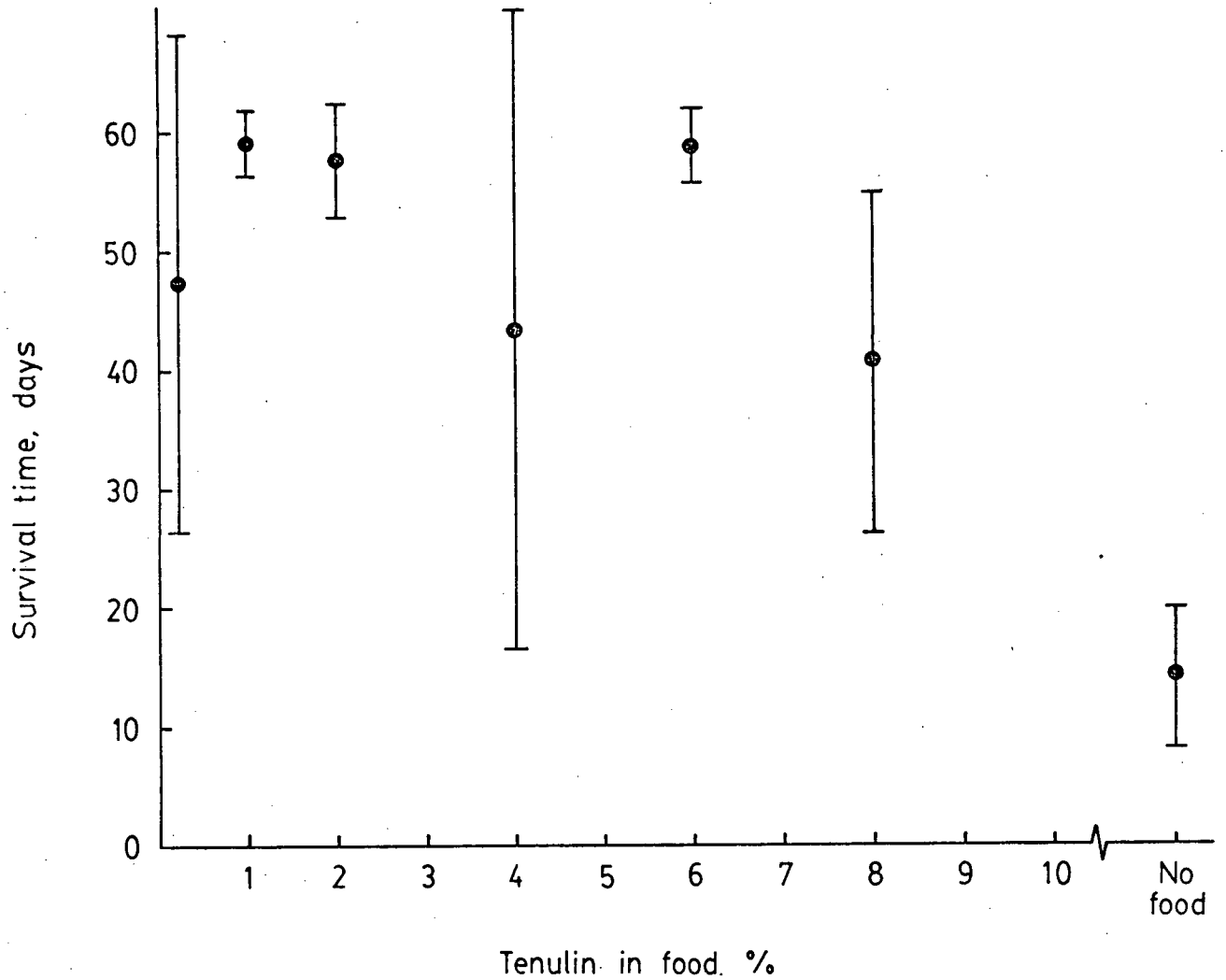


Table 31. Functional groups of pseudoguaianolides which were studied for their effects on survival of flour beetles.

Sesquiterpene lactone	Functional groups: α-methylene-γ-lactone	Functional groups: cyclopentenone	Effect on beetles
Parthenin	+	+	+
Helenalin	+	+	+
Coronopilin	+	-	+
Tenulin	-	+	-

CHAPTER 4

Cross-reactivity between sesquiterpene lactones related to parthenin in parthenin sensitized guinea pigs

Introduction

Allergic contact dermatitis, known as delayed hypersensitivity or Type IV cell mediated hypersensitivity (Roitt 1971), is produced by contact of the skin with low molecular weight chemicals. The chemical (hapten) reacts in the skin with protein (carrier) forming an antigen which sensitizes blood lymphocytes. Rashes on the skin appear after re-exposure of the sensitized individual to the chemical usually within 24 to 48 hours.

Many members of the Compositae, including common weeds and cultivated plants, have been reported to cause allergic contact dermatitis (Mitchell 1969, Evans and Schmidt 1980). Chemicals responsible for this disease are sesquiterpene lactones which are known to be potent allergens (Mitchell 1975a).

Contact dermatitis evoked by *P. hysterophorus* became a widespread disease among people in India, after the introduction of this species in 1956. Parthenin is considered to be the major allergen of this plant (Lonkar et al. 1976), but also the recently discovered tetraneurin-A, hysterophorin (Sohi et al.

1979), and coronopilin (Picman et al. 1980) might play a role in causing Parthenium dermatitis.

Cross-reactions between various allergenic plant species are common. Patients who develop sensitivity to the lactone(s) of one species frequently exhibit cross-sensitivity to chemically related lactones of other species of Compositae (Mitchell et al. 1972, Mitchell 1975a). Highly sensitized individuals usually show a wider spectrum of cross-sensitivity than do weakly sensitized ones (Mitchell et al. 1975a, Roed-Petersen and Hjorth 1976). Only a few studies have been conducted on this complex phenomenon and the underlying mechanism of cross-sensitivity towards various sesquiterpene lactones is unknown. The study of cross-sensitivity is complicated by the fact that indviduals sensitized by identical compound(s) develop their own characterisic patterns of cross-sensitization (Baer 1954).

On the basis of tests with many sesquiterpene lactones, it has been established that the presence of an α-methylene group attached to the γ-lactone ring is a prerequisite for allergenic activity (Mitchell and Dupuis 1971, Mitchell et al. 1975a), with the exception of deacetoxymatricarin where the positive response might have been caused by impurities (Bleumink et al. 1976). Hydrogenation of the methylene group results in a loss of activity (Mitchell et al. 1970). Adducts involving the methylene function such as the alantolactone-amino acid adducts also showed loss of activity (Dupuis et al. 1974). However, it appears that the exomethylene on the lactone ring alone is not

always sufficient to cause allergenicity (Mitchell et al. 1970, 1971a,b, Mitchell and Dupuis 1971, Epstein et al. 1980). Negative results of patch tests to compounds other than sesquiterpene lactones which possess an unsaturated methylene group, including α-methylene-γ-butyrolactone, show that a lactone ring together with a sesquiterpene attached to it are immunological requisites in addition to a methylene group (Mitchell et al. 1972). However, because all known allergens belong to all skeletal clases of sesquiterpene lactones, structural differences in a skeleton between classes are not important (Mitchell et al. 1970). Comparison of other structural groups of sesquiterpene lactones (hydroxyl group, acetylated or formylated hydroxyl groups) and stereochemistry at the level of attachment of the lactone ring indicate that none of them is involved in activity (Mitchell et al. 1970).

In this study I investigated the relationship between cross-sensitivity and chemical structure of parthenin and related sesquiterpene lactones. The study was conducted on guinea pigs sensitized to parthenin. Guinea pigs are extensively used in immunology and studies of inflammatory reations since the physiological characteristics of their skin are similar to those of man (Wagner and Manning 1976).

Experimental

(1) Guinea pig sensitization

The experiments were carried out on six albino female guinea pigs which were fed with Guinea Pig Chow granules (Purina, St. Louis, Mo.) and water, supplemented with fresh lettuce and carrot. In the beginning of the experiments the animals were 5 months old, each weighing between 615-933 g.

During the first attempt at sensitization, 50 µl of 5% parthenin solution in 80% ethanol was applied daily for 18 days to the same area, approximately 1.5 cm in diameter, of the shaved flank of each animal. To ensure a proper application of the solution to the skin, newly grown hairs were shaved every 4 days with an electric shaver. The first erythema (+) occurred on the 5th-7th day of attempted induction of sensitization. On the 18th day all animals exhibited a strong inflammatory reaction (++++). The application of parthenin solution was stopped and the animals were allowed to recover completely. After 22 days the opposite flank of all animals were shaved and 5% parthenin (80% ethanol) applied. However, none of the animals responded to parthenin (checked after 24, 48, 72, and 96 hours). To sensitize the animals, the procedure was repeated with parthenin solution of a higher concentration. To avoid the lengthy procedure of shaving the animals, parthenin solution was applied this time

directly on the guinea pig ears which are almost without hairs. Fifty ul of 10% parthenin solution (in 80% ethanol) was applied daily for 15 days (except on the 6th and 12th day). On the 3rd day some animals developed slight erythema (+) at the place of application, and on the 6th day four of these animals exhibited a strong reaction (++++). The sensitization was then continued by solution application on the opposite ear of all animals until all exhibited the strongest reaction (day 15). Eighteen days later, after the animals completely recovered, a 10% solution of parthenin (as above) was applied on their shaved flanks. All guinea pigs responded positively 24 or 48 hours after the application (Table 32). The reaction of sensitized animals to parthenin was checked again after cross reactivity tests with the same results.

(2) Cross reactivity tests

Sesquiterpene lactones used in this study (Fig. 14) were obtained or prepared as described in Sections I and II. Challenges were performed with 50µl of 10% solutions of individual compounds which were applied on the shaved flanks of the animals. The reactions were recorded every 24 hours for several days. The intensity of the reactions were evaluated according to the following scoring symbols:

0 no reaction

(+) slight erythema

\+ distinct spotted erythema

++ almost confluent erythema

+++ distinct confluent erythema

++++ intense confluent erythema, exudation,
spreading into nearby areas.

The presence of positive reactions can induce other false positive reactions, so-called "angry back" (Mitchell 1975b). To avoid this "angry back syndrome", I applied only one compound at a time. The next sesquiterpene lactone was applied on the opposite flank of the animal and only after at least 3 days after a complete recovery from the previously tested compound. If a sesquiterpene lactone did not cause a positive reaction, a new lactone was applied also to the opposite flank at least 4 days later. All 6 animals were tested with each compound except for hymenin and hysterin, where a limited quantity allowed for the testing of 3 animals only.

Results and Discussion

The application of parthenin solution after the sensitization period resulted in a strong reaction in all animals (Table 32). All animals also reacted to coronopilin, and 2 out of 6 gave a positive reaction to damsin (Table 32). However, none of the animals tested responded to hymenin, hysterin, dihydroisoparthenin, and tetrahydroparthenin (Table 32).

The relationship between reaction of animals to tested lactones and the presence of various functional groups is summarized in Table 33. Hymenin (Fig. 14) differs from parthenin only in the configuration of the hydroxyl group on C1. This difference in stereochemistry alone resulted in a complete loss of reactivity of guinea pigs to hymenin.

Coronopilin (Fig. 14), which differs from parthenin by the absence of a double bond between C2 and C3, gave positive responses in all 6 animals, though generally weaker than parthenin (Table 32). The fact that the 6 animals greatly differed in their responses (Table 32) indicates that the absence of a double bond between C2 and C3 of parthenin leads to differing immunological reactions by different individuals. Damsin (Fig. 14), which differs from parthenin in two functional groups (Table 33), gave a weak positive reaction in 2 animals. Thus, compared to coronopilin, the additional change on the parthenin molecule (the absence of a hydroxyl group on C1) resulted in a further decrease in sensitivity of parthenin sensitized guinea pigs. Another change in a structure, as seen

in hysterin (Fig. 14; Table 33), resulted in a complete loss of sensitivity in the examined animals.

Dihydroisoparthenin and tetrahydroparthenin (Fig. 14), two hydrogenated products of parthenin which contain α-methyl on the γ-lactone ring, did not cause any reaction (Table 32). Since the animals did not give any response to dihydroisoparthenin, which possesses a double bond between C7 and C11, this functional group does not seem to play any role in cross-sensitivity in parthenin-sensitized guinea pigs.

The above results indicate that the presence of the α-methylene-γ-lactone moiety in sesquiterpene lactones is a prerequisite for cross-sensitivity of parthenin sensitized guinea pigs to the examined parthenin-related compounds. The presence of this group alone, however, is not sufficient for cross-sensitivity (see hysterin; Tables 32,33). This finding is consistent with Mitchell's (1975a) hypothesis on the role of the exomethylene on the lactone ring in allergic contact dermatitis.

On the basis of their results, Epstein et al. (1980) concluded that the presence of the methylene conjugated to the lactone ring does not alone determine cross-sensitivity or the lack of it, but that it is rather the presence of various additional groups on a skeleton, which hinder greatly the reactivity of the exocyclic methylene. My study, however, shows that as the number of any structural changes (either through the addition, deletion, or shifts of groups) in a parthenin molecule was increased (parthenin - coronopilin - damsin - hysterin), so the response of guinea pigs became weaker (Table 32). This

observation could be explained in terms of the immunological processes. It is known that individual organisms may form several different types of antibodies to a given antigen (Roitt 1977). Different types of antibodies present a copy of various parts of a given antigen and hence can fit only these parts (Roitt 1977). Therefore, as the number of structural changes in an antigen molecule increases, the number of different types of antibodies formed in response to a given antigen (e.g. parthenin-protein complex) which can fit a progressively more different allergen should become smaller. Hence the skin reaction of guinea pigs becomes weaker.

This explanation is also supported by results of Schlewer et al. (1978) who examined cross-sensitivity between sesquiterpene lactones from a liverwort Frullania sp. and several synthetic, structurally similar lactones. These authors reported that skin reaction of individuals tested to synthetic compounds was weaker and one compound, the α-methylene-γ-butyrolactone, was unreactive. Since the synthetic compounds tested possessed the exocyclic methylene on the lactone ring, the other structural features of these lactones must have also played a role in cross-sensitivity. Stampf et al. (1978) obtained similar results from cross-sensitivity tests between alantolacone and other similar sesquiterpene lactones.

There are two other possible sources of variation in response of an individual organism to a given allergen. First, since parthenin forms two adducts with certain thiols such as cysteine (Picman et al. 1979) and therefore presumably different

types of antigens, this is likely to further increase the number of different types of antibodies which present copies of various parts of a hapten (parthenin)-carrier (thiol-protein) complex. If the chemistry of individuals in terms of these thiol-proteins differ, then this is likely to cause a great degree of variation in a number of formed antibodies.

Second, in a given individual, parthenin might form a number of antigens with different proteins containing thiol groups. The number of antigens might further increase through various combinations of parthenin with various carriers in the case of a biadduct. These factors require further investigation.

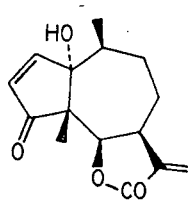
Also changes in stereochemistry of the parthenin molecule can lead to a complete loss of cross-reactivity, as in the case of hymenin (Table 32). This is in agreement with results of tests on parthenin sensitized humans (Subba Rao et al. 1978) and one instance of a patient who responded positively to hymenin but not to parthenin (Rodriguez et al. 1977). This differential immunological activity between two diastereoisomers is not unusual. It has been also documented for d-usnic acid which gave positive reaction in 6 patients, while l-usnic acid gave negative reactions in patch tests for allergenicity (Mitchell 1966).

The stereoisomeric specificity in allergic contact dermatitis can be explain by the fact that while the exomethylene on the lactone ring appears to be necessary for combining with skin proteins to produce allergens, the remaining part of the parthenin molecule may determine the specific

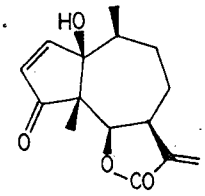
recognition by antibodies. Thus alterations in stereochemistry may result in a loss of activity to an otherwise chemically identical molecule (Subba Rao et al. 1978).

To conclude, results of this study support the general idea of the importance of the α-methylene-γ-lactone moiety in allergenicity. In addition, this study also suggests that any changes in the structure of parthenin appear to have additive effects of weakening of the allergenic response, presumably because of a smaller number of different types of antibodies involved.

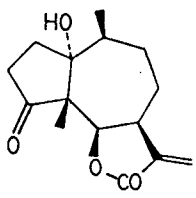
Fig. 14. Chemical structures of selected pseudoguaianolides used for cross-reactivity tests.



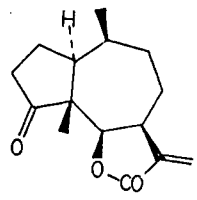
Parthenin



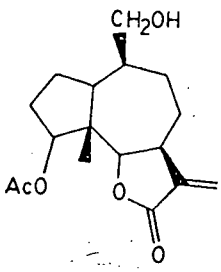
Hymenin



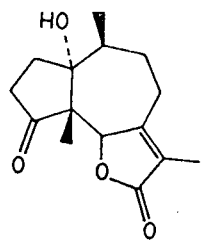
Coronopilin



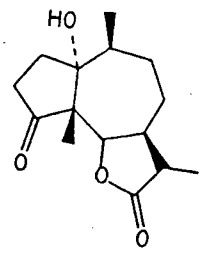
Damsin



Hysterin



Dihydroisoparthenin



Tetrahydroparthenin

Table 32. Cross-reactivity between sesquiterpene lactones related to parthenin in parthenin sensitized guinea pigs. Fifty ul of 10% ethanolic solutions were applied. Only the strongest reactions observed during 72 hours after applications were recorded.

	Challenge with *						
Animal	Parthenin	Hymenin	Coronopilin	Damsin	Hysterin	Dihydroiso-parthenin	Tetrahydro-parthenin
A	+++	0	++	(+)	NT	0	0
B	+++	NT	(+)	0	0	0	0
C	+++	0	(+)	0	NT	0	0
D	+++	NT	+++	(+)	0	0	0
E	+++	0	++	0	NT	0	0
F	+++	NT	(+)	0	0	0	0

* See text for scoring symbols used for evaluation of the intensity of reactions

NT= not tested

Table 33. Relationship between cross-reactivity in parthenin sensitized guinea pigs to parthenin and related sesquiterpene lactones and their chemical structure.

Sesquiterpene lactone	Functional groups							No. positive reactions/total
	α-methylene-γ-lactone	cyclo-pentenone	on C-1			double bond C7-C11	CH_3COO- on C-4	
			α-OH	β-OH	α-H			
Parthenin	+	+	+	-	-	-	-	6/6
Hymenin	+	+	-	+	-	-	-	0/3
Coronopilin	+	-	+	-	-	-	-	6/6
Damsin	+	-	-	-	+	-	-	2/6
Hysterin	+	-	-	-	+	-	+	0/3
Dihydroiso-parthenin	-	-	+	-	-	+	-	0/6
Tetrahydro-parthenin	-	-	+	-	-	-	-	0/6

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APPENDIX. Chemical structures of sesquiterpene lactones examined in this study.

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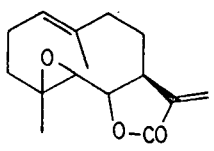

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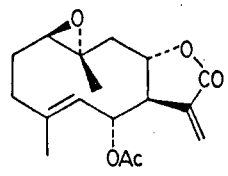

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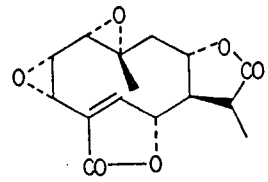

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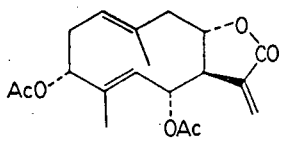

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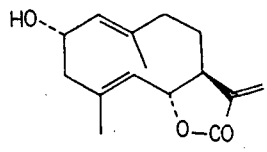

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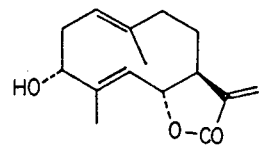

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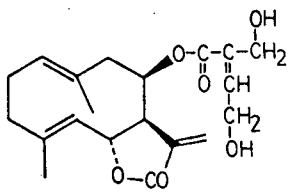

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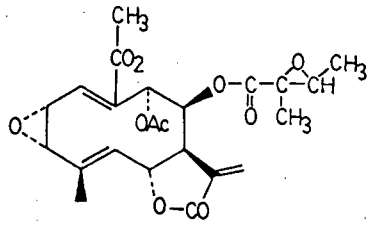

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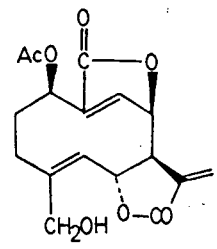

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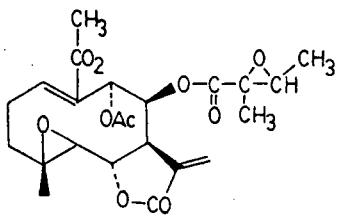

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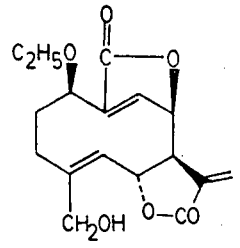

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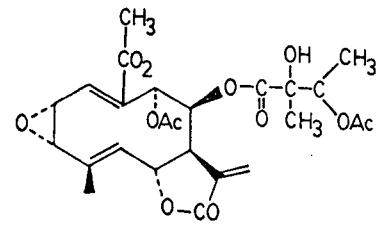

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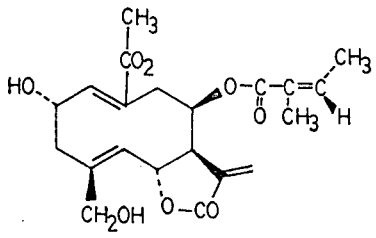

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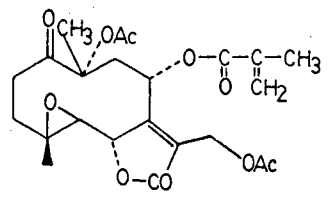

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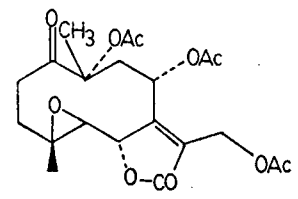

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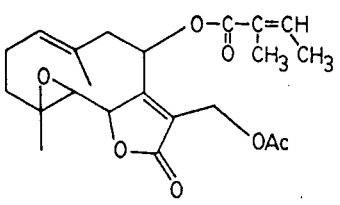
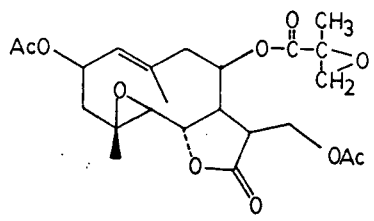
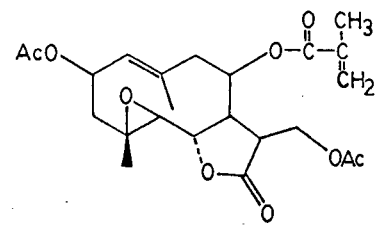


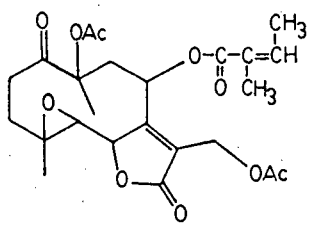
16 Marginatin



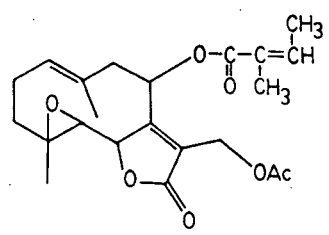
17 Glaucolide-D



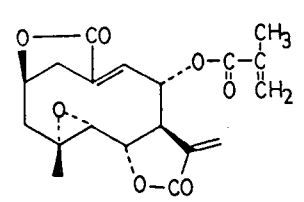
18 Glaucolide-E



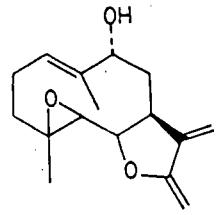
19 Glaucolide-F



20 Glaucolide-G

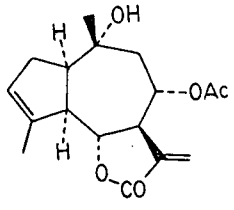


21 Elephantopin

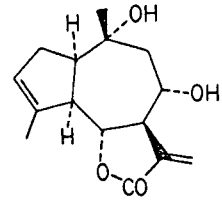


22 Parthenolide, 9-α-OH

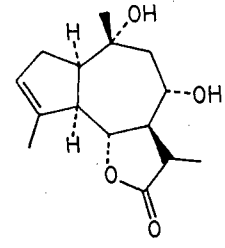
Guaianolides:



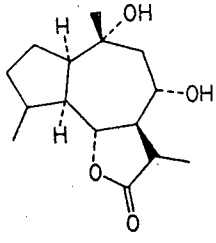
23 Cumambrin-A



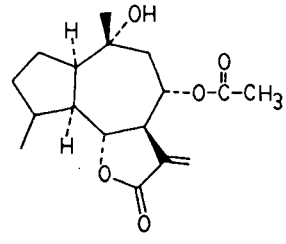
24 Cumambrin-B



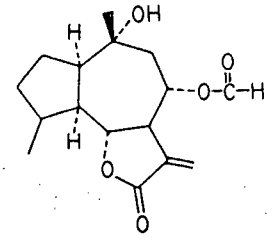
25 Cumambrin-B, dihydro-



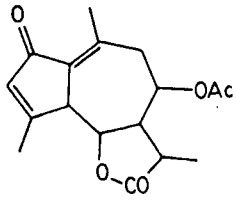
26 Cumambrin-B, tetrahydro-



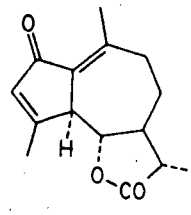
27 Cumambrin-B, acetate



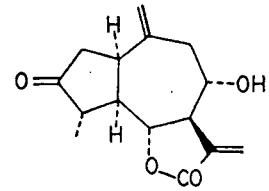
28 Cumambrin-B, formyl



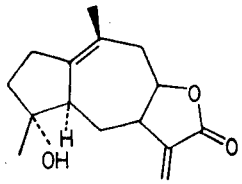
29 Matricarin



30 Matricarin, desacetoxy-

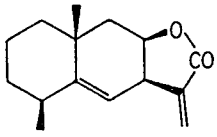


31 Grossheimin

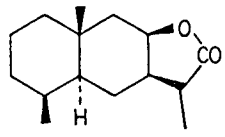


32 Ivalin, pseudo-

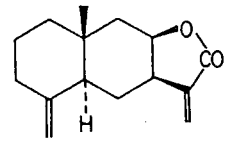
Eudesmanolides:



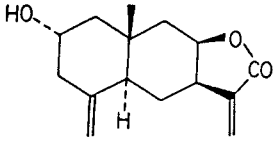
33 Alantolactone



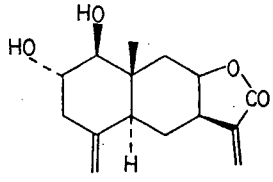
34 Alantolactone, tetrahydro-



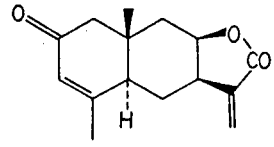
35 Alantolactone, iso-



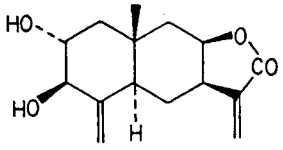
36 Ivalin



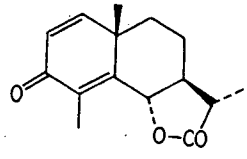
37 Ivasperin



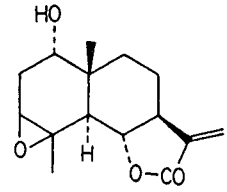
38 Pinnatifidin



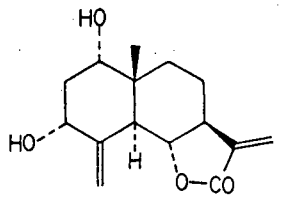
39 Pulchellin-C



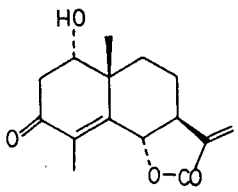
40 α-Santonin



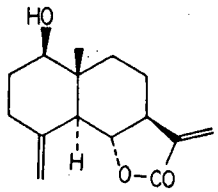
41 Ludovicin-A



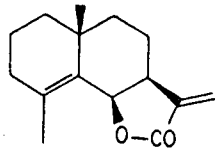
42 Ludovicin-B



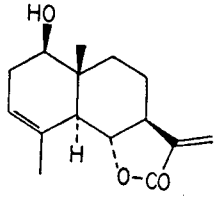
43 Ludovicin-C



44 Reynosin

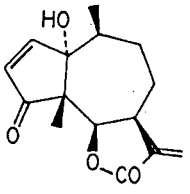


45 Frullania lactone

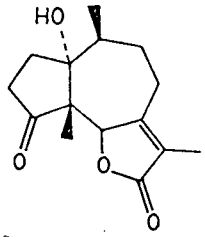


46 Santamarine

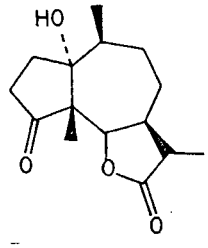
Pseudoguaianolides:



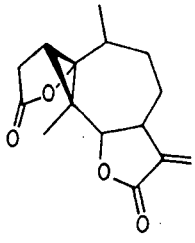
47 Parthenin



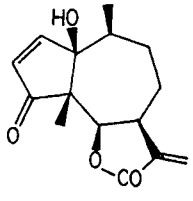
48 Parthenin, dihydroiso-



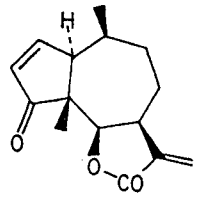
49 Parthenin, tetrahydro-



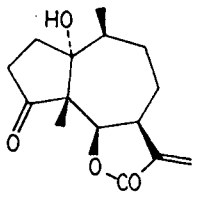
50 Parthenin, photolytic product



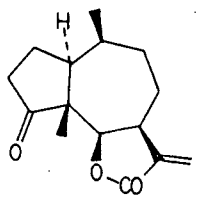
51 Hymenin



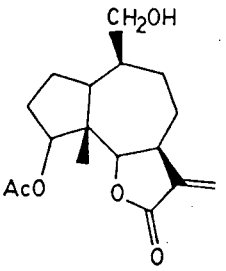
52 Ambrosin



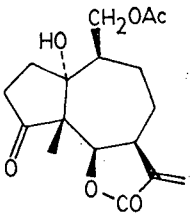
53 Coronopilin



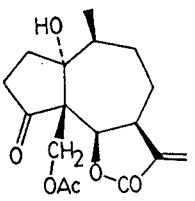
54 Damsin



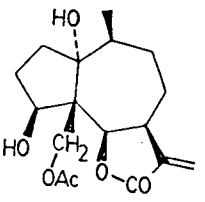
55 Hysterin



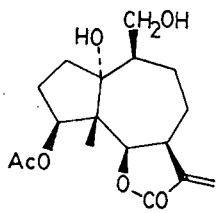
56 Tetraneurin-A



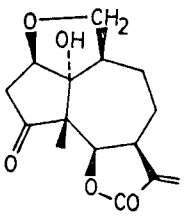
57 Tetraneurin-B



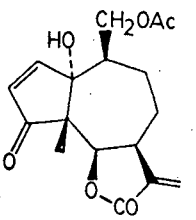
58 Tetraneurin-D



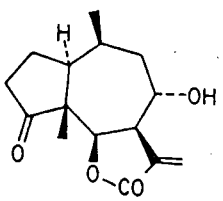
59 Tetraneurin-E



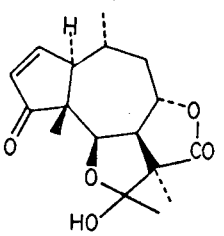
60 Conchosin-A



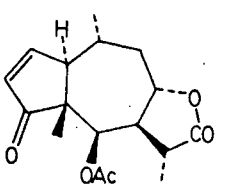
61 Conchosin-B



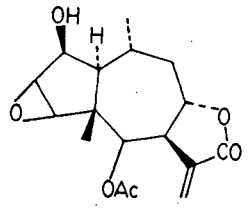
62 Confertiflorin, desacetyl-



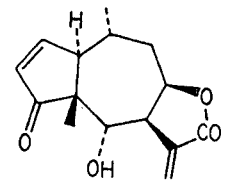
63 Tenulin



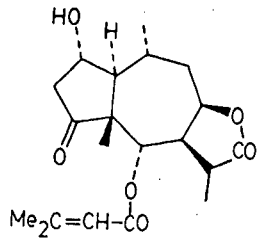
64 Tenulin, iso-



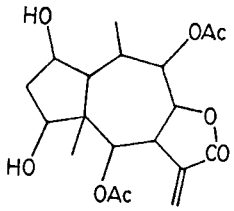
65 Gaillardilin



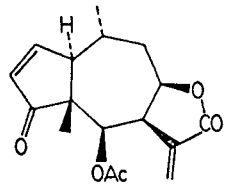
66 Helenalin



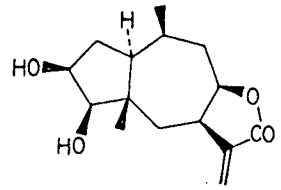
67 Flexuosin



68 Spathulin

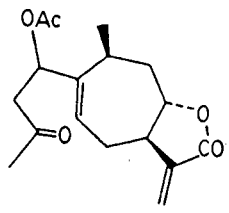


69 Balduilin

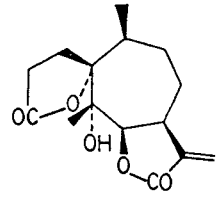


70 Cumanin

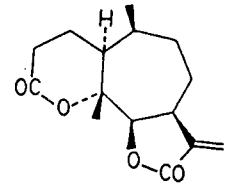
Other sesquiterpene lactones:



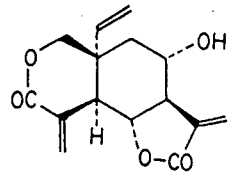
71 Xanthinin



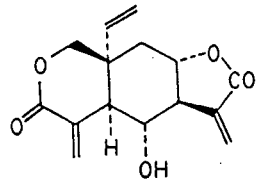
72 Psilostachyin



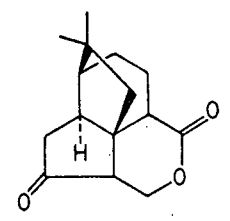
73 Psilostachyin-C



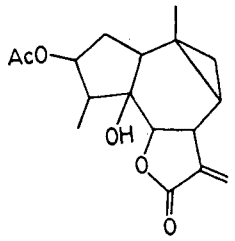
74 Vernolepin



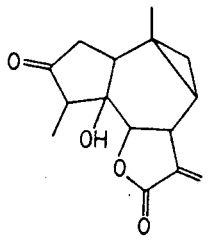
75 Vernomenin



76 Quadrone



77 Axivalin



78 Ivaxillarin