SUMMARY

This Thesis is divided into four distinct and self-contained parts whose unifying theme is implicit in the general title "Chemical Studies of Some Natural Products".

Part I discusses possible biogenetic routes to the vesicant principle cantharidin in which it is considered that this compound is terpenoid in origin. It indicates the expected radio-active labelling patterns that would be encountered should feeding experiments with ^{14}C labelled mevalonic acid to the beetle Meloe proscarabeus L. lead to the incorporation of radio-activity in the cantharidin shown to be synthesised by this insect. Chemical degradations of the theoretically possible labelled cantharidins designed to distinguish between them are discussed and a survey of the occurrence of cantharidin within the family Meloidae is given.

Part II describes an attempt to prepare, from a consideration of certain theoretical factors which are briefly discussed, a compound with potentially

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high neuromuscular blocking activity, 3α, 17α-bis (trimethylammonium)-5-α-androstane, by S_N^2 replacement of the methanesulphonyloxy groups of 3β, 17β-dimethanesulphonyloxy-5α-androstane with trimethylamine.

Part III describes a physico-chemical approach to the elucidation of the structure of the sesquiterpene γ-lactone, aristolactone occurring in Aristolochia reticulata L and A. serpentaria L. Successful degradation to the parent hydrocarbon germacrane (as a mixture of diastereoisomers) is described - these results permitting unequivocal assignment of the basic carbon skeleton of aristolactone. Re-examination of accumulated chemical evidence together with a number of physical measurements (I-R and n.m.r.) are employed to give a probable structure for aristolactone. A cyclization of methyl oxoaristate, a γ-keto ester derived from aristolactone, has been effected giving rise to a new derivative possessing an as yet undetermined bicyclic system.

Part IV describes the elucidation of the structures of the quaternary alkaloid petaline chloride and its methine base, lecticine - compounds isolated from

Leontice leontopetalum L. An attempt to synthesise one of the degradation products derived from the methine base leonticine is described but the projected synthesis failed at the last stage.

Chemical Studies of Some Natural Products

A Thesis Submitted To The University of Glasgow

For the degree of

DOCTOR of PHILOSOPHY

in the

Faculty of Science

by

Sidney J. Smith, B.S.P., M.Sc. (Sask.)

December, 1963.

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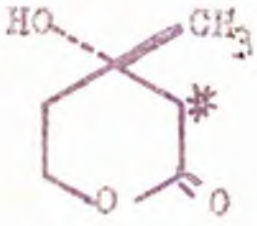
TABLE OF CONTENTS

The Biogenesis of the Vesicant Principle Cantharidin

I N T R O D U C T I O N

In 1956 the important observation was made by Tovormina, Gibbs and Hoff[1] that mevalonic acid lactone (I) labelled in the 2 - position with ^{14}C (in all formulae an asterisk denotes a ^{14}C isotope) gave rise to ^{14}C labelled cholesterol (II) in rat liver homogenates. Shortly afterwards Folkers' group[2] reported that mevalonic acid lactone could replace acetate as the growth factor for Lactobacillus acidophilus, and within a very short time work by several schools, namely those of Bloch[3], Rabinowitz[4], Cornforth and Popjak[5], and Lynen[6], conclusively demonstrated that mevalonic acid is a key precursor of the triterpene squalene (III) from which all naturally occurring steroids are derived.



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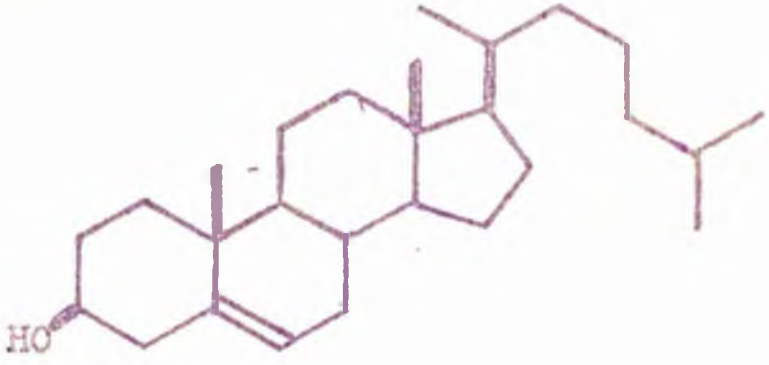

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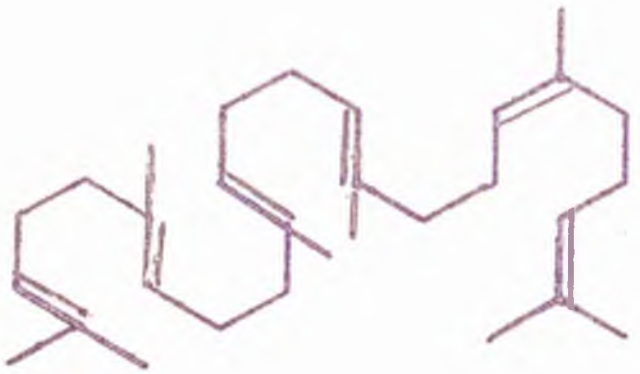


III

Accordingly a great deal of attention was focussed on terpenoid biogenesis[7-10] and, as a result of a number of important tracer experiments, a rational basis was provided for the isoprene rule[11]. This was then modified and expanded into the biogenetic isoprene rule[12] which states that the carbon skeleton of terpenoids is either formally divisible into "isoprene units" linked in a regular "head-to-tail" manner or is derived in a simple fashion from such a skeleton or skeletons through processes of condensation, bond migration, or loss of carbon atoms.

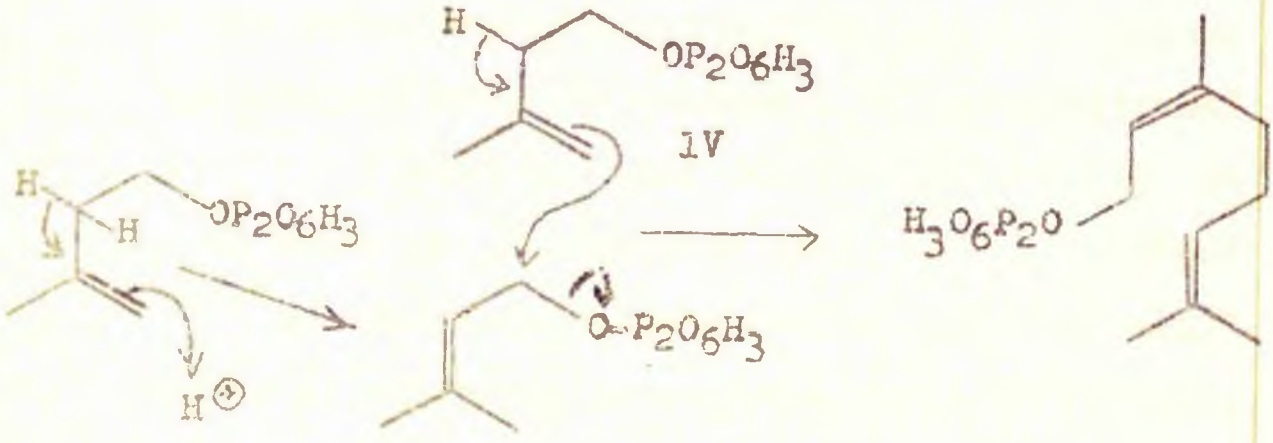
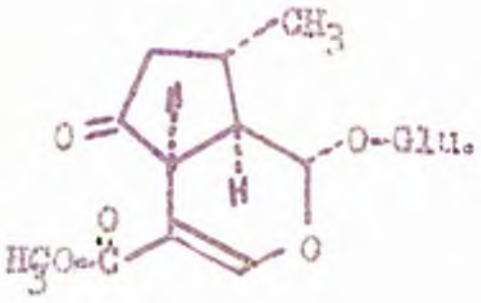


Figure 1.

Satisfactory rationalizations of the elaboration in nature of the major groups of terpenoids have now been put forward[13,14]. Thus, condensation of 3 acetate units through a mechanism involving malonate yields phosphorylated mevalonic acid which in turn gives rise to the two key intermediates isopropenylpyrophosphate (IV) and the isomeric dimethylallylpyrophosphate (V) which may be identified as the 5-carbon fragments responsible for the validity of the isoprene rule. Condensation of these compounds[15], as shown in figure 1, then affords geranylpyrophosphate[6,16,17] (VI), which is thus the monoterpene prototype in nature. Geranylpyrophosphate can then either undergo further condensation with isoprenoid pyrophosphates to form higher terpenoids, or it can undergo a variety of biochemical transformations to give the various classes of monoterpenoids.

Although the evidence concerning the biogenesis of monoterpenoids belonging to the long established groups is unequivocal, there exist in nature certain compounds for which conclusive proof of anisoprenoid biogenesis has still not been adduced. One such group

is the"cyclopentanoid monoterpenes". This includes the plant glycosides verbenalin(VII)[18], aucubin(VIII)[19], and asperuloside(IX)[20]; the sapogenin genipin(X)[21]; nepetalactone(XI)and nepalic acid(XII)isolated from catnip[22]; and the structurally related lactones iridomyrmecin(XIII)[23-25] isoiridomyrmecin (XIV)[24,25], and iridodial XV[24-26] which occur in ants belonging to the genus Iridomyrmex.



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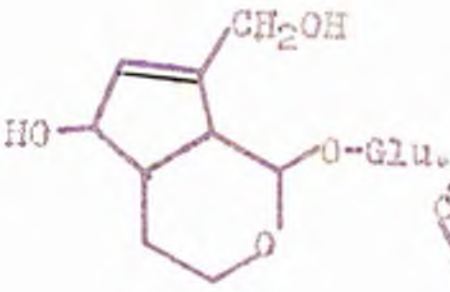

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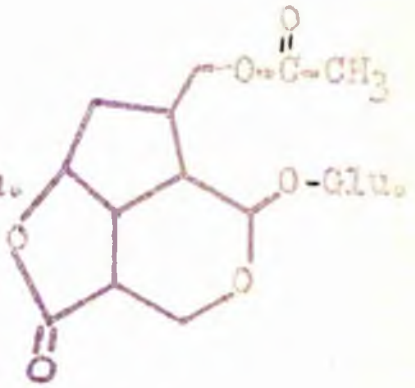

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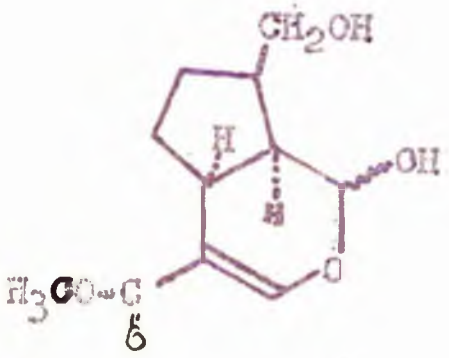

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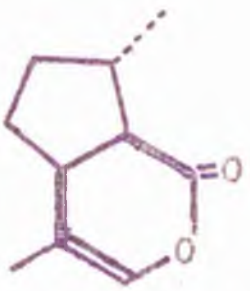

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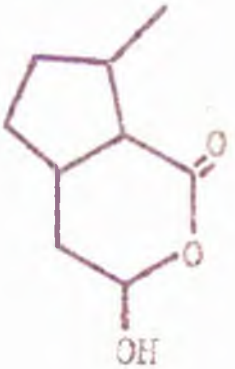

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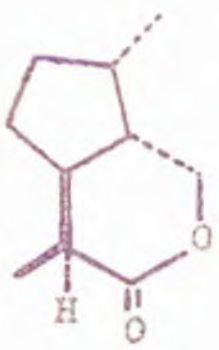

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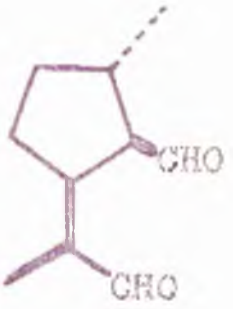
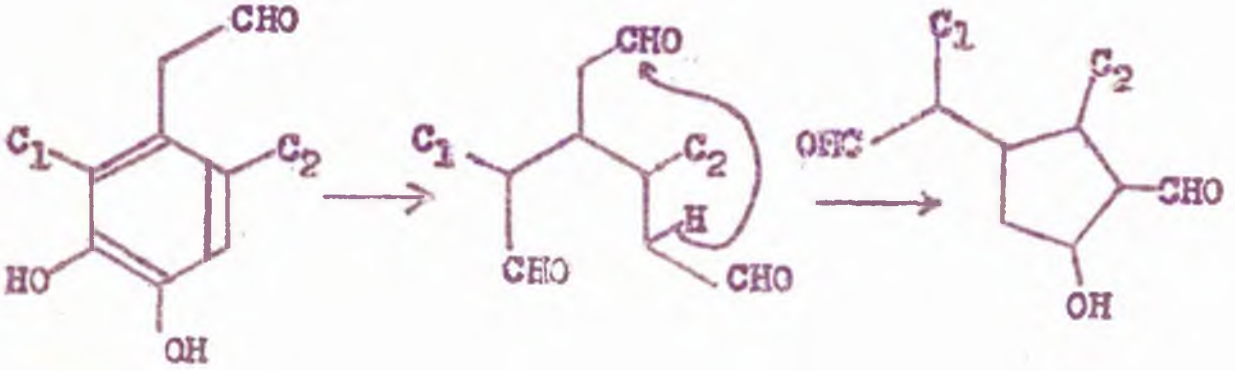


XV

As has been pointed out by Wiesner and his co-workers[27], the fact that these compounds obey the isoprene rule could possibly be fortuitous, and they could well arise in nature in a manner other than via the isoprene route. Wiesner's group suggest as a possible pathway a Woodward fission[28] of an alkylated phenylacetaldehyde as indicated in scheme A (formulae XVI to XVIII).

SCHEME A.



XVI XVII XVIII

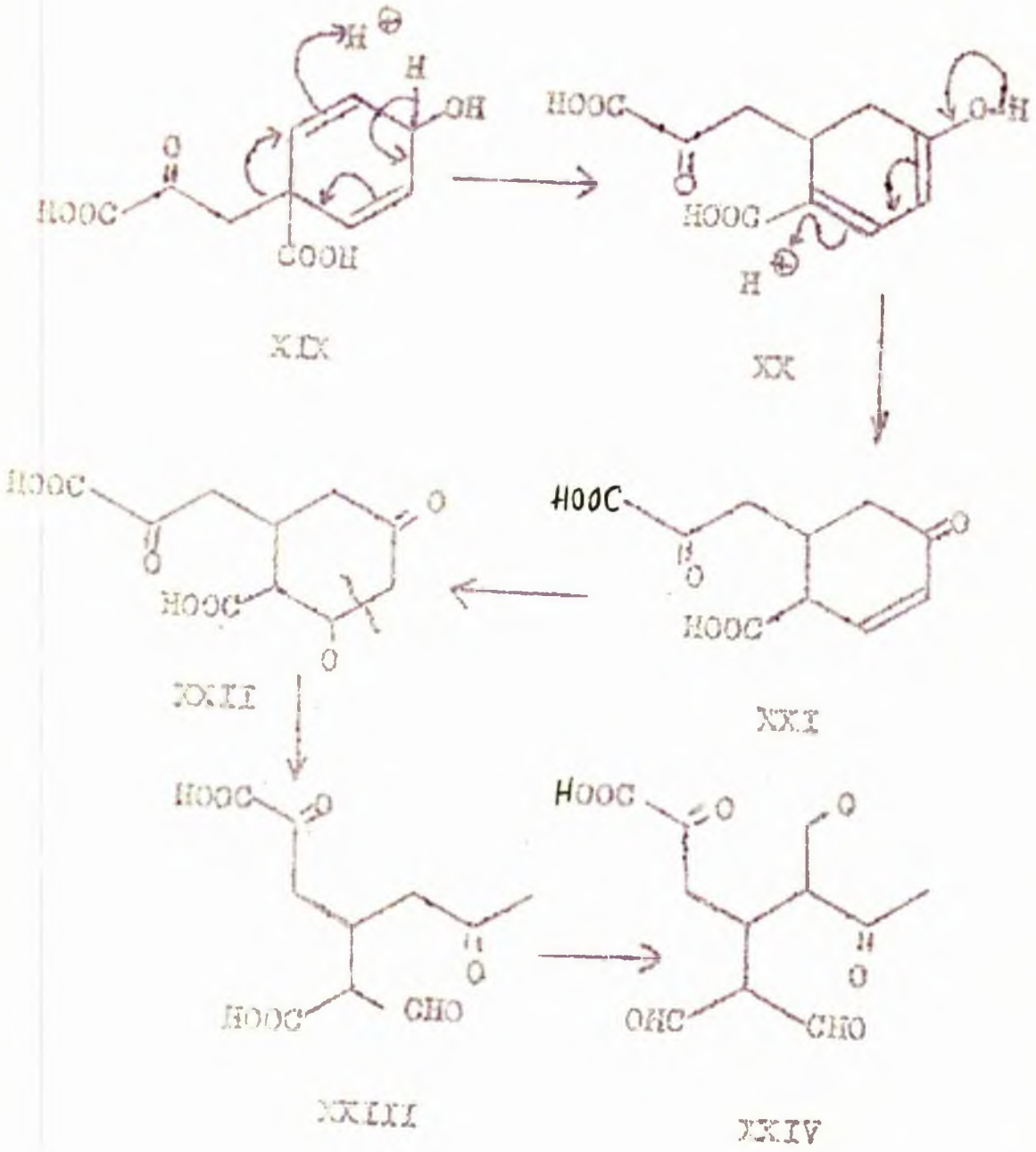
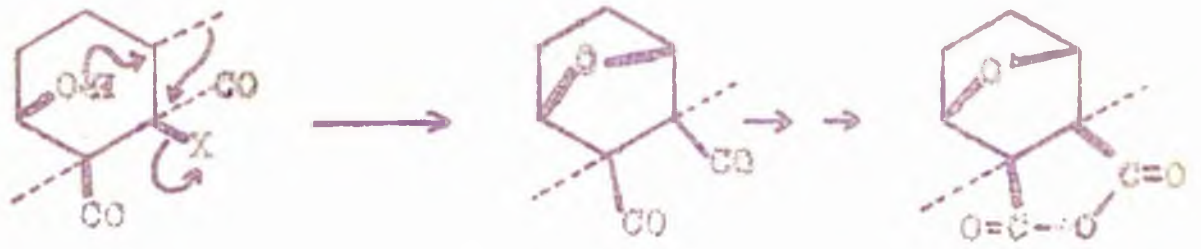


Figure 2

However other possibilities also exist as suggested by schemes which were originally put forward in connection with the biogenetic origin of the non-tryptamine portion of the complex indole alkaloids[29]. Although these schemes are now known not to be involved in indole alkaloid biogenesis[30] it is still possible that similar mechanisms could be involved in the formation of the "cyclopentanoid monoterpenes". For example, Wenkert[31] has suggested that the glycosides VII to IX (and by implication the lactones and derivatives X to XV) could be formed from the unit XXIV which in turn could arise from the rearrangement, hydration and retro-aldolization of prephenic acid[31,32], followed by condensation with a formaldehyde unit, as outlined in figure 2. Wenkert[31] however emphasises that although there is great structural similarity between the prephenic acid derivatives and the "cyclopentanoid monoterpenes", this similarity may be only fortuitous. Radio-tracer studies would be expected to throw more light on the biosynthetic pathway of these interesting compounds, but up to the present no such studies

would appear to have been reported.

A further compound which may be monoterpenoid in nature is cantharidin (XXVII) the vesicant principle occurring in certain beetles belonging to the family Meloidae. Inspection of the formula of this compound would certainly show it to be consistent with a tail-to-tail condensation of isoprene units. However, a mechanism which would explain this unusual carbon skeleton in terms of normal head-to-tail coupling of isoprene units followed by a methyl group migration has been very recently put forward by Martin-Smith and Khatoon[33]. These authors suggest that cantharidin could conceivably arise from a suitably substituted β-cyclocitral derivative by some such process as is shown in sequence XXV to XXVII. 1,2-Methyl shifts of the type postulated have many analogies in nature[8,34,35]. It is to be noted that formula XXVII shows the relative stereochemistry of cantharidin with the anhydride ring cis to the oxide bridge - not an absolute stereochemistry - since cantharidin is a non-optically active molecule, having a plane of symmetry.

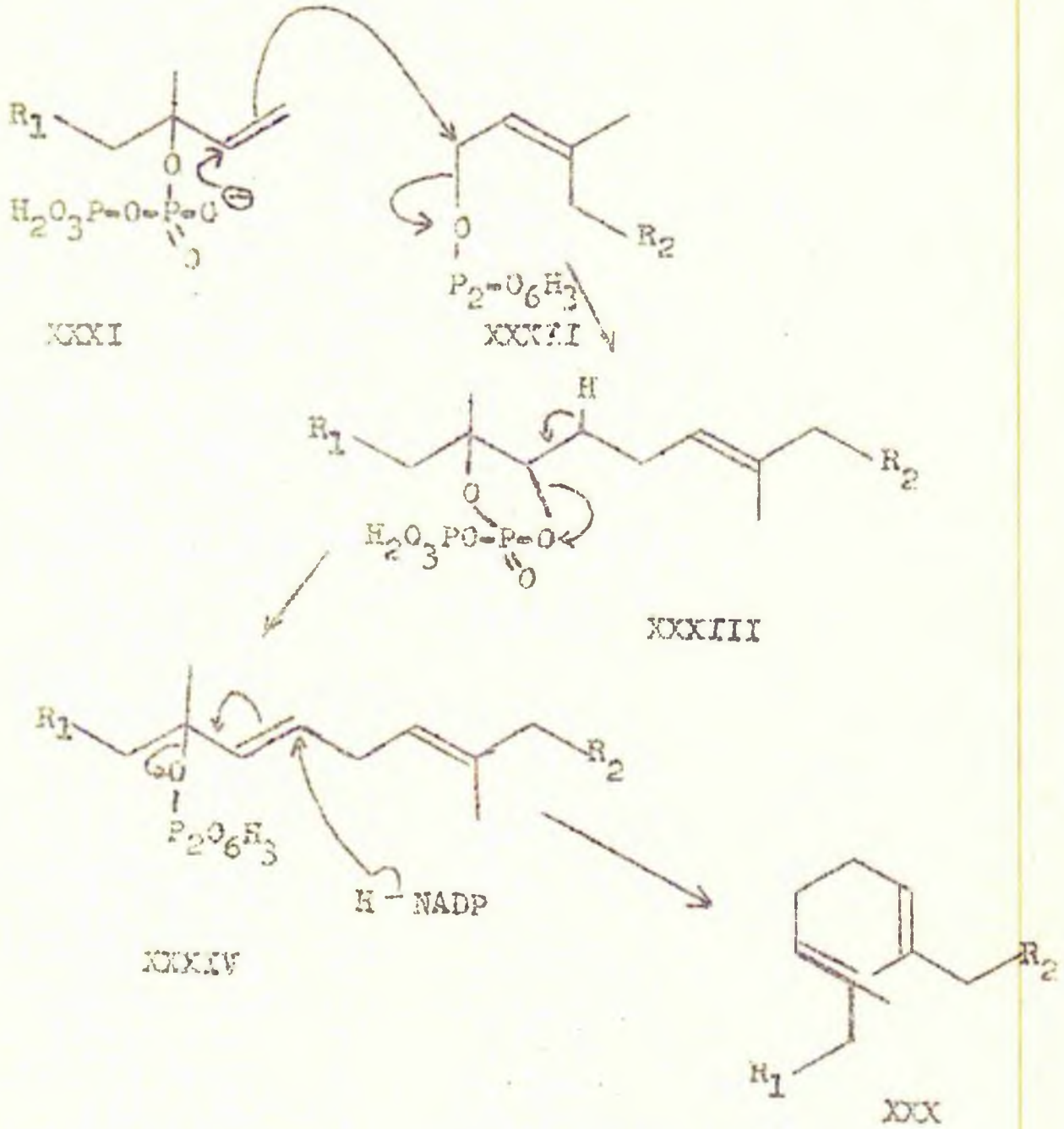


XXV XXVI XXVII

It is to be noted that very recently Dean[36] has suggested that the oxide bridge in cantharidin could arise in nature via peroxide formation (as in ascaridole) followed by reduction to the tetrahydrofuran ring system.

That cantharidin might indeed arise by the tail-to-tail condensation of two isoprene units can perhaps be considered from analogy with the tail-to-tail condensation, either between one molecule of farnesylpyrophosphate (XXVIII) and one molecule of nerolidylpyrophosphate (XXIX) or between two molecules of farnesylpyrophosphate

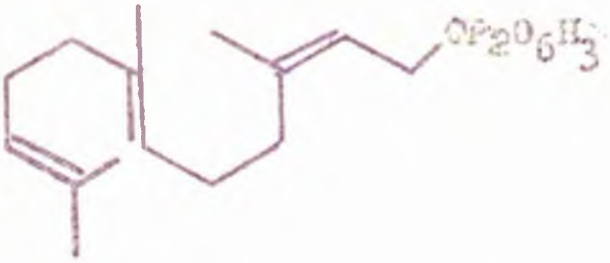
(XXVIII) to form squalene (III; XXX, $R_1=R_2=$ $CH_3-C(CH_3)=CH-CH_2-CH_2-C(CH_3)=CH-CH_2-$) [6,9,10,37-41].



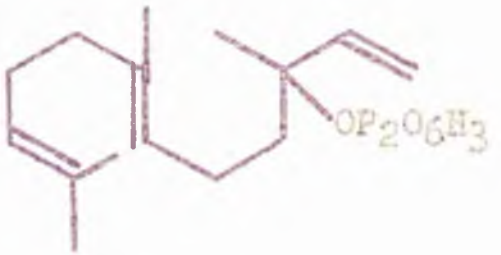
Squalene biogenesis: $R_1=R_2=$ $CH_3-\overset{CH_3}{\overset{|}{C}}=CH-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}=CH-CH_2-$

Hypothetic Cantharidin biogenesis: $R_1=R_2=$ H-

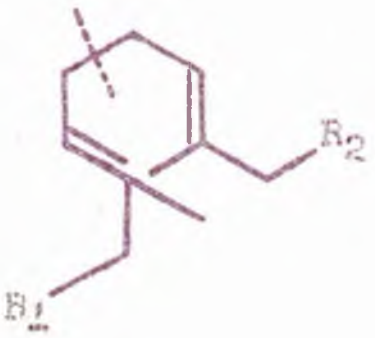
Figure 3.



XXVIII

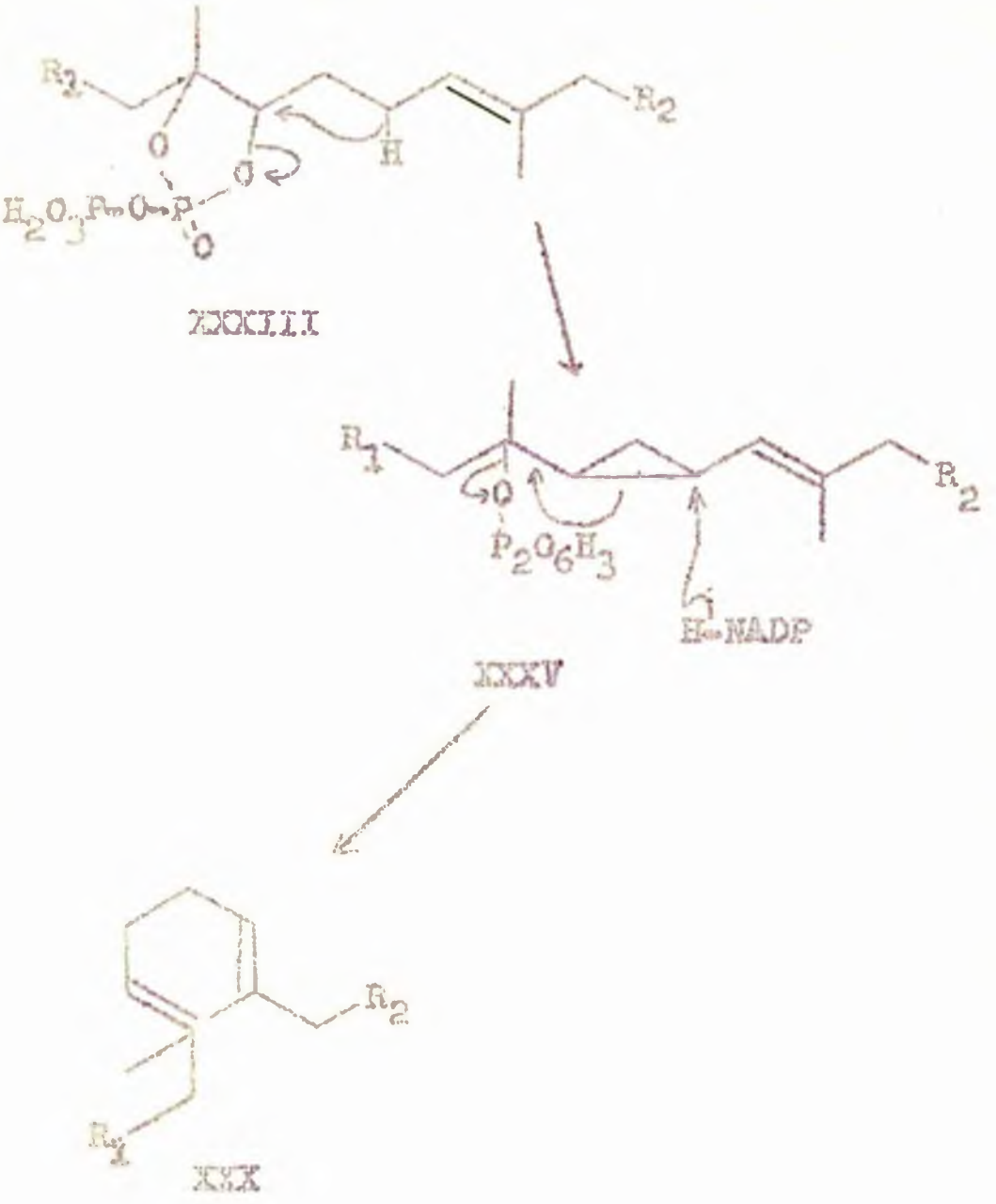


XXIX



XXX

The Cornforth and Popjak school[41] has postulated that the tail-to-tail condensation of the two fifteen carbon units takes place via a nucleophilic process, analogous to that involved in the biosynthesis of geranyl- and farnesyl- pyrophosphates[37]. Thus nerolidyl- and farnesyl- pyrophosphates are considered to condense to form the cyclic phosphate ester XXXIII, as shown in figure 3. Following an elimination reaction the resulting intermediate XXXIV is postulated to suffer reduction by reduced nicotinamide-adenine dinucleotide phosphate (H-NADP) to form squalene and it would seem quite



Squalene biogenesis: $R_1 = R_2 = CH_3-\overset{CH_3}{\overset{|}{C}}=CH-CH_2-CH_2-\overset{CH_3}{\overset{|}{C}}=CH-CH_2-$

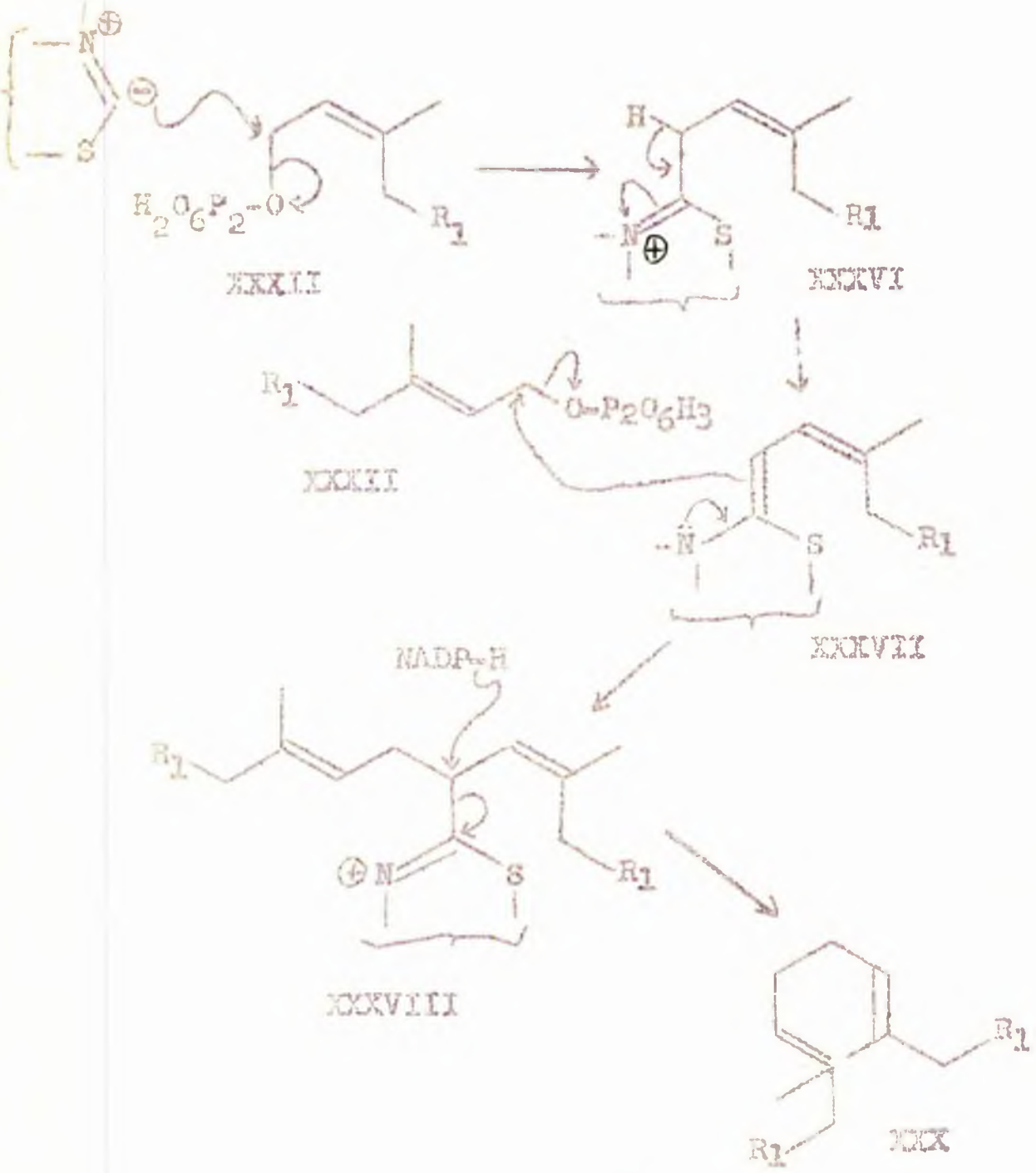
Hypothetic cantharidin biosynthesis: $R_1 = R_2 = H-$

Figure 4

possible that an acyclic precursor of cantharidin could be formed in much the same way.

The scheme outlined in figure 3 fully covers the known experimental observations with respect to the biogenesis of squalene although, as has been pointed out[41], there is some doubt as to the formation of nerolidylpyrophosphate in the liver[42,43]. Nevertheless the occurrence of optically active nerolidol in plants supports the possibility of enzymatic isomerization of a farnesol derivative to this tertiary alcohol.

The scheme shown in figure 3 assumes the elimination of a proton (in XXXIII) attached to what was originally C-1 of the farnesylpyrophosphate half of the molecule (i.e. a carbon atom originally derived from C-5 of mevalonic acid) but this does not necessarily have to be the case. The proton loss could occur from what was originally C-2 of the farnesyl moiety (XXXIII, figure 4)[41]. After condensation of the nerolidylpyrophosphate (XXXI) with farnesylpyrophosphate (XXXII) to yield XXXIII, the molecule could undergo cyclopropane ring formation (XXXV) with the proton elimination



Squalene biogenesis: $R_1 = CH_3-C(CH_2)=CH-CH_2-CH_2-C(CH_2)=CH-CH_2-$

Hypothetic cantharidin biogenesis: $R_1 = H-$

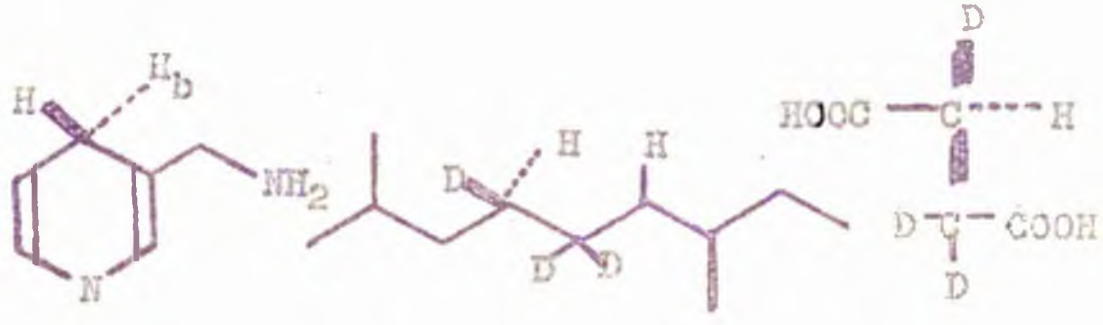
Figure 5

occurring from the carbon atom β to the pyrophosphate bearing carbon, rather than from the adjacent carbon as proposed in figure 3. Reductive cleavage of this cyclopropanoid intermediate by H-NADP with the concerted elimination of the pyrophosphate anion would give rise to squalene (XXX), as shown in figure 4.

An alternative mechanism for the tail-to-tail coupling of sesquiterpene units in the biogenesis of squalene which does not involve nerolidylpyrophosphate was suggested to Cornforth and Popjak[41] by Professor R.B. Woodward. This mechanism (figure 5) involves the coupling of farnesylpyrophosphate (XXXII) with the ylide of thiamine pyrophosphate giving XXXVI, which would be followed by the elimination of a proton to yield the complex XXXVII. The condensation of this intermediate with another molecule of farnesylpyrophosphate(XXXII) would result in the formation of the intermediate XXXVIII. This mechanism is analogous to the acyloin type of condensation involved in the elaboration of acetoin[44]. Reduction of XXXVII by the reduced

form of nicotinamide - adenine dinucleotide phosphate (H-NADP) would yield squalene (XXX) and at the same time regenerate the ylide of thiamine pyrophosphate.

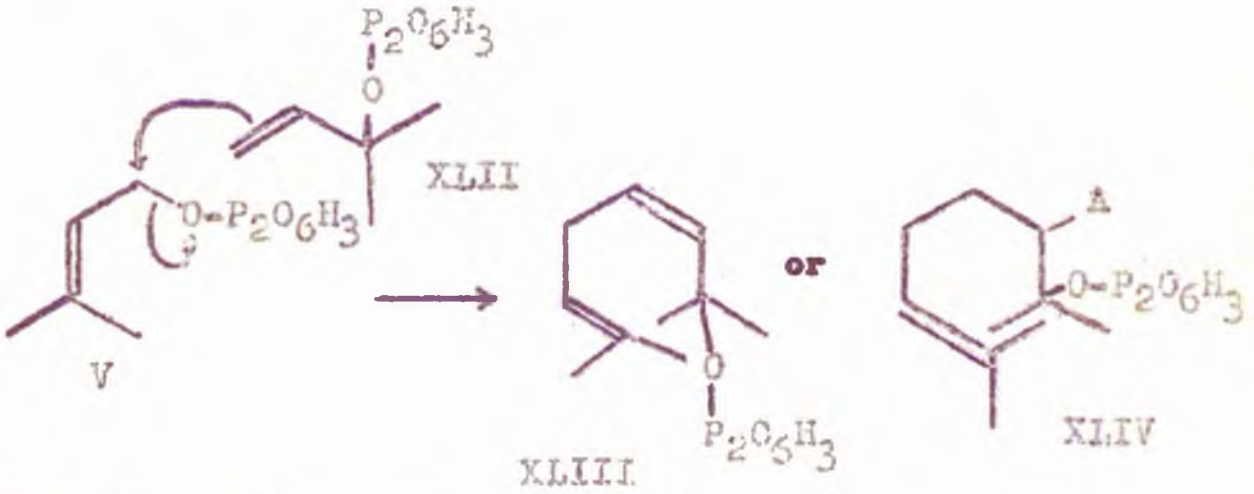
Popjak, Low and Moore[45] proposed that the condensation of the two C15 units was a stereo-specific process, and partial proof of their hypothesis was soon adduced[46] from the observation that during "hydrogen transfer" only the hydrogen atom from the "b" side of the reduced nicotinamide coenzyme was involved[47] (H_b in partial structure XXXIX). Complete proof was provided[48] by the elegant correlation of the stereochemistry at C-11 and C-12 in trideuterated squalene (partial structure XL) with that of S-trideuterated succinic acid (XLI)



XXXIX XL XLI

It is to be anticipated that direct experimental evidence will eventually indicate which of the mechanisms shown in figures 3,4, and 5 is actually involved in squalene biogenesis.

Should a tail-to-tail condensation parallel to one of those shown in figures 3,4, or 5, be occurring in the biogenesis of cantharidin, condensation between one molecule of 3 - methyl butenyl-3-pyrophosphate (XLII) and one molecule of α,α- dimethylallylpyrophosphate or between two molecules of α,α-dimethylallylpyrophosphate (V) would be expected to give rise to the possible intermediates XLIII or XLIV, where "A" is an attacking nucleophilic species.



The alternative condensation of the butenylpyrophosphate XLII with isopropenylpyrophosphate (IV)

would give the corresponding double bond isomer of XLIII or XLIV. Ring closure of XLIII or XLIV could then afford cyclohexanoid monoterpenes with a tail-to-tail isoprene coupling.

Radio-active tracer studies employing 2-^{14}C mevalonic acid would be expected to distinguish between the head-to-tail (with rearrangement) and the tail-to-tail mechanisms just outlined as possible biosynthetic routes for cantharidin. Accordingly attention was focussedon approaches to this end.

It was first necessary to secure a suitable organism with which to undertake the proposed research. A survey of the literature revealed that cantharidin is of widespread occurrence in the family Meloidae, especially within the genera Cyaneolytta, Epicauta, Lytta, Meloe, and Mylabris, the more well authenticated sources of cantharidin being shown in Table 1. For reasons to be outlined in the discussion, Meloe proscarabeus was selected as the most likely potential candidate for the proposed research.

TABLE I

SOURCES OF CANTHARIDIN

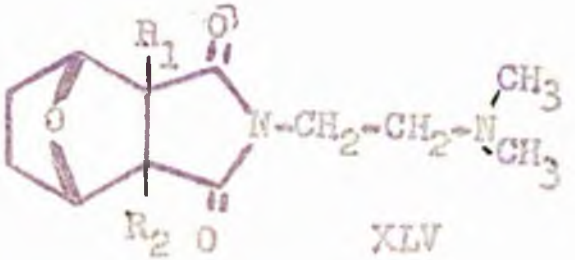
Organism	Percentage Cantharidin	Reference
Cissites cephalotes Ol. (C. maxillosa)	-	49
Cyaneolytta gigas F. (Lytta gigas)	-	50
C. signifrons Fahr. (Lytta coelestina)	1.89	51
C. violacea Brandt (Lytta violacea)	-	50
Decapotama lunata Pall. (Mylabris lunata)	1.0	51
Fletica wahlbergia Fahr.	0.32	51
Epicauta adspersa Klug. (Lytta adspersa)	0.38 - 0.41	52
E. femoralis Fr. (Cantharis femoralis)	1.7 - 3.5	53
E. korbani Mars.	0.40 - 1.75	54
E. hirticornis Haag (Cantharis hirticornis)	2.02	55
E. pennsylvannica Deg. (Lytta strata)	-	50
E. ruficeps Ill.	-	56
E. velata Gerst. (Cantharis velata)	2.73	51
E. vittata F. (Lytta vittata Cantharis vittata)	0.4 - 1.33	50, 57, 58
Moria debyi Fairm.	-	49
Lydus trimaculatus Fischer	-	50
Lytta conspicua Waterh. (Mylabris conspicua)	-	50
L. sanguinea Haag (Hycchys sanguinea)	-	59
L. vesicatoria L. (Cantharis vesicatoria)	wings & alytra 0.082 head & antennae 0.088 legs 0.091 thorax & abdomen 0.24	58, 60
Macrobasis albida Say	up to 4.6	61
N. cinerea F. (Lytta cinerea)	-	50

TABLE I

SOURCES OF CANTHARIDIN

Organism	Percentage Cantharidin	Reference
Meloe angusticollis Say	-	50
M. majalis L.	-	50,62
M. proscarabeus L.	-	50,62
M. variegatus Donov. (Mylabris variegata)	-	50
M. violaceus Marsh	-	50
Mylabris balteata Pall. (M. punctum)	0.193	60
M. bifasciata De Geer	1.02	50,63
M. calida Pall. (M. maculata)	-	50
M. cichorii L.	0.40 - 1.5	51,55,58,64,66
M. colligata Redt.	-	50
M. Crocata Pall. (M. duodecimpunctata)	-	50
M. dicincta Bertol.	-	59
M. holosericea Klug.	1.3	51
M. macilenta Mars.	-	67
M. oculata Thumb	0.615	51
M. phalerata Pall. (M. sidae)	1.0 - 1.2	59
M. pustulata Thumb	0.33- 2.9	64,68
M. quadripunctata L. (M. melanura)	9.2 (dry)	69,70
M. quatuordecimpunctata Pall.	0.49	71
M. tripartita Gerst.	-	59
M. variabilis Pall.	19.28 (dry)	70

The earlier interest in the occurrence of cantharidin can be traced to its former medicinal application as a topical vesicant and counter-irritant but due to its pronounced renal toxicity[72,73,74,75], it is no longer employed in this way. The exact function of cantharidin in the insect has not been established although its insecticidal properties may point to a defensive function[56,76,77] Volker[78] has pointed out that in the case of Lytta vesicatoria appreciable concentrations of cantharidin are present only in sexually mature individuals and so this fact may indicate that it has a sexual role. However, cantharidin is known to be distributed throughout the wings, head, legs, and abdomen of this species[60] and so it is not confined to the sex organs. At present cantharidin is neither of chemical nor pharmaceutical interest. Nevertheless recently a renewed interest has been shown in the biochemical mechanism of cantharidin acantholysis[79] and also some synthetic analogues of the type XLV have been claimed to exhibit anti-hypertensive properties[80].



XLV

Discussion

As outlined above it first became necessary to obtain a species of beetle suitable for radio-tracer experiments. The beetle Meloe proscarabeus L. was chosen since it was large enough for intraperitoneal injection of radio-active material, and was of local occurrence. The early literature makes reference to the isolation of cantharidin from this beetle and from the reddish droplets it secretes when handled [50,62]. In view of the inaccessibility of some of the original work, and in view of the fact that no recent investigations have been reported on this beetle, together with the ambiguity surrounding certain early identifications of cantharidin (especially the confusion of this compound with pederin[31]), it was deemed necessary to first confirm the occurrence of cantharidin in Meloe proscarabeus.

Adult specimens of *M. proscarabeus* collected at Loch Ardinning, Stirlingshire, in May 1962, were killed in the laboratory by means of chloroform. The red droplets which were secreted just prior to death were collected and examined separately. The droplets were taken up in hot ethanol and after removal of the solvent the oily residue was sublimed by heating to 90°/0.05 mm, thus affording a colourless crystalline sublimate. The material was shown to be cantharidin by comparison with an authentic sample, there being no melting point depression (sealed tube) on admixture of the two specimens. The infra-red spectra in K Cl disc were completely superposable and identical with the published spectrum[82] Cantharidin was also obtained from both freshly killed and pulverized and from dried and pulverized bodies of the beetles. It was found to be present to the extent of 0.137°/₀ of the total body weight and 0.364% of the dried body weight.

Radio-active tracer studies on *Meloe proscarabeus* employing 2-^{14}C mevalonic acid would be expected to

pin-point the cantharidin biosynthetic pathway. Incorporation of the labelling would indicate that cantharidin is indeed terpenoid in origin, rather than being formed, for instance, from shikimic acid *via* the theoretical *seco*-prephenate-formaldehyde intermediate XXIV postulated by Wenkert[31]. Degradation of the labelled cantharidin molecule would then be expected to give a distinction between a head-to-tail or a tail-to-tail coupling of isoprene units. Although the symmetry of the cantharidin molecule (XXVII) does not make it strictly an ideal compound with which to conduct tracer biogenetic experiments, the labelling patterns which would arise from the alternative coupling pathways would be different, as seen below.

HEAD-TO-TAIL CONDENSATION AS A POSSIBLE MECHANISM FOR CANTHARIDIN BIOSYNTHESIS

If the biogenesis of Cantharidin were to involve a normal head-to-tail condensation of isoprenoid units as in the biogenesis of geraniol[6,15,16,17], injection of *Meloe proscarabeus* with 2-^{14}C mevalonic acid should after its 2- stage phosphorylation to XLVI[83] give rise to 1-^{14}C-isopropenylpyrophosphate (XLVII) and

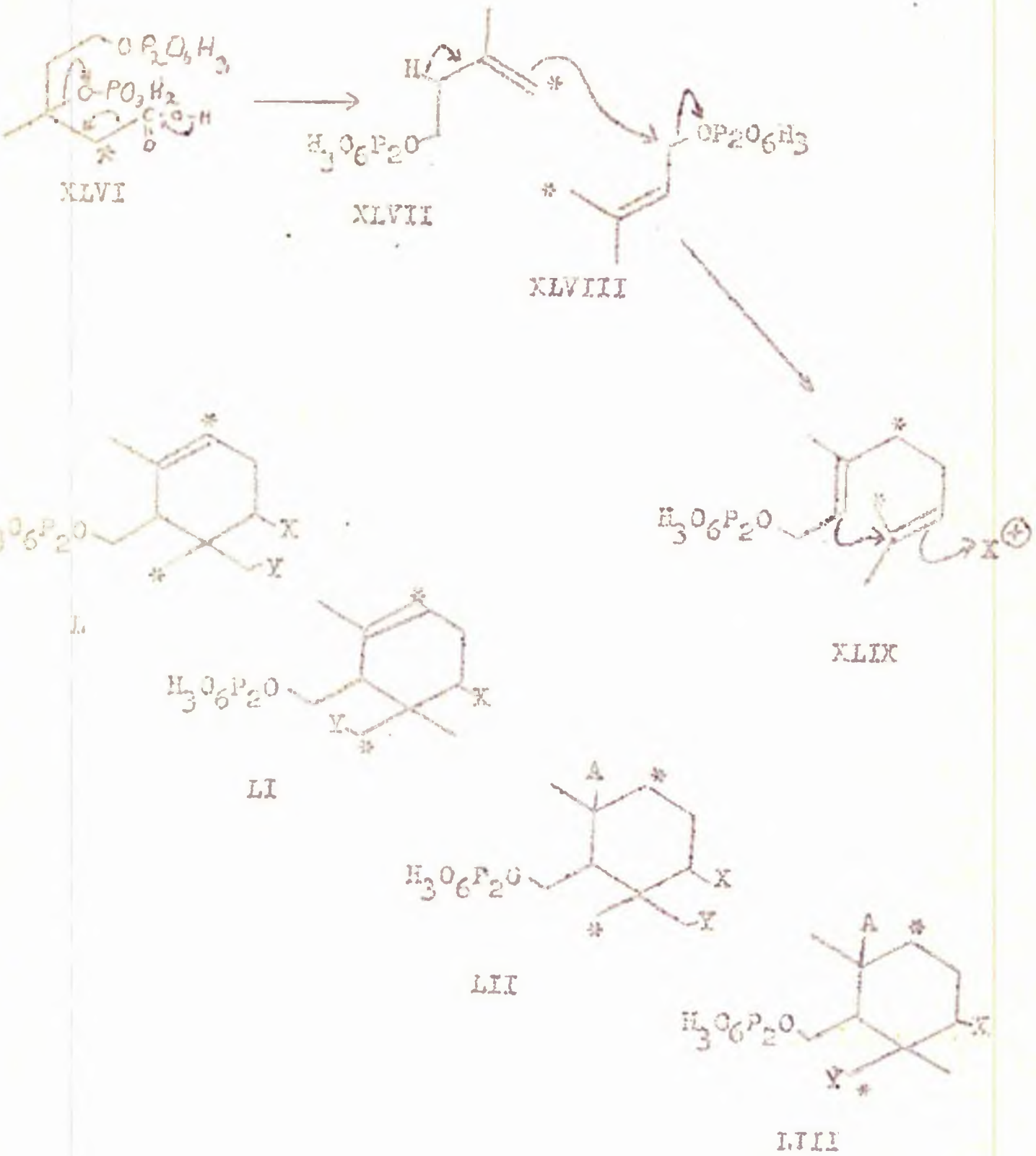
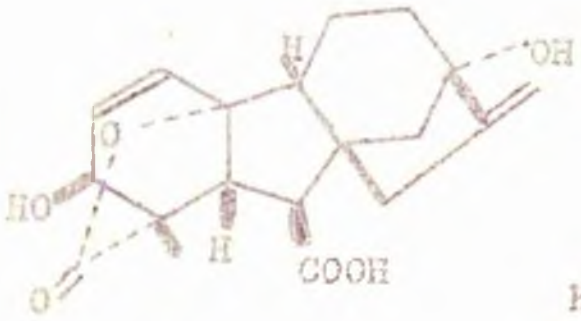


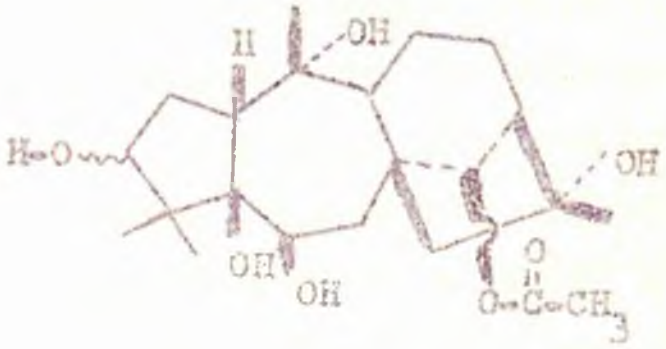
Figure 6

4-^{14}C-3-methylbut-2-enyl-1-pyrophosphate (α-methyl-α-^{14}C-methyl-allylpyrophosphate) (XLVIII) which would be expected to condense to give the isotopically labelled geranylpyrophosphate (XLIX) by the accepted mechanism[10,37,42] as shown in figure 6. This compound(XLIX) or subsequent derivatives might then be expected to undergo specific hydroxylations at allylic positions, the particular sites of attack presumably being uniquely determined by specific enzymes.

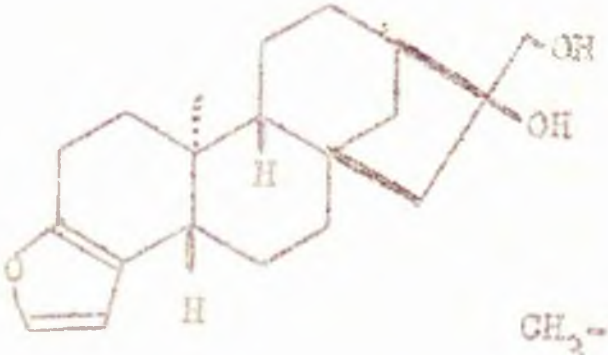
Cyclization of geranylpyrophosphate itself, geraniol or some allylically hydroxylated derivative to the β-cyclocitral skeleton can be envisaged as occurring by attack of an electrophilic species on the isopropylidene double bond of XLIX, with concerted attack from the π electrons of the second double bond, effecting ring closure. The resulting compound would belong to one of four possible types L to LIII, where "X" represents the attacking electrophilic species, "Y" an oxygen function resulting from an earlier allylic hydroxylation, and "A" a nucleophilic species (e.g. hydroxyl ion) completing the cyclization sequence.



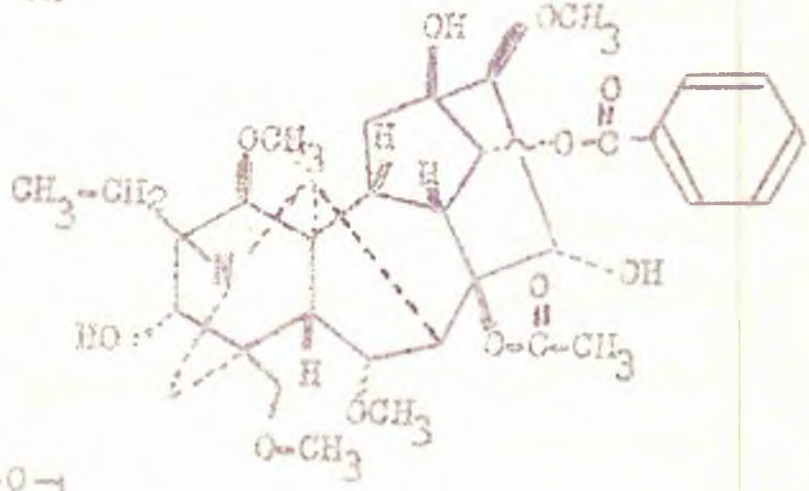
LXV



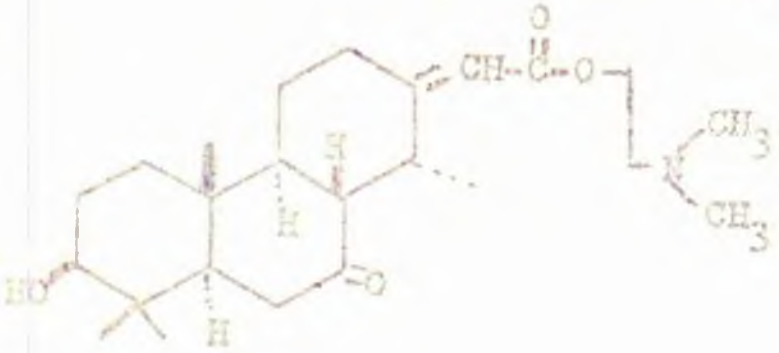
LV



LVI



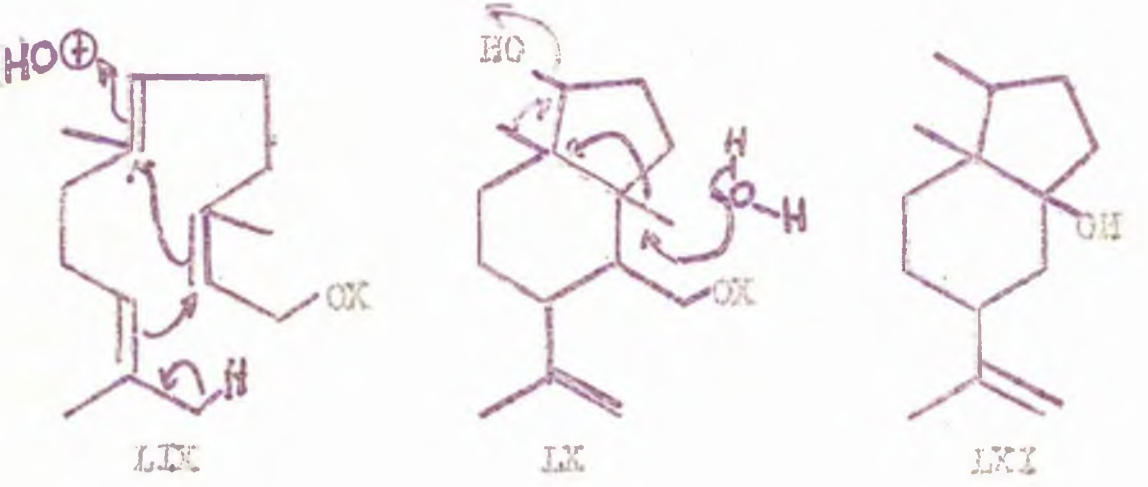
LVII



LVIII

In the cyclizations of geranyl- and farnesyl-pyrophosphates in plants to yield cyclic mono- and sesquiterpenes respectively, the initiating electrophilic species "X" would appear to be invariably a proton[11,13,14,84]. On the other hand in the cyclizations of squalene giving rise to tetracyclic, pentacyclic, or onocerin types of triterpenes, the electrophilic species "X" would appear invariably to be the equivalent of $OH^{\oplus}$, in both plants and animals[85], this species being known to involve molecular oxygen[86]. The cyclizations of geranylgeranylpyrophosphate leading to the various types of diterpenoids appear to involve both mechanisms. A proton would seem to be involved in most cases but certain diterpenoids appear to utilize $OH^{\oplus}$ as evidenced by the occurrence in nature of a number of 3- oxygenated diterpenoids. Examples are gibberellic acid (LIV)[87], the grayanotoxins[88-90] (e.g. andromedotoxin, LV) [illegible] (LVI)[91], and certain diterpenoid alkaloids such as aconitine LVII[92] and the Erythrophleum alkaloids[93-95] an example of the last group being cassaine (LVIII).

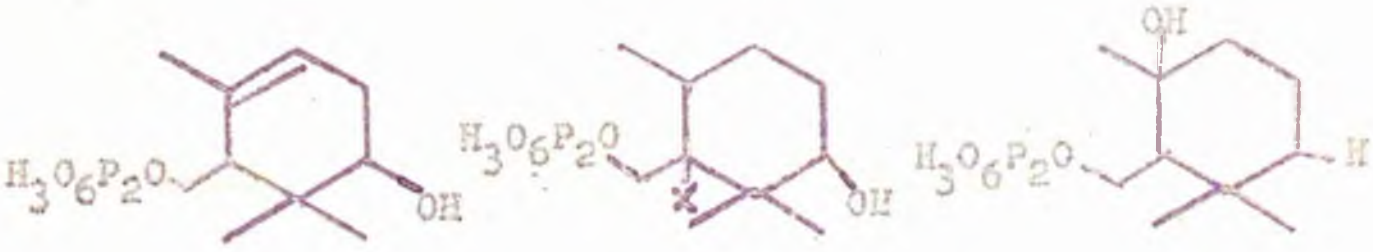
It is also to be noted that Cross[96] has suggested that the sesquiterpenoid picrotoxinin (LXI) could arise by attack on LIX by a hydroxonium ion, followed by hydroxide ion attack on the product LX causing a concerted double 1,2-methyl shift with elimination.



LIX LX LXI

Should beetles belonging to the family Meloidae possess an enzyme system utilizing $OH^{\oplus}$ as the electrophilic cyclizing agent in cantharidin biogenesis, a compound such as LXII might well be formed which on conversion into LXIII could readily give rise to the biogenetic sequence XXV to XXVII proposed by Martin-Smith and Khatoon[33]. At the same time intermediate LXIII could be derived from compounds such as LXIV where the cyclization has been initiated by a proton and followed by dehydrations, allylic oxidations, reductions, etc. From the number of

steps required it might appear that such a route was less likely than the one involving initial attack by $OH^{\oplus}$, but the possibility cannot be disregarded.



LXII LXIII LXIV

It can be seen that compounds L and LI or LII and LIII differ from each other only with respect to whether it is the unlabelled or the labelled carbon atom which suffers oxidative allylic attack in the proposals outlined. Since it may be reasonably assumed that the isomerization of XLVII to XLVIII (Figure 6) is stereospecifically controlled by an enzyme, the labelled methyl group in XLVIII would be expected to be solely cis or solely trans to the methylene groups bearing the pyrophosphate function, and randomization of the isotope label would not be expected. Similarly any allylic hydroxylation (in XLIX for example) under enzymatic control would be expected to give rise to solely an L or LII type, as distinction would be made between the methyl

group cis to the long chain at the other end of the double bond and the methyl group trans to it. Thus a mixture of the L and LII type would not be expected and one or the other would be expected as the sole product. However there is no a priori method of predicting which would be formed. The labelling pattern in cantharidin itself would then be LXV or LXVI.

LXV

LXVI

It should be noted, however, that Arigoni[13] has stressed the point that the specificity of enzyme systems appears to decrease rapidly with the number of carbon atoms present in the substrate molecule and so the validity of the above assumptions must remain in some doubt until further biochemical information becomes available.

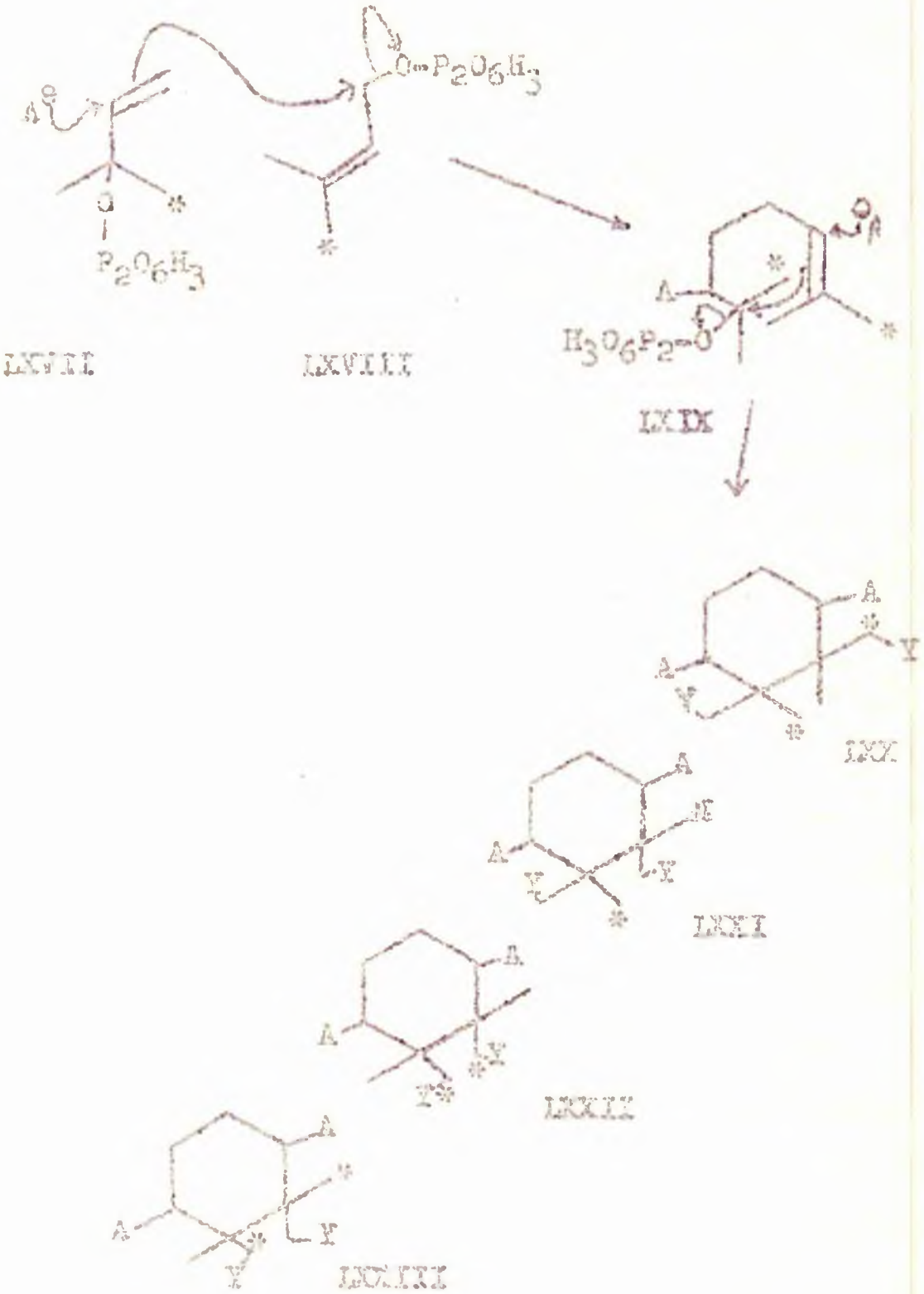


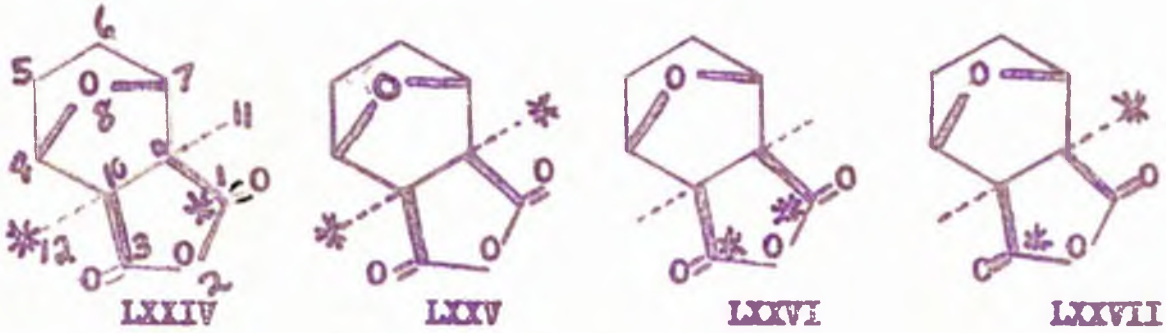
Figure 7

TAIL-TO-TAIL CONDENSATION AS A POSSIBLE MECHANISM FOR CANTHARIDIN BIOSYNTHESIS

Should cantharidin arise in nature through a tail-to-tail coupling mechanism such as those previously indicated in figures 3,4, and 5, any cyclization step must involve an anti-Markownikoff attack. Such an anti-Markownikoff addition would seem highly unlikely. However it is to be noted that the formation of the tetra- and penta- cyclic triterpenes from squalene does involve an anti-Markownikoff addition in the formation of ring C, although here the concerted nature of the total electron movements obviously provides sufficient driving force to over-ride the one unfavourable ring closure. It may also be noted that an anti-Markownikoff cyclization has been postulated in the proposed mechanism of picrotoxinin biogenesis (LIX to LXI)[96].

Several intermediates are possible from the condensation of LXVII with LXVIII, or of two molecules of LVII by the Woodward mechanism, one of which is shown in LXIX (figure 7). However, all would be expected to lead, by anti-Markownikoff cyclization, to one of the four basic types shown in LXX to LXXIII,

where "Y" is an oxygen function resulting from an earlier allylic oxidation. Once again no prior prediction of the actual position adopted by the ^{14}C label is possible and the resulting cantharidin would be one of LXXIV to LXXVII.



LXXIV LXXV LXXVI LXXVII

Proposed Research

Unfortunately the extreme winter of 1962-1963 caused the non-appearance of Meloe proscarabeus in May 1963 and despite many field trips no specimens could be found upon which to carry out labelling experiments. In outline, the approach was to have been as follows.

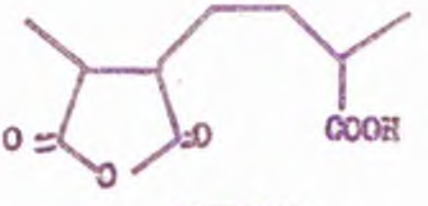
Live adult specimens of Meloe proscarabeus were to have been made to deplete their body stores of cantharidin through induction of its secretion by irritation of the beetles. Aqueous 2-^{14}C mevalonic acid lactone was then to have been

injected intraperitoneally and after a suitable interval the beetles milked for newly synthesised cantharidin, the process being repeated successively. The collection of the cantharidin - containing droplets and their subsequent concentration and sublimation, was to have been performed in an identical manner to that used in the identification of cantharidin already achieved on the specimens collected in May 1962. Later the beetles would have been killed and their bodies worked up for further quantities of cantharidin. It would then have been established whether or not the cantharidin had incorporated ^{14}C from the mevalonic acid lactone. If so, the level of radio-activity would have been ascertained so that suitable dilution with unlabelled cantharidin could be carried out. Then degradative experiments based on the known chemistry of cantharidin would have been performed on the diluted material.

Examination of formulae LXV and LXVI shows that a head-to-tail coupling of isoprene units in cantharidin would result in half the radio-activity being located in the cyclohexane ring and half in the

ring substituents. Tail-to-tail coupling on the other hand would result in all the activity being located in the ring substituents (formulae LXXIV to LXXVII). Thus the degradative scheme must include a procedure for distinguishing between the cyclohexane ring and the substituent carbon atoms.

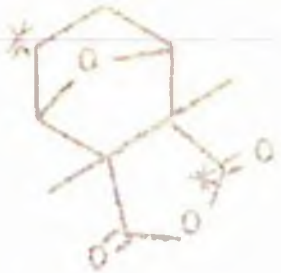
Kuhn-Roth oxidation of cantharidin would *a priori* be expected to yield two moles of acetic acid and two moles of carbon dioxide with removal of all ring substituents. Unfortunately, however, this method has been found in general to give unsatisfactory results with highly substituted C-methyl compounds[97,98]. For example the substituted methylsuccinic anhydride LXXVIII a degradation product of vitamin B_{12} gives values consistent with only one C-methyl group[99].



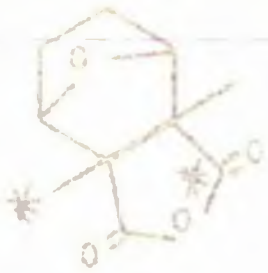
LXXVIII

During work on the structural elucidation of cantharidin, Piccard[100] and later Gadamer[101] heated

LXV



LXVI

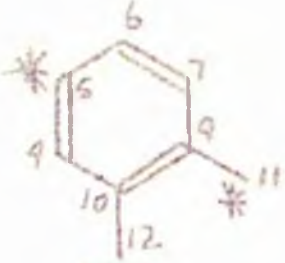


LXXIV

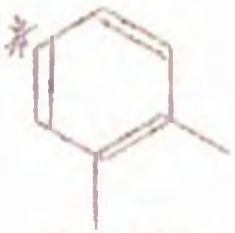
LXXV

LXXVI

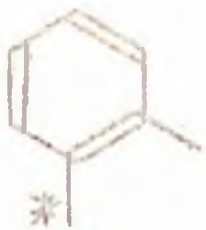
LXXVII



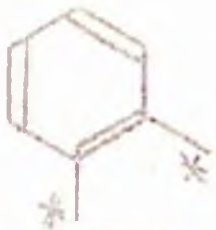
LXXX a



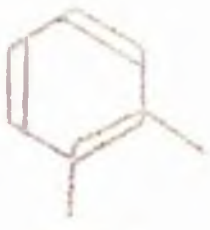
LXXX b



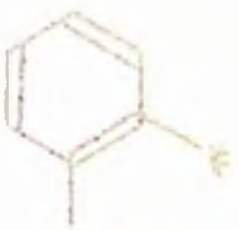
LXXX c



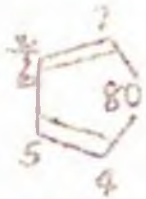
LXXX d



LXXX e



LXXX f



LXXXII a

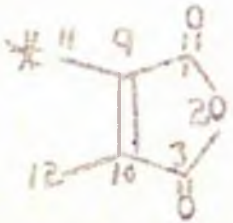
LXXXII b

LXXXII c

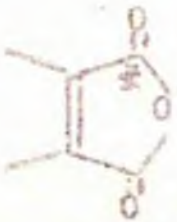
LXXXII d

LXXXII e

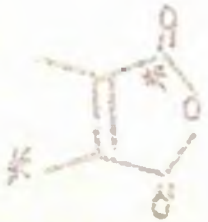
LXXXII f



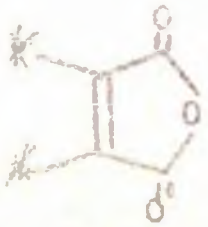
LXXXIII a



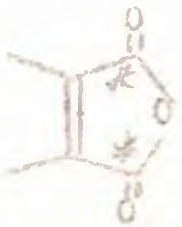
LXXXIII b



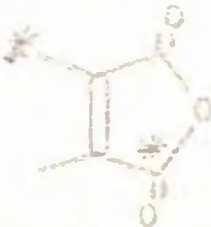
LXXXIII c



LXXXIII d



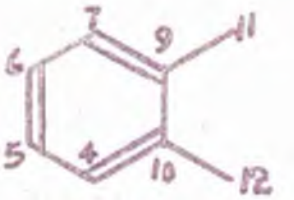
LXXXIII e



LXXXIII f

Figure 8.

cantharidin with phosphorus pentasulphide and obtained as product o-xylene (LXXIX) (numbering corresponds to the cantharidin molecule).



LXXIX

Should this degradative scheme be followed, however, the symmetry of the cantharidin molecule coupled with the symmetry of the o-xylene molecule would necessitate additional experiments. This is apparent when it is noted (Figure 8) that the six possible labelled cantharidins give rise to five labelled and one isotope-free o-xylenes (see LXXX - a to f). Production of the isotope-free form would conclusively point to pattern LXXVI in cantharidin and thus a tail-to-tail coupling occurring in Meloe proscarabeus. Production of labelled o-xylene would not per se be unambiguous. Thus double labelling in o-xylene (LXXX-a or-d) would point to either LXV or LXXV leaving the biosynthetic pathway undefined. Further the presence of single labelling in the o-xylene (as in LXXX b,c, or f) would not distinguish between LXVI, LXIV, or LXVII.

Similarly the modified degradation procedure devised by Piccard[100] and Gadamer[101] of dry distilling cantharidin with soda-lime leading to the formation of cantharene (dihydro-o-xylene; LXXXI) would also provide an ambiguous result.

LXXXI

A considerably more useful degradative pathway, especially if employed in conjunction with o-xylene formation, would seem to be catalytic fission (reversed Diels-Alder reaction) of cantharidin. Heating cantharidin with 20% palladium on charcoal is reported[102] to result in the formation of furan and dimethylmaleic anhydride, which in the case of labelled cantharidin would give rise to one of LXXXII a to f and LXXXIII a to f, respectively (Figure 8). Presence of labelling in the furan (LXXXII a LXXII b) would conclusively indicate that the head-to-tail coupling route was involved in cantharidin biosynthesis. This information coupled with the number of isotopic carbon labels found in the o-xylene, would pin-point the labelling pattern, two isotopes indicating LXV, and one, LXVI. Similarly the absence of ^{14}C in the

furan indicates that a tail-to-tail mechanism is occurring in cantharidin biogenesis. This conclusion together with knowledge of the number of isotope labels in o-xylene will show the exact route, no label in the aromatic compound indicating LXXVI, and two labels, LXXV. Should the o-xylene contain one label a distinction in the fine mechanism could not be made, but the tail-to-tail biosynthetic route for cantharidin would be confirmed.

Experimental

Melting points were taken in sealed capillary tubes in a heated block, and are uncorrected. Sublimations were carried out in horizontal tubes employing a Towers heating unit. Infra-red spectra were measured on a Perkin-Elmer Infracord.

Cantharidin from the secretion of Meloe proscarabeus L.

The droplets (5 m.g.) secreted by the M. proscarabeus prior to death were dissolved in boiling ethanol and transferred to a sublimation tube. Removal of the solvent under reduced pressure and sublimation of the oily residue at 90°/0.05 mm afforded white crystalline needles (1.3 mg.). The needles were washed with petroleum ether (40-60°) and dried in vacuo to give material m.p. 212-214° (sealed tube) identical with that obtained below from the body of the same insect and identical with authentic cantharidin.

Cantharidin from the body of M. Proscarabeus.

a. A freshly killed adult specimen (1.07g.) was frozen with dry ice and pulverized in a mortar with a pestle. A small quantity of 10% w/v HCl was added to ensure liberation of all combined cantharidin[103]

and the remains were transferred to a sublimation tube with the aid of chloroform. After first heating to 60°/0.05 mm Hg for 1 hour to remove highly volatile matter, the temperature was raised to 90° whereupon cantharidin (2 mg.) sublimed as silky white needles. After washing with petroleum ether (40-60°) these had m.p. 214-216° (sealed tube), undepressed on admixture with authentic cantharidin. The infra-red spectrum in KCl disc showed prominent peaks at 1840, 1770, 1235, 1005, 995, 890 and 850 cm^{-1}, and was completely superposable with that of authentic cantharidin.

b. A freshly killed specimen of M. proscarabeus weighing 0.626g. immediately after death was dried at 50° for 3 hours and then stored in a vacuum desiccator for 3 days. The dried carcass (0.346g.) was pulverized and treated as above, to afford, on sublimation, cantharidin (1 mg.) again having m.p. 214-216° and the correct infra-red spectrum.

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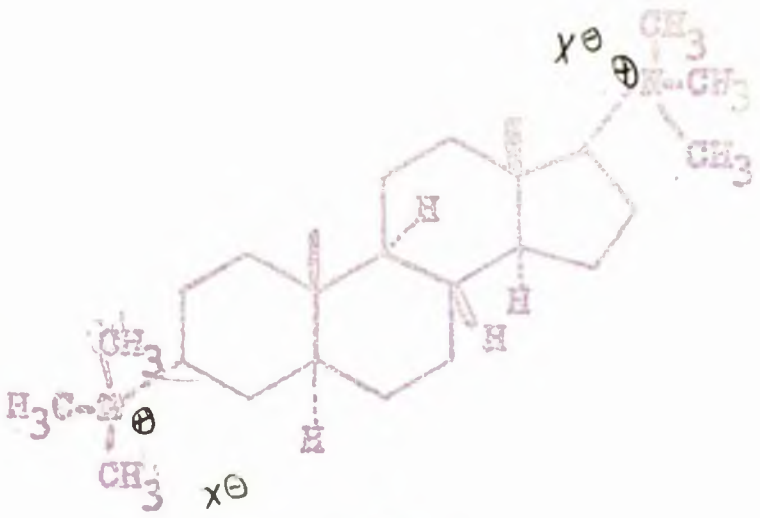
ATTEMPT TO PREPARE 3α,17α-Bis(trimethylammonium)-5α-Androstane Dimethanesulphonate By Direct Reaction of 3β,17β-Dimethanesulphonyloxy -5α-Androstane With Trimethylamine

The development and refinement within recent years of such concepts as the receptor theory of drug action[1], the metabolite displacement theory[2], the concept of bioisosterism[3], the supporting moiety theory[4], and the concept of drug latentiation[5], coupled with an increased understanding of the ultimate biochemical, physiological, and pharmacological mechanisms involved in drug action, holds out promise of a possible departure from the empiricism traditionally inherent in the synthesis of new drugs required for specific clinical purposes, with an accompanying increase in the rationality of approach to this problem. Indeed the planned synthesis of antimetabolites has been termed "the revolution in pharmacology"[6], while conclusions drawn from the receptor theory recently led to the introduction of a new class of anabolic steroids[7]. The presently described attempt to prepare 3α, 17α-bis (trimethylammonium) - 5α- androstane (I) represents an application of certain theoretical deductions in an attempt to achieve a rational approach to the synthesis of new neuromuscular blocking agents as outlined below.



I

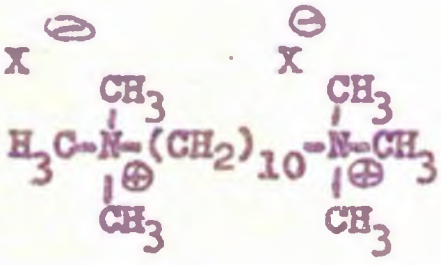
Although neuromuscular blocking properties are exhibited by a wide variety of quaternary salts as first shown by the classical work of Crum Brown and Fraser in 1869[8], potent activity would appear to be characteristic of certain compounds in which there are two or more quaternary centres. This fact has given rise to proposals of a "two-point attachment" theory[9,10], in which it is postulated that bisquaternary compounds interact simultaneously with two anionic receptor sites - sites which are normally concerned in the translation of nerve impulses into muscular contraction through the action of acetylcholine - on the outer surface of the muscle end plate of voluntary muscle[11]. A somewhat modified version of

the two-point attachment idea has been advanced by Waser[12] who regards the receptor as a roughly circular pore with anionic centres around the inner rim. Bisquaternary salts are then assumed to straddle this pore and achieve two point attachment at opposite ends of the chord which the molecule thus makes with the cross section of the circular pore. At the same time, however, not all workers accept a two point attachment and it has been proposed[13] that only one cationic head of a bisquaternary salt is actually involved in interaction with the acetylcholine receptors, the second cationic centre serving to repell incoming acetylcholine molecules. These ideas have become known as the "adumbration theory".

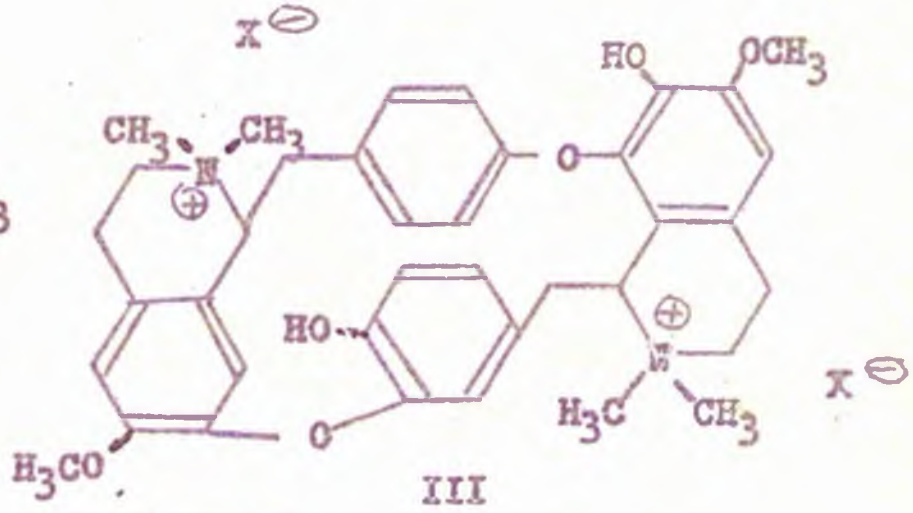
Should the "two-point" attachment theory be correct, it is of great importance that the interanionic distance of the receptor system be accurately determined since such information would prove invaluable in the design of new molecules capable of acting as potent neuromuscular blocking agents. Indeed considerable attention has been devoted to this problem, all deductions being necessarily made from considerations of the molecules of compounds known to be potent

neuromuscular blocking agents, since direct study of the receptors themselves has not proven to be feasible although claims have been made for the isolation of a protein material showing many of the characteristics to be expected of the acetylcholine receptor[14]. Unfortunately, however, the great bulk of such studies has been with conformationally non-rigid molecules, such as the polymethylene bisammonium compounds, d-tubocurarine, and their closely related analogues. It was assumed in these studies that the flexible pharmacon was adsorbed at the receptor in the conformation showing minimal non-bonded interactions within the molecule and maximal charge separation - that is in the case of the polymethylene bisammonium salts, the fully staggered conformation. The fact that decamethonium (II) was the most potent of the polymethylene series was then taken as an indication that the anionic sites in the receptor were spaced at a distance of ca 14Å apart as this was the interonium distance in the fully staggered conformation of decamethonium. Moreover Paton and Zaimis[10] claimed that this distance of 14Å would also accomodate the flexible d-tubocurarine

molecule (III) despite the fact that examination of molecular models reveals that this molecule can have interonium distances varying from 6Å to only 12Å.



II



III

The different mechanisms of action of the two compounds - depolarizing block in the case of decamethonium and non-depolarizing (formerly termed competitive) block in the case of d-tubocurarine - wasnot considered significant in terms of the inter-anionic site distance.

There is no a priori reason for assuming that bisquaternary compounds are adsorbed on the receptor in their thermodynamically most favoured conformation. Indeed the influence of entropy would be expected to ensure that within a given population of bisquaternary molecules a number would exist in other conformations and these could easily be the active species. Again

although little is known concerning the exact nature of the forces involved in drug-receptor interaction it would seem safe to assume (by analogy with known physical and chemical processes) that energy must be supplied to the system drug and receptor in order to form the drug-receptor complex. One way in which the supplied energy could be taken up would be for the drug molecule to adopt a conformation of higher energy than the fully extended conformation.

That the originally proposed distance of 14Å between adjacent anionic sites in the receptor system cannot be correct is made quite apparent by the potent neuromuscular blocking properties of the fully rigid C-curarine I and toxiferine I[15], in which the interonium distance is fixed at 9.7Å [16] and of compounds such as cyclooctadecane - 1,10-bis (trimethylammonium) iodide[17] and the polymethylene bis (tropinium) halides[16] in which the maximal interonium distance is only just over 9Å as seen by inspection of models. Moreover, recent deductions as to the interonium distances of the polymethylene bisquaternary salts from studies of their conductance in water has led to the conclusion that the internitrogen distance in decamethonium (II) is ca 9.5Å [18].

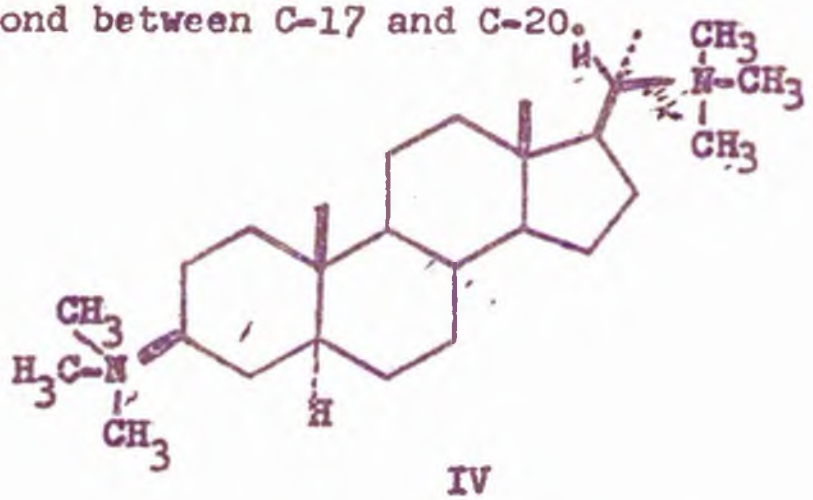
These results are of considerable interest in terms of the identity distance[19,20], or the distance between peptide bonds in a maximally extended peptide, which has the value of 3.61Å[19], and in terms of the distance between two turns of an α-protein helix which has a value of 5.5Å[21]. Making allowance for slight adaptation of the shape of the cellular proteins during drug receptor interaction (compare the induced fit theory of Koshland[22]), the interonium distances of the very active neuromuscular blocking agents can be seen to correspond closely to multiples of these distances. Thus considering the identity distance, the interonium distance of hexamethonium at 6.3Å[18] is somewhat less than 2 x 3.61, that of decamethonium at 9.5Å[18] is somewhat less than 3 x 3.61, whilst that of octadecamethonium, at which a second maximal neuromuscular blocking potency is reached[14] should be ca 14Å[18] which is somewhat less than 4 x 3.61Å.

It would appear that an exact fit at the anionic sites by the molecule of the neuromuscular blocking agent is necessary for depolarizing activity, for when steric hinderance to the approach of the cationic heads

to the receptor is increased by increasing the bulk of the substituents on the nitrogen atom, change to non-depolarizing activity is observed. In view of the non-depolarizing activity of hexamethonium it may therefore be that the molecules of this compound are less capable of an exact fit at the receptor system than are those of decamethonium. It is also of considerable interest that replacement of the trimethylammonium functions in the polymethylene bis (trialkylammonium)-series by triethylammonium groups leads to an increase in interonium distance as shown by conductance experiments[23].

In view of the fact that the conductance measurements show the interonium distance in decamethonium to be ca 9.5Å [18] whilst the rigid toxiferine I has an interonium distance of 9.7Å, it seemed of considerable importance to prepare other rigid bisquaternary salts having an interonium distance of this order and test whether or not such compounds would be potent neuromuscular blocking agents. Construction of molecular models shows that 3α,17α- bis (trimethylammonium) - 5α- androstane (I) has an interonium distance of 9.5Å

so this compound seemed an obvious compound to synthesise and test biologically. Moreover that it could be expected to possess a favourable combination of lipophilic to hydrophilic properties, upon which great stress has been laid as a factor in the determination of neuromuscular blocking activity[24], would be suggested by the potent activity present in the steroidal alkaloid malouetine (3β, 20α- bis (trimethylammonium) - 5α- pregnane) (IV)[25] which has an interonium distance of ca 11.5 ± 1.5Å, the range being due to free rotation of the bond between C-17 and C-20.



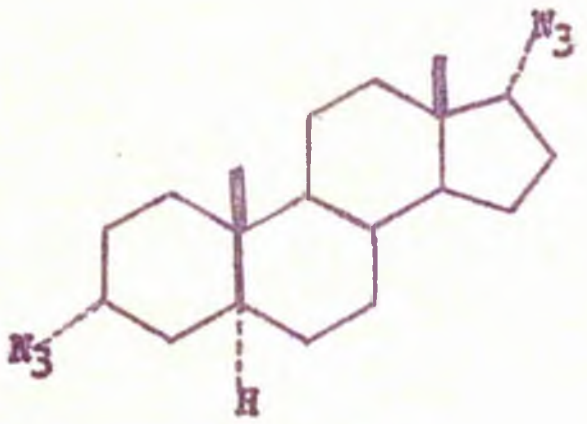
IV

The obvious route to the desired 3α, 17α-bis (trimethylammonium)-5α androstane appeared to be by way of nucleophilic displacements of the methane - sulphonyloxy groups of 3β, 17β- dimethanesulphonyloxy 5α- androstane by suitable nitrogenous nucleophilic

species as this would be expected to give inversion of configurations at both C-3 and C-17 if the reaction were S_N^2 at both centres. Nucleophilic displacement with inversion of the methanesulphonyloxy group in the 3β-position would not be expected to involve any complications in view of earlier work[26] but S_N^2 attack at C-17 could be expected to involve some difficulties as this position is a neopentyl position. However, recently, through the use of N-methyl-pyrrolidone : tertiary-butyl alcohol (19:1) as a solvent, a number of successful S_N^2 replacements of 17β- substituents have been reported in the steroid field[27]. Accordingly it was decided to employ a modification of this procedure in an endeavour to replace both methanesulphonyloxy groups in 3β, 17β-dimethanesulphonyloxy - 5α-androstane by 3α and 17α trimethylammonium functions through the direct action of trimethylamine. Earlier attempts employing dimethylamine on the 3β, 17β- dimethanesulphonyloxy- compound had failed to give the corresponding bis tertiary amine, 3α,17α- bis (dimethylamino) - 5α-androstane - replacement of the 3β-methanesulphonyloxy group only being achieved[28].

In the event a number of experiments employing different reaction conditions failed to give the required product. Water soluble materials, bitter in taste as would be expected of the desired product, consistently gave high nitrogen analyses and none of the crude products showed any neuromuscular blocking activity on the cat[29]. Subsequent work by another worker[30] in this laboratory directed towards the preparation of 3α,17α- diazido-5α- androstane (V) employing azide ion as the attacking nucleophilic species on 3β,17β-dimethanesulphonyloxy - 5α- androstane in accordance with the known superior yields resulting in the steroid field when this ion is employed in place of an amine[31] has shown that in the course of the reaction the N-methyl pyrrolidone is destroyed. Thus it could well be that the products with the high nitrogen content obtained in the present work are formed from the N-methyl pyrrolidone. In view of the promising results obtained with the azide ion[30] the current project was abandonned since the successful preparation of 3α,17α-diazido- 5α- androstane gives a ready route to the desired bis-quaternary salt <u>via</u> reduction to the di-primary

amine[32] and exhaustive methylation.



V

EXPERIMENTAL

Melting points were taken on a hot-stage melting point apparatus and are uncorrected. N-methyl pyrrolidone was dried over sodium hydroxide pellets and distilled, the fraction b.p. 201-203° being collected. Tertiary-butyl alcohol was distilled from sodium and the fraction b.p. 82-83° collected. The 3β,17β-dimethanesulphonyloxy-5α-androstane employed in these experiments was prepared by the sodium in ethanol reduction of 5α-androstane - 3,17-dione as described by Alauddin[28] Authenticity of the dimethanesulphonate so prepared was confirmed by comparison of infra-red spectra and undepressed mixed melting point, 150-152°, with an authentic sample. A number of experiments were performed of which the following is typical.

Attempted S_N2 Displacement of 3β, 17β-dimethanesulphonyloxy-5-α- Androstane by Trimethylamine.

3β,17β-dimethanesulphonyloxy-5α-androstane (0.45g) in N-methyl pyrrolidone (20 ml) containing tertiary butyl alcohol (0.5 ml.) and anhydrous trimethylamine (20 ml.) was placed in a pressure bomb (95 ml. capacity) the bomb closed and then heated at 206°C for 25 hr.

After cooling for 14 hr. the bomb was opened and the excess trimethylamine allowed to spontaneously evaporate. The reaction mixture was then transferred to a flask and the excess N-methyl pyrrolidone evaporated under reduced pressure. The brown residue thus remaining was extracted with distilled water (5x5 ml.) and the combined aqueous wash taken to dryness on a rotary film evaporator. The hygroscopic, bitter-tasting, tan-coloured crystalline residue was re-crystallized from ethanol/ether to m.p. 238-240° (softens ca 180°) (Found; N,6.44; $C_{27}H_{54}N_2O_6S_2$ requires: N,4.94%) Treatment of the quaternary compound with saturated ethanolic picric acid readily afforded the picrate m.p. 310° (decomp.) (Found: N,17.89; $C_{37}H_{52}N_8O_{14}$ requires: N,13.46%)

Other experiments employing modified conditions also afforded material showing high nitrogen analyses.

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The Structure of Aristolactone, A 10-Membered Carbon Ring Sesquiterpene Lactone from *Aristolochia reticulata* L. and *Aristolochia serpentaria* L.

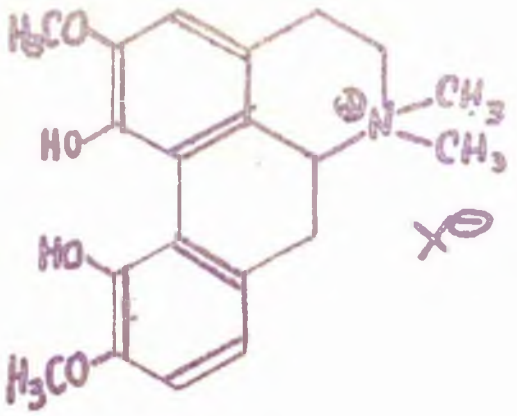
I N T R O D U C T I O N

The plant order Aristolochiales embraces three families, Rafflesiaceae, Hydnoraceae and Aristolochiaceae. The first two consist of parasitic species whilst the third, which is made up mainly of climbing plants with woody stems, is composed of some six genera containing about 200 species. Of these approximately 180 belong to the genus Aristolochia, a genus having world-wide distribution[1].

Aristolochia species are known to have been used in folk medicine since about the fourth century B.C.[2,3] their use which was held in high esteem by the ancient Greek, Roman, and Hebrew physicians being varied. Thus they are reputed to have been employed in child-birth, in the treatment of cancer, wounds, ulcers, abscesses, fevers, asthma and epilepsy, as bitter tonics and purgatives, and for treating snake-bite[4]. The Chinese are reported to have used A. contorta and A. kempferi in treating similar afflictions. Various species of Aristolochia were apparently also used by the early North American Indians as a snake-bite remedy[6,7,8] and to this day Aristolochia species are still employed

in this way in Brazil [9] and Mexico[10], although examination of the extractives of a number of species showed them to be ineffective as antidotes to snake poisoning[11].

An indication of the possible value of Aristolochia species in child-birth was given by Shaw's isolation of an unidentified alkaloid from A. elegans which produced uterine contractions[12]. Later the aporphine alkaloid magnoflorine (I) was isolated from A. debilis[13], A. kempferi[14] and A. clematitis[15,16] but no reports of its pharmacological properties appear to have been published. Extracts of Aristolochia species have also been shown to be bacteriostatic towards Staphylococcus aureus[17] and other bacteria[18]. Very recently Kupchan and Doskotch[19] have reported that aristolochic acid (II) (a constituent of a number of Aristolochia species) exhibits tumour-inhibiting activity against adrenocarcinoma 755, in mice, thus providing a renewed interest in the genus.



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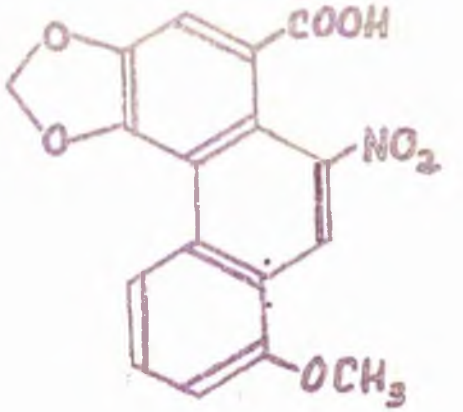
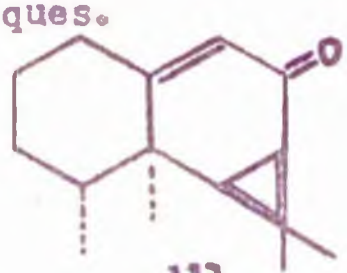


II

Another interesting compound recently isolated from the genus by Furukawa and Soma[20] is the sesquiterpenoid ketone, aristolone (III) which occurs in A. debilis and which is probably derived from the maliane skeleton(IV) via a 1,2 - methyl shift. The stereochemistry of aristolone was determined by Buchi, Greuter, and Tokoroyama[21] employing nuclear magnetic resonance techniques.



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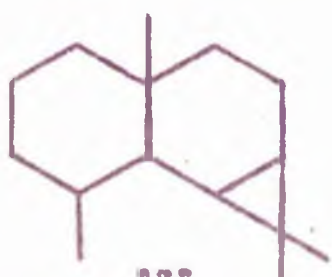


IV

Apart from the compounds just mentioned, the very extensive chemical studies which have been performed on the genus Aristolochia have resulted in the identification of a large number of other

organic constituents. A summary of the constituents so far isolated from Aristolochia species is given in Table 1.

T A B L E 1.

SPECIES	CONSTITUENT	FIG.	FORMULA	m.p. °C	REF.
A. argentina Griseb.	aristolochine		$C_{15}H_{28}NO_3$	265	>22
	palmityl phytosterolin		$C_{42}H_{74}O_2$	82	
	aristinic acid		$C_{18}H_{13}NO_7$	275	
	aristolic acid		$C_{15}H_{11}NO_7$	260-270	
	aristidinic acid		$C_{18}H_{13}NO_7$	260	
	aristolochic acid I.	II	$C_{17}H_{11}NO_7$	290	
A. bracteata Retz.	Aristolochic acid I.		$C_{17}H_{11}NO_7$	287-292	23,24. *
A. clematitis L.	magnoflorine	I	$C_{20}H_{24}NO_4$	248-249 (iodide)	15,16
	aristolochine		$C_{32}H_{22}N_2O_{13}$	215	2
	choline	V	$C_5H_{14}NO$		15
	aristolochic acid I.	II	$C_{17}H_{11}NO_7$	287-292	25
	aristolochic acid II.				26
	nor-aristolochic acid	VI	$C_{16}H_{19}NO_6$	209	27

SPECIES	CONSTITUENT	FIG.	FORMULA	m.p. °C	REF.
A. cymbifera Mart. (A. grandiflora Gomes)	neutral compound		$C_{18}H_{28}O$	137	28
	crocetin dimethylester	VII	$C_{22}H_{28}O_4$	211-212	
	isobixin		$C_{25}H_{30}O_4$	215	
	allantoin	VIII	$C_4H_6N_4O_3$	221	
	A. cymbifera acid		$C_{20}H_{32}O_2$ or $C_{21}H_{34}O_2$	107	
	β-sitosterol	IX	$C_{29}H_{50}O$	140	*
A. debilis Sieb et Zucc	aristolochic acid I	II	$C_{17}H_{11}NO_7$	290	29,30
	debelinic acid		$C_{18}H_{13}NO_7$	350	31
	aristolochic acid "C"		$C_{16}H_{19}NO_7$	280	32
	aristolactam	X	$C_{17}H_{11}NO_7$	305	32
	magnoflorine	I	$C_{20}H_{24}NO_4$	245	14
	aristolone	III	$C_{15}H_{22}O$		20
A. indica L.	aristolochine		$C_{17}H_{19}NO_3$	215	33
	iso-aristolochic acid		$C_{17}H_{11}NO_7$	275	
	phytosterolin				
	ishwarene		$C_{15}H_{24}$		
	ishwarone		$C_{15}H_{22}O$		
	ishwarol		$C_{15}H_{24}O$		

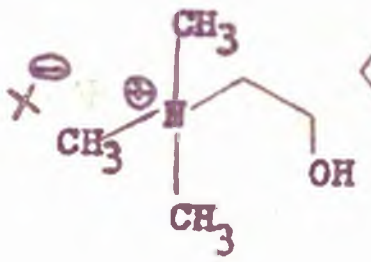
SPECIES	CONSTITUENT	FIG	FORMULA	m.p. °C	REF.
A. indica L.	allantoin	VIII	$C_4H_8N_4O_3$		33
	oleic acid		$C_{18}H_{34}O_2$		
	linoleic acid		$C_{18}H_{32}O_2$		
	palmitic acid		$C_{16}H_{32}O_2$		
	stearic acid		$C_{18}H_{36}O_2$		
	lignoceric acid		$C_{24}H_{48}O_2$		
	cerotic acid		$C_{26}H_{52}O_2$		
	glycerol		$C_3H_8O_3$		
	ceryl alcohol				
	phytosterol			137-138	
	aristolochic acid I.	II	$C_{17}H_{11}NO_7$	290	33,34
A. kempferi Willd.	magnoflorine	I	$C_{20}H_{24}NO_4$		13
	aristolochic acid I.	II	$C_{17}H_{11}NO_7$	290	14
A. longa L.	aristolochic acid I	II	$C_{17}H_{11}NO_7$	290	34
A. maxima Jacq.	aristolochic acid I	II	$C_{17}H_{11}NO_7$	290	35

SPECIES	CONSTITUENT	FIG	FORMULA	m.p. °C	REF.
A. pandurata Jacq.	aristolochic acid I	II	$C_{17}H_{11}NO_7$	290	35
A. reticulata L.	Terpene		$C_{10}H_{16}$		36
	acetic acid		$C_2H_4O_2$		37
	maleic acid				
	oxalic acid				
	aristolochine				
	D(+)Glucose				
	water-insoluble acid		$C_5H_9O_2$	approx 65	
	(-) - borneol	XI	$C_{10}H_{18}O$		38
	(-) -Δ^4carene	XII	$C_{10}H_{16}$		
	aristolactone		$C_{15}H_{20}O_2$	111	
	quaternary alkaloid		$C_{17}H_{20}NO_3Cl$		34
	iso-rhamnetin	XIII	$C_{16}H_{12}O_7$	318-322	
	aristolochic acid I	II	$C_{17}H_{11}NO_7$	290	
	aristo-red	XIV	$C_{19}H_{15}NO_6$		
	allantoin	VIII	$C_4H_8N_4O_3$	221	39
	β-sitosteryl-1-β-D-glucoside		$C_{35}H_{60}O_6$		
	reticulene	XV	$C_{15}H_{24}$		

SPECIES	CONSTITUENT	FIG.	FORMULA	m.p°C	REF.
A rotunda	aristolochine		$C_{32}H_{22}N_2O_{13}$	215	40
A. serpentaria L.	borneol	XI	$C_{10}H_{18}O$		41
	β-sitosterol	IX	$C_{29}H_{50}O$	140	42
	β-sitosteryl-1-β-D-glucoside		$C_{35}H_{60}O_6$		39,42
	aristolochic acid I	II	$C_{17}H_{11}NO_7$	290	34,35
	aristo-red	XIV	$C_{19}H_{15}NO_6$		39
	aristolactone		$C_{15}H_{20}O_2$	111	39
A. sipho L'Herit	aristolochic acid	II	$C_{17}H_{11}NO_7$	290	43

* present work

† quoted as aristolochic acid I by Boit, "Ergebnisse Der Alkaloid-Chemie Bis 1960", Akademie Verlag, Berlin, 1961, p. 271.



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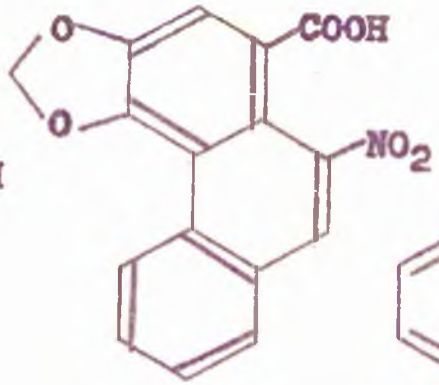

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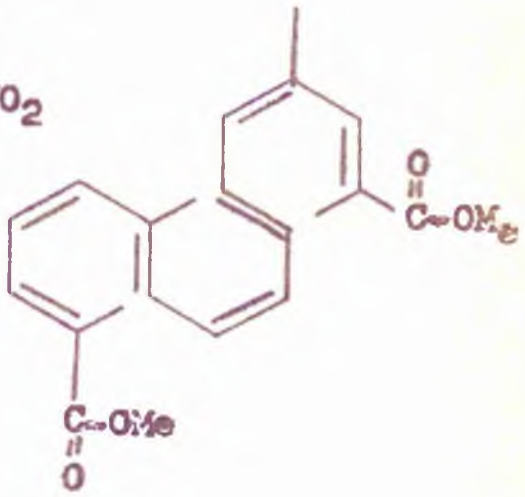

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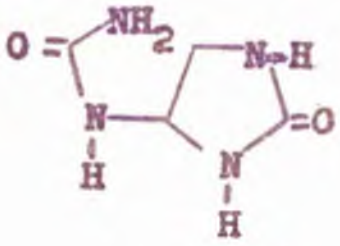

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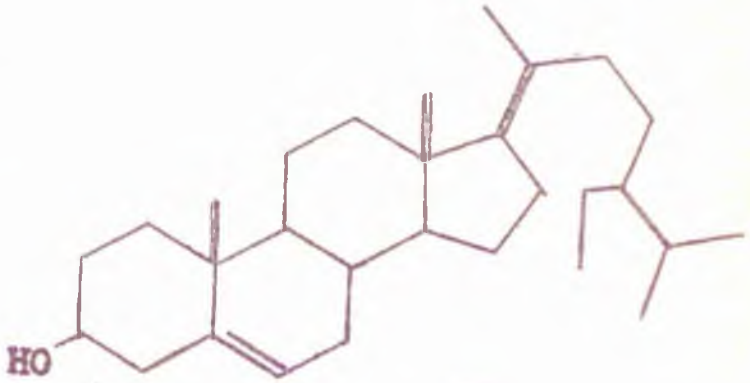

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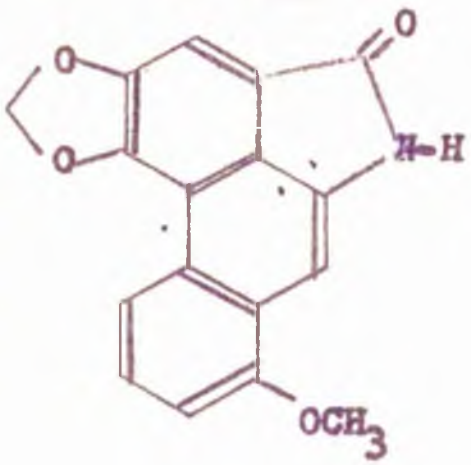

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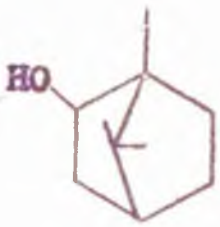

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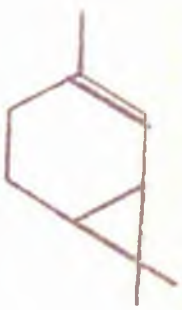

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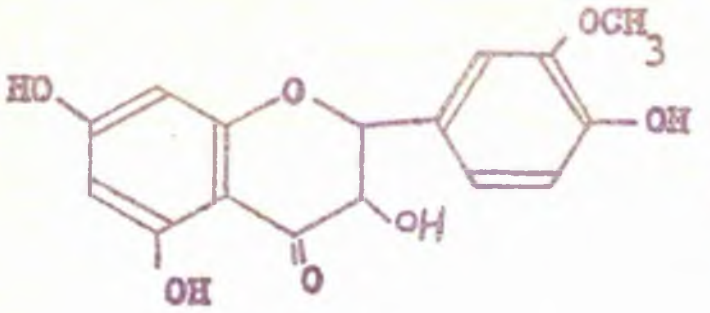

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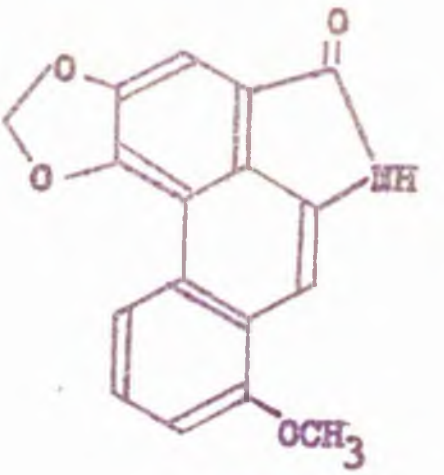

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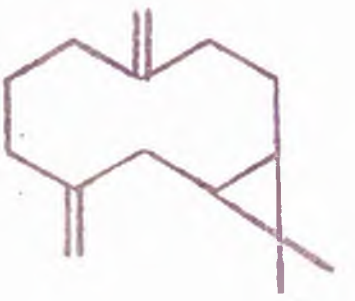


XV

In the course of a general investigation of representative Aristolochia species, Stenlake and Williams isolated from the petroleum ether extractives of A. reticulata[38,44] and later from the corresponding fraction of A. serpentaria[45] a crystalline lactone which they designated aristolactone. Although their investigations resulted in the tentative assignment of a structure for aristolactone, there were several inconsistencies in the experimental data necessitating further work on the problem.

Accordingly the present investigation was undertaken and in the event resulted in a revised structure for this compound.

Aristolactone, m.p. 110.5 -111°, $[\alpha]_D^{14}$ +156.4 (EtOH) was assigned the empirical formula $C_{15}H_{20}O_2$ on the basis of elemental analyses and Raast molecular weight determination. Hydrogenation established the presence of three double bonds. Calculation of double bond equivilents ($C_nH_{2n+2-m}O_{15}H_{32}$; $\frac{32-20}{2}=6$) thus showed that aristolactone could contain only one ring other than the lactone ring. The presence of the lactone ring was established by the hydrolysis of aristolactone to an hydroxy-acid containing all the carbon atoms of aristolactone in quantitative yield. Aristolactone was initially thought to exhibit an ultraviolet absorption maximum at 211 mμ (ε,11,500)[30,44,47] but later Steele[46] using more accurate techniques showed aristolactone to exhibit an absorption maximum with ε,11,500 at 205 mμ. Ozonolysis of aristolactone yielded formaldehyde (collected as the dimedone derivative)[45,46] thus indicating the presence of at least one vinylidene group.

Careful hydrogenation of aristolactone over palladium on charcoal in ethanol[44,47] afforded a dihydroderivative, m.p. 79-80·5°, $[\alpha]_D^{17}$-77, (EtOH), λ 209 mμ (ε,7800) which on ozonolysis no longer gave formaldehyde. When hydrogenation of aristolactone was carried out using Adam's catalyst, 2 moles of hydrogen were rapidly absorbed yielding an oil whose properties were indicative of a mixture of double bond isomers. Exhaustive hydrogenation of aristolactone over prereduced platinum oxide[44,47] gave a total hydrogen uptake of 3 moles, to yield a crystalline compound, $C_{15}H_{26}O_2$, m.p. 103·5-104°, $[\alpha]_D^{17}$+3.0° (EtOH) initially termed hexahydroaristolactone[44,47] but later termed isohexahydroisoaristolactone[45]. The hexahydrocompound, unlike aristolactone, was found to be stable to cold alkali, but was hydrolysed by hot alkali to a mixture of two hydroxy-acids. The major product $C_{15}H_{28}O_3$ exhibited m.p. 86-87°, and $[\alpha]_D^{16}$+16° (Et OH), while the minor hydroxy-acid, which was isolated in only trace amounts, had m.p. 121-122°. The hexahydro-compound itself could be isolated in two forms, needles, m.p. 99-100° and platelets m.p. 101-102°. Since both showed identical

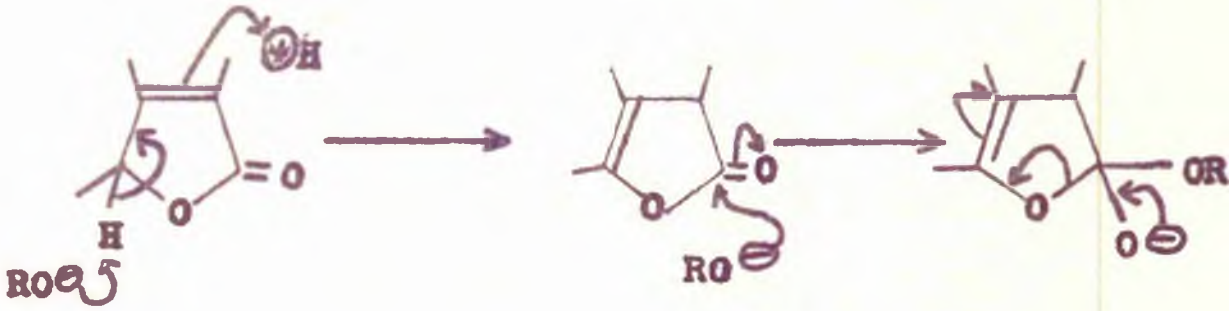
infrared spectra and optical rotations, and on hydrolysis afforded the same mixture of hydroxy acids it was concluded that the two forms of the hexahydrolactone (which on admixture gave an elevated melting point, 103-104°) were dimorphs and not stereoisomers, which might well be expected to arise on reduction of the three double bonds present in aristolactone. The large shift in optical rotation encountered in the reduction products of aristolactone was attributed to one or more asymmetric centres being in close association with a double bond [44,48].

Williams[44,47] observed that treatment of aristolactone with cold potassium hydroxide in ethanol also produced a marked change in optical rotation. The initial increase which reached a maximum of $[\alpha]_D$ +317° in 50 minutes, was shown to occur without the consumption of alkali, whereas the subsequent decrease in rotation was accompanied by an uptake of one mole of base. Work-up of the reaction mixture at the point of maximum rotation afforded a keto-ester which was designated ethyl oxoaristate. Subsequently it was discovered that the use of methanolic potassium hydroxide afforded

the corresponding methyl ester in superior yield[44,47]. Formation of the keto-ester was attributed to attack by alkoxide ion on a proposed αβ-unsaturated lactone this being followed by hydrolysis of the ester function under the further influence of hydroxide ion in accord with scheme A (partial structures XVI to XX).

The keto ester, ethyl oxoaristate, $C_{17}H_{26}O_3$ had m.p. 56-57°, $[\alpha]_D$+ 317° (EtOH) λ_{max} 1726 and 1168 cm^{-1} (ester) and 1704 cm^{-1} (shoulder, ketone) in paraffin mull, and exhibited a low intensity ultraviolet ketone maximum at 291 mμ (ε,250). Methyl oxoaristate had m.p. 68-69°, $[\alpha]_D$+ 342° (EtOH)[47]. Hydrogenation of methyl oxoaristate over prereduced platinum oxide afforded methyl dihydrooxoaristate, $C_{16}H_{26}O_3$, m.p. 68-68.5°, $[\alpha]_D^{17}$ + 152°, (EtOH) λ_{max} 290 mμ (ε,160) and end absorption at 210 mu (ε,3600)[45]. Further reduction gave the fully saturated tetrahydro derivative as an oil[44,46]. Similarly hydrogenation of ethyl oxoaristate afforded a dihydro derivative, m.p. 65-66°, $[\alpha]_D$+ 131°, λ_{max} 287 mμ (ε,52) and end absorption at 208 mμ (ε,3570)[45]. On further reduction it gave the fully saturated tetrahydro derivative, again as an oil[44,46].

Steele[45,46] questioned the original proposals



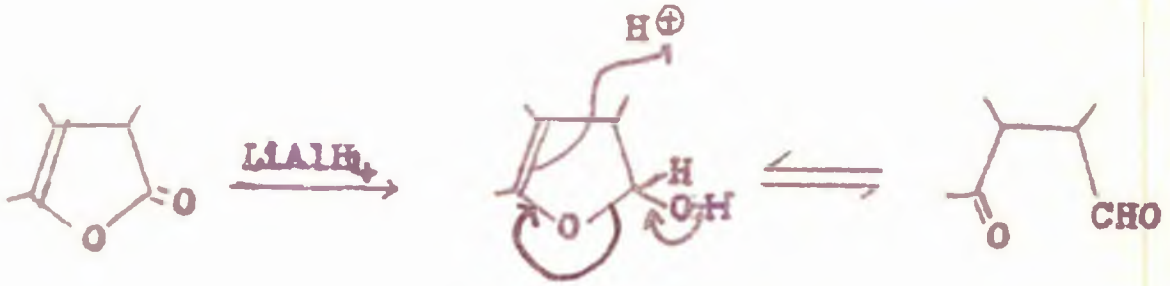
XVI XVII XVIII

XX XIX

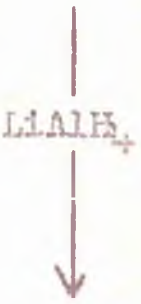
S C H E M E A

in which aristolactone was considered to be an $\alpha\beta$-unsaturated lactone, and pointed out that a β,γ-unsaturated lactone would be more consistent with the observed ultraviolet maximum at 205 mμ, since an $\alpha\beta$-unsaturated lactone would be expected to exhibit a maximum at 215-225 mμ[49]. This reassignment of the lactone double bond of aristolactone $\beta\gamma$- to the lactone, as shown in partial structure XVII still permitted rationalization of the formation of methyl- and ethyl-oxoaristates (XVII to XIX in scheme A). In addition, this new assignment of the double bond explained the negative Legal test[50] and the non-formation of an ammonia adduct from aristolactone, (a reaction typical of $\alpha\beta$-unsaturated lactones[51,52]). Further, the presence of a $\beta\gamma$-double bond explained the absence of pyruvic acid in the oxonolysis products of aristolactone[44,46] and the partial iodine values given by this compound[53].

Steele[45,46] adduced what he considered to be better evidence for the presence of a β,γ-unsaturated γ- lactone system in aristolactone when he obtained what was concluded to be a



XVII XXI



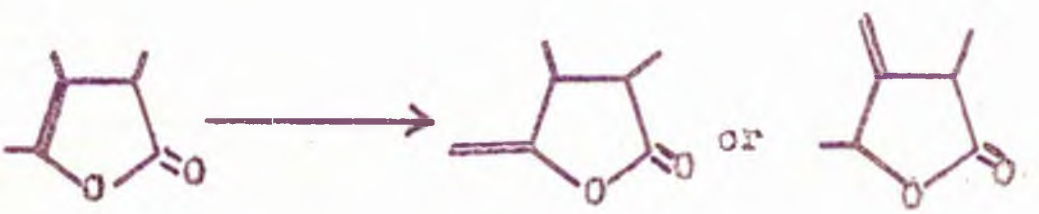
XXIII XXII

S C H E M E 3

1,4- ketal from the lithium aluminium hydride reduction of aristolactone. An $\alpha\beta$- unsaturated, γ-lactone would have given rise to an allylic 1,4- diol. The presumed ketal was assigned the name oxoaristaldehyde[45]. Analysis indicated the empirical formula $C_{15}H_{22}O_2$. The compound had m.p. 197-198°, $[\alpha]_D^{16.5}$ +82° (EtOH) ν_{max} 1752, 1733 cm^{-1}(shoulder) and λ_{max} 284 mμ (ε,58), and was assigned partial structure XXI (scheme 3). Lithium aluminium hydride reduction of aristolactone under slightly more vigorous conditions yielded a compound named oxoaristool, $C_{15}H_{24}O_2$, m.p. 245-246° which was assigned the partial structure XXII (scheme 3). On lithium aluminium hydride reduction of the hexahydro-derivative of aristolactone, Steele[46] obtained the expected 1,4-diol, $C_{15}H_{30}O_2$, m.p. 106-107°, $[\alpha]_D^{20}$ +18.7° (EtOH) partial structure XXIII (scheme 3).

On treating aristolactone with a number of acidic reagents both Williams[44] and Steele[46] obtained isoaristolactone, $C_{15}H_{20}O_2$, m.p. 90-91°, $[\alpha]_D^{19}$ - 44° (EtOH) λ_{max} 209 mμ (ε,11,200) and 272 (ε,640). This isomer was affected by base only under very vigorous conditions, a

fact which was interpreted to mean that the double bond considered to be $\beta\gamma$-to the lactone carbonyl in aristolactone was the site of isomerization. The large change in optical rotation accompanying the formation of isoaristolactone ($[\alpha]_D$ +156→$[\alpha]_D$ -44°) was assumed to arise from the generation of a new asymmetric centre. Hydrogenation of isoaristolactone was found to afford the same dihydro- and hexahydro-derivatives as were obtained from aristolactone. This was taken as support for the hypothesis that rearrangement of the double bond $\beta\gamma$-to the lactone carbonyl in aristolactone also occurred on hydrogenation of this compound as illustrated by the conversion of partial structure XVII into XXIVa or XXIVb.



XXII XXIVa XXIVb

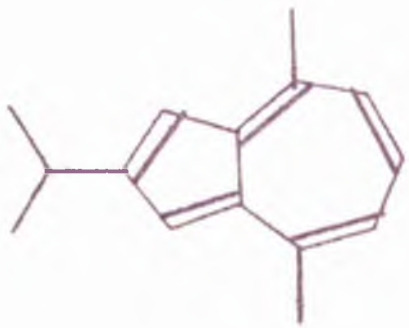
Partial structure XXIVb was eliminated on the grounds that the action of lithium aluminium hydride

on isoaristolactone afforded a compound, $C_{15}H_{22}O_2$, $[\alpha]_D$- 50.1(EtOH) considered to be a ketone as it exhibited λmax 290 (ϵ,30) and end absorption at 209 mμ (ϵ,4960). Such a product might be expected to arise from XXIVa but not from XXIVb which would form a diol.

In view of the identical reduction products obtained from aristolactone and isoaristolactone the nomenclature of the dihydro- and hexahydro- derivatives was amended to dihydroisoaristolactone and hexahydroisoaristolactone respectively.

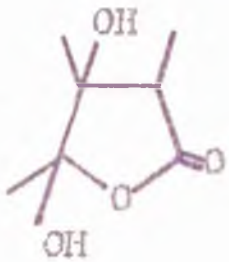
Williams[44] carried out a number of dehydrogenation experiments on aristolactone employing palladium on charcoal, but was unable to demonstrate the presence of either azulenic or naphthalenic products. However, benzenoid absorption in the ultraviolet was observed in the dehydrogenation products of both aristolactone and hexahydroisoaristolactone, although none of these products was isolated in pure form. Steele[46] using a slightly modified dehydrogenation procedure on certain semisolid residues obtained as by-products from the preparation of various derivatives of

aristolactone, isolated a purple azulenic product. This azulene, aristazulene, exhibited ultraviolet absorption maxima at 245, 279, 289, 333 and 348 mμ and visible light absorption maxima at 553, 561, 585, 593 and 642 mμ. In 50% sulphuric acid aristazulene showed absorption maxima at 227, 269, and 374 mμ. Steele drew the firm conclusion that aristazulene was a 2,4,8- trisubstituted azulene since its spectrum was virtually identical with the published spectra of typical 2,4,8- trisubstituted azulenes, in both the ultraviolet[54,55] and the visible[55,56,57] ranges. He also drew attention to the possibility that aristazulene was identical with vetivazulene (XXV)[54,57].



XXV

When Williams[44] treated aristolactone with potassium permanganate in ice-cold acetone he obtained a neutral crystalline product, $C_{15}H_{22}O_4$, m.p. 158.5 - 160°, $[\alpha]_D$ +128°, concluded to be a 1,2- diol. Since sodium metaperiodate oxidation of this product afforded no formaldehyde it was concluded that the diol had not formed at the site of a vinylidene double bond but that it was derived from the double bond placed β,γ-to the lactone carbonyl. Further support for this contention was considered to be provided by the facts that there was little change in the optical rotation in passing from aristolactone to the diol, that the lactone ring was intact, and that the iodine values were in good agreement with the presence of two double bonds. The diol was accordingly represented by partial structure XXVI.



XXVI

Subsequent hydrogenation of the diol by Steele[46] afforded two compounds, termed dihydrodihydroxy-aristolactone, m.p. 135-136°, end absorption at

210 mμ (ε,3000), and the fully saturated tetrahydrodihydroxyaristolactone, $C_{15}H_{26}O_4$, m.p. 123-124°, $[\alpha]_D^{20}$ +32.2 (EtOH).

From the chromic acid oxidation of aristolactone, Williams[44] isolated acetic acid in quantity indicative of the presence of two $>C-CH_3$ groups. Also formed were an unidentified keto-acid, and succinic acid. Since the presence of glutaric acid, an expected oxidation product of partial structure XXVII, could not be detected in the oxidation products, structures possessing this unit were eliminated from consideration as possible structures for aristolactone

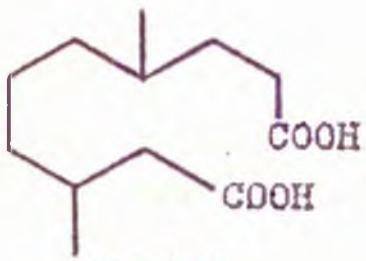
$$C-CH_2-CH_2-CH_2-C$$

XXVII

From the chromic oxide oxidation of isoaristolactone Steele[46] was able to isolate formic acid (derived from a vinyl group), acetic acid (derived from $C-CH_3$ groups), and succinic acid. In addition an unidentified dibasic acid thought to contain 8 to 10 carbons on the basis of its behaviour on paper chromatography was found to

be present in the reaction products.

After treating methyl tetrahydro-oxoaristate with hot nitric acid, Steele isolated another dicarboxylic acid which he characterized as the silver salt, $C_{12}H_{20}O_4Ag_2$. This dibasic acid was considered to be 3,7-dimethyldecanedioate (XXVIII) solely on the grounds that it had an Rf. value greater than that of pimelic acid (XXIX) when subjected to paper chromatography.[46]

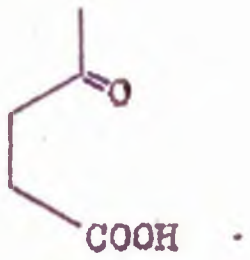


XXVIII

XXIX

Both Williams[44] and Steele[46] found that ozonolysis of aristolactone yielded formaldehyde (60% of theoretical yield based on one vinyl group) as the only volatile fragment. The non-volatile residues contained two keto-acids, one of which was suggested by Williams[44] on the basis of paper chromatographic studies to be laevulenic acid (XXX). Steele[46] however pointed out the similarity of its Rf. value to that of acetoacetic acid (XXXI), although the known

instability of this compound would make its isolation highly unlikely.



XXX

XXXI

The second keto-acid was not characterized, but was found to yield acetaldehyde on reozonolysis. No acetone or pyruvic acid could be detected in the ozonolysis products.

Similarily ozonolysis of isoaristolactone[44] gave formaldehyde (53% of theoretical yield based on one vinylidene group) as the only volatile product. Dihydroisoaristolactone, however, gave no volatile carbonyl containing fragment on ozonolysis, indicating the absence of a 1,1- disubstituted double bond in that compound.

On ozonolysis, ethyl oxoaristate[44] also yielded formaldehyde (40% of theoretical yield based on one vinylidene group) as the only volatile product. The non-volatile residues were not characterized. Ethyl dihydro-oxoaristate afforded no volatile fragments on ozonolysis

indicating the absence, as in dihydroisoaristolactone, of any vinyl double bond. Identical observations were made for methyl dihydrooxoaristate, although here the oily non-volatile product, b.p. 220-240 °/0.4 mm Hg showing positive reactions for a methyl ketone gave good analyses for $C_{16}H_{24}O_5$.

The non-formation of acetone or 2,2-dimethylacetaldehyde in the ozonolysis of all these aristolactone derivatives precluded the presence of an isopropylidene (XXXII) or a 2-methylpropylidene (XXXIII) group.

XXXII

XXXIII

The infrared spectrum of aristolactone in carbon tetrachloride solution was stated[144] to exhibit a band at 1770 cm^{-1} typical of a γ-lactone and this absorption was said to shift to 1780 cm^{-1} in hexahydroisoaristolactone but the present work (*vide infra*) employing a Unicam S.P. 100 instrument

showed that these frequency assigments were not accurate. The fact that the ester stretching vibrations at 1064 and 1034 cm^{-1} in aristolactone were replaced by a single band in the hexahydro-isoderivative at 1167 cm^{-1} was taken[44] to indicate that hydrogenation had caused marked changes in the lactone environment. Bands at 1650 and 890 cm^{-1} in the infrared spectrum of aristolactone were attributed to vinylidene absorption, and peaks at 840, 800 (weak) and 782 cm^{-1} were assigned to the two other double bonds. These bands were absent in the fully saturated hexahydroisoaristolactone[44].

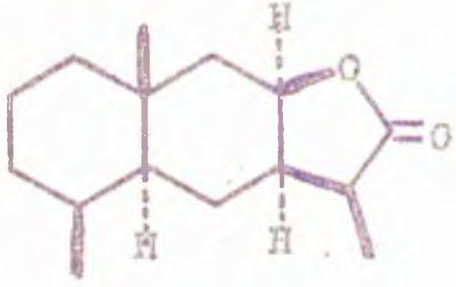
The infrared spectrum of isoaristolactone in carbon tetrachloride solution also showed typical vinylidene bands at 1656 and 892 cm^{-1} equal in intensity to those of the parent lactone. However peaks attributed to the other double bonds in aristolactone were absent in the iso-compound, being replaced by new bands at 833 and 815 cm^{-1} which were assigned to a newly formed trisubstituted double bond. Dihydroisoaristolactone contained these new bands but the vinylidene absorption was

absent, an observation consistant with the non-formation of formaldehyde on ozonolysis of this compound[44].

Ethyl oxoaristate (liquid paraffin mull) was found to exhibit infrared maxima at 1726 and 1186 cm^{-1} (ester group) - the former peak showing a shoulder at 1704 cm^{-1} (ketone carbonyl). Methyl oxoaristate showed similar absorptions. Examination of the ethylenic absorption region of methyl oxoaristate in carbon tetrachloride solution showed the presence of vinylidene absorption at 1650 and 890 cm^{-1} and a new trisubstituted double bond peak at 813 cm^{-1}. In addition the bands at 840, 800, and 782 cm^{-1} in the infrared spectrum of aristolactone and isoaristolactone were present in the infrared spectrum of methyl oxoaristate though reduced in intensity[44].

Williams[44] observed that the infrared spectrum of hexahydroisoaristolactone showed marked similarity to the spectrum of tetrahydroalantolactone (XXXIV), especially with respect to the absorptions in the 1000 and 950 cm^{-1} regions, but the two compounds were found not to be

identical (although of course this could have been attributable to stereoisomerism).



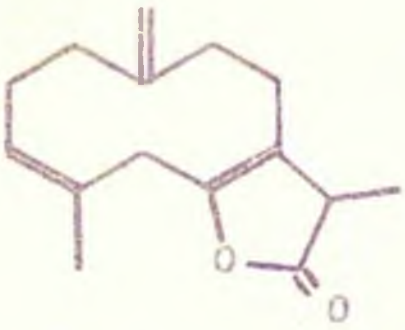
XXXIV

Absorption in these regions had been regarded by Marrison[58] as typical of cyclohexane rings leading Williams to conclude that aristolactone contained a cyclohexane ring. Further evidence that aristolactone might contain a six-membered ring system was adduced by Williams on the grounds that the two bands at 1008 and 951 cm^{-1} in aristolactone were replaced by a single band at 1100 cm^{-1} in methyloxoaristate, observations paralleling those of Lecompte[59] on cyclohexanone derivatives.

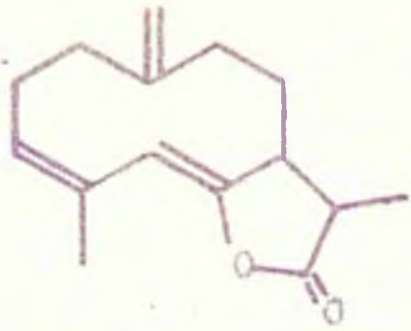
Williams[44] accordingly examined all possible C_{15} structural formulae possessing cyclohexane ring systems and a γ-lactone which obeyed the isoprene rule[60] but found no structure for aristolactone among these which would accommodate all the accumulated experimental evidence.

Steele[46] reexamining the infrared evidence drew attention to the work of Prelog and his group[61,62] wherein bands quoted by Williams[44] as being typical of a cyclohexane ring system were found also to be present in the spectra of cyclononane or cyclodecane and their derivatives. This information coupled with the discovery at about that time of a number of sesquiterpenoid lactones containing a cyclodecane ring[63,64,65] suggested to Steele[46] that aristolactone might well be derived from a 9 or 10 membered ring system. The monocyclic nature of aristolactone together with the formation of a 2,4,8- trisubstituted azulene on dehydrogenation made the cyclodecane system seem more likely, in light of the isoprene rule[60].

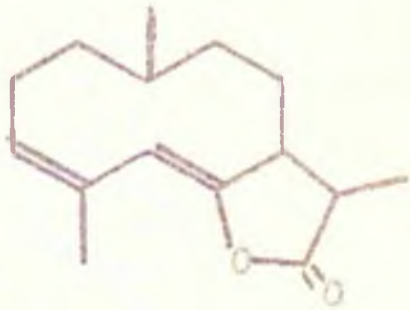
Steele[46] accordingly postulated aristolactone as XXXV, isoaristolactone as XXXVI, dihydroisoaristolactone as XXXVII, hexahydroisoaristolactone as XXXVIII, methyl oxoaristate as XXXIX, and dihydro- and tetrahydromethyl oxoaristate as XL and XLI respectively.



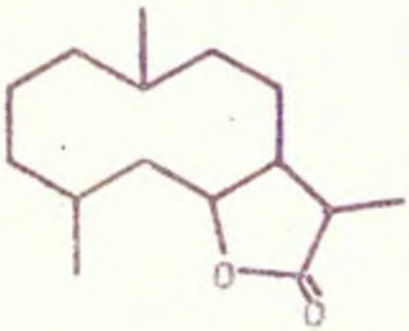
XXXV



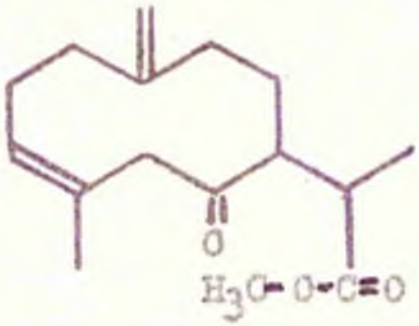
XXXVI



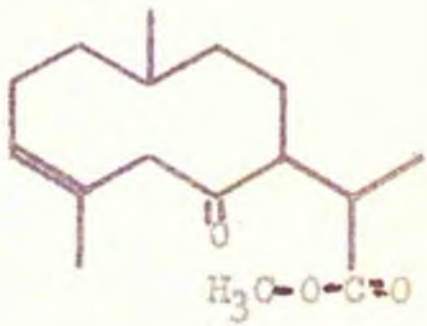
XXXVII



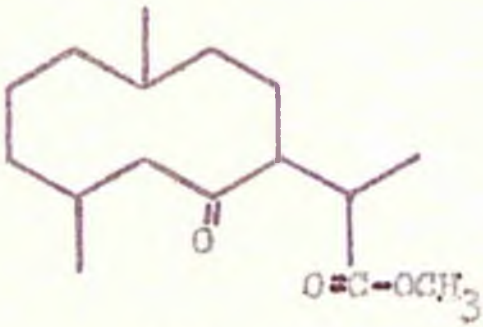
XXXVIII



XXXIX

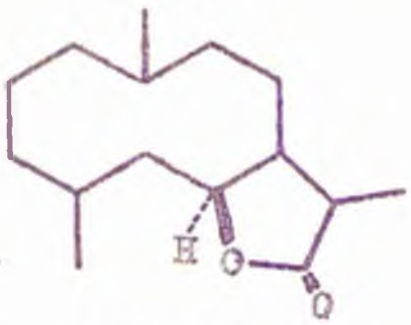


XL



XLI

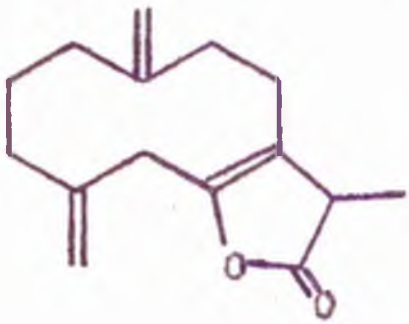
From a consideration of the Klyne-Beckett lactone rule[66], Steele[46] further suggested the partial stereochemistry shown in XXXVIIIa for hexahydroisoaristolactone.



XXXVIIIa

Upon a re-examination of the evidence the structure of aristolactone XXXV was revised by Stenlake, Steele and Williams[45] to(XLII)which contains two vinylidene groups. Since dihydro-isoaristolactone(XXXVII)contains no vinyl groups, formation of the same dihydro-compound from both aristolactone and isoaristolactone was suggested to involve reduction of one vinyl group with concurrent rearrangement of the second vinyl group to a trisubstituted double bond, as in structure XXXVII. Thus the nomenclature of the dihydro- and hexahydro-lactone was altered to isodihydroisoaristolactone and isohexahydro-isoaristolactone. A similar rearrangement was postulated to occur in the formation of methyl

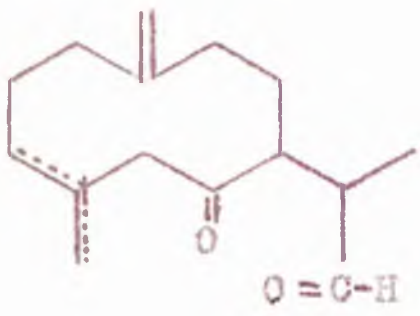
dihydro- and ethyl dihydro-oxoaristates.



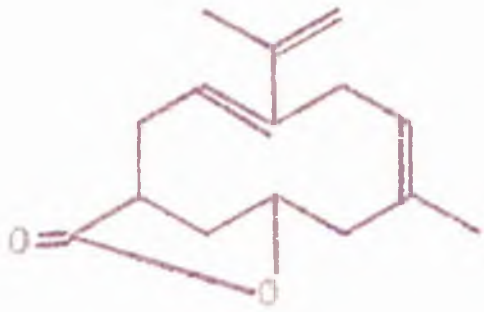
XLII

However, acceptance of structure XLII leaves certain conflicting evidence. For example, the anomalous infrared carbonyl absorption maxima in oxoaristaldehyde (XLIII) and iso-oxoaristaldehyde[45] is unexplained. Similarly the low infrared absorption values for the proposed enol lactone function in aristolactone XLII coupled with the absence of a frequency shift in the fully saturated isohexahydroisoaristolactone XXXVIIIa is not readily rationalized on the basis of structure XLII. It is of interest that a degradation product of arctiopicrin of proven structure XXXVIII[67] is obviously not identical with the hexahydro-lactone. However as there are five asymmetric centres in this compound giving rise to 2^5 theoretical stereoisomers

This evidence is not necessarily conclusive. Low formaldehyde values on ozonolysis of aristolactone and its derivatives are not explained on structure XLII[68] nor are structures alternative to XLII precluded. Thus it was felt necessary to continue work on this problem so that the structure of aristolactone could be firmly established. This work has resulted in the assignment of structure XLIV to aristolactone.



XLIII



XLIV

D I S C U S S I O N

As outlined in the introduction, certain unsatisfactory features in the evidence upon which the earlier structural proposals for aristolactone and its derivatives had been based necessitated further studies on the constitution of these compounds. Initially, all known derivatives were subjected to a re-investigation of their infra-red spectra employing a Unicam model S.P. 100 double beam spectrophotometer equipped with an S.P. 130 sodium chloride prism - grating double monochromator operated under vacuum conditions. These studies brought to light certain errors in frequency values in the earlier studies. Accordingly, all the infra-red frequencies quoted in this section (unless specification is made to the contrary) are those of the present determinations wherein measurements were made in dilute carbon tetrachloride solution. Aristolactone and certain of its derivatives were also subjected to nuclear magnetic resonance (n.m.r.) study in deuterated chloroform employing tetramethylsilane as internal standard. The instrument used was the Perkin-Elmer nuclear magnetic resonance spectrometer run at

40 megacycles per second.

The spectral studies quickly proved the structure XLII previously assigned to aristolactone to be untenable, and when taken in conjunction with new chemical evidence permitted structure XLIV to be assigned to aristolactone.

Despite the extensive nature of the earlier work on aristolactone and its derivatives[38,39,44-47,50], no attempt had been made to secure the parent hydrocarbon. The assumption that aristolactone was derived from the germacrane skeleton, inferred from considerations of double bond equivalents and from hydrogenation and dehydrogenation evidence, had never been unequivocally proven. It therefore seemed that degradation of a suitable derivative, to the parent hydrocarbon would be a logical first step. The obvious line of attack appeared to lie in the preparation of the di-p-toluenesulphonate (ditosylate) or the di-methanesulphonate (dimesylate) of the so-called tetrahydroisoaristo-6,12-diol which had been previously prepared by the action of lithium aluminium hydride on hexahydroiso-aristolactone[45]. Hydrogenolysis of either disulphonate

by means of lithium aluminium hydride would then be expected to afford the parent hydrocarbon. This was indeed found to be the case although the hydrogenolysis of the disulphonate proved more complex than had been anticipated. The diol, m.p. 106-107° was prepared as previously described[45] and converted into the dimesylate, m.p. 79-81°, in good yield by treatment with a 2.2 molar ratio of methanesulphonyl chloride in pyridine at 0°. This derivative was chosen in preference to the ditosylate because of the greater ease of mesylate formation under mild conditions[69]. Further, the mesylate function is known to be a poorer leaving group than the tosylate group[70] and so is less likely to suffer nucleophilic replacement by a pyridine molecule to give a water soluble pyridinium salt as an unwanted by-product during the sulphonate ester formation - an important consideration in the present case where the starting diol was available only in small quantity[45]. The dimesylate was found to be highly unstable quickly, exhibiting a pronounced fall in melting point which was accompanied by the appearance of a new absorption band at ca 1660 cm^{-1}

in the infra-red, attributable to the generation of a double bond *via* elimination of methanesulphonic acid. Lithium aluminium hydride reduction of the freshly prepared dimesylate afforded a colourless oil which unexpectedly gave a positive tetranitromethane test for the presence of a double bond. Unsaturation was confirmed by the presence of a sharp band at 1660 cm^{-1} in the infra-red spectrum of the oil. Gas-liquid chromatography (g.l.c.) employing a 50 metre capillary column coated with polypropylene glycol as the stationary phase showed the oil to consist of three components (table 2). After catalytic hydrogenation of the unfractionated oil over platinum oxide in ethanol (hydrogen uptake: 0.49 mole based on $C_{15}H_{30}$), g.l.c. employing the same capillary column showed the complete disappearance of the first peak (the numbering of peaks is in order of increasing retention time), diminution of the second, enhancement of the third, and the appearance of two new peaks (table 2). The infra-red spectral examination of the crude product showed that unsaturated material was still present. Accordingly the material was

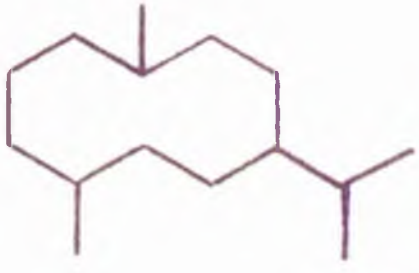
subjected to further hydrogenation over highly active Adam's catalyst in ethanol (hydrogen uptake: 0.39 mole based on $C_{15}H_{30}$). This afforded a product which g.l.c. indicated to be free of the first two peaks present in the original product obtained from the dimesylate (table 2). Since the infra-red spectrum also showed the absence of unsaturation the first two peaks just discussed can now be assigned to unsaturated components. Further, it can be deduced that the third peak present in the original mixture is due to a saturated component, more of which is formed by catalytic reduction of the unsaturated material appearing as peak number one. At the same time reduction of the compound represented by peak number two gives rise to the saturated isomers represented by peaks four and five. Thus treatment of the dimesylate with lithium aluminium hydride gives rise both to hydrogenolysis and to elimination products in the ratio 15.5:84.5. Gas-liquid chromatography of an authentic sample of synthetic germacrane (XLV) employing the same capillary column under the same conditions showed the presence of three isomers, the retention times of which each coincided with that of one of the saturated hydrocarbons derived from

aristolactone. Admixture of the saturated hydrocarbons derived from aristolactone with the synthetic germacrane followed by g.l.c. confirmed that both materials contained the same three isomers. Further the infra-red spectra of both mixtures run as liquid films were superposable.

	SAMPLE	RELATIVE PERCENTAGE OF PEAKS* (BY TRIANGULATION)				
		1	2	3	4	5
1	$LiAlH_4$ Hydrogenolysis Product	14.4	70.1	15.5	-	-
2	Partial Reduction	-	36.5	29	12	22.5
3	Full Reduction	-	-	30.1	20.8	49.1
4	Authentic Synthetic Germacrane	-	-	25.7	50.0	24.3

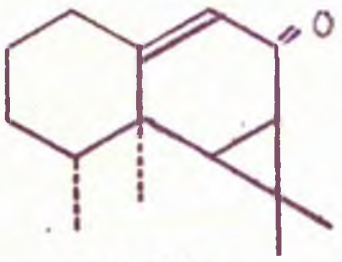
*Peak numbers assigned in order of increasing retention time.

TABLE 2

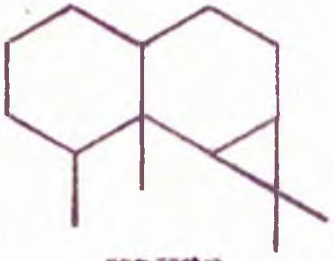


XLV

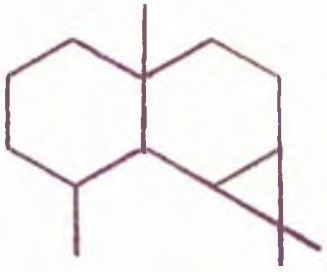
Thus it was unequivocally established that aristolactone unlike the sesquiterpene ketone aristolone, occurring in Aristolochia debilis[20] and having structure XLVI[21], is not a derivative of calarane (XLVII) (which can be envisaged as arising in nature via a 1,2-methyl shift in a maliane (XLVIII) derivative). Since rationalization of the isomers of Table 2 is best made in terms of the structure of aristolactone, account of their formation is left until page 125.



XLVI



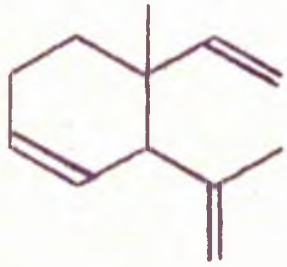
XLVII



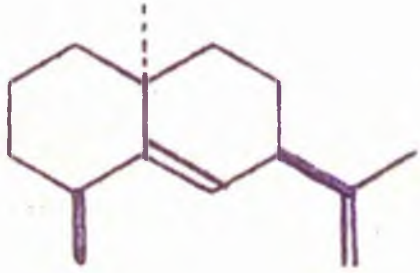
XLVIII

Comparison of the n.m.r. spectra of isoaristolactone[47] and methyl oxoaristate[47] with those of their dihydro derivatives shows the conversion of an isopropenyl group into an isopropyl group. Thus absorptions representing overlapping doublets which appear as barely resolved triplets with intensity 3 protons at 8.14 and 8.33 τ respectively in the former compounds are replaced by

a pair of superposed doublets (J=6c.p.s) of total intensity 6 protons at 8.94 and 9.11τ respectively in the dihydro compounds. As is to be expected the complex absorption pattern in the 5τ region is reduced in intensity by 2 protons. The values of 8.14 and 8.33τ for the isopropylene methyl protons in isoaristolactone and methyl oxoaristate is in good agreement with the range 8.1 - 8.4τ previously assigned to the methyl group of an isopropenyl function[71,72]. Furthermore this evidence for the presence of an isopropenyl group in isoaristolactone and methyl oxoaristate is in good agreement with the n.m.r. absorptions observed with geijerene (XLIX)[72] and the decalin derivative L[21]. The methyl group of the isopropenyl function in aristolactone itself is seen as a barely resolved apparent triplet of intensity 3 protons at 8.17τ.



XLIX



L

The superposed doublets at 8.94τ in dihydroisoaristolactone and at 9.11τ in the dihydro derivative of methyl oxoaristate assigned to the gem dimethyl group protons are also in excellent agreement with the absorption to be expected from an isopropyl group[71,73,74]. The n.m.r. evidence is also in full agreement with the infra-red evidence. Thus the absorptions in aristolactone, isoaristolactone, and methyl oxoaristate at 3075 cm^{-1} and 895 cm^{-1} which are characteristic of 1,1,-disubstituted ethylenes[72,75a,76,77] are absent in the corresponding dihydro compounds which instead exhibit doublets in the 1385 and 1375 cm^{-1} region which are typical of gem dimethyl groups[75b].

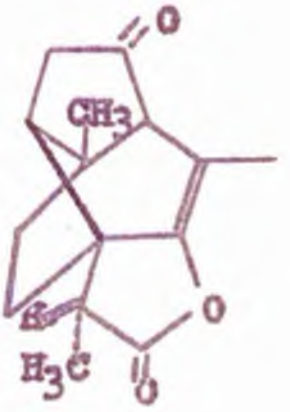
The presence of the isopropenyl function in aristolactone necessitates that the lactonic carbonyl function be derived from one of the ring methyl groups of germacrane. This conclusion, incidentally is consistent with the observations of Professor V. Herout[78] who has pointed out that the only known lactones derived from the germacrane skeleton in which the lactone carbonyl function is derived from the isopropyl group occur in plants of the

family Compositae to which Aristolochia species do not belong.

The infra-red absorption of the lactone carbonyl at 1765 cm^{-1} in both aristolactone and isoaristolactone, at 1764 cm^{-1} in dihydroisoaristolactone, and at 1769 cm^{-1} in hexahydroisoaristolactone, enables the lactone ring to be designated as saturated and γ- (75c, 79-82) in all four compounds. These absorption frequencies rule out the possibility that any of the three compounds, aristolactone, isoaristolactone, or dihydroisoaristolactone, is an α,β-unsaturated lactone or unsaturated lactone having an exocyclic double bond from the α-position (such compounds being known to absorb in the range 1760 to 1740 cm^{-1} [75c,83]. Further confirmation is given by the lack of ultra-violet absorption attributable to an α,β-unsaturated lactone in these compounds[45,47]. All unsaturated lactones having a double bond from the α-carbon atom must exhibit true conjugation as a consequence of the planar nature of the 5-membered lactone ring. Similarly the lactone carbonyl absorption frequencies of aristolactone and its derivatives mentioned above are inconsistent with any of these compounds being an enol lactone. Unsaturated

lactones with the double bond in the β,γ- position or having an exocyclic double bond from the β- position are known to absorb near 1800 cm^{-1}, while the absorption frequency is lowered 20-40 cm^{-1} to the range 1780-1760 cm^{-1} on reduction to the corresponding saturated γ-lactone[75c]. For example, α- angelica- lactone (LI) exhibits lactone carbonyl absorption at 1799 cm^{-1} when the infra-red spectrum is measured in chloroform (1806 cm^{-1} when measured in carbon tetrachloride), whereas the derived saturated γ- lactone exhibits carbonyl absorption at 1775 cm^{-1}, measured in chloroform[84]. Again parasantolide (LII) exhibits lactone carbonyl absorption at 1792 cm^{-1} whilst its saturated derivatives show this maximum at 1764 cm^{-1} [85].

LI



LII

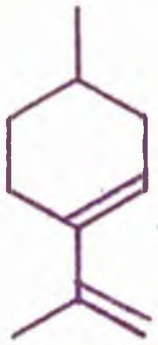
That the lactone ring of the aristolactone series is derived from a secondary alcohol is confirmed on two counts. Firstly aristolactone gives rise to the keto-ester methyl oxoaristate[45,47] and secondly an apparent triplet (J=8 c.p.s.) of intensity 1 proton at 5.2τ is present in the n.m.r. spectrum of hexahydroisoaristolactone, and this can be assigned to a single proton on the carbon atom bearing the alcoholic oxygen atom[86-88]. This same absorption is discernible in the n.m.r. spectra of aristolactone, isoaristolactone and dihydroisoaristolactone although it merges with vinylic proton absorption. This absorption is absent from the n.m.r. spectrum of methyl oxoaristate.

The absence of any proton resonance above 8.6τ in the n.m.r. spectra of aristolactone, isoaristolactone, and methyl oxoaristate indicates that none of these compounds contain a methyl group bound to a carbon atom bearing hydrogen. Thus the second ring methyl group of germacrane must form part of a trisubstituted double bond system in all three compounds (no tetrasubstituted double bond in association with a ring methyl group being possible in the germacrane skeleton). Resonance of the protons of this methyl group

as expected[72-74,82;88,89] appears as barely resolved doublets of intensity 3 protons at 8.52τ, 8.41τ, and 8.40τ in aristolactone, isoaristolactone, and dihydroisoaristolactone respectively, at 8.46τ in methyl oxoaristate, and at 8.52τ in the latter's dihydro-derivative. In the fully saturated hexahydroisoaristolactone absorption from the protons of this methyl group appears as part of the doublet (J = 6 c.p.s.) of intensity 9 protons at 9.06τ.

The 5τ region of the n.m.r. spectra of aristolactone and isoaristolactone shows absorption from three vinylic protons in both compounds. A fourth vinylic proton absorbing at ca 3.2τ is also present. On the basis of the total n.m.r. absorption of 20 protons there must be a third double bond present in addition to the isopropenyl double bond and the double bond associated with the ring methyl group in both compounds which, this n.m.r. evidence now shows, must be trisubstituted. Since the infra-red and ultra-violet spectra, as already discussed, have eliminated the possibility that either compound could be an α,β - unsaturated or an enol lactone, this last

double bond must lie in association with the isopropenyl group. The absence of conjugated double bond absorption in the ultra-violet similar to that found for 3,8(9)-p-menthadiene (LIII; λmax 233.5; ε,19000)[90], must depend upon the inability of the diene system in the aristolactone series to assume a planar nature due to non-bonded interactions arising from the geometry of the 10-membered ring system. This point will be discussed in more detail on p p. 146-147.

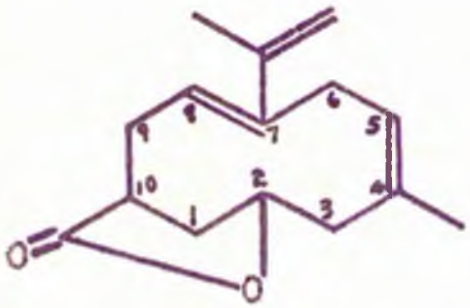


LIII

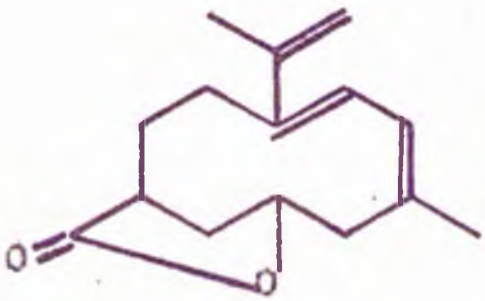
The n.m.r. evidence would further indicate that it is this last double bond in association with the isopropenyl group which is involved in the rearrangement of aristolactone to methyl oxoaristate[45,47]. Thus the n.m.r. spectrum of this keto-ester shows that the isopropenyl group and the methyl group trisubstituted double bond system are unchanged from aristolactone, whilst the very low field proton appearing as a triplet

(J=3 c.p.s.) at 3.26τ in aristolactone is not present in methyl oxoaristate.

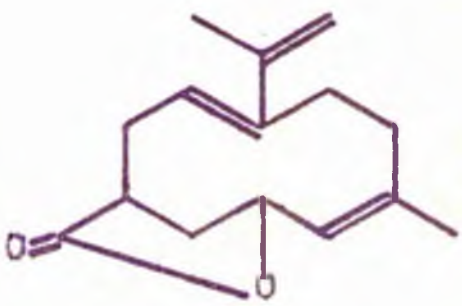
The evidence thus far therefore reduces the possible formulae for aristolactone to six viz LIVa to LIVf (numbering refers to the germacrane skeleton) without taking into account geometrical isomerism about double bonds, while hexahydroisoaristolactone would be LVa or b without taking stereochemistry into account.



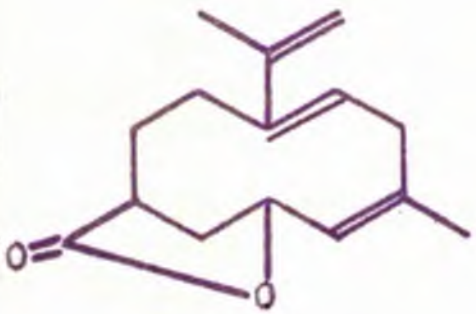
LIVa



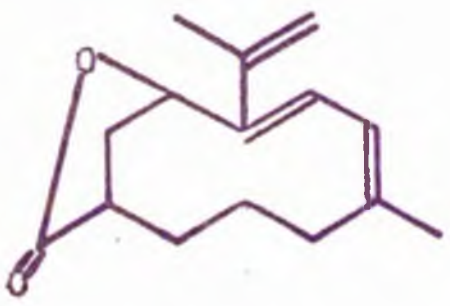
LIVb



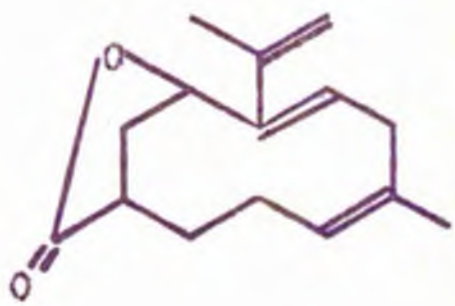
LIVc



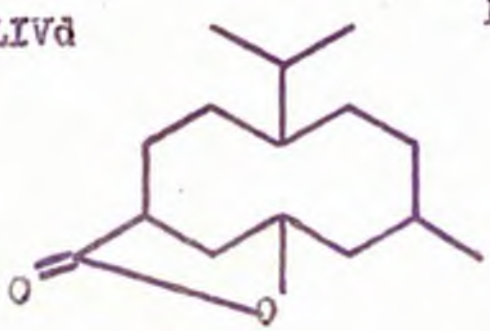
LIVd



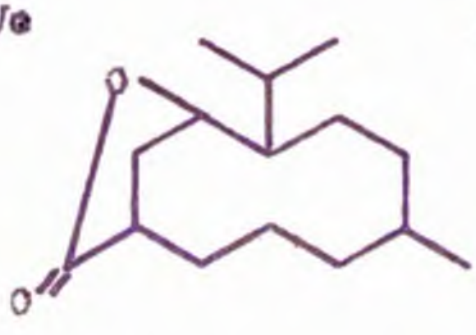
LIVe



LIVf



LVa

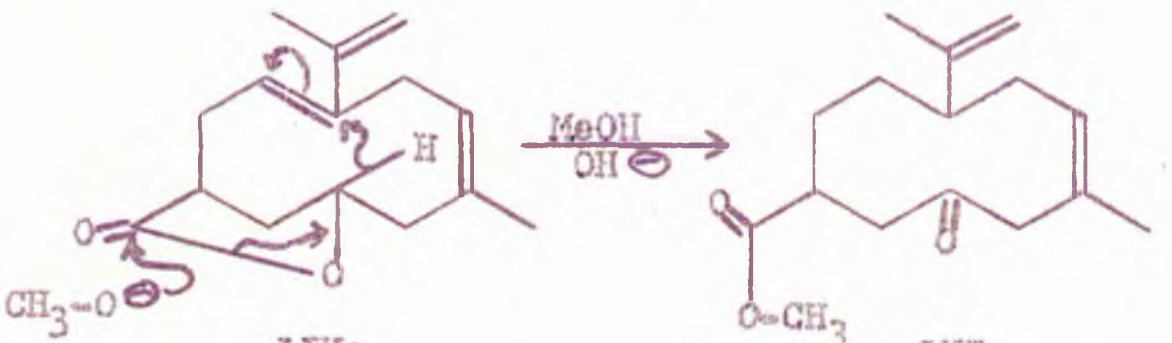


LVb

Structures LIVc to LIVf inclusive for aristolactone, and with them structure LVb for hexahydroisoaristolactone, may be immediately eliminated on the basis that no rational mechanism is apparent which would account for the facts that the keto-ester, methyl oxoaristate is β,γ- and not $\alpha\beta$ - unsaturated[45] and that it still retains a double bond in association with the ring methyl group as shown by its n.m.r. spectrum. Moreover, the isopropenyl double bond is known not to be involved in the conversion of methyl oxoaristate under the influence of base into an $\alpha\beta$- unsaturated keto-acid[45] since its dihydro derivative in which this function is converted into an isopropyl group on treatment with base also affords an $\alpha\beta$- unsaturated keto-acid[45].

The formation of methyl oxoaristate from LIVa or LIVb can be rationalized on the basis of parallel transannular hydride shifts as shown in LIVa going to LVI wherein the ring ethylenic link associated with the methyl group remains β,γ- to the ketonic carbonyl group. Such transannular interactions are not without parallel in 10-membered ring systems[91,92]

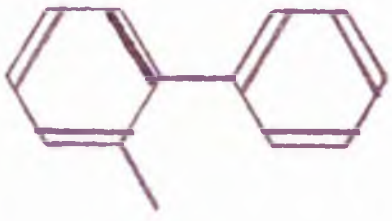
although such transannular hydride shifts are normally acid catalysed, not base catalysed, and are completed by an elimination, not an addition. The present mechanism, however, has certain features akin to those of S_N2' reactions with the eliminated hydride ion resubstituting in the molecule across the ring - this geometrically favoured substitution being offered as a driving force for the original elimination.



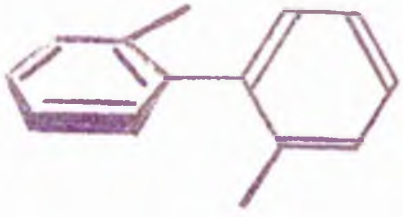
LIVa LVI

The alternative structure LIVb for aristolactone cannot be eliminated on the basis of the absence of diene conjugation in the ultra-violet since should the 10-membered endocyclic diene be cis-cis no true conjugation is possible as has already been shown for the cis-cis isomer of cyclodeca - 1,3-diene itself[93] although for the cyclodeca - 1,3-diene in which one double bond is cis and the other trans true conjugation was demonstrated[93]. In the case of aristolactone

however the lactone bridge could so twist the molecule from the planar that electron delocalization would not be possible with respect to an endocyclic cis, trans diene. A somewhat analogous effect is observed for the "K" band of biphenyls. Biphenyl itself exhibits λ_{max} 249 mμ (ε,15,000)[44], while 2-methylbiphenyl (LVII), where the methyl group forces the two benzene rings slightly out of plane with resultant lowering of band position and intensity, has λ_{max} 237mμ(ε,10500)[94]. In a more extreme case the two ortho-methyl groups of ditolyl (LVIII) force the two rings into planes at right angles to one another so that no high wave length absorption apart from benzenoid absorption appears[95].



LVII



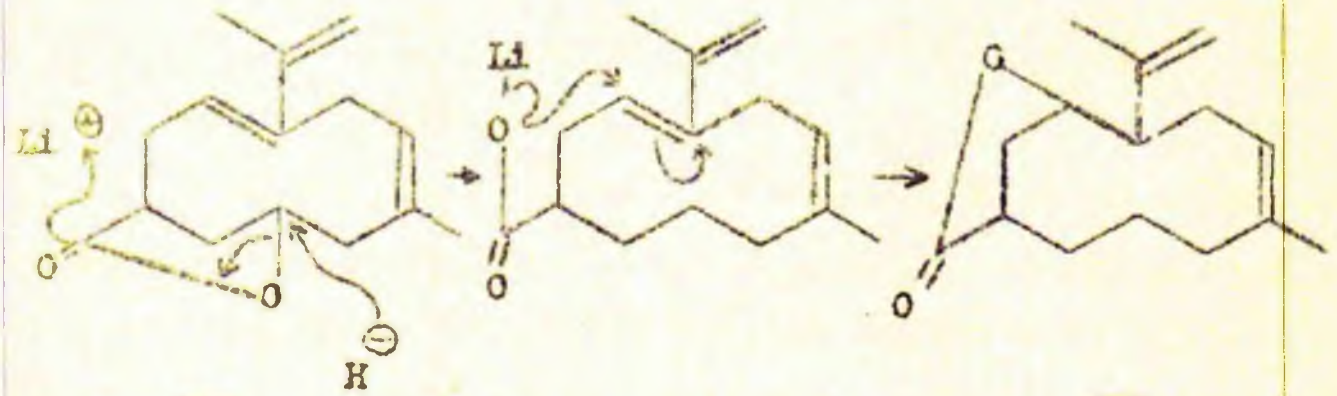
LVIII

The n.m.r. spectra of none of the three compounds, aristolactone, isoaristolactone, and dihydroiso-aristolactone show vinylic protons with coupling constants in the range 8-14 c.p.s., as would be

expected from adjacent protons such as on C-5 and C-6 in LIVb. Indeed the J value of 1 c.p.s. in the low field vinylic proton triplet in these compounds would point to structure LIVa for aristolactone, since either of the isolated ethylenic links therein could reasonably give rise to such a coupling pattern[71,73,82,89]. The placing of the double bond bearing the isopropenyl group as in LIVa and not as in LIVb can, however, be done with virtual certainty as a result of a reinvestigation of the so-called "oxoaristaldehyde" and "isooxoaristaldehyde" which are formed by treating aristolactone and isoaristolactone respectively with lithium aluminium hydride[45]. The n.m.r. spectra of these two compounds clearly show that they are not aldehydes as previously suggested[45] whilst the absence of hydroxyl absorption in the infra-red together with a carbonyl absorption band at 1765 cm^{-1} in both derivatives indicate them to be saturated γ-lactones. Their nomenclature has accordingly been changed to dihydroneoaristolactone and dihydroneoisoaristolactone respectively. This is in accordance with their lower oxidation state as indicated by the presence of n.m.r. absorption from only 3 vinylic protons

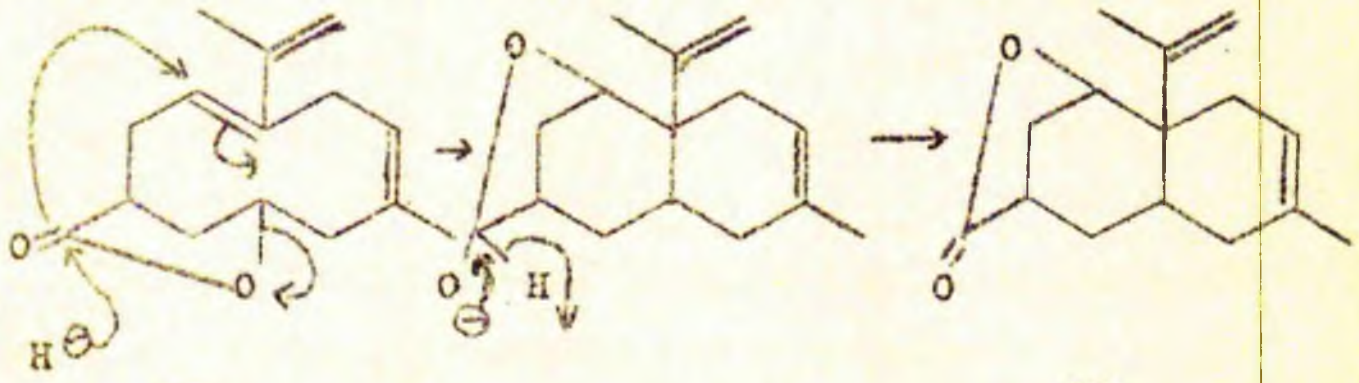
in the 4.5 to 5.5 τ region. The formation of these two new lactones, like the formation of methyl oxoaristate is accompanied by the loss of the low field vinylic proton appearing at 3.26 τ in the n.m.r. spectrum of aristolactone. Inspection of molecular models shows that in certain of the geometrical isomers possible with structure LIVa the vinylic proton on C-8 lies over the lactone carbonyl group. The de-shielding effect of the carbonyl group on this proton could therefore account for its low field absorption at 3.26 τ. That a new point of attachment has arisen for the acyloxy oxygen in going to the dihydroneo-lactone series (and that this point may be on C-8) is certainly not contraindicated by the n.m.r. spectra. In aristolactone, as already mentioned, the oxygen bearing methine proton gives rise to a triplet at ca 5.2 τ (J = 8 c.p.s.) whereas in the dihydro-neoaristolactone series the corresponding proton affords a doublet (J = 8 c.p.s.) at ca 6 τ which would indicate a change in the lactone ring system.

The formation of dihydroneoaristolactone by the action of lithium aluminium hydride on aristolactone as represented by LIVa may be considered to occur by one



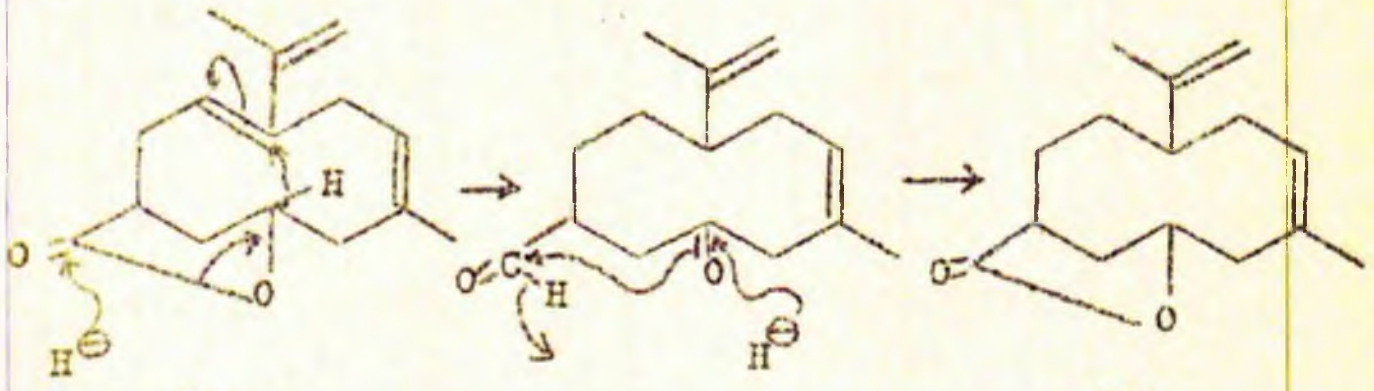
LIVa LIX

SCHEME C



LIVa LX

SCHEME D



LIVa LXI

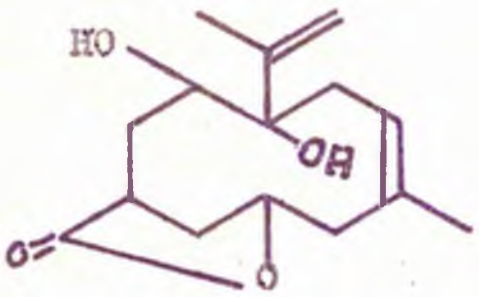
SCHEME E

of schemes C, D or E, making the product one of LIX to LXI. Alternative structure LIVb for aristolactone could only give rise to dihydroneoaristolactone via a scheme analogous to E in order to account for the formation of a γ- lactone. Scheme E may be eliminated entirely and with it alternative structure LIVb for aristolactone since in addition to the mechanism involving the highly unlikely loss of a hydride ion from an aldehyde, the mechanism is completely parallel to that involved in the formation of methyl oxoaristate from aristolactone, - the attacking species being a hydride ion in one case and a methoxide ion in the other - and it is known that isoaristolactone, which undergoes an analogous reaction with lithium aluminium hydride, will not undergo keto-ester formation[45]. Thus the structure of aristolactone is confirmed as LIVa, and dihydroneoaristolactone must be one of the products arising from either scheme C or D.

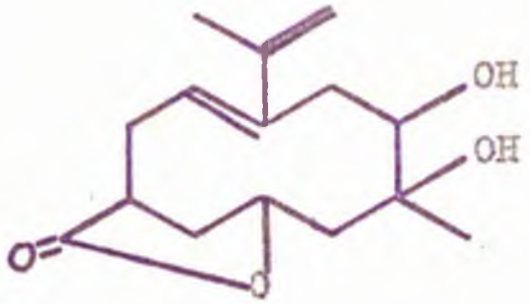
The carbon and hydrogen analytical values as well as the proton count in the n.m.r. spectrum of dihydroneoaristolactone indicates that it contains 22 hydrogen atoms (as is also true for dihydroneoisoaristolactone). The decalin derivative LX which would arise from the

mechanism in scheme D, can only accommodate 20 protons, and so scheme D must be ruled out and structure LXI, which fits all the available physical evidence, accepted for dihydroneoaristolactone.

When aristolactone (LIVa) is treated with neutral potassium permanganate[45] two products are obtained. That formed in major yield is an oily carboxylic acid containing a methyl ketone group, whilst the minor product which is obtained crystalline can be concluded to be a 1,2- diol since it forms an isopropylidene derivative $C_{18}H_{26}O_4$ m.p. 120-121°, $\varepsilon_{210m\mu}$=103, on treatment with excess dry acetone in the presence of p-toluenesulphonic acid[96]. That the formation of this 1,2- glycol (which was originally termed "6,7-dihydroxyaristolactone" [45]) does not involve the isopropenyl double bond of aristolactone was shown by the absence of formaldehyde as a product when the diol was treated with sodium meta-periodate and from the retention of vinylidene absorption at 3068 and 895 cm^{-1} in the infra-red. Thus the 1,2- glycol can be formulated as LXII or LXIII.

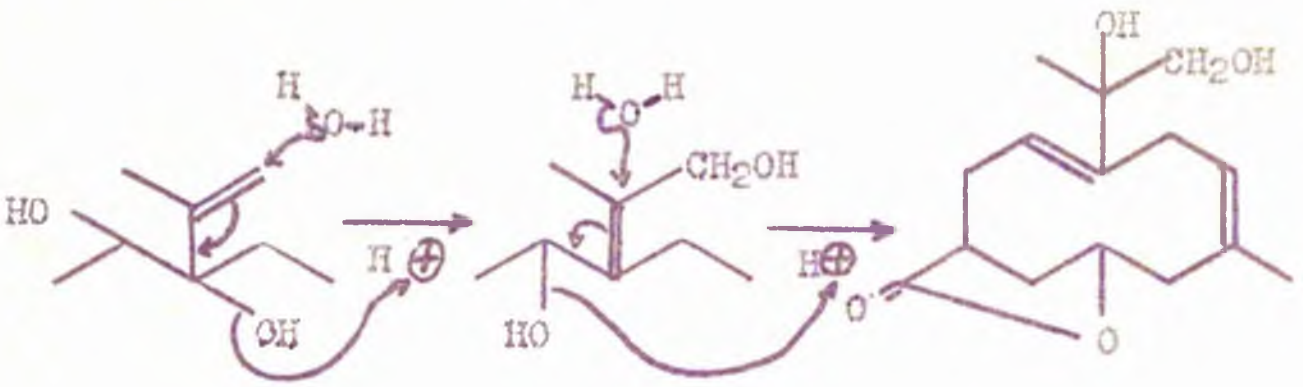


LXII



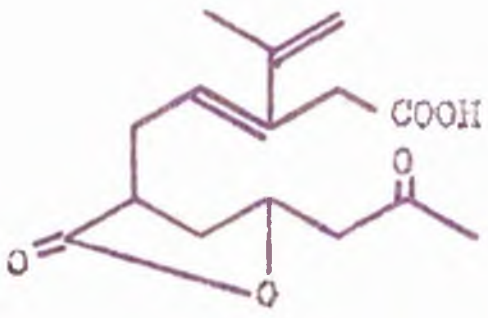
LXIII

Structure LXIII would a priori appear to be the more likely alternative since it is reported[45] that the diol is stable to mineral acid. Even if a rearrangement analogous to that involved in the conversion of aristolactone into isoaristolactone (which concerns the 4:5 double bond - see page141)does not occur as it would be expected to do, diol LXII would still be expected to undergo allylic rearrangement in acid medium by a mechanism such as shown in partial structures LXIV and LXV and LXVI. Product LXV is not a 1,2- glycol and product LXVI is the same compound as would result if initial diol formation had taken place on the isopropenyl vinylidene group - known not to be the case. Hence it would appear that the crystalline product of neutral permanganate oxidation is LXIII.



LXIV LXV LXVI

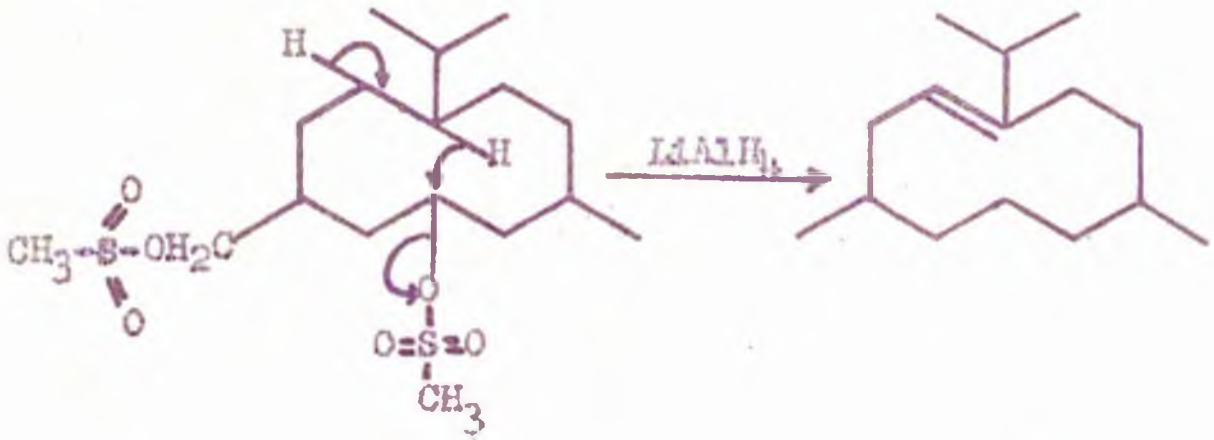
Final proof of structure LXIII follows from the fact that the oily carboxylic acid formed as the major product in the permanganate oxidation of aristolactone (LIVa) possesses a methyl ketone function in accordance with structure LXVII which is readily derived from LXIII. Interestingly the infra-red spectrum of the 1,2- glycol (measured in potassium chloride disc) shows a well resolved split lactone carbonyl peak, the two component peaks being of equal intensity and occurring at 1768 cm^{-1} and 1745 cm^{-1}, which could well arise from hydrogen bonding in the crystal lattice.



LXVII

The present studies have shown that hexahydroiso-aristolactone (LVa) when subjected to g.l.c. over 0.5% Apiezon M on acid washed celite, exhibits a main peak with a shoulder on the high retention time side. Unfortunately, however, complete resolution could not be obtained with this column at other temperatures or with a column packed with silicone on celite and further work employing other columns is necessary in order to achieve complete separation of the isomers. It is nevertheless clear that two stereoisomers are present in hexahydroisoaristolactone (LVa), thus confirming previous evidence wherein hydrolysis of hexahydroisoaristolactone afforded both a crystalline and an oily hydroxy acid - the latter being present in only small amounts[45]. The occurrence of two isomers in hexahydroisoaristolactone can be rationalized if stereospecific reduction of one double bond and non-stereospecific reduction of the other were to occur during its formation from dihydroiso-aristolactone. The stereoisomerism would be expected at C-4 since inspection of models of various geometrical isomers fitting formula LIVa indicates that the high steric impedence of the lactone ring on one side of the 7:8 double bond would lead to stereospecific

reduction at this site. The formation of two germacrenes and one germacrane from the action of lithium aluminium hydride on the two diastereoisomeric dimethanesulphonates prepared from the mixed hexahydroisoaristo - 2,14- diols (LXVIII) can be explained by invoking a transannular hydride shift and elimination of the elements of methanesulphonic acid as an alternative to hydrogenolysis giving rise to germacrenes LXIX. Analogous eliminations during complex metal hydride reductions have been encountered in the steroid field[97] and with phenyl substituted butanols[98].



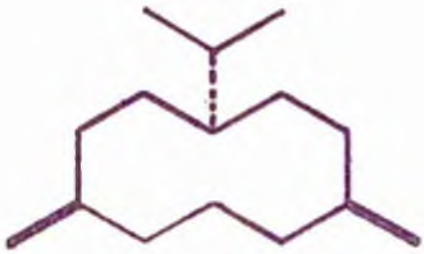
LXVIII LXIX

Should one of the diastereoisomeric dimethanesulphonates give rise solely to one germacrene isomer whilst the other gives rise to the second germacrene and the germacrane, the occurrence of three hydrocarbons in the lithium aluminium hydride reduction product as proven by gas-liquid chromatography would be completely accounted

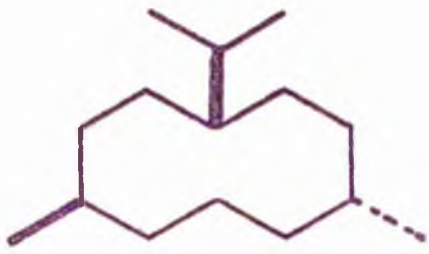
for in terms of the results presented in table 2.

The elements of symmetry present in the germacrane molecule reduces the number of possible isomers from the usual 2^n, where n = the number of asymmetric carbon atoms. Indeed examination of models indicates that the germacrane structure can exist as a total of two non-optically active stereoisomers LXX and LXXI and one pair of enantiomorphs LXXII and LXXIII. The latter would of course not be resolved by gas-liquid chromatography and so synthetic germacrane should exhibit three peaks on g.l.c., as was indeed observed.

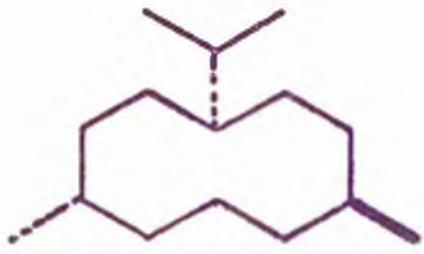
LXX



LXXI



LXII



LXXIII

Complete reduction of the mixture of the two germacrenes and the germacrane resulting from the action of lithium aluminium hydride on the mixed diastereoisomeric methanesulphonates prepared from the mixed hexahydroiso-aristo- 2,14- diols would then give germacranes in which stereoisomerism was possible on both C-4 and C-7 - the stereochemistry at C-10 being uniquely defined as that in aristolactone. Stereoisomerism at both C-4 and C-7 would, however, give rise to three germacrane isomers viz LXX, LXXI and one of the two enantiomorphs LXXII or LXXIII, thus accounting for the observed composition of the germacranes derived from aristolactone(LIVa). Moreover as can be seen from the results summarized in table 2, the germacrene with lowest retention time gives rise to the germacrane with the lowest retention time, whilst the germacrene with the highest retention time gives rise to the two germacranes having the higher retention times.

So far nothing has been said concerning the possibilities of geometrical isomerism about the endocyclic double bonds of the aristolactone series.

However important evidence concerning the configurations of these double bonds is perhaps available from the differences in behaviour of methyl oxoaristate and its dihydro-derivative towards the action of refluxing acetic anhydride, as discovered in the present work. Thus methyl oxoaristate on treatment with boiling acetic anhydride affords a neutral crystalline compound of m.p. 112-113°, $[\alpha]_D$ +164° (EtOH) analysing for $C_{15}H_{20}O_2$. This compound showed weak end absorption in the ultra-violet (ε,950 at 210 mμ) and a low intensity maximum at 275 mμ (ε,30). Catalytic hydrogenation of this derivative (hydrogen uptake: 2 moles) afforded the fully saturated tetrahydro-derivative, m.p. 70-72°. The infra-red spectrum of the unsaturated compound, in dilute carbon tetrachloride solution, was devoid of hydroxyl absorption and exhibited a split carbonyl peak of near equal intensities at 1788 and 1779 cm^{-1} (in potassium chloride disc the lower frequency band is only barely apparent) which would indicate the presence of a saturated γ-lactone function[75c] and the absence of keto or ester functions. Since the spectrum was measured in dilute carbon tetrachloride solution (4 mg /ml) the split carbonyl band cannot have its origin in

intermolecular hydrogen bonding of the type demonstrated in LXXIV or in dipole interactions of the type shown in LXXV.

LXXIV

LXXV

The carbonyl doublet would not be expected to arise from the presence of configurational isomers since any lactone formation by the action of acetic anhydride on methyl oxoaristate could reasonably be expected to be stereospecific. However, the split carbonyl peak could be expected to arise from either Fermi resonance (the coupling between the overtone of a low frequency absorption with the fundamental stretching frequency of the lactone carbonyl)[99-101] or from a "hot transition" (wherein a low frequency vibration is excited from both the ground state and an upper energy level)[99]. In so far as Fermi resonance is solvent dependent and concentration independent, the carbonyl absorption of the product obtained from the action of acetic anhydride on methyl oxoaristate, was studied in tetrachloroethylene solution and in acetonitrile solution at different concentrations. The results

clearly indicated that the double carbonyl peak does indeed arise from Fermi resonance. Thus in place of the two peaks at 1788 and 1779 cm^{-1} in carbon tetrachloride solution, the spectrum measured in tetrachloroethylene solution (0.26 mg/70 µl; 0.49 mm microcell) showed a high intensity peak at 1791 cm^{-1} with a shoulder on the low intensity side at 1782 cm^{-1} whilst the spectrum run in acetonitrile solution showed a high intensity peak at 1772 cm^{-1} with a shoulder on the high frequency side at 1783 cm^{-1} - the relative intensities being independent of concentration. The small frequency shifts observed are in accord with other studies of Fermi resonance[100].

The n.m.r. spectrum of the unsaturated product from the action of acetic anhydride on methyl oxoaristate shows the presence of 20 protons so it can be concluded on the basis of double bond equivalents (C_nH_{2n+2} ; $\frac{32-20}{2} = 6$) two being present as double bonds as evidenced by quantitative hydrogenation and absence of unsaturation in the product as shown spectroscopically; a third being utilized in lactone ring formation, with a fourth being present in the lactone carbonyl) that the

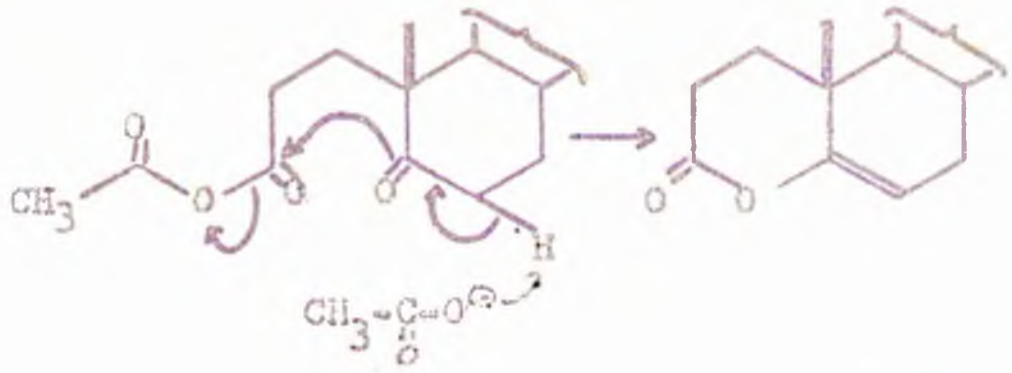
derivative is bicyclic in addition to the lactone ring. The infra-red spectrum of this compound exhibited in addition to the typical vinylidene C-H stretching band of aristolactone and its derivatives at 3073 cm^{-1} a new band at 3091 cm^{-1} which may be assigned to a second vinylidene group[72,75a76,77], the C-H stretching of other ethylenic groups being known to absorb in the range 3055-3010 cm^{-1} 75a,102. The presence of two vinylidene groups would appear to be confirmed by the n.m.r. spectrum which exhibits 4 vinylic protons in the range 5.1 to 5.3 τ. The absence of absorption in the range 8.4 to 8.5 τ typical of the trisubstituted double bond methyl group on C-4 in other aristolactone derivatives, and the absence of any absorption from protons of a methyl group bound to a carbon atom bearing hydrogen would further support the conclusion that the C-4 methyl group had undergone isomerization to a vinylidene group. At the same time a barely resolved triplet ($J = 0.5$ c.p.s.) of intensity 3 protons at 8.27 τ comparable to the analogous absorptions from the methyl group of the isopropenyl function at 8.33 τ in methyl oxoaristate (LVI), at

8.17τ in aristolactone (LIVa) and at 8.14τ in isoaristolactone, would indicate that the isopropenyl function was intact in the new lactone. Further, the absence of resonance attributable to a proton on an oxygen bearing carbon atom would indicate that the lactone acyloxy oxygen is bound to a tertiary carbon atom. In summation then, the action of acetic anhydride on methyl oxoaristate (LVI) affords a bicyclic compound possessing in addition a γ- lactone ring derived from a tertiary hydroxyl group, and two vinylidene groups.

The dihydro-compound obtained by catalytic hydrogenation of methyl oxoaristate (LVI), and which still retains a trisubstituted double bond in association with the methyl group on C-4, on treatment with boiling acetic anhydride yields a neutral crystalline product, m.p. 166-167°, $[\alpha]_D$-74° (EtOH) analysing for $C_{17}H_{26}O_4$, and exhibiting λmax 215 mμ (ε,154) and 254 mμ (ε,69). The analytical figures for this compound thus indicate a net replacement of methoxide ion in the starting material by acetoxy ion, but the infra-red spectrum (measured in potassium chloride disc with a Perkin-Elmer Infracord) clearly shows

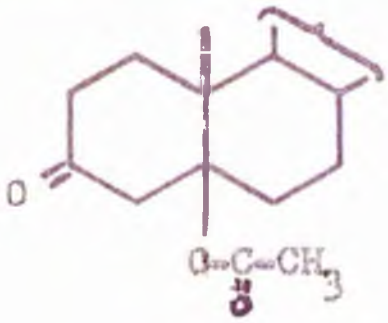
that it is not an anhydride by the absence of peaks in the 1850-1800 cm^{-1} region. Moreover there is no generation of a vinylidene group corresponding to that observed in the case of methyl oxoaristate as evidenced by the absence of absorption at ca 3075 cm^{-1} and 990 cm^{-1}. Further, no absorptions ascribable to a hydroxyl group are present but the infra-red spectrum exhibits peaks at 1768 cm^{-1} and 1735 cm^{-1}. The first of these is assignable to a saturated γ-lactone function[75c] whilst the second when taken in conjunction with a C-O stretching mode at 1245 cm^{-1} can be assigned to an acetate group.

The generation of such an acetoxy lactone from a γ-keto ester by the action of acetic anhydride would appear to have its closest analogy in the formation of enol lactones by the action of acetic anhydride on keto acids. Woodward[103] has suggested that the latter reaction involves attack by an acetoxy ion on a proton attached to a carbon atom α- to the keto group with concerted elimination of acetoxy ion from the mixed anhydride which is first formed, as is shown in the conversion of partial structure LXXVI into LXXVII.



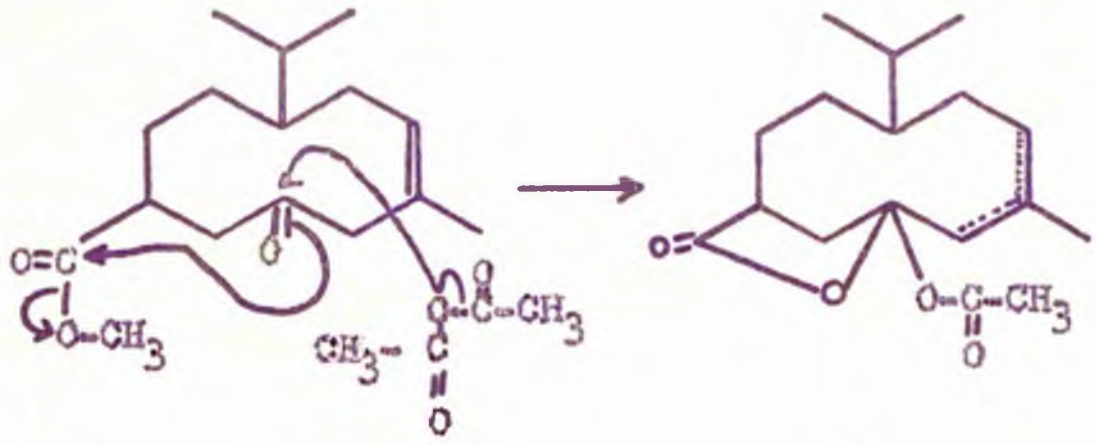
LXXVI LXXVII

At the same time he expected the compound represented by partial structure LXXVIII to be formed as a second product on the grounds of alternative attack by acetoxy ion on the carbon atom of the carbonyl group[104] although in actual fact none of this substance could be detected in his reaction product.



LXXVIII

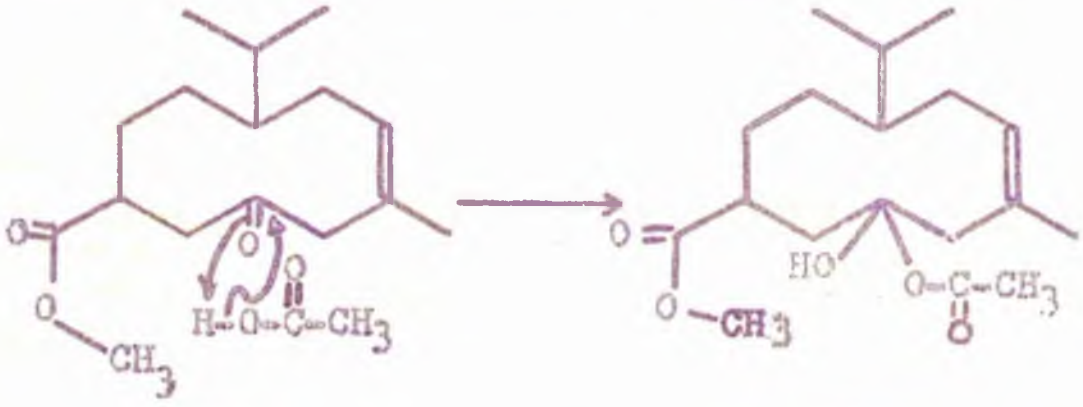
If however in the case of the dihydro derivative of methyl oxoperistate(LXXIX), attack on the carbonyl carbon atom were to occur in this very manner satisfactory account of the properties of the product can be given as shown in the conversion of LXXIX into LXXX.



LXXIX LXXX

Further evidence in support of a structure such as LXXX for this compound comes from the nature of the product formed by the action of glacial acetic acid on the dihydro derivative of methyl oxoaristate, which in turn under the influence of boiling acetic anhydride is converted into the same compound as represented by LXXX (mixed m.p., infra-red spectrum and optical rotation). When treated with glacial acetic acid at room temperature for 48 hours the dihydro derivative of methyl oxoaristate afforded a neutral crystalline compound $C_{18}H_{30}O_5$, m.p. 90.5-91°, $[\alpha]_D$- 73.91° (EtOH) and ε,600 at 210 mμ. The infra-red spectrum (in potassium chloride disc measured on the Perkin-Elmer Infracord) indicated hydroxyl O-H stretching at 3400 cm^{-1} and C-O stretching at 1130 cm^{-1}, indicative of the presence of a tertiary hydroxyl group[75d].

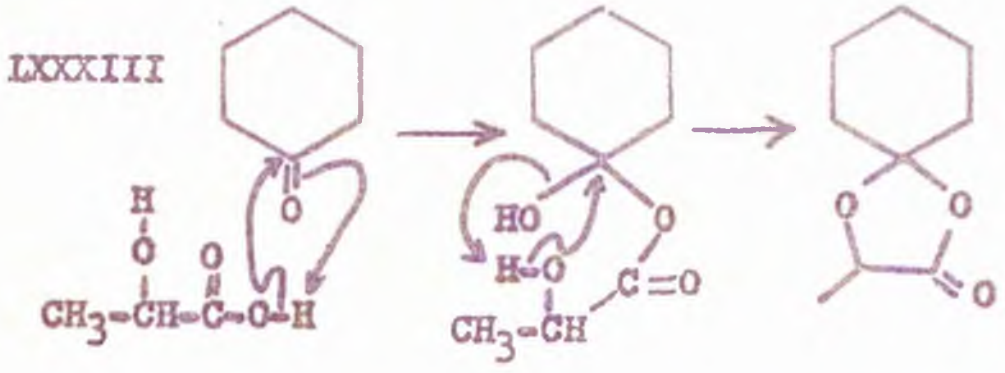
A carbonyl band at 1720 cm^{-1} is assignable to the methyl ester, while a second band at 1705 cm^{-1} when taken in conjunction with a C-O stretching mode at 1260 cm^{-1} would indicate the presence of an acetate group[75d] - that is the elements of acetic acid appear to have been added to the starting compound. This can be rationalized by formation of the monoacetate of the gem diol derived from the keto carbonyl group (LXXIX ⟶ LXXXI). The inductive effect of the hydroxyl group in LXXXI would be expected to lower the infra-red absorption frequency of the acetate carbonyl[85] in agreement with the observed facts.



LXXIX LXXXI

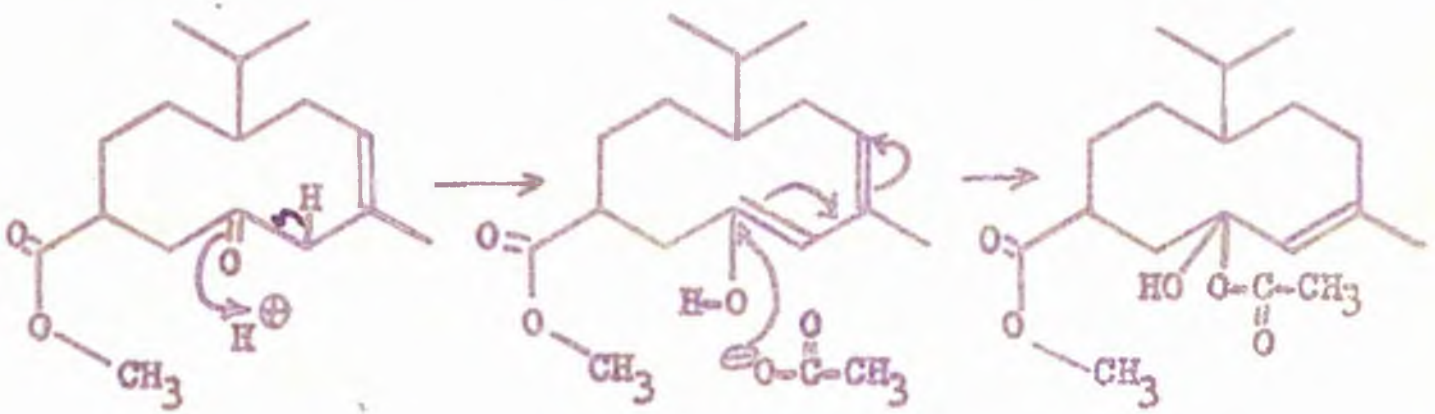
An interesting reaction which may be taken for an analogy is given by the formation of LXXXIV by the action of lactic acid(LXXXII) on cyclohexanone (LXXXIII) in the presence of an acid catalyst. This cyclic

ketal (LXXXIV) may be envisaged as arising by some such mechanism as shown below.



LXXXIII LXXXII LXXXIV

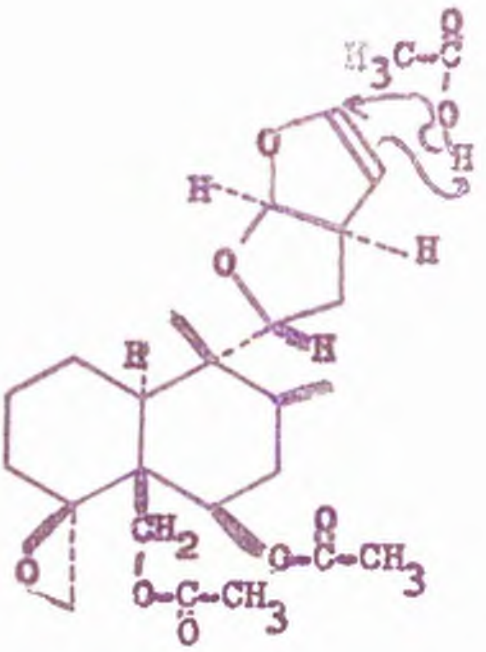
An alternative mechanism for the net addition of the elements of acetic acid to the oxo group of the dihydro derivative of methyl oxoaristate (LXXIX) involving enolization and a double bond shift is portrayed in the sequence LXXIX LXXXV LXXXVI.



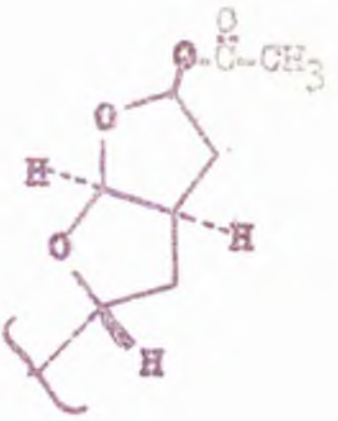
LXXIX LXXXV LXXXVI

Such nucleophilic attack by the acetoxy ion on the enol carbon atom is not unlike the hemiacetal

acetate formation found with the dihydrofuran ring in clerodin (LXXXVII going to partial structure LXXXVIII)[105], or the addition of carboxylic acids to dihydropyran (LXXXIX) giving rise to tetrahydropyranyl -2- esters (XC)[106].



LXXXVII



LXXXVIII

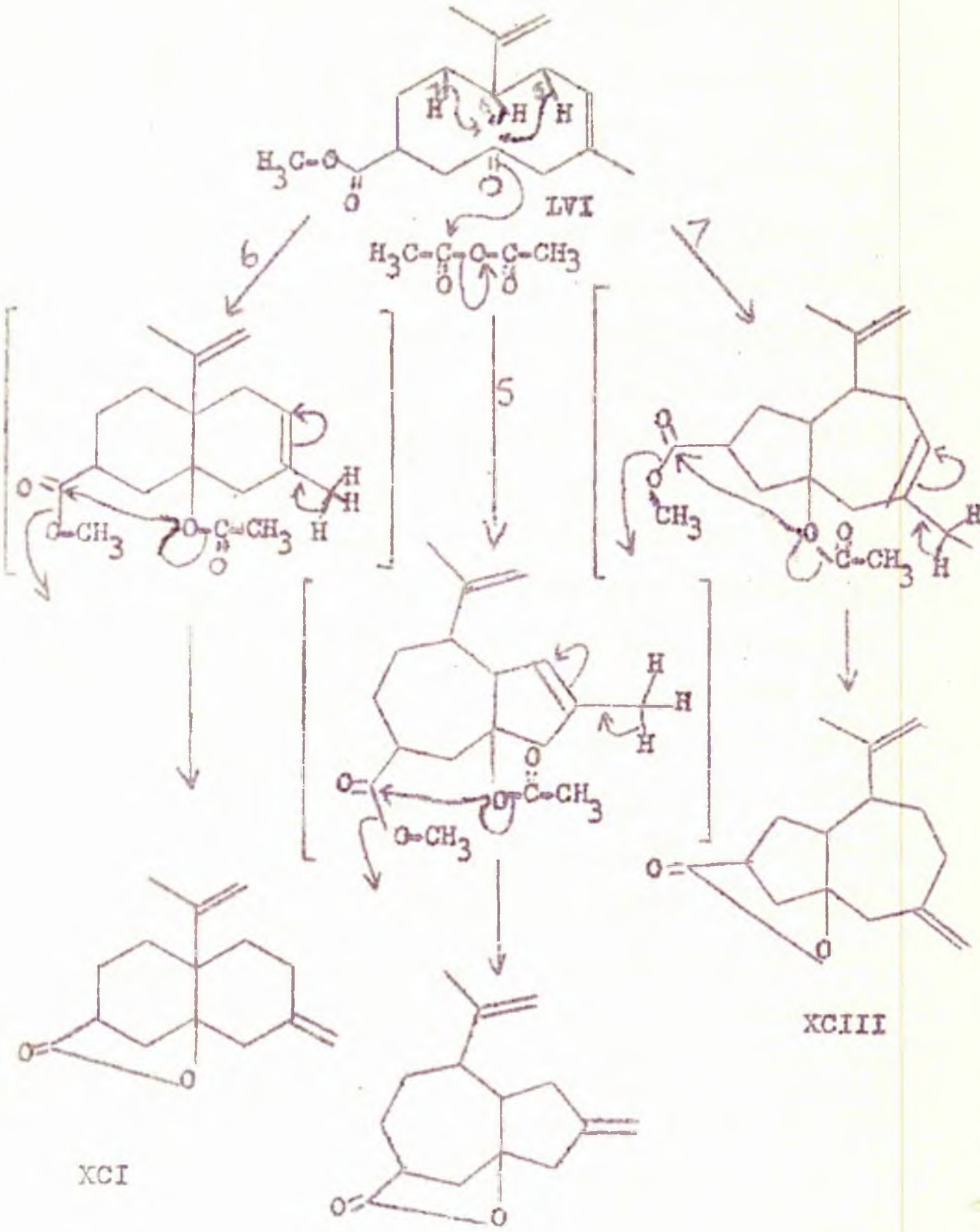
LXXXIX XC

As is true of such tetrahydropyranyl esters, the hydroxy acetate derived from the dihydro derivative of methyl oxoaristate (LXXIX) was found to be unstable to acids, dilute acetic acid itself causing decomposition. Until degradation studies have been carried out on the hydroxy acetate however, distinction between structures LXXXI and LXXXVI cannot be made, although the latter would be expected to readily generate an α,β-unsaturated ketone through loss of the elements of acetic acid. Nevertheless either structure on treatment with acetic anhydride would be capable of losing the elements of methanol to give a lactone acetate of the type represented by LXXX.

The formation of a new carbocyclic ring by methyl oxoaristate under the influence of acetic anhydride whilst the same reagent gives rise to a reaction of a completely different type in the case of the dihydro derivative can only have its explanation in differences in the nature of the 4:5 double bond in the two compounds. Certainly direct attack on either the oxo or carbomethoxy groups would not take such different courses without an underlying reason.

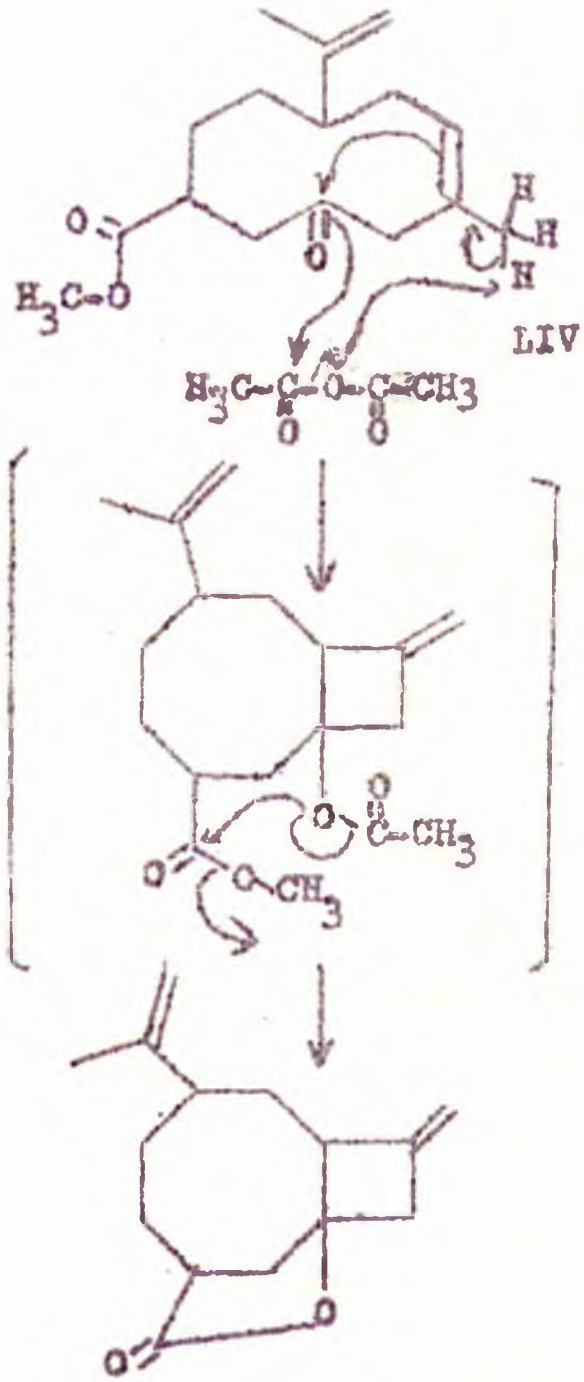
The presence of an isopropenyl group in methyl oxoaristate (LVI) and its absence in the dihydro compound (clearly shown by infra-red and n.m.r. spectroscopy) can in no way affect the issue since the isopropenyl group is retained unchanged in the product formed from methyl oxoaristate as evidenced by infra-red and n.m.r. studies. Moreover the double bond must be 4:5 in methyl oxoaristate and in its dihydro compound since both compounds are not $\alpha\beta$-unsaturated ketones and both are isomerised to $\alpha\beta$- unsaturated ketones by base[45,47].

Since the dihydro derivative of methyl oxo-aristate is prepared under conditions strictly analogous to those known to convert aristolactone into dihydroisoaristolactone, it is to be concluded that an identical isomerization is occurring in both cases and that "methyl dihydrooxoaristate" is more properly to be termed methyl dihydroisooxoaristate. In the original preliminary communication announcing the revised structure of aristolactone[107] (a reprint of which is included as appendix 2), it was assumed that the conversion of the aristolactone series into



Scheme F

the isoaristolactone series involved migration of the 4:5 double bond into the 3:4 position in the absence of other evidence. However it is now clear that the iso series must differ from the original series through geometrical isomerism about the 4:5 double bond although unambiguous assignment of configuration to this double bond in the aristolactone and isoaristolactone series cannot be made until the actual bicyclic skeleton present in the product of the action of acetic anhydride on methyl oxoaristate has been determined. Should the ring closure to the bicyclic compound involve loss of a proton from C-6, C-7, or C-8 (scheme F), inspection of models shows that a transannular reaction is only possible with the 4:5 - double bond in the cis configuration and so it would be cis in methyl oxoaristate (LVI), aristolactone (LIVa) and dihydroneo-aristolactone (LIX), and trans in methyl dihydro-isooxoaristate (LXXIX), isoaristolactone, dihydroiso-aristolactone and dihydroneoisoaristolactone. However, should the generation of the bicyclic ring system involve attack by the π electrons of the 4:5 double bond on the carbonyl carbon atom as shown in scheme G (an attractive mechanism in so far as it involves spontaneous



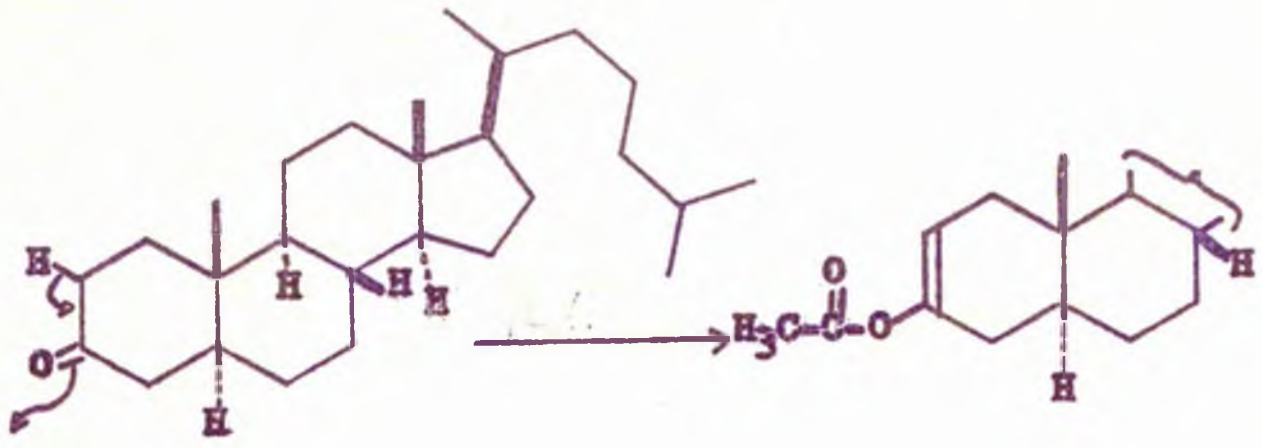
LIV

XCIV

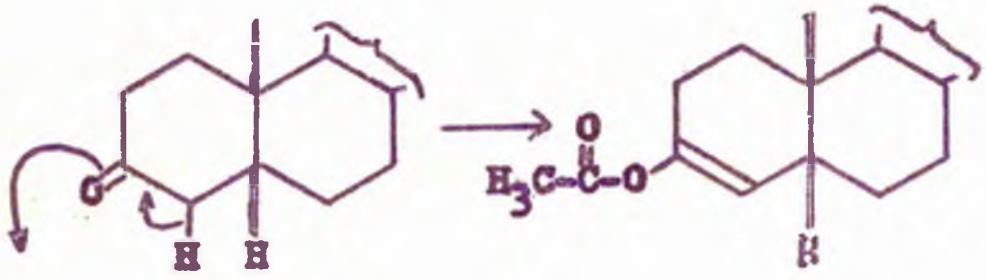
Scheme G

generation of the second vinylidene group), inspection of models would show that no distinction can be made between a cis double bond or a trans double bond in the 4:5 position of methyl oxoaristate, and so the configurations as listed above could be reversed.

Should scheme F be operative, in view of the known greater stability of double bonds exocyclic to a 5-membered ring[108] it might be considered that structure XCII is more likely than XCI or XCIII. Should the compound prove to be XCI, the generation of the exocyclic vinylidene group in this decalin derivative might possibly be rationalized by analogy with the direction of enolization in 3-keto steroids. In the case of these latter compounds, where the A/B ring fusion is trans (XCV) enolization generates the double bond in the 2:3 position[109] as shown in XCVI, whilst where the A/B ring fusion is cis, enolization generates the double bond in the 3:4 position (XCVII → XCVIII)[109]. The same phenomenon is also evidenced by the 2-bromination of 3-oxo trans A/B steroids and the 4-bromination of 3-oxo cis A/B steroids[110].



XCV XCVI



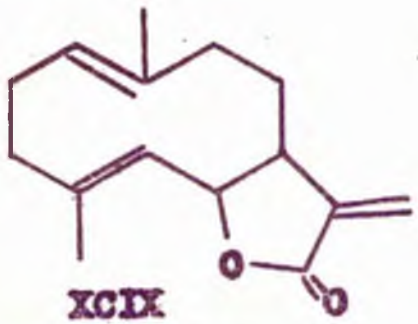
XCVII XCVIII

Thus should the intermediate in pathway 6 of scheme F be a cis fused decalin, an unfavourable configuration would result[111] with the double bond being endocyclic, so that allylic rearrangement to the exocyclic position might not be unexpected.

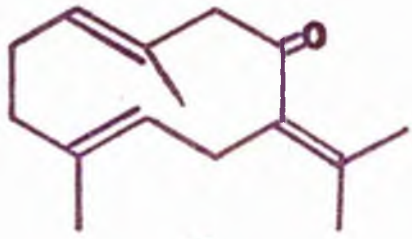
Although J values are of little assistance in distinguishing between the two possibilities, an

indication that the 4.5 double bond may be trans in aristolactone and cis in isoaristolactone (i.e. the mechanism shown in scheme C is involved in the action of acetic anhydride on methyl oxoaristate) follows from the positions of the resonance from the 3 protons of the methyl group on C-4 in aristolactone and isoaristolactone (at 8.52 τ and 8.41 τ respectively) since Bates and Gale[112] have shown that the resonance from the protons of the methyl group substituted to a cis ring double bond in costunolide (XCIX) absorb some 0.07 τ lower than the corresponding methyl group protons in germacrone (C) where the methyl group is substituted on a trans double bond. Other examples with acyclic compounds and caryophyllene derivatives further support the generalization that protons of a methyl group on a cis double bond absorb at a lower field than protons of methyl groups on trans double bonds. However the values of the C-4 methyl proton absorption at 8.48 τ in methyl oxoaristate and at 8.52 τ in methyl dihydroisooxoaristate show a shift in the opposite direction to that with aristolactone and isoaristolactone. Unfortunately superposition of both methyl absorption peaks in the n.m.r. spectrum

of dihydroneoaristolactone prevents the acquisition of further evidence from the neolactone series. Thus no definite assignment of double bond configuration to the aristolactone and isoaristolactone series can be made with any degree of certainty from consideration of the position of the absorption of the protons on the C-4 methyl group.



XCIX



C

The inability of isoaristolactone to undergo the transannular hydride shift involved in the formation of methyl oxoaristate (LVI) from aristolactone (LIVa) can of course be explained by the changes produced in the geometry of the 10-membered ring as a result of the isomerization of the 4:5 double bond, whatever the configurations in the two compounds.

Unambiguous assignment of configuration to the 7:8 double bond in aristolactone (LIVa) and the various derivatives in which it occurs, is again not possible since examination of molecular models

shows that whether this 7:8 double bond be cis or trans, certain conformations are possible with the 4:5 double bond cis or trans in both series (when variation in the fusion of the lactone ring is also considered) which are relatively strain free and permit of the transannular hydride shift involved in the formation of methyl oxoaristate (LVI), and the deshielding of the C-8 proton by the lactone carbonyl group. However, it would appear that there is greater restriction to true conjugation between the 7:8 double bond and the isopropenyl double bond where the former is trans than where it is cis. It may be noted that models can be made where both the 4:5 and 7:8 double bonds have the trans configuration.

Incidentally, the assignment of new formulae to the aristolactone series as reported in this thesis necessitates a complete re-interpretation of the ultraviolet spectral comparisons made earlier by Steele, Stenlake, and Williams[113] but this will not be attempted here.

From the above it can be seen that in order to complete the aristolactone problem, the following need to be done:-

1. Conclusively establish the constitution of the diol formed by potassium permanganate oxidation of aristolactone.

2. Establish the carbon skeleton of the neoaristolactone series. This may be determined by first catalytically reducing the neo lactones to their corresponding fully saturated compounds and then comparing this product with hexahydroisoaristolactone (although because of the possibility of stereoisomerism non identity of the products with hexahydroisoaristolactone does not mean that they necessarily have the new lactone system, as portrayed in LIX). By treating these fully saturated compounds with lithium aluminium hydride, the corresponding diol would be formed which after mesylation and lithium aluminium hydride hydrogenolysis (and if necessary catalytic hydrogenation as employed in the conversion of hexahydroisoaristolactone into germacranes) would yield the parent hydrocarbons which could then be identified.

3. Isolate once more and characterise the second product from the lithium aluminium hydride reduction of aristolactone i.e. the compound previously termed "oxoaristool"[45].

The infra-red spectrum in carbon tetrachloride

solution of a specimen of this compound prepared by earlier workers on the aristolactone problem showed carbonyl absorption at 1759 cm^{-1} which can only be assigned to an ester or lactone carbonyl function, and two hydroxyl stretching peaks at 3605 cm^{-1} (non hydrogen bonded) and 3544 cm^{-1} (hydrogen bonded). Since it is impossible to have both an ester function and a hydroxyl function in this compound which analyses for $(C_{15}H_{24}O_2)_n$ [45] when n=1, it is apparent that the compound must be polymeric - the most likely case is that it is dimeric (i.e. n=2) and that it possesses one ester function and two hydroxyl groups in agreement with the double hydroxyl absorption in the infra-red. Moreover the relative intensity of the C=O stretching absorption as compared with those of the C - O stretching absorptions in the 1100-1000 cm^{-1} region would further support this interpretation. Its dimeric nature would also be indicated by its melting point of 245-246° which is considerably higher than the melting points of the other known aristolactone derivatives. The n.m.r. spectrum does not permit an accurate proton count, but it is not inconsistent

with the presence of 44, 46 or 48 protons of which 12 are present as methyl groups attached to vinylic positions (superposed absorption at 8.42 τ) and of which 6 or 7 are present as vinylic protons (complex absorption in the 5 τ region). At least 2 protons (and perhaps 3) attached to carbon atoms bearing oxygen are present as indicated by complex absorption at ca 6 τ.

Lack of material prevented further study of this compound and until more is available it would seem unwise to engage in predictions of its structure. It would appear however, that its formation does not involve a simple partial reduction of lactone groups to afford hemiacetals as is observed in the picrotoxinin series[114].

4. Establish the carbon skeleton of the product of the action of acetic anhydride on methyl oxoaristate employing similar methods to that described in point 2. Should this compound prove to be XCII it is to be noted that selenium dehydrogenation would be expected to give 2,5-dimethyl-8-isopropylazulene (which would not appear to be so far known).

5. Establish the carbon skeleton of products arising from the action of glacial acetic acid and acetic anhydride on methyl dihydroisooxoaristate, employing similar methods.

6. Completely resolve hexahydroisoaristolactone by gas-liquid chromatography and determine the percentage of each isomer present as a check that they are in the ratio of 7:3 as calculated from the results shown in table 2.

7. All these compounds should be subjected to n.m.r. spectroscopy as well as infra-red spectroscopy in dilute carbon tetrachloride solution on the Unicam model S.P.100, where such work has not been previously performed.

8. It might also be profitable to convert methyl oxoaristate through into germacrane as a double check on its carbon skeleton.

To fully establish the stereochemistry of aristolactone and its derivatives (both with respect to the geometry of the double bonds and with respect to the fusion of the lactone ring) it would appear that the best available method involves an X-ray crystallographic study of certain

key compounds as heavy atom derivatives. For instance the presence of 3 double bonds in aristolactone and isoaristolactone might conceivably lead to crystalline silver nitrate adducts[115]. Again the addition of the elements of acetic acid to methyl dihydroisooxoaristate to afford a crystalline product would suggest that the corresponding compound prepared from bromoacetic acid might well be a suitable derivative for X-ray study. Other suitable derivatives could conceivably be prepared by the addition of such electrophilic reagents as nitrosyl bromide, hydrogen bromide, or iodine monochloride to a variety of unsaturated aristolactone derivatives available.

In addition to the work on aristolactone certain preliminary screenings were also performed in the present work on two Aristolochia species previously studied by other workers[23,24,28] viz A. cymbifera (syn. A. grandiflora) and A. bracteata, to ascertain whether these species, as well as A. reticulata and A. serpentaria, contained aristolactone. It was shown that they did not. However certain other constituents were isolated. From A. bracteata,

aristolochic acid (VI) was obtained in 0.008% yield whilst A. cymbifera afforded what appeared to be β-sitosterol (IX) on the basis of mixed melting point and infra-red spectra (measured as the potassium chloride disc). However as sitosterols nearly always occur in admixture in nature[116] it is necessary to carry out further work for example, conversion into the methyl ether and then gas-liquid chromatography as described by Clayton[117] before identification of this material can be made with certainty. Also some preliminary work was done on the "water insoluble acid" previously reported by Stenlake and Williams[38]. This was shown to be dibasic and to fit the formula $C_{20}H_{28}O_4$, by molecular weight determinations employing boiling point elevation measurements. Kuhn-Roth determination indicated that the compound contained 3 C-CH_3 groups. Generation of the dimethyl ester by treatment of the acid with diazomethane gave a compound analysing for $C_{22}H_{32}O_4$. Preliminary ozonolysis studies on both the dibasic acid and its dimethyl ester gave indistinct and variable results so that no conclusions as to the nature of the diacid can be made. Further work on

this compound should initially be directed towards confirming its chemical individuality by employing gas-liquid chromatography of the dimethyl ester.

E X P E R I M E N T A L

Melting points were determined on a hot-stage melting point apparatus and are uncorrected. Ultra-violet spectra were measured on an Optica CF-4 recording spectrophotometer and on a Hilger and Watts U-V spek. Infra-red spectra were measured on an Unicam model S.P 100 instrument as described in the discussion, or on Perkin-Elmer 237 or Infracord instruments. Optical rotations were determined on a Bellingham and Stanley polarimeter employing a 1 decimetre cell. Gas-liquid chromatographic analyses were carried out on a Perkin-Elmer Fractometer run at 138° (employing a 50 metre capillary column coated with polypropylene glycol as stationary phase). Microanalyses were carried out by the microanalytical laboratory of the Royal College of Science and Technology and by Drs. Weiler and Strauss, Oxford. Catalytic hydrogenations were performed in a Towers microhydrogenation apparatus at atmospheric pressure and room temperature. Horizontal tube distillations were carried out in a Towers heating unit.

Isoaristolactone, dihydroisoaristolactone and hexahydroisoaristolactone were prepared as previously

described in the literature[47], as were dihydroneoaristolactone ("oxoaristaldehyde") and dihydroneoisoaristolactone ("isooxoaristaldehyde")[45].

Hexahydroisoaristo-2.14-dimethanesulphonate (LXVIII)

To hexahydroisoaristo-2,14-diol, m.p. 106-107° (formerly termed "tetrahydroisoaristo-6,12-diol") prepared as previously described[45] (1.00g; 3.66 m mole) in dry pyridine (10 ml) at 0°, was added dropwise methanesulphonyl chloride (1.15g; 8.05 m mole) and the reaction mixture allowed to stand at room temperature for 36 hr. Addition of crushed ice precipitated crystals which were collected by filtration and dried. Recrystallization from ether/petroleum ether afforded colourless needles of the dimethanesulphonate m.p. 79-81° (0.90g), which quickly suffered a pronounced fall in melting point (Found: C,51.19; H,7.62, S,15.45. $C_{17}H_{34}O_6S_2$ requires C,51.23; H,8.60; S,16.09%). ν_{max} (in KCl disc) 1330 and 1175 cm^{-1} (sulphonate).

Conversion of the Dimethanesulphonate into Diastereoisomeric Germacranes (XLV)

The dimethanesulphonate prepared as described above (0.85g) in dry ether (50 ml) was treated with excess of

lithium aluminium hydride (0.8g) and the mixture heated under reflux for 6 hours. Excess of complex metal hydride was decomposed by the slow addition of dil. HCl to the reaction mixture. The resultant acidic solution was exhaustively extracted with ether and the ethereal solution washed with water and dried over anhydrous sodium sulphate. Concentration of the ethereal solution afforded a colourless oil (0.321g) a sample of which gave a strong positive reaction to tetranitromethane indicating the presence of unsaturated material (confirmed by a band at ca 1660 cm^{-1} in the infra-red spectrum). G.l.c. (see table 2) indicated that the oil was composed of three compounds. The unfractionated oil (0.32g) in ethanol (11 ml) was then hydrogenated over platinum oxide (0.08g) until hydrogen uptake ceased (total uptake: 0.49 mole hydrogen). The catalyst was removed by filtration and after concentration of the filtrate the oily residue remaining was distilled in a horizontal tube at 90°/0.05 mm Hg, the entire distillate being collected as one fraction. Since the infra-red spectrum and the g.l.c. trace (see table 2) indicated that unsaturated material was still present, the crude product was subjected to rehydrogenation this

time employing highly active Adam's catalyst (uptake: 0.39 mole hydrogen). Distillation of the fully saturated oily product at 90°/0.05 mm Hg afforded a mixture of three components as shown by g.l.c. The three peaks present were each found to correspond in retention time to one of the three peaks obtained from a sample of synthetic germacrane (confirmed after admixture of the two samples).

Aristolactone diol Acetonide.

Aristolactone diol (formerly termed "6,7-dihydroxy-aristolactone") prepared by neutral potassium permanganate oxidation of aristolactone as previously described[45] (0.090g) in dry acetone - benzene (50 ml to 20 ml) to which p-toluenesulphonic acid (100 mg) had been added, was heated under reflux for 20 hours following a procedure developed by Takeda, Kubota and Shimaoka[96] for the preparation of the acetonides of sapogenins. The reaction mixture was neutralized by 15% sodium bicarbonate solution and the organic solvents were removed by distillation under reduced pressure. The aqueous residue was exhaustively extracted with benzene, the benzene solution washed with water, dried (Na_2SO_4), and concentrated affording crystalline material

m.p. 126-128° (0.1g.) Chromatography over ferric oxide[118] with chloroform as eluant gave nearly colourless crystals which upon recrystallization from methanol exhibited m.p. 128-129° Subsequent recrystallizations from methanol induced a fall in m.p. to 120-122° which could not be elevated by repeated recrystallization or sublimation. The acetonide melting at 120-122° was subjected to analysis (Found: C, 69.69; H, 8.85. $C_{18}H_{36}O_4$ requires C, 70.55; H, 8.85%) ε 103 at 210 mμ.

Formation of the Bicyclic Derivative of Methyl oxoaristate Under the Influence of Acetic Anhydride.

Methyl oxoaristate prepared from aristolactone by the action of methanolic potassium hydroxide as previously described[47] (0.12g) in acetic anhydride (10 ml) was heated under reflux for 2 hours. The yellowish crystalline mass (0.11g) remaining after distillation of the acetic anhydride under reduced pressure was recrystallized from aqueous acetone affording the cyclization product as colourless needles m.p. 112-113°, $[\alpha]_D^{18.5}$ +164.1° (c, 1.02 in ethanol) (Found: C, 76.99, 77.07; H, 8.40, 8.67. $C_{15}H_{20}O_2$ requires C, 77.54; H, 8.68%) ε 950 at 210 mμ.

ν_{max} (in CCl_4 solution) 3091 and 3073 (vinylidene) and 1788 and 1778 (γ-lactone) 1656 cm^{-1} (vinylidene).

Catalytic reduction of the cyclized product in ethanol over Adam's catalyst (uptake: 1.8 moles hydrogen) afforded the tetrahydro derivative m.p. 70-72° shown spectroscopically to be fully saturated.

Lactone Acetate (LXXX) Derived from Methyl dihydroiso-oxoaristate.

Methyl dihydroisooxoaristate previously termed methyl oxoaristate prepared as in the literature[45] (0.97g.) in acetic anhydride (20 ml) was heated under reflux for 4.5 hr. after which time the acetic anhydride was removed in vacuo. The crystalline residue was recrystallized from petroleum ether (40-60°) giving colourless needles of lactone acetate m.p. 166-167°, $[\alpha]_D^{25}$ - 63°(c 1.23 in ethanol) (0.31g.) (Found: 69.39, 68.84; H, 8.95, 8.80. $C_{17}H_{26}O_4$ requires C,69.36; H,8.90%).

λ_{max} 215 mμ (ε,15⁴.)

ν_{max} (CCl disc) 1765 (γ-lactone) 1725 (acetate) and 1248 cm^{-1} (acetate).

Carbon and hydrogen values were highly variable duplicate analyses of the same sample giving different

values. Other analyses were: C, 68.32, 67.90, 68.15, 68.23 H, 8.87, 9.13, 8.74, 8.87%

Hydroxy Acetate Derivative (LXXX or LXXXVI) of Methyl dihydroisooxoaristate.

Methyl dihydroisooxoaristate[45] (0.68g) containing traces of colloidal platinum oxide) in glacial acetic acid (40 ml) was allowed to stand at room temperature until the fall in optical rotation had ceased (48 hr.). Careful addition of water precipitated the product which was collected by filtration, washed with water and dried under vacuum. The hydroxy acetate derivative m.p.80-82 was recrystallized from aqueous ethanol to constant m.p. 90.5-91°, $[\alpha]_D^{18}$ - 73.91° (c,1.0 in ethanol) (Found: C,65.82, H,9.21. $C_{18}H_{30}O_5$ requires C, 66.24; H,9.24%)

ε, 239 at 210 mμ

λ_{max} (KCl disc) 3350 (hydroxyl) 1726 (methyl ester) 1703 and 1260 (acetate) and 1135 cm^{-1} (tertiary alcohol).

The hydroxy acetate when treated with refluxing acetic anhydride was converted into the same lactone acetate derivative of methyl dihydroisooxoaristate as described previously as shown by mixed m.p., optical rotation and infra-red spectra.

Isolation of β- Sitosterol From Aerial Parts of A. cymbifera.

Dry powdered aerial parts of A. cymbifera (500g.) were percolated with petroleum ether (40-60°) until the percolate was colourless (total volume of menstruum, 3 l). Concentration of the solution yielded a heavy green oil (3.2g), which after seeding with aristolactone and storing in a refrigerator for an extended period of time, failed to deposit crystalline aristolactone as was always observed with corresponding extractives of A. serpentaria and A. reticulata. The oil was chromatographed over a ferric oxide[118]: cellulose (1:2.5) column eluting with petroleum ether (40-60°). Concentration of the various fractions obtained showed that later fractions contained a crystalline material. This was collected and re-crystallized from petroleum ether (40-60°) until colourless and then sublimbed. The material showed m.p. and mixed m.p. 138-140° with β-sitosterol. The substance gave a positive Liebermann-Burchard test.

Isolation of Aristolochic Acid from A. bracteata.

Powdered dried A. bracteata (270g) was percolated with petroleum ether (40-60°) until the menstruum was

colourless (total volume: 2 l) Removal of the solvent and seeding the residue with aristolactone failed to afford any of this lactone. The marc was air dried and then percolated with ethanol after maceration for 24 hr. Concentration of the percolate (I L) afforded a dark green oil (4.4g) which was treated with hot water. Trituration of the water-insoluble material with ethanol dissolved the oily matter present and left out of solution tacky crystals. Recrystallization to constant m.p. 276-278° gave aristolochic acid (0.1g) as bright yellow needles, identical with an authentic sample (mixed m.p., U-V., I-R.).

"Water-Insoluble Acid"

Water insoluble acid[38] obtained as an oil (0.3g) was distilled six times in a horizontal tube at 105°/0.05 mm Hg affording a pale yellow oil (Found: C, 71.80, 72.00; H, 8.87, 8.96; molecular weight 303; C-CH_3 10.05. $C_{20}H_{30}O_4$ requires C, 71.85; H, 9.04%; 334.44; equivalent to 2.23 C-CH_3). ν_{max} (liquid film) 1700 (carboxylic acid) 1630 (shoulder) and 940 cm^{-1} (broad; carboxylic acid).

Esterification of "Water Insoluble Acid"

Water insoluble acid (1.2g) in ether (15 ml) was

treated with excess of ethereal diazomethane. Concentration of the ethereal solution and distilling the resulting oil in a horizontal tube at 90°C/0.05 mm Hg afforded the dimethyl ester (Found, C,73.18; H,9.17. $C_{22}H_{34}O_4$ requires C,72.90; H,9.45%).

ν_{max} (liquid film) 1715 (ester) and 1630 cm^{-1} (double bond).

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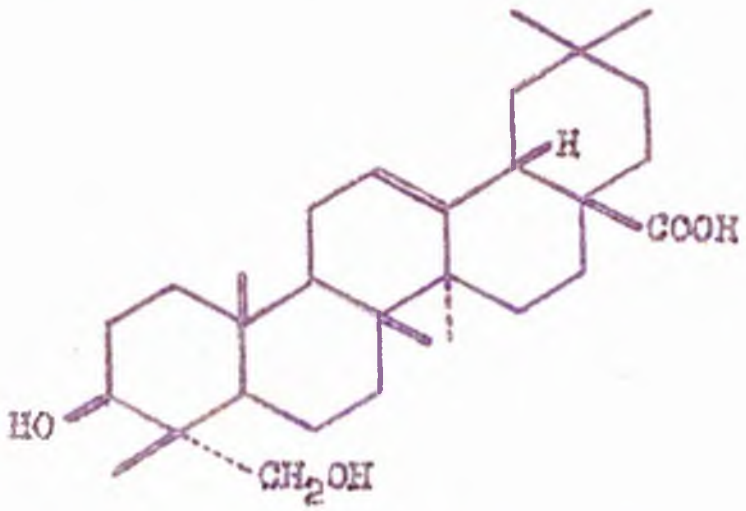
The Structure of Petaline Chloride, an Alkaloid Isolated From *Leontice leontopetalum* L.

HISTORICAL INTRODUCTION

Certain plants belonging to the genus Leontice (family Berberidaceae) have a long history of application in folk medicine, which in the case of two species can be traced back to the early Greeks. One of these species, referred to by Gunther[1] as Leontice chrysogonum (syn., leontopetalon) was employed by the ancients as a remedy for snake-bite and in the treatment of sciatica. The other, which was probably Leontice leontopetalum (syn., chrysogonon), they reputedly used for treating "bitings of the shrew mouse". In more recent times the people of eastern Mediterranean countries have employed the tubers of Leontice leontopetalum as a soap substitute[2,3,4,5], a snake-bite remedy[3], a corrective for overdoses of opiates[2] and in the treatment of epilepsy[3,6]. Yet despite this long established use in folk medicine few scientific studies of Leontice species, apart from some Russian work, appear to have been undertaken. Accordingly in 1955 an investigation of Leontice leontopetalum - a species thought to have been first introduced into Great Britain in 1597[7] - was undertaken in these

laboratories[8,9,10,11].

Using a modification of a method developed by Power and Salway[12], McShefferty[10] isolated a saponin from the tubers of L. leontopetalum. This saponin, which is doubtlessly the agent responsible for the soap-like activity of the tubers[2,3,4,5], analysed for $C_{69}H_{112}O_{36}\cdot 5H_2O$, and had m.p. 236-238°, and $[\alpha]_D$ +15.1°. It gave a cosanacetate, $C_{109}H_{152}O_{56}$, m.p. 155-156°, $[\alpha]_D$ +19.9°. Acid hydrolysis of the saponin afforded the sapogenin, $C_{30}H_{48}O_4$, m.p. 333-334° and $[\alpha]_D$ +78°, which was shown to be identical with hederagenin (I). The sugar portion of the saponin was found to consist of four D-glucose and three L-arabinose units[10].



From the petroleum ether extractives of the tubers of L. leontopetalum, McShefferty[10] isolated

ceryl alcohol, and a compound concluded to be a 3-β-hydroxy-Δ^7-sterol, $C_{29}H_{46}O$, m.p. 155.5-156°. Examination of the fatty acids in the extractives indicated the presence of palmitic, stearic, oleic and linoleic acids, and probably n-hexacosanoic acid. Glycerol was also shown to be present. In addition, McShefferty[10] isolated three alkaloids from the aqueous acid extraction of the defatted plant material.

Alkaloids had been isolated previously from other species of the genus Leontice. In 1932 Orekhov and Konovalova[13,14] demonstrated the presence of at least four alkaloids in the tubers of L. ewersmanni Bge. (which earlier had been mistakenly identified by Hooker Jr. and Thomas as L. leontopetalum[15]), a plant found in Persia and Turkestan. These authors showed that the total basic fraction of L. ewersmanni amounted to 0.4% of the dry weight of tubers. One alkaloid which was designated leontamine was isolated as an oil, b.p. 118-119°/ 4mm, $[\alpha]_D$ +2.53 and n_D 1.5113 and gave an analysis in agreement with the empirical formula $C_{14}H_{20}N_2$. The second base, the crystalline leontidine had m.p. 116-118°, but Orekhov and Konovalova

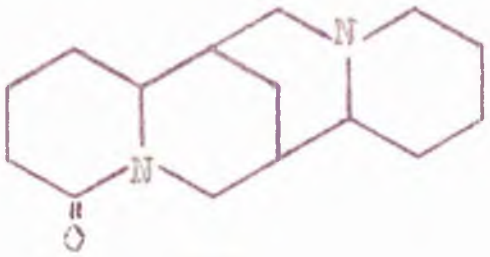
quote no empirical formula for this compound in their papers[13,14]. The third alkaloid was obtained only as a picrate, m.p. 176-178°, whilst the fourth was not isolated, but only inferred to be present.

Later Yunusov and Sorokina[16] re-examined the alkaloids from the tubers of L. ewersmanni and the formula $C_{15}H_{20}N_2O$ was assigned to leontidine and its $[\alpha]_D$ was quoted as - 188·7°. Attempts were made to show that leontamine was the optical antipode of sparteine (syn., pachycarpine) (II) a base present in the aerial parts of the plant[16] but not in the tubers. However the experimental evidence failed to confirm this assumption.

II

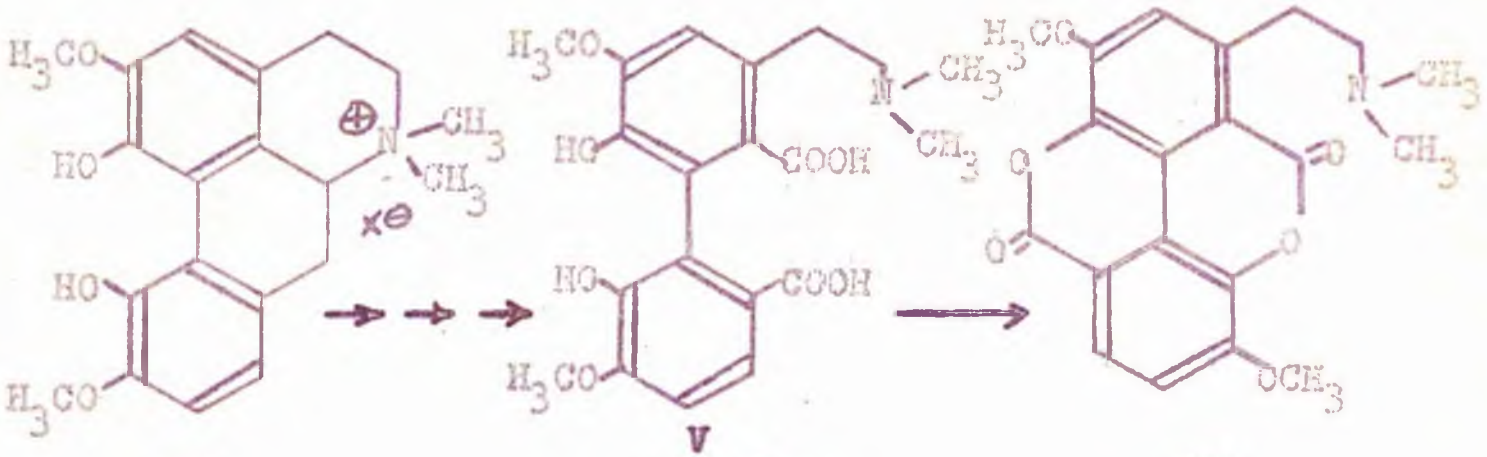
Yunusov and Sorokina[16] isolated a further alkaloid, $C_{15}H_{24}N_2O$, m.p. 103-104°, from the tubers, which they designated leontine. The aerial portions (0.87% alkaloids) were found to

contain leontidine, sparteine (II), and d-lupanine (III).

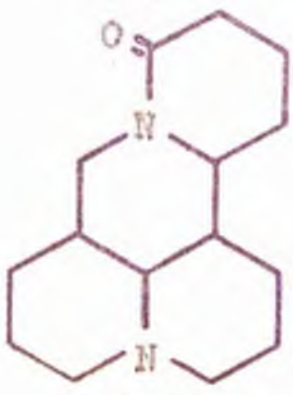


III

Working on the same species, Platonova, Kuzovkov, and Massagetov[17,18] isolated two further bases, tapsine (VI) the dilactone of tapsinic acid (V) (which biogenetically may be derived from the aporphine alkaloid magnoflorine (IV) by a Hofmann-type degradation and oxidation), and isoleontine, $C_{15}H_{24}N_2O$, m.p. 107-108°, $[\alpha]_D$ -78.2°, thought to be related to matrine (VII).



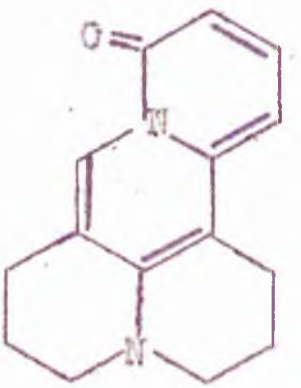
IV V VI



VII

No further alkaloids were detected in any part of the plant[19,20].

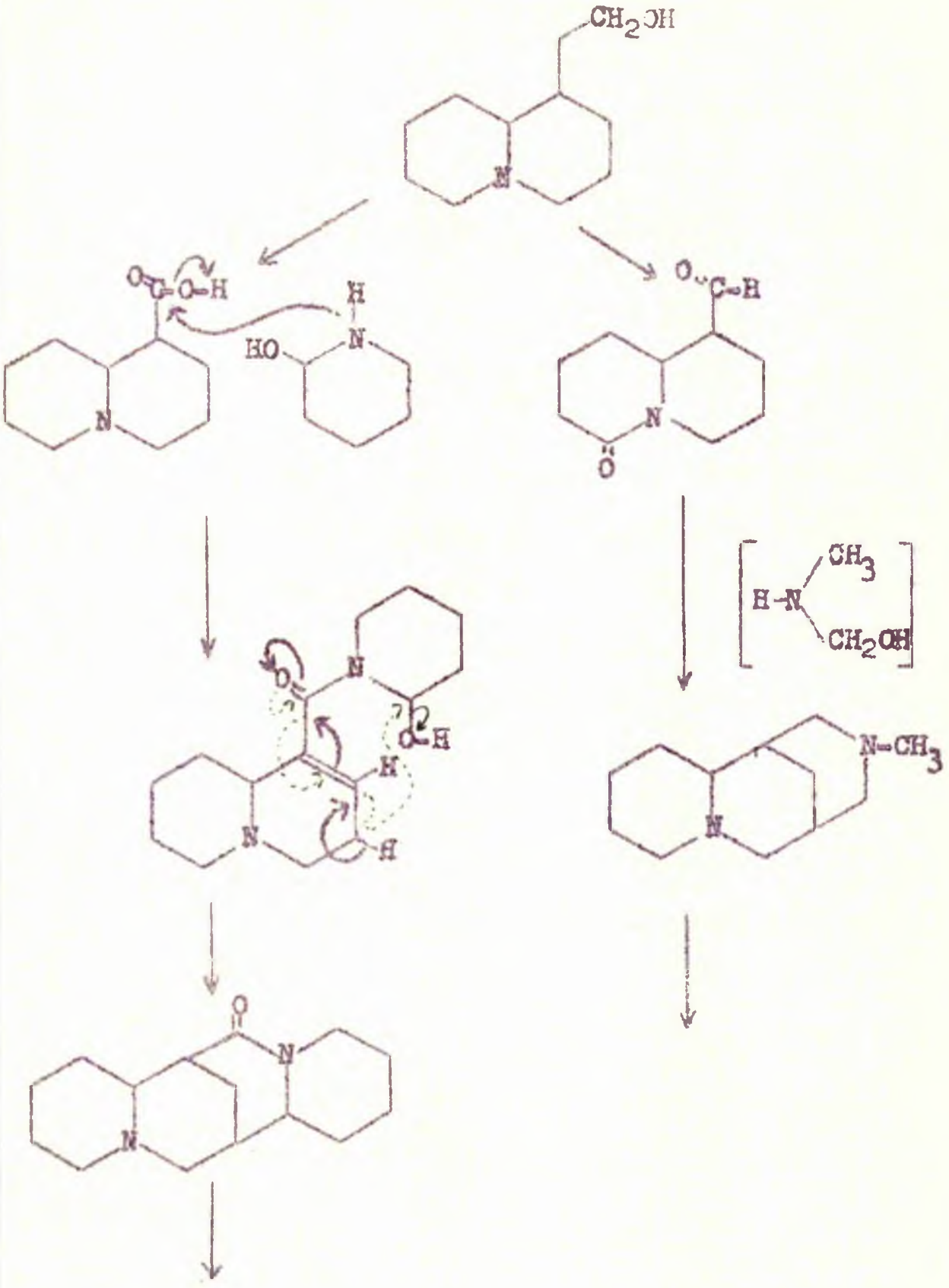
Recently, Rulko and Proskurnina[21] confirmed that leontine was a stereoisomer of matrine by dehydrogenation to octadehydromatrine (VIII).



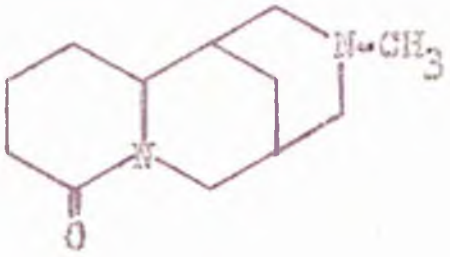
VIII

The pharmacological properties of the alkaloids from L. ewersmanni appear not to have been reported, although the saponins from the tubers of this plant, like all saponins, have been shown to exhibit in vitro haemolytic activity[22].

Yunusov and Sorokina also investigated L. alberti Bge.[16] a rare plant found in the mountainous regions of central Asia. From it they isolated an unidentified liquid base, a small amount of a crystalline alkaloid, m.p. 180-183° and methylcytisine (IX).



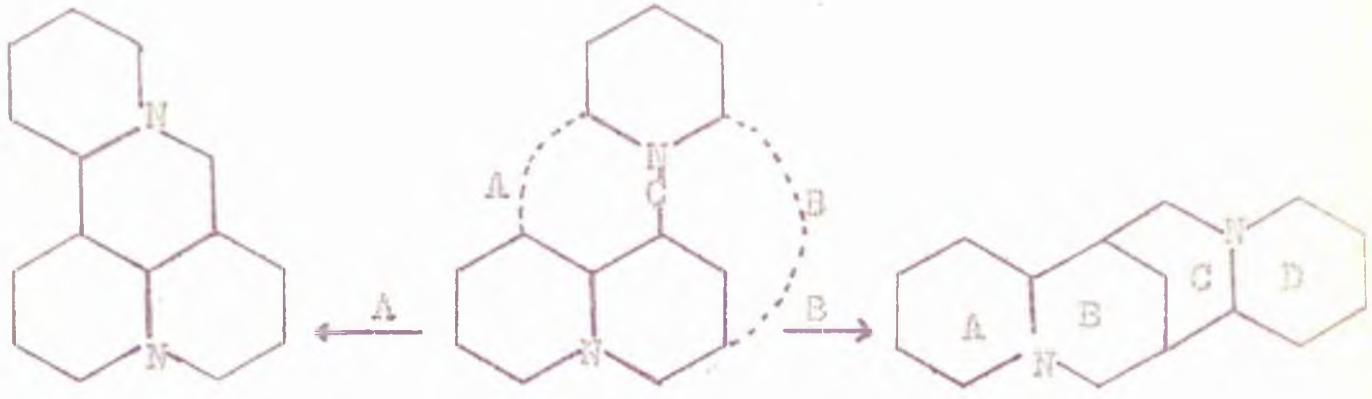
SCHEME 1.



IX

To explain the frequent occurrence of quinolizidine alkaloids belonging to the lupanine, cytisine, sparteine, and matrine series in the same plant, Yunusov and Sorokina[16] suggested a scheme (akin to that portrayed in scheme I) of skeletal interconversions using lupinine (X) as the basic unit.

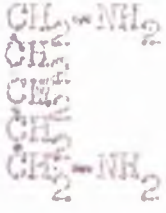
If an intermediate of type XI were to be formed from the condensation of lupinine with a piperidine derivative, it is readily seen that ring closure via route A would give rise to the matrine skeleton (XII) whilst ring closure via route B would lead to the sparteine skeleton (IX).



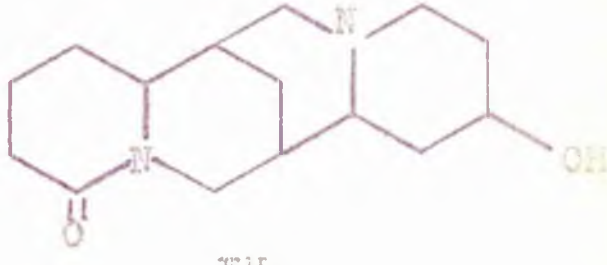
XII XI II

Radio-active tracer studies employing ^{14}C have shown that lupinine (X) (formed from lysine (XIII) via cadaverine (XIV) is incorporated into sparteine (II)[23] lupanine (III), 13-hydroxylupanine (XV)[24] in Lupinus angustifolius, and matrine (VII)[25] in Sophora tetraptera.

XIII



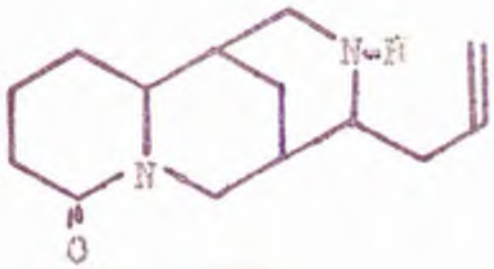
XIV



XV

More recent studies however, have indicated that the biogenesis of the tetracyclic alkaloids of the sparteine series involves the initial formation of the more highly oxidised members (13-hydroxylupanine (XV) and angustifoline (XVI)) which are then converted into

sparteine (II). Thus a preformed piperidine ring is not involved in the formation of rings C and D in this alkaloid.



XVI

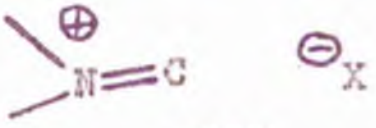
A recent examination of Leontice odessana by Kolisnichenko[27] led to the demonstration of the presence of five alkaloids, comprising 2.6% of the plant material. However, none of the individual alkaloids were characterized chemically.

Of the three bases isolated from L. leontopetalum during the earlier work in these laboratories[8,10] one, a non-crystalline base had physical constants and empirical formula $C_{14}H_{26}N_2$, (b.p. 118-120°/4 mm, $[\alpha]_D$ +2.78°, EtOH, n_D 1.5117) in agreement with those cited by Orekhov and Konovalova for leontamine[13,14], the constitution of which is still unelucidated. Direct comparison of specimens was not made but it was assumed that they were identical. The specimen obtained by McShefferty was shown to be fully saturated both by chemical and physical means[10]. The second base isolated was named leonticine,

$C_{20}H_{25}NO_3$, m.p. 118.5-119.5°, $[\alpha]_D$ 0(EtOH) and was obtained in 0.018% yield from the tubers. Although it exhibited the same melting point, leonticine was obviously not identical with leontidine, $C_{25}H_{20}NO_2$, isolated from Leontice ewersmanni[13,14,16]. Leonticine slowly decolourized acidic potassium permanganate but attempts at catalytic hydrogenation were unsuccessful.

The third alkaloid, a water-soluble quaternary base, was isolated as the pink micro-crystalline reineckate, $C_{20}H_{22}O_3N\left[Cr(SCN)_4(NH_3)_2\right]$, m.p. 179-181°. Decomposition of the reineckate by the silver sulphate barium chloride method of Dutcher[28] gave the quaternary chloride as deep yellow deliquescent scales, m.p. 140-143°. This quaternary salt, designated petaline chloride, was shown by elemental and functional group analysis to be $C_{18}H_{16}ONCl(OCH_3)_2$. It was found to be optically active, $[\alpha]_D^{20}$ +11.3° (H_2O), and exhibited ultraviolet absorption maxima at 224 mμ (ε, 20,576), 280 mμ (ε,11,600) and 328 mμ (ε,334). Petaline chloride was reported to give indistinct spot tests for the presence of a phenolic hydroxyl group but it gave positive tests typical of the berberine type of alkaloid[29]. Petaline

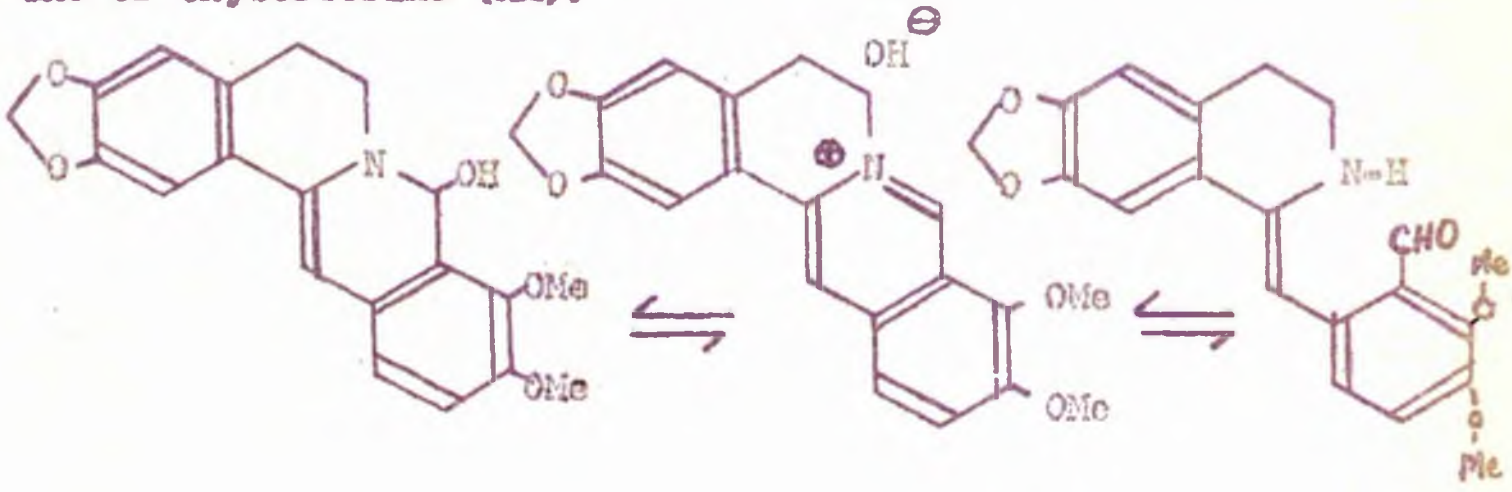
chloride was readily converted into the corresponding quaternary picrate, chloroplatinate and sulphate. It was reported to undergo reduction with zinc and dilute hydrochloric acid, and with hydrogen over Adam's catalyst in aqueous acetic acid (uptake: one mole) to yield dihydropetaline chloride $C_{20}H_{24}NO_3Cl \cdot 2H_2O$, m.p. 122-125° $[\alpha]_D$ -16.7° (H_2O) with λ_{max} 224 (ε,15,364) and 280 mμ (ε,11,580). McShefferty[10] concluded, on evidence which was by no means unequivocal, that petaline chloride was a pseudoquaternary base (partial structure XVII) whose double bond appeared not to be affected by hydrogenation although isomerization appeared to have taken place about the optical centre, as indicated from the inverted optical rotation.



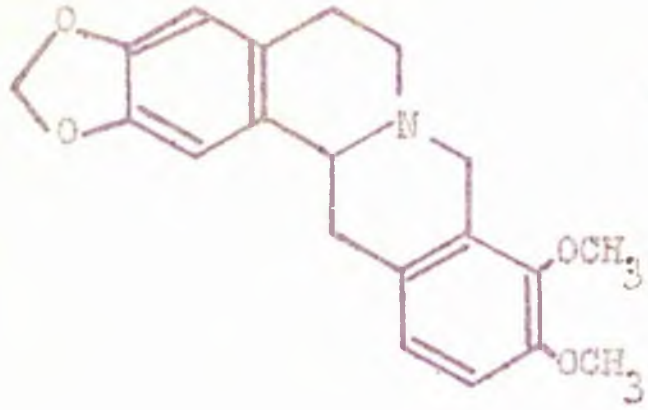
XVII

The oxidation of petaline chloride with alkaline potassium permanganate was stated to give inconclusive results. Petaline chloride was found to be extremely unstable to alkali, yielding black tars. In one experiment[10] on treatment with excess 4% barium hydroxide, petaline chloride

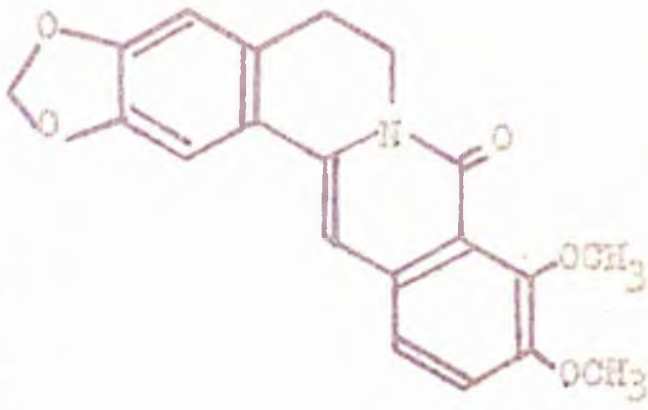
afforded a 30% yield of leonticine, m.p. 117.5-118.5°, together with another substance, m.p. 105-110°, exhibiting reactions typical of a ketone or aldehyde. This carbonyl compound, designated oxypetaline chloride gave what were regarded as unsatisfactory analyses, both as the free base and as the 2,4-dinitrophenylhydrazone and no formula was assigned to it. It was further suggested that leonticine was tetrahydroanhydropetaline[10]. For analogy, comparison was made with the work of Gadamer[30,31] and Perkins[32] as modified by Faltis[33]. In this work three molecules of berberine (ammonium form, XVIII) are converted, in the presence of alkali, into two moles of tetrahydroanhydroberberine (XIX) and one of oxyberberine (XX).



XVIII



XIX



XX

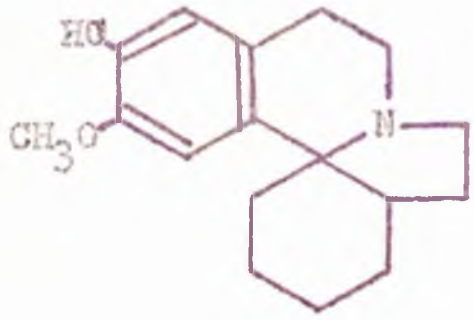
On the basis of this analogy the second product of the action of barium hydroxide on petaline chloride should have been a lactam incapable of 2,4-dinitrophenylhydrazone formation and not a ketone or aldehyde. On the basis of ultraviolet spectral comparisons, McShefferty[10] suggested that petaline chloride was an isoquinoline alkaloid. Further he suggested that the tertiary base, leonticine was an artefact resulting from the action of alkali on petaline chloride during the work-up of the plant, a conclusion supported by the present investigation (vide infra).

Petaline chloride was tested pharmacologically by Ahmad and Lewis[34] who claimed it to be a more potent convulsant than leptazol although at low dosage levels petaline chloride was found to reduce the

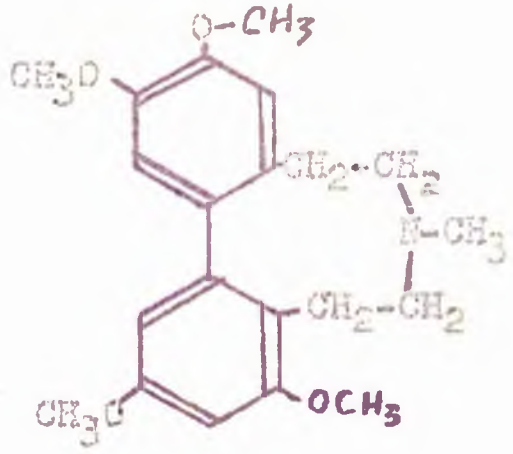
convulsant activity of leptazol and exhibited muscle relaxant activity.

DISCUSSION

A survey of the work previously performed with petaline and leonticine as outlined in the introduction revealed the conspicuous absence of any attempted Hofmann degradation of any quaternary salt derived from leonticine. Since this reaction has proven to be of great value in the structural elucidations of a variety of alkaloids (as with aporphine alkaloids such as isocorydine methchloride[35], the erythrina alkaloids such as tetrahydroerysodine (XXI)[36] and in the structural elucidation of the interesting alkaloid protostephanine (XXII)[37], isolated from Stephania japonica[38] in addition to the classical structural elucidations of the tropine and pomegranate alkaloids), it seemed a logical first step to apply the Hofmann degradation to leonticine which the earlier work would indicate was the methine base of petaline. This last deduction follows from a consideration of the method of formation of leonticine from petaline, by the action of base on the latter compound[10] and from its analytical figures.



XXI



XXII

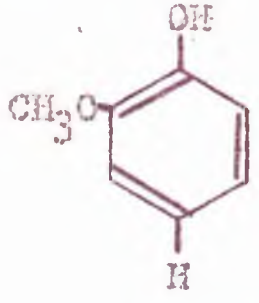
Indeed employing milder conditions than used by the earlier workers[10], that is passage over Amberlite IRA-400 anionic exchange resin, the conversion yield of petaline chloride into leonticine was raised to 65% while petaline reinckate was converted directly into leonticine in ca 40% yield.

Quaternization of leonticine by the action of methyl iodide in dry acetone readily afforded the corresponding quaternary iodide $C_{21}H_{28}O_3NI$, m.p. 169-171° (addition of 1 mole methyl iodide) in good yield. This was subjected to passage over IRA-400 anion exchange resin but Hofmann degradation failed to occur - the product being the corresponding leonticine

methohydroxide m.p. 150-155°. Treatment of the methohydroxide with 5% sodium ethoxide in ethanol afforded a nitrogen-free methine m.p. 111-113°, $[\alpha]_D = 0°$ analysing for $(C_6H_6O)_n$. Molecular weight determination by the mass spectographic method showed the molecular weight to be 282 thus fixing the formula of the nitrogen-free product as $C_{18}H_{18}O_3$. This product therefore has been formed from leonticine by the net elimination of the elements of trimethylamine and water. That the lost nitrogen containing fragment was indeed trimethylamine was shown by the isolation of the volatile fragment as its picrate. The picrate thus isolated was identical with authentic trimethylamine picrate (mixed m.p., infra-red spectra, and analyses).

The infra-red spectrum of the nitrogen-free fragment from the Hofmann degradation of leonticine methohydroxide measured in carbon tetrachloride solution (2.07 mg/6 ml) clearly showed an intramolecularly hydrogen bonded hydroxyl group as evidenced by the position of the O-H stretching frequency at 3545 cm^{-1} $(\varepsilon, 144)$[39a]. That the same intramolecular

hydrogen bonding was present in leonticine was shown by the retention of this absorption at 3535 cm^{-1} (ε,137) in the infra-red spectrum of this compound measured in carbon tetrachloride (2.44 mg/6ml). These two results taken in conjunction with the fact that methoxyl determinations show the presence of two methoxyl groups in both leonticine and the nitrogen free degradation product (thus accounting for all the oxygen atoms present in both compounds) shows that the -C-H group is adjacent to a methoxyl group. Petaline, leonticine and the nitrogen-free Hofmann degradation product all give a positive Gibb's test[40,41] for a phenol possessing a free para position, thus showing the fragment XXIII to be present in all three compounds.



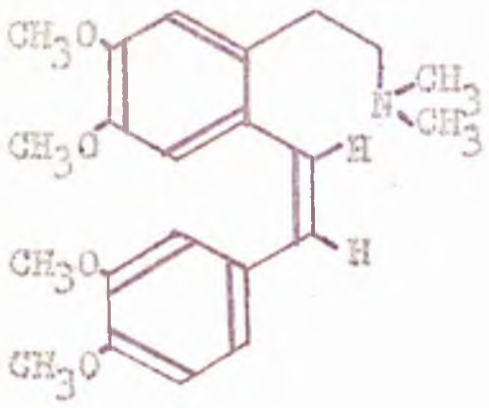
XXIII

The nuclear magnetic resonance spectra of leonticine and its nitrogen-free degradation product (as acetate) clearly shows the conversion of the system XXIV into XXV

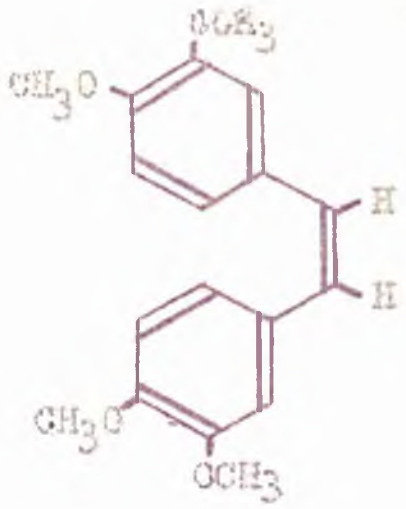
XXIV XXV

Thus absorption in leonticine of intensity 6 protons at 7.65 τ typical of methyl groups substituted on nitrogen[35,42] and complex methylene absorption of intensity 4 protons in the range 7.0-7.6 τ are replaced in the nitrogen-free degradation product (as acetate) by doublets of intensity 1 proton at 4.5 τ (J=17 c.p.s.) and 4.9 τ (J=10 c.p.s.), each peak showing fine splitting, typical of the vinylidene protons on the styryl double bond[35,42,43]. Further the infra-red spectrum of the nitrogen-free degradation product (in potassium chloride disc) exhibits maxima at 3086 cm^{-1} and 905 cm^{-1} typical of a vinylidene double bond[39b]. Such absorption was absent in the infrared spectrum of leonticine.

The ultra-violet spectrum of leonticine exhibited λmax 216 mμ (ε, 23,400) and 299 mμ (ε, 21,100) which would not be incompatible with a stilbene derivative, being very similar to the absorption from the cis-methine base XXVI of laudanosine which has λmax 215 mμ (ε, 23400) and 294 mμ (ε, 10,960)[44] and 3,3′, 4,4′-tetramethoxystilbene (XXVII) which has λ214 mμ (ε, 26,900) and λmax 303 mμ (ε, 13,500)[45].



XXVI

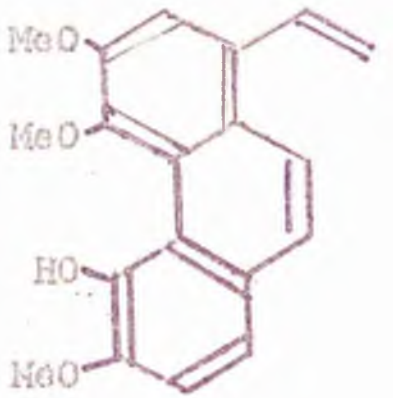


XXVII

The ultra-violet spectrum of leonticine run in N. HCl was virtually unchanged showing maxima at 216 mμ (ε,29,100) and 239 mμ (ε,23,500) thus indicating that leonticine is not an α,β-unsaturated amine (enamine)[46].

The ultra-violet absorption spectrum of the Hofmann degradation product of leonticine exhibits λmax 209 mμ (ε27,000), 269 mμ (ε,23,900) and 305 mμ (ε,21,100). The first and last maxima may be attributed to a cis-stilbene system whilst the maximum at 269 mμ is not unlike the contribution of the styryl vinylidene group in the phenanthrene derivative (XXVIII), of the aporphine alkaloid chakranine, which accounts for the maximum in its spectrum at 259 mμ (ε,37,150). As with the degradation product XXVIII, the maximum at 269 mμ in the ultra-violet spectrum of the Hofmann degradation product of leonticine measured in

N KOH is virtually unaltered appearing at 272 mμ (ε, 21,700)

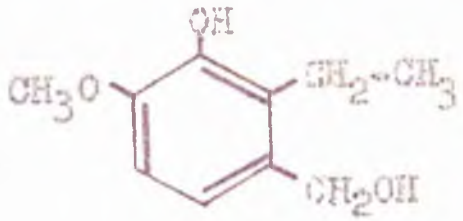


XXVIII

Ozonolysis of the nitrogen-free Hofmann degradation product of leonticine afforded p - methoxy benzaldehyde, identified by comparison of its infra-red spectrum with that of authentic material and by lack of mixed melting point depression in the 2,4-dintrophenylhydrazones prepared from the two specimens, thus identifying one half of the stilbene system. However the nature of the remainder of the molecule could not be determined from this experiment owing to the intractability of the material. The formation of p- methoxybenzaldehyde on ozonolysis, incidentally, would explain the strong odour of p-methoxy benzaldehyde, encountered during attempted Kuhn-Roth estimation of C-methyl groups in leonticine.

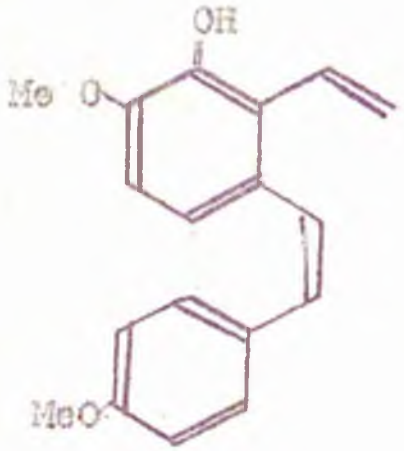
Ozonolysis of leonticine methiodide in ice-cold ethanol readily permitted the separation of the water soluble quaternary salt formed from one half of the stilbene from the p-methoxybenzaldehyde (identity again confirmed) formed from the other. This salt was not characterized as such but was subjected to Hofmann degradation to generate the substituted styrene and the resulting nitrogen-free oily product was hydrogenated over Adam's catalyst in ethanol. The resulting oil thus obtained showed no vinylidene absorption in the infra-red (measured in carbon tetrachloride solution; 2.85 mg/10 ml) indicating full reduction of the styryl double bond generated during the Hofmann elimination. Moreover no carbonyl absorption was present but two O-H stretching bands were present. The high frequency hydroxyl absorption at 3615 cm^{-1} (ϵ,22) can be assigned to the benzylic hydroxyl group formed by the reduction of the aldehyde group generated from the stilbene double bond on ozonolysis, whilst the low frequency absorption at 3547 cm^{-1} (ϵ,112) can be assigned to the phenolic hydroxyl group intramolecularly hydrogen bonded to the adjacent methoxyl group.

The constitution of this hydroxy-, methoxy-, hydroxymethyl- ethyl- benzene is limited to the one formula XXIX.

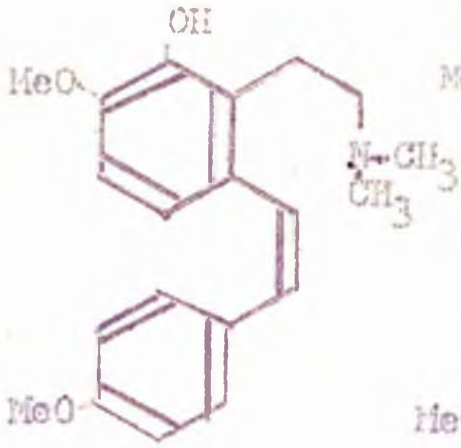


XXIX

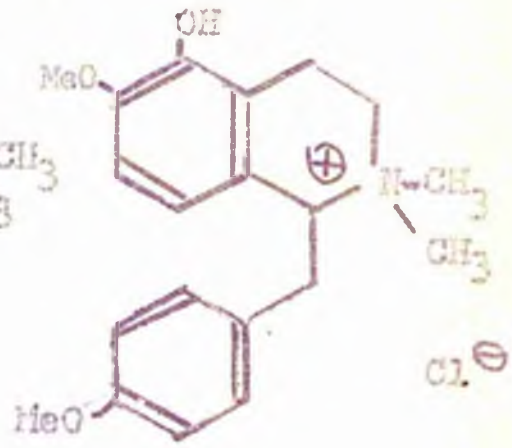
This stems from the fact that it has a free position para to the hydroxyl group (positive Gibbs test in accord with the similar positive tests given by petaline, leonticine and the methine derived from leonticine methiodide) and that there is no intramolecular hydrogen bonding of the benzylic hydroxyl group as would occur if this function were ortho to either the phenolic hydroxyl group on the methoxyl group (as evidenced by its absorption frequency of 3615 cm^{-1}) when the need for the ethyl group and the hydroxymethyl group to be ortho to one another is taken into account. From this it follows that the structure of the nitrogen free degradation product of leonticine methohydroxide is XXX, that of leonticine is XXXI and that of petaline, XXXII.



XXX



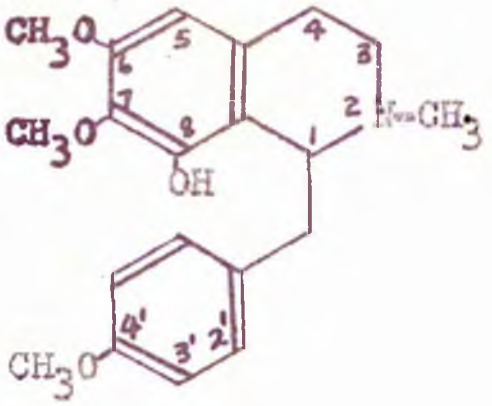
XXXI



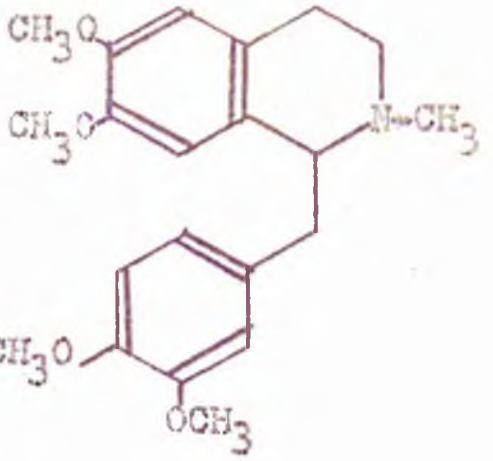
XXXII

Petaline (XXXII) being the only compound in the above series with a centre of asymmetry would be expected to be the only compound showing optical activity as indeed is the case.

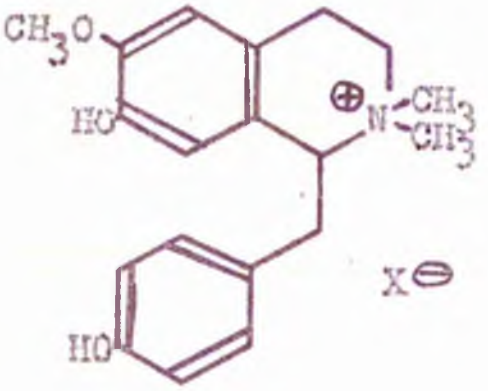
The substitution pattern of the benzene ring in the tetrahydroisoquinoline moiety of petaline (XXXII) is of interest biogenetically. Normally the substitution pattern is such that oxygen functions occur at two, three or four of the positions, 6, 7, 8, 3' and 4' of the benzyl tetrahydroisoquinoline nucleus as for example in the alkaloids corpaverine (6,7,8,4') (XXXIII) laudanosine (6,7,3',4') (XXXIV) magnocurarine (6,7,4') (XXXV) and the dioxygenated (6,4') alkaloid XXXVI[47], reflecting their biogenesis from hydroxyphenylalanines[43].



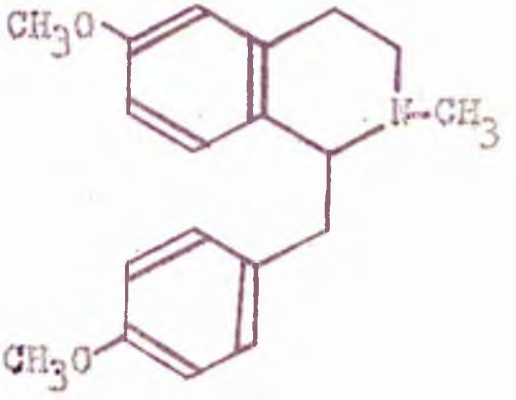
XXXIII



XXXIV



XXXV



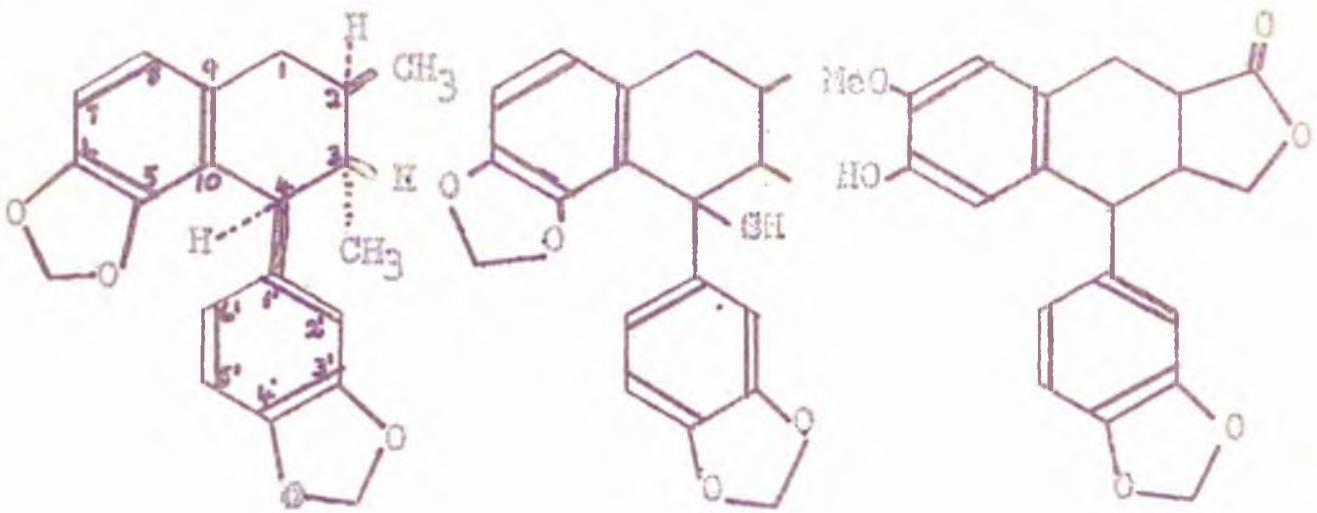
XXXVI

Indeed it would appear that no benzyltetrahydroisoquinoline alkaloid bearing an oxygen function at C-5 has so far been reported[53,54] and so petaline would seem to be of a unique substitution pattern.

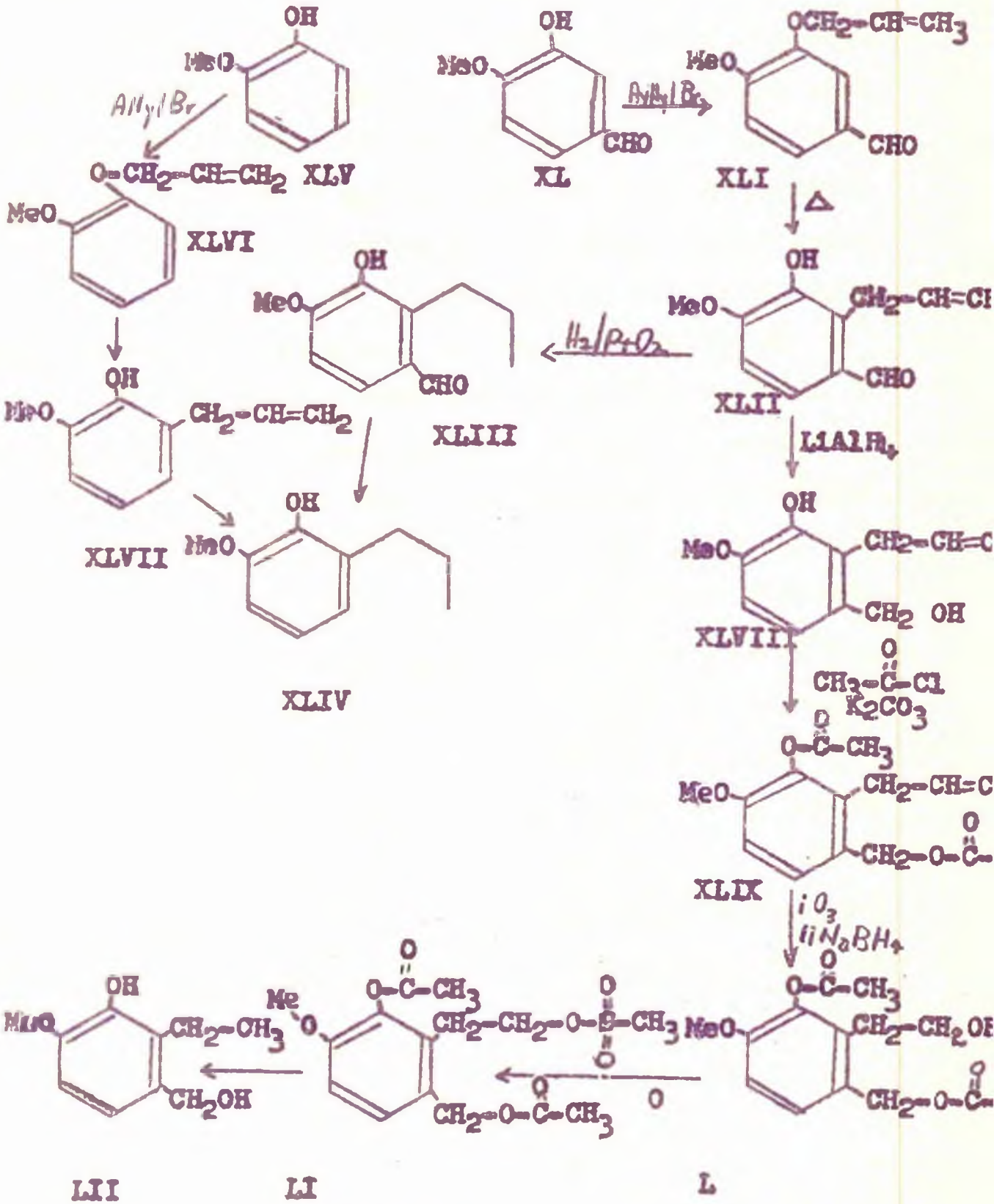
A possible explanation of the oxygenation pattern in the benzene ring of the tetrahydroisoquinoline

nucleus of petaline would perhaps be that loss of hydroxyl from C-7 of a precursor could occur through a quinonoid intermediate (compare corresponding loss of hydroxyl from the precursor of volucrisporin[55,56]) followed by the introduction of oxygen ortho to the phenolic group on C-6 prior to methylation of the latter[56].

It is perhaps also of some interest in connection with the unusual oxidation pattern present in petaline that recently otobain (XXXVII)[57] and hydroxyotobain (XXXVIII)[58] have been shown to possess a 5,6-dioxygenated substitution pattern, which is different from that normally pertaining in lignans which are usually 6,7-dioxygenated (plus, occassionally, 5-oxygenated)[59] as in, for example, conidendrin (XXXIX)[60].



XXXVII XXXVIII XXXIX


OH
MeO
Allyl Br
O-CH2-CH=CH2
XLV
MeO
XLVI
OH
MeO
CH2-CH=CH2
XLVII
OH
MeO
CHO
XL
AlMgBr
OCH2-CH=CH3
MeO
CHO
XLI
Δ
OH
MeO
CH2-CH=CH
CHO
XLII
H2/Pt+O2
OH
MeO
CHO
XLIII
LiAlH4
OH
MeO
CH2-CH=C
CH2 OH
XLVIII
CH3-C-Cl
K2CO3
O-C-CH3
MeO
CH2-CH=C
CH2-O-C
XLIX
i O3
ii NaBH4
O-C-CH3
MeO
CH2-CH2 OH
CH2-O-C
L
OH
MeO
CH2-CH3
CH2OH
LII
Me
O
O-C-CH3
CH2-CH2-O-P-CH3
O
CH2-O-C-CH3
O
LI

SCHEME 2.

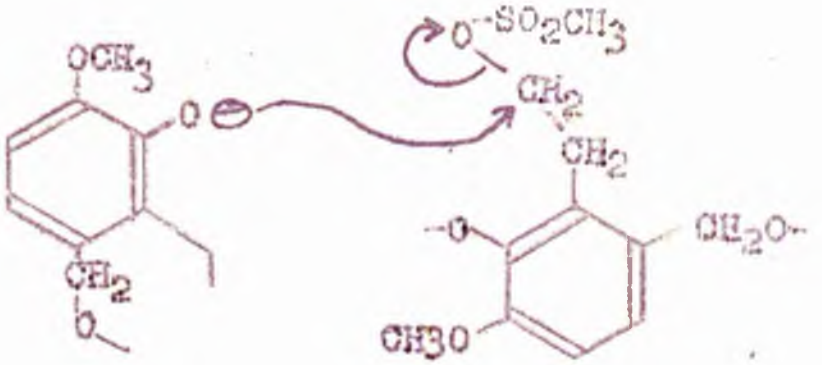
Otobain, which thus appears to be a case where the hydroxyl on C-7 responsible for ring closure at C_{10}, para to it, has been lost and an oxygen function has been inserted at C-5, represents a situation strictly paralleling that in petaline-*viz* loss of an oxygen (from perhaps C-7) and insertion of oxygen on the other side of a second oxygen ortho to that lost.

In order to clinch the evidence for the structure of petaline (XXXII) it was necessary to confirm the structure of XXIX by chemical means. It was therefore decided to attempt the synthesis of authentic material of this structure by an unambiguous route. The pathway chosen is shown in scheme 2. Thus isovanillin (XL) was chosen as a suitable starting material and was converted into the corresponding allyl ether XLI by boiling under reflux with a 1 molar ratio of allyl bromide in acetone containing excess of anhydrous potassium carbonate[49]. The product XLI b.p. 136°/1mm Hg was subjected to Claisen re-arrangement[50] by heating to 300°C for 5 minutes (oil-bath) and fractionally distilled, the fraction b.p. 142-143°/1mm Hg of product XLII being collected.

The authenticity of XLII was established by catalytic reduction of the vinylidene double bond followed by decarbonylation of the resultant 2-n-propyl iso-vanillin XLIII over 5% Palladium on charcoal by adaptation of a method described by Hawthorne and Wilt[51]. The product XLIV was identical (infra-red spectra of liquid films) with that obtained by the catalytic reduction of o-eugenol (XLVII) (possessing the correct physical properties prepared from guaiacol (XLV) by Claisen rearrangement of its allyl ether XLVI[49].

Lithium aluminium hydride reduction of 2-allylisovanillin (XLII) in dry ether smoothly converted this compound into the corresponding alcohol XLVIII which after chromatography on neutral alumina was acetylated by the action of acetyl chloride in dry acetone in the presence of excess of anhydrous potassium carbonate. Acetylation was shown to be quantitative by the absence of hydroxyl absorption in the infra-red spectrum of the oily diacetate (XLIX. Ozonolysis of the diacetate in ice-cold ethanol followed by reductive cleavage of the ozonide with

sodium borohydride[52] afforded the phenyl ethyl alcohol L. This compound was readily converted into the methanesulphonate derivative LI which was directly hydrogenolysed by the action of lithium aluminium hydride in dry tetrahydrofuran. Considerable quantities of high boiling material were present in the product which might indicate the occurrence of side reactions such as that depicted in LIII, and no material corresponding in properties to that obtained from the degredation of petaline could be isolated.



LIII

EXPERIMENTAL

Melting points were determined on a hot-stage melting point apparatus and are uncorrected. Ultra-violet spectra were measured on an Optica CF-4 recording spectrophotometer. Infra-red spectra were measured on an Unicam model SP100 equipped with a model S.P.130 sodium chloride prism-grating double monochromator operated under vacuum conditions and the Perkin-Elmer model 237 Infra-Red spectrophotometer. Optical rotations were determined on a Bellingham and Stanley polarimeter employing a 1 decimetre cell. Microanalyses were carried out by the microanalytical laboratory of the Royal College of Science and Technology, and by Drs. Weiler and Strauss, Oxford. Catalytic hydrogenations were effected in a Towers hydrogenating apparatus and were performed at room temperature and pressure.

Petaline reineckate was isolated from dry powdered Leontice leontopetalum extractives as previously described[8].

Conversion of Petaline Reineckate Leonticine (XXXI)

1. Petaline reineckate (5g.) in acetone (2) ml) was introduced onto a column of anhydrous Amberlite resin IRA-400 (in OH form) (250g) and eluted with dry ethanol until all coloured material had been washed through. After concentration of the eluate, the brown semi-solid mass was taken up in benzene (50 ml) and the resultant solution filtered through neutral alumina (10g.) to afford a colourless solution. Concentration of this solution under reduced pressure followed by recrystallization of the solid residue from acetone afforded leonticine m.p. 121-123°, $[\alpha]_D = 0°$, as colourless needles (Found: C, 73.58; H, 7.76; N, 4.61; N-CH_3, 13.24, 11.72; -OCH_3, 19.40, 17.56, 16.98; active H, 0.330. Calculated for $C_{20}H_{25}O_3N$: C, 73.38; H, 7.70; N, 4.28; N-CH_3, 17.72; -OCH_3, 18.96; 1 active hydrogen, 0.308%). λ_{max} 216 mμ (ε, 28,400) and 299 mμ (ε, 21,100); in N HCl 216 mμ (ε, 29,100) 289 mμ (ε, 23,500); ν_{max} (CCl_4) 3535 cm^{-1} (ε, 137) (intramolecularly bonded hydroxyl).

11. Petaline chloride (7.2g.) in ethanol (25 ml) was eluted through an Amberlite IRA-400 anionic exchange column (OH form). Work-up of the eluate as described above afforded leonticine m.p. 121-123° (4.8g; 65%).

Leonticine Methiodide.

Leonticine (3.1g.) in dry acetone (50 ml) was treated with methyl iodide (4 ml) and the solution heated under reflux for 2.5 hr. The crystals which were deposited were collected by filtration and dried affording 4.02g product. The methiodide was recrystallized to constant m.p. 169-171° (Found: C, 53.69; H,6.89; N,3.1 $C_{21}H_{28}O_3NI$ requires c,53.73; H,6.2g; N,2.98%)
λmax 217 mμ (ε,63,000) and 297 mμ (ε,24,200)

Hofmann Degradation of Leonticine Methiodide.

Leonticine methiodide (3.6 g.) in methanol (60 ml) was slowly eluted down an Amberlite IRA-400 anionic resin column (OH form) (60 g.). The dark brown eluate was taken to dryness yielding dark needles shown to be leonticine methohydroxide m.p. 150-155° (2.66g).

Methohydroxide (1.0g.) in 5% sodium ethoxide in ethanol (25 ml) was heated for 2.5 hours under nitrogen in a closed system, the effluent gas passing through saturated ethanolic picric acid. Water (10 ml) was

added to the reaction mixture which was then made acidic with acetic acid and then exhaustively extracted with benzene. Washing the benzene solution with water and drying over anhydrous sodium sulphate afforded orange crystals (0.63g.) Recrystallization from ether/ petroleum ether (40-60°) gave Hofmann degradation product (XXX) m.p. 111-113° $[\alpha]_D$ 0°, (Found: C, 76.66; H, 6.78; O-CH_3, 13.42, 11.98; molecular weight by mass spectrograph, 282. $C_{18}H_{18}O_3$ requires: C, 76.58; H, 6.42; 2-OCH_3 require 21.95% mol. wt. 282). λ_{max} 209 mμ (ε, 27,000) 269 mμ (ε, 23,900) and 305 mμ (ε 21,100) in N KOH, 254 mμ (ε 21,800), 272 mμ (ε, 21,700) and 365 mμ (ε, 9300).

ν_{max} (CCl_4 solution) 3545 cm^{-1} (ε 144) (intramolecularly bonded hydroxyl.

Treatment of the Hofmann degradation product (1.1g) with 10% BF_3 in methanol complex (35 ml) on a water-bath for 20 minutes and decantation of the reaction mixture into crushed ice precipitated crystals which were collected, dried, and recrystallized from benzene/ petroleum ether (40-60°) to constant melting point 168-170°, thus affording the nitrogen-free product

methyl ether (0.84g.) (Found: C,77.28; H,6.57. $C_{19}H_{20}O_3$ requires, C,77.00; H,6.80%).

The crystals present in the ethanolic picric acid solution employed as trap for the volatile nitrogenous fragment eliminated during the Hofmann degradation of leonticine methohydroxide were collected by filtration and recrystallized from ethanol and exhibited m.p. 209-211° undepressed on admixture with authentic trimethylamine picrate (Found: C,37.85; H,4.22; N, 19.26. Calculated for $C_9H_{12}N_4O_7$ C,37.51; H,4.20; N, 19.45%). As expected the infra-red spectrum of the trimethylamine picrate derived from the Hofmann degradation of leonticine methohydroxide was completely superposable with that of authentic trimethylamine picrate.

Ozonolysis of the Hofmann Degradation Product of Leonticine Methohydroxide

Employing the published procedure of Bauer, Birch and Ryan[61] nitrogen-free methine (0.3g.) in ethanol(25ml). containing conc. sulphuric acid (0.5 ml) at 0°C was rapidly ozonized for 20 minutes and the flask was then flushed with oxygen for 25 minutes. Excess 5% aqueous ferrous sulphate (25 ml) was added to decompose the ozonide and

then the reaction mixture was exhaustively extracted with ether (6 x 15 ml). After washing with water, the ethereal extractives were dried over anhydrous sodium sulphate and concentrated to dryness. The resultant brown oil (0.1g.) exhibited an infra-red spectrum superposable with that of authentic p-methoxybenzaldehyde whilst the m.p. on admixture of the two 2,4 - dinitrophenylhydrazones, from derived material and authentic p-methoxylbenzaldehyde, was undepressed, m.p. 260-261°. On standing the oil deposited crystals which were collected by filtration and washed with ice-cold ethanol giving m.p. 184°, undepressed on admixture with authentic p-methoxybenzoic acid. The infra-red spectrum of the crystals was superposable on that of p-methoxybenzoic acid.

Chromatography of the oil, over neutral alumina failed to afford successful isolation of a second fragment. Ethyl acetate extraction of the aqueous residue from the ether extraction also failed to afford further material.

Ozonolysis of Leonticine Methiodide

Leonticine methiodide (0.3g.) in ethanol (25 ml) at 0°C was rapidly ozonized for 20 mins., and the flask then flushed with oxygen for 25 mins. The ozonide was hydrogenolysed over Adam's catalyst. Removal of the catalyst by filtration and dilution of the reaction mixture with distilled water (25 ml) precipitated a brownish oil which was removed by extraction of the aqueous solution with ether. Work-up of the ethereal solution as above resulted in the isolation of further p-methoxybenzaldehyde again identified as above. The aqueous solution was taken to dryness on a rotary-film evaporator. The hydroscopic crystalline residue was treated with 5% sodium ethoxide in ethanol (20 ml) and the mixture heated under reflux for 1.5 hr. After dilution of the reaction mixture with water and acidification with glacial acetic acid, the reaction mixture was exhaustively extracted with ether. Washing and drying the ethereal solution followed by concentration to dryness afforded a yellowish oil (ca 50 mg.). The oil was hydrogenated over highly active Adam's catalyst in ethanol. The catalyst was

removed by filtration, the ethanolic solution concentrated under reduced pressure and the resultant oil twice distilled in a horizontal tube at 85°/0.04 mm Hg, affording a yellow oil (15 mg). ν_{max} (carbon tetrachloride solution) 3615 (ε, 22) (benzylic hydroxyl) and 3545 cm^{-1} (ε112) (intramolecularly hydrogen bonded hydroxyl).

Conversion of Isovanillin (XL) into 2-Allyl-isovanillin (XLVIII)

Isovanillin (120g.), allyl bromide (115g.) and powdered anhydrous potassium carbonate (90g.) in dry acetone (300 ml) placed in a 1l flask fitted with an efficient stirrer, were heated under reflux for 8 hours. Acetone (ca 200 ml) was taken off under reduced pressure and water (300 ml) was added. The reaction mixture was extracted with ether (6 x 100 ml) and the ethereal solution washed with 10% sodium hydroxide (2 x 50 ml) followed by water until the wash was neutral, then three times more. The ethereal solution was concentrated to dryness thus affording a yellow oil (147.5 g; 97.4%).

The infra-red spectrum indicated the absence of

hydroxyl absorption in the 3500 cm^{-1} range with the appearance of a new ether (allyl) band at 1030 cm^{-1}

The allyl ether (XLI) (147.5g.) was heated to 300°C (oilbath temperature 312°C) for 5 minutes. After cooling the rearrangement product was distilled under vacuum, the fraction b.p. 142-143°/ 1 mm Hg. being collected thus affording 2 - allyl-isovanillin (XLII) (108g; 73.3%) as a pale yellow oil. Chromatography of the product over neutral alumina with ether as eluant and distillation of the oil so obtained b.p. 142°/1mm afforded a low melting solid (Found, C,68.21; H,6.40; $C_{11}H_{12}O_3$ requires C,68.74; H,6.30%) ν_{max} (liquid film) 3400 (hydroxyl) 3080 (vinylidene) 1085 cm^{-1} (hydroxyl).

Elution of the column with ether: ethanol (10:1) afforded a small amount of a second aldehyde, presumed to be 6-allyl-isovanillin, which was not characterised.

Authentication of 2-Allyl-isovanillin (XLII)

2-Allyl-isovanillin (1g.) was hydrogenated over Adam's catalyst in ethanol (hydrogen uptake: 1 mole) Removal of the catalyst by filtration and concentration of the solvent afforded the dihydro derivative as

evidenced by absence of the vinylidene band at 3080 cm^{-1} and the ethylenic double bond peak at ca 1640 cm^{-1} in the infra-red spectrum. Decarbonylation of the dihydro compound by the palladium on charcoal procedure developed by Hawthorne and Wilt[51] (CO collected: 0.8 mole) afforded 2-methoxy-6-n-propylphenol (XLIV). Identity of this compound was confirmed by infra-red spectral comparison with dihydroeugenol (XLIV), prepared by reduction of synthesised o-eugenol having the correct physical constants[49], the spectra being superposable.

Conversion of 2-Allyl-isovanillin (XLII) into 2-allyl-3-acetyloxy-3-methoxybenzylacetate (XLIX)

2-Allyl-isovanillin (48g.) in dry ether was treated with excess of lithium aluminium hydride (22g.) and heated under reflux for 19 hours. Destruction of the excess of complex metal hydride by the slow addition of dilute HCl and exhaustive extraction of the acidic reaction mixture with ether gave a dark yellow ethereal solution. After washing with water and drying over anhydrous sodium sulphate, the ethereal solution was taken to dryness. The resulting dark brown 2 - allyl-isovanyllyl alcohol (XLVIII) (47.5g; 98%) was filtered

through neutral alumina. Concentration of the ethereal eluate afforded the product as a pale yellow oil. Attempts to purify the 2-allyl-isovanillyl alcohol by high vacuum distillation were unsuccessful due to apparent polymerization of the material. The chromatographed material had ν max (in carbon tetrachloride solution) 3590 (ϵ,36) (benzylic hydroxyl) 3555 (ϵ,203) (intramolecularly hydrogen bonded hydroxyl), 3090 (vinylidene) and 1640 cm^{-1} (vinylidene).

2-allyl-isovanillyl alcohol (XLVIII) (7.5g.) acetyl chloride (10 ml) and anhydrous potassium carbonate (8.5g.) in dry acetone (125 ml.) were heated under reflux for 17 hr. After cooling, water (200 ml.) was slowly added and the mixture exhaustively extracted with ether. The ethereal solution was washed with water, dried (Na_2SO_4) and concentrated to dryness. The oily yellow diacetate XLIX could not be induced to crystallize. Thus the product was chromatographed on neutral alumina with ether as eluant. The pale yellow oil obtained after concentration of the eluate was negative to Gibb's reagent[40,41].

ν max (liquid film) 3090 (vinylidene) 1765 (shoulder; acetate) 1760 (acetate) and 1635 cm^{-1} (vinylidene).

Conversion of 2-Allyl-3-acetyloxy-4-methoxybenzyl acetate XLIX into 2-Hydroxymethyl 3-Acetyloxybenzyl acetate (L) by Reductive Cleavage of the Intermediate Ozonide.

Allyl compound (XLIX) 2.6g.) in ethanol (80 ml) at 0°C was rapidly ozonized for 35 mins. and the reaction solution flushed with oxygen for 40 mins. Excess of sodium borohydride (2.6g.) was added portion-wise, in a procedure analogous to that developed by Sousa and Blum[52], and the reaction mixture kept at 0°C for 14 hrs. Excess of borohydride was decomposed by the careful addition of dilute acetic acid. Water (150 ml) was added to the slightly acidic solution and the resultant solution extracted with ether. After washing the ethereal solution with water followed by drying over anhydrous magnesium sulphate, the ethereal solution was concentrated to dryness. The phenylethyl alcohol (L) (2.3g) gave a negative Gibb's test[40] whilst the infra-red spectrum exhibited no maxima in the ranges 3100-3050 cm^{-1} and 1700-1630 cm^{-1}.

Methanesulphonate ester (LI) Formation of 2-β-hydroxyethyl-3-acetyloxy-4-methoxybenzyl acetate (LI) and Hydrogenolysis to the Proposed 2-Ethyl-3-hydroxy-4-methoxy-benzyl alcohol

2-β-hydroxyethyl-3-acetyloxy-4-methoxy-benzyl acetate (L) (1.63g.) in dry pyridine at -5°C was treated with methanesulphonyl chloride (0.66 ml) and the mixture kept at -5°C for 19 hr. The reaction mixture was then poured onto crushed ice. The semi-solid precipitate was extracted with ethyl acetate. Washing the ethyl acetate solution with dil HCl followed by water and drying (Na_2SO_4) afforded a clear brown solution. Concentration of the solution gave crystalline methanesulphonate derivative (LI) (1.5g.) m.p. 103-106°, νmax (KCl disc) 1765 and 1740 (acetate) 1415 (methanesulphonate, 1230 (acetate), 1245 (acetate) and 1170 cm^{-1} (methanesulphonate).

The methanesulphonate derivative (LI) was not purified as such but was dissolved in tetrahydrofuran (40 ml), excess of lithium aluminium hydride (1.3g) added portion-wise, and the mixture heated under reflux for 42 hr. Excess of lithium aluminium hydride was destroyed by the careful addition of dil HCl and

the resultant acidic solution was extracted with ether (7 x 25ml). After washing with water the ethereal solution was dried and concentrated to dryness affording a brown resinous mass (1.1g). The resinous material was extracted with ether (10 x 1 ml), the ethereal solution concentrated and the brown-oily residue distilled at 102°/0.05 mm Hg thus affording a yellowish oil, in very small yield, not showing identity of infra red spectra with the product (XXIX) obtained from petaline.

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