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I. BIOMIMETIC SYNTHESES OF BIS-INDOLE ALKALOIDS

II. SELECTIVITY IN THE REDUCTION OF STEROIDAL ANHYDRIDE ADDUCTS

by

David Eugene Burke

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Chemistry)
in The University of Michigan
1972

Doctoral Committee:

Assistant Professor Philip W. Le Quesne, Chairman
Associate Professor M. David Curtis
Associate Professor Seyhan N. Ege
Associate Professor Rolf Hartung
Assistant Professor Joseph P. Marino

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DEDICATION

To Flora, Christine, Michael and Steven.

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to Dr. Philip Le Quesne for his encouragement, guidance, and above all, patience during the course of this work.

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CHAPTER I

INTRODUCTION

The great surge of activity in the area of indole alkaloid chemistry in the past fifteen years has led to the accumulation of much information on the structures and chemistry of this constantly increasing group of natural products.[1] It was soon recognized that the non-tryptophane derived part of a large majority of the indole alkaloids is a nine or ten carbon unit. The origin of this nine or ten carbon portion of these alkaloids was the subject of much interesting speculation by many workers.[2-5] Finally, Arigoni, Battersby, Scott and their groups[6,7] provided insight into the biosynthesis of these alkaloids, particularly with respect to the non-tryptamine portion, and unambiguously established using labeled precursors that the terpenoid unit is derived from secologanin (1), a hypothesis first postulated by Thomas[2] and Wenkert.[5]

Recently Schmid and collaborators[8] have proposed a "biogenetic" classification of the indole alkaloids built up from tryptamine and secologanin or modified secologanin. Schmid divides the alkaloids into five major classes according to the modified secologanin moiety present. Within each of the five major classes are subclasses defined by the number of bonds between the secologanin

and tryptamine moiety. A brief description of the five major classes is given below (Scheme I).

Class 1 alkaloids possess an unrearranged secologanin skeleton (2), e.g., ajmalicine (8).

Cleavage of the bond between C-15 and C-16 via process a forms a derivative of (2), in which C-16 is linked to C-2 of tryptamine, giving the skeleton for the alkaloids of class 2, e.g., 16,17-dihydrosecodine-17-ol (9).

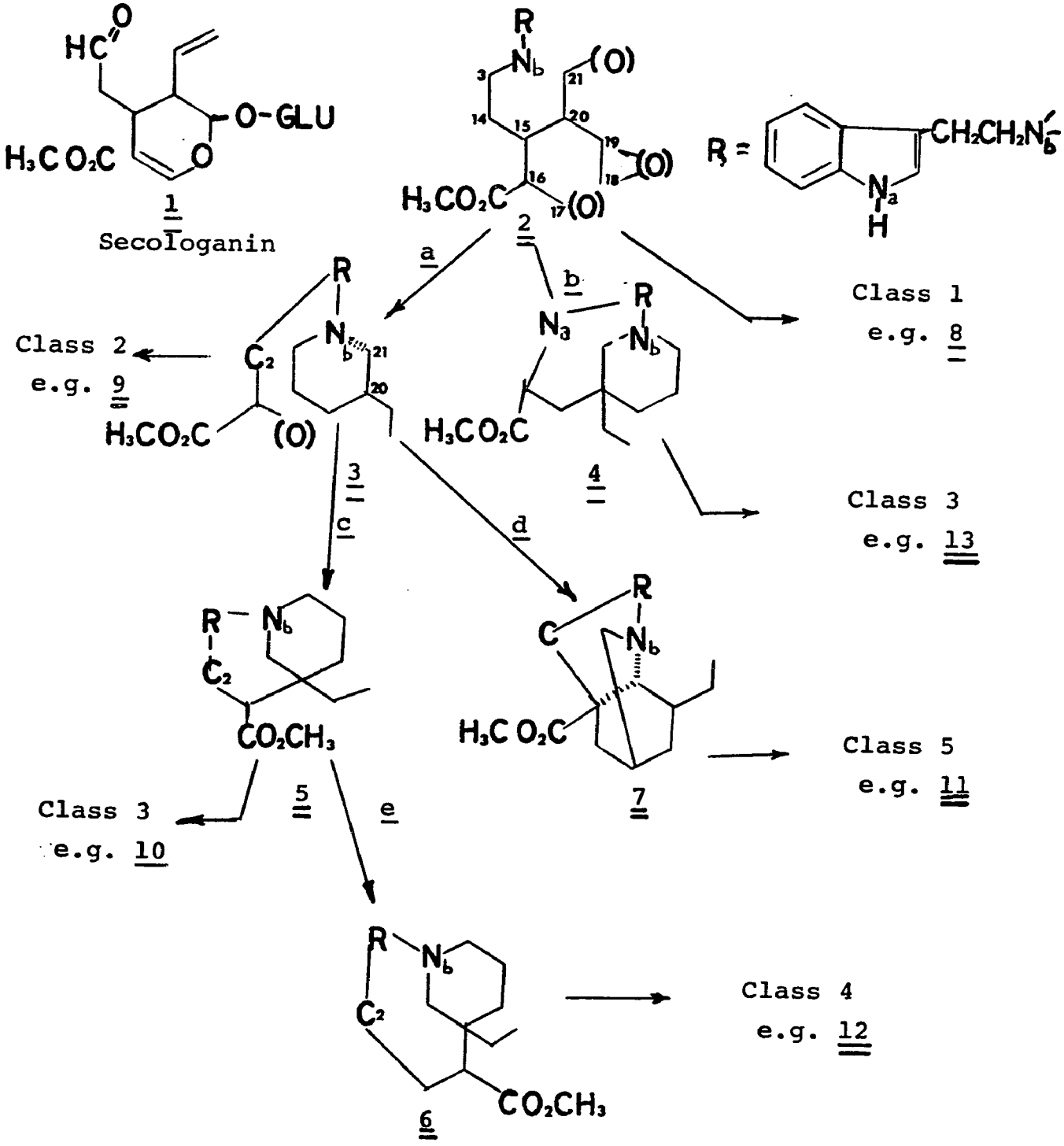
Process a and rearrangement b give the skeleton (4) found in some alkaloids of class 3, e.g., (13). Process a and rearrangement c from (3) give the rest of the class 3 alkaloids, e.g., (10). Both classes 4 and 5 possess the same rearranged skeleton and differ only in the mode of attachment to the tryptamine moiety.

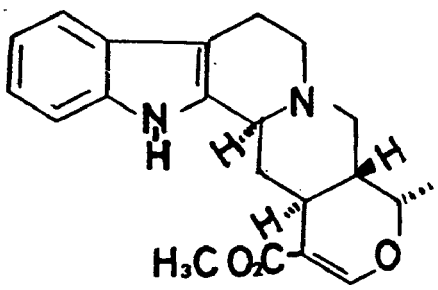
Rearrangement e of (5) gives the unit (6) which comprises class 4 alkaloids e.g., (12).

Attachment of C-17 to G-14 in (3) (process d) gives the skeleton (7) which is characteristic of class 5 alkaloids e.g., (11).

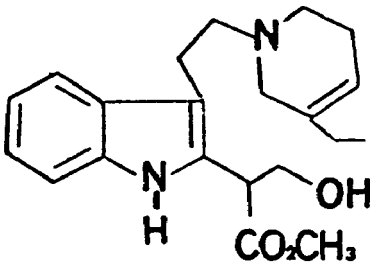
The validity of the above scheme will undoubtedly be ascertained by future labeling studies and by phytochemical investigations.

SCHEME I

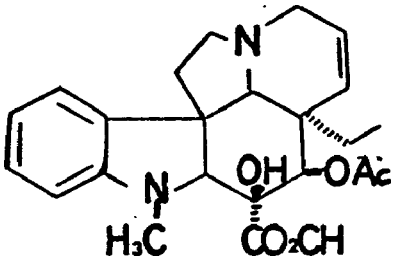

1
Secologanin
2
a
b
c
d
e
3
4
5
6
7
Class 1
e.g. 8
Class 2
e.g. 9
Class 3
e.g. 13
Class 3
e.g. 10
Class 4
e.g. 12
Class 5
e.g. 11



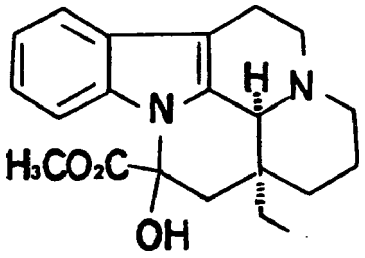
8 Ajmalicine



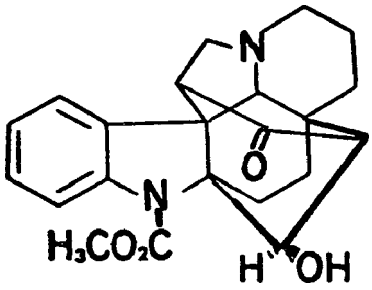
9 Dihydrosecodin-17-ol



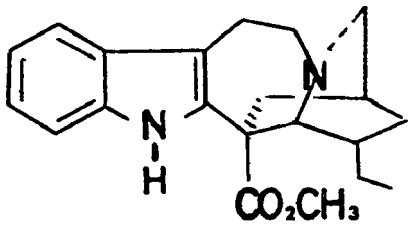
10 Vindoline



13 Vincamine



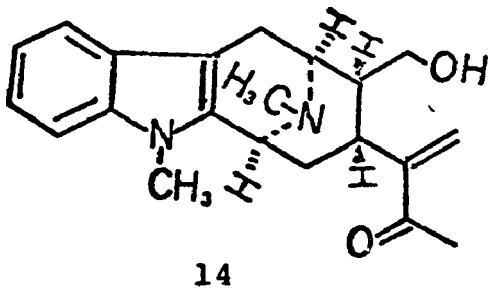
12 Fruticosine



11 Catharanthine

Alstonia Indole Alkaloids

The genus *Alstonia* consists of some thirty-nine species and twelve varieties which were classified into five sections by Monachino[9] (Table 1). The alkaloids of the *Alstonia* species, currently numbering approximately thirty-five, whose structures have been elucidated, can be divided into two groups: those that contain a macroline unit (14) and those that do not. *Alstonia* alkaloids have been the subject of three recent reviews,[10,11,12] and therefore only the macroline containing alkaloids will be summarized briefly here. There have been reported to date eleven alkaloids containing the macroline moiety; seven monomeric indoles (Table 2) and four "dimeric" or bis-indole alkaloids (Table 3). Although only approximately ten of the *Alstonia* species have been examined, eight of the macroline-containing alkaloids have thus far been found in only three *Alstonia* species: *A. muelleriana*, *A. spectabilis* and *A. macrophylla*. The isolation of three "macroline type" alkaloids (23), (24) and (25) from plants other than *Alstonia* species has recently been reported.



14

TABLE 1

Botanical Classification of the Genus Alstonia (Apocynaceae)

Section Winchia

A. glaucescens
A. rostrata
A. pachyarpa

Section Pala

A. scholaris
A. scholaris var. velutina
A. Boonei De Wild
A. congensis Engl.
A. angustiloba Mig.
A. pneumatophora Backer ex L.G.
A. pneumatophora var petiolata Monachino
A. spatula Blume
A. actinophylla (A. Cunn.)

Section Blaberopus

A. venenata
A. venenata var pubescens Monachino
A. neriifolia D. Don
A. neriifolia var glabra Monachino
A. Sebusi
A. Sebusi var szemaoensis
A. Mairei Leville
A. Curtisii King and Gamble
A. rupestris Kerr
A. yunnanensis Diels

Section Monuraspermum

A. angustifolia Wall
A. angustifolia var annamensis Monachino
A. angustifolia var latifolia
A. parvifolia Merrill
A. spectabilis R. Br. *
A. Muelleriana Domin *
A. ophioxyloides F. Muell
A. macrophylla Wall *

*Species from which macroline containing alkaloids have been isolated.

TABLE 1(cont.)

A. Brasii Monachino
A. glabriflora Markgraf
A. linearis Benth

Section Dissuraspermum

A. constricta
A. lanceolata van Heurck
A. Vieillardi van Heurck and Muell. Arg.
A. Roeperi van Heurck and Muell. Arg.
A. plumosa La Bill.
A. vitiensis Seem
A. vitiensis var novo-ebudica Monachino
A. Reineckeana Lauterb
A. montana Turrill
A. montana var filiformis Monachino
A. costata
A. Deplanchei van Heurck and Muell. Arg.
A. Lenormandi van Heurck and Muell. Arg.
A. Lenormandi var coriacea (Panch ex S. Moore) Monachino
A. Lenormandi var lanceolifera
A. Legouixiae van Heurck and Muell. Arg.
A. saligna S. Moore
A. comptonii S. Moore

Macroline alkaloids are placed by Schmid[8] in Class 1 (alkaloids containing a non-rearranged secologanin skeleton of type (2), group 1.9(sarpagine group) and subgroup 1.9.2 (macroline alkaloids). Scheme II shows a hypothetical biological conversion of the skeleton (26) to macroline (14).

The elucidation of the structure of macroline[13,14] was an essential preliminary to solving the structure of

TABLE 2

Monomeric Macroline Type Alkaloids*

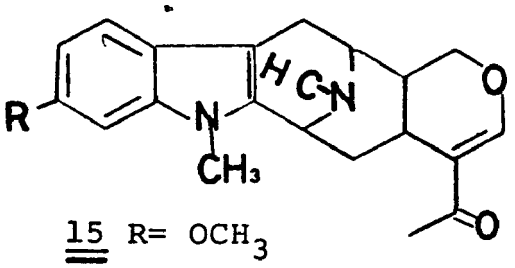
Name	Str.	Source	Ref.
Alstophylline	15	A. macrophylla	37
Macrosalhine	16	A. macrophylla	38,39
Alstonisine	17	A. muelleriana	40,41,42
Alstonerine	18	A. muelleriana	10,40
Talcarpin	23	Pleiocarpa talbotii	36
Talpinin	24	P. talbotii	36
Suaveoline	25	Rauwolfia suaveolens	44

TABLE 3

Dimeric Macroline Type Alkaloids*

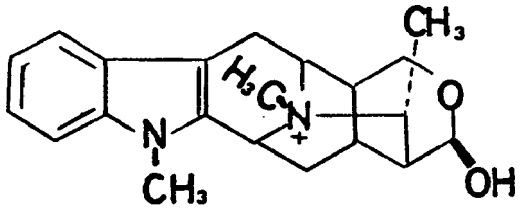
Name	Str.	Source	Ref.
Villalstonine	19	A. spectabilis	13,43
		A. macrophylla	13,43
		A. muelleriana	40
Macralstonidine	20	A. macrophylla	43,46
Alstonisidine	21	A. muelleriana	40,48
Macralstonine	22	A. macrophylla	43,45
		A. muelleriana	47

*Structures on Page 9 and 10.



15 R= OCH_3

18 R= H



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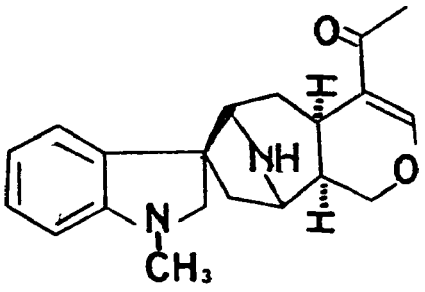

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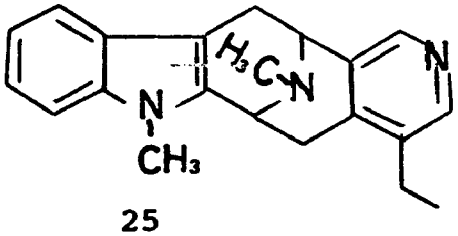

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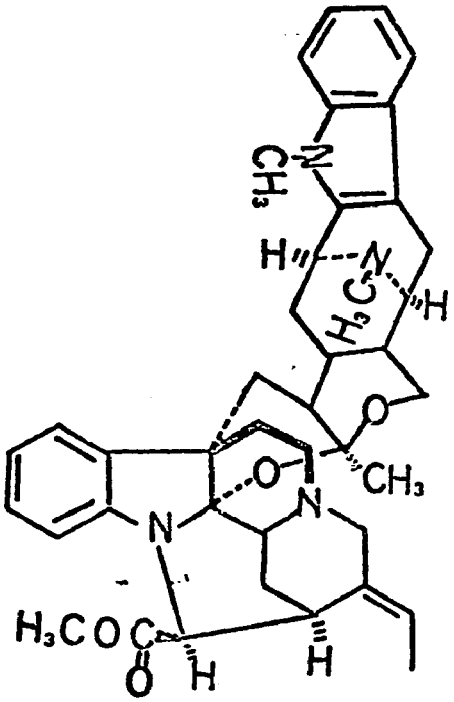

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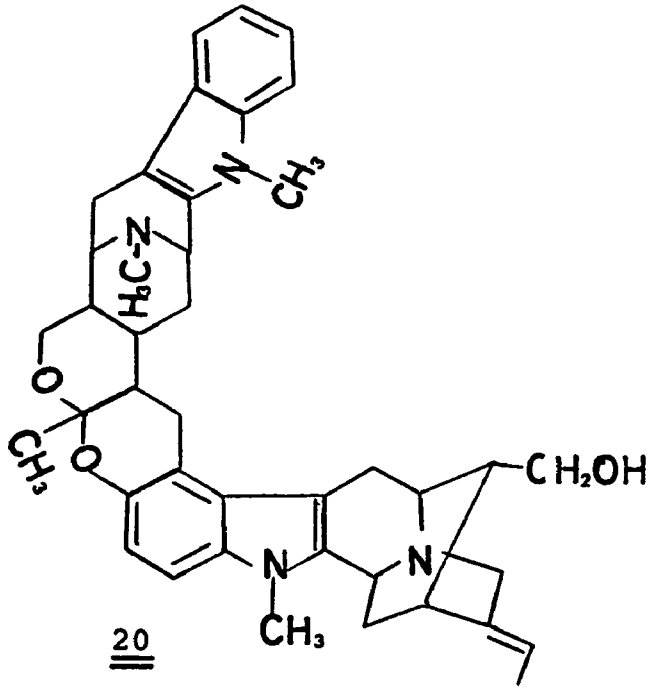

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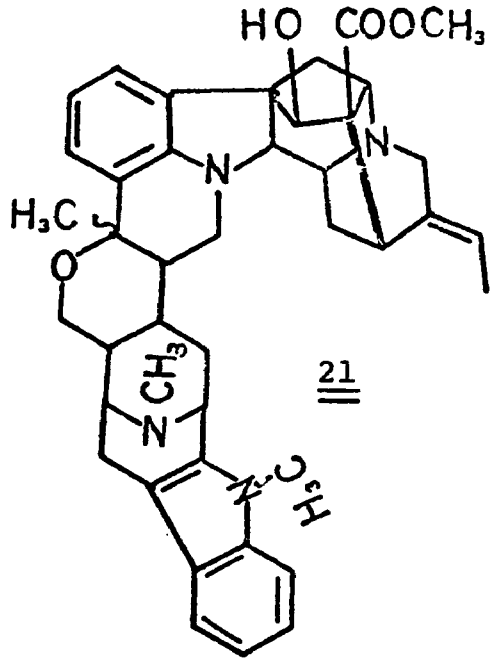

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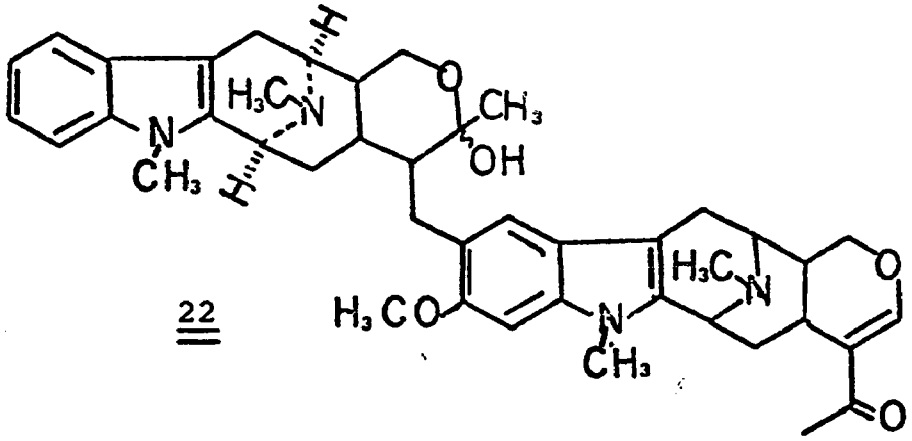

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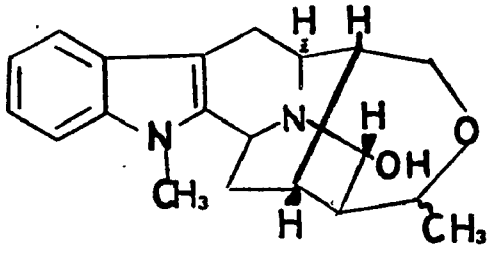

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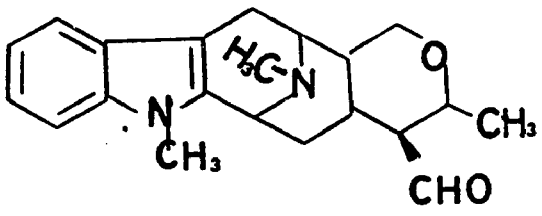

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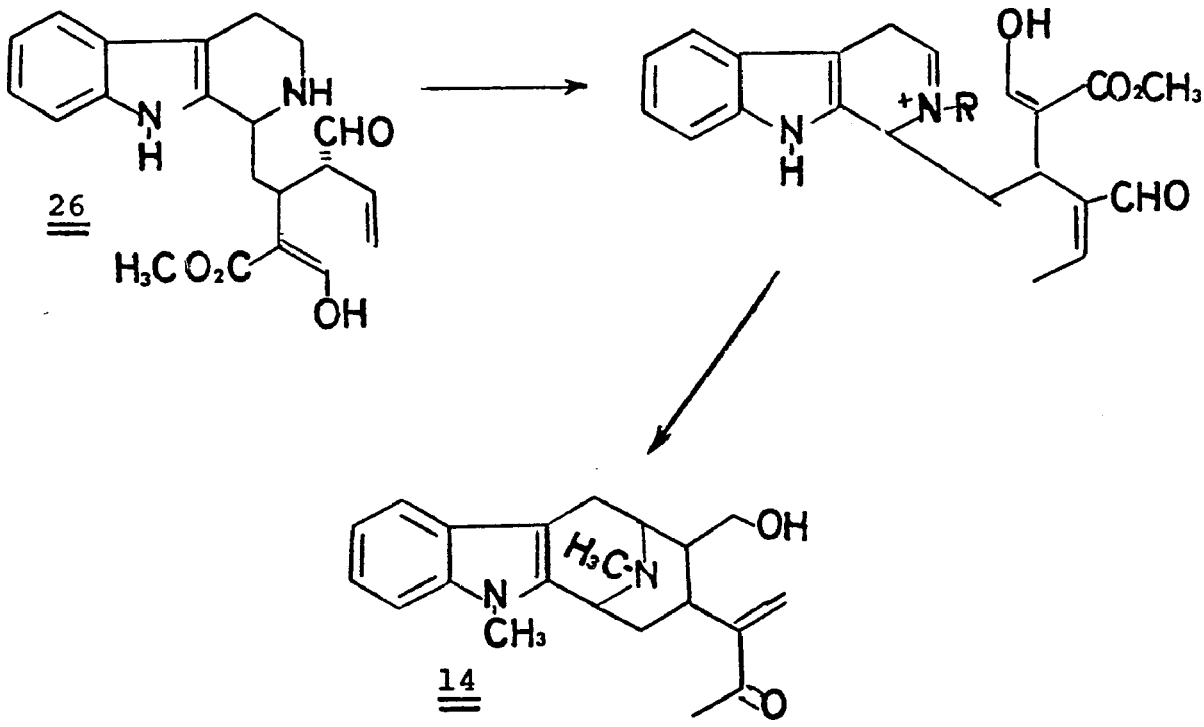
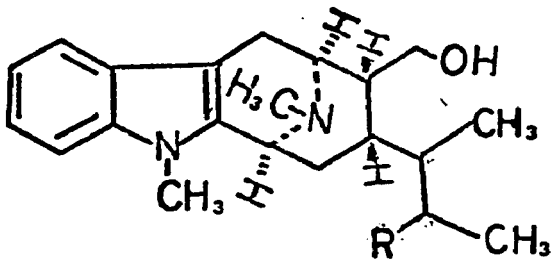
SCHEME II

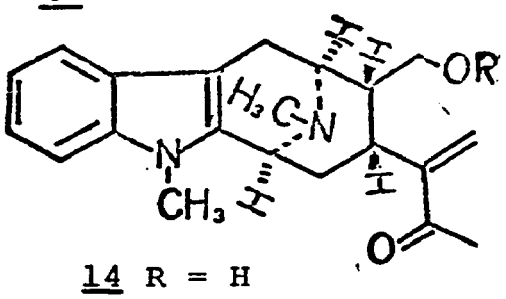


many of the alkaloids found in A. macrophylla and A. muelleriana. Macroline has not been found as a free alkaloid but is obtained as a degradation product from villalstonine (19). When villalstonine is treated with trifluoroacetic anhydride-trifluoroacetic acid mixture, the acetal function is cleaved and villamine (27) is one of the cleavage products.[13,14] On heating at 250°C, 0.01 Torr villamine undergoes a retro Diels-Alder reaction to give pleiocarpamine (28) and macroline (14). The N.M.R.



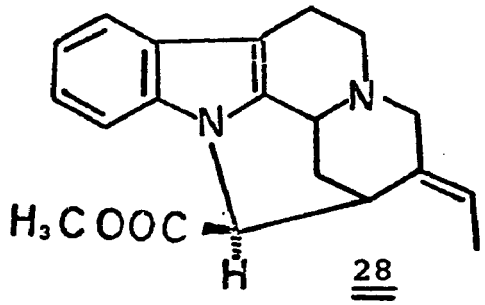
29 R = O

30 R = OH, O



14 R = H

31 R = CH_3CO-



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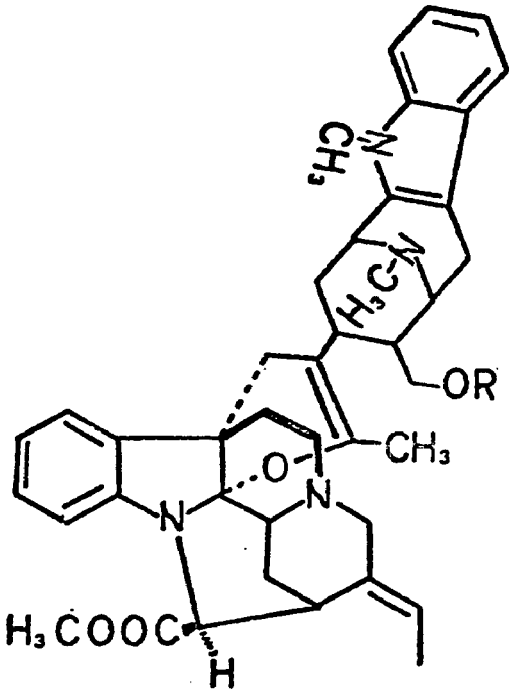


27 R = H

32 R = CH_3CO-

and I.R. spectra of macroline indicated an α,β-unsaturated ketone group, which was supported by hydrogenation to dihydromacroline (29) and lithium aluminum hydride reduction to macrolinol (30). The N.M.R. spectrum also indicated two N-methyl groups and a methyl ketone group while the U.V. indicated an indole chromophore. Attempts at acetylation of macroline yielded only intractable tars, but O-acetyl macroline (31) could be prepared by pyrolysis of

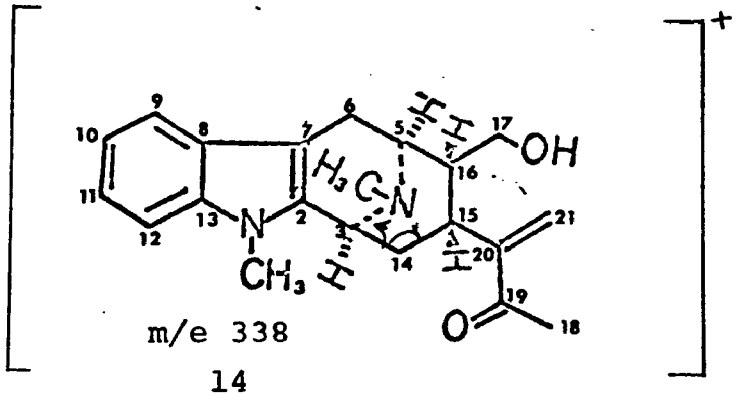
O-acetyl villamine (32).

The tentative assignment of structure (14) to macroline gave a working hypothesis on which to rationalize the mass spectrum.[13] The mass spectrum of macroline indicates the occurrence of two prominent fragmentation processes. The first of these leads to the β-carbolinium ion (33) at m/e 197 via two pathways (Scheme IIIA).

The second fragmentation undergone by macroline (Scheme IIIB) starts with a retro Diels-Alder reaction in ring C, after which the 14,15 bond is allylic to both the 2,3 and 20,21 bonds. Fission of the 14,15 bond gives the ion (34) at m/e 170 while hydrogen migration and fission of the 15,16 bond gives the ion (35) at m/e 251 which in turn gives rise to ions (36) at m/e 208, (37) at m/e 181 and (38) at m/e 182. However the most convincing evidence in support of structure (14) for macroline was provided by the mass spectrum of the two epimeric degradation products of ajmaline, (39) and (40).

Treatment of 4-methyl-desoxydihydro-chanoajmaline (41) and 4-methyl-desoxydihydro-chanoisoajmaline (42) with lead tetraacetate gave 4-methyl-desoxydihydro-chanoajmalol B(40) and 4-methyl-desoxydihydro-chanoisoajmalol B(39) respectively.[15] The mass spectra of these two products were almost identical to the mass spectrum of macroline,

SCHEME IIIA[13]

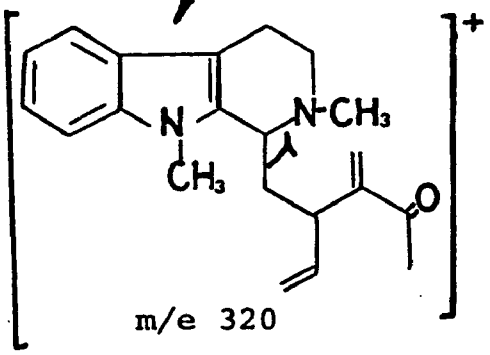


m/e 338

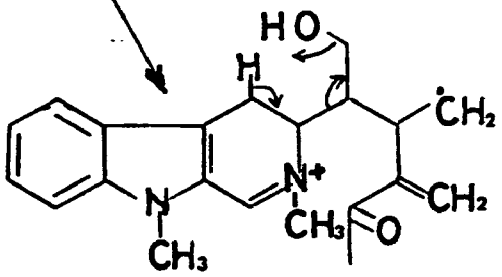
14

$-H_2O$

Cleavage of 3-14 bond

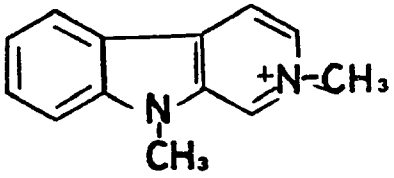


m/e 320



m/e 338

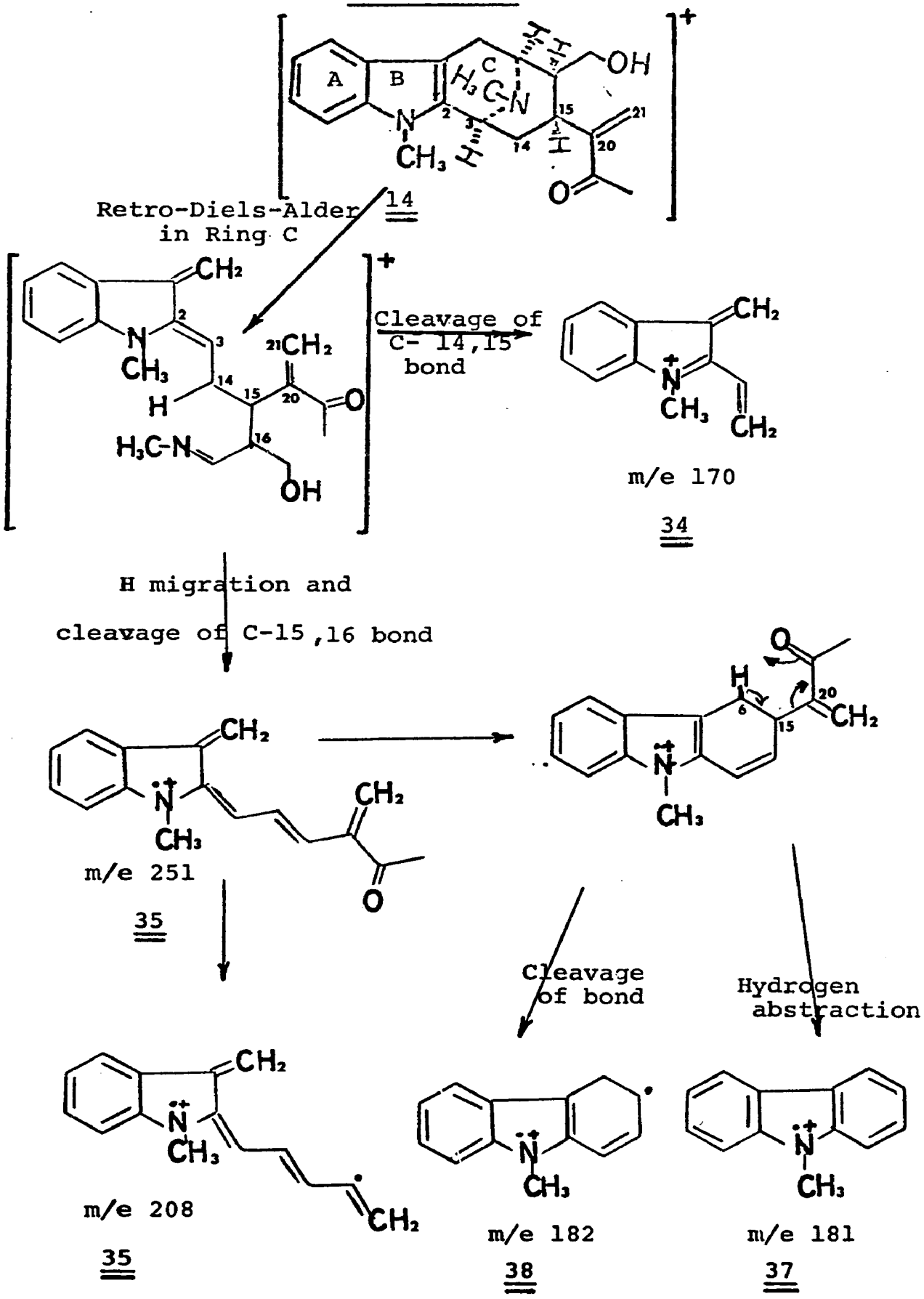
Abstraction of C-6 H



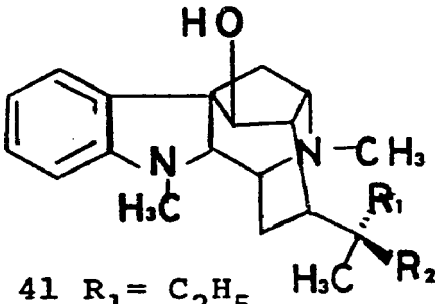
m/e 197

33

SCHEME IIIB[13]

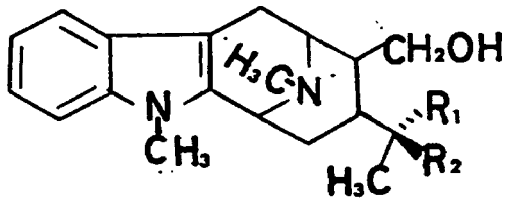


the only difference occurring in the relative intensities of the corresponding peaks. Since the eludication of the structure of macroline, which was the key to the determination of the structure of villalstonine, macroline has been found as "one half" in all of the bisindole alkaloids found in Alstonia species (Table III).



41 R_1= C_2H_5
R_2= H

42 R_1= H; R_2= C_2H_5



39 R_1= H; R_2= C_2H_5

40 R_1= C_2H_5; R_2= H

BisIndoles Which Have Been Synthesized

The synthesis of bisindole alkaloids is a major challenge to organic chemists. Because of the large molecular size of many of the bisindoles, the syntheses for the most part have hitherto been partial, - usually emphasizing the joining together of the two halves of

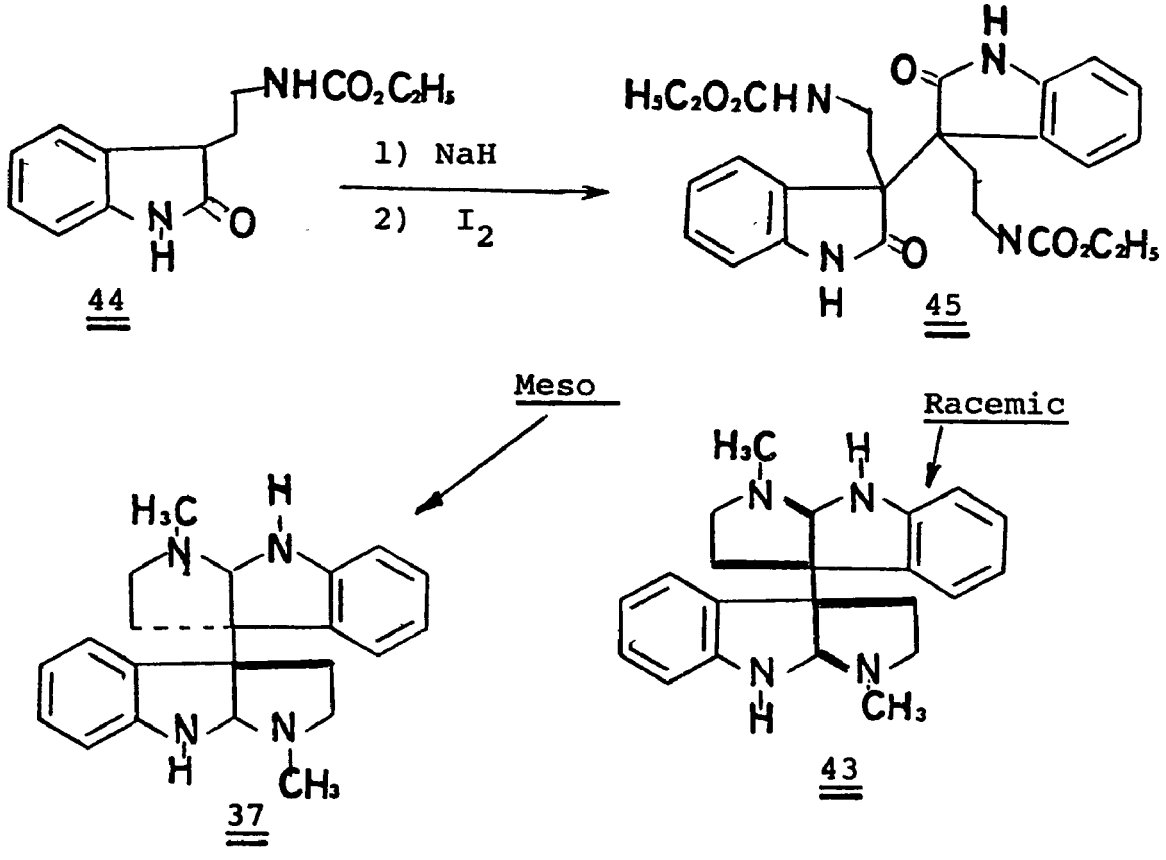
the alkaloid. The structures, properties and syntheses of the known bisindole alkaloids have been the major concern of a recent review by Schmid[16] and collaborators and thus the syntheses will be discussed only briefly here.

Calycanthaceous Alkaloids

Chimonanthine and Folicanthine

The synthesis of the bisindole alkaloid chimonanthine (43) has been accomplished in two ways. The first reported synthesis by Hendrickson and co-workers[17] (Scheme IV) involved oxidative dimerization of N-carbethoxy-oxytryptamine (44) to give both meso and racemic (45). The

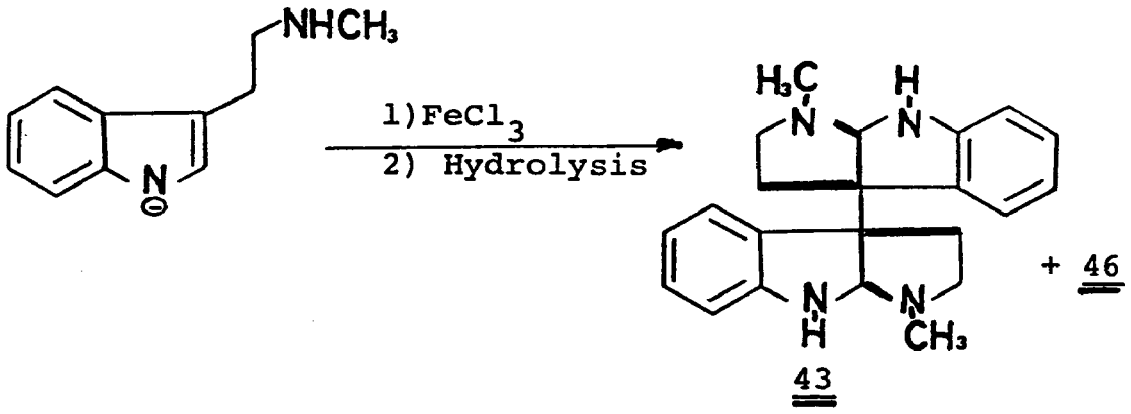
SCHEME IV



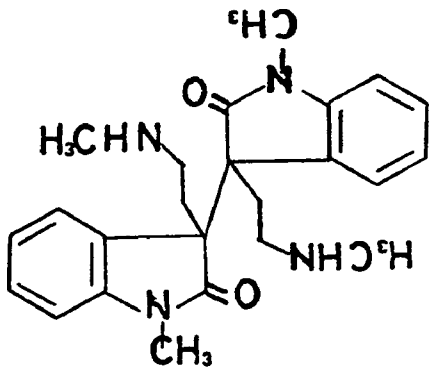
reductive cyclization of racemic (45) with lithium aluminum hydride gives racemic chiomonanthine (43) in low yield while meso (45) gives meso chimonanthine (46).

The second approach to the synthesis of chimonanthine was accomplished by Scott and co-workers[18] and proceeds *via* oxidative dimerization of the Grignard derivative of N_b-methyl tryptamine to give β,β-coupling in a one step synthesis of racemic and meso chimonanthine (Scheme V).

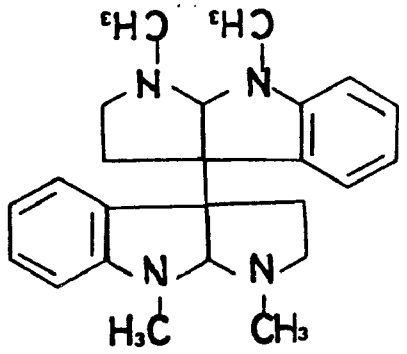
SCHEME V



Folicanthine (47), isolated by Eiter[19] has been synthesized by Hino[20,22] using a similar approach in which the bis oxindole (48)[21] was reductively cyclized with lithium aluminum hydride to give (±)folicanthine.



48



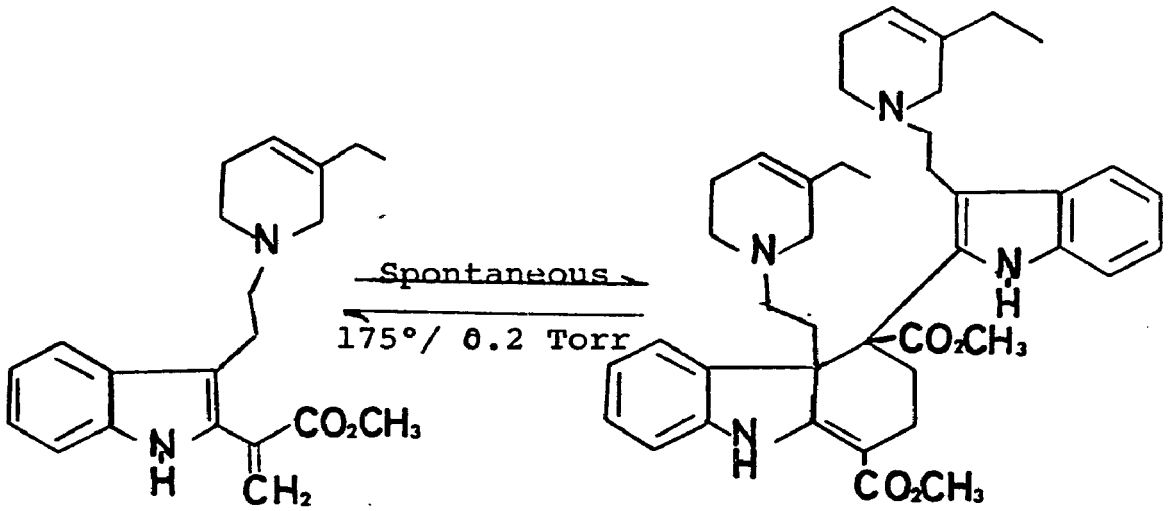
47

Presecamine Alkaloids

Presecamine

The presecamines were first isolated by Smith and co-workers from Rhazya stricta.[23] Presecamine (49) is readily formed spontaneously in vitro by dimerization of secodine (50) also found in R. stricta[24] (Scheme VI). The ease with which secodine dimerizes to presecamine and the fact that there is some evidence that the presecamines are largely racemic has given rise to the question as to whether the presecamines are artifacts of isolation. Presecamine is also readily thermalized to secodine at 175°C and 0.2 Torr.

SCHEME VI



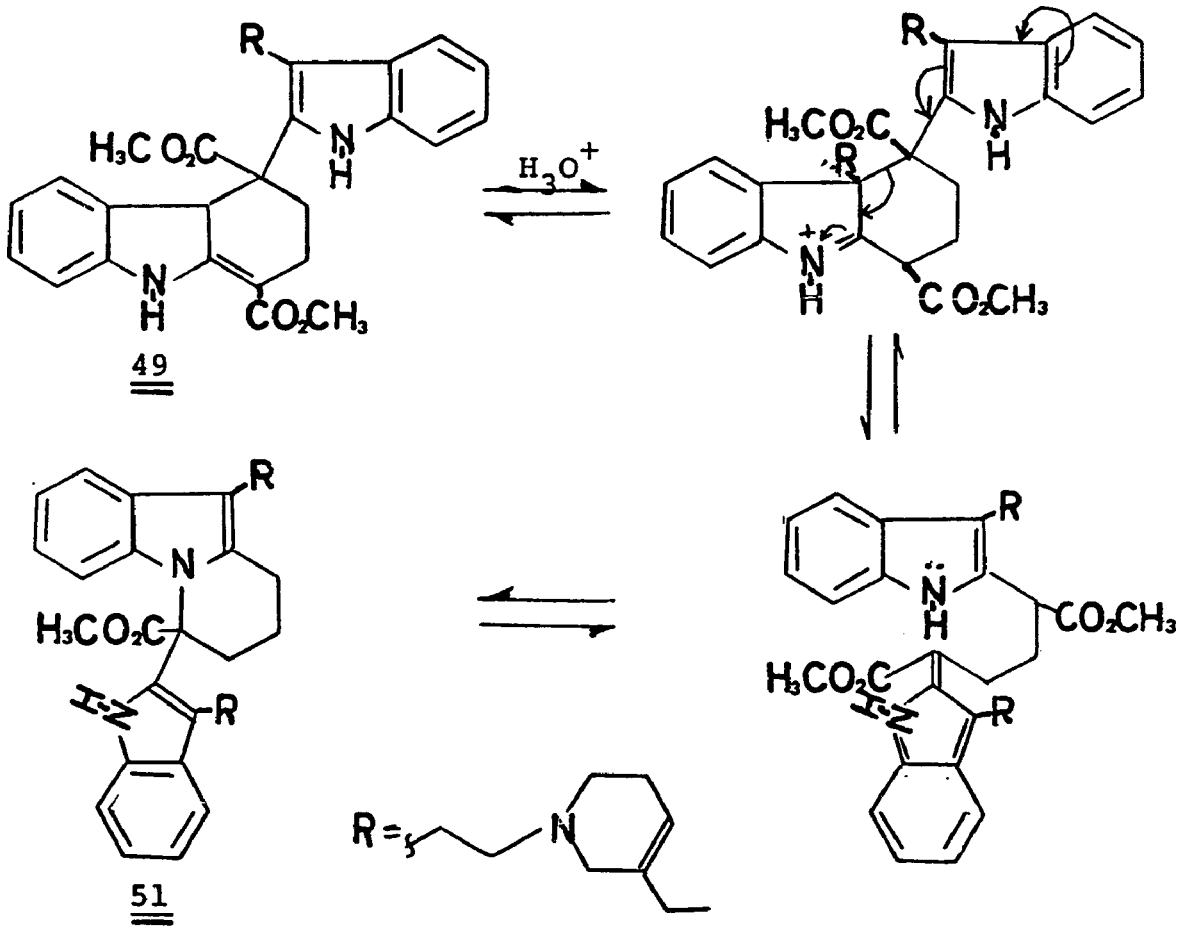
50 49

Secamine Alkaloids

Secamine

The secamines were isolated from Rhazya stricta and Rhazya orientalis by Smith and co-workers in 1968[25] and by Saki from Amsonia elliptica.[26] The synthesis of secamine (51) was readily accomplished by spontaneous and quantitative rearrangement of presecamine (49) on standing in aqueous 2 N hydrochloric acid for fifteen minutes[23] (Scheme VII).

SCHEME VII



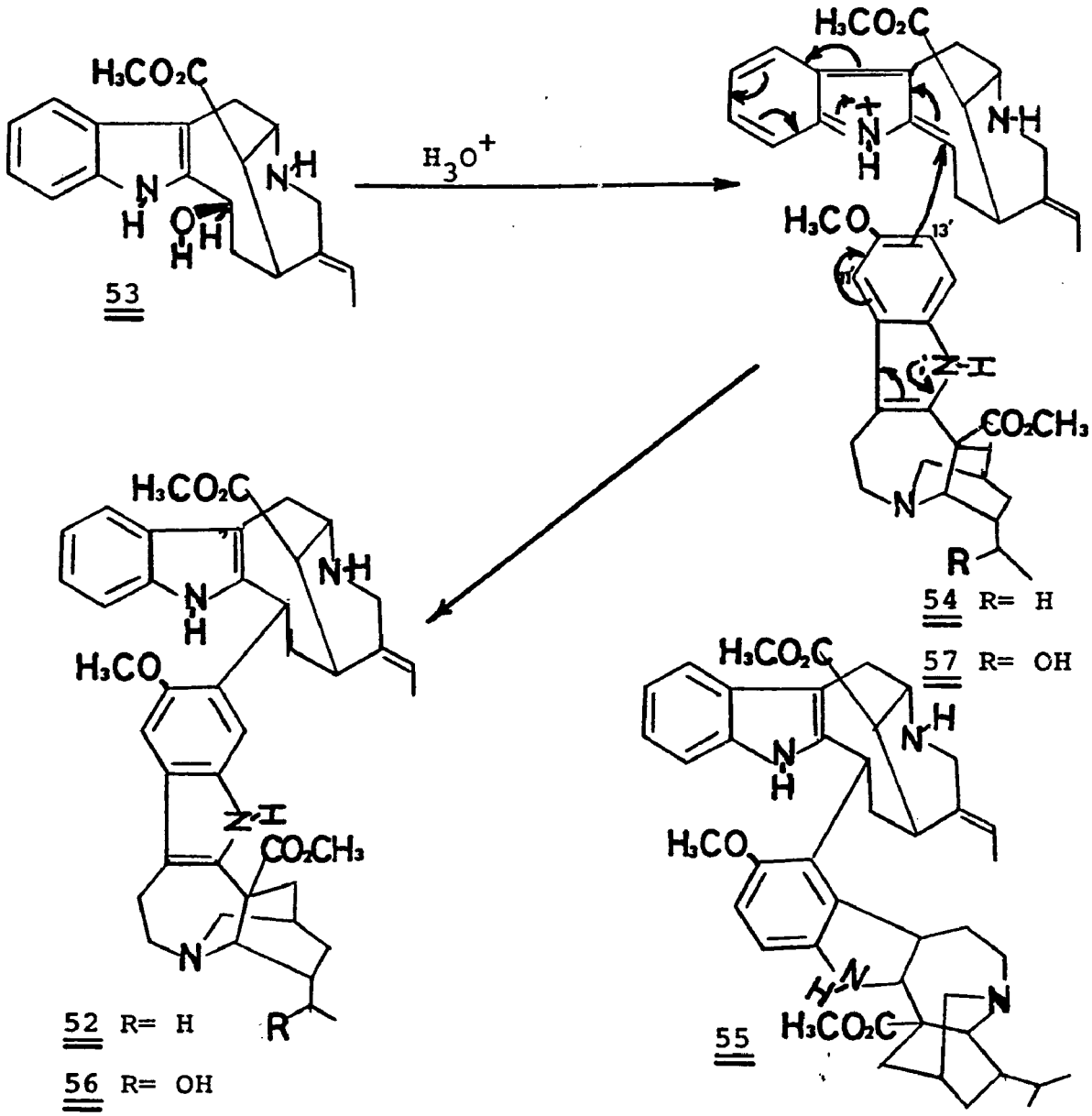
Voacamine Type Alkaloids

Voacamine and Voacorine

Voacamine (52) was first isolated from Voacanga africana[27] and subsequently from other species[28] and genera.[29] The structure of voacamine was elucidated by Buchi and co-workers in 1964,[30] and involved degradation and a partial synthesis. The synthesis was accomplished

by refluxing, under nitrogen, equimolar amounts of vobasinol (53) and voacangine (54) in 1.5% methanolic HCl for 7 hours (Scheme VIII). If somewhat milder conditions are employed another bisindole, voacamidine (55) which arises from the Mannich condensation on C-11' of voacangine is also formed.

SCHEME VIII



It is interesting to note that when (55) is subjected to more concentrated acid treatment it isomerizes to (52).

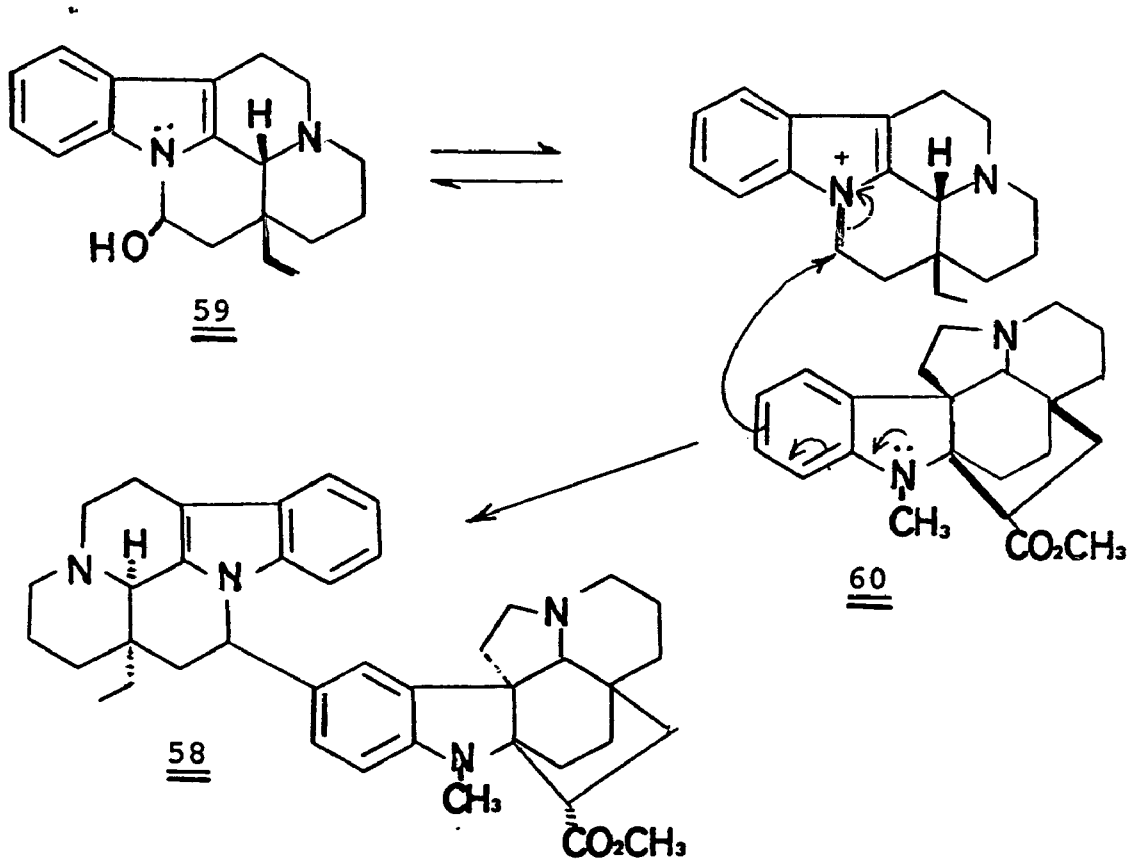
Voacorine (56) also a dimeric alkaloid found in V. africana, was prepared from vobasinol (53) and voacangarine (57) using the same procedure as for voacamine. Whether the other isomer corresponding to condensation at the C-11' of voacangarine was also obtained was not reported.

Pycnanthine Alkaloids

Pleiomutine

Pleiomutine (58) was originally isolated by Schmid and Kump in 1961 from Pleiocarpa mutica Benth.[31] The structure elucidation and partial synthesis was accomplished independently and simultaneously by Biemann[32] and Schmid.[43] The condensation was accomplished by refluxing together for eight hours eburnamine (59) and pleiocarpinine (60) in 2% aqueous hydrochloric acid to give a 60% yield of pleiomutine (58) (Scheme IX).

SCHEME IX

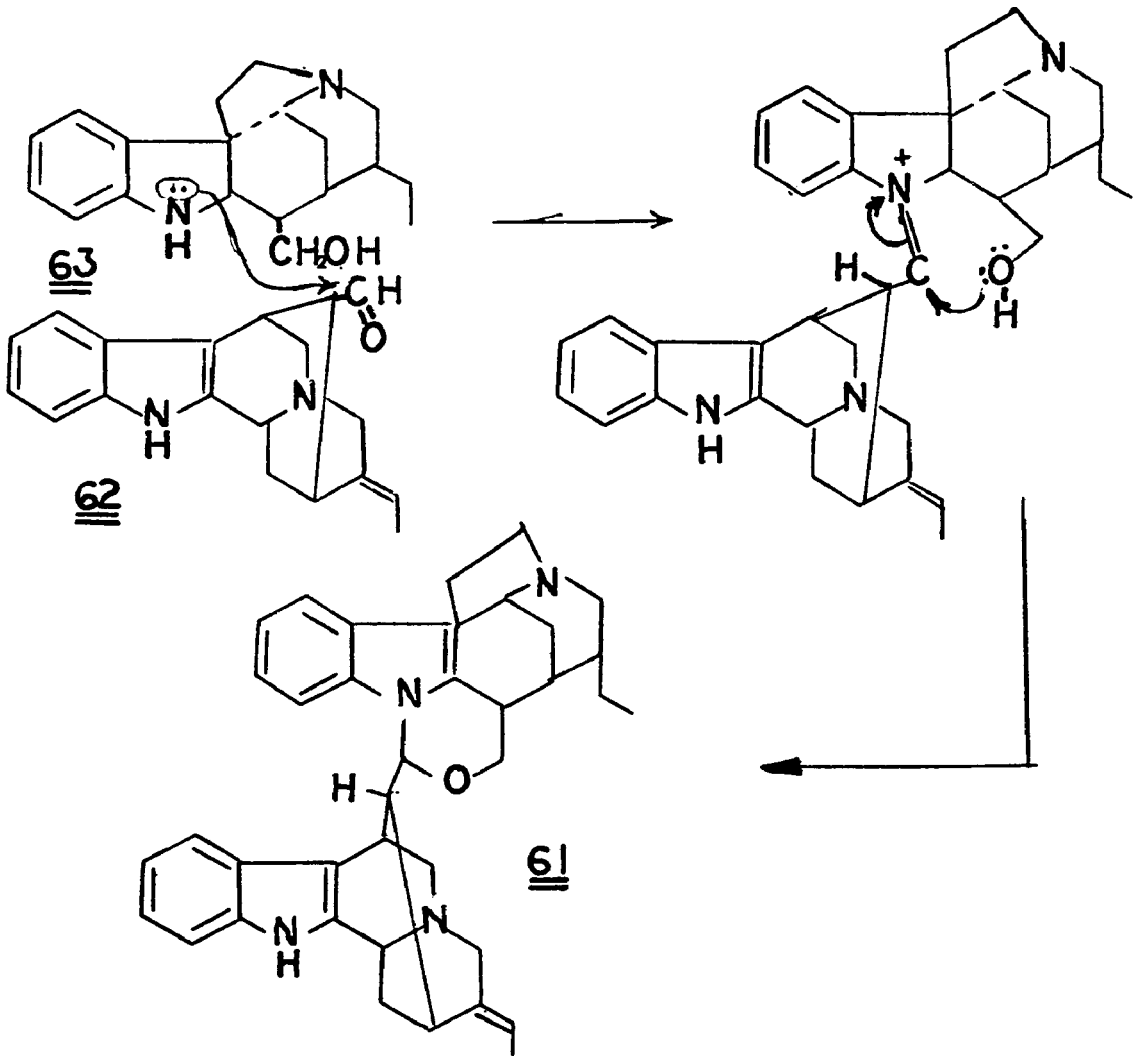


Geissolosimine and Geissospermine

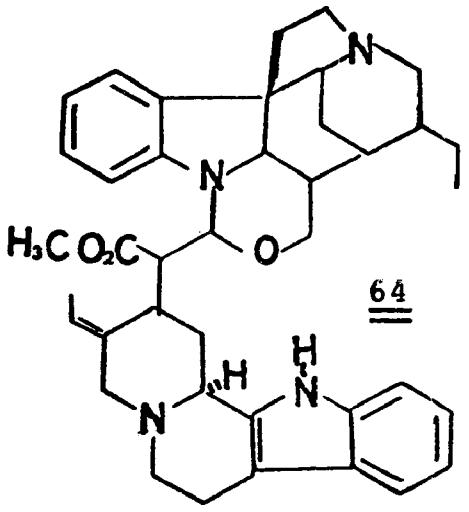
The structure and partial synthesis of geissolosimine (61) found in Geissospermum vellosii were reported by Rapaport[34] in 1962. The partial synthesis was accomplished by stirring vellosimine (62) and geissoschizoline chloroformate (63) together at room temperature for two days in 1.5 N acetic acid (Scheme X).

Geissospermine (64) was partially synthesized in

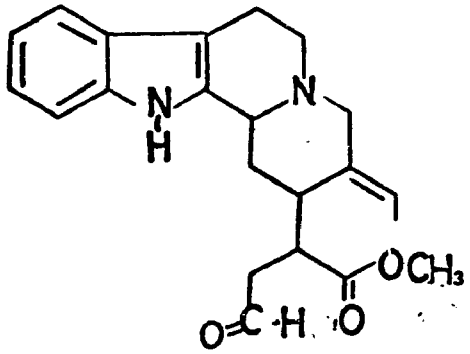
SCHEME X



a similar manner from condensation of geissoschizoline (63) with geissoschizine (65).[35]



64



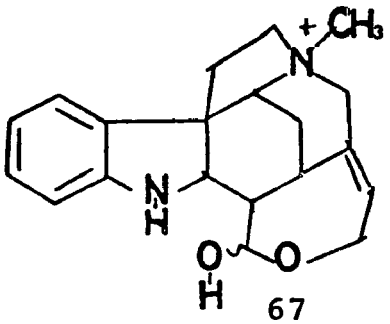
65

Calabash-Curare Alkaloids

C-Toxiferine-I

The synthesis of C-toxiferine-I (66), one of the most medicinally important of the curare alkaloids, has been accomplished by two groups. The first synthesis of C-toxiferine-I, reported by Schmid and coworkers,[49] consisted of heating at 70°C in absence of air N_b-metho-Wieland-Gumlich aldehyde (67) in acetic acid/sodium acetate solution to give C-toxiferine-I (66) in 25% yield. The major products were caracurin-V methochloride (68) and acetylated toxiferine-I. An improved synthesis described by Battersby[50] involved heating N_b-metho-Wieland-Gumlich aldehyde in pivalic acid at 120°C in the

absence of air to give in one step, 72% of (66) after direct crystallization.



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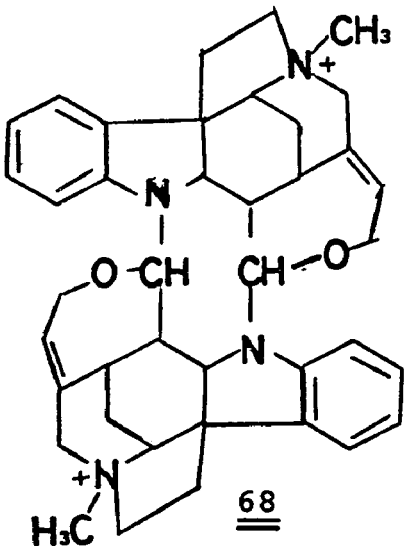
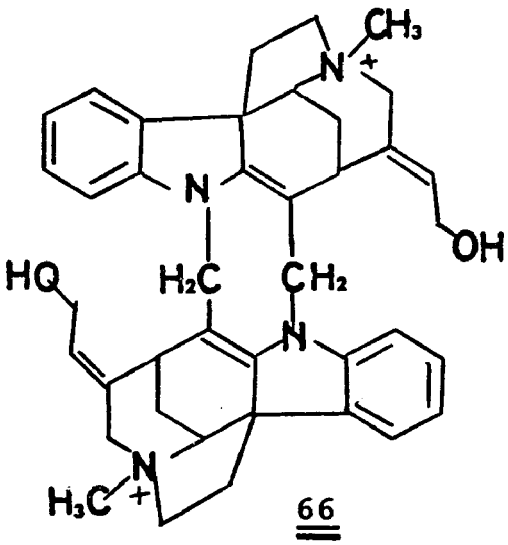


68



66

Biomimetic Syntheses

The synthesis of natural products by nonenzymatic biomimetic means has been the goal of many researchers. These types of syntheses take place via the presumed pathway and use the same or very closely related materials

and reagents *in vitro* that are presumed to be used *in vivo*. Many of the syntheses of the bisindoles described above may, therefore, be called biomimetic since simple reagents such as H_3O^+ are used to effect dimerization and enzymes are not essential. The number of generally recognized biomimetic reactions in this sense is, however, quite small. In addition to biomimetic syntheses, some researchers have concentrated on enzyme models to duplicate reactions of enzymes.[74,75,76]

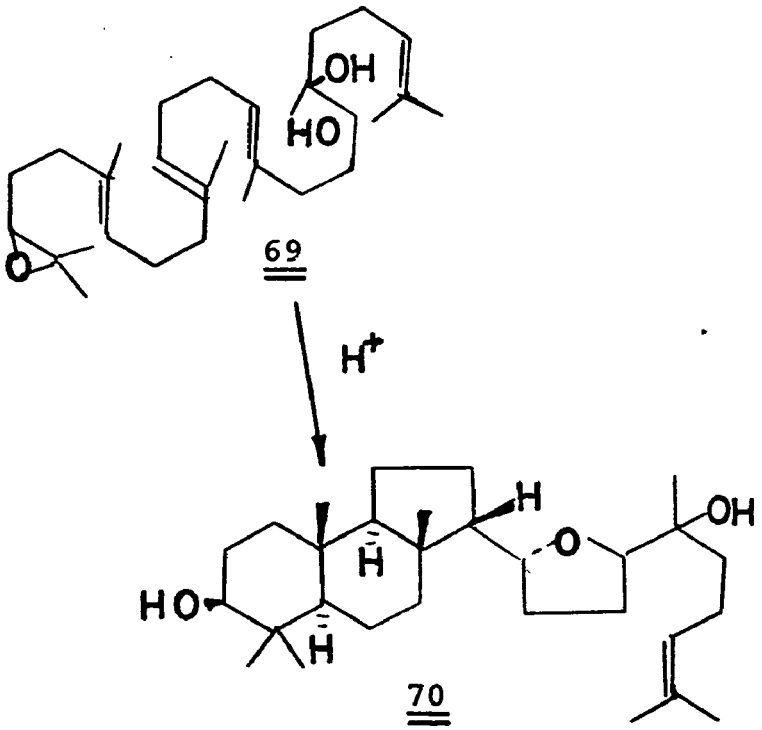
Two of the best known examples of biomimetic reactions outside of the indole alkaloid field are probably cyclization of polyolefins to polyterpenes[51,52] and oxidative coupling of phenols.[53] These are illustrated briefly.

Polyolefin Cyclizations

The first reported example of the formation of a natural product from a polyolefin was Sharpless' cyclization of squalene 2,3 oxide derivative (69) to malabraieanediol[54] (70) using picric acid as the proton source to effect cyclization (Scheme XI). The yield was reported to be seven per cent in the cyclization step which is remarkable considering that the molecule has nine asymetric centers, six of which must be formed stereo-

specifically during the course of cyclization. Other cyclizations of polyolefins to give natural product systems in the absence of enzymes have also been reported.[52,55,56]

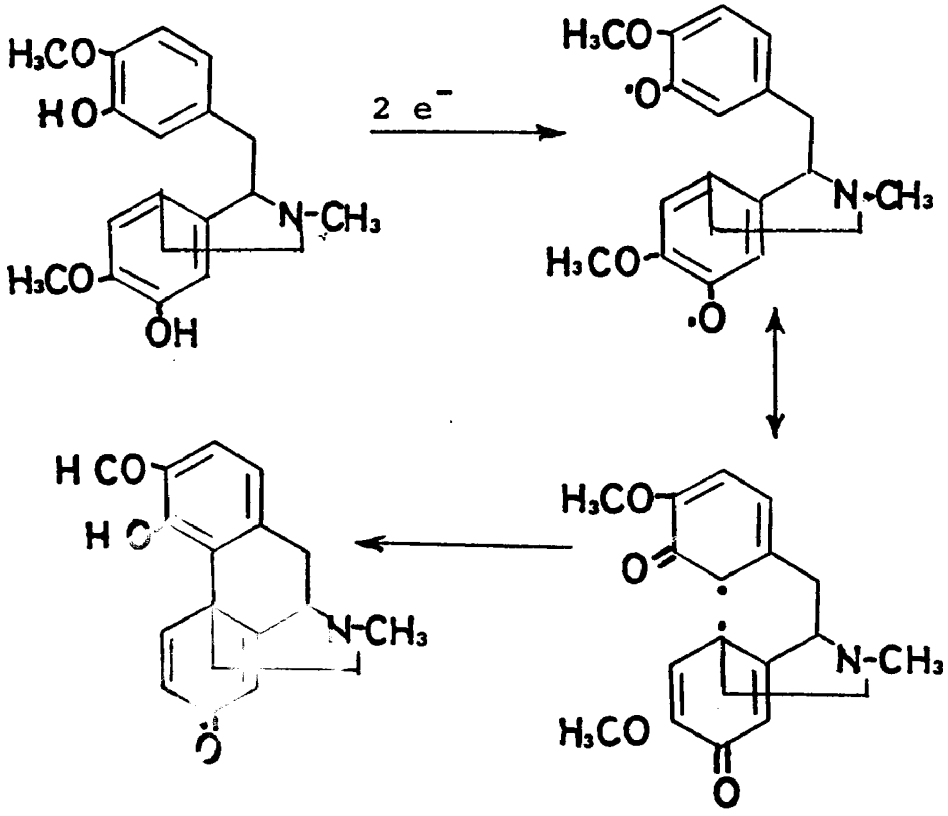
SCHEME XI



Oxidative Phenol Coupling

The oxidative coupling of phenols has long proposed as the biosynthetic route employed in the formation of certain benzylisoquinoline alkaloids.[57,58,59] The non-enzymatic synthesis of benzylisoquinoline alkaloids has been accomplished by phenol oxidation and other routes[53] to give naturally occurring alkaloids.[60,61] (Scheme XII)

SCHEME XII

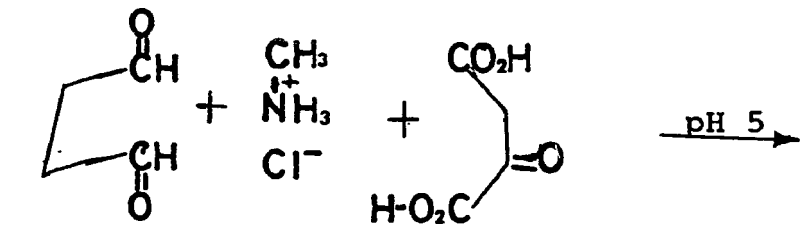


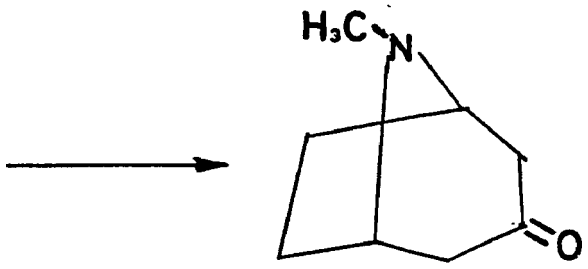
Synthesis of Tropanones

While the synthesis of the tropanone skeleton (71) is not a biomimetic reaction, it is included here because of its great historical importance as the germinal chemistry in the field of biomimetic synthesis. Robinson in the 1920's[62] demonstrated that in aqueous solution and at "physiological" pH, the tropane skeleton could be formed in high yield from succindialdehyde, methylamine hydro-

chloride and acetone dicarboxylic acid. (Scheme XIII) Since the conditions employed, pH 5 and room temperature, were similar to conditions in cell sap, this gave rise to much speculation that this was indeed the true biosynthetic route to tropanes in the plant. Later workers, however, have proven by tracer studies that this is not the case.[63-66]

SCHEME XIII





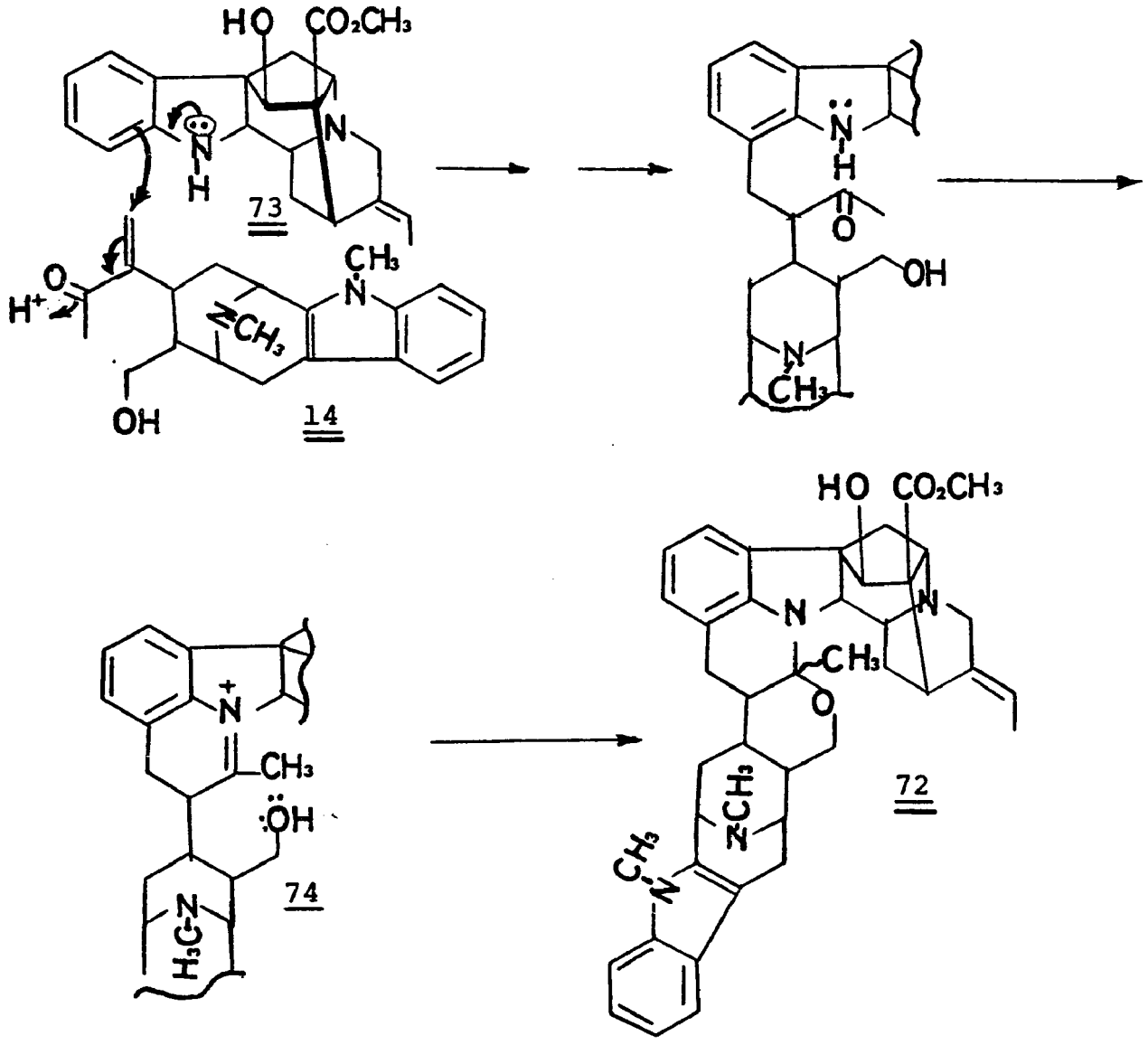
71

Initiation of Present Work

When the structure of alstonisidine was proposed to be (72) by workers in our research group,[10,48] it was proposed to attempt a possibly biogenetic partial synthesis of alstonisidine. Quebrachidine (73), one half of the

molecule was available to us and macroline (14), the other half, could be obtained by degradation of villalstonine (19) which was readily available. The synthesis proposed C-alkylation of the aromatic ring followed by Schiff base formation and attack on the Schiff base (74) by the primary alcohol function on macroline (Scheme XIV).

SCHEME XIV

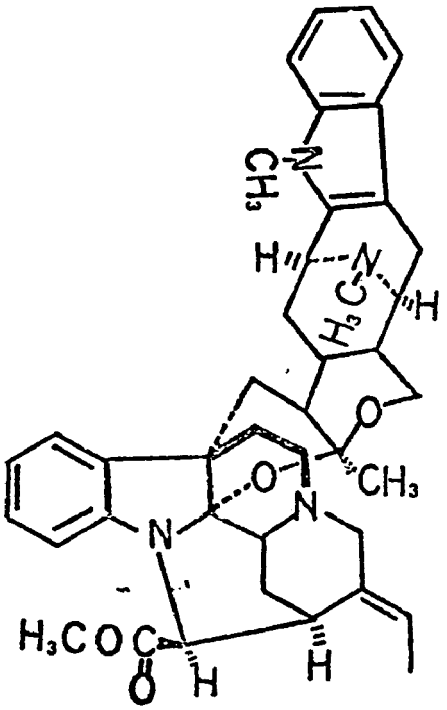


The results of the attempted synthesis of (72) are discussed below.

CHAPTER II

RESULTS AND DISCUSSION

Before beginning the partial synthesis of alstonisidine, the two "halves" of the molecule had to be obtained. One presumed half, quebrachidine, (73) was available to us through the courtesy of Dr. N. Neuss of Lilly Research Laboratories, but macroline (14) had to be prepared by the degradation of villalstonine. A procedure for the preparation of macroline has been described by Schmid in his elegant paper on the structure of villalstonine (19).[14] The procedure consists of the cleavage of the villalstonine acetal function to give villamine (27) and pyrolysis of villamine to macroline and pleiocarpamine (28).



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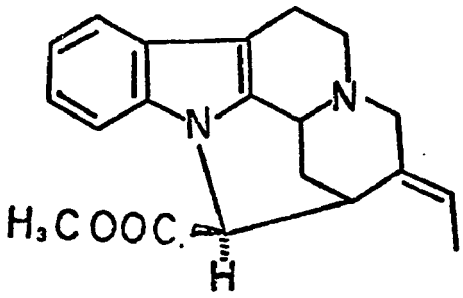

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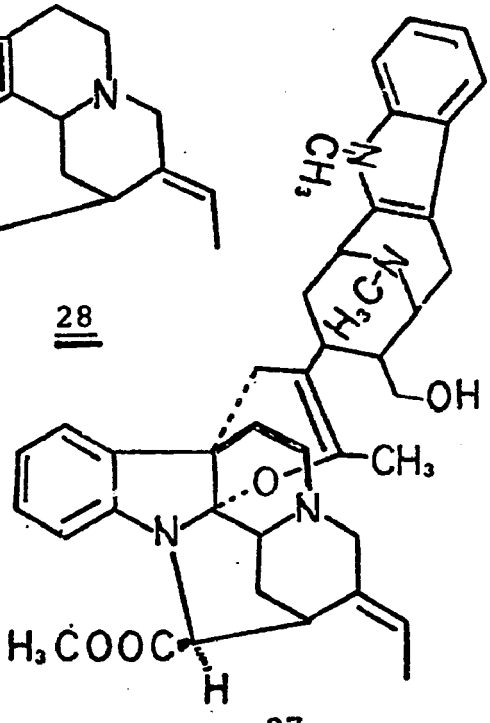

27

Isolation of Villalstonine

Since the synthesis was anticipated to require several hundred milligrams of macroline and the degradation reactions to macroline are low yield transformations, this necessitated the isolation of villalstonine in large quantities. We had a large batch of A. muelleriana bark available thorugh the courtesy of Dr. J. A. Lamberton, C. S. I. R. O., Melbourne, Australia, and obtained our villalstonine from this.

The isolation of villalstonine has been reported by several workers,[40,43,50] but an improved procedure, a modification of that described by Schmid,[50] was developed. The crude methanolic extract was acidified with dilute sulfuric acid and filtered. The filtrate was basified with concentrated aqueous ammonia and extracted with chloroform. It was found unnecessary to divide the alkaloids into "strong" and "weak" bases.[45] The dark brown residue obtained on evaporation of the dried chloroform extract was chromatographed on aluminum oxide and eluted with chloroform until all alkaloids were removed, as shown by tlc. The first portions of the eluate contained almost pure villalstonine while the later fractions contained villalstonine along with other alkaloids present in the extract. The fractions containing villalstonine

contaminated with the other alkaloids were combined, rechromatographed on highly activated alumina and eluted with benzene and benzene/chloroform until the villalstonine was washed off, as indicated by tlc. To obtain the other alkaloids present, the polarity of the solvent was increased and the remaining alkaloids were washed from the column. These alkaloids were set aside for investigation by other workers. In this manner the large quantities of villalstonine that were required could be obtained quickly and efficiently.

Conversion of Villalstonine to Villamine

In the procedure described by Schmid,[14] for the cleavage of the acetal functions, villalstonine is dissolved in trifluoroacetic anhydride and trifluoroacetic acid is generated *in situ* by the addition of water to the mixture. However, in our hands this method gave inconsistent and unreproducible results. Better and more consistent results were obtained when trifluoroacetic acid was added to a solution of villalstonine in trifluoroacetic anhydride. This method avoided the severe local over-heating encountered when water was added to the alkaloid/anhydride mixture and afforded better control of the reaction. Another product obtained from the reaction in equal or greater amounts

as judged by tlc, in addition to villamine is a compound of unknown structure called villoin. Villoin is, unfortunately, unstable and reverts back to villalstonine on heating or chromatography.[14] Schmid reports the purification of villamine by column chromatography on silica gel and elution with one percent methanol in chloroform. However, when silica gel, activity I, was tried the villamine obtained was always heavily contaminated with villalstonine. After experimentation with other adsorbents (florisil, alumina and adsorbosil) it was finally determined that activity IV silica gel would effect the separation of villamine from the more polar villoin. The separation of villamine requires a delicate balance of solvent polarity and adsorbent activity. If the adsorbent is highly active, the villoin reverts to villalstonine at too rapid a rate and the villalstonine, being relatively nonpolar, elutes simultaneously with villamine. If the adsorbent is too inactive no separation of villamine from villoin is achieved. The villamine obtained by column chromatography was always contaminated slightly with villalstonine and was freed from traces of villalstonine by careful crystallization from acetone. The yields of purified villamine were disappointingly low, seldom getting above twenty percent.

Pyrolysis of Villamine to Macroline

Schmid[14] carried out the pyrolysis of villamine by heating villamine in a kugelrohr at 200-280°C, 0.01 Torr and collecting pleiocarpamine and macroline as distillate. When this procedure was attempted no macroline was obtained. The failure is probably due to the lack of proper equipment rather than a poor procedure and the procedure probably could have been made to work by modifying the equipment available and optimizing the experimental conditions. Fortunately, however, another method being tried simultaneously proved to be highly successful.

It was decided to attempt the pyrolysis of villamine in a sealed evacuated tube primarily for two reasons. First, it would eliminate the need for a vacuum pump being connected to system throughout the reaction and secondly it would allow the pyrolyzing conditions to be more easily and carefully controlled. In addition, it would allow the pyrolysis of a greater number of samples in a shorter time than the kugelrohr method.

A 100 x 13 mm, pyrex test tube was drawn out such that a 3 to 4 cm. long bulb was formed at the end. The sample was introduced into the lower bulb, evacuated, sealed at the constriction and heated in a carefully controlled

oil bath. The pyrolysis conditions ultimately used were determined after considerable trial and error. If the sample was heated at too high a temperature, i.e., above 280°C no macroline was obtained. If the temperature was too low, below approximately 230°C the rate of decomposition of villamine is too slow. The conditions eventually used represent a compromise between those giving rapid decomposition of villamine to pleiocarpamine and macroline and those leading to too rapid further decomposition of the generated macroline. The reaction was finally performed at 245°C to 255°C for 90 seconds. The size of the sample is also important because in order to effect rapid decomposition of villamine the sample has to be distributed in a noncrystalline film on the walls of the sealed tube. A sample size of approximately 10 to 15 mg. was the maximum that could be efficiently pyrolyzed.

Acoording to Schmid,[14] the separation of pleiocarpamine from macroline can be effected by treatment of the pyrolyzed mixture with excess methyl iodide for five minutes at 20°C. Pleiocarpamine forms a water soluble methiodide while the macroline does not. However, this method was not tried because silica gel analytical tlc using acetone as a developing solvent showed that macroline could be separated easily and efficiently from the other

components of the reaction. The conditions employed for analytical tlc were transferred directly to silica gel preparative tlc with excellent separations. Yields of macroline using the above procedure were approximately 48%.

Implications of Model Studies

While work was in progress for the preparation of macroline, studies were being carried out by Dr. Cook, another worker in our research group, on model systems for the synthesis of alstonisidine, then proposed to have structure (72).[10] Since the biosynthesis (Scheme XIV) was proposed to proceed by C-alkylation of the aromatic ring ortho to the secondary amine of quebrachidine by the enone moiety of macroline, the first model reaction tried by Cook was reaction of methyl vinyl ketone with N-methyl aniline. However, the only product obtained was the Michael-type adduct (75). Numerous catalysts and conditions were tried but no other reaction product than (75) was ever isolated. The reaction of indoline with methyl vinyl ketone was also investigated by Cook but again only the Michael-type adduct (76) was isolated. When (76) was heated in aqueous acid a new product, 6-methyl-lilolidine (77), arising from alkylation on the

contaminated with the other alkaloids were combined, rechromatographed on highly activated alumina and eluted with benzene and benzene/chloroform until the villalstonine was washed off, as indicated by tlc. To obtain the other alkaloids present, the polarity of the solvent was increased and the remaining alkaloids were washed from the column. These alkaloids were set aside for investigation by other workers. In this manner the large quantities of villalstonine that were required could be obtained quickly and efficiently.

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A 100 x 13 mm, pyrex test tube was drawn out such that a 3 to 4 cm. long bulb was formed at the end. The sample was introduced into the lower bulb, evacuated, sealed at the constriction and heated in a carefully controlled

oil bath. The pyrolysis conditions ultimately used were determined after considerable trial and error. If the sample was heated at too high a temperature, i.e., above 280°C no macroline was obtained. If the temperature was too low, below approximately 230°C the rate of decomposition of villamine is too slow. The conditions eventually used represent a compromise between those giving rapid decomposition of villamine to pleiocarpamine and macroline and those leading to too rapid further decomposition of the generated macroline. The reaction was finally performed at 245°C to 255°C for 90 seconds. The size of the sample is also important because in order to effect rapid decomposition of villamine the sample has to be distributed in a noncrystalline film on the walls of the sealed tube. A sample size of approximately 10 to 15 mg. was the maximum that could be efficiently pyrolyzed.

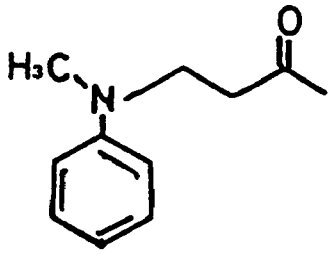
According to Schmid,[14] the separation of pleiocarpamine from macroline can be effected by treatment of the pyrolyzed mixture with excess methyl iodide for five minutes at 20°C. Pleiocarpamine forms a water soluble methiodide while the macroline does not. However, this method was not tried because silica gel analytical tlc using acetone as a developing solvent showed that macroline could be separated easily and efficiently from the other

components of the reaction. The conditions employed for analytical tlc were transferred directly to silica gel preparative tlc with excellent separations. Yields of macroline using the above procedure were approximately 48%.

Implications of Model Studies

While work was in progress for the preparation of macroline, studies were being carried out by Dr. Cook, another worker in our research group, on model systems for the synthesis of alstonisidine, then proposed to have structure (72).[10] Since the biosynthesis (Scheme XIV) was proposed to proceed by C-alkylation of the aromatic ring ortho to the secondary amine of quebrachidine by the enone moiety of macroline, the first model reaction tried by Cook was reaction of methyl vinyl ketone with N-methyl aniline. However, the only product obtained was the Michael-type adduct (75). Numerous catalysts and conditions were tried but no other reaction product than (75) was ever isolated. The reaction of indoline with methyl vinyl ketone was also investigated by Cook but again only the Michael-type adduct (76) was isolated. When (76) was heated in aqueous acid a new product, 6-methyl-lilolidine (77), arising from alkylation on the

aromatic ring, was isolated. The isomer, 4-methyl-lilolidine (78), could be prepared by reaction of a functionalized methyl vinyl ketone (79) with indoline to give (80). This methoxy derivative could then be cyclicized to 4-methyl lilolidine by heating in polyphosphoric acid. On reaction of methyl vinyl ketone with quebrachidine and reduction with sodium borohydride, a compound was obtained whose NMR spectrum in the aromatic region resembled 6-methyl-lilolidine more than 4-methyl-lilolidine.



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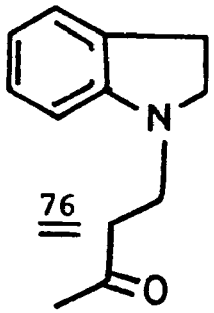

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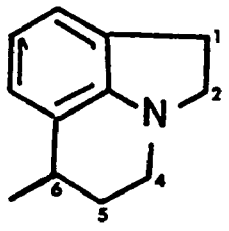

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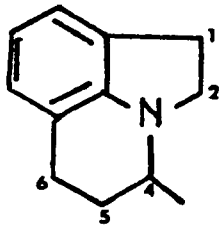

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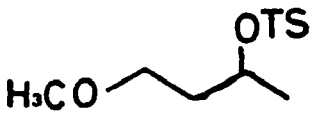

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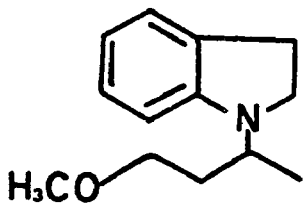
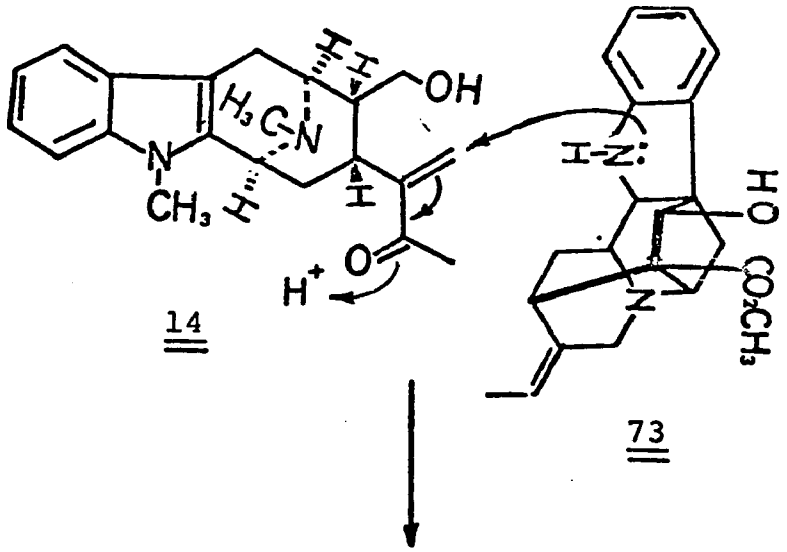


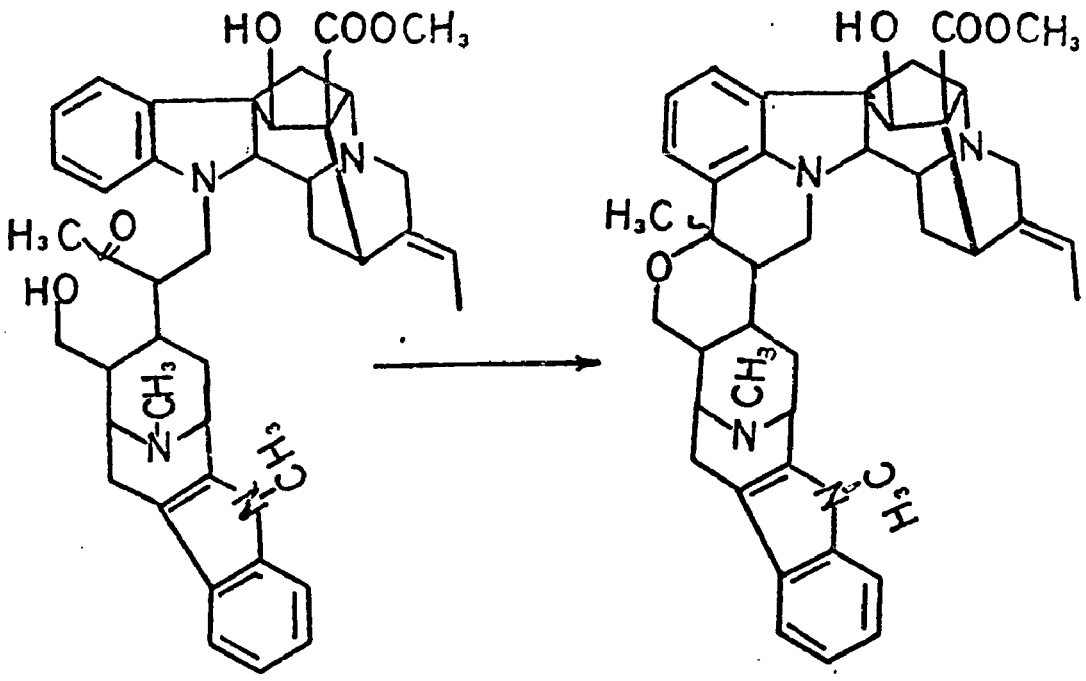
80

It thus became readily apparent that reaction scheme XIV would not be realized in the laboratory. Our goals then became to synthesize the isomer (21) of alstonisidine. This was not without interest in itself because the physiological properties of this "unnatural" dimer could be investigated and might show interesting results. In addition, since (21) as a possible structure for alstonisidine had not been unequivocally eliminated during the structure investigation of alstonisidine,[10] the properties of (21), if significantly different from natural alstonisidine, would further support the structural assignment of (72) to alstonisidine. Thus it was expected that reaction of quebrachidine (73) with macroline (14) would give the Michael-type adduct (81) which might be cyclized to (21) on treatment with acid (Scheme XV).

SCHEME XV



14 73



81 21

Synthesis of Alstonisidine

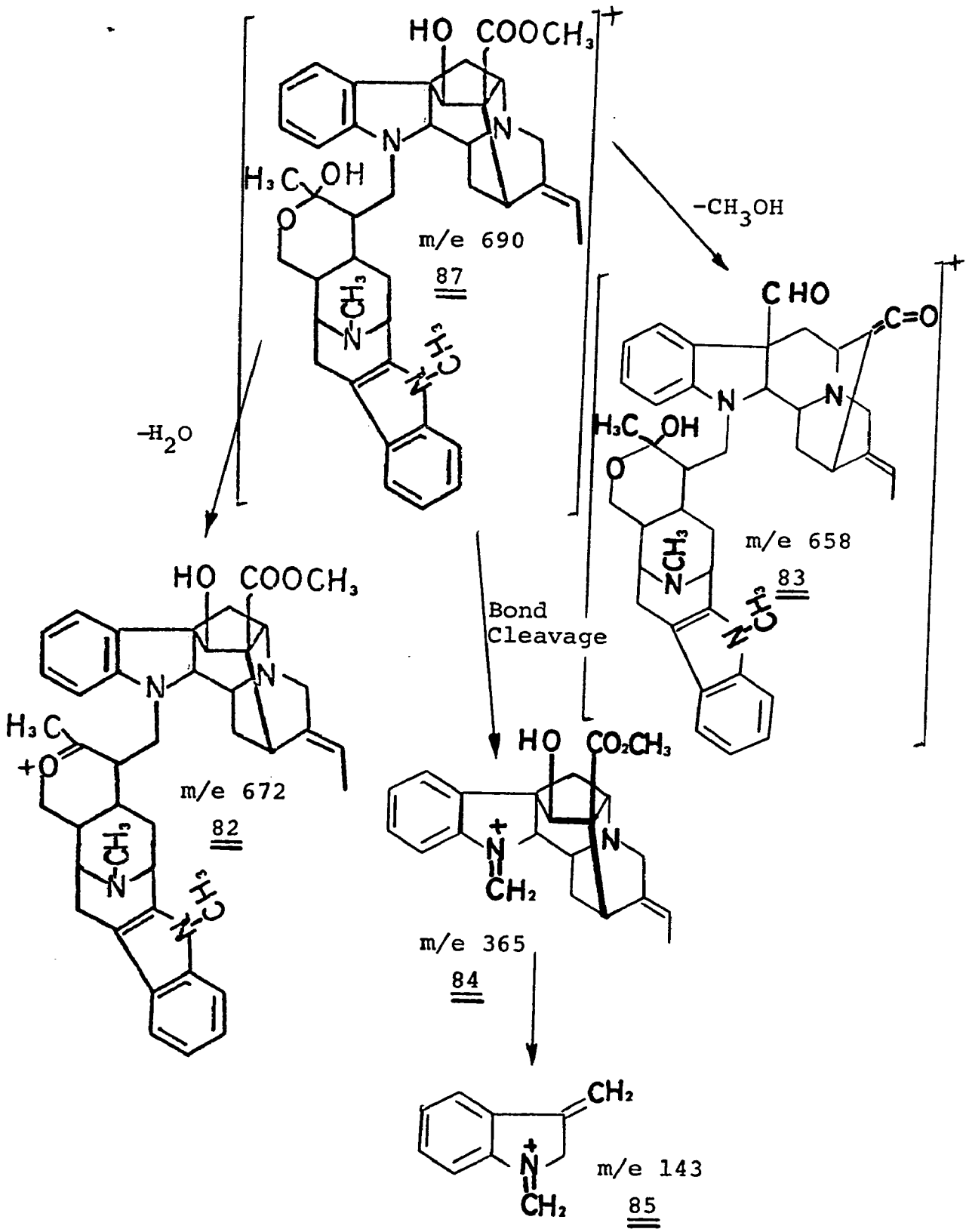
The Michael-type adduct was prepared by stirring macroline and quebrachidine together in 0.2 N aqueous hydrochloric acid at 20°C. Analysis (tlc) of small aliquots of the solution after a few hours showed the presence of three components: macroline, R_f 0.7, Ce(IV) spray bluish orange; quebrachidine, R_f 0.4, Ce(IV) spray, orange; and a new spot, R_f 0.32 Ce(IV) spray, reddish purple. Stirring was continued until tlc analysis showed the

absence of macroline. Macroline itself was found to remain unchanged even after several days of stirring in 0.2 N aqueous hydrochloric acid. The adduct was isolated after work up, by chromatography on silica gel preparative tlc plates using acetone as the developing solvent. The addition of quebrachidine to macroline was also tried in glacial acetic acid and in acetonitrile containing pyridine and pyridinium acetate. Both of these reaction media failed to give any additional product.

The adduct is a fairly labile compound and undergoes decomposition on heating and slowly during chromatography. For this reason and since the R_f of quebrachidine is not much greater than that of the adduct, the compound was not obtained absolutely free of quebrachidine, minute traces of this compound being detectable in the adduct by tlc.

The mass spectrum (Scheme XVI) has a molecular ion peak at m/e 690 ($C_{42}H_{50}N_4O_5$, mol. wt. 690). Loss of water gives the ion (82) at m/e 672, while loss of methanol[48] from the quebrachidine moiety gives the ion at m/e 658 (83). Another important peak is due to the ion (84) at m/e 365. This ion arises from cleavage of the carbon-carbon bond β to the indoline nitrogen in quebrachidine.

SCHEME XVI

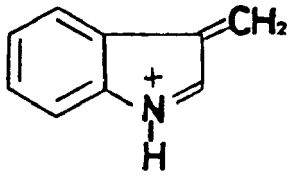

HO COOCH3
H3C OH
m/e 690
87
-CH3OH
-H2O
CHO
C=O
m/e 658
83
HO COOCH3
Bond
Cleavage
m/e 672
82
HO CO2CH3
m/e 365
84
CH2
m/e 143
85

Further decomposition of (84) gives rise to the ion at m/e 143 (85). This ion is the analog of (86), m/e 130, one of the principal ions observed in the mass spectrum of quebrachidine.[67] The largest peaks in the spectrum, occuring at m/e 352 and m/e 320, are to a small extent due to contamination of the sample with quebrachidine. It is significant, however, that a peak at m/e 338 (macroline) is present which indicates that the labile adduct is probably undergoing a retro Michael reaction on the hot inlet. The peaks below m/e 352 are generally characteristic of quebrachidine and macroline.

The I.R. of the adduct shows a carbonyl at 1735 cm^{-1} (ester) but none at ca. 1705 cm^{-1} that would indicate the presence of a ketone. A sharp band at 3440 cm^{-1} and a broad band at 3575 cm^{-1} indicated the presence of a non-bonded and bonded hydroxyl and the characteristic indoline band at 1610 cm^{-1} was present. This led to the conclusion that the compound was in the form of a hemiacetal (87), the ease of acetal formation being greatly enhanced when the carbonyl is no longer conjugated.

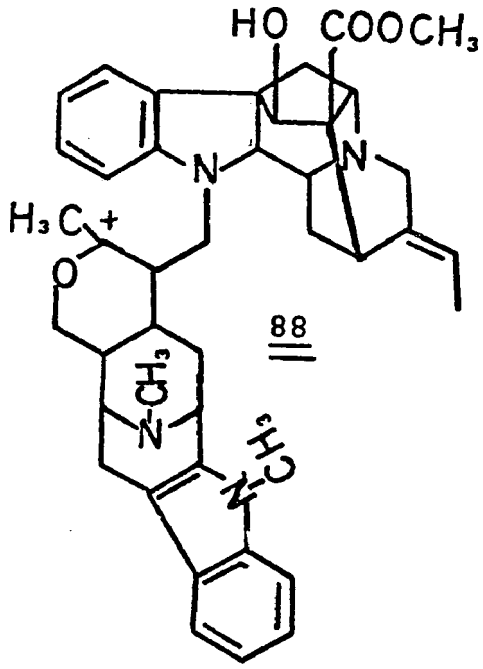
To effect alkylation on the aromatic ring, a reagent was needed that would cause the elimination of -OH from the hemiacetal forming a carbonium ion (88) which would attack the ring or a reagent which would coordinate with

hemiacetal so that an S_N2 type displacement by the aromatic ring would occur. The first attempt to effect cyclization was simply heating the adduct to approximately 80°C in 0.2 N aqueous hydrochloric acid. This, however, caused the decomposition of the molecule and no identifiable products were observed.



m/e 130

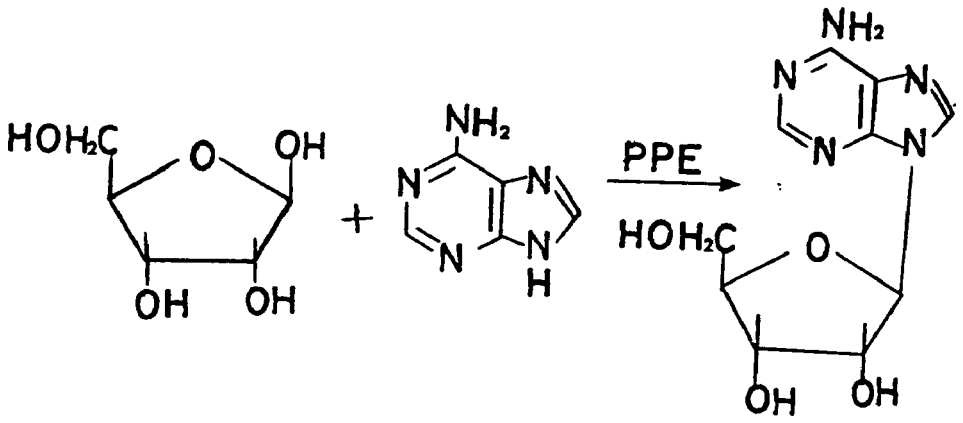
86



88

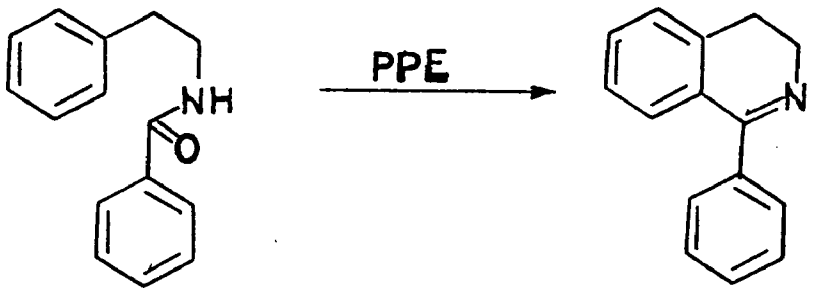
Another reagent tried was polyphosphate ester (PPE).[68] This reagent has found successful applications in nucleoside chemistry because of its selective action on the aldehyde function of sugars[69] (Scheme XVII). The reagent

SCHEME XVII



has also found success in effecting cyclizations in the Bischler-Napieralski reaction[70] (Scheme XVIII). The

SCHEME XVIII

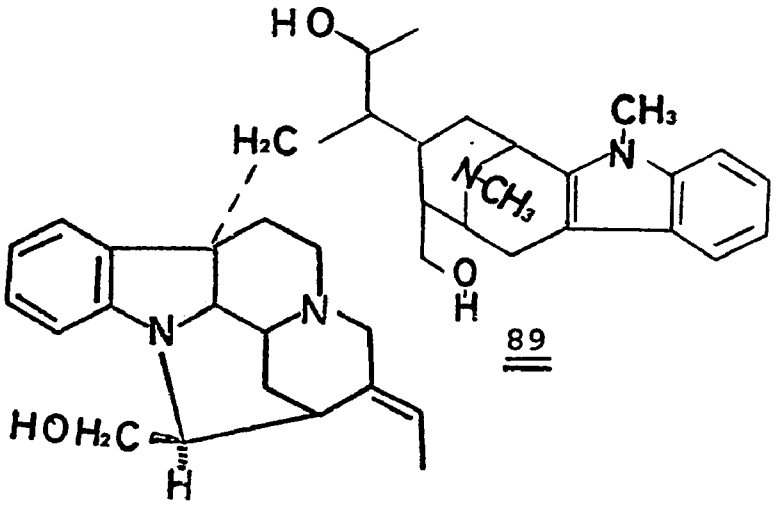


cyclization attempt was carried out by stirring a solution containing PPE for 18 hours at room temperature. When

the reaction was worked up, tlc showed only an orange spot due to quebrachidine plus small amounts of highly polar materials attributed to decomposition products.

A mild Lewis acid, zinc chloride, was also tried without success. After stirring an ethereal solution of the Michael adduct with excess zinc chloride for several hours at room temperature only starting material was detected after workup.

Success was finally achieved using boron trifluoride etherate to effect the cyclization. Stirring an ethereal solution of the Michael adduct with an excess of boron trifluoride etherate for seven hours at 0°C gives a single product having the same R_f and giving the same color (cherry red) with Ce(IV) spray as natural alstonisidine. While this phenomenon was quite surprising, it was thought at first to be merely coincidental because the two isomeric structures (21) and (72), differing only in the junction of the two "halves" of the alkaloid, might be expected to behave similarly in chromatography. However, thoughts of mere coincidence were quickly abandoned when the I.R. and 100 MHz N.M.R. spectra of the synthetic alkaloid were superimposible on those of natural alstonisidine. In addition the mass spectrum of the synthetic and natural alkaloid were virtually identical. With this new information some of the original structural proposals have to be revised.



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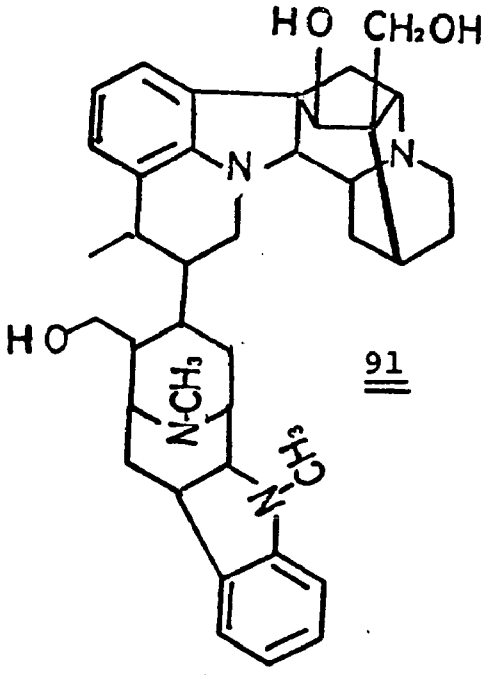
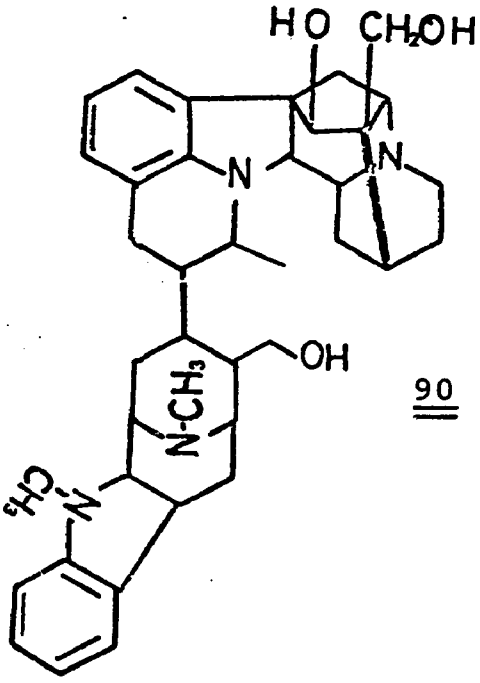


91

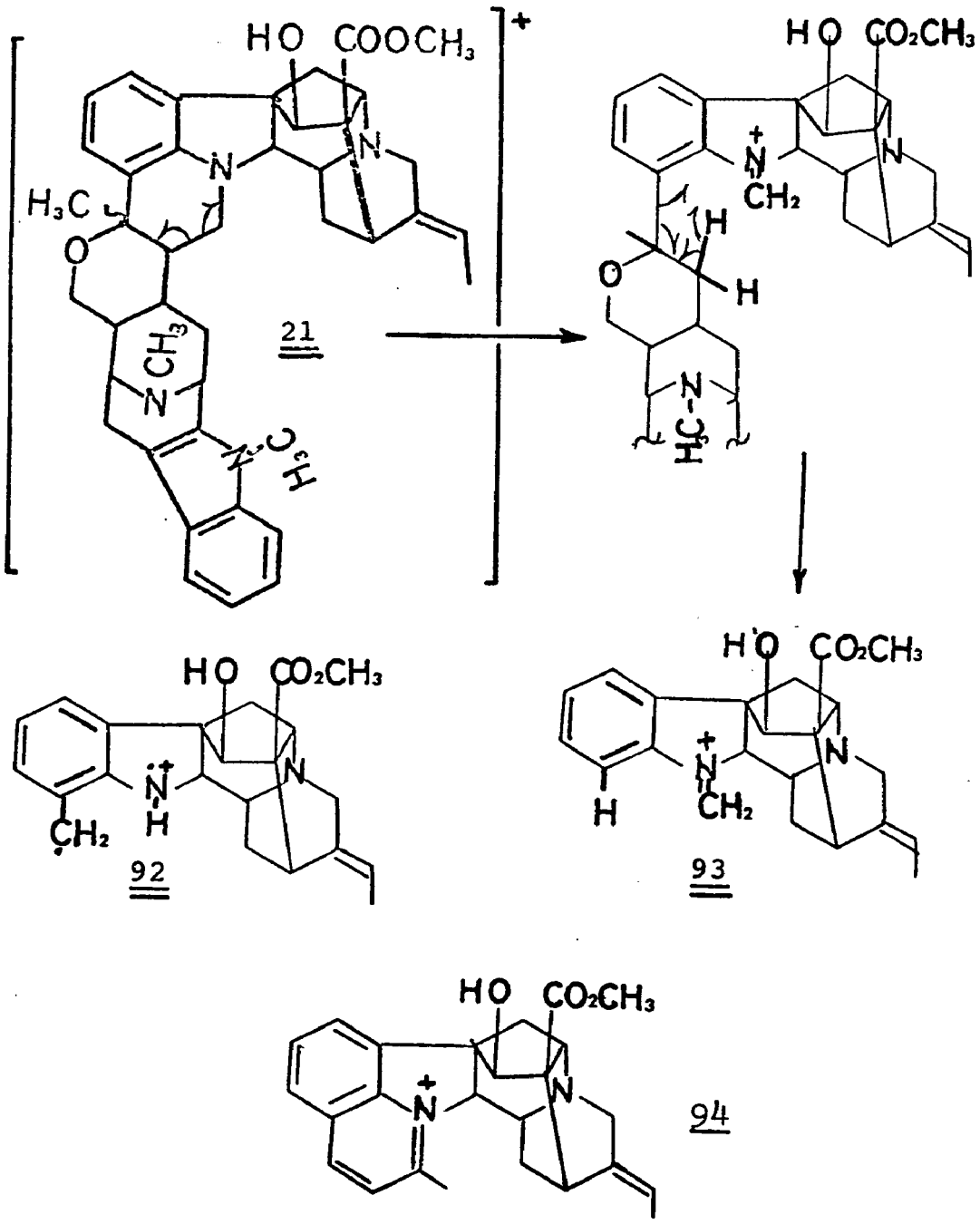


90

All of the experimental data obtained by Cook and used to assign the structure (72) for alstonisidine are also consonant with (21) for alstonisidine. The proof of an amino ketal in alstonisidine was based on an identical reaction used by Schmid[14] to prove the presence of an amino-acetal function in villalstonine. Vigorous reduction of villalstonine (lithium aluminum hydride in refluxing tetrahydrofuran) gave the triol (89) and under the same conditions alstonisidine also gave a triol assigned structure (90) by Cook.[10] However, under these conditions, tertiary benzyl ethers may also undergo hydrogenolysis.[72] Thus the triol (90) is reassigned structure (91). In addition, the structures of some of the mass spectral fragments and the pathways by which they arise must also be revised.

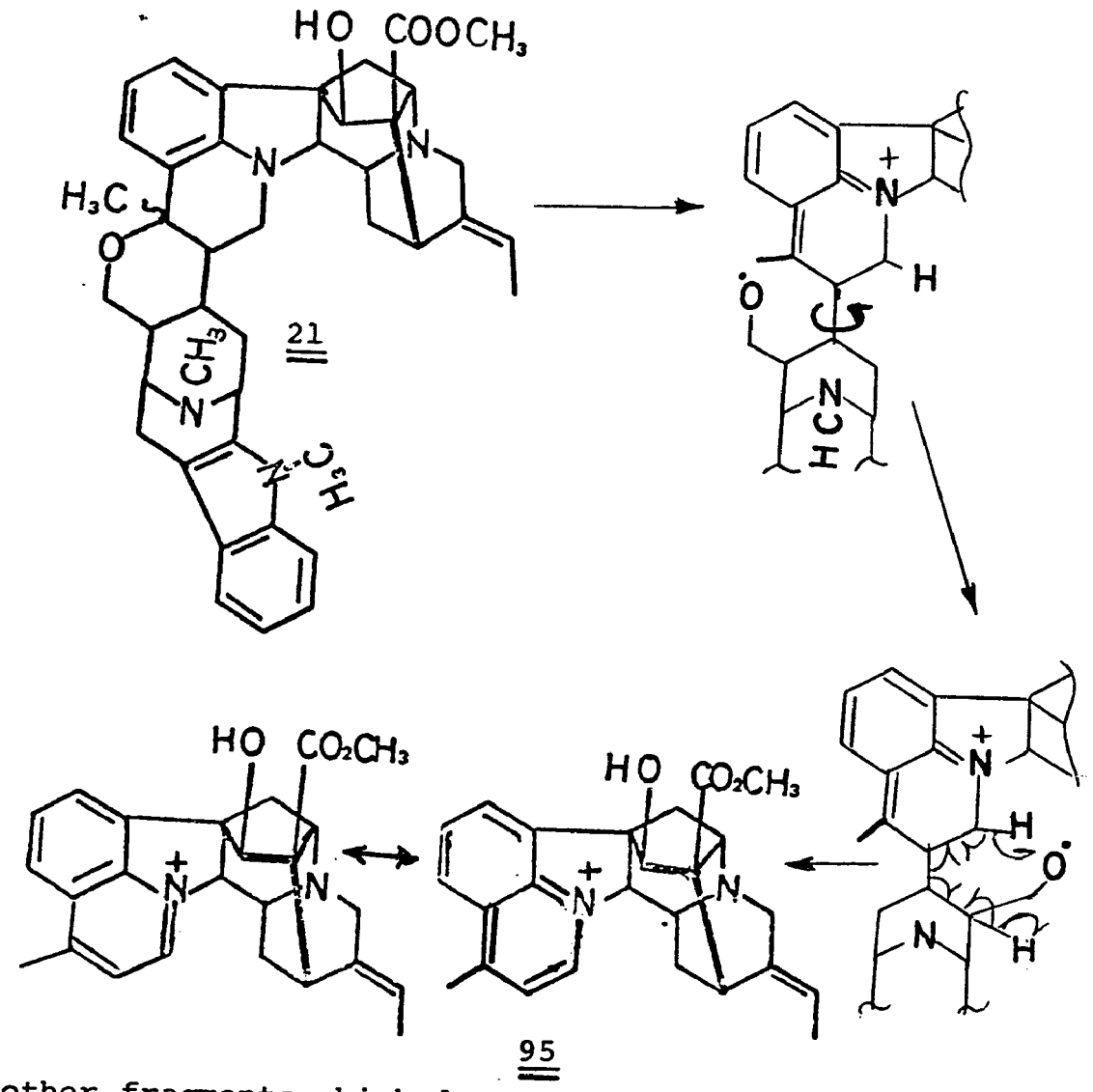
The ion at mass m/e 365 originally assigned structure (92) and proposed to arise from a retro Diels-Alder reaction is reassigned structure (93) and arises by bond cleavage and 1,3 hydrogen migration (Scheme XIX). The ion at m/e 403 previously assigned structure (94) is reassigned structure (95) and probably arises by initial cleavage of the benzyl ether carbon-oxygen bond in (21) to give (96). Bond rotation and hydrogen abstraction (Scheme XX) gives (95).

SCHEME XIX



The ion at m/e 450 previously assigned to (97) is reassigned to (98). The originally assigned structures of the

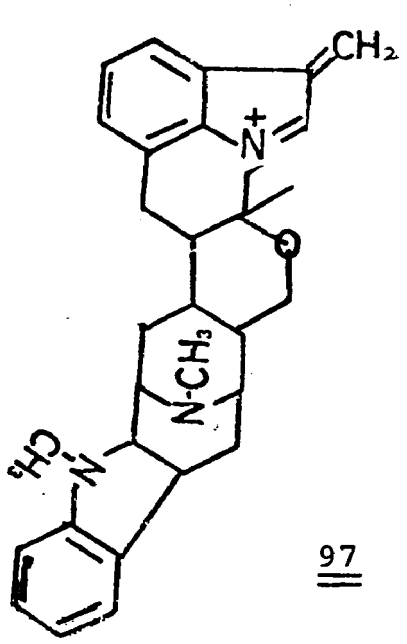
SCHEME XX



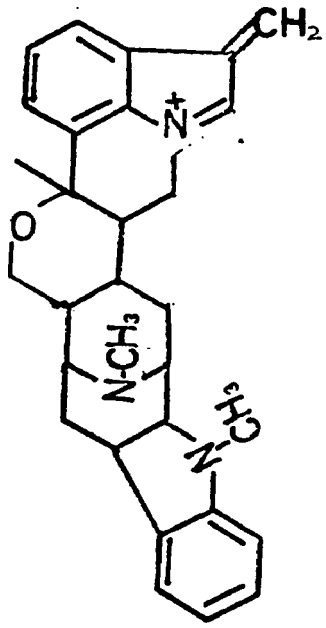
21 95

other fragments which do not contain the junction remain unchanged.

This synthesis constitutes proof of the structure of alstonisidine and proof of the stereochemistry at all but two of the asymmetric centers.



97



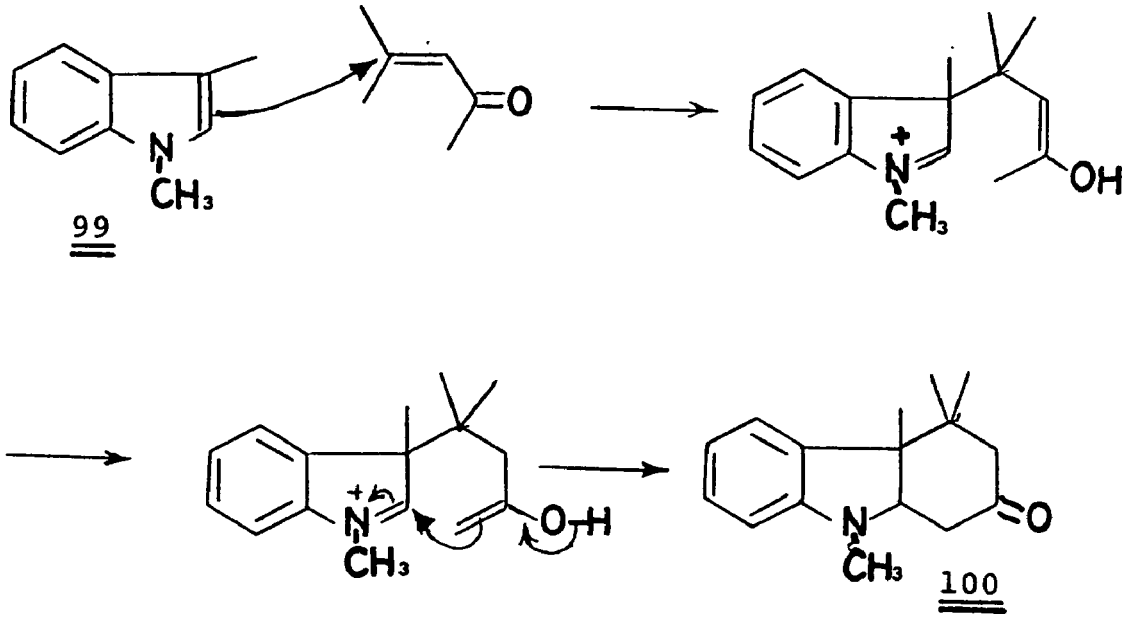
98

Synthesis of Villalstonine

With the successful synthesis of alstonisidine accomplished, it was decided to determine if the "dimerization" could be generalized to other "macroline"-containing bisindoles. A logical extension seemed to be to attempt the synthesis of villalstonine (19). This was done primarily for two reasons. First, the starting materials were readily obtained, and secondly, model-type reactions done by Robinson in 1955[73] suggested a good probability of success. When Robinson stirred N-methylskatole (99) with an excess of mesityl oxide in aqueous ethanol saturated

with hydrogen chloride, (100) was formed (Scheme XXI).

SCHEME XXI

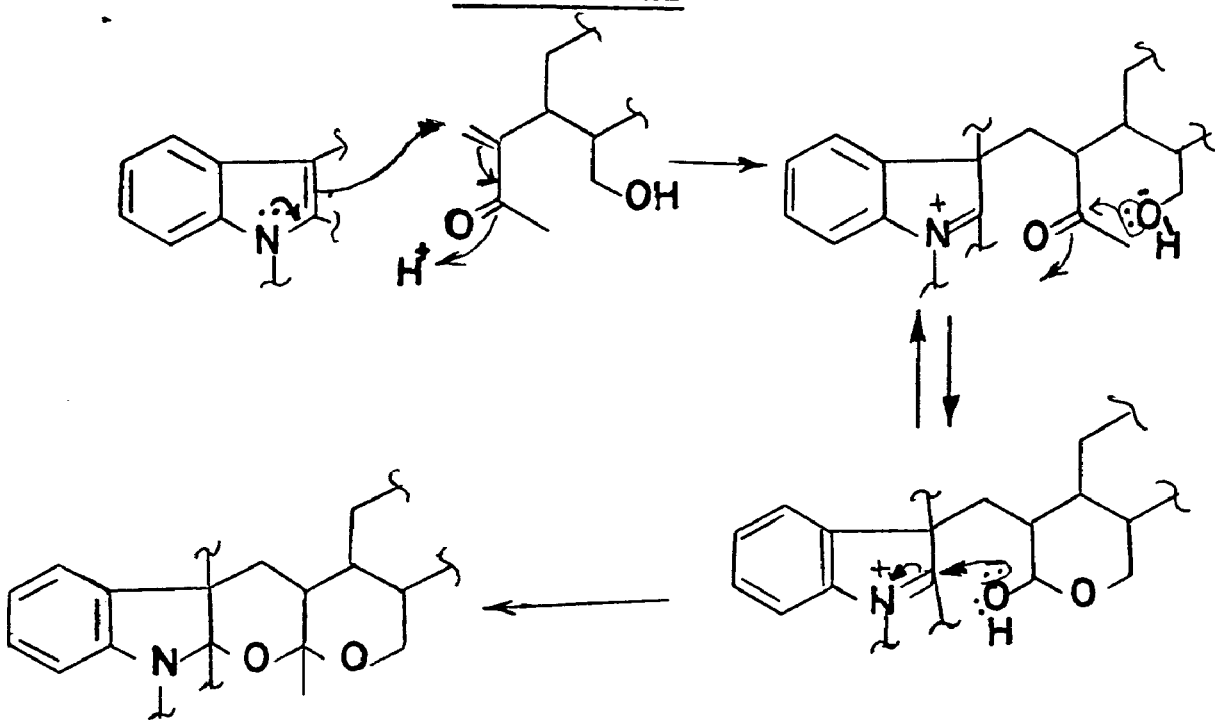


99 100

While the synthesis of villalstonine would not involve such a reaction of an enol with an iminium ion, it was felt, based on experience with the Michael adduct (87), that hemiacetal formation or the complete double cyclization would be faster than enolization and thus that no enol would be effectively available to attack the iminium species. A similar biogenetic route to the *in vivo* formation of villalstonine has been proposed by Schmid.[13] (Scheme XXII).

Pleiocarpamine (25) one half of the bisindole, was prepared from villalstonine itself by stirring in 70%

SCHEME XXII



perchloric acid as described by Schmid.[14] Only pleiocarpamine can be isolated from the reaction mixture, the macroline portion being completely destroyed. The attempted synthesis of villalstonine was carried out by simply stirring an aqueous 0.2 N hydrochloric acid solution of macroline and pleiocarpamine (slight molar excess). After eighteen hours at 20°C, analytical tlc of the reaction showed the presence of two spots; one with the same R_f as villalstonine and one corresponding to pleiocarpamine. No other products were observed. After work up, the recovered material was chromatographed on a two mm silica

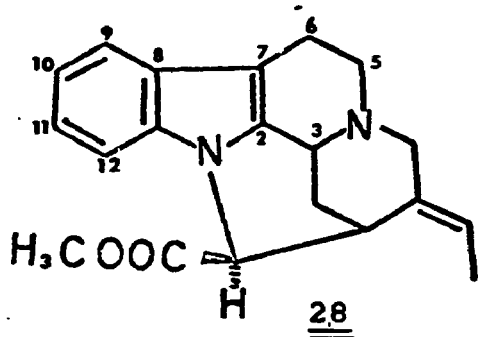
gel preparative tlc plate and developed with acetone. To ensure no contamination with pleiocarpamine, a narrow center portion of the band corresponding to villalstonine was removed and extracted with diethyl ether to give an amorphous solid. Crystallization of small amounts of villalstonine is quite difficult, as observed by other workers,[43] and attempts to crystallize the amorphous material were soon abandoned.

The I.R. of crystalline and amorphous villalstonine in KBr are different in that crystalline material shows two bands in the carbonyl region at 1775 cm^{-1} and 1705 cm^{-1} while amorphous material shows bands at 1775 cm^{-1} and 1735 cm^{-1}. The I.R. (KBr) and 100 MHz N.M.R. ($CDCl_3$) spectra of the synthetic material were identical in every respect with that of amorphous authentic villalstonine.

The $[\alpha]_D$ of the synthetic material, unfortunately, was only +57° ($CHCl_3$) while the $[\alpha]_D$ of crystalline authentic material was +79°. However, the low rotation is thought to be due to tenaciously held solvent in the amorphous matrix. To test this hypothesis, a sample of crystalline villalstonine was dissolved in a small amount of chloroform and placed under vacuum to rapidly remove the solvent. After three hours at 20°C, 0.01 Torr, the $[\alpha]_D$ of the amorphous solid was +67°. This indicates that chloroform is tenaciously held by amorphous villal-

stonine and that the low $[\alpha]_D$ of the synthetic material is due to solvent present in the sample. This solvent-holding property has also been observed by other workers.[43]

The synthesis of villalstonine involves the formation of four asymmetric centers and two rings. The fact that these centers are formed in the proper stereochemistry is probably due to the profound chirality of the reactants. Pleiocarpamine (28) is available for attack only on the α-face since access to the 2-7 bond is blocked from the upper or β-face (28a). Macroline (14) can attack only in one mode and the ring closes such that steric interactions are at a minimum. Macroline itself does not undergo dimerization because the 2-7 bond is sterically blocked to attack from both the upper and lower faces of the molecule.



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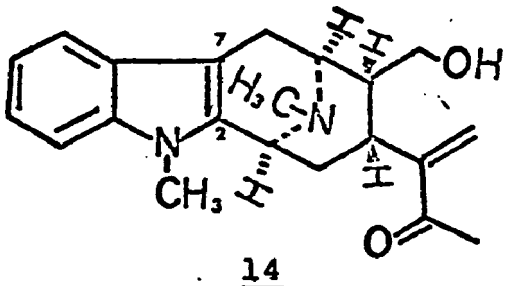
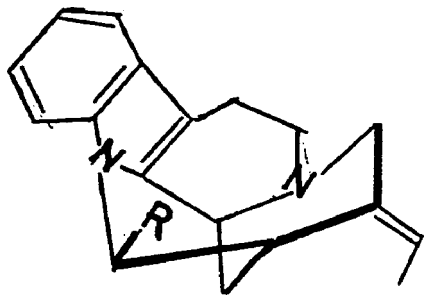


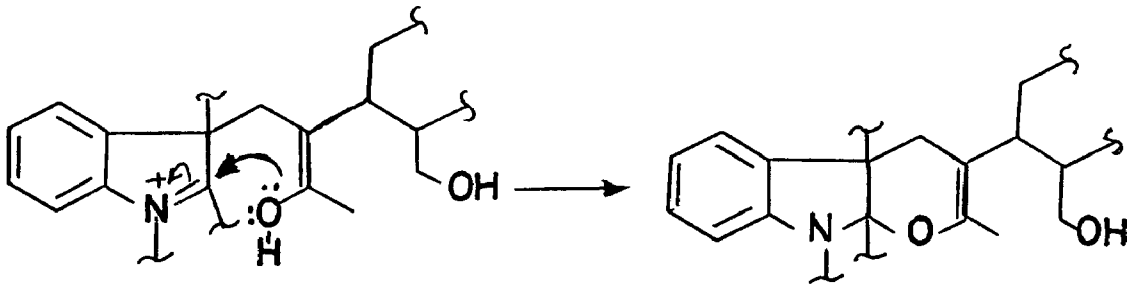
14



28a R= CO_2CH_3

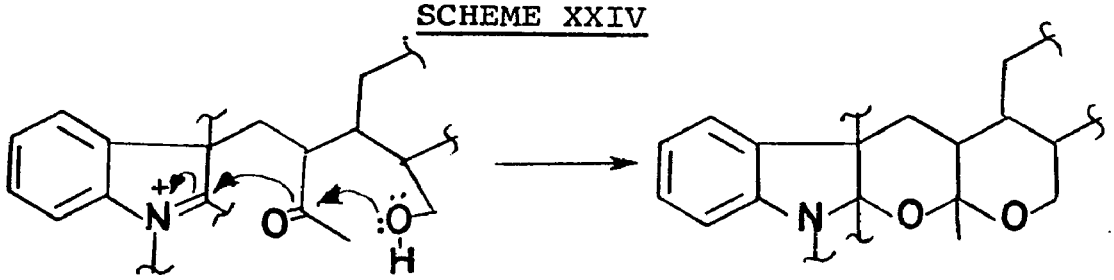
If the reaction between macroline and pleiocarpamine had taken place in the same sense as that between N-methyl skatole and mesityl oxide (Scheme XXI), villamine would have been detected in the reaction mixture since villamine does not cyclize to villalstonine under the reaction conditions. The fact that villamine is not formed during the reaction rules out step wise attack by the oxygen of the enol on the iminium (Scheme XXIII). Thus the cyclization step is either simultaneous with the attack on the carbonyl by the alcohol function (Scheme XXIV) or

SCHEME XXIII



involves a hemiacetal intermediate similar to that proposed by Schmid.

SCHEME XXIV

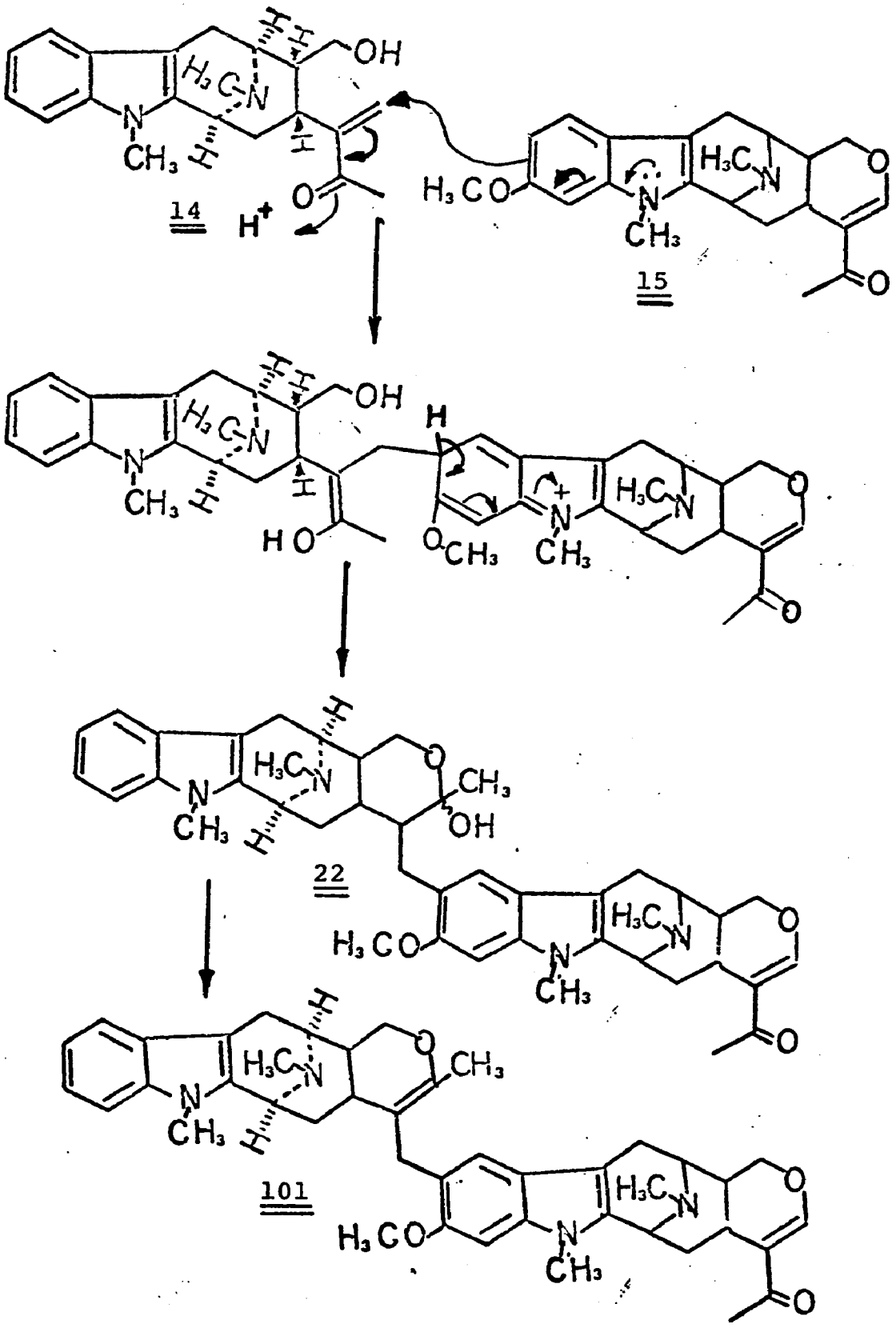


To further extend this type of synthesis, we investigated the synthesis of macralstonine (22), an alkaloid first reported by Sharp[43] and whose structure was elucidated by Schmid.[45] Unfortunately, due to insufficient amounts of starting materials, only preliminary results have been obtained thus far.

Macralstonine is thought to arise by attack of C-10 of alstophylline (15) on the enone moiety of macroline (14). (Scheme XXV). This position, being para to amine and ortho to a methoxy, is especially activated. Alstophylline, formally a functionalized macroline, does not undergo reaction at C-7 in the same sense as pleiocarpamine because, like macroline, the 2,7 bond is sterically blocked.

A slight excess of alstophylline was stirred with macroline in 0.2 N aqueous hydrochloric acid for forty-eight hours. After work up of the reaction, analytical tlc showed two spots; one with the same R_f as alstophylline and the other the same R_f as macralstonine. The reaction products were chromatographed on a 0.5 mm silica gel preparative tlc plate and the band at R_f 0.15 was excised and extracted with ether. The mass spectrum of the sample did not show a peak at m/e 704, the molecular ion of macralstonine, but had an intense peak at m/e 682 ($M^+ - H_2O$) due to anhydromacralstonine (101). The remainder of

SCHEME XXV



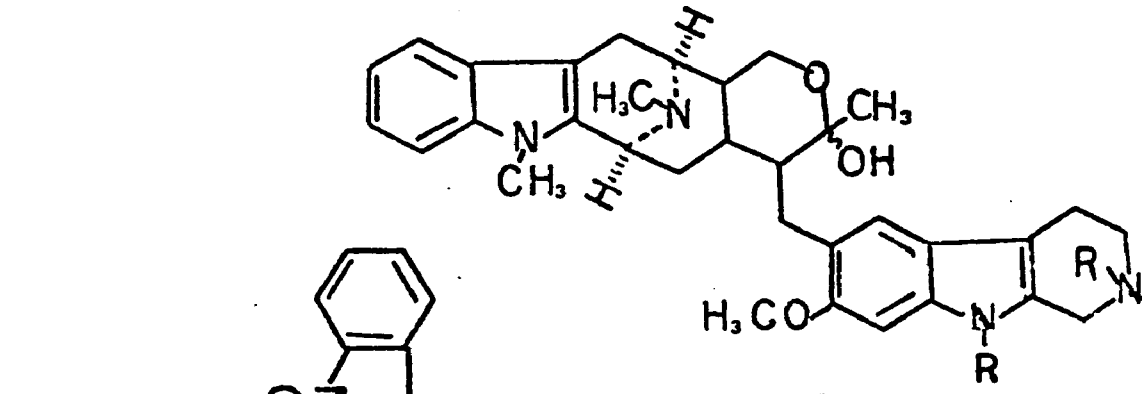
the mass spectrum was almost identical with that of authentic macralstonine except for three peaks at m/e 640, 440 and 429. The origin of these peaks is unknown and may be due to contamination of the sample. Authentic macralstonine also did not give a molecular ion when run under the same conditions as the synthetic sample. The absence of a molecular ion is probably due to the conditions under which the sample was run.

The synthesis of macralstonine, obviously, must be further studied and the product more thoroughly characterized. Before this can be done, however, additional alstophylline must be obtained from some source. Two possibilities exist; the first is the procurement of A. macrophylla bark and extraction of the alstophylline. The second and more immediately available is the degradation of macralstonine itself,[45] small amounts of which are available from Professor Elderfield's investigations, to alstophylline. The degradation of macralstonine can be accomplished by stirring in perchloric acid at room temperature, a procedure similar to the degradation of villalstonine to pleiocarpamine.

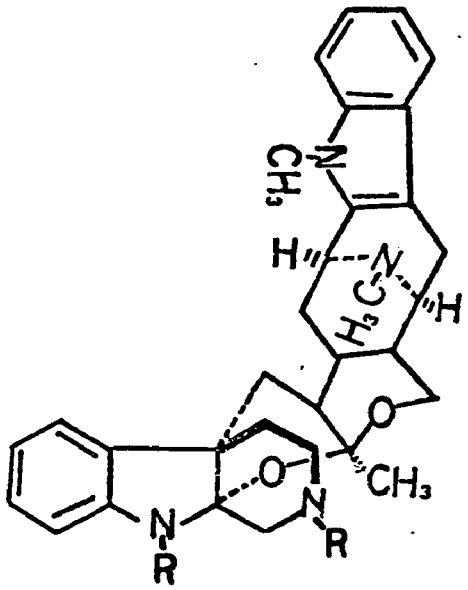
Conclusions and Further Work

It is obvious, based on this work and the results of others outlined in the introduction, that certain bisindole alkaloids can be synthesized in vitro without the necessity of enzymes. The syntheses discussed in this work are the first of the Alstonia bisindoles to be synthesized and also seems likely to be the actual pathway followed in the plant.

Macralstonine has been shown to have potent hypotensive activity,[71] and thus the preliminary indications that macralstonine can be synthesized are most encouraging for future work. For example, it would be interesting to determine whether the activity of macralstonine is due to the uniqueness of its whole structure or due to macroline and the type of juncture between the monomers. Simpler derivatives using macroline could be made in which macroline has been added to the 10 position of 11-methoxy hexahydro-β-carboline derivatives to give a macralstonine-like junction (102). Macroline could also be added to the 2,7 position of tetrahydro-β-carboline to give a villalstonine-like union (103).

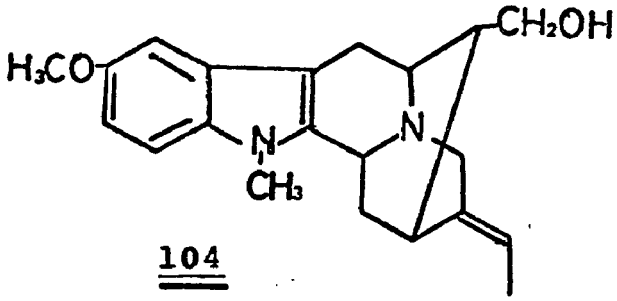


102

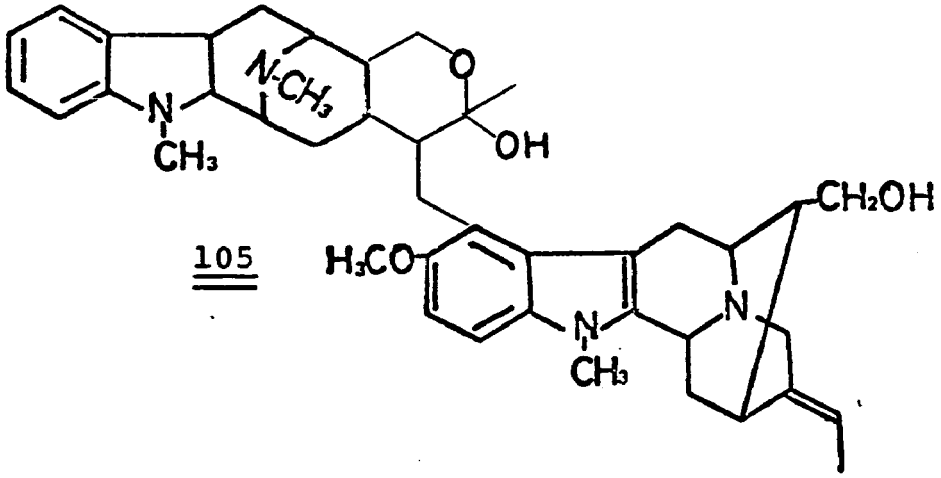


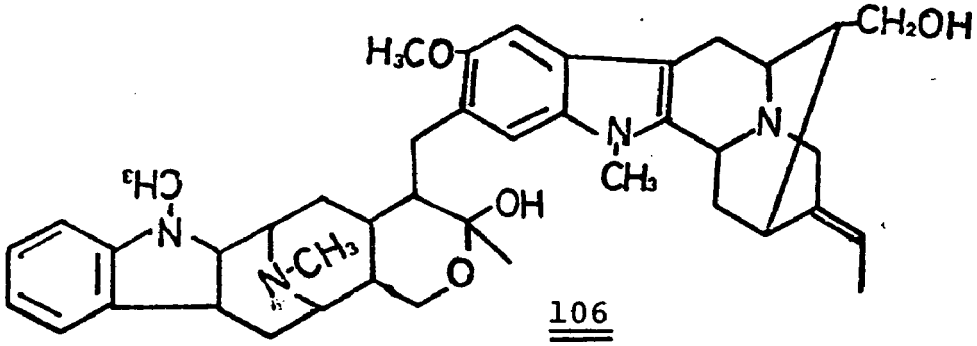
103

A particularly interesting synthesis would be the reaction of macroline with 0-methyl-N_a-methyl sarpagine (104) to give (105) and/or (106). These bis indoles from two naturally occuring indoles have not been reported as yet but there is no reason not to expect their existence. The 0-methyl-N_a-methyl sarpagine can be obtained by methylating sarpagine, a degradation product of macralstonidine. In addition, the synthesis of macralstonidine (20) itself should be attempted as a logical extension of the synthesis of villalstonine and macralstonine.


H3CO
CH2OH
N
CH3
N

104


N-CH3
O
N
CH3
OH
105
H3CO
CH2OH
N
N
CH3


H3CO
CH2OH
N
N
CH3
OH
N-CH3
O
106

CHAPTER III

EXPERIMENTAL

Extraction of the Alkaloids of Alstonia muelleriana.[10,40] The bark (220 lb.) was mixed with heptane (120 l.) and allowed to soak for six days. The heptane was withdrawn from the bottom of the pot through glass wool. The heptane extract was then concentrated to a small volume. The bark was then soaked, with occasional warming and mixing, in methanol (80 l.) for five days. The methanol (actually a two phase system at this point containing some residual water and heptane) was concentrated to about 8 liters and transferred to gallon bottles. The concentrate weighed approximately thirty-five pounds.

Separation of the Alkaloids from the Nonbasic Components of the Bark Concentrate.[45] To a solution of the crude concentrated extract (300 g.) in methanol (1200 ml.) was added 0.018 N H_2SO_4 (2 l.) and the mixture stirred. The precipitate that formed was removed by filtration through Celite with the aid of water aspirator vacuum. The filtrate was divided into two parts and each made basic (pH 10) with aqueous ammonia and extracted with chloroform (3 x 500 ml.). The chloroform extracts were

combined, dried ($MgSO_4$) and evaporated to dryness to give a brown foam (70 g.).

Preliminary Column Chromatography of the Crude Alkaloids Obtained from the Above Experiment. A solution of the crude alkaloid foam (50 g.) in chloroform (75 ml.) was placed on a column of Merck alumina (450 g. directly from bottle) packed in chloroform. The column was eluted with chloroform to give the following results. The R_fs were determined by analytical tlc on Eastman silica-gel Chromatogram sheets using acetone as a developing solvent. The developed chromatograms were visualized by spraying with a solution of 50% vol/vol aqueous sulfuric acid saturated with ceric sulfate and the color reactions (CR) noted.

Fractions 1-3 (100 ml. each). No alkaloids present.

Fraction 4 (100 ml.), wt. 3.9 g. Alkaloids present: villalstonine, R_f 0.4, CR violet; alstonerine, R_f 0.49, CR blue-violet; alstonisine, R_f 0.57, CR blue-violet.

Fraction 5 (100 ml.), wt. 10.4 g. Alkaloid present: unknown, R_f 0.2, CR violet; villalstonine, R_f 0.42; alstonerine R_f 0.49; alstonisine, R_f 0.57.

Fraction 6 (100 ml.), wt. 8.1 g. Alkaloids present: unknown, R_f 0.076, CR violet; alkaloid K, R_f 0.11, CR turquoise-blue; unknown, R_f 0.2; villalstonine, R_f 0.42; alstonisidine, R_f 0.43 CR cherry-red; alstonerine, R_f 0.49; alstonisine, R_f 0.57.

Fraction 7 (100 ml.), wt. 4.7 g. Alkaloids present: unknown, R_f 0.076; alkaloid K, R_f 0.11; unknown R_f 0.20; villalstonine R_f 0.42; alstonisidine R_f 0.43; alstonerine R_f 0.49; alstonisine R_f 0.57.

Fraction 8 (100 ml.), wt. 2.2 g. Alkaloids present: Same as fraction 7.

Fraction 9 (100 ml.), wt. 1.4 g. Alkaloids present: unknown R_f 0.076; alkaloid K, R_f 0.11; unknown R_f 0.19; villalstonine, R_f 0.38; alstonisidine, R_f 0.42.

Fraction 10 (200 ml.), wt. 1.6 g. Alkaloids present: alkaloid K, R_f 0.11; villalstonine, R_f 0.30.

Fraction 11 (200 ml.), wt. 0.9 g. Alkaloids present: alkaloid K, R_f 0.11, villalstonine, R_f 0.27.

Fractions 12 and 13 (200 ml. each), wt. 0.3 g. Alkaloids present: highly polar material that remains at the origin, CR violet.

Total weight of alkaloids obtained was 33.5 g.

The apparent gradual variation in the R_f's quoted for villalstonine arises because villalstonine tends to overload on tlc in large concentrations. Its preponderance in the extract causes this effect.

This chromatographic procedure removes much unidentified non-alkaloidal material from the extract and enables the

separation of the alkaloids to be conducted efficiently as described below. The non-alkaloidal material is highly polar and can be washed off the column with methanol to give an intractable gum.

Isolation of Villalstonine (19). The dried alkaloid residue (4.0 g.) obtained from the above preliminary column chromatography was placed on a column of Merck alumina (75 g. heated at 200° for 12 hours) packed in benzene. The column was eluted in 40 ml. fractions with benzene, 600 ml.; 10% chloroform/benzene, 100 ml.; 20% chloroform/benzene, 100 ml.; 30% chloroform/benzene, 100 ml.; and 40% chloroform/benzene, 100 ml. The fractions containing only villalstonine (Nos. 3-37) as judged by analytical tlc (silica-gel, acetone developed) were combined and evaporated down to give 2.8 g. of villalstonine as an amorphous foam. Dissolving the amorphous foam in a minimum of hot acetone and subsequent cooling gave crystalline villalstonine whose properties were identical with those previously recorded.[14,40]

Beginning with fraction 38, when the other alkaloids present began to elute as judged by analytical tlc, the solvent was changed to 100% chloroform and the remaining alkaloids along with a small amount of villalstonine were

washed from the column. The fractions from the chloroform elution were combined and evaporated down to give a tan foam (1.2 g.).

Amorphous villalstonine gradually turns pink at room temperature; but in its crystalline form stays pure white indefinitely, especially when kept at 5°.

Preparation of Villalstonine Dihydrochloride. To 20 g. of villalstonine in diethyl ether (150 ml.) diethyl ether saturated with anhydrous hydrogen chloride was added drop wise until precipitation of the dihydrochloride was complete. The solvent was removed under vacuum and the precipitate further air dried to give a white powder (22 g.) which slowly turned brown after several days exposure to air.

Preparation of Villamine (27). Trifluoroacetic acid (2 ml.) was added during 2 minutes with stirring to a solution of villalstonine dihydrochloride (1.0 g.) in trifluoroacetic anhydride (13 ml.) at 20°. After stirring for 10 minutes more, solvents were removed under reduced pressure at 20°. Crushed ice and excess ammonia were then added and the mixture extracted with chloroform. The organic layer was dried (potassium carbonate) and concentrated. The residue was chromatographed on silica gel

(Woelm, activity IV, 13 g) and the column eluted with 1% methanol in chloroform. Analytical tlc (SiO_2 gel, acetone) disclosed three components: unchanged villalstonine (R_f 0.36 Ce(IV) purple), villamine (R_f 0.32, Ce(IV) purple), and villoin (R_f 0.15, Ce(IV) purple).[13] which readily reverted to villalstonine on heating or prolonged column chromatography. Those fractions containing mostly villamine were carefully crystallized from the minimum amount of hot acetone: 200 mg. villamine with properties identical in every respect with those recorded,[13] were obtained.

Preparation of Macroline (14). Villamine (510 mg) was dissolved in a little chloroform and by evaporation under N_2 was distributed equally as a film on the inside walls of 38 Pyrex test-tubes (10 cm x 13 mm), which had previously been drawn out to give a lower bulb 4 cm long to contain the villamine. The tubes were evacuated to 0.01 mm Hg, sealed off, and placed in batches for 90 sec. in an oil bath maintained carefully at 245-250°. After cooling the tubes were broken open and the contents washed out with chloroform. The product was then chromatographed on a 2 mm. silica gel preparative tlc plate, and developed twice with acetone. The macroline band (R_f 0.7; Ce(IV) pinkish-blue) was excised and the adsorbent extracted at 20° with acetone. Concentration of the acetone extract

gave macroline (14), recrystallized from ether: mp 207-210° (lit.13, mp. 211-213°) (126 mg; 48%), of identical properties to those described.[13]

Attempted Formation of the Michael Adduct (87) in Glacial Acetic Acid. Macroline (5 mg.) and quebrachidine were stirred in glacial acetic acid (1.0 ml.) for 18 hours at 20°C. The solution was diluted with water, made basic with aqueous ammonia solution and extracted with chloroform. Analytical tlc of the dried (K_2CO_3) extract showed only macroline (R_f 0.7 CR, pinkish-blue) and quebrachidine (R_f 0.42, CR, orange).

Attempted Formation of the Michael Adduct (87) in Acetonitrile and Pyridine. Macroline (5 mg.) and quebrachidine (8 mg.) were dissolved in 2 mls. of acetonitrile. Pyridine (5 dps.) and glacial acetic acid (2 dps.) were added and the solution stirred for 18 hours at 20°C. The solution was concentrated under vacuum to a gum and partitioned between water and chloroform. Analytical tlc of the dried (K_2CO_3) chloroform layer showed only the presence of macroline and quebrachidine.

Synthesis of the Michael Adduct (87). Quebrachidine (73) (52 mg.) was dissolved in 0.2 N hydrochloric acid

(5 ml.), and macroline (14) (39 mg.) added. After 72 hours stirring at 20°C, the mixture was basified with ammonia and extracted with chloroform. The chloroform layer was quickly dried (potassium carbonate), concentrated, and chromatographed on a 2 mm preparative SiO_2 gel plate (3 developments with acetone). Three bands were seen; two from quebrachidine and macroline, and one very polar one. The most polar band (R_f 0.3, Ce(IV), reddish-violet) was excised, powdered, and extracted at 20° with acetone-methanol (1:1) to give the adduct (87) (35 mg.) as a foam: ir ν^{KBr} 3570, 3420 (O-H), 1735 cm^{-1} ester C=O; mass spectrum M^+ m/e 690 (calcd. for $C_{42}H_{50}N_4O_5$ mol. wt. 690); the spectrum run by direct inlet at 250° and 20 e.v. gave peaks at m/e 692(7), 691(25), 690(M^+, 31), 674(4), 673(13), 672(25), 659(3), 658(3), 647(1), 481(3), 480(2), 383(2), 381(4), 368(4), 367(4), 366(20), 365(74), 364(28), 354(4), 353(25), 352(100), 340(3), 339(8), 338(13), 326(3), 325(4), 324(2), 323(6), 322(25), 321(100), 320(17), 316(3), 309(6), 308(8), 307(5), 277(3), 275(3), 270(4), 265(2), 264(2), 263(2), 262(3), 261(2), 260(2), 257(2), 251(5), 247(4), 246(15), 239(4), 238(2), 237(2), 236(3), 229(3), 226(3), 225(3), 224(3), 223(5), 222(33), 221(5), 211(2), 210(5), 209(3), 208(3), 207(2), 199(9), 198(10), 197(22), 190(16), 182(8), 181(20), 171(3), 170(5), 162(4), 158(4), 157(4). The adduct gradually decomposed to macroline and quebrachi-

dine on storage, on treatment with more concentrated aqueous hydrochloric acid, and on tlc alone (acetone-silica gel).

Preparation of Polyphosphate Ester (PPE).[68] Phosphorus pentoxide (3 g.) was added to chloroform (3 ml.) and diethyl ether (6 ml.) and refluxed for 18 hours. A clear viscous solution was obtained. This solution was used directly in the cyclization reactions.

Attempted Cyclization of the Michael Adduct (87) with Polyphosphate Ester (PPE). The Michael adduct (2.4 mg.) was dissolved in 1 ml. of chloroform and 1 ml. of the PPE solution was added. The mixture was stirred for 18 hours at room temperature. The reaction was diluted with water and made basic with aqueous ammonia solution. The mixture was shaken in a separatory funnel until the excess PPE was hydrolyzed. The chloroform layer was removed and dried (K_2CO_3). Analytical tlc showed an orange spot with the same R_f as quebrachidine (0.32) and a small purple spot that remained at the origin.

Attempted Cyclization of the Michael Adduct (87) with Zinc Chloride. The Michael adduct (10 mg.) was dissolved in diethyl ether (3 ml.). On addition of zinc chloride

(5 mg.) a white precipitate formed. After stirring for 8 hours at 20°C, the reaction was diluted with water, made basic with aqueous ammonia and extracted with chloroform. Analytical tlc of the dried (K_2CO_3) chloroform layer showed only the presence of starting material.

Cyclization of the Michael Adduct (87) to Alstonisidine (21). The Michael adduct (14) (50 mg) was dissolved in ether (10 ml.) at 0° and freshly distilled boron trifluoride etherate (1.0 ml.), and the mixture stirred for 7 hours. Workup as above gave a product which analytical tlc (silica gel, acetone) showed to be alstonisidine, with only traces of other unidentified components. This product was chromatographed on a 2 mm preparative silica gel plate (acetone, two developments) and the alstonisidine-containing band excised and extracted (Soxhlet) with ethyl acetate to give pure alstonisidine as a powdery solid (34 mg.): R_f 0.47 (silica gel, acetone) 0.53, (silica gel, benzene 7.75: ethyl acetate 2: diethylamine 0.25), with a cherry-red color with Ce(IV); this behavior was identical with that of authentic alstonisidine. The infrared (KBr) and 100 MHz nmr spectra of the synthetic and authentic alkaloids were superimposable, and the mass spectra (MS-902, run sequentially) were identical; $[\alpha]_D$ -144° (c, 0.335 in $CHCl_3$) for the synthetic sample; for authentic base $[\alpha]_D$

$-133°$ (c, 0.208 in $CHCl_3$).

Attempted Reaction of Macroline (14) and Quebrachidine (73) with Boron Trifluoride-Ethyl Ether. Macroline (3 mg.) and quebrachidine (5 mg.) were mixed in a solution of freshly distilled boron trifluoride etherate (0.1 ml.) and ether (1 ml.) at 0°. After stirring for 6 hours, the mixture was worked up *via* ammonia basification and chloroform extraction. Analytical tlc (silica gel, acetone) showed only the starting alkaloids; no other compounds were detected.

Test for Stability of Macroline under Acid Conditions. Macroline (5 mg.) was dissolved in 0.2 N HCl and allowed to stand at 20-25°C. After 24 hours half of the solution was removed, basified with aqueous ammonia to pH 10, extracted with chloroform and dried (K_2CO_3). The residue obtained was identical with macroline.

After 48 hours at 20-25°C, the remainder of the solution was worked up as described above. Analytical tlc showed one spot with identical R_f and color reaction with Ce(IV) spray as macroline. The infrared spectrum of the residue was identical with that of macroline.

Synthesis of Villalstonine (19). Macroline (33 mg.) was added to a solution of pleiocarpamine (37 mg) in o.2 N

aqueous hydrochloric acid (3 ml.) and the mixture stirred at 20° for 18 hours. After basification with ammonia the solution was worked up as above. Analytical tlc (silica gel, acetone) showed that all the macroline had been consumed and that only pleiocarpamine (R_f 0.25; Ce(IV) violet) and villalstonine (R_f 0.39) were present. Villalstonine was isolated by preparative layer chromatography on half a 2 mm silica gel plate (acetone as developing solvent). A narrow band containing pure villalstonine was excised and extracted (Soxhlet) with ethyl acetate. The extract on concentration gave pure villalstonine (25 mg., 38%). The villalstonine obtained was amorphous. It was chromatographically indistinguishable from authentic material in the two systems employed for alstonisidine. (R_f 0.63 in benzene 7.75: ethyl acetate 2: diethylamine 0.25, SiO_2 gel). Its 100 MHz nmr spectrum was superimposable on that of the authentic material. It had the characteristic ir spectrum (KBr) of amorphous villalstonine,[40] and its spectrum was superimposable on that of a sample of amorphous base prepared by rapid evaporation in vacuo at 20° of a freshly-prepared chloroform solution of crystalline authentic base. The synthetic sample has an $[\alpha]_D$ + 59°, lit.[14] + 79°. The amorphous base likely contains tenaciously held solvent; this is reflected in the $[\alpha]_D$ + 79° ($CHCl_3$) of crystalline material, while the authentic amorphous base, prepared as above and main-

tained in vacuo at 20° for 3 hours had $[\alpha]_D$ +67° ($CHCl_3$).

Attempted Cyclization of Villamine (27) to Villalstonine (19). Villamine (3 mg.) was dissolved in 0.2 N HCl (3 ml.) and stirred for 8 hours. Analytical tlc showed only the presence of unchanged villamine and no trace of villalstonine.

Reaction of Alstophylline with Macroline to form Macralstonine (22). Macroline (21 mg.) and alstophylline (15) (23 mg.) were dissolved in 0.2 N HCl (3 ml.) and stirred for 48 hours at 20°. The solution was diluted with water, made basic with aqueous ammonia and extracted with chloroform. The dried (K_2CO_3) chloroform layer was concentrated, placed on two 0.5 mm silica gel tlc plates and developed twice in acetone. The major band at R_f 0.15 was removed and extracted with ethyl ether (Soxhlet) to give 20.9 mg. of amorphous gummy material. The synthetic product was chromatographically identical with natural macralstonine on silica gel tlc in two solvent systems: acetone (R_f 0.53) and benzene/ethyl acetate/diethyl ether, 7.75/2/0.25, (R_f 0.45). The mass spectrum run by direct inlet at 275° and 20 e.v. gave peaks at m/e 688 (8 x 0.1), 687(23 x 0.1), 686(58 x 0.1), 673(5), 622(12), 671(10), 658(6), 657(4), 656(8), 655(5), 641(7 x 0.1), 640(15 x 0.1), 626(4), 625(4), 618(6), 617(15), 616(8), 577(4), 572(3),

571(8), 570(2), 562(3), 548(9), 547(20), 528(3), 521(3), 520(7), 503(4), 488(4), 487(21), 486(59), 470(5), 440(12), 430(3), 429(7), 380(10), 379(23), 371(4), 370(6), 369(16), 368(3), 367(8), 366(14), 355(8), 338(4), 322(8), 321(5), 320(4), 309(8), 308(32), 307(15), 297(4), 296(6), 295(16), 281(5), 252(7), 251(20), 250(7), 239(5), 225(5), 224(3), 223(4), 222(4), 221(15), 213(11), 212(60), 211(5), 210(5), 209(5), 208(3), 207(9), 199(20), 198(67), 197(100), 183(5), 182(5), 181(13), 171(9), 170(44), 158(5), 127(6), 113(5), 112(10), 111(5).

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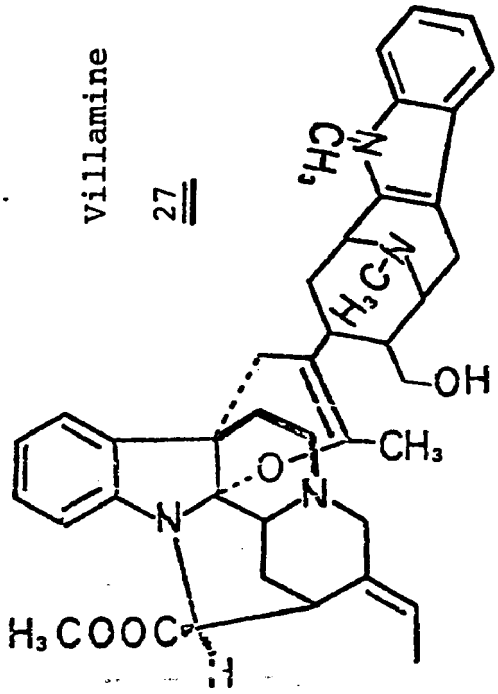
APPENDIX

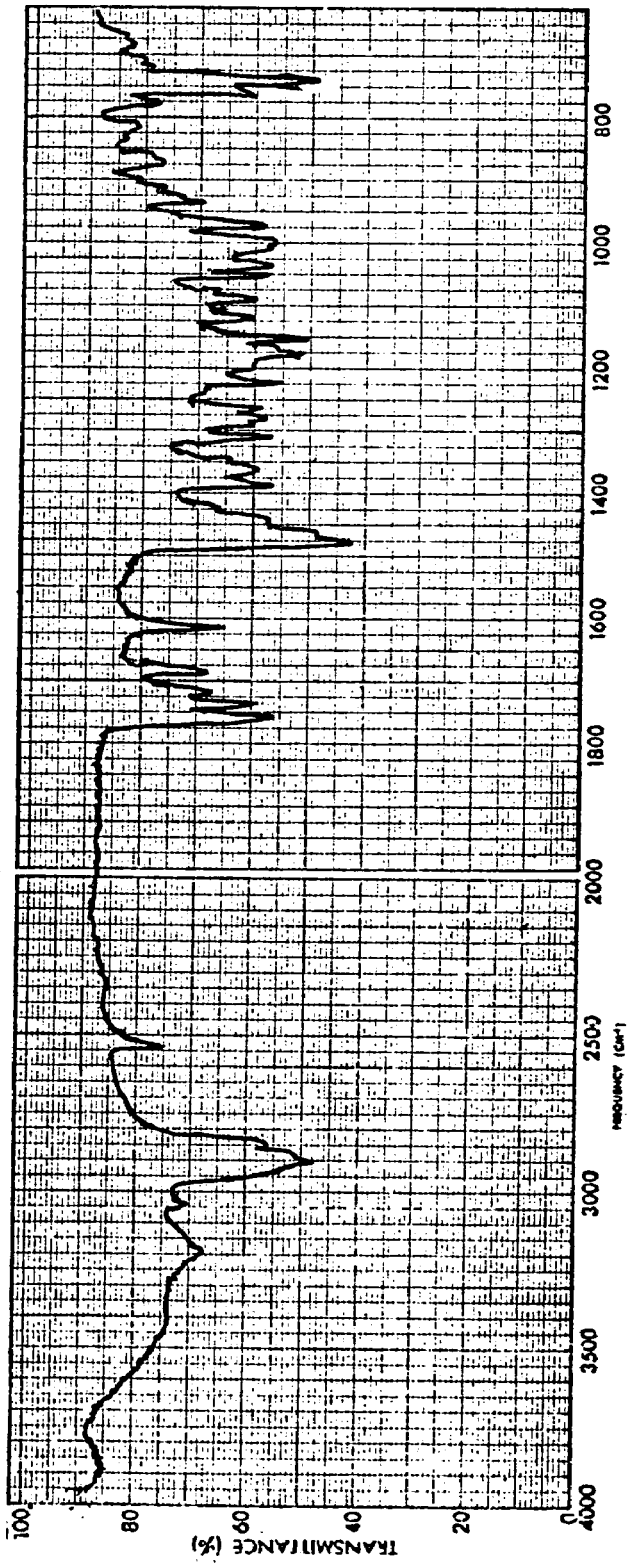
NMR, INFRARED AND MASS SPECTRA OF SELECTED COMPOUNDS*

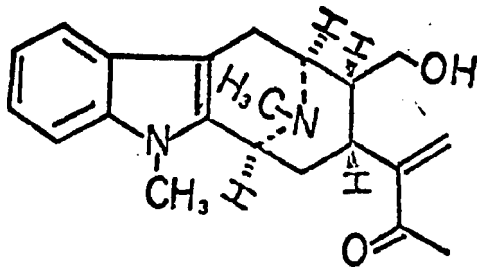
*The nmr and infrared spectra were obtained by reducing the originals with a pantograph.

Villamine

27

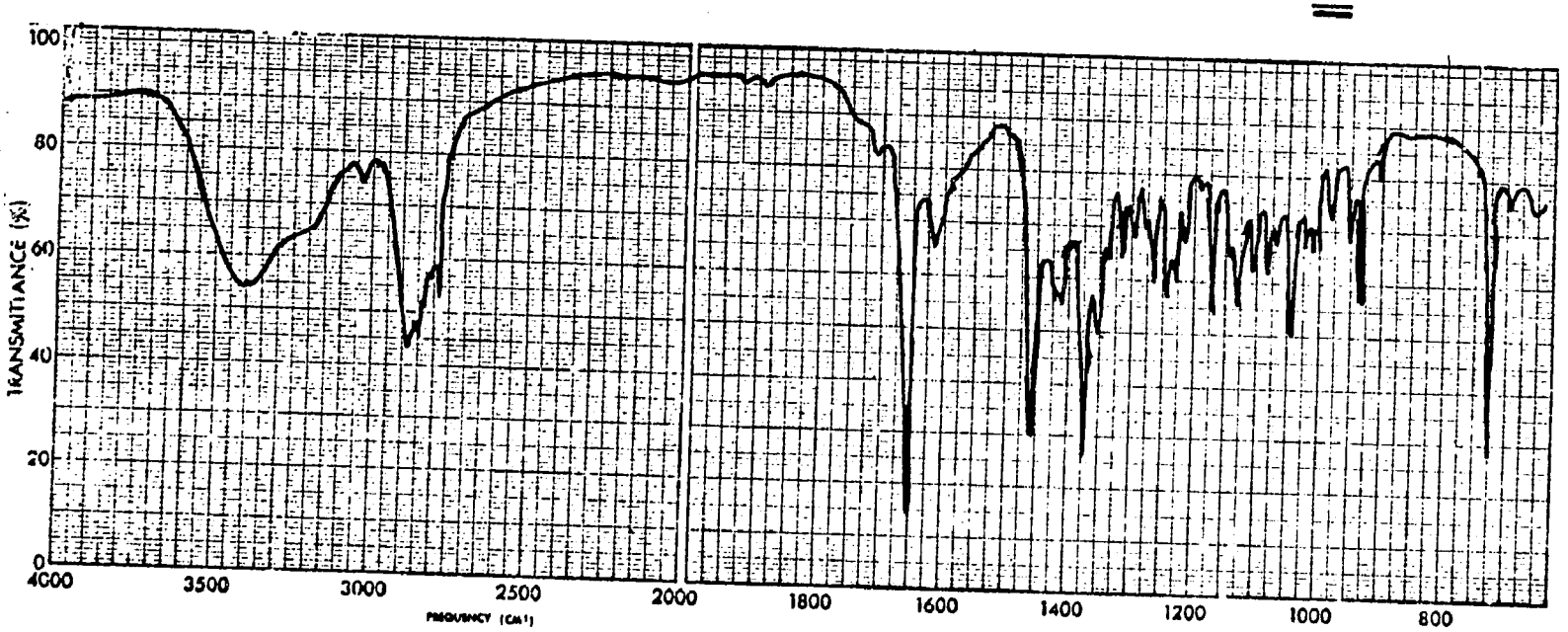


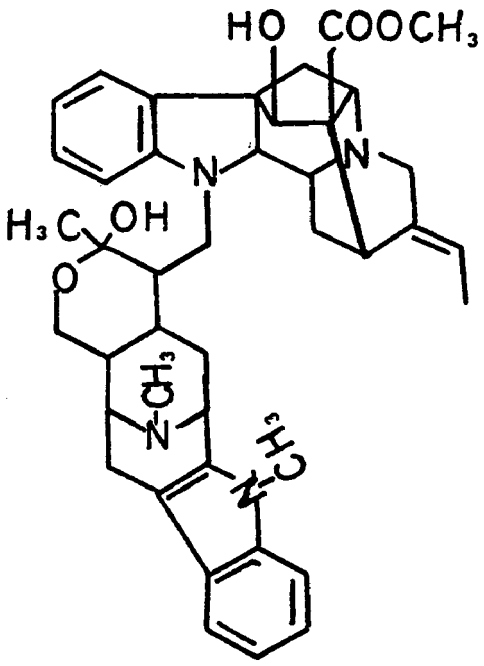




Macroline

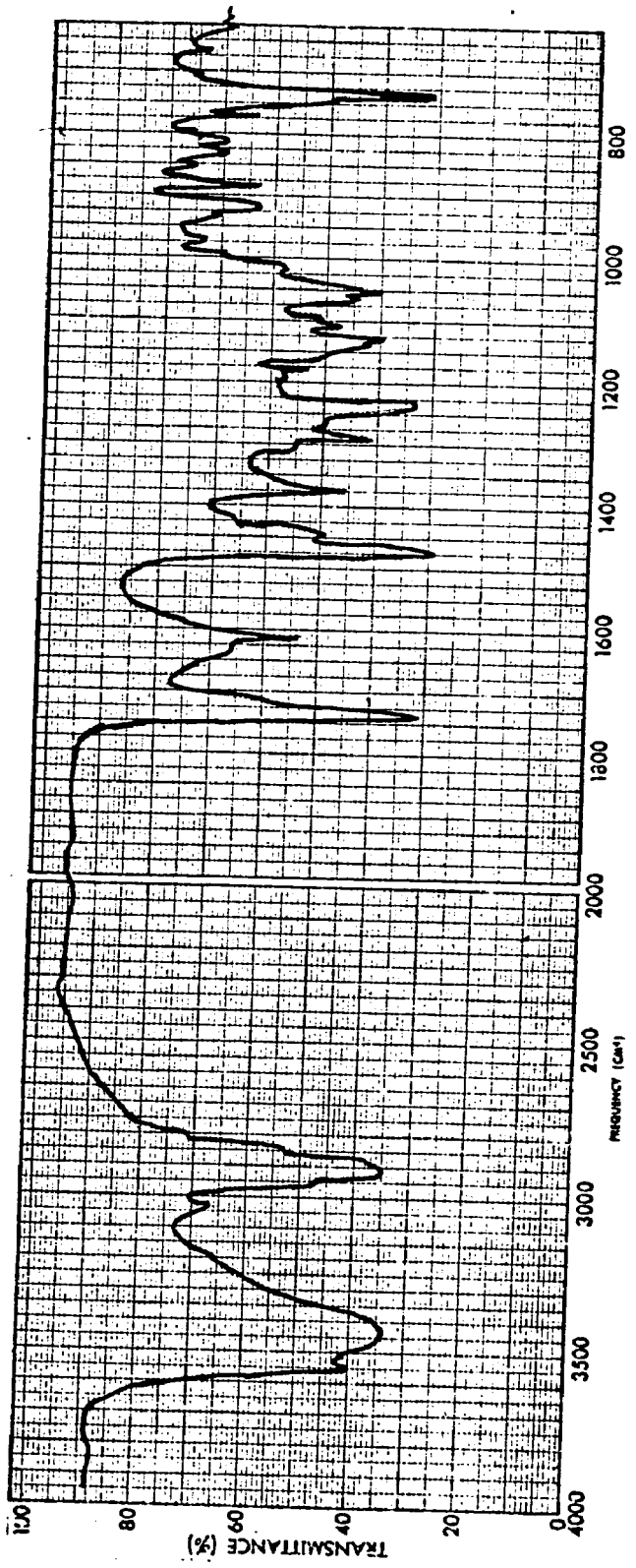
14

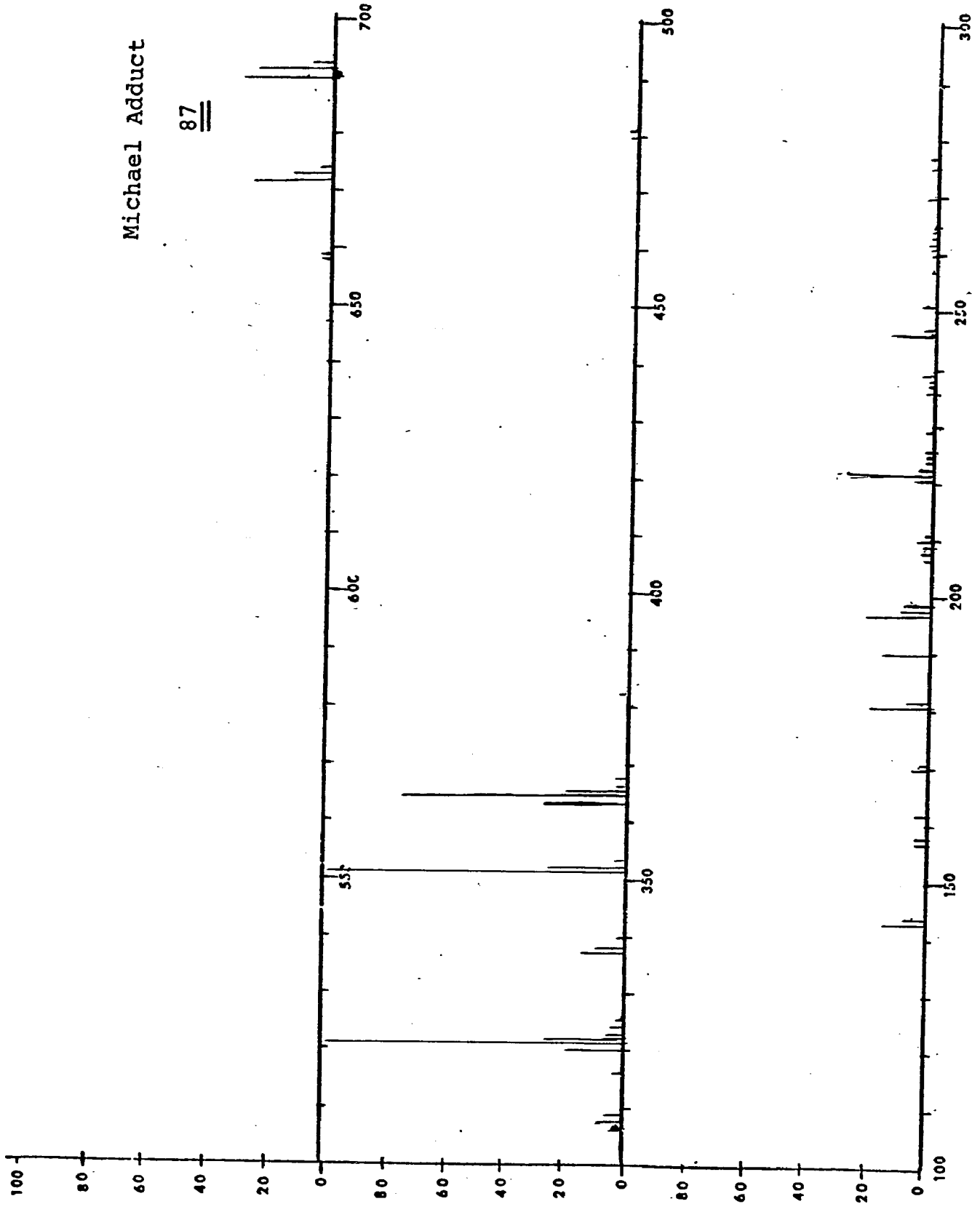




Michael Adduct

87




Michael Adduct
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650
700
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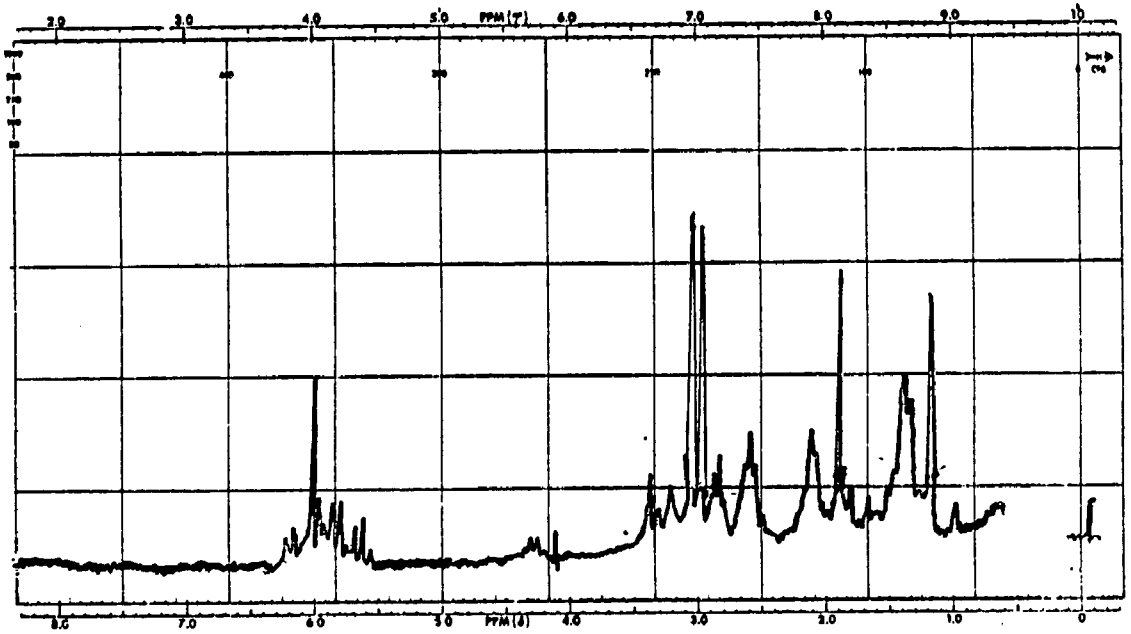
150
200
250
300



Natural Alstonisidine

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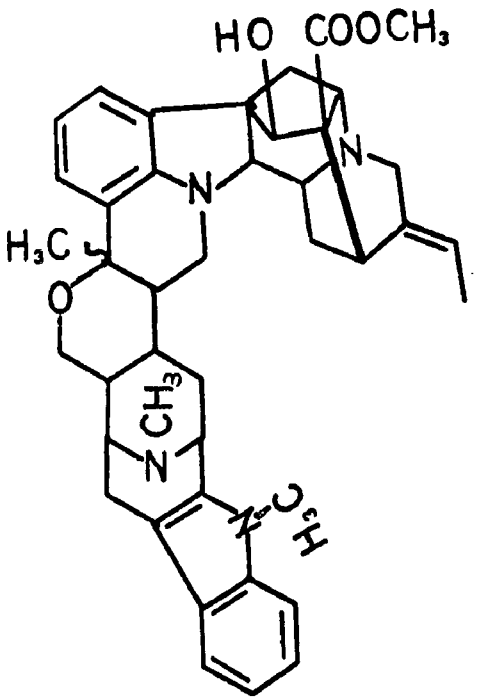

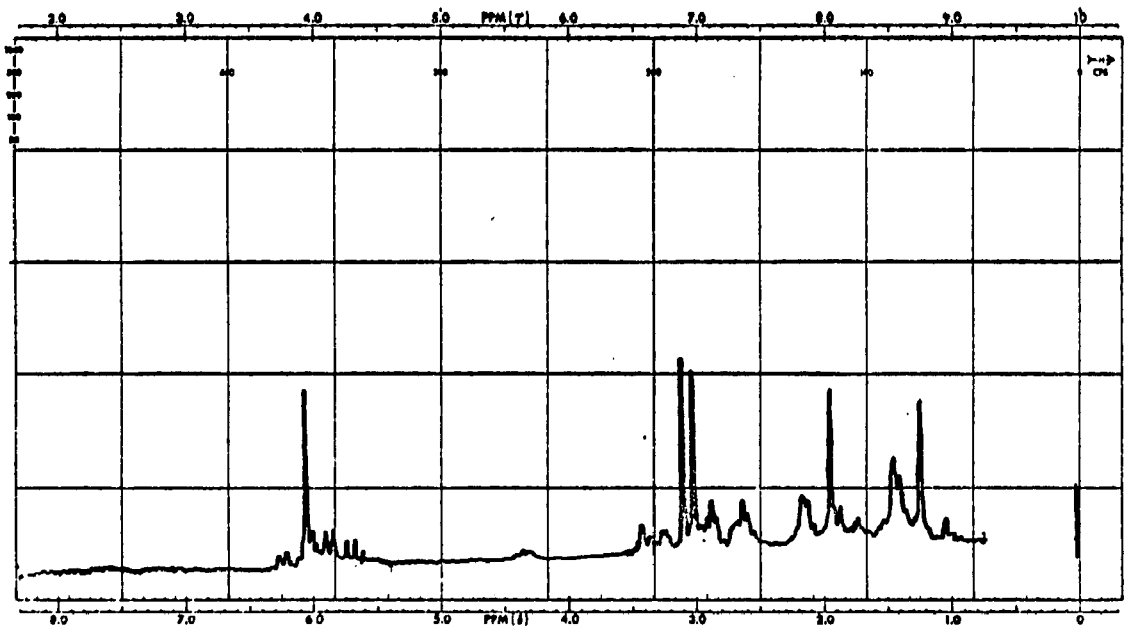

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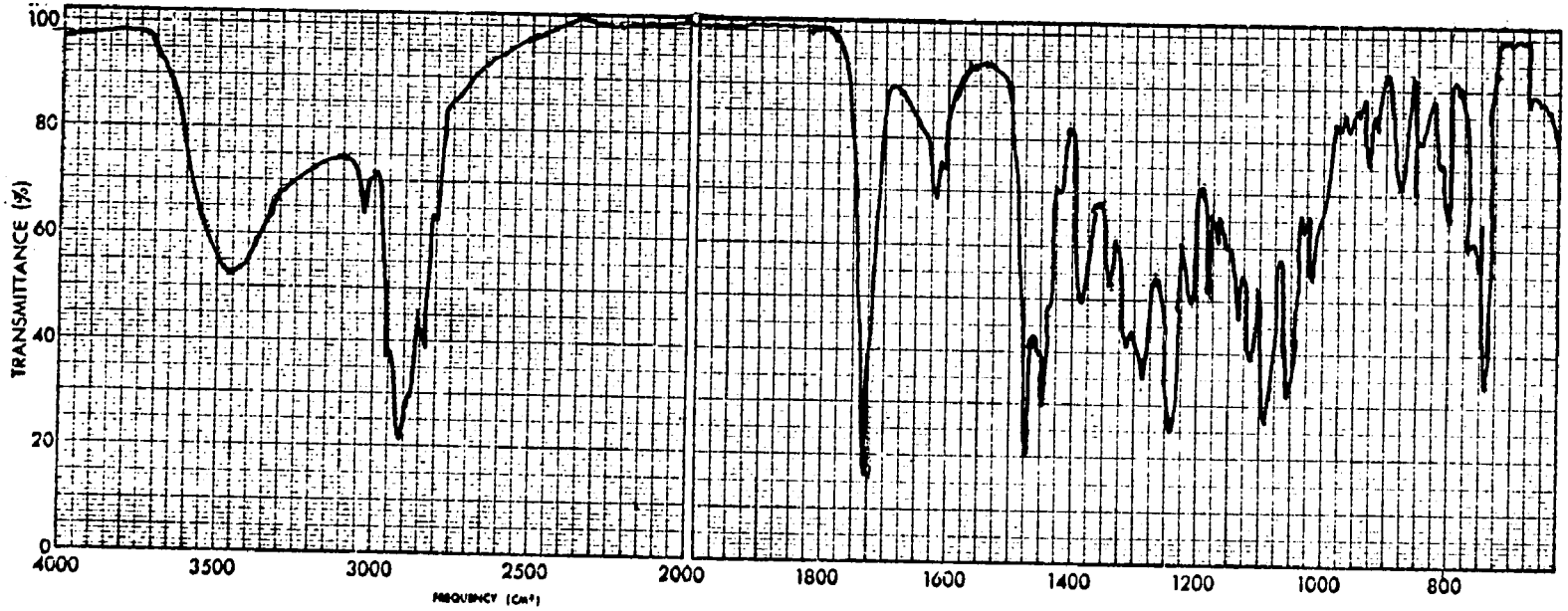
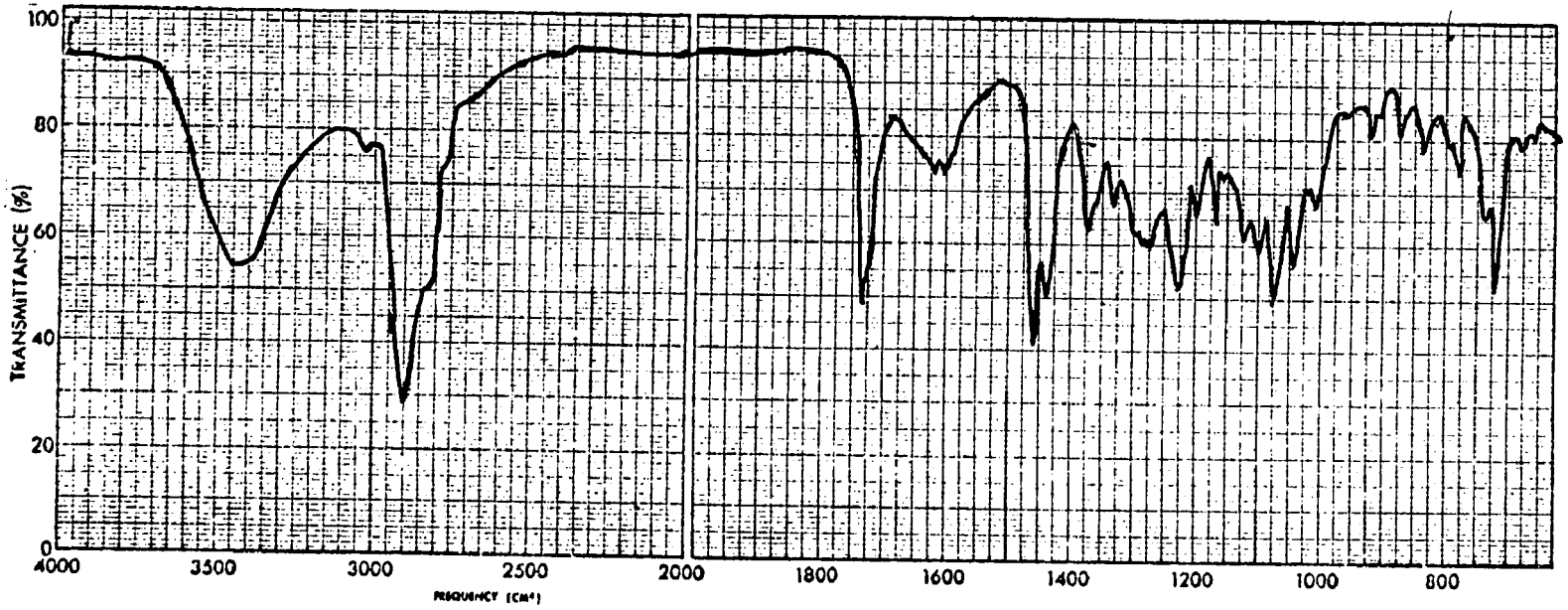
(100 MHz)



Natural

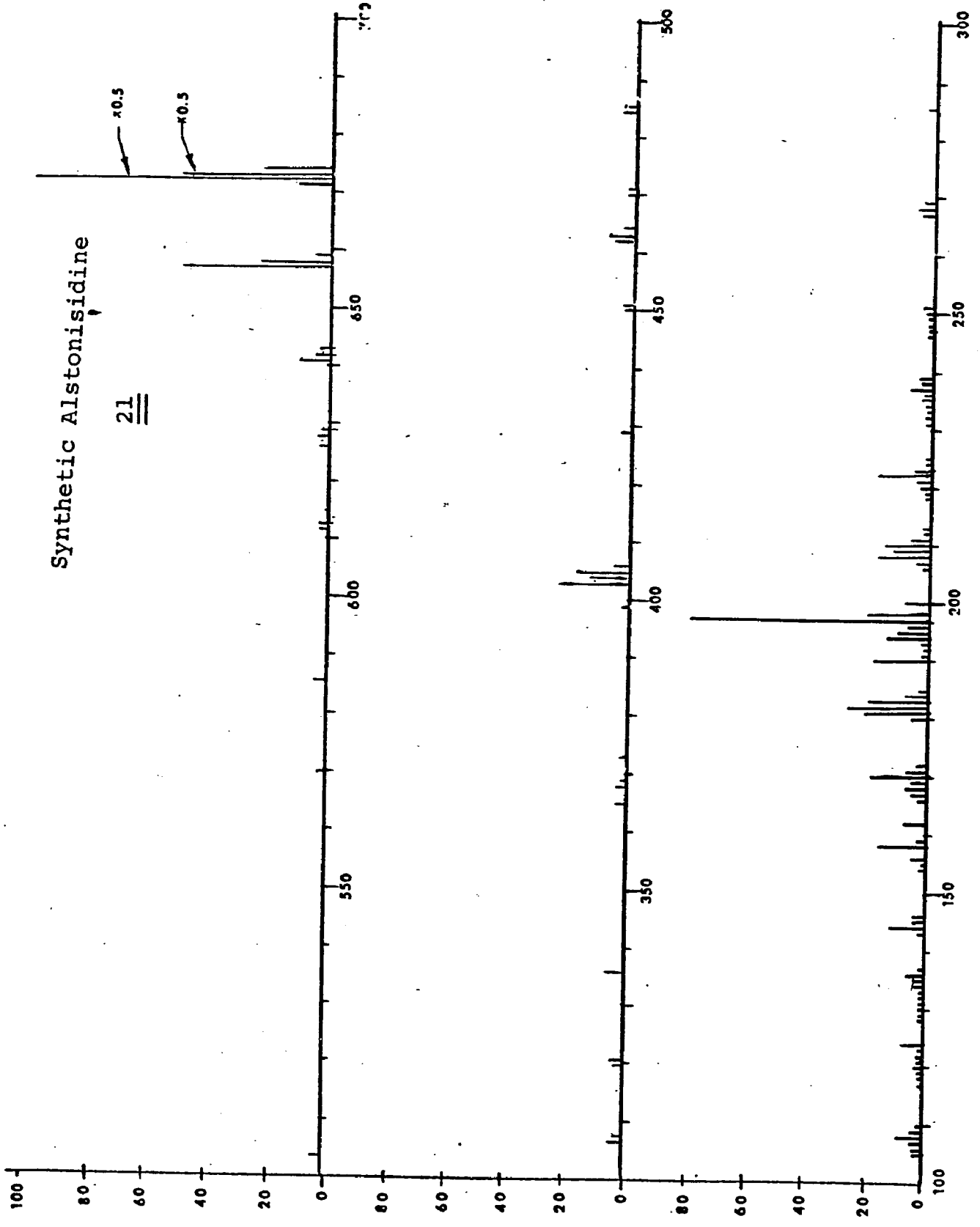
Alstonisidine

21



Synthetic

Alstonisidine


Synthetic Alstonisidine
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x0.5
x0.5
100
80
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20
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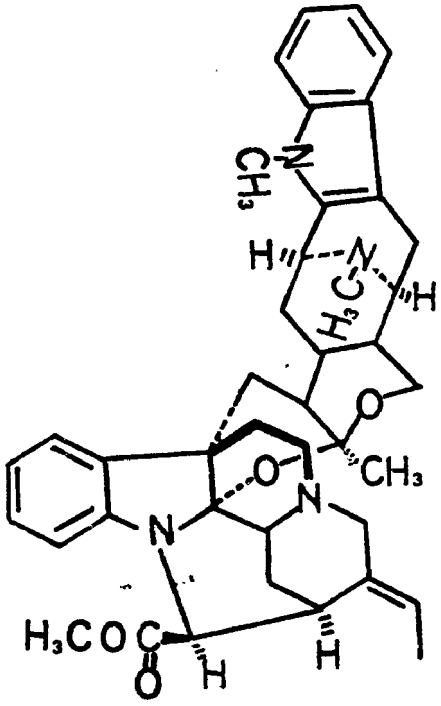
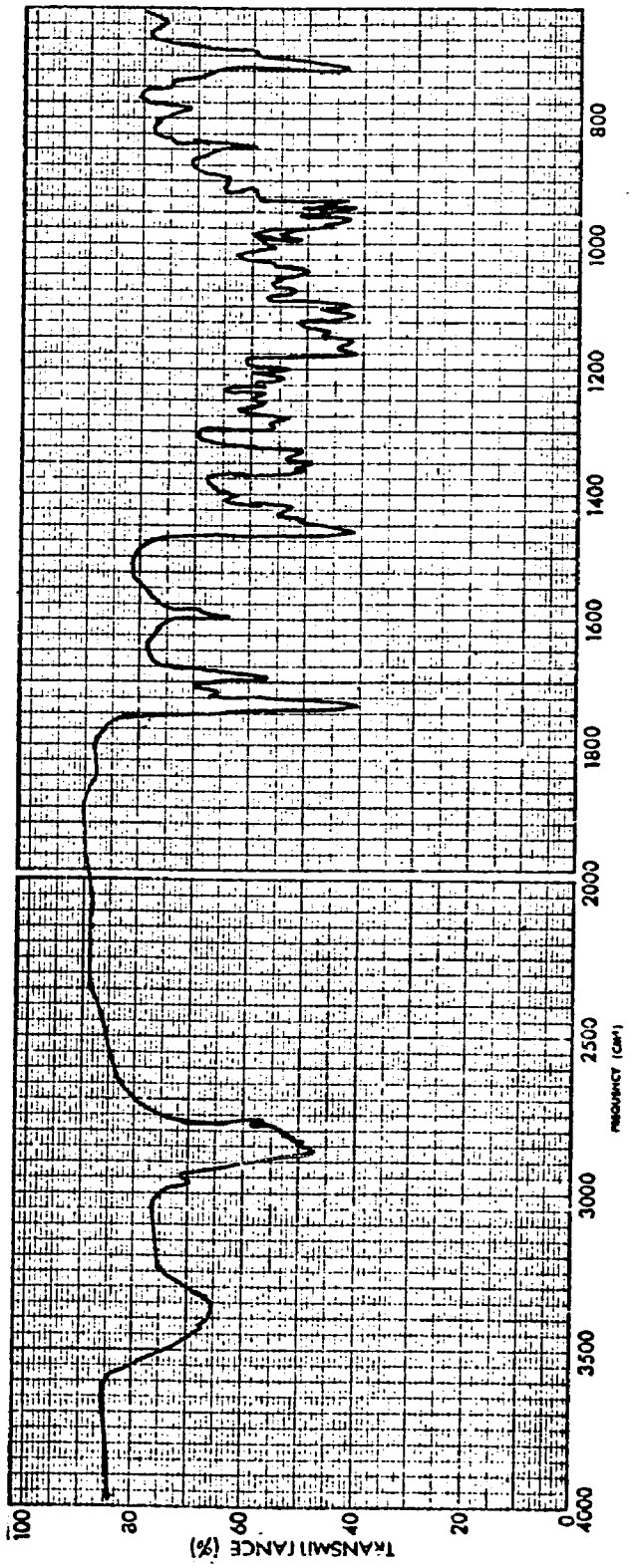
550
600
650
350
400
450
500
150
200
250
300

Natural

Villalstonine 19

(Crystalline)

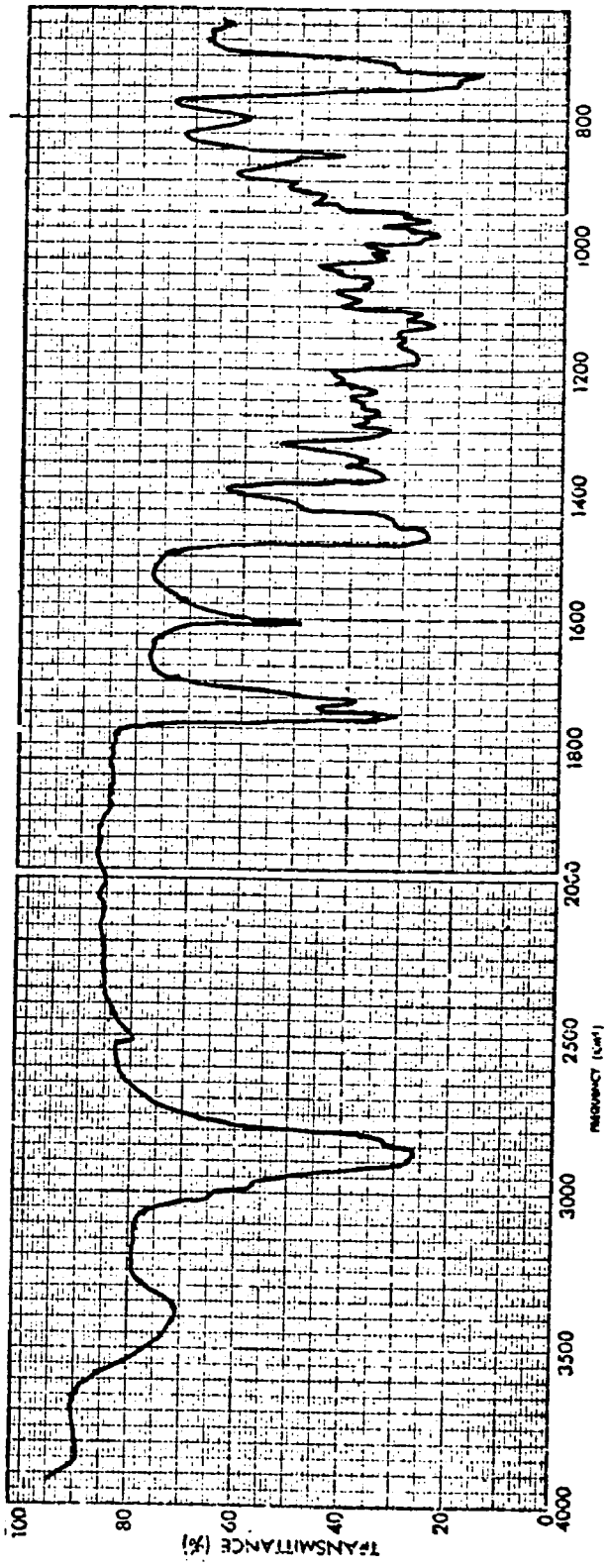




Natural

Villalstonine 19

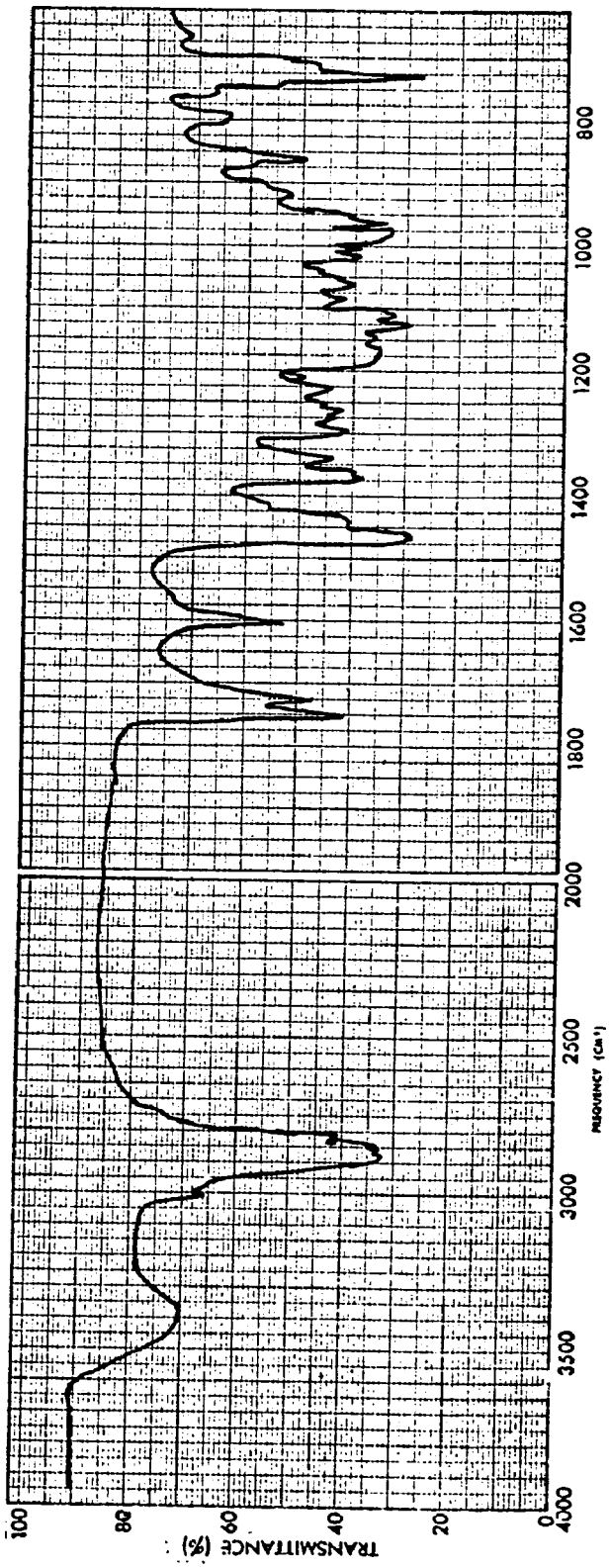
(Amorphous)

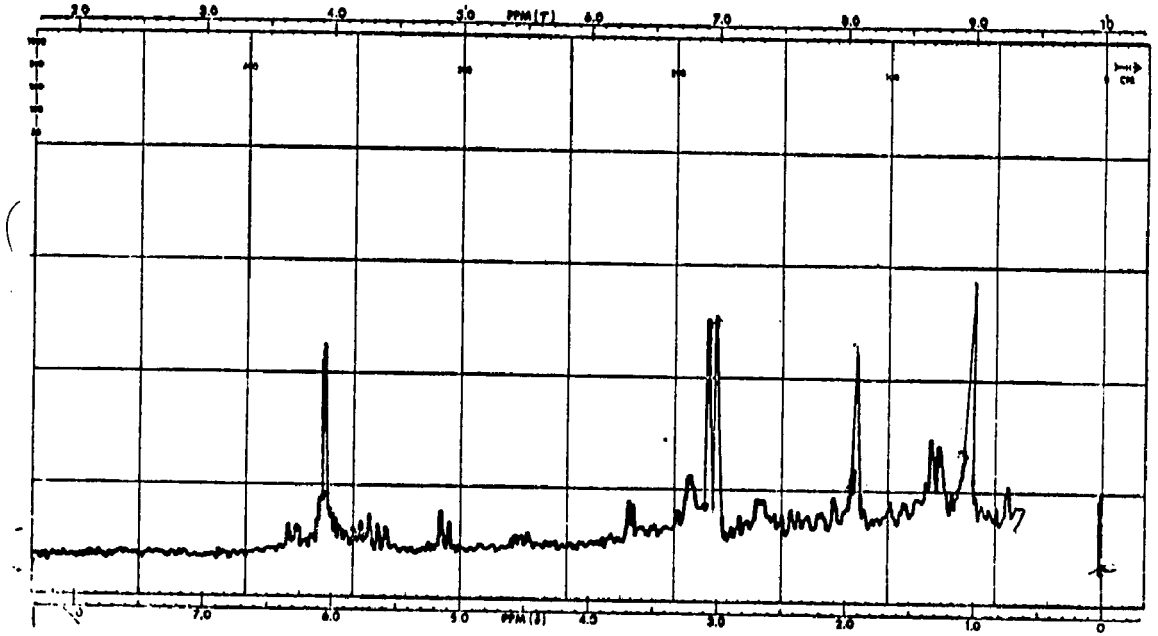


Synthetic

Villalstonine 19

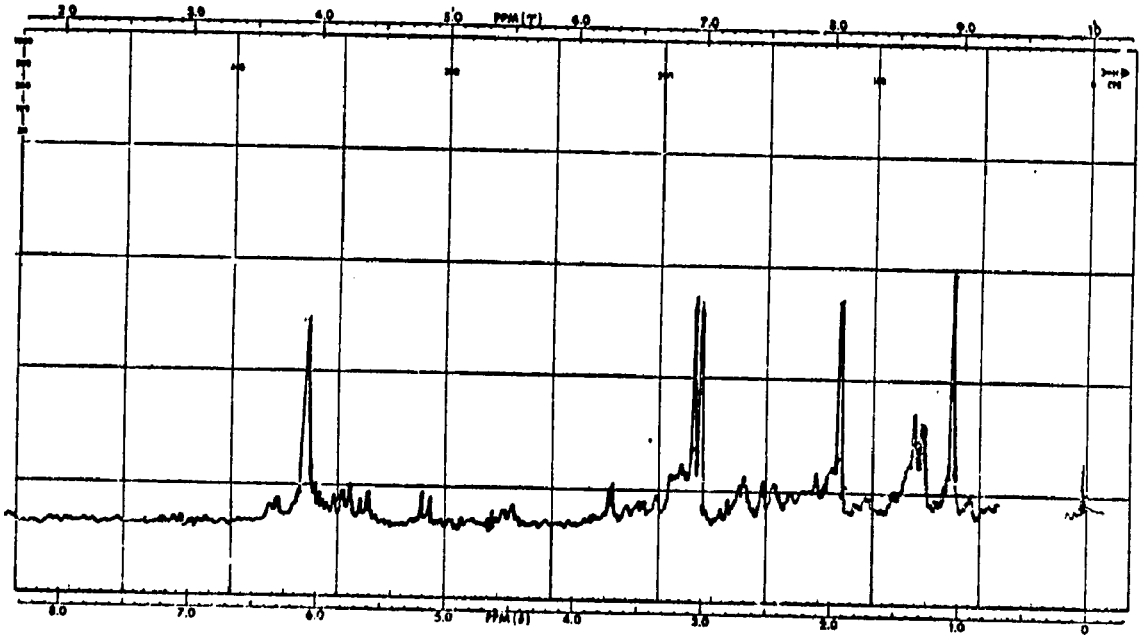
(Amorphous





Synthetic Villalstonine

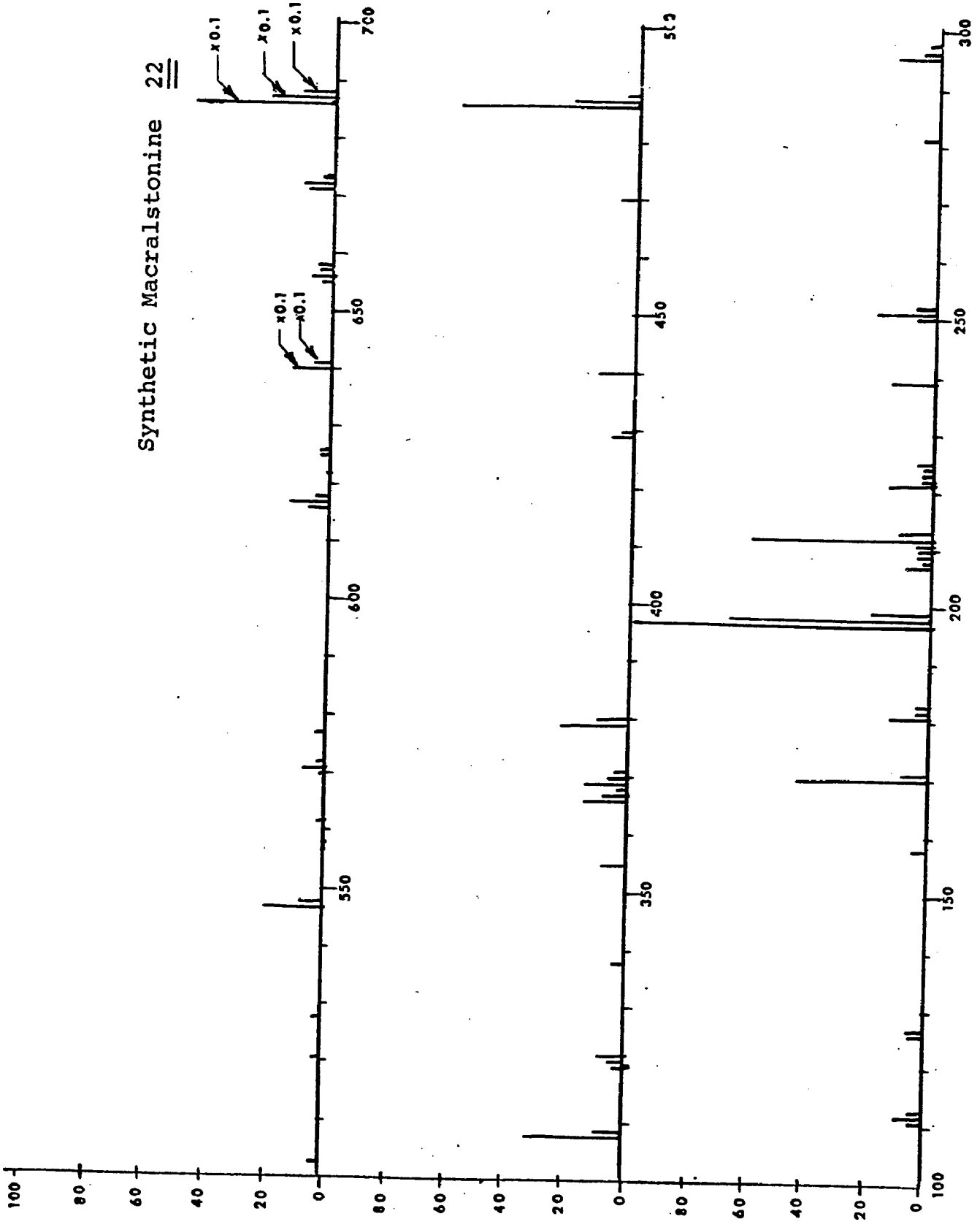
(100 MHz) 19



Natural Villalstonine

(100 MHz) 19

Synthetic Macralstonine 22



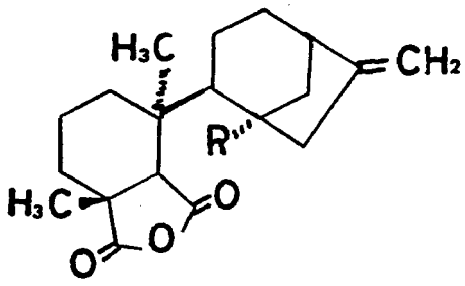
PART II

SELECTIVITY IN THE REDUCTION OF STEROIDAL ANHYDRIDE ADDUCTS

CHAPTER I

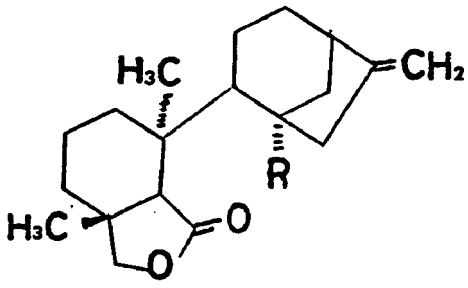
INTRODUCTION

The formation of lactones by reduction of cyclic anhydrides with complex metal hydrides is a relatively recent development. The first reported attempt was in 1949 when Chaikin and Brown[1] reported that carboxylic acid anhydrides were only very slightly reduced with sodium borohydride. No reduction products were reported by these authors. However, in 1963, two reports of the reduction of a cyclic anhydride to a γ-lactone were made. In the first example, fujenal (1) and fujenoic acid (3) gave the lactone derivatives (2) and (4) respectively on reduction with sodium borohydride or with lithium aluminum hydride.[2]



1 R= CHO

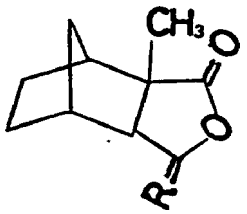
3 R= COOH



2 R= CH_2OH

4 R= COOH

In the second example exo-2-methylnorbornane-endo-2,3-dicarboxylic anhydride (5) was reduced with sodium borohydride to the lactone (6).[3]



5 R=O

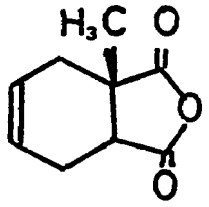
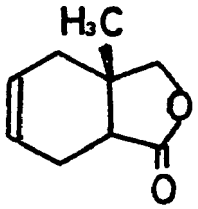
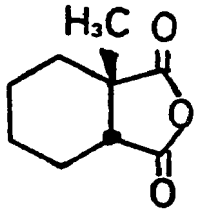
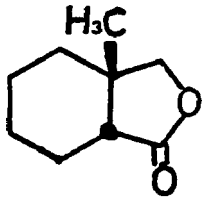
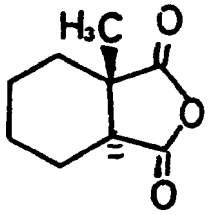
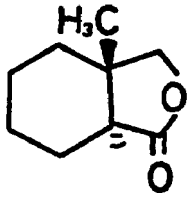
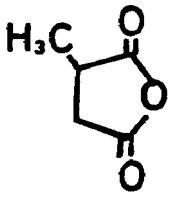
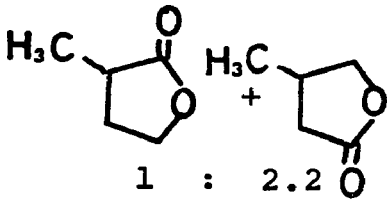
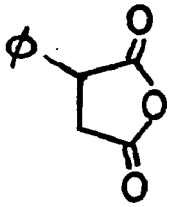
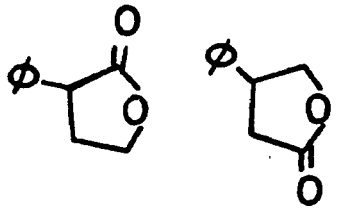
6 R=H_2

Since these two instances, there have been other scattered reports of the reduction of cyclic anhydrides to lactones with complex metal hydrides.[4-7]

In 1967, Bloomfield and Lee[8] studied the reductions of cyclic carboxylic acid anhydrides with lithium aluminum hydride as a possible general route to γ-lactones or to diols. The reductions were carried out at low temperature (-55°) in tetrahydrofuran and gave good yields of lactonic materials (Table (Table 1). In most cases a high degree of selectivity was observed in that the carbonyl adjacent to the more highly substituted carbon was preferentially reduced. To explain this specificity, the authors proposed that the less hindered carbonyl oxygen initially complexes with a solvated cation and in the process becomes the more hindered one. Attack at the other carbonyl by an alumino-hydride ion produces an aldehyde which is

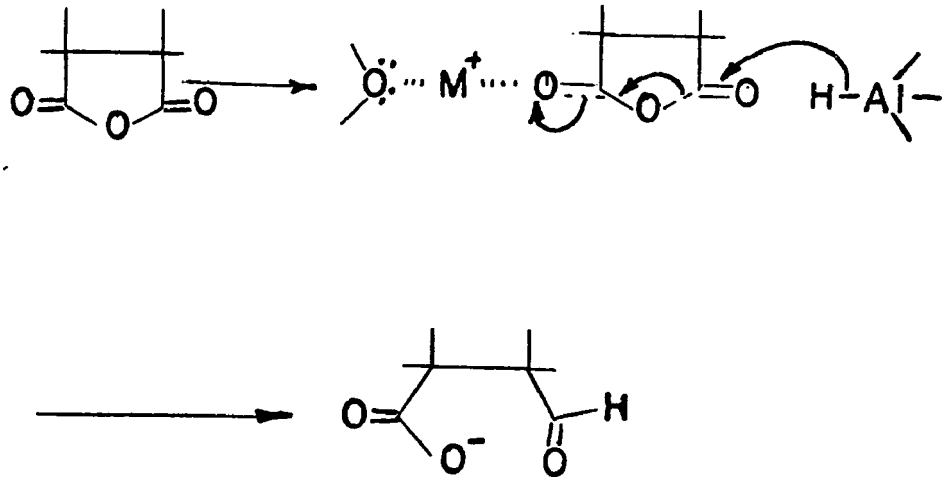
TABLE 1

Reduction of Cyclic Carboxylic Acid Anhydrides with $LiAlH_4$

Anhydride	Reduction Product	Yield %
		75
		80
		85
	 1 : 2.2	70
	 2 : 3	72

subsequently rapidly reduced to the alcohol. Acid work up of the reaction then causes spontaneous formation of the lactone. This amounts to a 1,4 hydride addition to the O=C-O-C=O system (Scheme I). However, there was no experimental evidence presented to support the hypothesis.

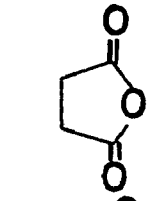
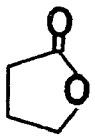
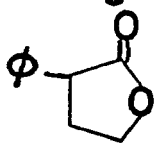
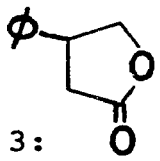
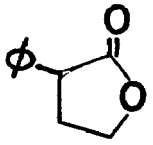
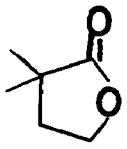
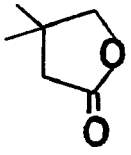
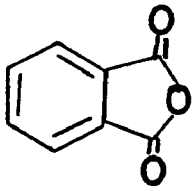
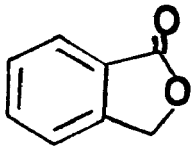
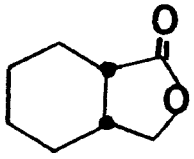
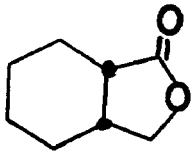
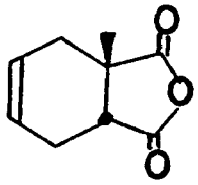
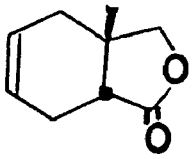
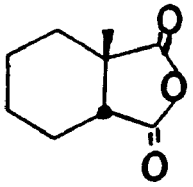
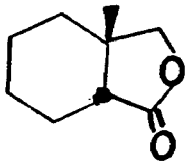
SCHEME I



In 1970, the reduction of cyclic carboxylic acid anhydrides with sodium borohydride was also reinvestigated by Bailey and Johnson[9] as a general method for the synthesis of lactones. These workers studied the reduction of a series of cyclic anhydrides in tetrahydrofuran or dimethyl formamide at room temperature and found that lactones were formed in good to excellent yield (Table 2). They also

TABLE 2

Reduction of Cyclic Carboxylic Acid Anhydrides with $NaBH_4$

Anhydride	Reduction Product	Yield %
		57
	 3:  2	67
		74
		97
		76
		65
		80

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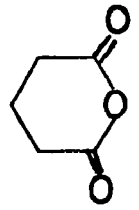
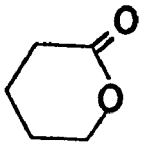
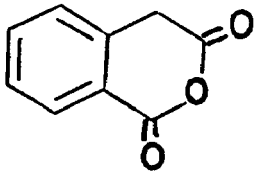
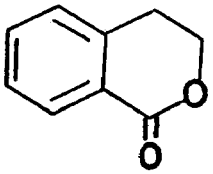
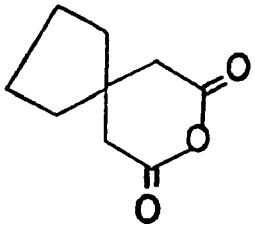
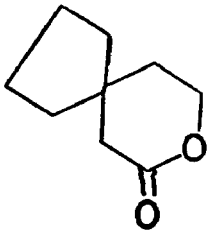
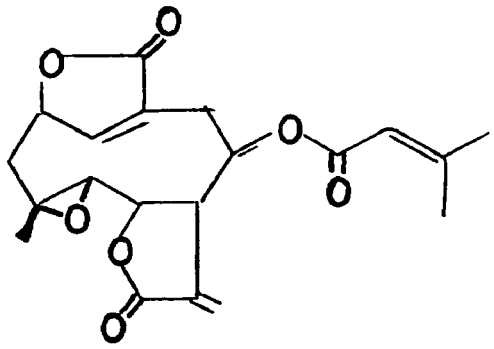
		67
		55
		68

found that, like lithium aluminum hydride, sodium borohydride also preferentially reduces the carbonyl adjacent to the more highly substituted carbon.

To date there has been notably little use of the reduction of cyclic anhydrides for the formation of a lactone function in the synthesis of natural products possibly because the method is relatively new. The importance and occurrence of the lactone functional group in natural products, e.g., terpenoids and steroids is very great, as shown in compilations of these compounds.[10,11,12]

For example, among terpenoids[13-17] (e.g., 7) and steroids[18,19] (e.g. 8) that show anti-tumor activity or outstanding toxicity (e.g., the aflatoxins[20] 9) the lactone function is an important structural feature.



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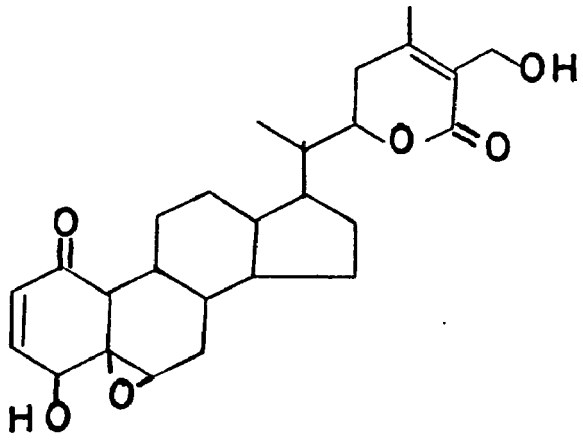

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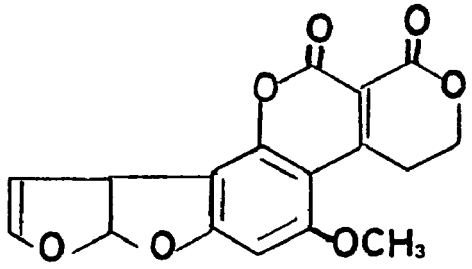

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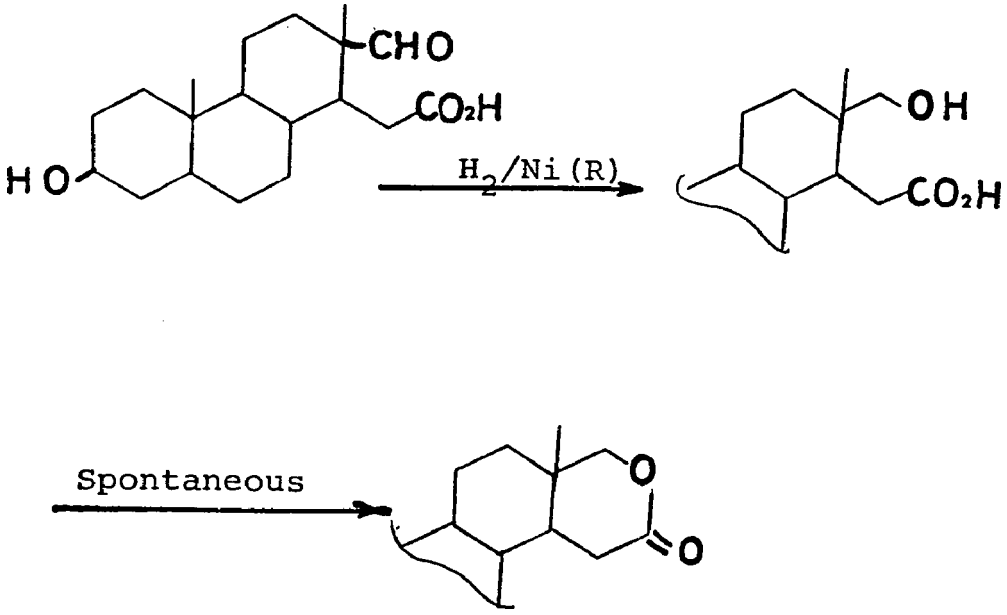
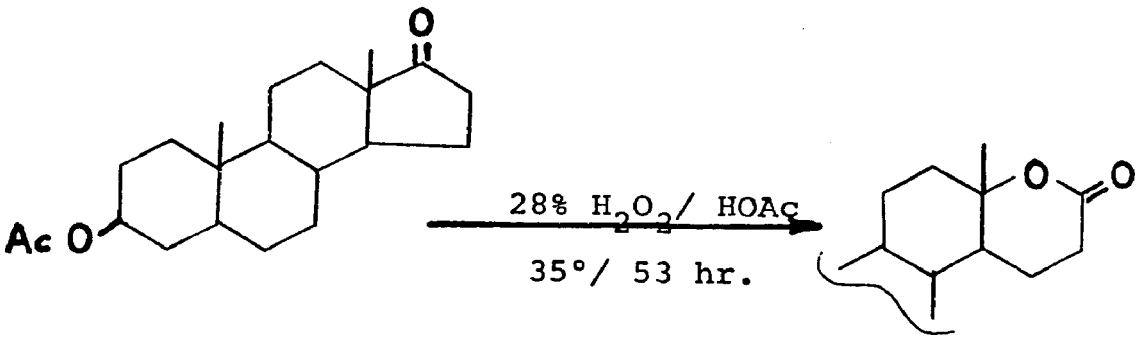
In steroidal synthesis, the formation of the lactone function has usually been accomplished by two methods: displacement reactions (as for example in Scheme II)[21] or by direct oxygen insertion reactions such as the

SCHEME II

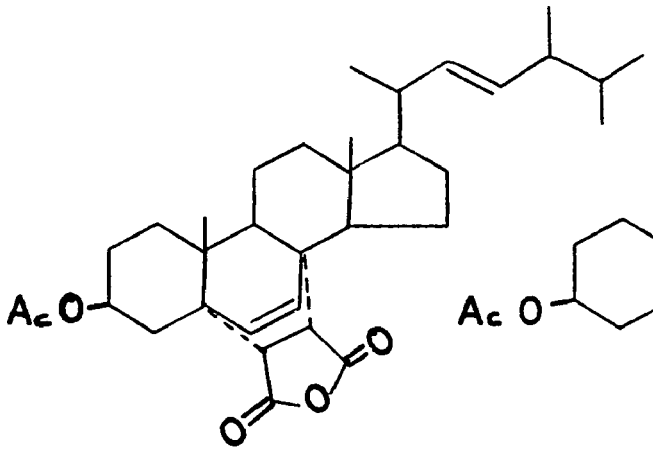


Baeyer-Villiger reaction[22] (Scheme III).[23]

SCHEME III



The only reported formation of a steroidal lactone by a complex metal hydride reduction has been the reduction of the Diels-Alder maleic anhydride adduct of ergosteryl acetate[24,25,26] (10) to the lactone (11) by sodium borohydride and lithium aluminum hydride.[27] With both reagents (11) was the only lactone produced. Reduction of (10) with



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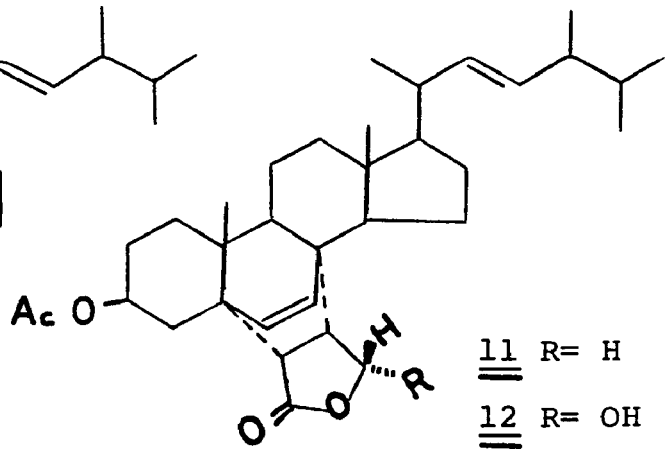
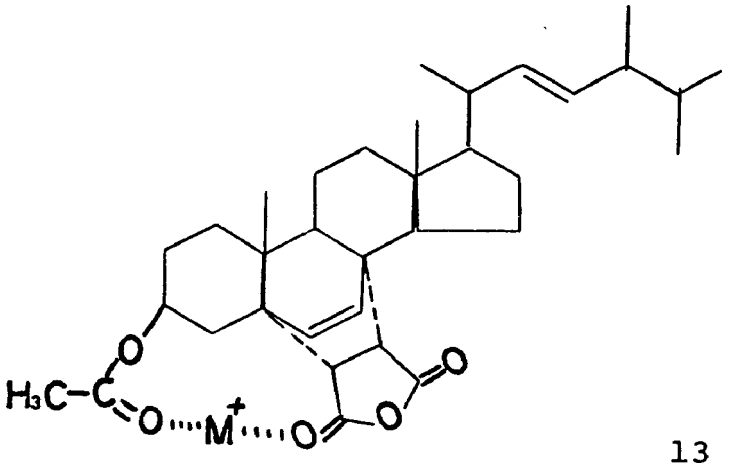


11 R= H

12 R= OH

lithium tri-t-butoxy alumino hydride gave the lactol (12). This high degree of specificity was ascribed to an intramolecular complex involving the acetate carbonyl, the metal ion and an anhydride carbonyl (13). Reduction would then involve 1,4 addition of a hydride ion to the anhydride as proposed by Bloomfield and Lee.[8]

The high degree of selectivity found in the above reductions represents a potentially useful method of



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selectively introducing a lactone function. However, the general synthetic utility of this method remains to be determined.

Initiation of Present Work

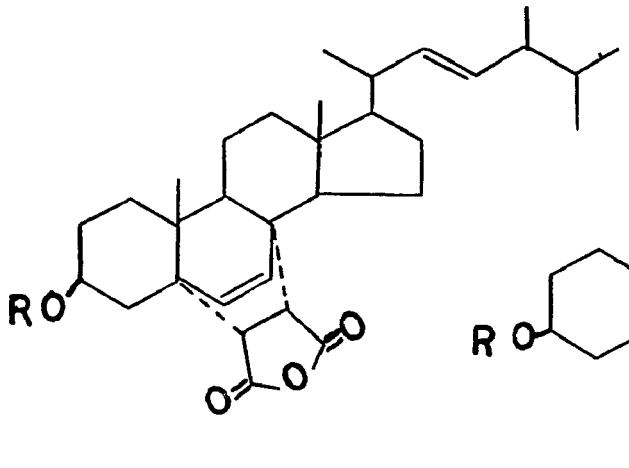
Since an intramolecular complex involving the acetate group was proposed to account for the complete specificity in the reduction of (10), it was proposed to carry out reductions in the absence of this group and to determine the ratios of lactones produced. In this manner there could be no intramolecular complex and any selectivity observed would have to arise from the intrinsic differences in the environments of the carbonyls or from the formation

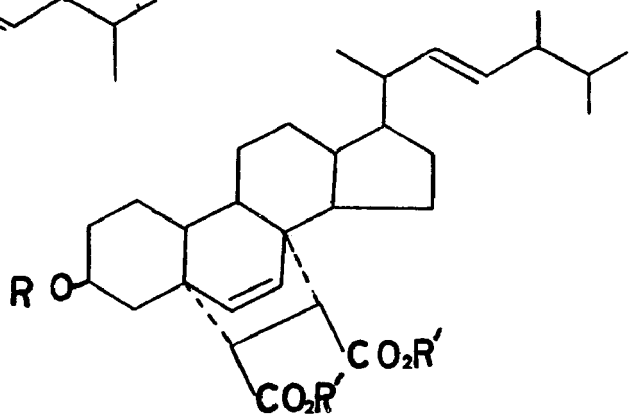
of preferred intermolecular complexes. The group chosen to replace the acetate was a methoxy group, primarily for two reasons. First the group would be inert under the reaction conditions employed; and secondly, because of its small size it should not exert significant steric effects on the course of the reaction.

CHAPTER II

RESULTS AND DISCUSSION

The anhydride (14) whose reduction was to be studied, was to be prepared from the known anhydride (10). Preparation of (14) directly from ergosteryl methyl ether via a Diels-Alder reaction with maleic anhydride was considered but rejected for the following reasons. First would be the problem of separating the desired adduct from isomeric Alder-ene adducts (there are two "ene" adducts formed with ergosteryl acetate and maleic anhydride).[25,26] Secondly, since the yield of Diels-Alder adduct (10) is only approximately ten per cent, there was no reason to expect that the ergosteryl methyl ether yield would be any greater. Thirdly the conversion of (10) to (14) would involve well known and simple high yield transformations and give products of known stereochemistry.





10 R = CH_3CO

14 R = CH_3

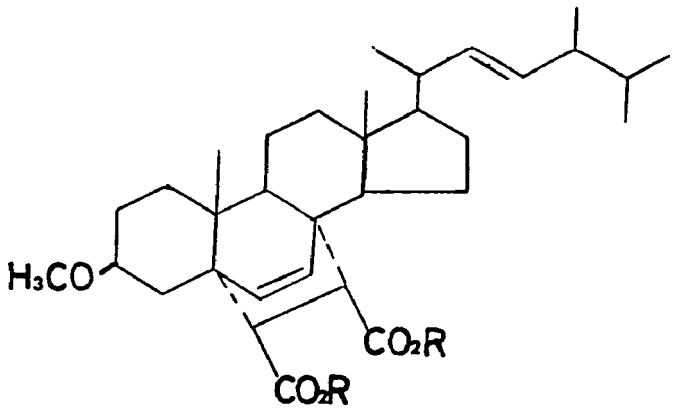
15 R = R' = H

16 R = H; R' = CH_3

The Inhoffen adduct (10) was prepared by the method described by Jones and Thomas[15,26] and hydrolyzed in five per cent methanolic potassium hydroxide to the diacid[25] (15). Treatment of the diacid with diazomethane[28] gave the dimethyl ester (16) [95% yield from the anhydride (10)].

The formation of the methyl ether with diazomethane was chosen because the reaction time is very short and the reaction can be carried out at room temperature or below. The preparation of methyl ethers with other alkylating agents such as methyl iodide or dimethyl sulfate usually involves heating and the presence of a strong base. In addition, work up from the diazomethane reaction would be easy and clean. The use of diazomethane to prepare methyl ethers has been described using boron trifluoride, fluoroboric acid or aluminum chloride as catalysts.[29,30] The method using aluminum chloride was chosen primarily because of the reportedly higher yields obtained and simpler work up.[30] To effect alkylation the dry diester (16) was suspended in anhydrous diethyl ether and cooled to 0°. Anhydrous aluminum chloride was added followed by anhydrous ethereal diazomethane until the yellow color of excess diazomethane persisted. As the alkylation proceeded the slightly soluble diester gradually dissolved as the highly soluble methyl ether (17) was formed. Work up of the reaction

gave an oil which crystallized in stout colorless prisms (m.p. 92-93°) from a concentrated methanol solution (87% yield).



17 R = CH_3

18 R = H

The saponification of the diester (17) to the diacid (18) was anticipated to be quite straight forward but unfortunately proved to be quite difficult. The diester was completely unaffected by several hours of refluxing in ten per cent methanolic potassium hydroxide and only partial hydrolysis was obtained by refluxing in a ten per cent solution of potassium hydroxide in ethylene glycol/water (4:1) or in a fifteen per cent solution of potassium hydroxide in glyme/water (8:1). Saponification was achieved by dissolving the diester in warm propylene glycol followed by addition of a large excess of fifty per cent aqueous potassium hydroxide and refluxing for three to four hours. The hydrolysis had to be carried out under an atmosphere of nitrogen to prevent severe yellowing and darkening of

the reaction. The diacid (18) was recovered by pouring the cooled mixture into a large volume of dilute aqueous hydrochloric acid and filtering off the precipitated acid.

The formation of the anhydride (14) from (18) was readily accomplished by heating in acetic anhydride. The crystals were filtered off and dried over potassium hydroxide under vacuum (88% yield). The NMR spectra of the anhydrides (14) and (10) are quite similar (see Table 1) and thus establish that the stereochemistry of the two anhydrides is identical.

TABLE 3

NMR Spectra of Anhydrides (10) and (14) (τ Units)

Protons Attached to	Anhydride (10)	Anhydride (14)
C-6, C-7 H	AB q, 3.86, 4.28 ($J_{6,7}$ = 8 Hz)	AB q, 3.83, 4.49 ($J_{6,7}$ = 8 Hz)
C-22, C-23 H	m, 4.90	m, 4.87
C-1', C-2' H	AB q, 6.58, 7.18 ($J_{1',2'}$ = 9 Hz)	AB q, 6.58, 7.17 ($J_{1',2'}$ = 9 Hz)
CH_3COO	s, 8.00	- - -
CH_3O	- - -	s, 6.58
C-18 CH_3	s, 9.28	s, 9.28
C-19 CH_3	s, 8.98	s, 8.96

Reduction of the anhydride with sodium borohydride in refluxing dioxane gave two new compounds (R_f, 0.57 and 0.50, 20% diethyl ether/benzene) along with very polar materials. Column chromatography on activated alumina in benzene gave a rapidly eluting fraction which contained the new components. Further elution with benzene/methanol gave only highly polar materials. Concentration of the fractions containing the two new components gave an oil which solidified on standing. I.R. of the solid showed a carbonyl at 1750 cm^{-1} (γ lactone). The separation of the two lactones was accomplished by preparative tlc on silica gel using n-pentane/diethyl ether (9:1) as developing solvent. After nine developments, two well resolved bands were excised and extracted (soxhlet) to give two lactones in virtually equal amounts. Proof that the two lactones are isomeric was that both give the same molecular ion (M^+ 494), have the same elemental analysis and give the same diol (19) on reduction with lithium aluminum hydride.

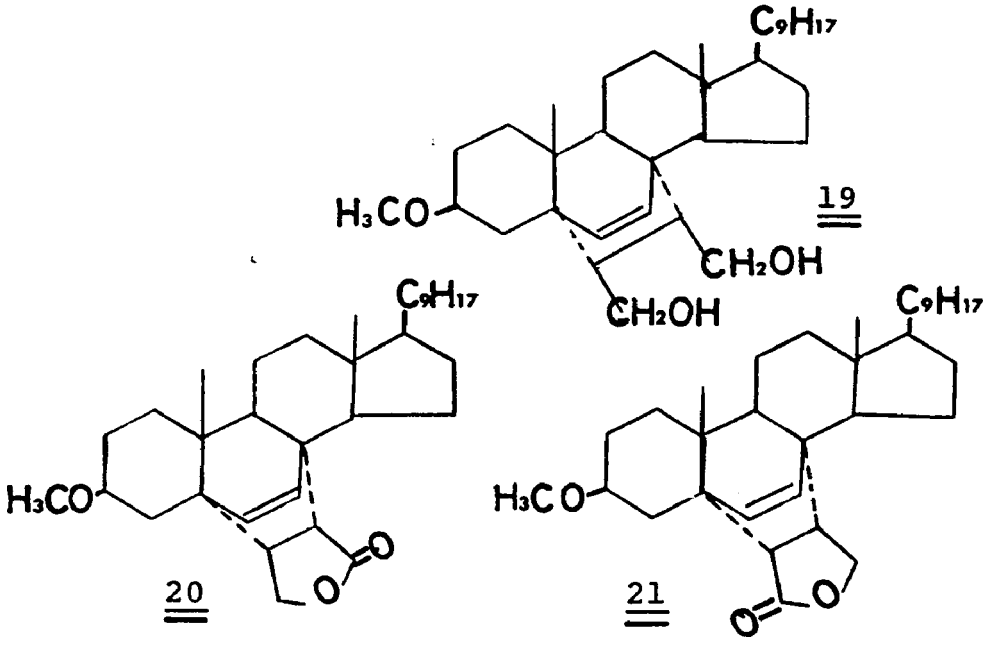
While the NMR's of the two lactones are slightly different (see Table 2), there is only one characteristic feature that substantially indicates one structure over the other and that is that in one lactone the C-3 methoxy is deshielded by approximately 0.1 p.p.m. more than in the other. When Dreiding models of the two isomers are

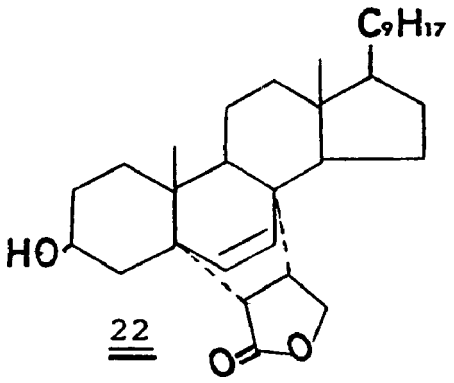
constructed it is found that in lactone (20), faster moving on tlc, the methoxy group is directly behind and in line with the nodal plane of the lactone carbonyl and should thus be more deshielded relative to the methoxy in lactone (21).[31] The assignment of the structures of the two lactones was made primarily, however, from chemical correlation and ORD/CD spectroscopy. Methylation of the known lactone (22)[27] with diazomethane and aluminum chloride gave a lactone identical with the lactone (21). In accord with this, the ORD of (21) showed a negative Cotton effect and was superimposible on the ORD curve of the acetoxy lactone (11). The CD of the lactone (21) was also negative as predicted from the lactone sector rules.[32] In contrast, the CD of lactone (20) was positive as predicted by the lactone sector rules. The ORD curve of lactone (20) was more complex than that of (21). The curve begins with negative rotation but becomes positive forming a weak peak at 230 nm then again becomes negative showing a trough at 224 nm. Instances similar to this are known where the ORD curve is complex and ambiguous while the CD of the same compound is relatively simple and definitive.[38] This complexity has been ascribed to an effect of the combined asymmetry of the whole molecule.[39]

TABLE 4

NMR Spectra of Lactones (20) and (21) (τ units)

Protons Attached to	Lactone (21)	Lactone (20)
C-6, C-7	AB q, 3.86, 4.32 (J_{AB} = 8 Hz)	AB q, 3.94, 4.24 (J_{AB} = 8 Hz
C-22, C-23	m, 4.88	m, 4.89
C-3' (2H)	m, 6.1	- - -
C-4' (2H)	- - -	m, 6.1
C-1'	d, 7.64 (J = 9 Hz)	m, 6.3
CH_3O	s, 6.72	s, 6.63
C-2'	m, 6.24	d, 7.08 (J = 10 Hz)
C-18	s, 9.28	s, 9.24
C-19	s, 8.97	s, 8.98





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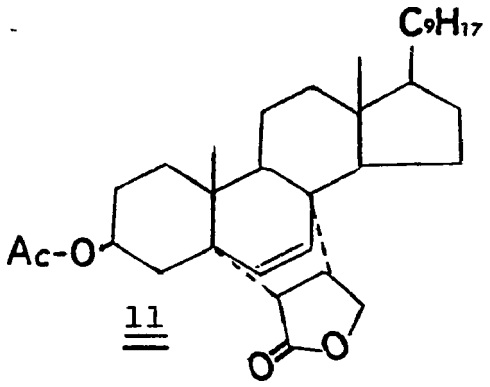

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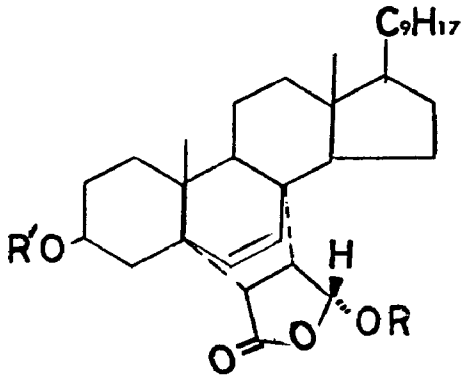
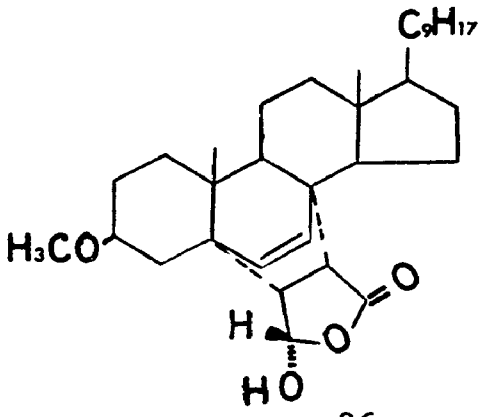
Reduction of the anhydride (14) with lithium aluminum hydride in tetrahydrofuran at -55° gave both lactones but lactone (21) greatly predominated (13:1). With sodium aluminum hydride in tetrahydrofuran at -55°, the selectivity was somewhat less (10:1) but still great.

Reduction of (14) with lithium tri-t-butoxyaluminohydride in tetrahydrofuran at 0° gave mainly one lactol (23). The structure of (23) was assigned from comparison of its spectra [and those of its acetate (24)] with those of the acetoxy lactol (25) and from its reduction by sodium borohydride to the lactone (21). The isomeric lactol (26) was formed in such small amounts that its structure was assigned on the basis of its polarity in tlc and its reduction to the lactone (20) with sodium borohydride.





26

23 R = H, R' = CH_3

24 R = CH_3CO, R' = CH_3

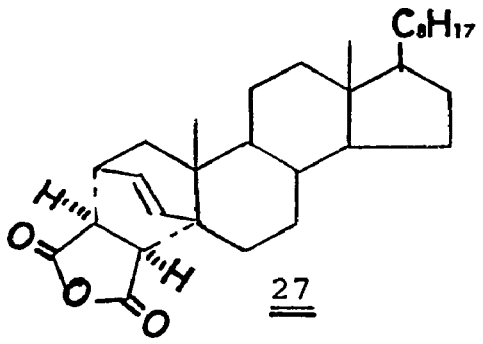
25 R = CH_3CO, R' = CH_3CO

The selectivity found in the reduction of the anhydride (14) was somewhat unexpected since Dreiding models do not indicate a large difference in the steric accessibility of the two carbonyl groups (the C-3' carbonyl is slightly more hindered by rings C and D). The pronounced selectivity observed with lithium aluminum hydride at -55° probably, therefore, reflects a preferred complex formation involving the slightly less hindered C-4' carbonyl, a lithium cation and a carbonyl group from another molecule. The small lithium cation would be highly solvated thus adding to the effective steric bulk of such a complex and enhancing the selectivity of its formation. The lesser selectivity

of sodium aluminum hydride reduction may reflect lesser solvation of the metal cation in the complex. The lack of selectivity with sodium borohydride is probably due to the high temperatures (90°) used which would lessen the selectivity of formation and the stability of the intermolecular complexes. The reductions studied by Bailey, and Johnson[9] in which high selectivity was found with sodium borohydride were carried out in either tetrahydrofuran or dimethylformamide at 0° to 25°. At these lower temperatures any solvated complexes would be more stable and thus effective in blocking reduction at the complexed carbonyl.

In order to investigate this mechanism further, it was decided to carry out analogous reductions on the anhydride (27), a Diels-Alder adduct of maleic anhydride and $\Delta^{2,4}$-cholestadiene (28). The Diels-Alder reaction between maleic anhydride and $\Delta^{2,4}$-cholestadiene was first reported by Stavely and Bergmann[33] but the adduct was reported as the diacid and not as the anhydride. The preparation of $\Delta^{2,4}$-cholestadiene by vacuum distillation of cholesterol from alumina was also described by these authors. However, in our hands after several attempts

only the $\Delta^{3,5}$ isomer (29) was ever obtained.



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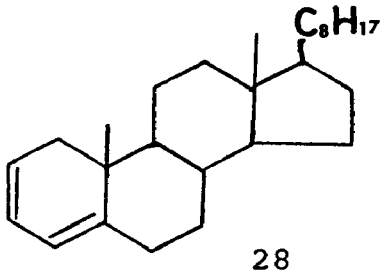

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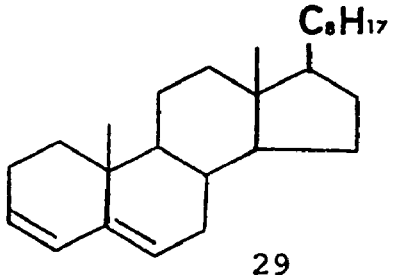

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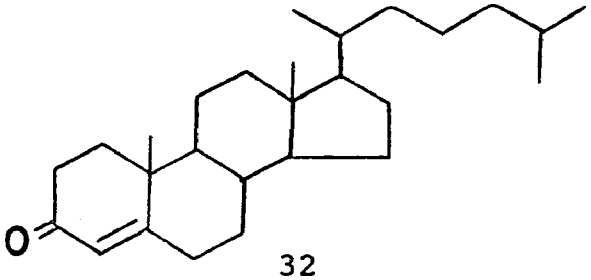
Since this method of dehydration of cholesterol gave no desired product, it was decided to pyrolyze the benzoate of Δ^4-cholesterol (30) in an attempt to obtain (28). The pyrolysis of esters of carboxylic acid is a cis elimination reaction (Scheme IV) and has been known to give good yields of olefins.[37] Δ^4-Cholesterol (31) was prepared by the reduction of Δ^4-cholestene-3-one (32), prepared by the method of Fieser,[36] with lithium aluminum hydride[35] or with sodium borohydride.

The elimination reaction was initially tried by refluxing in ethylene glycol but $\Delta^{3,5}$-cholestadiene was the only hydrocarbon product isolated. The $\Delta^{3,5}$-isomer



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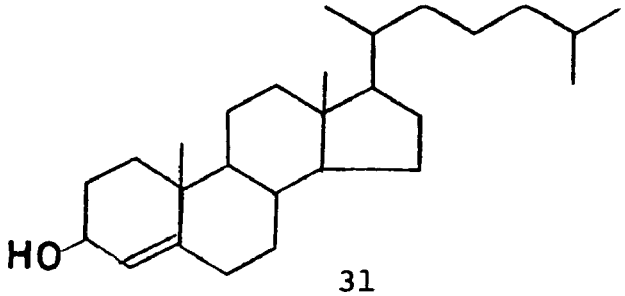

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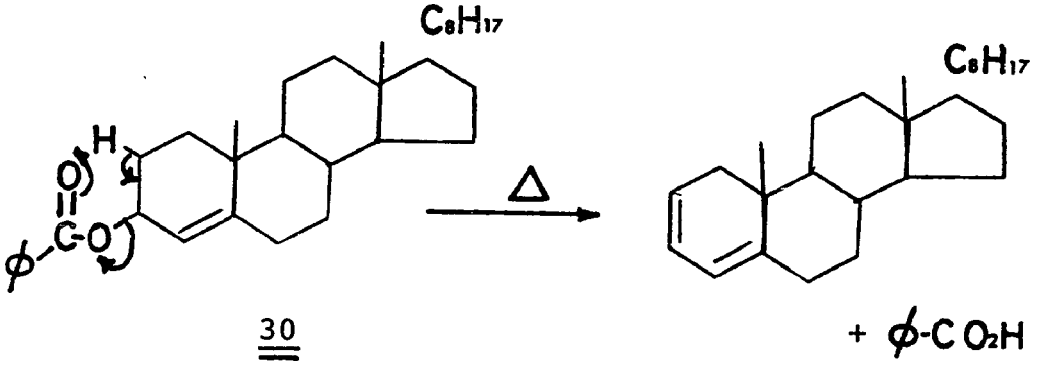
SCHEME IV



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was thought to arise by isomerization of the $\Delta^{2,4}$-cholestadiene by the benzoic acid. When the reaction was repeated with added base (diethylene triamine) to remove the benzoic acid, a small amount of the $\Delta^{2,4}$-isomer was formed, but the main product was $\Delta^{3,5}$-cholestadiene. This approach was, therefore, abandoned when a much simpler method of preparing $\Delta^{2,4}$-cholestadiene was found.

Since the described preparation of $\Delta^{2,4}$-cholestadiene involved dehydration of cholesterol and necessitated a double bond migration from the five to the four position, it seemed logical that Δ^{4}-cholesterol should itself dehydrate to $\Delta^{2,4}$-cholestadiene in good yield. Heating Δ^{4}-cholesterol with basis alumina under vacuum gave a hydrocarbon product which was approximately 85% $\Delta^{2,4}$ isomer as determined by UV spectroscopy.

Refluxing the hydrocarbon (N_2) mixture with maleic anhydride in dry xylene gave $\Delta^{3,5}$-cholestadiene, maleic anhydride and two new components; one, the major component R_f 0.55, present in much greater proportion than the other, the minor component, R_f 0. 45. Chromatography on silica gel afforded the major component free of contamination by the minor component. Unfortunately, the minor component could not be obtained free of the major component by any chromatographic means employed. However, the minor

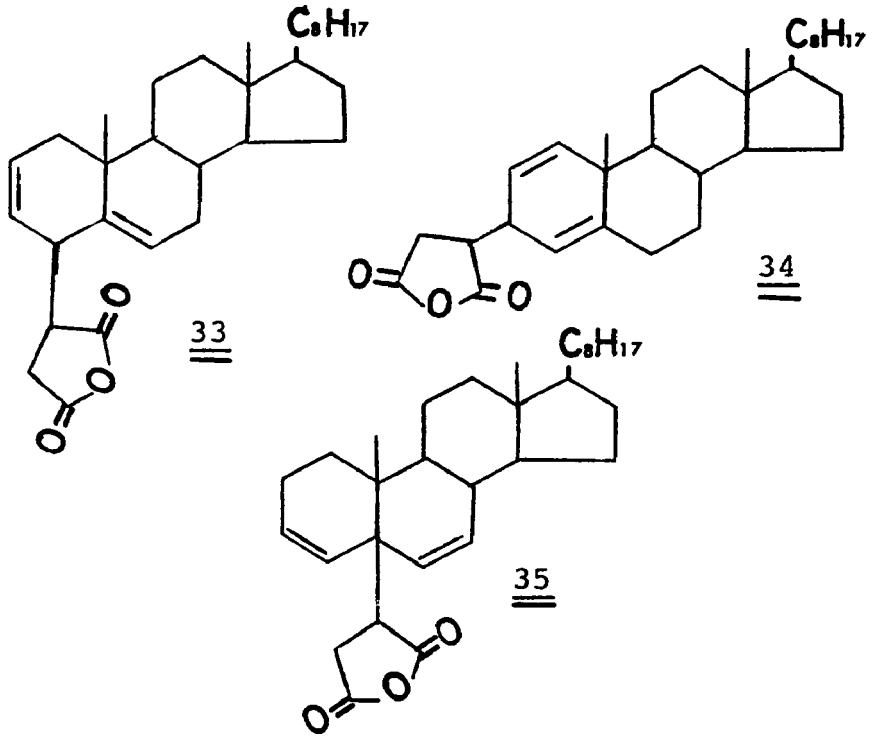
component is considerably less soluble in petroleum ether/acetone than the major component and could be obtained pure, after chromatography had removed most of the major adduct, by careful crystallization.

The major component ($C_{29}H_{46}O_3$) was assigned structure (27) on the following evidence. The mass spectrum showed a molecular ion peak at m/e 466 (calculated for $C_{29}H_{46}O_3$, 466) and the infrared showed strong anhydride carbonyl bands at 1835 and 1756 cm^{-1} (KBr). The vinyl region of the NMR shows an AB quartet (C-3 and C-4) at δ 6.1, τ 3.9, (J = 9 Hz) with the low field doublet of the quartet further split into a doublet of doublets by C-2 (J = 7 Hz). Irradiation of the C-2 proton (at δ3.1, τ6.9) caused the collapse of the complex vinyl pattern to a clean AB quartet.

The orientation of the anhydride moiety is assigned to be α on the basis of the strong shielding of the C-19 methyl group. In this α-orientation, models show that the shielding cone of the 3,4-double bond faces directly towards the C-19 methyl causing the C-19 methyl to resonate at δ0.89, τ9.11. This value is 0.05 p.p.m. higher field than the C-19 methyl of the Diels-Alder adduct of ergosteryl methyl ether and maleic anhydride (14) in which it is known that the α-orientation of the anhydride moiety causes the C-19 to be in the shielding region of the 6,7-

double bond. If the orientation were β, the C-19 methyl would be expected to resonate at much lower field, since models show that it would be deshielded by the 2,3-double bond. Analogous arguments have been used by Huisman et. al., to allocate the α-endo structure to Diels-Alder adducts of 3-acetoxycholesta-5,6,9(11)-triene with maleic anhydride[41] and by Thomas et al., in their structure determination of the Inhoffen adduct [25] of ergosteryl acetate and maleic anhydride.

The structure of the minor component has not been thoroughly investigated and is still unknown as yet. The compound, $C_{29}H_{46}O_3$, is an anhydride adduct as evidenced by carbonyl bands at 1835 and 1765 cm^{-1} in the IR (KBr) and is isomeric with the Diels-Alder adduct (27) as evidenced by the mass spectrum (M^+ 466) and combustion analysis. The normal "ene" adducts[40] expected from $\Delta^{2,4}$-cholestadiene are (33) and (34). These structures, however, do not agree with the NMR spectrum of the compound. The NMR has two vinyl protons at 6.13 while (33) requires three vinyl protons and (34) requires four vinyl protons. The adducts expected from "ene" reactions with $\Delta^{3,5}$-cholestadiene, (33) and (35) also do not agree with the NMR data.



33 34 35

The possibility that the minor component may be an isomerized "ene" adduct has been considered but not yet pursued exhaustively. That there is no conjugation in the system is shown by the lack of any significant ultraviolet absorption (λ_{max} 235, ε 200) other than that of the anhydride carbonyls.

The reduction of the Diels-Alder adduct (27) has been carried out but no conclusive results have been obtained. Reductions with sodium borohydride in refluxing dioxane gave two products (by tlc). Column chromatography of the material from this reduction separated the two components from the other materials. The IR of the mixture

showed carbonyl absorption at 1735 cm^{-1} which is characteristic of normal esters of δ-lactones but not of γ-lactones. Attempts to isolate the compounds by preparative tlc were not successful. The project has been postponed at this stage because it seems clear that unanticipated differences between this system and the ring B diene adduct systems explored previously are great enough to warrant a fresh future investigation.

Conclusions

The selective reduction of anhydrides is a potentially useful synthetic method of preparing the γ-lactone function. The general utility of this method, however, needs to be explored further. During such investigations additional data relating to the validity of our mechanistic proposals will undoubtedly become available.

CHAPTER III

EXPERIMENTAL

Preparation of Ergosteryl Acetate. Ergosterol (Aldrich, 50 g) was dissolved in acetic anhydride (500 ml) and the solution refluxed for one hour. The hot solution was poured slowly into a large volume of water (2.5 l) and allowed to stand 24 hours; the yellow solid was filtered off with suction and washed with water. The damp filter cake was recrystallized from ethyl acetate containing sufficient methanol to prevent separation of the water. Ergosteryl acetate (40 g) was obtained as colorless plates (80% yield). m.p.179-180°, Lit. 181.

Preparation of the Inhoffen Adduct (10).[24,26] Ergosteryl acetate (40 g) and maleic anhydride (13 g) were added to dry xylene (300 ml) and refluxed (N_2) for 18 hours. The solution was concentrated under reduced pressure to a thick syrup and further heated (100°) under vacuum (0.1 Torr) for one hour to remove excess maleic anhydride. The yellowish glassy residue was dissolved in hot acetone (60 ml) and allowed to stand for two days whereupon the Diels-Alder adduct (10) crystal-

lized from solution as colorless cubes (5.4 g). m.p. 217-218°, Lit.[24,26] 216°.

Preparation of the Hydroxy Dimethyl Ester (16).

The Inhoffen adduct (10) (5.0 g) was added to 5% methanolic potassium hydroxide (100 ml) and refluxed for 45 minutes. The solution was poured into water (500 ml), acidified with dilute HCl and extracted with ether (3 x 100 ml). The ether solution was dried ($MgSO_4$) and reduced in volume to 100 ml. Ethereal diazomethane was added slowly until the yellow color due to excess diazomethane persisted. The excess diazomethane was destroyed by dropwise addition of acetic acid and the ether solution concentrated under reduced pressure. Upon cooling the dimethyl ester crystallized as colorless needles (4.9 g). m.p. 163-164°, Lit.[26], 164-166°.

Methylation of the Hydroxy Dimethyl Ester (16).

Ethereal diazomethane was gradually added at 0° (N_2) to a solution of the hydroxy dimethyl ester (16) (13 g) in anhydrous ether (250 ml) containing aluminum chloride (0.2 g) until the yellow color persisted. Further aluminum chloride (0.2 g) was then added and the solution stirred at 0° for 30 min. Excess diazomethane was destroyed by dropwise addition of acetic acid, and 2 N

HCl was then added. The ether layer was dried (Na_2SO_4) and concentrated to an oil, which crystallized from a concentrated solution in methanol, giving the methoxy dimethyl ester (17) (12 g) as stout prisms: m.p. 92-93°; $[\alpha]^{23}D$ -54° (c 1.0, $CHCl_3$); IR ν^{KBr} 1748 cm^{-1} (ester C=O); nmr τ3.76, 4.01 (2H, AB q, J = 8 Hz, C-6, C-7 H), 4.85 (2H, m, C-22, C-23 H), 6.45, 6.55 (3H each, singlets, $COOCH_3$), 6.63, 7.21 (2H, AB q, J = 10 Hz, C-1', C-2' H), 6.73 (3 H, singlet, C-3 OCH_3), 8.96 (3H, s, C-19 CH_3), 9.23 (3H, s, C-18 CH_3). Mass spectrum: 555(2), M^+ 554(5), 524(2), 523(4), 522(2), 411(7), 410(24), 409(15), 408(16), 379(12), 378(62) x 0.5, 377(100) x 0.5, 364(3), 363(7), 338(2), 337(5), 285(2), 253(3), 251(3), 225(2), 211(3), 199(2), 197(2), 189(2), 185(2), 171(2), 169(2), 161(2), 159(6), 158(2), 157(7), 145(10), 143(2), 135(2), 133(2), 131(2), 125(13), 123(2), 121(2), 119(3), 117(2), 113(16), 111(2), 109(4), 107(5), 105(2), 97(2), 93(4), 91(2). Anal. Calcd. for $C_{33}H_{54}O_5$: C, 75.77; H, 9.81.

Found: C, 75.67, H, 9.88.

Synthesis of the Methoxy Anhydride (14). The methoxy dimethyl ester (17) (6 g) was dissolved in warm propylene glycol (90 g) (N_2) and after gradual addition of 50% aqueous potassium hydroxide (17.4 g) the mixture was heated

under reflux (N_2) for 3-4 hours. The reaction mixture was poured into a large volume of 2 N HCl which caused an acidic product (5.8 g) to be precipitated.

This material (2 g) was heated under reflux with acetic anhydride (15 ml) for 1 hour. Cooling the mixture caused crystallization of the anhydride (14) (1.7 g), essentially pure. One recrystallization from acetic anhydride gave the analytical sample as needles: m.p. 169-170°; $[\alpha]^{23}_D$ -32° (c 1.0, $CHCl_3$); IR ν^{KBr} 1850, 1775 cm^{-1} (anhydride C=O); nmr given fully in text. Mass Spectrum: 509(2), M^+ 508(6), 465(8), 411(8), 410(20), 409(14), 384(4), 383(15), 182(10), 381(32), 380(5), 378(5), 377(15), 368(4), 367(4), 366(4), 363(5), 356(5), 355(15), 352(5), 351(15), 337(5), 326(4), 325(5), 326(4), 325(5), 323(4), 313(4), 312(18), 311(43), 310(4), 285(17), 284(33), 283(53), 282(9), 281(6), 280(4), 279(8), 269(4), 254(7), 253(32), 252(10), 251(17), 239(5), 237(8), 227(4), 225(6), 213(5), 212(4), 211(19), 209(5), 199(6), 198(4), 197(10), 196(5), 195(10), 194(37), 193(5), 192(20), 189(6), 187(5), 185(10), 181(6), 173(8), 171(12), 170(5), 169(11), 167(7), 166(13), 161(10), 159(18), 157(28), 155(15), 149(10), 147(10), 145(20), 144(10), 143(25), 142(10), 141(12), 138(10), 137(14), 135(11), 133(20), 131(22), 130(10), 129(22),

128(11), 126(15), 125(75), 124(17), 123(37), 121(19), 119(27), 118(12), 117(58), 116(17), 115(10), 111(27), 110(20), 109(60), 108(12), 107(55), 101(10), 100(12), 99(19), 98(12), 97(54), 96(59), 95(100), 94(23), 92(10), 91(45).

Anal. Calcd. for: $C_{33}H_{48}O_4$: C, 77.91; H, 9.51.
Found: C, 77.87, H, 9.37.

Reaction of the Methoxy Anhydride (14) with Sodium Borohydride. Sodium borohydride (250 mg) was added to a solution of the methoxy anhydride (14) (1.7 g) in freshly distilled dioxane (17 ml) and the mixture heated under reflux for 3 hours. The solution was let cool, diluted with water and extracted with ether. The ether layer was dried ($MgSO_4$) and concentrated to a clear oil, which was chromatographed in benzene on alumina (Fisher, alumina for chromatography, 50 g). Elution with benzene rapidly gave a lactonic fraction (by IR, 0.7 g). This material was chromatographed on two E. Merck 2.0-mm silica gel preparative layer plates. Each plate was developed nine times with pentane-ether (9:1), giving two well-resolved bands (UV visualization). Each band from both dried plates was excised, powdered, and mixed with the equivalent from the other plate. The adsorbents were then exhaustively extracted (Soxhlet) with ether.

The ethereal extract of the material from the slower moving band crystallized on concentration to give the methoxy lactone (21) as needles (275 mg): m.p. 161-163°; $[\alpha]^{23}_D$ -87° (c 1.0, $CHCl_3$); ORD, negative Cotton effect, $[\alpha]_{MeOH}$ -4200° at 215 nm, the first extreme; λ_0 208 nm; CD, negative, trough not observable under the conditions employed; IR ν^{KBr} 1760 cm^{-1} (γ-lactone; nmr given in text. Mass spectrum: 495(8), M^+ 494(20), 451(5), 412(7), 411(33), 410(100), 397(4), 396(8), 379(4), 378(15), 177(38), 370(5), 369(14), 368(7), 367(20), 364(4), 363(12), 354(4), 341(7), 338(5), 337(12), 313(8), 312(22), 286(4), 285(15), 284(13), 283(7), 254(5), 253(14), 251(4), 225(4), 211(8), 199(4), 197(7), 189(4), 185(5), 183(5), 180(5), 175(7), 171(5), 169(5), 161(4), 159(10), 158(4), 157(13), 155(5), 149(5), 147(5), 145(10), 144(6), 143(15), 142(5), 141(5), 137(5), 135(5), 133(7), 132(4), 131(20), 130(4), 129(12), 128(10), 127(3), 126(4), 125(32), 124(4), 123(7), 121(7), 119(12), 118(5), 117(19), 116(5), 115(10), 111(6), 109(17), 107(16), 105(40), 103(15), 87(6), 96(5), 95(15), 94(8), 93(20), 91(29).

Anal. Calcd for $C_{33}H_{50}O_3$: C, 80.11; H, 10.19.
Found: C, 80.30; H, 10.17.

The ethereal extract of the faster moving band also crystallized on concentration to give the methoxy lactone

(20) as needles (290 mg): m.p. 168-170.5°; $[\alpha]^{23}_D$ -9° (c 1.0, $CHCl_3$); ORD, positive followed by negative Cotton effects, peak at 230 nm, $[\alpha]_{MeOH}$ +200°, trough at 209 nm, $[\alpha]_{MeOH}$ -3450°; λ_0 224 nm; CD, positive Cotton effect, peak at 229 nm, $[\theta]_{MeOH}$ + 5000°; IR ν^{KBr} 1750 cm^{-1} (γ-lactone); nmr given in text. Mass spectrum: 495(5), M^+ 494(13), 481(4), 480(15), 479(43), 412(6), 411(33), 410(100), 379(5), 378(15), 377(6), 369(5), 367(5), 364(5), 363(15), 339(8), 337(18), 253(13), 211(6), 197(5), 195(4), 194(18), 185(6), 183(5), 175(10), 171(6), 161(7), 159(8), 158(5), 157(15), 155(5), 149(21), 147(5), 145(10), 144(5), 143(14), 141(5), 137(5), 135(6), 133(7), 131(11), 129(10), 126(5), 125(88), 119(13), 117(18), 111(10), 109(17), 107(15), 105(14), 97(12), 96(10), 95(15), 93(21), 91(17).

Anal. Calcd. for $C_{33}H_{50}O_3$: C, 80.11; H, 10.19.
Found: C, 80.23; H, 10.10.

Methylation of the Hydroxy Lactone (22) to the Methoxy Lactone (21). To a solution of the hydroxy lactone (22) (50 mg) in anhydrous ether (10 ml) at 0°, anhydrous aluminum chloride (10 mg) was added, followed by excess ethereal diazomethane until the yellow color persisted briefly. Further aluminum chloride and diazo-

methane solution were then added. After 30 minutes of stirring at 0°, glacial acetic acid was added dropwise to destroy excess diazomethane. Work-up as in the methylation of (17) described above gave a material shown by tlc to comprise the methoxy lactone (21) and a small amount of the hydroxy lactone (22). No methoxy lactone (20) was observed.

Reductions of Lactone (20) and (21) with Lithium Aluminum Hydride. The lactone (20) (50 mg) was heated under reflux in dioxane (5 ml) with lithium aluminum hydride (10 mg) for 4 hours. The reaction was quenched with aqueous dioxane and worked up via 2 N HCl and ether. The product was the methoxy diol 11 (20 mg), which crystallized from ethyl acetate hexane as needles: m.p. 190.5-191.5°; $[\alpha]^{23}D$ -60° (c 1.0, $CHCl_3$); ν^{KBr} 3400 cm^{-1} (OH).

Anal. Calcd for $C_{33}H_{54}O_3$: C, 79.46; H, 10.92.
Found: C, 79.32; H, 10.90.

Lactone (21) reduced in the same way, gave the same methoxydiol.

Reductions of the Methoxy Anhydride (14) with Lithium Aluminum Hydride and Sodium Aluminum Hydride. 1. With Lithium Aluminum Hydride. A solution of the methoxy an-

hydride (14) (500 mg) in tetrahydrofuran (5 ml) was added dropwise to a stirred solution of lithium aluminum hydride (40 mg) in tetrahydrofuran (5 ml) at -55° (acetone CO_2 bath). The mixture was let warm to -5° during 90 minutes and stirred at this temperature for 30 minutes after which it was acidified (6 N HCl) and partioned between water and ether. The organic material obtained by concentration of the dried (Na_2SO_4) ether layer was chromatographed as described for the reduction of (14) with sodium borohydride above. Lactones (21) and (20) were obtained pure in yields of 200 and 15 mg, respectively. They were identified by tlc and ir.

2. With Sodium Aluminum Hydride. From 525 mg of anhydride 14 and 100 mg of sodium aluminum hydride (Ventron), lactone (21) and (20) were obtained in yields of 275 and 30 mg, respectively, using the same procedure as above.

Reduction of the Methoxy Anhydride (14) with Lithium Aluminum Tri-tert-butoxyhydride. A solution of the anhydride (14) (1 g) in tetrahydrofuran (25 ml) was added dropwise to a stirred solution of lithium aluminum tri-tert-butoxyhydride (2.0 g) in tetrahydrofuran (25 ml) at 0°. After 5 hours at 0°, the reaction was quenched with excess 2 N HCl, and the mixture partitioned

between water and ether. The ether layer was dried ($MgSO_4$) and concentrated, and the organic product chromatographed on preparative silica gel plates as in experiments described above, except that pentane-ethyl acetate (10:1) was used as developing solvent. After four developments, three bands were discerned: one at the origin and two above it. Extraction of the lower two of these as above gave the same material which was the lactol (23) obtained from ether as fine needles (650 mg): m.p. 234-237° dec; $[\alpha]^{23}D$ -51° (c 1.0, $CHCl_3$): IR ν^{KBr} 3404, 3230 (OH), 1770 (γ-lactone), 1735 cm^{-1} (aldehyde and acid C=O); ν^{CHCl_3} 3300 (OH), 1765 cm^{-1} (γ-lactone). Anal. Calcd for $C_{33}H_{50}O_4$: C, 77.60; H, 9.87.
Found: C, 77.68; H, 9.69.

Treatment of this compound (50 mg) with acetic anhydride (2 ml) under reflux (N_2) for 3 hours and removal of solvent under reduced pressure gave a slowly crystallizing oil, which after two recrystallizations from ethyl acetate-hexane gave the pure lactol acetate (24) as needles: m.p. 170-172°; IR ν^{KBr} 1780 (γ-lactone C=O), 1755 cm^{-1} (lactol acetate C=O); nmr τ 3.69, 4.12 (2H, AB quartet, J = 10 Hz, C-6, C-7 H), 3.87 (1H, broadened singlet C-3' H), 4.82 (2H, m, C-22, CH23 H), 6.63 (3H, singlet, C-3 OCH_3), 7.85 (3H, singlet, lactol CH_3COO), 8.91

(3H, singlet, C-19 CH_3), 9.24 (3H, singlet, C-18 CH_3).

Anal. Calcd for $C_{35}H_{52}O_5$: C, 76.04; H, 9.48.

Found: C, 76.13; H, 9.50.

The highest band in the preparative tlc from the reduction was present in very small amount. Analytical tlc of this band showed it to contain two compounds, one identical with the methoxy lactone (21). Reduction of a very small sample of this mixture with sodium borohydride in ethanol and work-up as above gave (tlc) a mixture containing methoxy lactone (21) and (20) only.

Similar sodium borohydride reduction of the pure lactol (23) gave the methoxy lactone (21).

Reduction of Δ^4Cholesten-3-one with Lithium Aluminum Hydride to Δ^4Cholesterol.[35] Lithium aluminum hydride (0.6 g) was added to anhydrous diethyl ether (150 ml) and stirred for 10 minutes at 25°. A solution of Δ^4-cholesten-3-one (10 g) in anhydrous diethyl ether (50 ml) was added slowly during 35 minutes. The reaction was stirred for an additional hour at 25°, quenched cautiously with water and the precipitated aluminum hydroxide filtered off. The ether solution was dried ($MgSO_4$) and evaporated under reduced pressure to give a mixture of 3α and 3β-Δ^4-cholesterol (10 g), m.p. 126-128°. Tlc of the mixture

showed it to be predominantly one isomer as reported[35] with small amounts of the 3α isomer. Since the isomers also form a 1:1 molecular complex (m.p. 141°)[35] no attempt was made to separate the isomers and the mixture was used in subsequent reactions.

Reduction of Δ^4-Cholesten-3-one with Sodium Borohydride to Δ^4-Cholesterol. Sodium borohydride (2.0 g) was added to iso-propyl alcohol (300 ml) and stirred for 10 minutes. Δ^4-Cholesten-3-one (20 g) was added in one portion and the reaction stirred for 18 hours at 20°-25°. The solution was slowly acidified with 2 N HCl to pH 4 or 5, concentrated under reduced pressure to approximately one third of its original volume and diluted with a large volume of water. It was then extracted with ether, dried ($MgSO_4$) and concentrated under reduced pressure to give a mixture of 3α and 3β-Δ^4-cholesterol (19.2 g) which was used without further purification for the preparation of $\Delta^{2,4}$-cholestadiene.

Preparation of the Benzoate of Δ^4-Cholesterol. To a solution of Δ^4-cholesterol (2.5 g) in pyridine (20 ml) was added benzoyl chloride (1.7 g). The solution was stirred at 25° for 1.5 hours and then heated on a steam bath for 30 minutes. Water was added dropwise to the

hot solution until it was cloudy. Slow cooling to room temperature gave a solid product which was filtered off and recrystallized from ethyl acetate to give 1.7 g of the presumed 3β-benzoate, m.p. 124-126°.

Pyrolysis of Δ^4-Cholesteryl Benzoate. A solution of Δ^4-cholesteryl benzoate (0.25 g) in ethylene glycol (10 ml) containing diethylenetriamine (0.11 g) was refluxed for three hours. Work-up by pouring into water and ether extraction gave a yellow oil (0.2 g). Chromatography on Fisher basic alumina (5 g) and elution with petroleum ether (20-60°) gave 0.1 g of a colorless oil which solidified slowly on standing.

The per cent of $\Delta^{2,4}$-cholestadiene present was determined by UV spectroscopy by assuming that the solutions obey Beers Law and making use of the fact that at the λ_{max} of each compound (237 nm, $\varepsilon = 2 \times 10^4$ for $\Delta^{3,5}$-cholestadiene and 265 nm, $\varepsilon = 6.3 \times 10^3$ for $\Delta^{2,4}$-cholestadiene) there is insignificant overlap of the absorption curves.

$$\frac{c_1}{c_2} = \frac{A_1 \varepsilon_2 l_2}{A_2 \varepsilon_1 l_1}$$

A_1 = observed absorption at 265 nm ($\Delta^{2,4}$isomer)
A_2 = observed absorption at 237 nm ($\Delta^{3,5}$isomer)

$$= \frac{A_1}{A_2} \times \frac{2 \times 10^4}{6.3 \times 10^3}$$

$$= \frac{A_1}{A_2}(3.17)$$

$$\%\Delta^{2,4}\text{-isomer} = \frac{(c_1/c_2)}{1+\frac{c_1}{c_2}} \times 100\% = \frac{\frac{A_1}{A_2}(3.17)}{1+\frac{A_1}{A_2}(3.17)} \times 100\%$$

ε_1 = molar absorptivity of $\Delta^{2,4}$isomer
ε_2 = molar absorptivity of $\Delta^{3,5}$isomer
$l_1 = l_2$ = cell length (1 cm)

Using the above method the solid was approximately 25% $\Delta^{2,4}$-cholestadiene.

Dehydration of Δ^4-Cholesterol with Aluminum Oxide. Δ^4-Cholesterol (3.0 g) was ground together with Fisher basic alumina (4.5 g) which had been freshly activated by heating at 200° for 24 hours. The mixture was then heated at 170-180° for 45 minutes, 0.1 Torr, let cool and extracted (Soxhlet) with ether. Removal of the ether under reduced pressure gave a clear oil which was chromatographed on activity I alumina (10 g) in petroleum ether. Elution with 200 ml of petroleum ether gave an oil (2.6 g)which slowly solidified on standing; m.p. 59-65°. Using the method described in the previous experiment, the solid was determined to be 85% $\Delta^{2,4}$-cholestadiene.

Reaction of $\Delta^{2,4}$-Cholestadiene with Maleic Anhydride. Maleic anhydride (1.5 g) was added to xylene (250 ml)

and the mixture distilled under nitrogen until 50 ml of xylene was collected. The cholestadiene (3.3 g, 75% $\Delta^{2,4}$-isomer) were added and the solution refluxed (N_2) for 18 hours. The solution was concentrated under reduced pressure to a thick syrup, which was heated at 100°under vacuum (0.1 Torr) for one hour to remove excess maleic anhydride. Tlc of the residue showed the presence of two new components, R_f 0.45 and 0.55, the latter present in much greater proportion than the former. The thick glassy amber residue was dissolved in a minimum amount of petroleum ether and placed on a silica gel column (45 g). The column was eluted with petroleum ether (300 ml); 25% benzene/petroleum ether (200 ml); 50% benzene/petroleum ether (600 ml); 75% benzene/petroleum ether (400 ml); and 5% acetone/benzene (100 ml). Tlc analysis showed cholestadiene in fractions 6 through 9 and the major component (R_f 0.55) in fractions 20-40. The minor component R_f 0.45 was present in fractions 41-55 along with small amounts of the above major component.

The fractions containing only the major component (20-40) were combined and the solvent removed under reduced pressure. The residue was recrystallized from petroleum ether as white fine needles (1.6 g); m.p.

123-124° and 149-150°, $[\alpha]_D$ = +86°, c 0.23 ($CHCl_3$), IR (KBr): 1835, 1765 cm^{-1} (anhydride C=O), UV λ_{max} 230 nm, ε = 2560, nmr,δ 6.18, 5.96 (2H, ABX, J_{AB} = 8 Hz, J_{AX} = 7 Hz, C-3, C-4 H), 3.61, 3.07 (2H, AB_q, J = 9 Hz, C-3', C-4'); 3.07 (1H, d, J = 7 Hz, C-2), 0.89 (3H, s, C-19 CH_3), 0.66 (3H, s, C-18 CH_3). Mass spectrum 467(14), M^+ 466(42), 451(4), 423(2), 369(8), 368(41), 367(100), 353(4), 341(2), 327(2), 336(3), 313(5), 312(18), 298(2), 297(4), 290(2), 285(3), 284(4), 283(9), 247(4), 245(3), 228(5), 227(15), 214(3), 213(9), 212(4), 211(9), 199(2), 198(2), 197(2), 185(3), 177(8), 163(4), 159(3), 157(5), 150(6), 149(5), 147(3), 146(3), 145(5), 143(6), 137(2), 136(2), 135(9), 133(3), 131(10), 126(4), 123(6), 122(3), 121(7), 119(5), 117(5), 111(3), 109(12), 108(7), 107(13), 106(6), 105(15), 104(2), 97(6), 95(20), 93(10), 91(14). Anal. calcd for $C_{29}H_{46}O_3$: C, 79.78; H, 9.94.

Found: C, 79.77; H, 9.97.

The fraction containing the minor component (contaminated slightly with the major component) 41-55, were combined and concentrated to an oil. Careful crystallization from petroleum ether containing a small amount of acetone gave the pure minor component as colorless plates (0.2 g) m.p. 230-235°, $[\alpha]_D$ +90 (c 0.1, $CHCl_3$); IR (KBr) 1835, 1765 cm^{-1} (anhydride C=O);

NMR 6.26s, 6.21s, (2H), 3.14, s, 3.06 m (2H), 0.93 (3H s, C-19), 0.63(3H, s, C-18); UV λ_{max} 235 nm, ϵ = 200. Mass Spectrum: M^+ 466(4), 451(2), 400(2), 384(3), 369(3), 368(4), 367(6), 326(2), 313(2), 312(8), 311(9), 297(4), 283(3), 247(5), 245(2), 229(3), 228(2), 227(5), 220(2), 218(2), 217(3), 215(2), 214(2), 213(5), 212(3), 211(6), 205(6), 199(3), 185(4), 178(3), 177(10), 175(4), 173(3), 171(5), 169(3), 163(6), 161(5), 159(6), 158(3), 157(8), 155(4), 151(4), 150(10), 149(15), 148(5), 147(10), 146(6), 145(10), 144(5), 143(12), 142(3), 141(5), 137(7), 136(7), 135(20), 134(6), 133(12), 132(5), 131(19), 130(4), 129(10), 128(3), 127(2), 126(5), 124(4), 123(18), 122(15), 121(24), 120(9), 119(20), 118(10), 117(20), 116(3), 115(4), 111(12), 110(8), 109(40), 108(23), 107(54), 106(27), 105(70), 104(11), 103(3), 100(3), 99(6), 98(5), 97(30), 96(14), 95(90), 94(28), 93(80), 92(19), 91(100).

Anal. Calcd for $C_{29}H_{46}O_3$: C, 79.78; H, 9.94.
Found: C, 79.77; H, 9.88.

Reduction of Anhydride (27) with Sodium Borohydride.

The anhydride (350 mg) and sodium borohydride (77 mg) were added to 10 ml of freshly distilled p-dioxane and refluxed for three hours. The reaction was let cool, acidified to pH 1 with 5 N HCl and extracted with ether. The ether layer

was washed with 3% potassium carbonate solution and dried ($MgSO_4$). Removal of the ether under reduced pressure gave a colorless gum which chromatographed on a Woelm neutral alumina column (Activity I, 1.5 x 24 cm) in 50% benzene/petroleum ether. Elution with benzene (100 ml), 10% ethyl acetate/benzene (100 ml) and 20% ethyl acetate/benzene (200 ml) gave the desired products (0.1 g), as analyzed by tlc on silica gel, in fractions 16-38 (10 ml each). Tlc analysis of the recovered material showed two components in approximately equal quantities. IR of the mixture showed prominent bands at 2960 (C-H), 1735 (carbonyl C=O), 1380 and 1200 cm^{-1}. All attempted separations on preparative tlc using 9/1 n-pentane/ethyl ether, 50/50 benzene/petroleum ether or benzene failed to resolve the two components.

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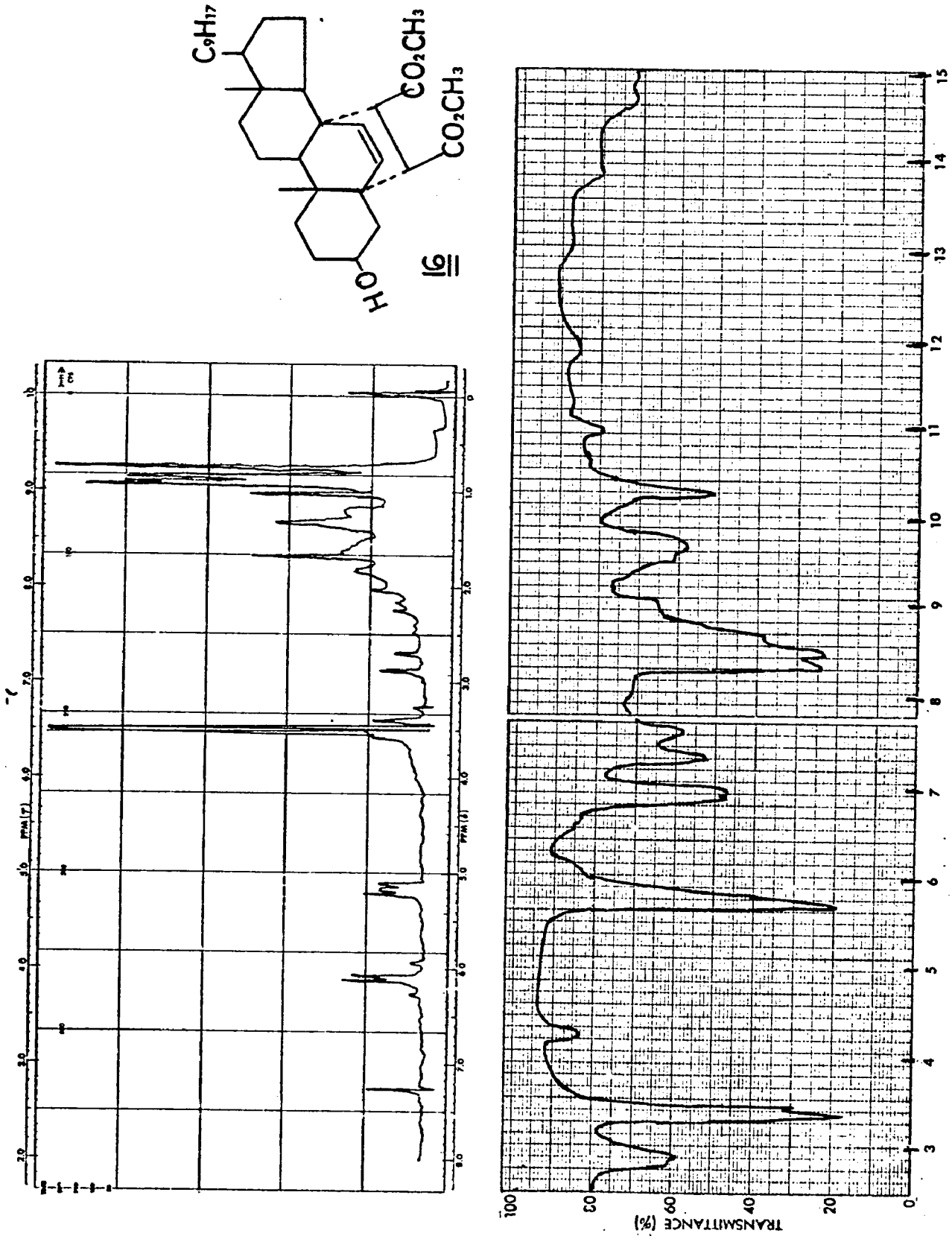
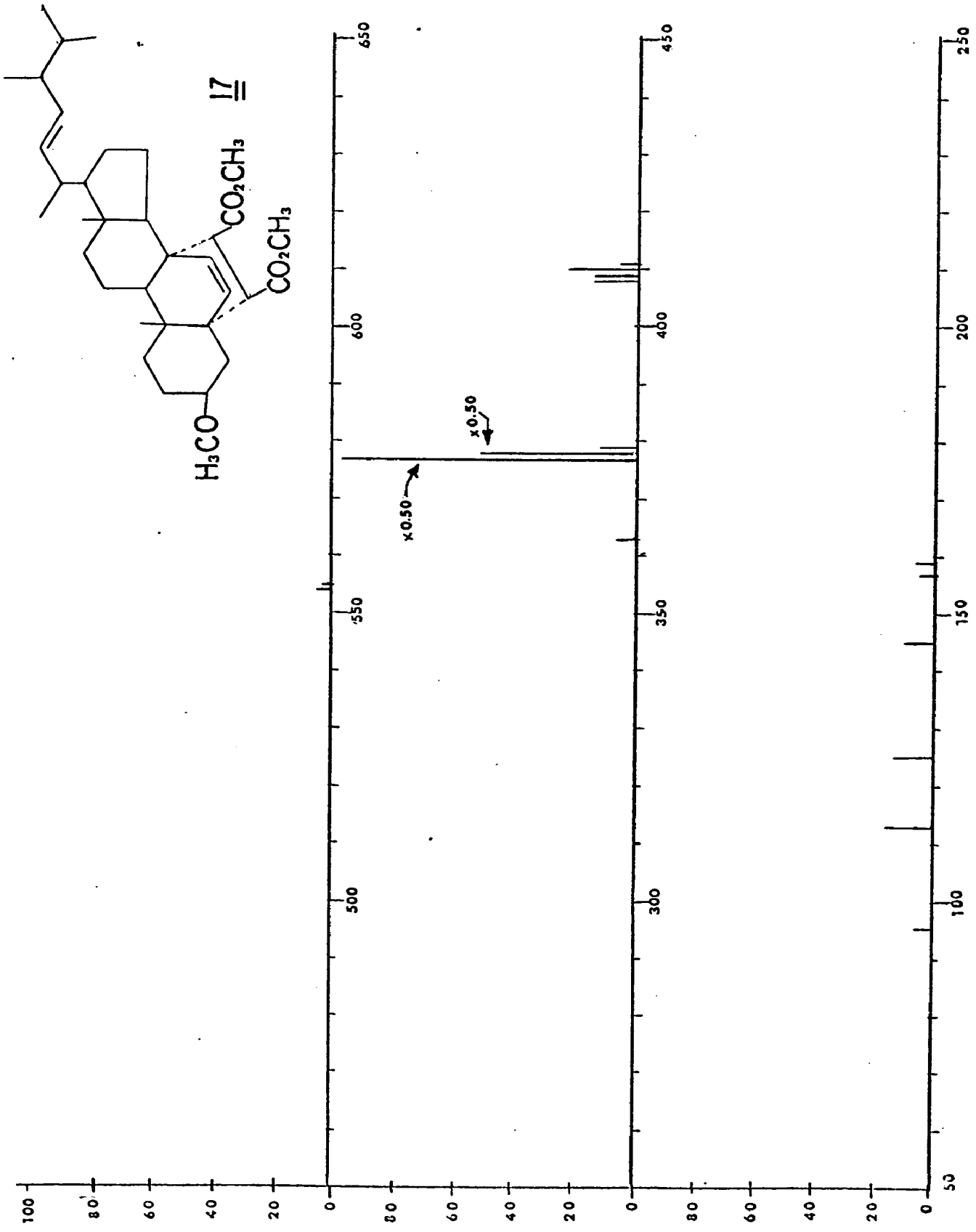
APPENDIX

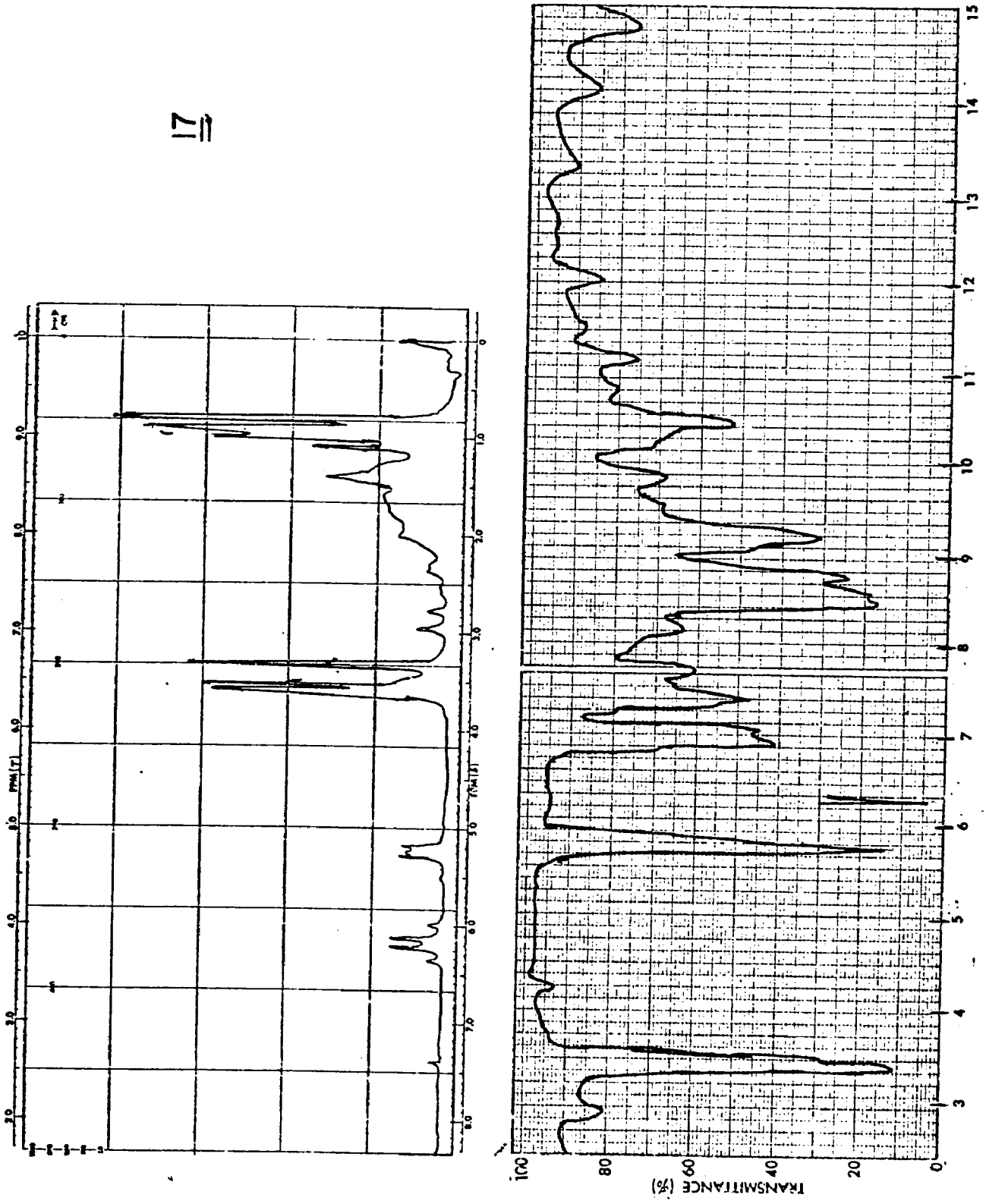
NMR, INFRARED AND MASS SPECTRA OF SELECTED COMPOUNDS*

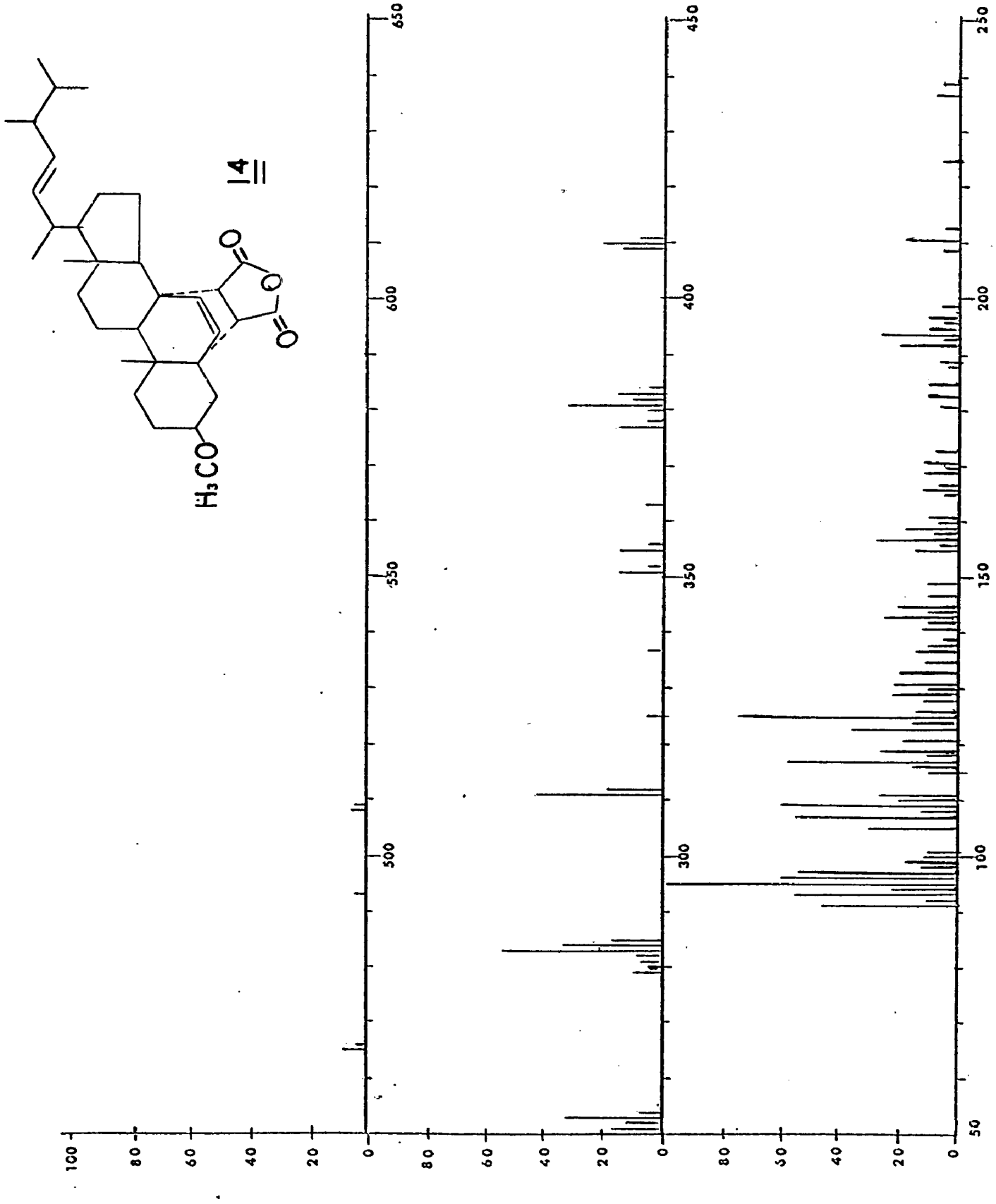
*The nmr and infrared spectra were obtained by reducing the originals with a pantograph.


C9H17
CO2CH3
CO2CH3
HO
16
TRANSMITTANCE (%)


H3CO
CO2CH3
CO2CH3
17
x0.50
x0.50

17




14
H3CO
O=O
O=O
100
80
60
40
20
0
80
60
40
20
0
80
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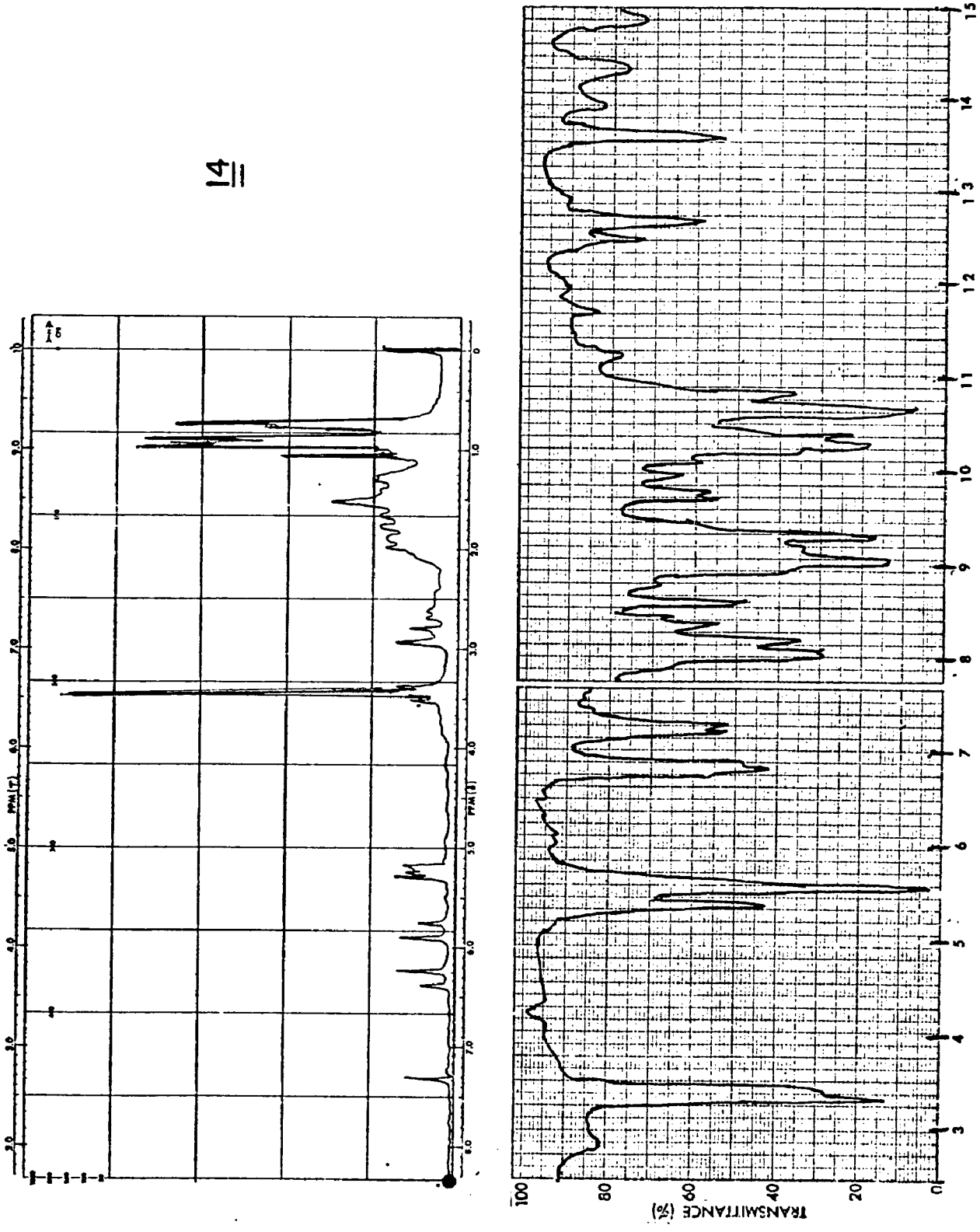
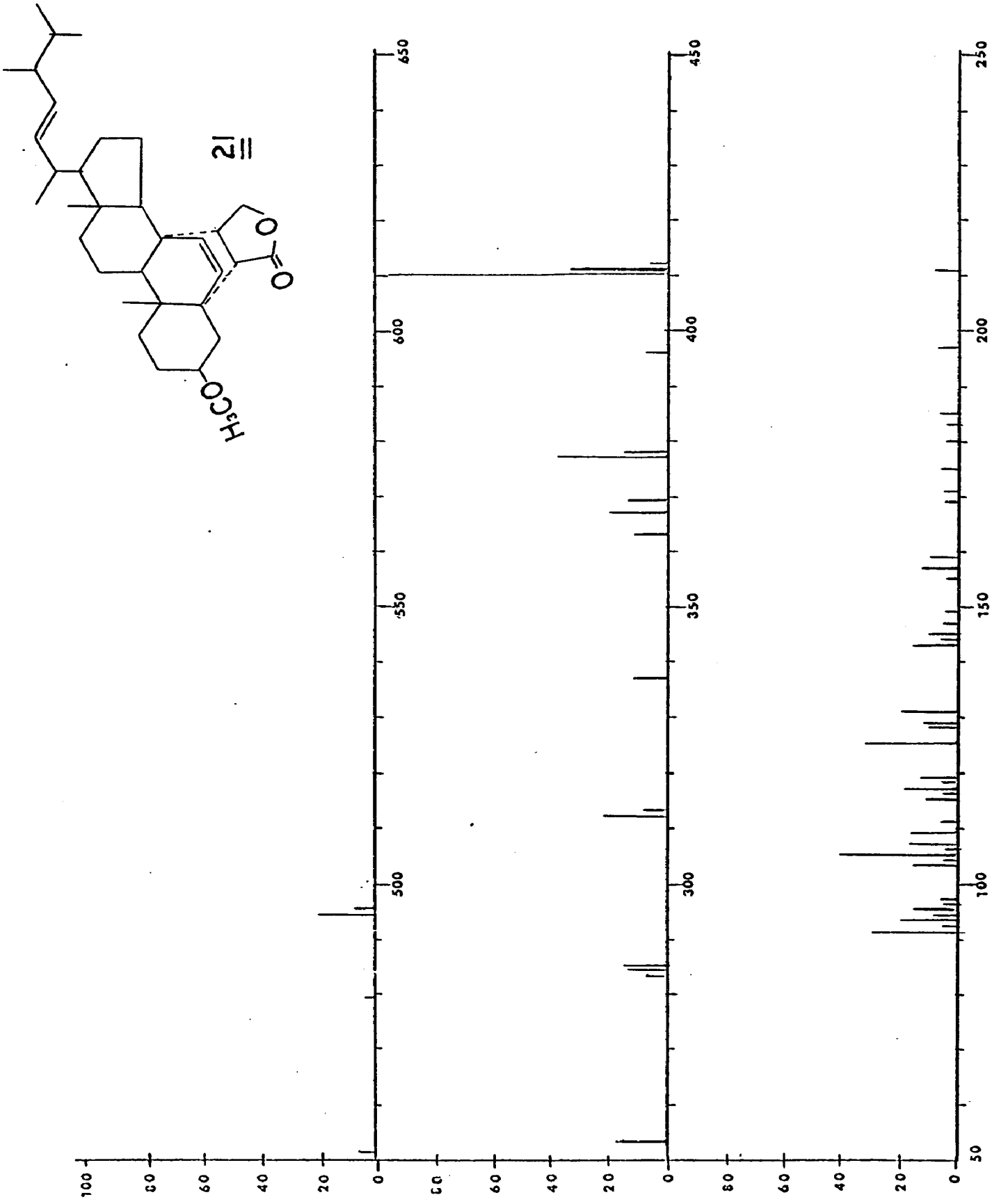
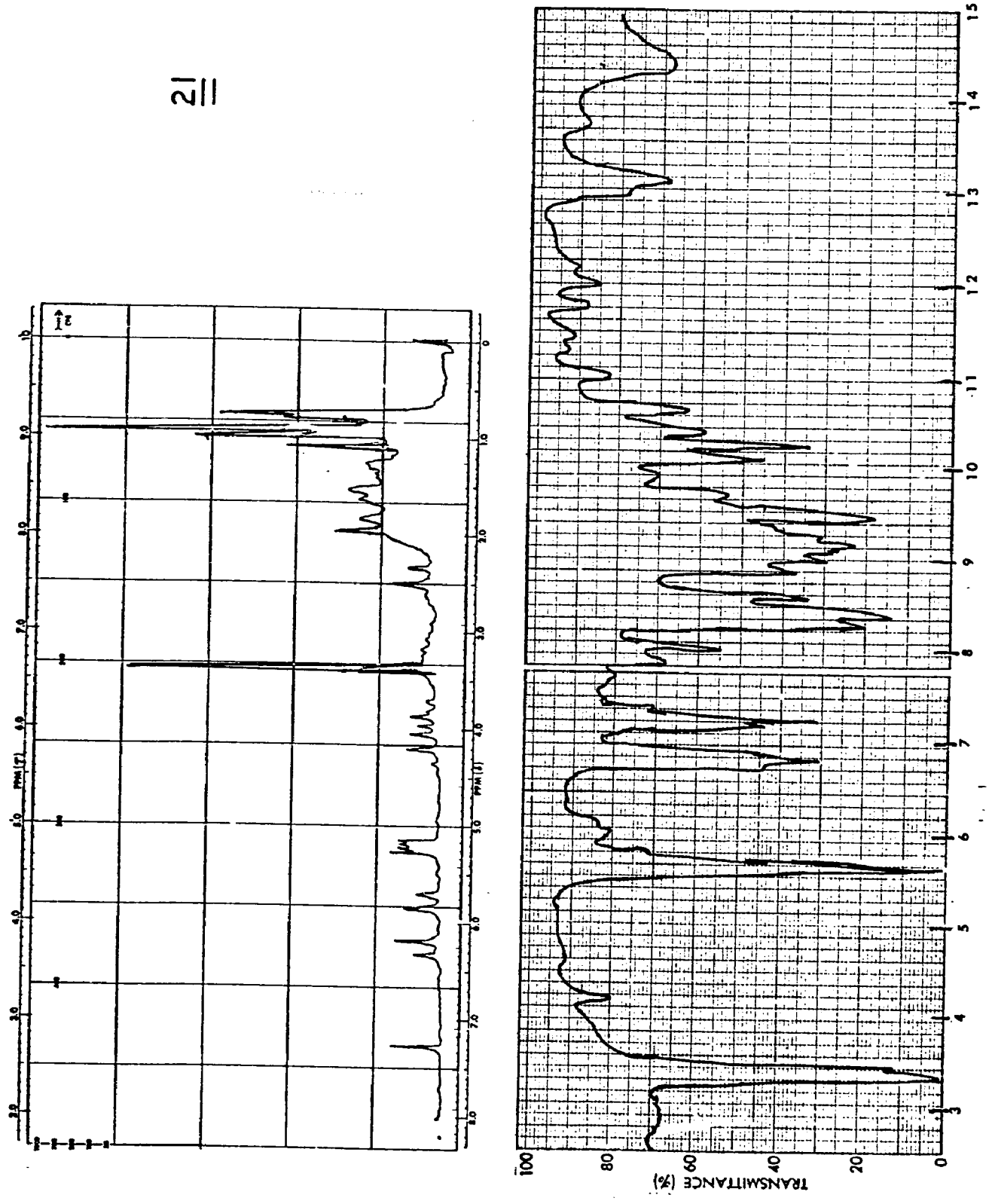
500
550
600
650

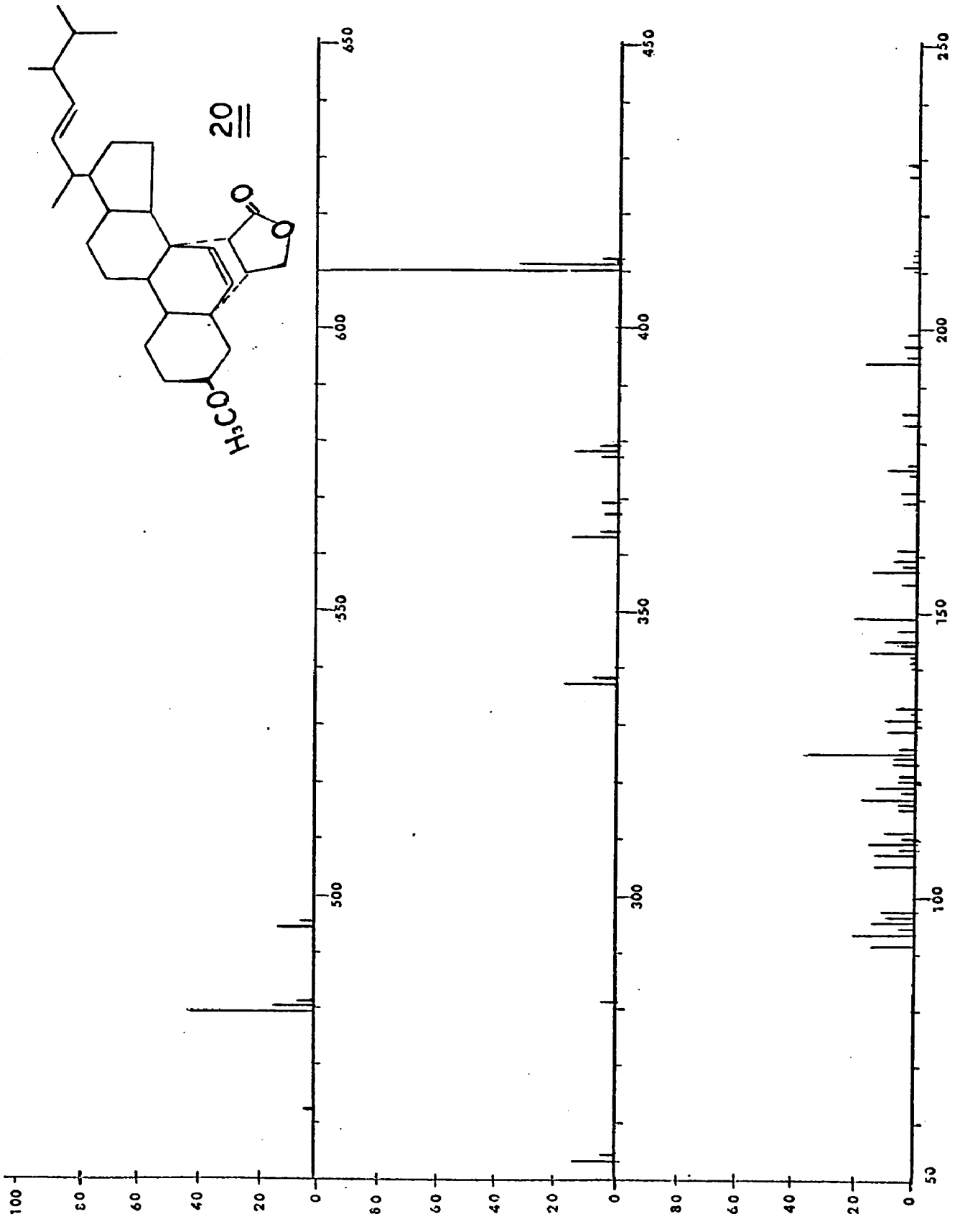
14




21
O
O
H3CO
100
80
60
40
20
0
500
550
600
650
300
350
400
450
100
150
200
250
50

21

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H3CO
O
O
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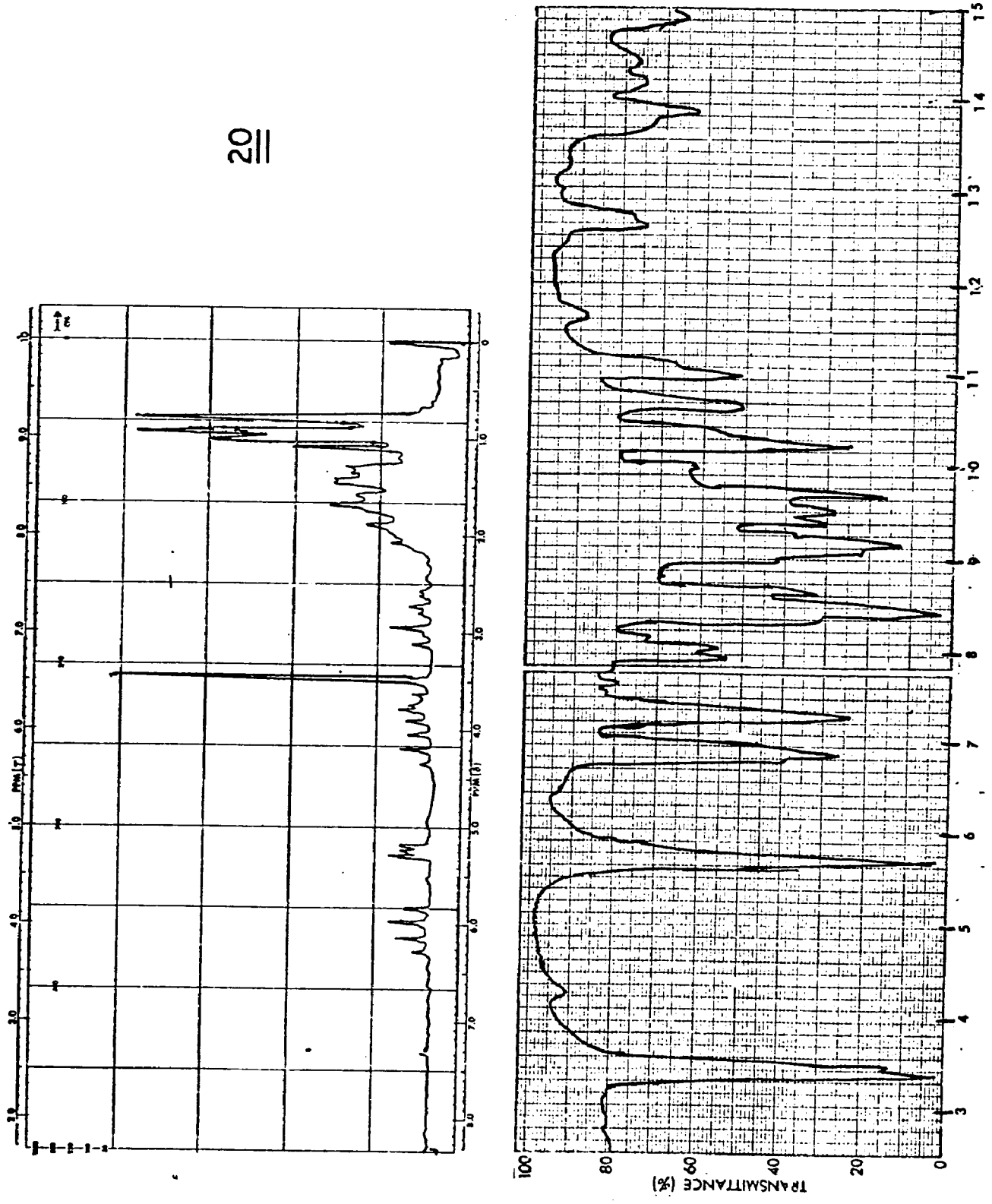
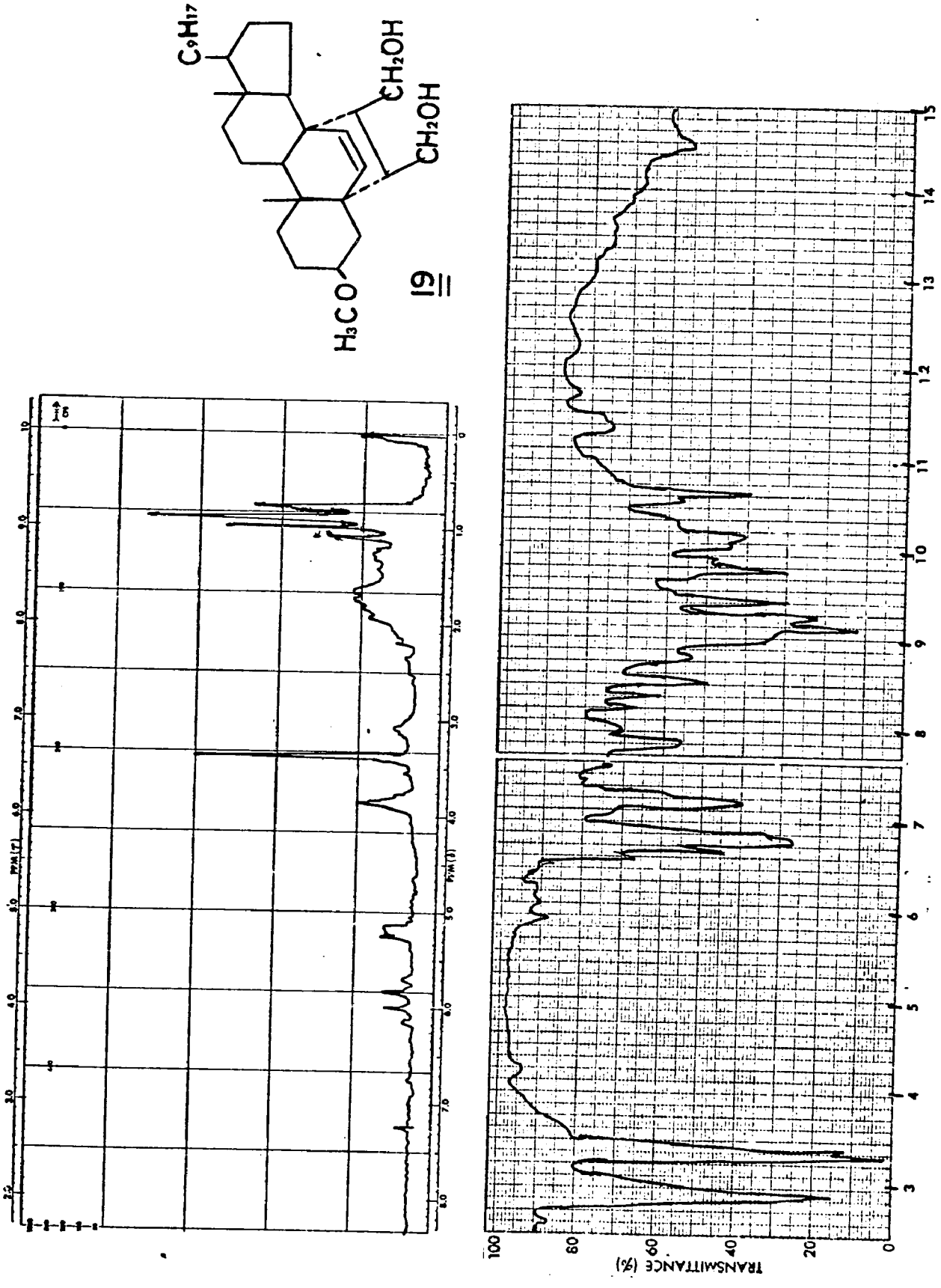
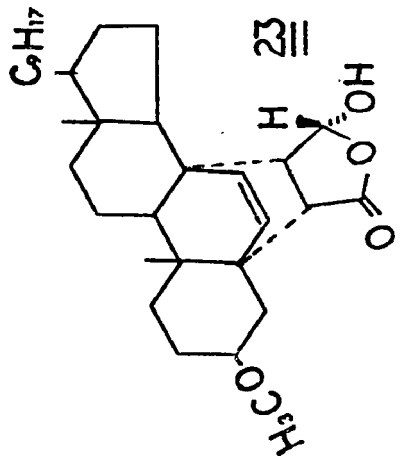
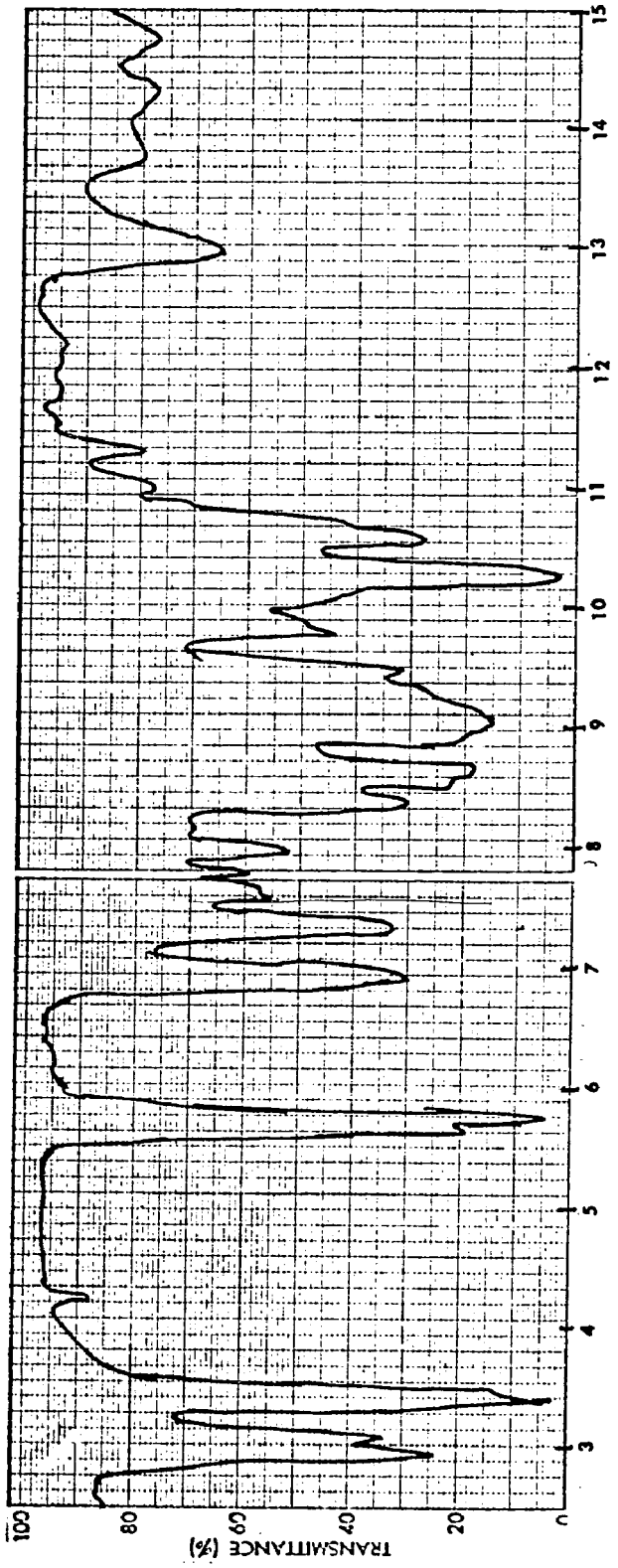
200
150
100
50

20




C9H17
CH2OH
CH2OH
H3CO
19
TRANSMITTANCE (%)
100
80
60
40
20
0
PPM(τ)
PPM(δ)


C9H17
23
H
HO
O
O
H3CO


TRANSMITTANCE (%)
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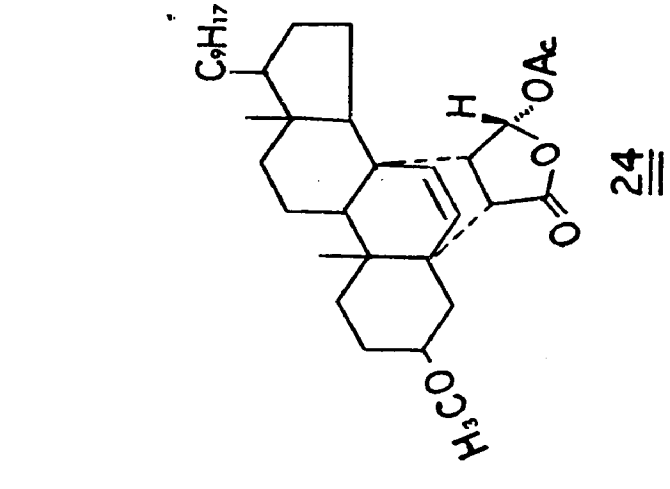
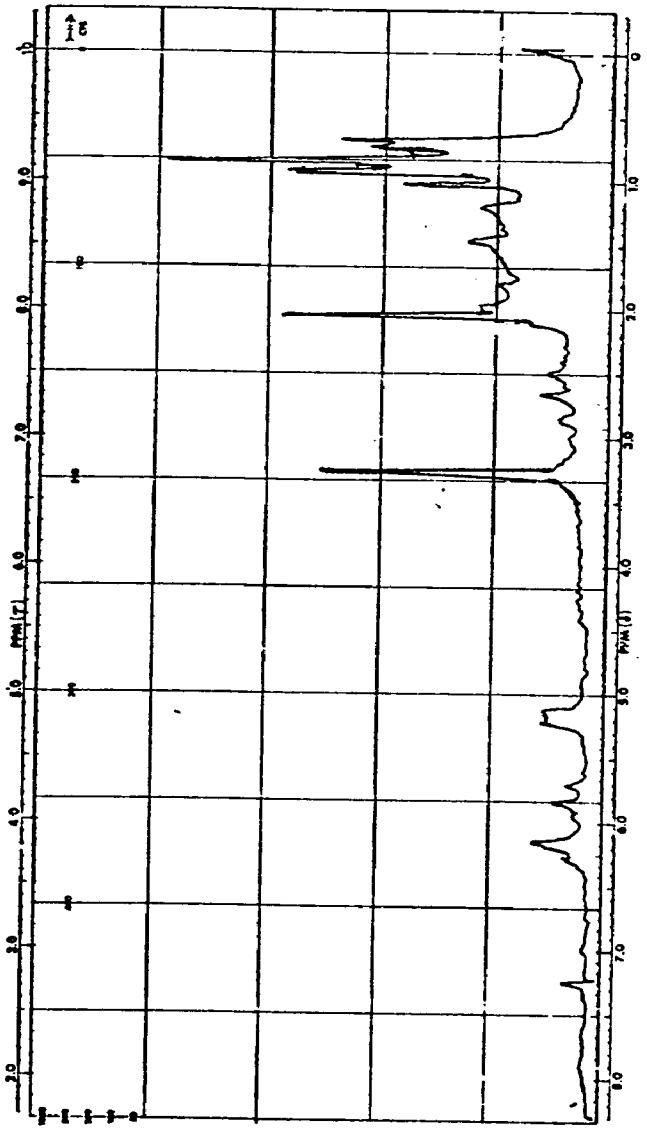
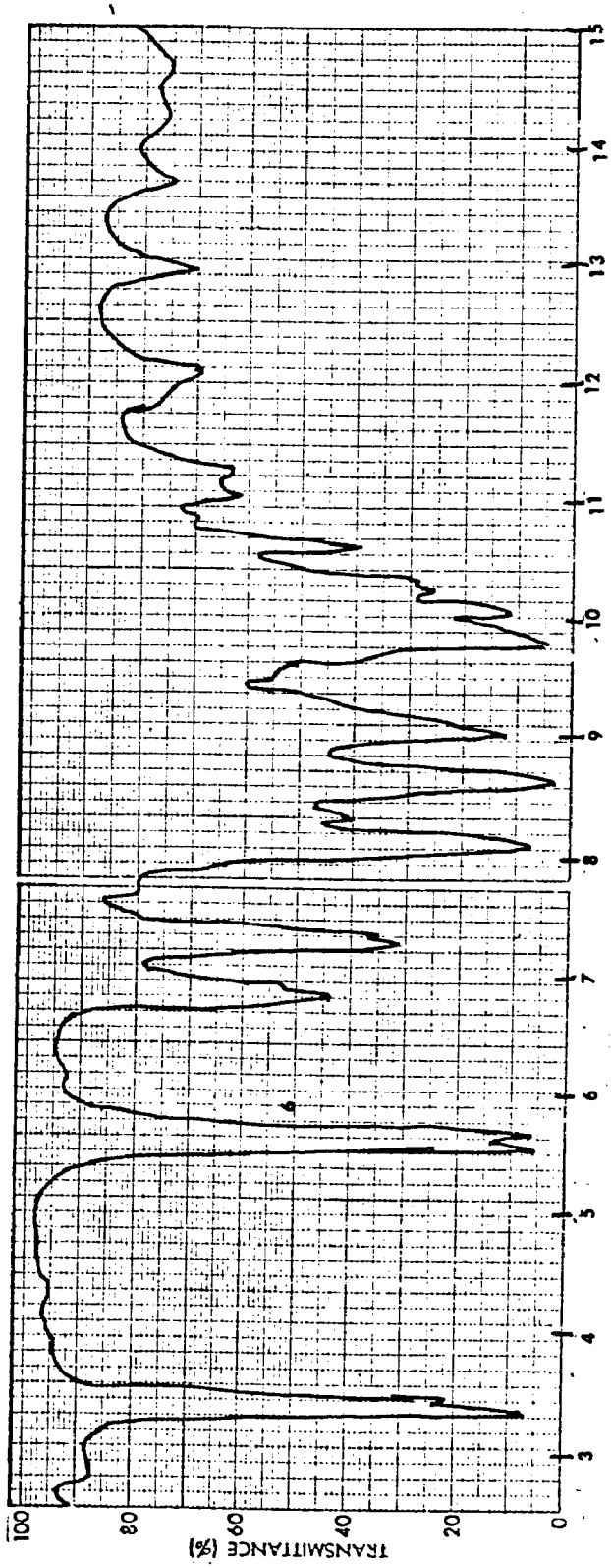
7
8
9
10
11
12
13
14
15


C_9H_{17}
H
OAc
O
O
H_3CO

24


PPM(τ)
PPM(δ)


TRANSMITTANCE (%)
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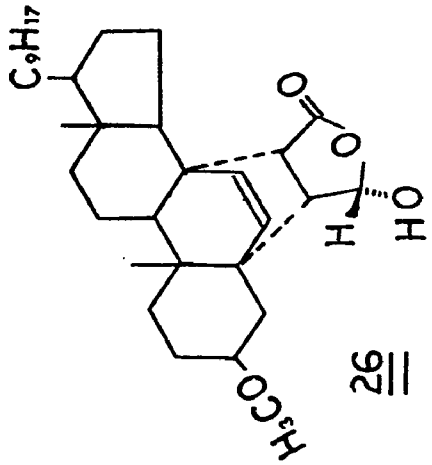
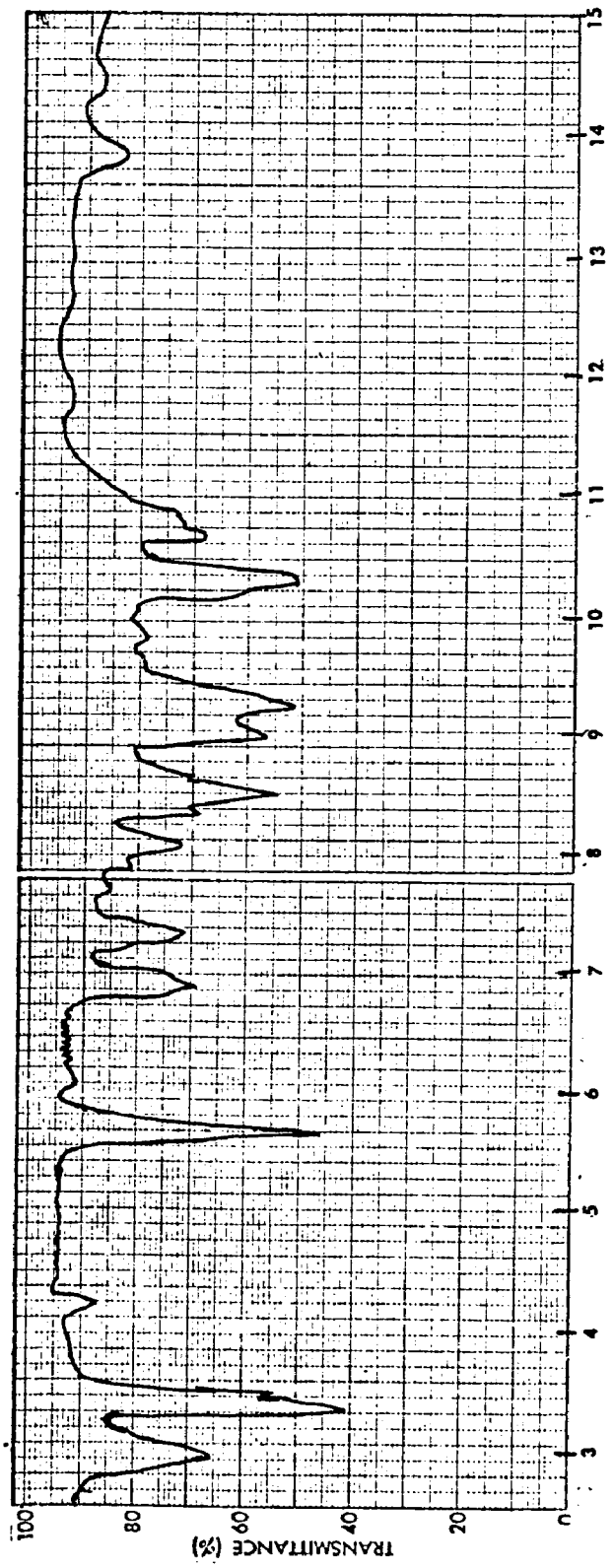
14
15


C9H17
O
O
H
HO
H3CO

26


TRANSMITTANCE (%)
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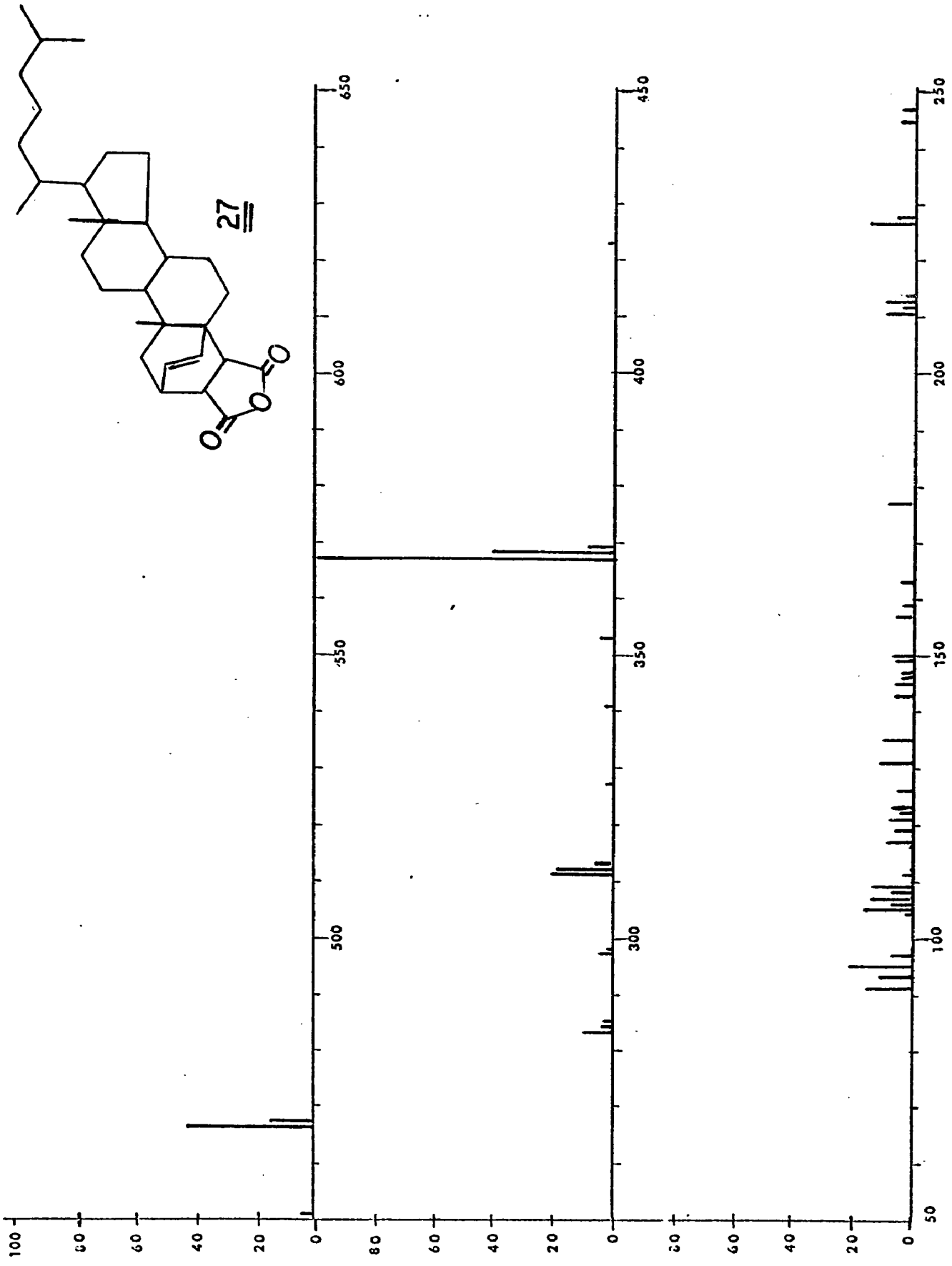
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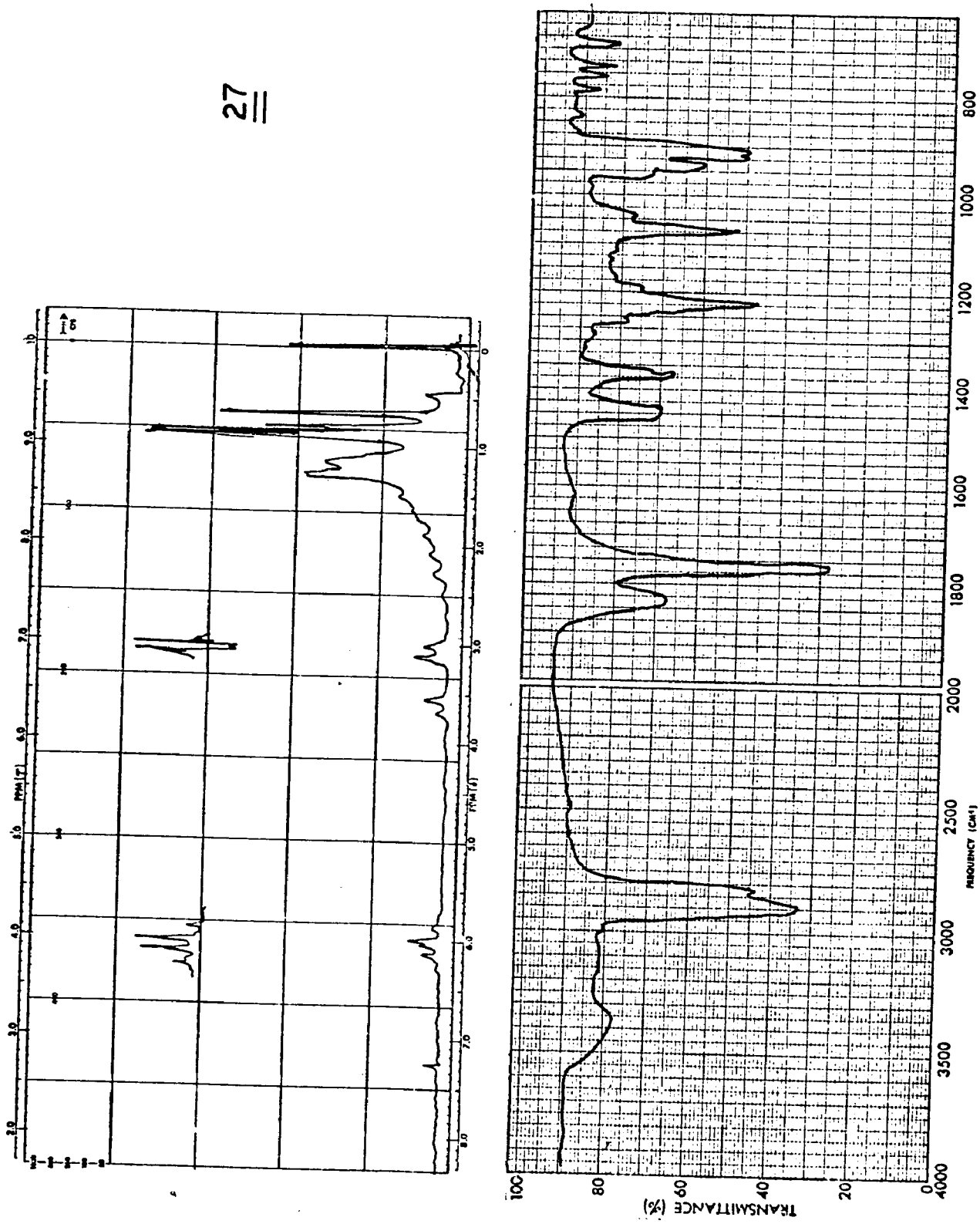
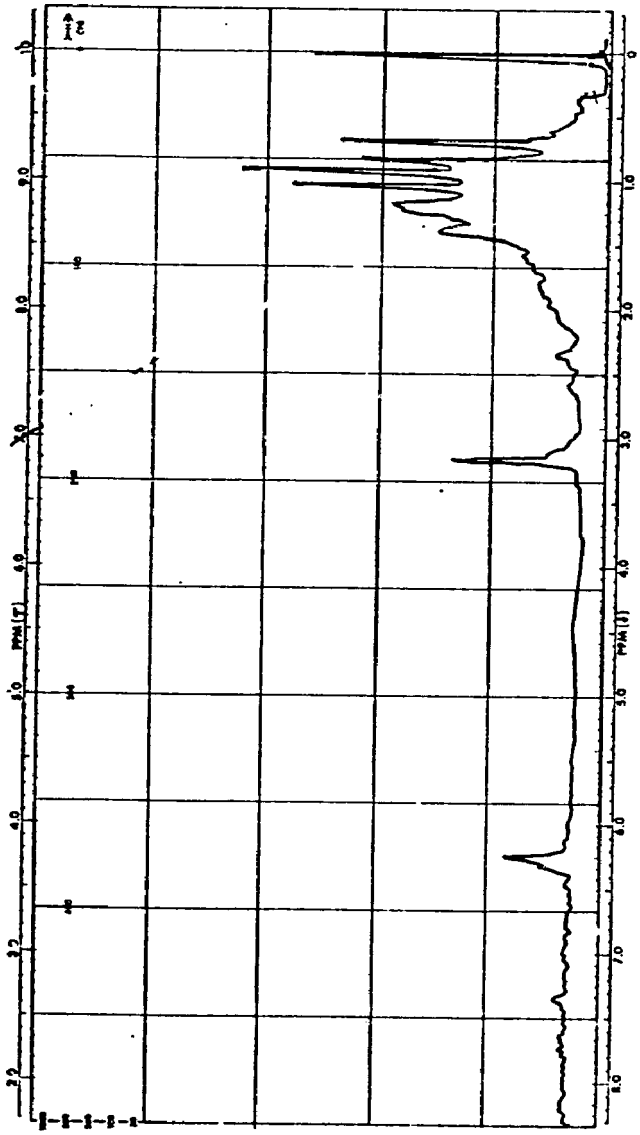
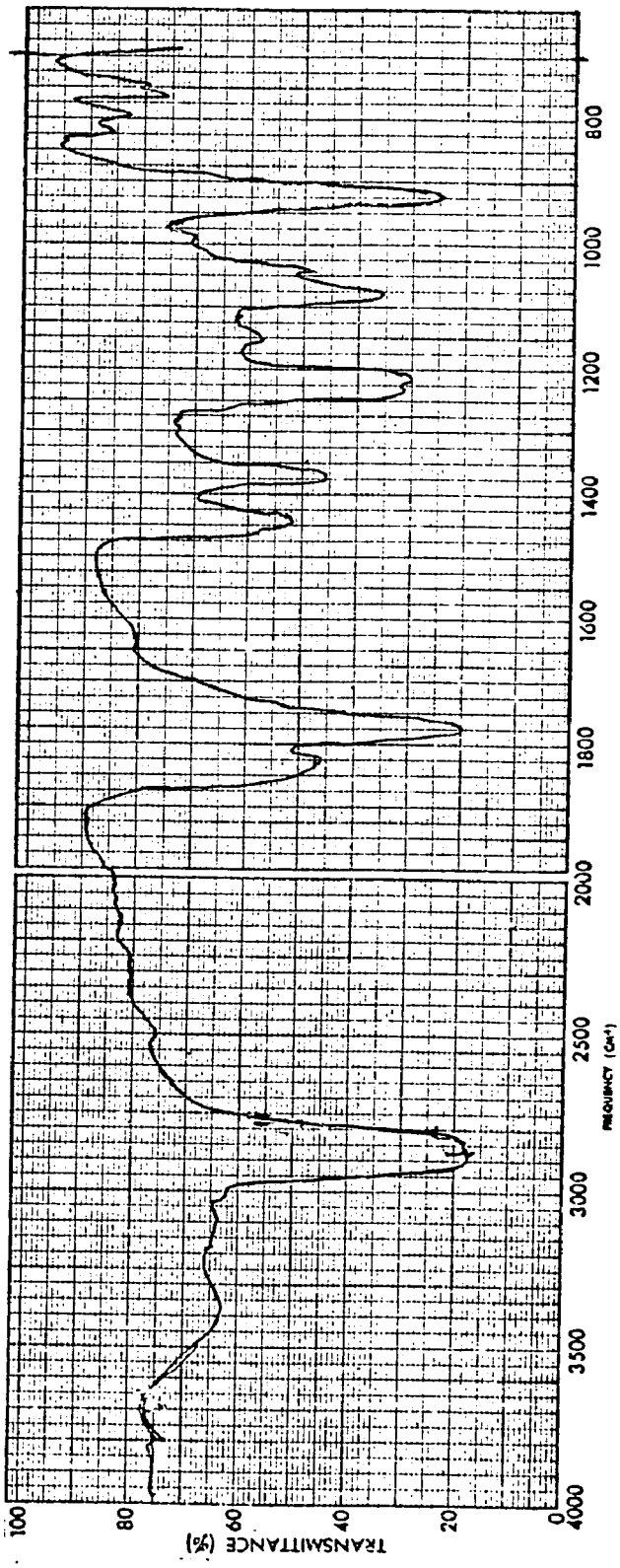
100
80
60
40
20
0

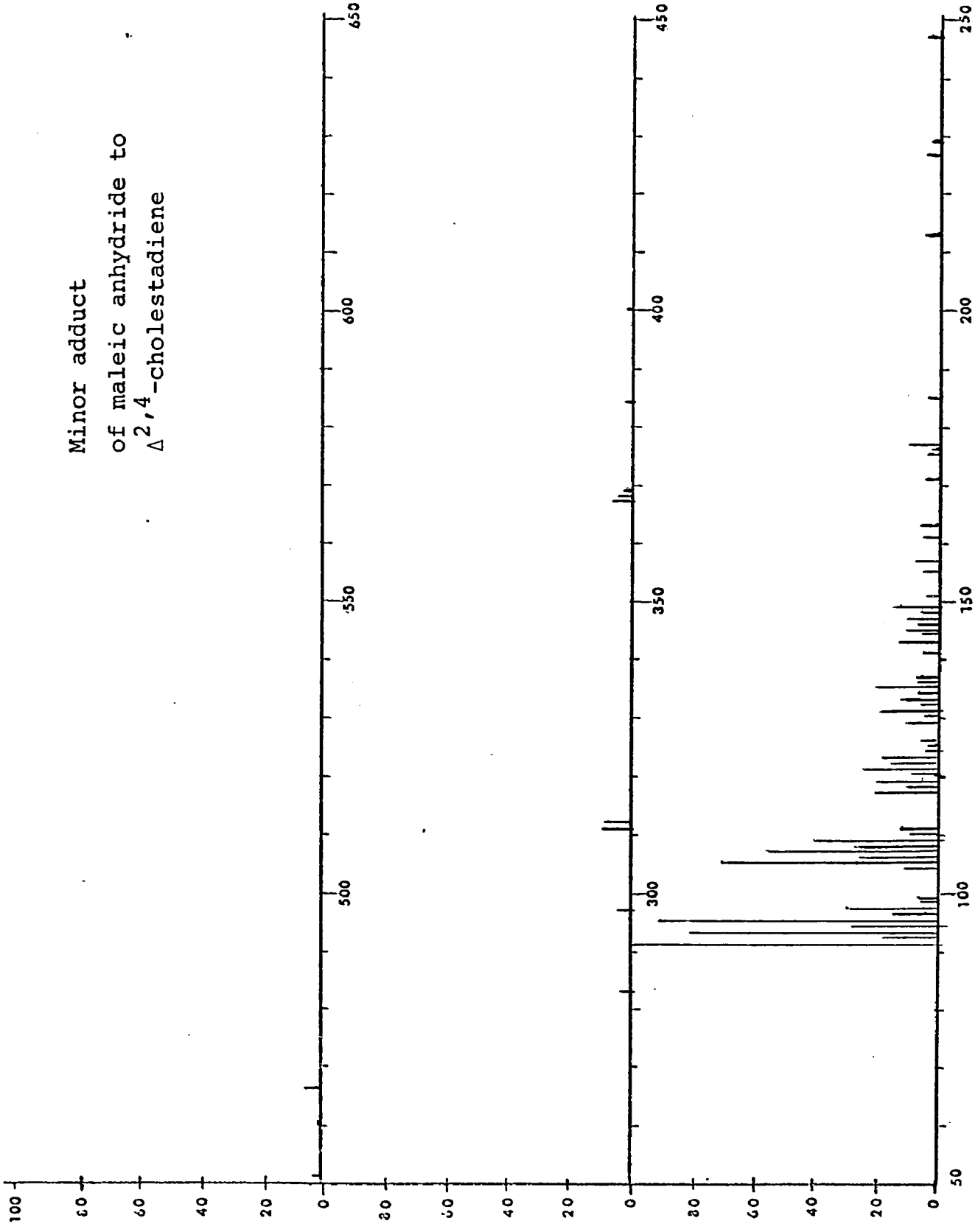
27



Minor adduct
of maleic anhydride to
$\Delta^{2,4}$-cholestadiene







Minor adduct
of maleic anhydride to
$\Delta^{2,4}$-cholestadiene

INDEX OF COMPOUNDS

PART I

Name	Structure Number	Page
O-Acetyl Macroline	31	12
Ajmalicine	8	4
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Alstonisidine	21	10,43
Alstonisine	17	9
Alstophylline	15	9,61
Anhydromacralstonine	101	61
Carcurin-V	68	27
Catharanthine	11	4
Chimonanthine	43	17
C-Toxiferine-I	66	27
Dihydromacroline	29	12
Dihydrosecodine-17-ol	9	4
Eburnamine	59	24
Folicanthine	47	19
Fruticosine	12	4
Geissolosimine	61	25
Geissoschizine	65	26
Geissoschizoline	63	25
Geissospermine	64	26
Macralstonidine	20	9
Macralstonine	22	10,61
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4-Methyl-desoxydihydro-chanoajmalol-B	40	16
4-Methyl-desoxydihydro-chanoisoajmaline	42	16
4-Methyl-desoxydihydro-chanoisoajmalol-B	39	16
4-Methyl-lilolidine	78	41
6-Methyl-lilolidine	77	41
N-Methyl skatole	99	55
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Secologanin	1	3
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Talcarpin	23	10
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Vincamine	13	4
Vindoline	10	4
Voacamidine	55	22
Voacamine	52	22
Voacangine	54	22
Voacorine	56	22
Vobasinol	53	22
Wieland-Gumlich Aldehyde	67	27

PART II

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Aflatoxin	9	106
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Fujenal	1	100
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