A THESIS

ENTITLED

'STUDIES IN THE DITERPENOID FIELD'

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SUMMARY

The constitution and stereochemistry of ε-caesalpin, a furanoid diterpenoid from Caesalpinia bonducella, has been derived from chemical and spectroscopic evidence. The proposed structure was verified by x-ray analysis of a p- bromobenzoate derivative, which in addition provided the absolute configuration. An attempt to determine the absolute configuration of α-, β-, and δ- caesalpins by direct correlation with ε- caesalpin was unsuccessful. This research revealed several inconsistencies in the results of previous workers on the stereochemistry of these compounds, and evidence is presented which defines unambiguously the stereochemistry of the ring B substituents of α- (and hence β- and δ-) caesalpin. The structures of six of the minor constituents of Caesalpinia bonducella are discussed.

Extraction of the whole plant Andrographis paniculata afforded, in addition the known compounds andrographolide and neoandrographolide, three diterpenoid lactones which had not been previously isolated. The structures of these have been deduced from chemical and spectral data. The mass spectra of some andrographolide derivatives are discussed.

To Angie

ACKNOWLEDGEMENTS

I would like to record my sincere gratitude to Dr. J.D. Connolly for his advice, encouragement and friendship over the past three years. I am also indebted to Mr. J.M.L. Cameron, B.Sc., and his staff for micro-analyses, Mrs. S.P. Hamilton and Mr. J. Gall for recording n.m.r. spectra, Mrs. F. Lawrie for solution infra-red spectra, and Dr. J. Roberts for mass spectra. The patience of Mrs. G. Finlay, who typed the manuscript, is gratefully acknowledged.

CONTENTS

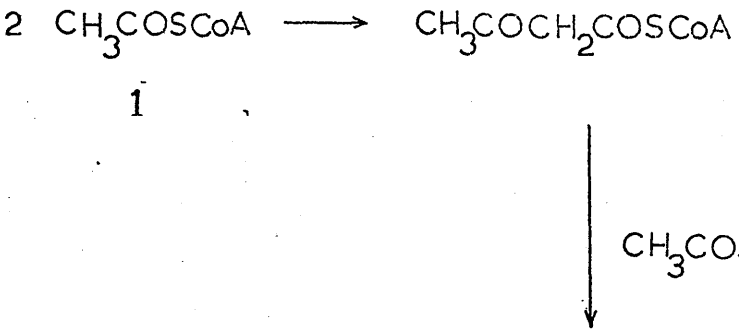
INTRODUCTION

The study of natural products in general, and terpenoids in particular, has long been a subject of fascination for organic chemists. The wealth of information derived from studying the diverse structural types and biosynthetic pathways has played a significant role in the development of general chemical concepts. In the synthetic field, natural products have provided a stimulus and challenge which has led to the undertaking of synthetic routes to molecules of awesome proportions and stereochemical complexity.[1]

Structural elucidation, with its inherent tendency to diverge, has provided many interesting inroads into the chemistry of alicyclic compounds in general. Despite the vituperative assertions of Cookson,[2] this type of research will continue to be pursued with undiminished vigour, owing to Nature's unique ability to fabricate new structures of the most bizarre and exotic quality. If one also considers the physical organic chemists' exploitation of the terpenoid framework as a tool in the study of both carbonium ion[3] and photochemical[4] reactions, one may appreciate the general influence which these structures have had on the evolution of modern organic chemical theory.

Although the biogenetic origin of the terpenoids has been subjected to intense scrutiny since the last century,[5] it was not until 1921 that Ruzicka crystallised contemporary ideas in the publication of the Isoprene Rule. This recognised a fundamental underlying unity in the mode of

Scheme 1



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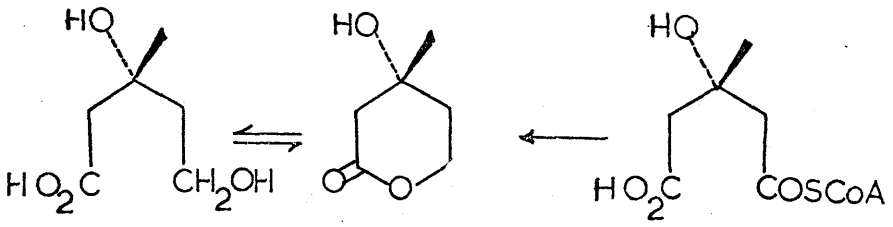

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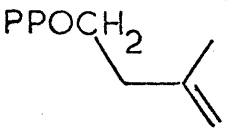

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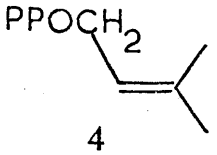

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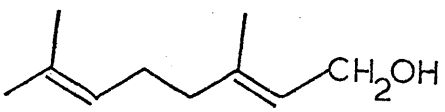

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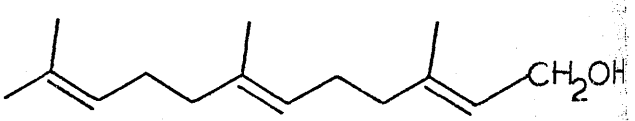

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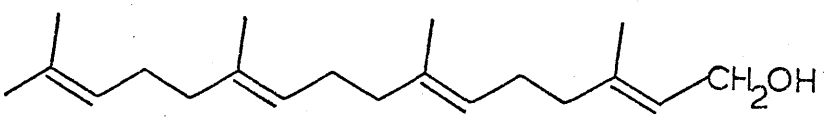

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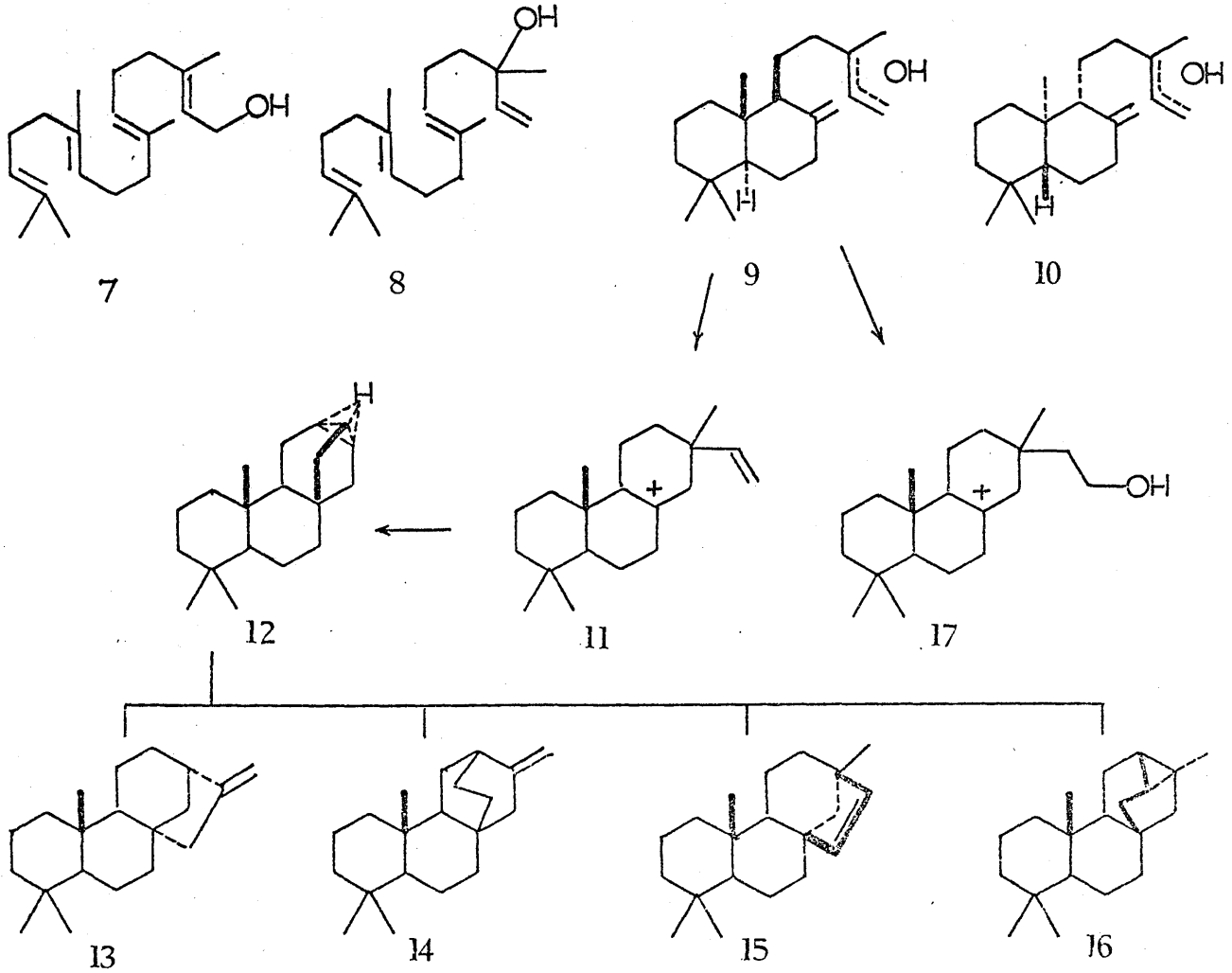
formation of even the most complex terpene structures, since each could be composed of isoprene units joined in a 'head to tail' fashion. However, the following years of extensive research unearthed several non-conformist carbon skeletons, and eventually culminated in the formulation of the celebrated Biogenetic Isoprene Rule.[6] This rule rationalised the biosynthesis of the most aberrant terpenoids as proceeding via mechanistically feasible rearrangements of the 'regular' polyisoprenoids.

A comprehensive review of terpenoid biosynthesis would be beyond the scope of this thesis, besides which this has already been summarised on several occasions.[7] It should suffice for our purposes to discuss briefly the accepted biogenesis of the diterpenoids, particularly with reference to the labdanes and cassanes.

The recognition of acetic acid, in the form of acetyl coenzyme A, as the fundamental biogenetic progenitor of all terpenoids is now well established. By a sequence of Claisen-like condensations, acetyl coenzyme A (1) gives rise to mevalonic acid (2)[8] - the immediate precursor of the isoprene unit (scheme (1)). Subsequent condensations of the "active isoprenes"[9] isopentenyl pyrophosphate (3) and dimethylallyl pyrophosphate (4) form the corresponding pyrophosphates of geraniol (5), farnesol (6) and geranyl geraniol (7).

The cyclisation of geranyl geraniol to the diterpenoids probably conforms with the stereochemical postulates of Eschenmoser et al.[10] These were originally applied to triterpenoids, but in principle can be



7 8 9 10

12 11 17

13 14 15 16

Scheme 2

extrapolated to the other polyisoprenoids. The main conclusions derivable from Eschenmoser's work are

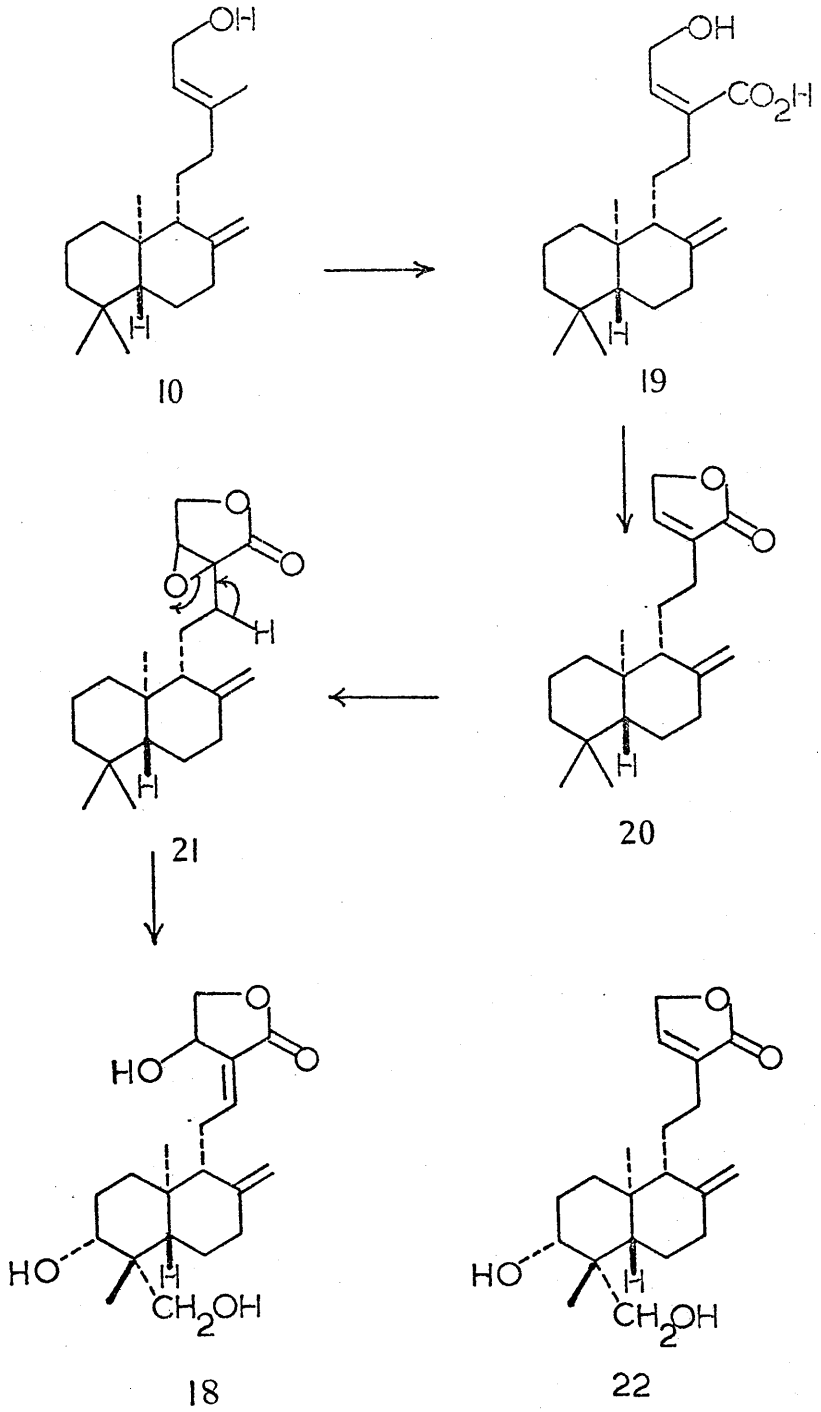
(i) The acyclic precursor is folded at the enzyme surface into a specific conformation.

(ii) Concerted cyclisation occurs by trans-planar additions to the double bonds.

(iii) All subsequent rearrangements and/or eliminations proceed in accordance with optimal stereoelectronic requirements, i.e. the affected groups are trans - antiparallel.

Cyclisation of geranyl geraniol or the isomer geranyl linalool can concur with the above demands to form the antipodal bicyclic alcohols (9) or (10). The accepted transformation of (9) into the tri- and tetracyclic diterpenes is indicated in scheme (2). It is noteworthy that the face-protonated "nortricyclonium" ion intermediate (12), originally proposed by Wenkert,[11] is now regarded with suspicion by some workers,[12] in view of the evidence in favour of an edge-protonated intermediate in norbornyl rearrangements.[13]

The great majority of naturally occurring labdanes are based on the bicyclic alcohol (9) with a trans-anti backbone and the absolute stereochemistry as written. There is, however, a small group of compounds of the enantiomeric series, that is, with the 10α, 5β configuration rather than 10β, 5α . (table (1)). Prominent in this group are the



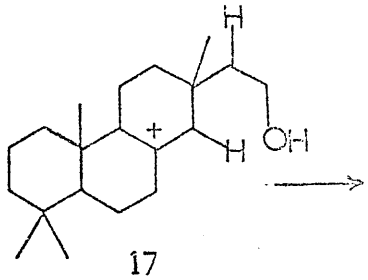
Scheme 3

diterpenoid lactone andrographolide (18) and its congeners, the chemistry of which is discussed in section 2 of this thesis.

The biogenesis of andrographolide can be considered to proceed from the 10α, 5β bicyclic alcohol (10). Although the origin of the lactone ring in terpenes has never been completely elucidated by tracer studies, it is generally assumed that its mode of formation is similar to that of the furan, for which biogenetic speculations have been advanced by Fetizon[14] and Wenkert.[15] Autoxidation of furans is known[14,16] to give unsaturated γ -lactones, and such a process is conceivably operative in the biosynthesis of andrographolide. A plausible alternative mechanism involves enzymic oxidation of the side chain of the bicyclic alcohol (10) followed by lactonisation, eventually leading to andrographolide via an intermediate epoxide (21).

Support for this mechanism is derived from the fact that deoxyandrographolide (22) has now been isolated from the same plant source (see section 2).

The cassane group of diterpenoids is in principle derived by rearrangement of the cation (17) (scheme (2)). The most extensively studied member of this group is cassaic acid (24), readily obtained by mild acid hydrolysis of the alkaloid cassaine (25).[17] In a recent review of the Erythrophleum alkaloids,[18] the structural and stereochemical elucidation of cassiac acid is presented, together with its correlation



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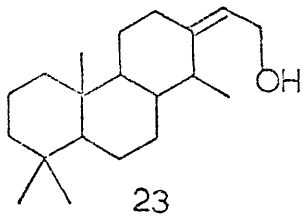

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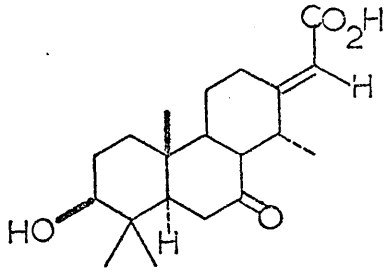

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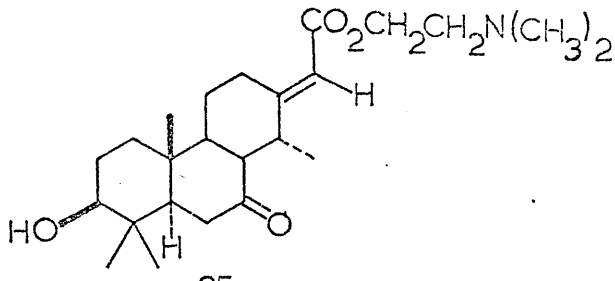

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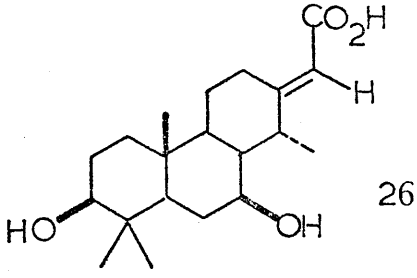
with the other members of this series : cassaidic acid (26), cassamic acid (27), erythrophlamic acid (28), and 6α- hydroxy - cassamic acid (29).



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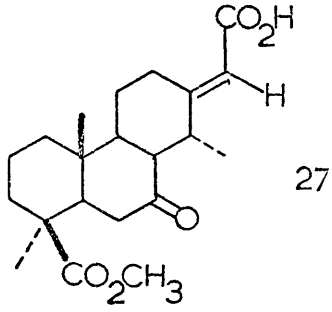

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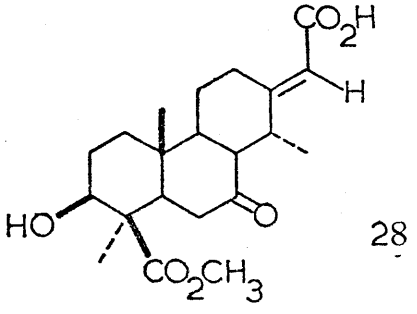

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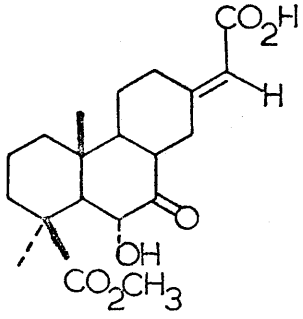

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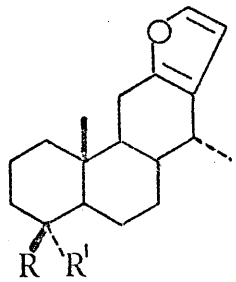
The structure of the first member of the furanoid cassanes, vinhaticoic acid (30), appeared

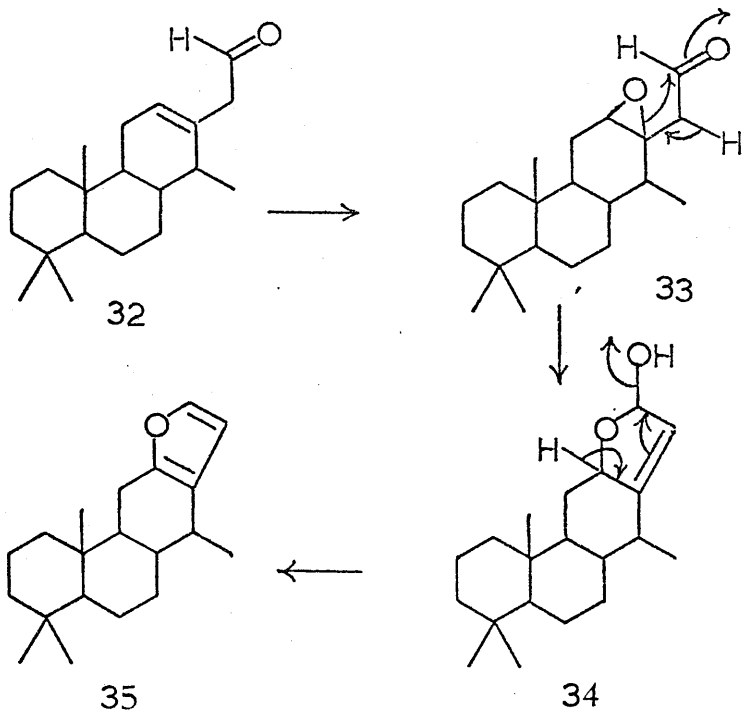
in 1953,[19] and was closely followed by the $C_{(4)}$ isomeric vouacapenic acid (31).[20] The generation of the furan ring of these and related



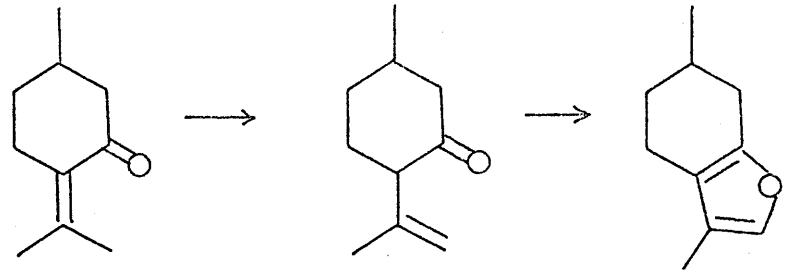
30, $R=CH_3$, $R'=CO_2H$

31, $R=CO_2H$, $R'=CH_3$

compounds has been postulated[14,15] to proceed by dehydration of the intermediate epoxide (33), formed by oxidation of the $\beta\gamma$-unsaturated aldehyde (32).



Support for this mechanism is obtained from the <u>in vitro</u> studies by Fetizon[14] on the formation of menthofuran (36) from pulegone (37).



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The seeds of Caesalpinia bonducella have yielded several furanoid diterpenes, the structures of which have been investigated by Canonica et al.[22] (page 14). The chemistry of these, and related compounds isolated in this laboratory, is discussed in Section 1.

TABLE 1.

COMPOUND	REFERENCE
EPERUANEDIOL	23
EPERUENEDIOL	24
EPERUANETRIOL	23
13-EPI-ENANTIO-MANOYL OXIDE	25
19-OH-13-EPI-ENANTIO-MANOYL OXIDE	24
EPERUIC ACID	26
15-OH-EPERUENOIC ACID	27
DIHYDROXYEPERUENOIC ACID	23
COPALIC ACID	28
ZANZIBARIC ACID	29
19-CARBOXY-13-EPI-ENANTIO-MANOYL OXIDE	24
EPERUENEDIOIC ACID	27
OZIC ACID	30
DANIELLIC ACID	31
POLYALTHIC ACID	32
LACTONE	27
ANDROGRAPHOLIDE	33
DEOXYANDROGRAPHOLIDE	34
11-KETO-DEOXYANDROGRAPHOLIDE	34
ANHYDROANDROGRAPHOLIDE	34

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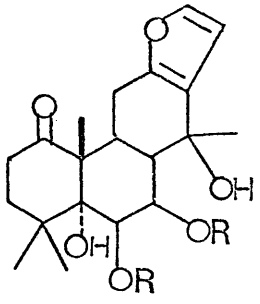
34. See Section 2.

SECTION 1

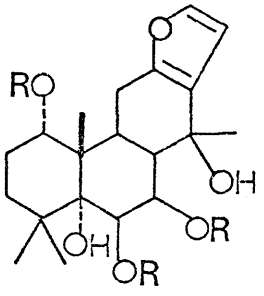
DISCUSSION

THE CONSTITUTION AND STEREOCHEMISTRY OF ε-CAESALPIN.

The seeds of Caesalpinia bonducella[1] have been subjected to intense chemical investigation by several research groups[2] since the first successful extraction of the bitter constituents was carried out by Ali and Khuda in 1960.[2a] Canonica et al in 1966 published a long and impressively detailed series of papers reporting the isolation and structural eludication of α-, β -, γ -, and δ -caesalpins. (1 - 4)[2e]



1, R = Ac
2, R = H

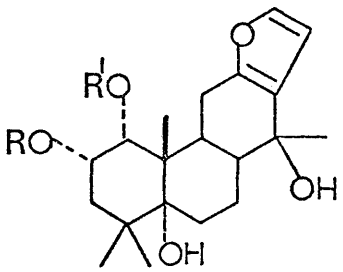


3, 2R = Ac, R = myristate
4, R = H

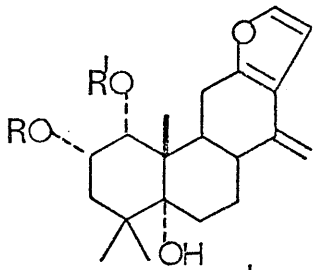
During the course of the present investigation a new furanoid diterpene, ε-caesalpin, was isolated from the same source and is formulated as 5 (or enantiomer) on chemical and spectroscopic evidence.

Analytical and mass spectral data indicated the molecular formula $C_{24}H_{34}O_7$ for ε-caesalpin, m.p. 191-194°, $(\alpha)_D$ + 2°. The n.m.r. spectrum shows signals attributable to a 2, 3 disubstituted furan ring (τ 2.77 and 3.61 ; both doublets, J = 2Hz), two secondary acetates (τ 8.10, 7.94 ($2\underline{CH_3}COO$-) ; 4.76, doublet and 4.7, multiplet (2-C$\underline{H}$OAc))

and four tertiary C-methyl groups (τ 8.73, 8.83, 8.85 and 8.94). The i.r. spectrum has acetate and hydroxyl absorption ($\nu_{max}^{CCl_4}$ 1758, 1745, 3596 cm^{-1}). There does not appear to be any >CH OH resonance in the n.m.r. spectrum of ε-caesalpin, but two sharp -C-OH signals at τ 7.07 and 8.36 disappear on exchange with deuterium oxide. The above evidence suggests that ε-caesalpin has two tertiary hydroxyl groups in addition to two secondary acetates and a furan ring and is therefore tricarbocyclic. The presence of a 2, 3 disubstituted furan and four tertiary C-methyl groups infers a normal or rearranged vouacapane[3] skeleton with a tertiary hydroxyl at $C_{(14)}$.



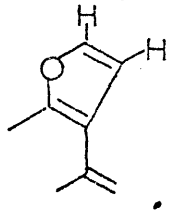
5 , R=R' = Ac
6 , R=R' = H
9 , R=Ac, R' = H



7, R = R' = H
8, R = Ac, R' = H

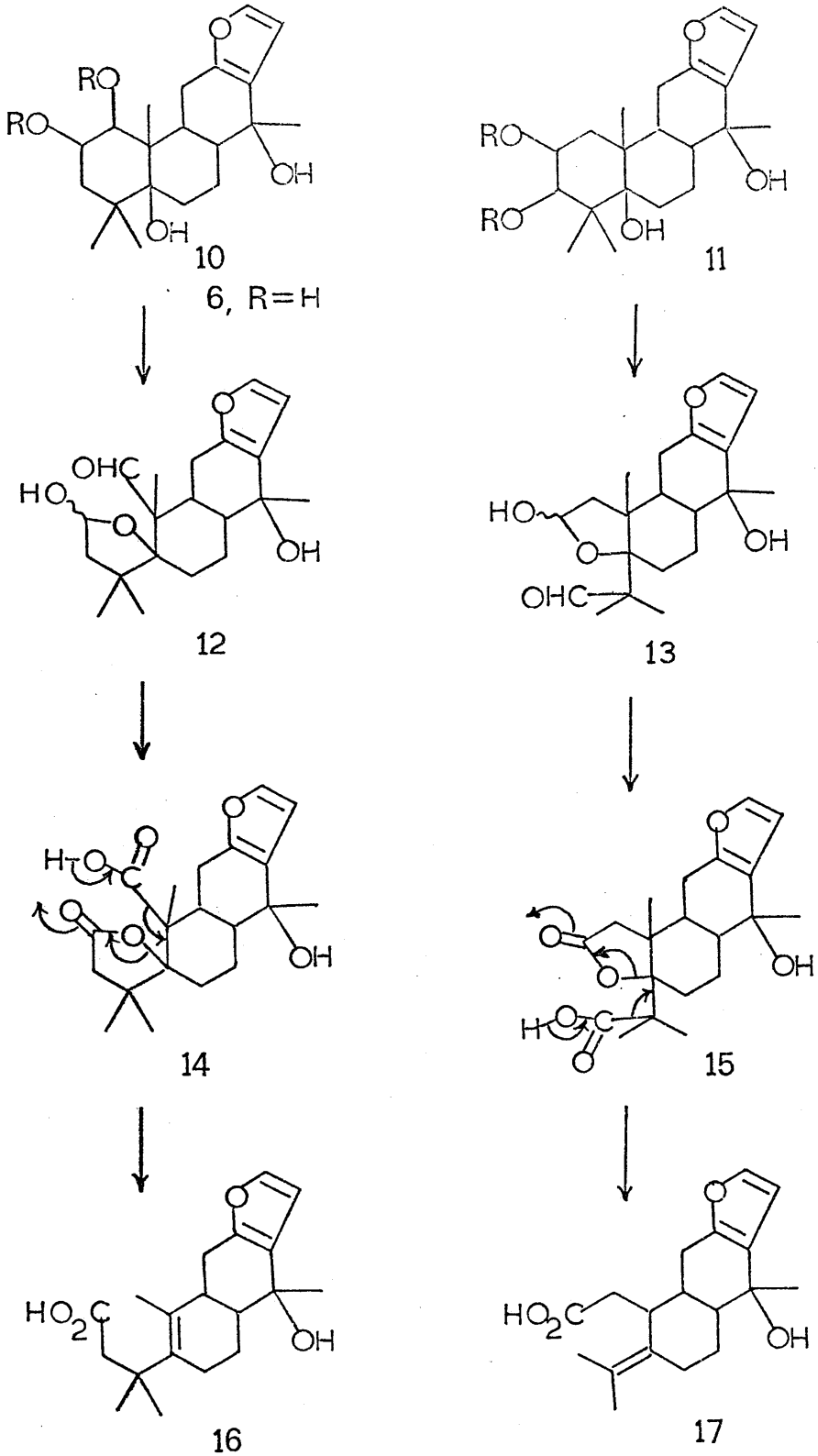
Treatment of ε-caesalpin with lithium aluminium hydride in ether yielded the crystalline tetraol 6, m.p. 194-196° and the corresponding anhydro - derivative 7, m.p. 183-185°, also characterised as the monoacetate 8, m.p. 203-205°, (τ 4.89, 5.09, diffuse singlets, $\underline{CH_2}=C$, disappearance of one C-methyl signal). The ultraviolet spectrum

of 8 in ethanol solution had bands at 232 nm. (ε 8, 600) and 213 nm (ε 9,500), which have been shown[2e,4] to be characteristic of a furan ring conjugated with another double bond. This evidence, coupled with the infra red absorption of 8 at 902 cm.$^{-1}$, establishes the part structure a for this compound and confirms the placing of one tertiary hydroxyl of ε-caesalpin at $C_{(14)}$.



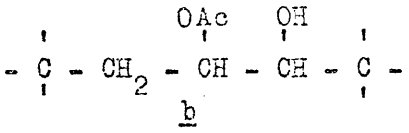
a

Under normal acetylation conditions the tetraol 6 was transformed into the monoacetate 9 m.p. 195-197°, $\nu_{max}^{CHCl_3}$ 3590, 3478 cm^{-1} (hydroxyl), 1737 cm^{-1} (acetate). The vicinal nature of the acetate and hydroxyl groups was demonstrated by double irradiation experiments on the n.m.r. spectrum of 9. The >CH OAc proton resonates as a double quartet (J = 12, 5, 2 Hz) at τ 4.74 and collapses to a clean quartet (J = 12, 5 Hz) on irradiation of the >CH OH resonance (doublet, J = 2Hz) at τ 6.30. In the reverse experiment, irradiation at τ 4.74 causes the >CH OH signal to collapse to a sharp singlet. The multiplicity of the >CH OAc proton requires the presence of an adjacent methylene group. Double irradiation studies show that the axial methylene proton appears as a partially obscured triplet (J = 13, 12 Hz) at τ 7.98 and the equatorial proton as a clean quartet (J = 13, 5 Hz) at τ 8.63. Since these protons are not further coupled, the above evidence leads to part structure b, a


RO
RO
OH
OH
10
6, R=H
RO
RO
OH
OH
11
OHC
HO
OH
12
HO
OH
OHC
13
HO
O
OH
14
OH
H
15
HO2C
OH
16
HO2C
OH
17

Scheme 1

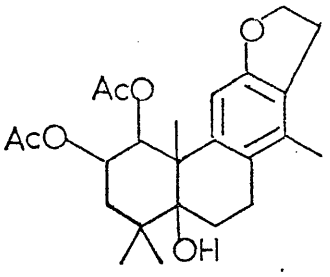
sequence which can be accommodated in two possible ways only in ring A of a vouacapane skeleton.



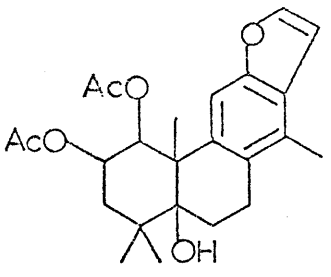
In an attempt to distinguish between the two possibilities 10 and 11 (scheme (1)) and in order to demonstrate chemically the vicinal relationship of the two secondary oxygen functions, the tetraol 6 was cleaved with sodium meta-periodate in aqueous methanol, affording a mixture of the epimeric hemiacetal aldehydes 12 or 13 ($\nu_{max}^{CHCl_3}$ 3604, 3468 cm.$^{-1}$ (hydroxyl), 1711 cm.$^{-1}$ (aldehyde) ; τ = 4.50 (multiplet, hemiacetal proton), -0.1 (singlet, -CH = O)). All attempts to oxidise this mixture of epimers to the corresponding γ - lactone-acid 14 or 15 were thwarted by the extremely facile dehydration and subsequent rearrangement in ring C of these compounds (vide infra). It had been anticipated that the expected decarboxylation of this acid to the olefin 16 or 17 would allow differentiation between the two possibilities for ε-caesalpin.

It was discovered that ε-caesalpin, on standing in chloroform for several days, was transformed into two products, which can be assigned structures 18 and 19. The dihydrobenzofuran 18, $C_{24}H_{32}O_6$, m.p. 210-211° still retained an intramolecularly bonded tertiary hydroxyl group ($\nu_{max}^{CCl_4}$ 3591 cm^{-1}). In the n.m.r. it had signals at τ 3.61 (singlet

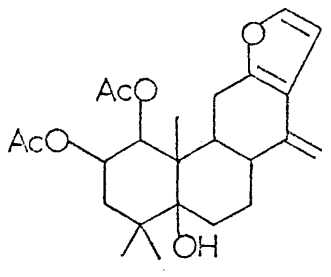
aromatic proton), 7.93 (singlet, Ar-$\underline{CH}_3$), 5.49 and 6.87 (mutually coupled



18

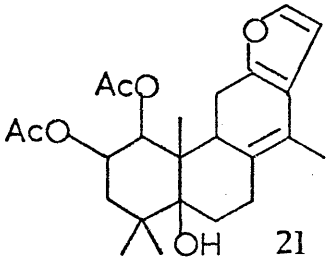


19



20

triplets, J = 9 Hz, dihydrofuran methylenes). It is envisaged that 18 arises from the initially formed dehydration product, the exomethylene diacetate 20, by acid catalysed rearrangement. This was supported by the treatment of ε-caesalpin with a strongly acidic reagent (viz., HCl in chloroform) which resulted in its conversion to the dihydrobenzofuran in quantitative yield. Under less acidic conditions the main product of the reaction was the benzofuran 19, m.p. 191-192° (λ max 251 nm. (ε 7500), 282 nm. (ε 2700), 292 nm. (ε 2800)),[5] which presumably arises by dehydrogenation of an intermediate such as 21 or 22



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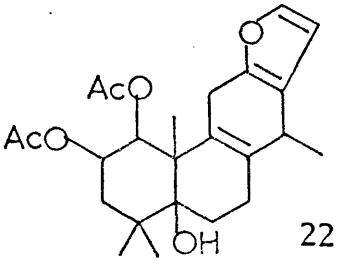


22

A perusal of the literature indicated several mechanistic possibilities for this dehydrogenation. These are:

(a) A reaction involving molecular oxygen and proceeding via a radical mechanism as described by Bromberg et al[6] for

dihydrophenanthrene (PH_2). It was found that the reaction was

$$PH_2 + O_2 \longrightarrow PH\cdot + HO_2\cdot$$

$$PH\cdot + O_2 \longrightarrow P + HO_2\cdot$$

first order with respect to both PH_2 and O_2.

(b) A reaction involving thermal elimination of hydrogen from the 1, 3 diene system in ring C of 21 to give the benzofuran.

(c) Thermal elimination of hydrogen from the 1, 4 diene system of 22.

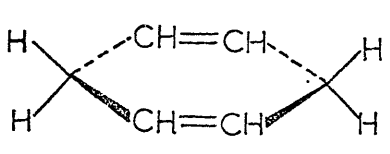
A decision between these possibilities in favour of the latter mechanism was made in the following manner. If molecular oxygen were involved, then any change in the oxygen concentration should be reflected in the product distribution between 18 and 19. However it was found that carrying out the acid treatment reaction both in the presence and in the absence of oxygen did not alter the relative yields of the two products. Thus mechanism (a) can not be operative.

Path (b) could be eliminated by consideration of the pertinent literature. It has been shown by the group theoretical approach of Longuet-Higgins and Abrahamson[7] that concerted elimination of general type A is only allowable when k is an odd integer. When k is even or zero,

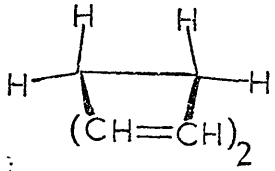
$$R - CH_2 - (CH = CH)_k - CH_2 - R \longrightarrow CH_2 = CH_2 - (CH = CH)_{k-1} - CH = CH_2 + R_2$$

A

concerted elimination is predicted to occur by way of a trans- mechanism which may be extremely difficult if k is small. The validity of these theoretical considerations has been conveniently demonstrated by the pyrolysis of 1, 4 - cyclohexadiene and 1, 3- cyclohexadiene. The former compound (k=1 system) undergoes a smooth unimolecular conversion to benzene and hydrogen in a process of low activation energy. The 1, 3 diene (k = 2 system) only forms these products at high temperatures (ca. 500-600°) in a reaction in which radical intermediates are implicated.[9] This evidence leads to the conclusion that the benzofuran 19 is formed by



k = 1

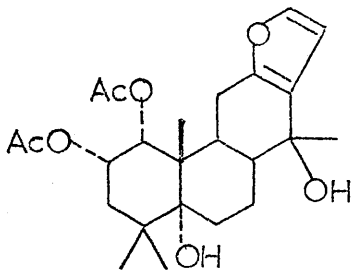


k = 2

a thermally induced dehydrogenation of the dihydrobenzofuran intermediate 22.

In the n.m.r. spectra of the aromatic compounds 18 and 19, one of the >CHOAc protons resonates as a doublet (J = 2Hz) at τ 4.13. This value represents a deshielding of 0.64 τ (relative to ε -caesalpin) owing to the introduction of the benzene ring. An inspection of models suggests that only the $C_{(1)}$ - β proton lies in the plane of the aromatic ring and would experience this effect. This provides a convenient way of deciding in favour of the 1, 2 - oxygenated ring A for ε -caesalpin 5,

rather than the otherwise possible 2, 3 system 23.



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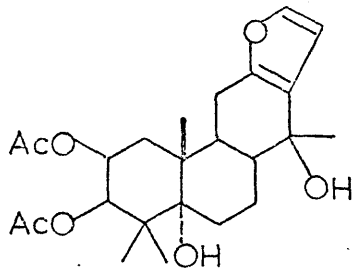
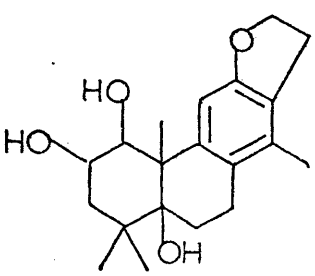


23

Cleavage of the triol 24, m.p. 263-265°, with sodium meta-periodate yielded the hemiacetal-aldehyde 25, m.p. 197-199° (τ = -0.03, singlet, (-CHO), 4.49 triplet (hemiacetal proton)), which was oxidised with Jones reagent to the corresponding γ - lactone 26, m.p. 289-292° ($\nu_{max}^{CCl_4}$ 1778 cm.$^{-1}$). This finally confirms the attachment of the remaining tertiary hydroxyl to $C_{(5)}$, and the evidence taken *in toto* with the assumption of a *trans* AB ring junction leads to structure 5 (or enantiomer) for ε -caesalpin.



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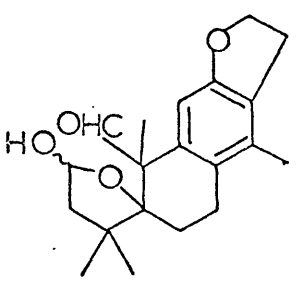

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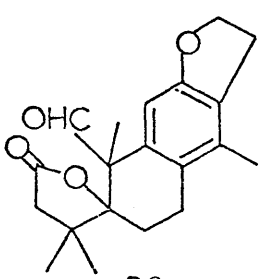
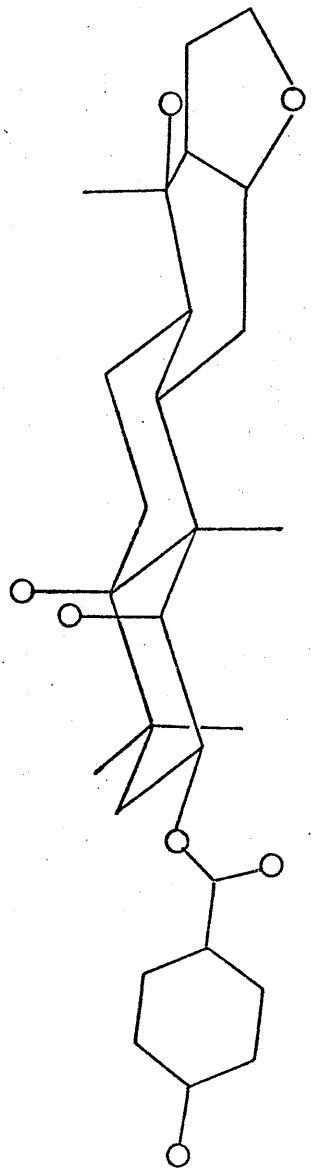
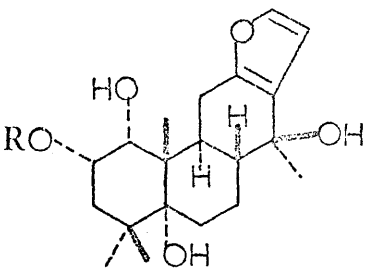


26

FIG 1



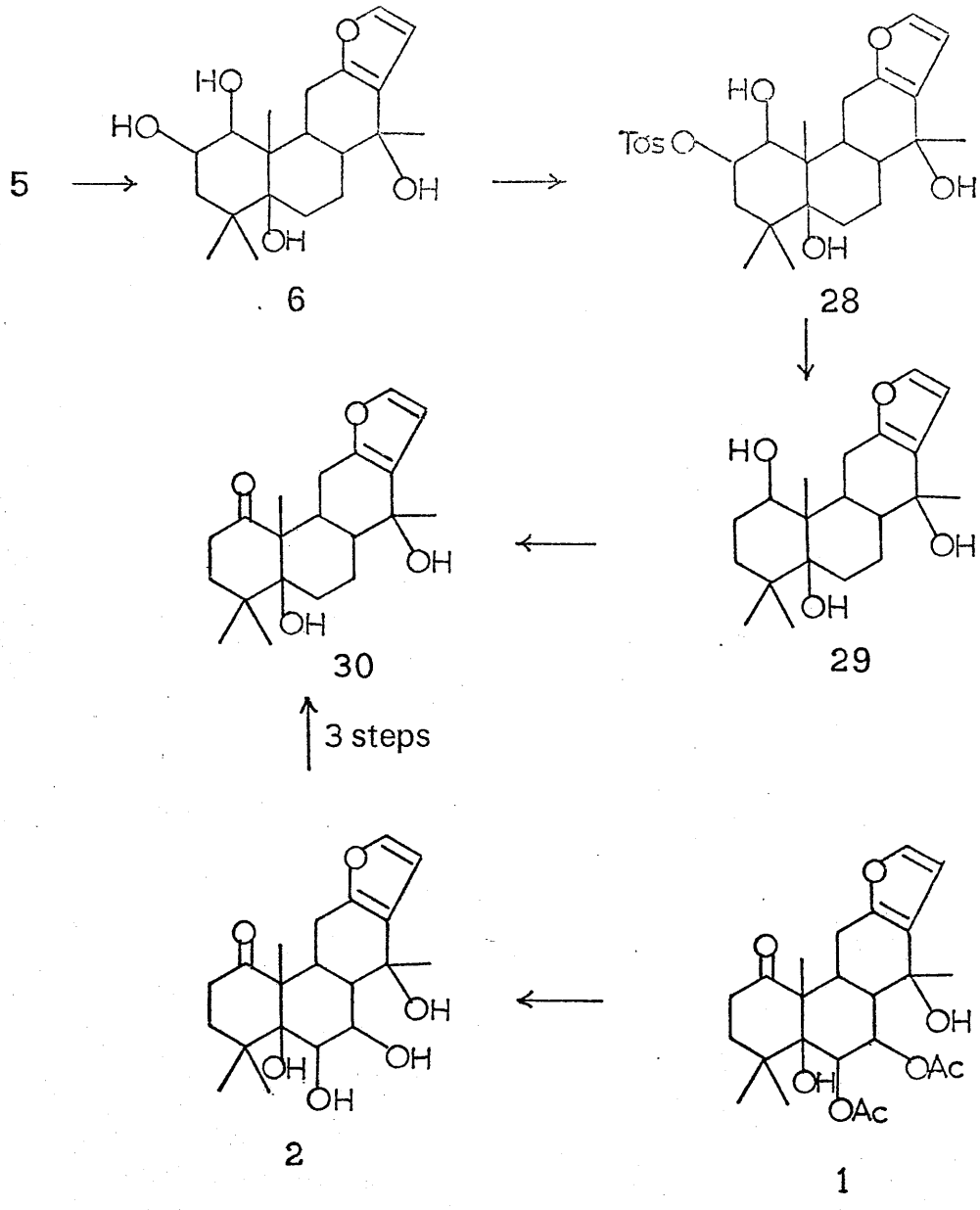
X-RAY ANALYSIS OF A p - BROMOBENZOATE OF ε - CAESALPIN.



27, R = p-Br-C_6H_4CO-

The p - bromobenzoate derivative 27 derived from ε - caesalpin crystallises in the monoclinic space group $P2_1$, as determined uniquely from systematic absences. The unit cell contains two moleculss of $C_{27}H_{33}O_6Br$ and has dimensions a=6.563, b=12.999, c=14.809 A° ; β = 94.50°. From equi-inclinatination Weissenberg photographs[12] taken along the a and b crystallographic axes with Cu Kα radiation some 3000 reflections were obtained. The structure was solved by the heavy atom method[13] and refined by block-diagonal least squares methods to an R - factor of 12.3%. Anomalous dispersion calculations allowed the absolute configuration shown in 27 to be determined from observed differences in intensities of 17 Bijvoet pairs[14] of reflections in an (h k l) precession photograph taken with Mo Kα radiation.

Figure (1) gives a view of the molecule down the b-axis andshows the molecular geometry. Rings A, B and C are fused in a trans-anti-trans manner with A and B in chair and C in half-chair conformations. The hydrogen of the $C_{(5)}$ axial hydroxyl is involved in an intramolecular bond (2.65 A°) with the axial hydroxyl group attached to $C_{(1)}$.


5
HO
HO
OH
OH
6
Tos O
HO
OH
OH
28
HO
OH
OH
29
OH
OH
30
3 steps
OH
OH
OH
2
OH
OAc
OH
OAc
1

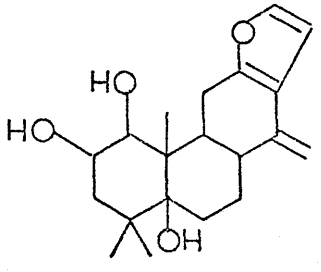
Scheme 2

THE STEREOCHEMISTRY OF α -, β -, AND δ - CAESALPINS AND THE ATTEMPTED CORRELATION WITH ε - CAESALPIN.

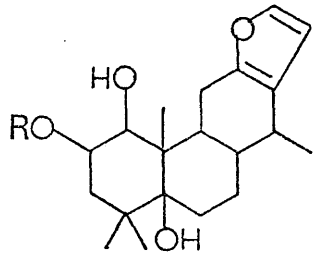
With the publication[11] of a totally unambiguous structure determination for ε-caesalpin, including the relative and absolute configuration(s) as derived from an x-ray analysis, several possibilities presented themselves for elucidation of the remaining stereochemical ambiguities in the other caesalpins.[2e] A viable prospect appeared to be the direct structural correlation of α - and ε - caesalpin, which, if successful, would fulfil the dual role of solving the absolute stereochemistry of α-caesalpin as well as providing an interesting academic exercise.

The method chosen for the inter-relation was the apparently innocuous one of converting both compounds to the intermediate 30 as shown in scheme (2). This project was undertaken in conjunction with Prof. L. Canonica at Milan, the intention being that each research group should approach the common intermediate 30 from its appropriate naturally occurring precursor 1 or 5. Work in both directions rapidly came to grief, however, with the production of intractable mixtures at stages 28 ⟶ 29 and 2 ⟶ 30, owing to the facile dehydration and rearrangement previously discussed. It was obvious at this point that some method had to be found which would circumvent this problem by eliminating any possibility of rearrangement in ring C. Accordingly, the tetraol 6 was dehydrated under mild conditions by stirring, in

acetone solution, with anhydrous copper sulphate. The resulting exomethylene compound 7, m.p. 183-185° (λ_{max} 232 nm. (ε 9000), 211-213 nm. (ε 9200)) was hydrogenated in ethanol solution with a trace of triethylamine to the $C_{(14)}$ epimeric mixture of triols 31. The constituent epimers here are present in the ratio 50 : 50 as determined



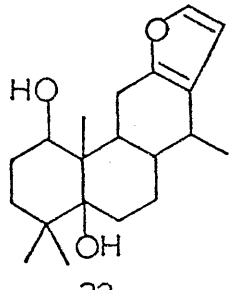
7



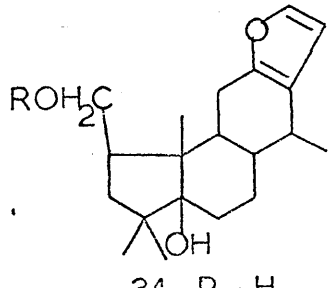
31 R = H
32 R = Tos

from the $C_{(15)}$-H n.m.r. signal. This ratio is as expected from examination of a molecular model of 7, which does not suggest preferential hydrogenation from any particular face.

Formation of the tosylate 32 was accomplished in the usual way without any difficulty, but the attempted hydrogenolysis to 33 encountered some problems. It had been anticipated that during the hydrogenolysis, which is known to require fairly strong conditions,[15] a certain amount of tosylate elimination might take place to the ring-contracted product 34.



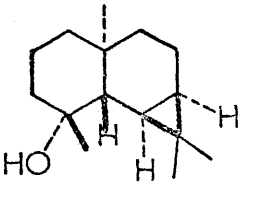
33



34, R = H
35, R = Ac

The molecular geometry of 32 places the $C_{(1)} - C_{(10)}$ bond exactly trans - antiparallel to the $C_{(2)} - O$ bond of the α - oriented tosylate. The system is therefore stereochemically set up for a pinacol type rearrangement[16,17], and the products of the hydrogenolysis would depend on the relative activation energies of the two competing reactions. In the event, the only products isolated from treatment of 32 with lithium aluminium hydride in ether were the triol 31, resulting from S - O cleavage[18] rather than the more commonly encountered C - O cleavage, and the A - nor primary alcohol 34, m.p. 158-160°. The structure of 34 was readily diagnosed from the n.m.r. spectrum, which had an AB quartet centred at τ 6.37 (J_{AB} = 12Hz) showing a small additional coupling with a multiplet at τ 8.10. These signals were attributed to the hydroxymethylene group coupling with the $C_{(2)}$ methine proton, an analysis which was confirmed by the downfield shift (Ca. 0.65 τ) of the quartet on formation of the primary acetate 35.

In retrospect, a parallel was found for these results in the work of Buchi et al,[19] who attempted the synthesis of the sesquiterpene alcohol epi-maaliol 36 by metal hydride reduction of the glycol monotosylate 37.



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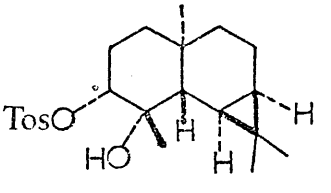
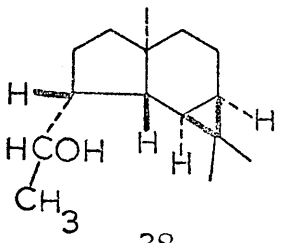


37

Their efforts were similarly rewarded with the production of one rearranged compound 38, and one resulting from S - O cleavage 39.



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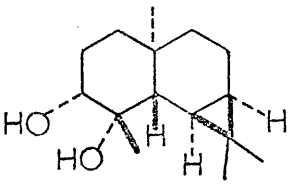
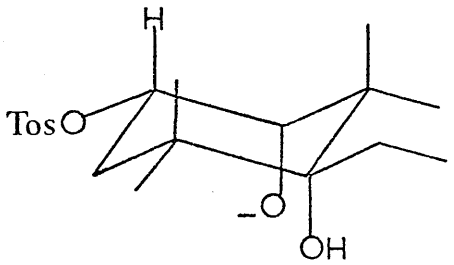


39

Clearly, then, an alternatively means had to be found to remove the offending $C_{(2)}$-hydroxyl. The first movement in this direction was an effort to prevent development of the oxy-anion 40 during the tosylate hydrogenolysis by protecting the $C_{(1)}$ hydroxyl as the tetrahydropyranyl ether.[23] The choice of this derivative was based on its known stability towards metal hydride reduction,[24] together with the fact that the alcohol can be regenerated by mild acid treatment.[23(a)] This approach was abandoned, however, owing to the sterically hindered nature of the $C_{(1)}$ axial hydroxyl, which precluded formation of the desired tetrahydropyranyl ether 41.



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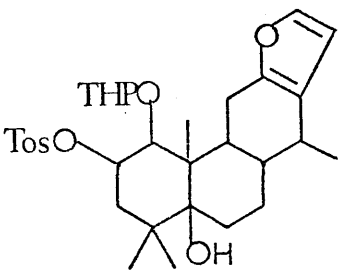
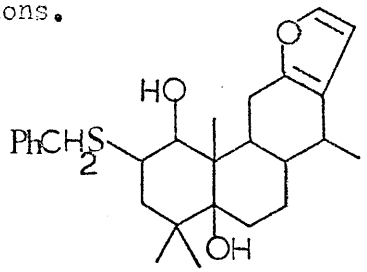


41

This defeat necessitated a thorough literature search to find other previously successful means for specific removal of oxygen functions.



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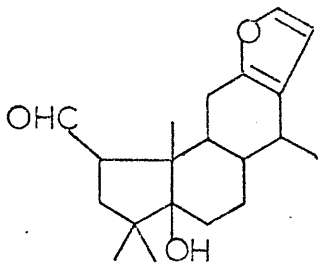
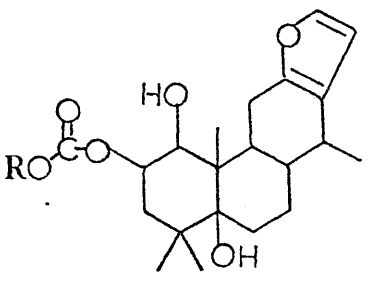


43

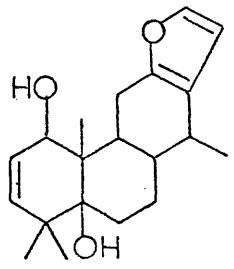
The first of these tried was the transformation of the tosylate into the benzyl thioether 42, the principle being that the powerfully nucleophilic benzyl mercaptide might react, albeit in minor account, by direct substitution of the tosylate group. Raney nickel desulphurisation of the derived thioether 42 would then furnish the desired desoxy caesalpin. Although it was anticipated that ring contraction would be the major competing reaction in the attempt to form 42, it was hoped that a minor amount of direct substitution product would be formed. The only isolable compound had an infra red spectrum showing carbonyl absorption at 1740 $cm.^{-1}$, and was presumed to be the ring contracted aldehyde 43.

The next general approach selected was pyrolysis of a suitable derivative of the triol 31. The mechanism of pyrolysis of esters, particularly xanthates[26] and carbonates,[27], has been shown to require a cis - configuration of the participating groups.[28] Later studies[27]

demonstrated by consideration of activation entropies, that the reaction involved a concerted cyclic process with a highly ordered transition state. Application of the above general principles to the pyrolysis of a caesalpin derivative of type 44 suggested that the reaction could lead either to the allylic alcohol 45 or to the enol form of the 1 - ketone 46. Molecular models indicated that the $C_{(2)}$ α - oxygen substituent of 44 was symmetrically disposed between the β - hydrogen atoms at $C_{(1)}$ and $C_{(3)}$, and thus the reaction should be able to proceed in both directions. The carbonate 44, formed from the triol 31 using



44 R=Et



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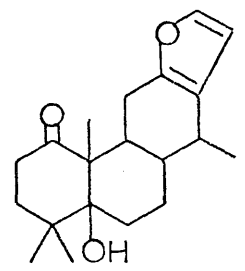
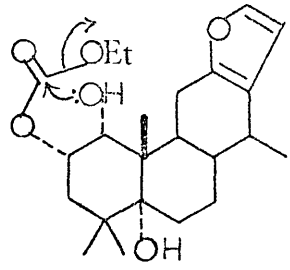


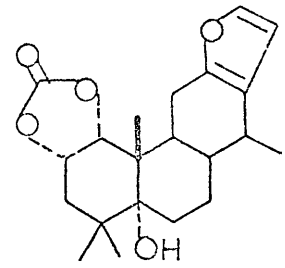
46

redistilled ethyl chloroformate and pyridine, was vacuum sealed into a pyrex tube and maintained at 250° in a heating block for one hour. The infra-red spectrum of the product, which was virtually homogeneous by t.l.c., still indicated the presence of a carbonyl group ($\nu_{max}^{CHCl_3}$ 1797 cm.$^{-1}$). One distinct possibility for the structure of this compound is the cyclic carbonate 47, which would certainly have carbonyl absorption in this region of the spectrum,[30] and could be easily formed

by intra-molecular displacement of ethoxide ion from 44.* However



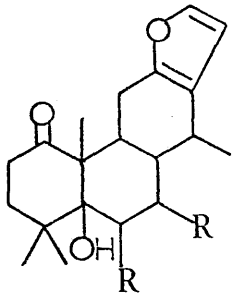
44



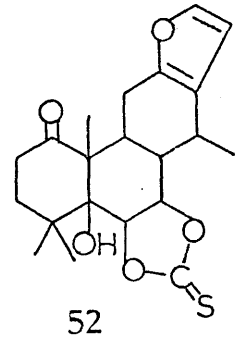
47

this remained unconfirmed, since lack of both time and material prevented complete characterisation.

Meanwhile, in Milan, out Italian colleagues were experiencing similar tribulations in their attempts to synthesize the intermediate 46 from deoxy - α- caesalpin 51. The 6, 7 diol system had shown

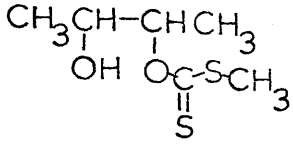


51, R= OH
46, R= H

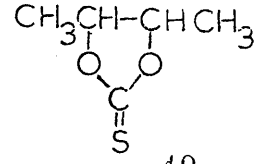


52

* Stevens and Richmond[29] have reported the analogous pyrolysis of the mono - S - methyl xanthate of 2;3 butanediol 48. The major product after extended heating was the cyclic thionocarbonate 49, but this was accompanied by some methyl ketone derived from the enol 50.



48



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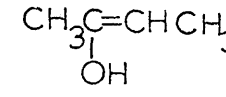
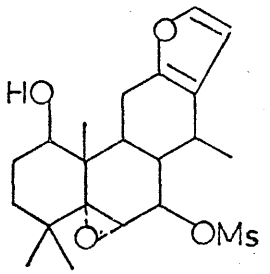


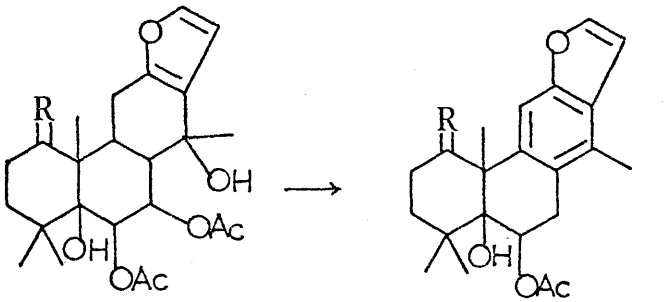
50

great reluctance to form the cyclic thionocarbonate 52 as described by Corey and Winter.[31] Thus the proposed conversion to 46 by treatment with triethyl phosphite followed by hydrogenation of the resultant 6, 7 double bond could not be realised. Another route, which involved hydrogenolysis of the epoxy-mesylate 53 had also been abandoned, for reasons which will be discussed later.



53

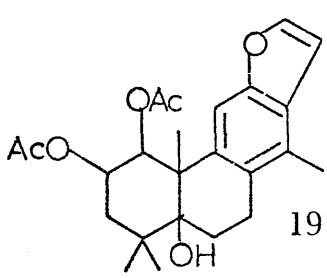
Simultaneous with the work on the caesalpin inter-relation, work was progressing in this laboratory which showed conclusively that α - caesalpin 1 and 1, 6, 7 triacetoxy - δ - caesalpin 54 could be smoothly transformed to benzofurans with concomitant loss of the $C_{(7)}$ oxygen substituent.



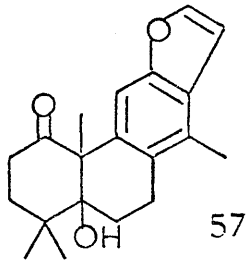
1 , R = O
54, R = H, OAc

55, R = O
56, R = H, OAc

This raised the possibility of utilising the stable aromatic compounds 19 and 55 (or 56) to relate ε - and α - caesalpin, by converting both to the benzofuran intermediate 57. This route would not necessarily simplify removal of the $C_{(2)}$ oxygen function from ε - caesalpin, but it could expedite the synthesis of 57 from the other direction, since this only required removal of the $C_{(6)}$ substituent from the benzofuran 56. Thus it was decided to pursue this scheme despite the obvious disadvantage of the loss of assymmetric centres.

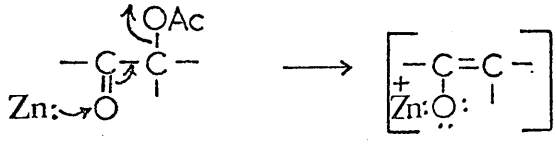


19



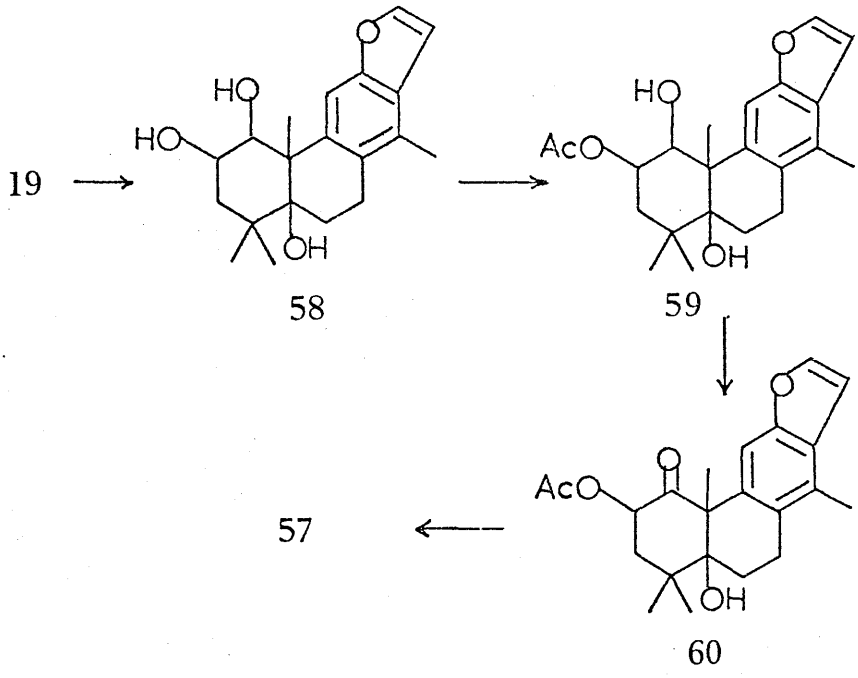
57

A survey of the literature unearthed several promising examples of de-acetoxylation reactions of α- ketol acetates with a wide variety of reagents. Woodward and his collaborators accomplished the de-acetoxylation of trans - 1 - acetoxy - 2 - keto - 10 - methyl - $\Delta^{3,6}$- hexahydronaphthalene by refluxing with zinc in acetic anhydride or xylene.[32] These authors proposed the mechanism:



This was later supported by the work of Rosenfeld and Gallagher,[33] who

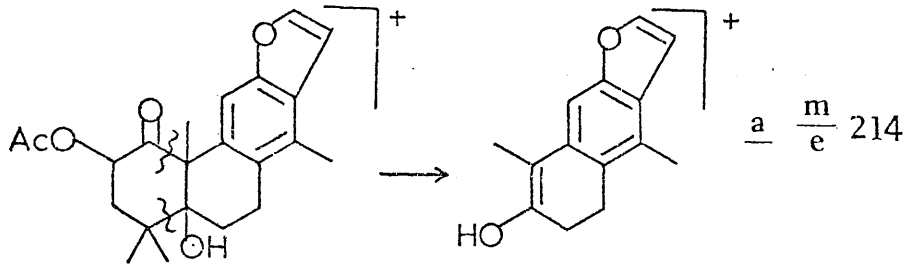
found in addition that axial acetates were removed more easily than their equatorial epimers.[34] This latter point struck an ominous note for the proposed reaction scheme, which included reductive removal of the 2 α- (equatorial) acetate from the keto-acetate 60.



The benzofuran 19 was treated with lithium aluminium hydride in ether, affording in good yield the triol 58, which was transformed in acetic anhydride/pyridine into the monoacetate 59, m.p. 210-211° (τ = 5.45 (broad singlet, >CH - OH), 4.59 (double quartet, J = 12, 5, 2 Hz, >CH OAc)). The keto-acetate 60, m.p. 174-178° was obtained in low yield (Ca.27%) by Jones oxidation of 59, the Sarett

reaction having previously proved ineffective. The constitution of 60 was readily verified from the n.m.r. spectrum, which exhibited, inter alia, a quartet (J = 12, 6 Hz) at τ 4.09, diagnostic of a >CHOAc proton being pulled downfield due to inductive electron withdrawal by the adjacent carbonyl group.

The mass spectrum of 60 is also compatible with the postulated structure. The base peak occurs at $\frac{m}{e}$ 214, and can be attributed to the ion a arising by cleavage of the $C_{(1)} - C_{(10)}$ and $C_{(4)} - C_{(5)}$ bonds.

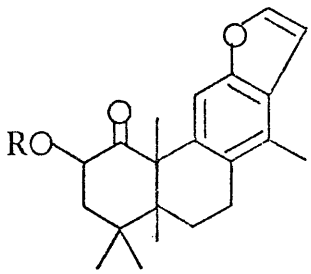


a $\frac{m}{e}$ 214

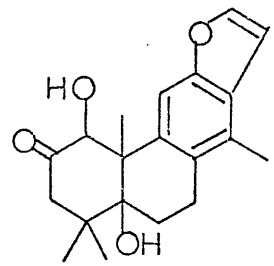
This fragmentation has previously been observed in the spectra of 1 - ketones with ring C aromatic.[70]

The keto-acetate 60, on treatment with zinc in refluxing acetic acid yielded an intractable mixture of products. Similar results were obtained when 60 was treated with a 2-mole excess of calcium in liquid ammonia, a method which had been eminently successful in de-acetoxylation reactions reported by Chapman et al[37,38]. A final effort was made using a modification of the chromous chloride reduction procedure described by Rosenkranz and Djerassi.[39] Under these milder conditions, the product comprised mainly returned starting material, but was accompanied by a small amount of the α - ketol 61.

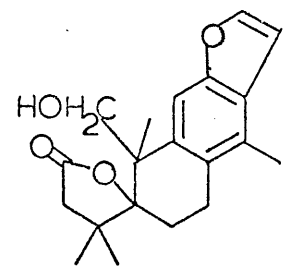
It is known[35] that a keto-acetate, on treatment with mild base or acid, is hydrolysed to the corresponding α- ketol, which can then isomerise under the reaction conditions. This suggested a method of removing oxygenation from the 2 - position by a Mozingo reaction on the ethylene dithioketal of 62.



60, R = Ac 64, R=Tos
61, R = H



62

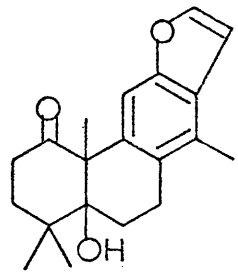


63

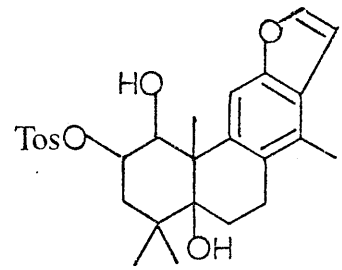
Treatment of the keto-acetate 60 with mild base, however, did not give the expected α- ketol 61 or 62, but instead provided a compound whose i.r. spectrum showed, in addition to hydroxyl absorption at 3560 $cm.^{-1}$, a strong band at 1784 $cm.^{-1}$. The possibility that this compound is the γ- lactone 63 gains support from the conspicuous absence of the sharp, characteristic ν (O-H) around 3590 $cm.^{-1}$ normally associated with the $C_{(5)}$ hydroxyl.

Acid hydrolysis of 60 afforded only the ketol 61, m.p. 175-177° ($\nu_{max}^{CCl_4}$ 1720 $cm.^{-1}$) distinguished from the other possible isomer 62 by n.m.r. (τ = 5.04 (quartet, J = 11, 7 Hz, >CH-OH)). This compound was stable to acid under the conditions of its formation, and isomerisation to the desired ketol 62 could not be effected.

The recalcitrant oxygen function was ultimately removed by treatment of the keto-tosylate 64 with chromous chloride in refluxing acetone.[39] An attempt to obtain the keto-tosylate directly by Jones



57



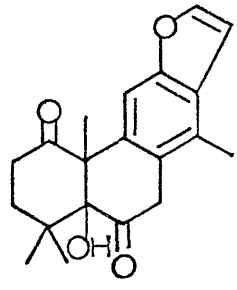
65

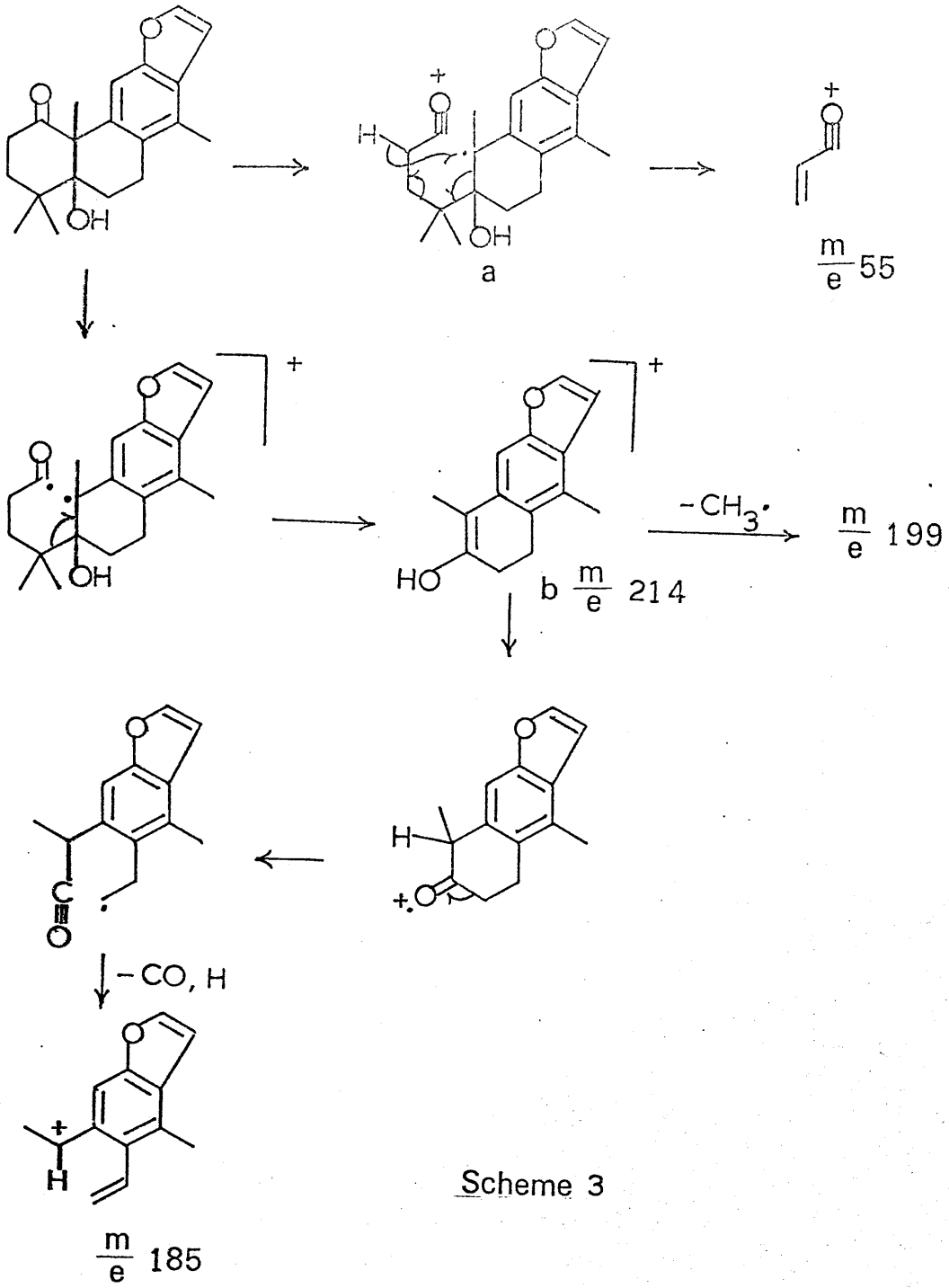
oxidation of the hydroxy compound 65 proved unsuccessful, but it was eventually derived by treatment of the ketol 61 with p - toluene - sulphonyl chloride in pyridine. The resultant tosylate 64 ($\nu_{max}^{CCl_4}$ 3568, 3480 cm.$^{-1}$(hydroxyl), 1740 cm.$^{-1}$ (carbonyl),* 1188, 1178 cm.$^{-1}$ (S = O)) was submitted without further purification to the chromous chloride reduction. It was most gratifying to be able to isolate from this reaction, in addition to starting material, a low yield of the elusive desoxy-caesalpin 57, m.p. 172-175°, $(\alpha)_D$ - 2.8° (chloroform) ;

* It is noteworthy that the carbonyl frequency of 1740 cm.$^{-1}$ is significantly higher than the expected value of 1720 cm.$^{-1}$ for a normal, strain free chair cyclohexanone. This value is also observed for the keto-acetate 60, and can be attributed to a direct dipolar interaction between the carbonyl group and the adjacent equatorially situated oxygen substituent. (Ref.40).

$\nu_{max}^{CCl_4}$ 3629 cm.$^{-1}$ (hydroxyl), 1712 cm.$^{-1}$ (ketone). The ν (O-H) frequency of 3629 cm.$^{-1}$ is interesting, since all other compounds in this series having a tertiary hydroxyl at $C_{(5)}$ display characteristically sharp absorption below 3595 cm.$^{-1}$. This indicates that in these compounds the -OH is strongly intramolecularly bonded. The molecular geometry of 57 rules out any possibility of intra-bonding: thus the $C_{(5)}$ hydroxyl is 'free' (apart from the less strong intermolecular association) and should exhibit the observed increase in stretching frequency. The unexpected polarity of the ketone 57 (which is more polar than the keto-tosylate 64) may also be rationalised on this basis, since the free hydroxyl may 'bond' to the silica, with a resultant reduction in R_f value going from 64 ⟶ 57.

The salient features of the n.m.r. spectrum are at τ = 9.07, 8.79, 8.50 (singlets, $3CH_3$ - $\overset{|}{C}$ -), 7.56 (singlet, Ar - CH_3), 7.2 (multiplet, $C_{(2)}$ methylene), 3.22 (singlet, aromatic proton). The chemical shift of the aromatic proton compares favourably with that of τ 3.17 observed for the diketone 66, subsequently obtained from 1, 6, 7 triacetoxy - δ - caesalpin.



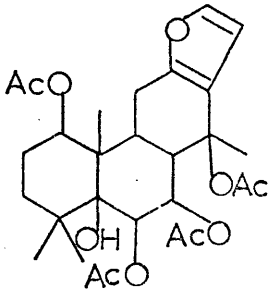


Scheme 3

The base peak of the mass spectrum occurs at $\frac{m}{e}$ 55. This peak is well established in the spectra of cyclic ketones,[42] and arises by concerted bond rupture of the initially formed α- cleavage product a (scheme (3)). The primary fragmentation leading to the other major peaks in the spectrum involves cleavage of the $C_{(1)} - C_{(10)}$ and $C_{(4)}-C_{(5)}$ bonds, with formation of the ion b at $\frac{m}{e}$ 214.[70]

ATTEMPTED SYNTHESIS OF THE BENZOFURAN INTERMEDIATE 57 FROM 1,6,7 - TRIACETOXY - δ - CAESALPIN.

It has already been mentioned that mild acid treatment of the triacetate 54 of δ - caesalpin resulted in the formation, via the exomethylene 67, of the benzofuran 56. The publication by Canonica



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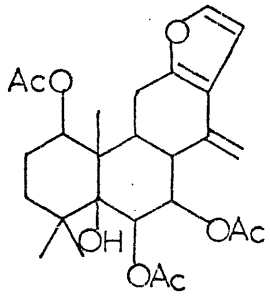
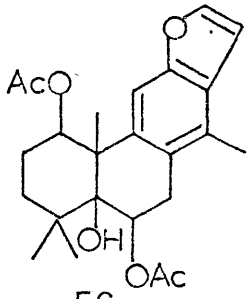
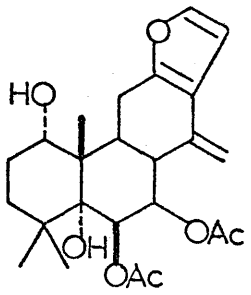


67



56

et al[2e] of the conversion of the hydroxy diacetate 68 into the corresponding 5 α , 6 α - epoxide 69 provided what was thought to be a reliable means of removing the functionality at the 6-position of 56.



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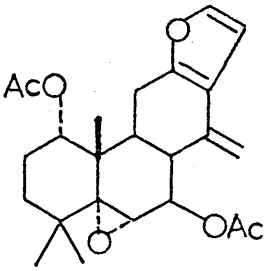
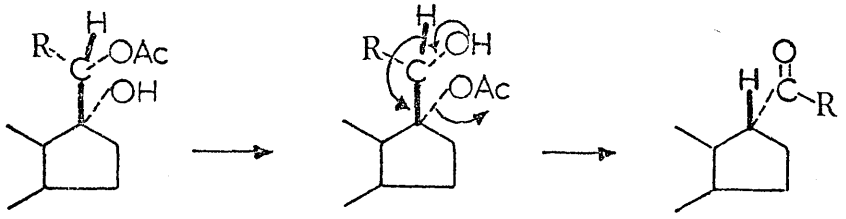
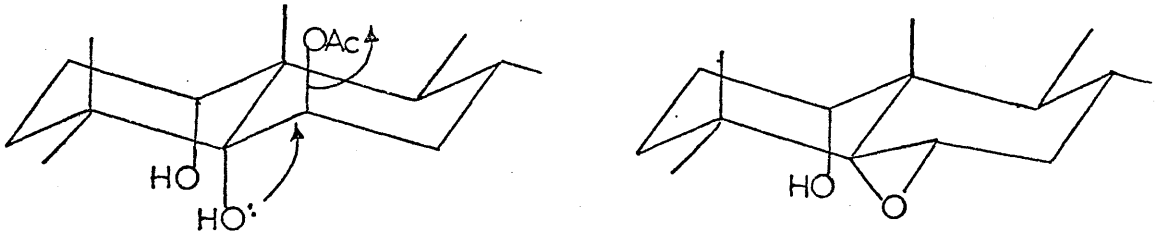


69

The method used by Canonica, known as the Serini reaction,[43] consisted of subliming the acetate 68 from zinc dust, whereupon the epoxide 69 was produced in low yield. This would appear to be an anomalous result for this reaction, since it has been shown[44,45] that the normal mechanism involves initial acyl migration to the tertiary hydroxyl, followed by pinacol rearrangement with stereospecific hydrogen transfer to give a ketone:

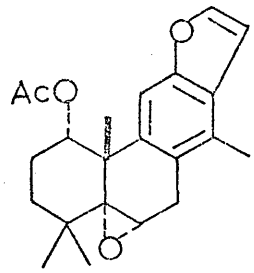


The Serini reaction, however, does not appear to be completely general,[46] and the epoxide 69 could conceivably result if the 6-acetate of the starting material 68 had the β - configuration.

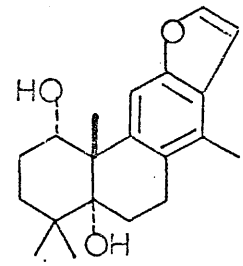


In principle, the same reaction, if applied to the benzofuran 56, should furnish the epoxide 70. Metal hydride reduction of a 5α, 6α - epoxide is known[47] to give mainly the tertiary alcohol, by preferential attack at the secondary carbon atom. Thus it seemed that our synthetic

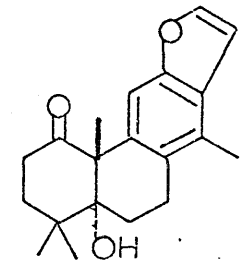
goal could be readily attained, as the above reaction sequence would yield, as a precursor to 57, the diol 71.



70

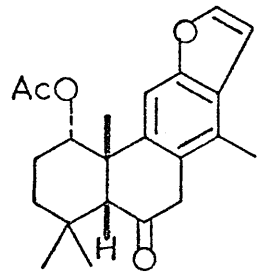


71

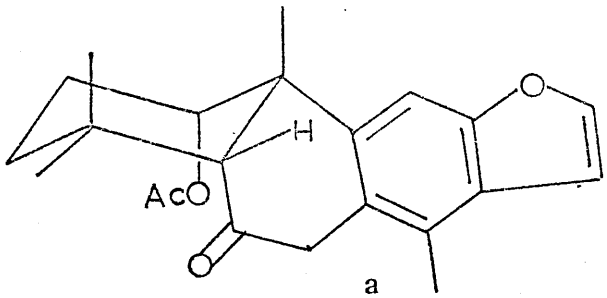


57

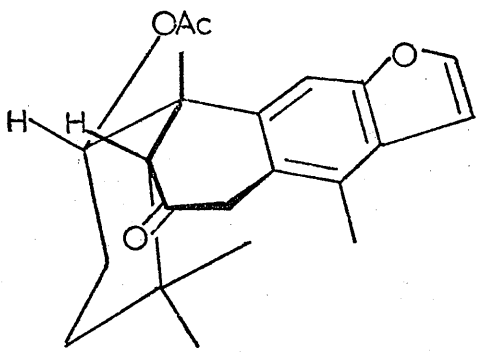
A pure sample of the benzofuran 56 was mixed with ten times its weight of zinc powder, and heated in a sublimation tube at 220°/0.3 mm. The products sublimed to the cold area of the tube, and were examined by t.l.c. None of the desired epoxide 70 was obtained. Analysis and mass spectroscopy of the main product, after chromatographic separation, indicated the molecular formula $C_{22}H_{26}O_4$. The infra-red spectrum, however, showed a strong absorption at 1714 $cm.^{-1}$, in addition to the expected acetate band at 1747 $cm.^{-1}$. Identification of this compound as the A/B cis-fused 6 - ketone 72 was made from the n.m.r. spectrum, which boasted a 3H singlet at τ 9.69. This can only be due to a methyl group which has penetrated the diamagnetic shielding zone of the benzene ring.



72



a



b

FIG 2

The Fieser model of 72 shows considerable conformational mobility, but the two most feasible situations, both with ring A in a chair and ring B in a half chair form, can be represented by a and b (Fig. 2).

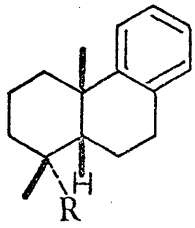
It can be readily seen that conformation a will be destabilised relative to b because of the strong 1, 3 diaxial interaction between the 4 β- and 10 β- methyls. Also, the 4 α-methyl of b is well within the shielding cone of the aromatic ring, thus allowing definitive assignment of the high field three proton singlet. Other features of the n.m.r. spectrum which are compatible with formulation b are the following:-

(i) a sharp singlet (2H) at τ 6.43 can be ascribed to the magnetically equivalent hydrogens of the $C_{(7)}$ methylene.

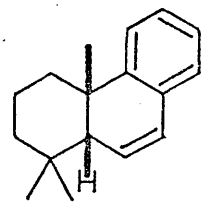
(ii) The >CHOAc proton now resonates as a pair of doublets (J = 12, 4 Hz) at τ 5.10. This represents an upfield shift of Ca. 0.78 p.p.m. with respect to the equivalent proton of the benzofuran 56, and demonstrates that the $C_{(1)}$-H bond has moved out of the plane of the aromatic ring. The change in the signal from a broad singlet to a pair of doublets lends support to the positioning of the $C_{(1)}$ hydrogen in a quasi - axial situation.

(iii) The $C_{(11)}$ aromatic proton is deshielded by 1.02 τ relative to the equivalent proton of 56, indicating its close proximity to the carbonyl of the $C_{(1)}$ acetate.

This conformational analysis lends additional weight to the findings of Fetizon[48] and Wenkert[49], who respectively studied the structurally related ring C aromatic compounds 73 and 74.

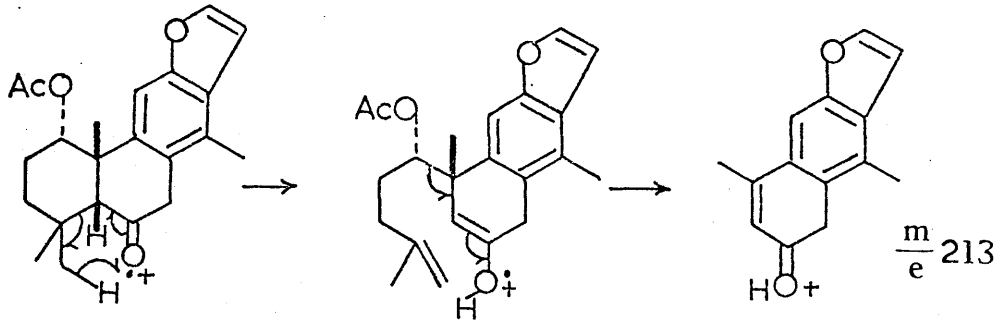


74 R CO_2Me
CH_2OH



73

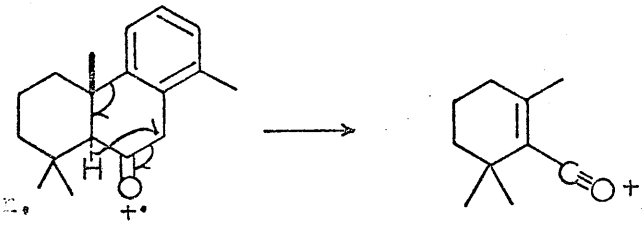
The mass spectrum of the 6 - ketone 72 has a base peak at $\frac{m}{e}$ 213, which arises by McLafferty rearrangement,[50] followed by cleavage of the allylic $C_{(1)} - C_{(10)}$ bond.



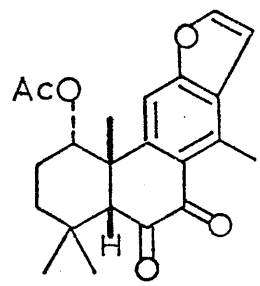
This can be adduced as confirmatory evidence of an A/B cis-fused ring

junction, since it is known[51] that the prevalent cleavage in the trans - case involves migration of the $C_{(5)}$ α - hydrogen. The mass spectrum does not have any significant peaks corresponding to this

fragmentation.

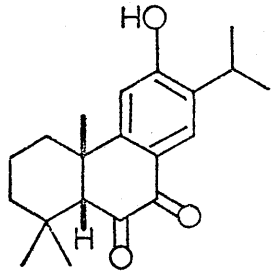


It was subsequently discovered that 72 underwent spontaneous aerobic oxidation to a product formulated as the α - diketone 75 (cf. ref. 54). The n.m.r. spectrum of 75, although similar to that of 72, showed several structurally significant differences. In particular, the 2H singlet representing the $C_{(7)}$ methylene was noticeably absent, and the $C_{(14)}$-methyl was deshielded relatively by 0.44 p.p.m. The obvious conclusion that oxidation of 72 had occurred at the doubly activated $C_{(7)}$ position was verified by analysis and mass spectroscopy.

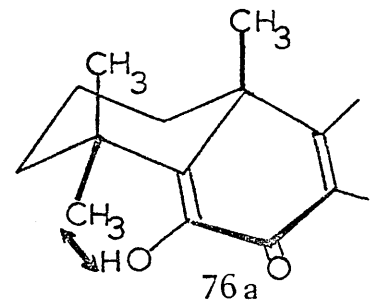


75

It seemed at first unusual that this compound should exist wholly in the di-keto form 75 (i.r. bands at 1750, 1228 cm.$^{-1}$ (acetate), 1730 cm.$^{-1}$ ($C_{(6)}$- ketone), 1689 cm.$^{-1}$ ($C_{(7)}$- ketone) - no hydroxyl absorption). However an analogous situation has been observed for the tricyclic diterpene xanthoperol* 76[52,56], the complete suppression of enolisation being attributed[53] to the strongly unfavourable non-bonded interaction between the equatorial $C_{(4)}$- methyl and the $C_{(6)}$ hydroxyl (arrows, 76 a).

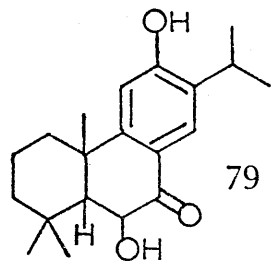


76

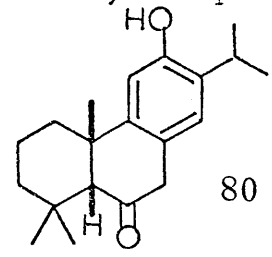


76 a

* Bredenberg has reported[52] that xanthoperol is only isolated as an artefact, and proposes, as a possible precursor, a compound having part structure 79.



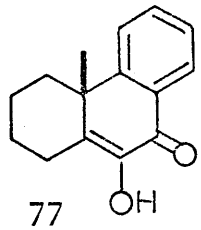
79



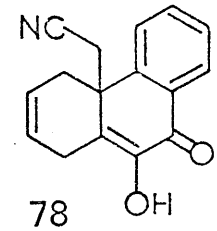
80

This hypothesis has never been verified, and in view of the facility of benzylic oxidation of 6 - ketones as previously discussed, compound 80 (6 - keto - 5 - iso - ferruginol) could be regarded as a more likely prospect for the naturally occurring precursor.

By contrast, the related compounds 77[54] and 78[55], in which there can be no peri-interaction, have been shown to exist in the enolic forms.

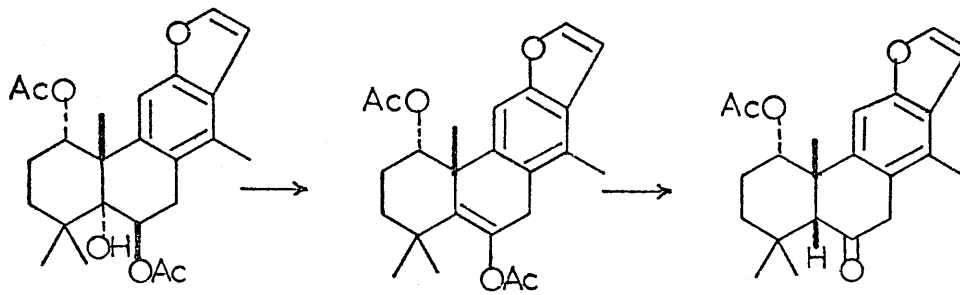


77



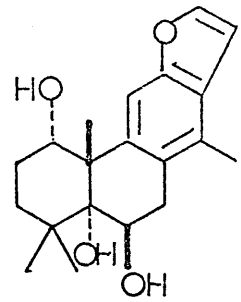
78

Meanwhile our Italian colleagues had also been examining the Serini reaction on the benzofuran 56. They reached a similar conclusion that the main product was the cis-fused 6 - ketone 72 and not the epoxide 70. From their previous results in the non-aromatic series (see page 43) they were convinced that the $C_{(6)}$ oxygen function had the β -configuration in α - and δ - caesalpin, and hence in the benzofuran 56. In order to explain the formation of the main Serini product, they proposed a mechanism which invoked pyrolytic elimination of the $C_{(5)}$ hydroxyl, followed by loss of ketene and equilibration to the more stable[57] cis-ring junction.

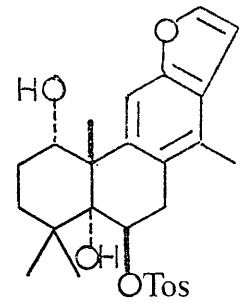


56 81 72

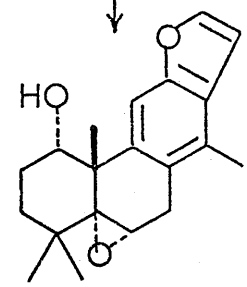
This must be regarded with some considerable scepticism, since



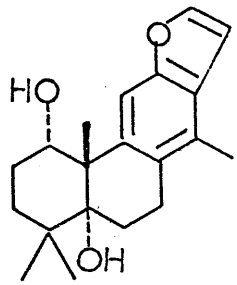
82



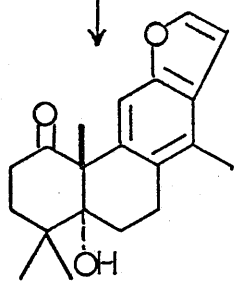
83



84



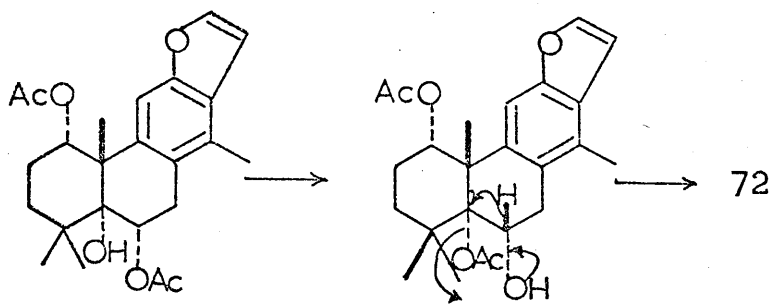
71



57

Scheme 4

the primary step is cis-elimination of water to form an enol acetate 81 which would experience a strong destabilising influence owing to the peri-effect (cf. page49). On the other hand if the 6 - substituent of 56 had the α - orientation, the 'normal' Serini mechanism would furnish the observed product 72.



56

At the same time as this work was progressing, a complementary line of research was being investigated which had also started with the basic premise that Canonica's original assignment of a 6 β - acetate was reliable. This research was directed towards synthesis of the benzofuran intermediate 57 via the hydroxy tosylate 83 (scheme (4)). Hydrogenolysis of the tosylate grouping should provide the diol 71, which could then be oxidised to the desired intermediate 57. Whether or not the hydrogenolysis proceeded through the epoxide 84 was immaterial, since the net result would be retention of the α - configuration at $C_{(5)}$.

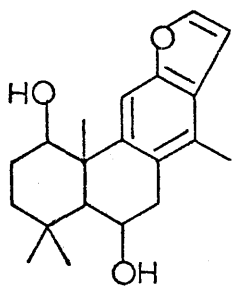
The triol 82 was obtained by lithium aluminium hydride reduction of the diacetate 56, and was allowed to stand overnight with p -

toluenesulphonyl chloride in pyridine. Two products were formed from this reaction, one of which had infra-red bands at 3570, 3480, 1177, 1187 cm.$^{-1}$ - diagnostic of the expected hydroxy-tosylate 83. On standing in chloroform solution, 83 gradually formed the other isolable reaction product with elimination of p - toluenesulphonic acid.* This was ostensibly the epoxide 84, $C_{20}H_{24}O_3$; $\nu_{max}^{CCl_4}$ 3594 cm.$^{-1}$ (hydroxyl), 1044, 1144 cm.$^{-1}$ (ether or oxide linkage). Since the n.m.r. spectrum was also in accord with the structure presented, lithium aluminium hydride reduction was carried out and proceeded smoothly with the production of a white crystalline solid, m.p. 201-203° presumed to be the diol 71. Analytical and mass spectral data confirmed the expected molecular formula $C_{20}H_{26}O_3$ for this compound. The n.m.r. spectrum showed the usual methyl signals at τ 8.96, 8.95, 8.71 and 7.62, while a complex system of multiplets around τ 7.0 was presumed to be an AB type system attributable to the protons attached to $C_{(6)}$ and $C_{(7)}$. The appearance of the >CH OH signal as a diffuse doublet (J = 5 Hz) at τ 5.76 rather than the normal broad singlet was only mildly disturbing.

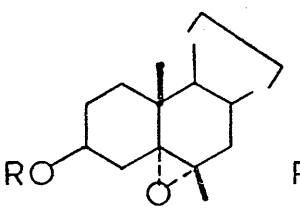
The first inkling of anything being amiss in the above reaction scheme came with the discovery that acetylation of 71 in refluxing acetic anhydride/sodium acetate gave a compound which had two secondary

* This process could be facilitated by treatment of the hydroxy tosylate, in ethanol solution, with mild base.

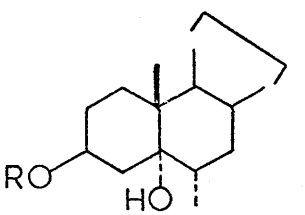
acetates (τ = 8.32, 8.06 (singlets, $2CH_3COO$-), 5.00(doublet, J = 3 Hz, $\geq$ CHOAc), 4.71 (double doublet, J = 7, 3 Hz, $\geq$CH OAc)). That this was a true derivative of the diol was shown by its reconversion to the parent compound on treatment with lithium aluminium hydride in ether. The unexpected formation of this diacetate raised several problems, not least of which was the positioning of the 'missing' $\geq$CH OH proton in the n.m.r. spectrum of the diol 71. The possibility that hydride attack on the epoxide 84 had given the secondary alcohol 85 rather than 71 was considered. This was later discounted on the grounds that reduction of the epoxide 86, which would appear to be a much more probable candidate for tertiary attack, had only provided the corresponding $C_{(5)}$ α - hydroxy compound 87.[59]



85



86

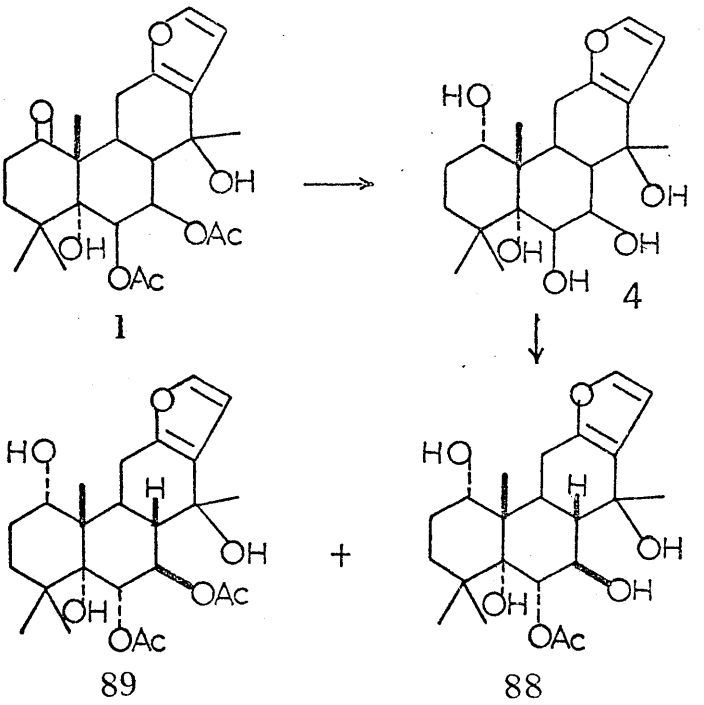


87

The above observations, together with the production of a cis-fused A/B ring junction from the Serini reaction, necessitated a complete re-appraisal of the stereochemistry of α - caesalpin.

To this end, α - caesalpin 1 was transformed into δ - caesalpin 4 by metal-hydride reduction.[2e] The resulting highly crystalline

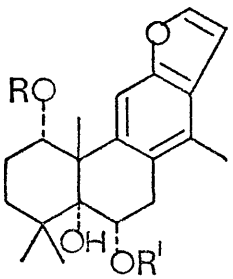
solid, m.p. 251° (reported[2e] 251°), was acetylated overnight in acetic anhydride/pyridine, affording a mixture of two acetates which, from the n.m.r. spectra, can be formulated as 88 and 89.



The monoacetate 88 displayed n.m.r. signals which were entirely consistent with the assignment of 6 α - , 7 β - orientations to the ring B substituents. The >CH OAc proton resonated as a doublet (J = 10 Hz) at τ 4.63, while the >CHOH proton appeared as a clean triplet (J = 10 Hz) at τ 5.70. Inspection of models of the various possible spatial arrangements of these two functional groups clearly indicated structure 88, with ring B in a slightly flattened chair, as a most likely prospect. The flattening effect referred to could be explained by a desire to alleviate, or at least

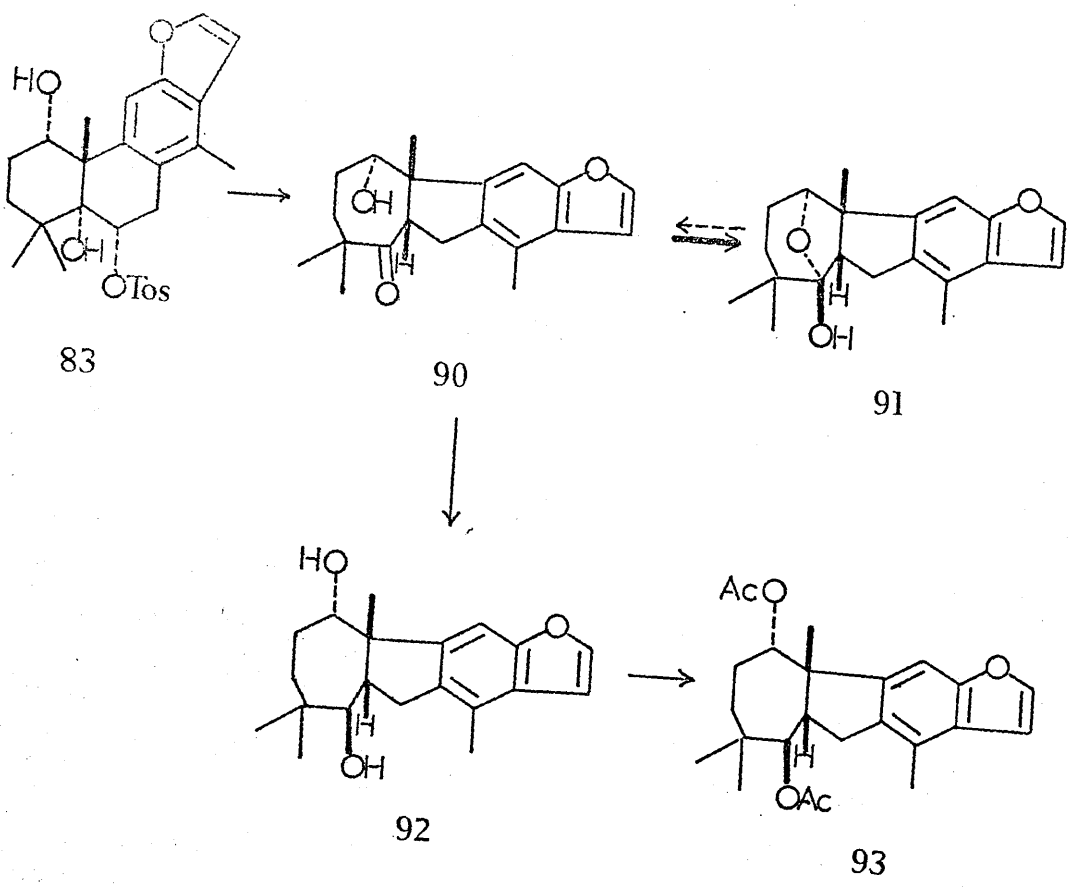
minimise the non-bonded interactions between the 7 β - hydroxyl and the 14 β -substituent, which may be considerable if both are in classical equatorial situations.

The Italian workers pointed out[58] that the observed coupling constants could also be reconciled with the dihedral angles involved in a ring B boat conformation with the oxygen substituents 6 β and 7 α . It seems unlikely, however, that this is the case, in view of the large "stem to stern" interaction involving the $C_{(10)}$ β - methyl which would result. We therefore consider that the monoacetate 88 (and hence α - and δ - caesalpin) has the 6 α , 7 β stereochemistry. Consequently the configuration of the 6 - oxygen substituent of the benzofuran diacetate 56 and tosylate 83, previously presumed to be β , should now be reversed.



56, R=R'
83, R=H R'=Tos

These stereochemical considerations led to the dénouement which would rationalise all of the anomalous experimental observations. On re-investigating the possible transformation products of the hydroxy-tosylate 83 (now with the 6 α - configuration) it was concluded that a pinacol type rearrangement must have occurred, in



83 90 91

92 93

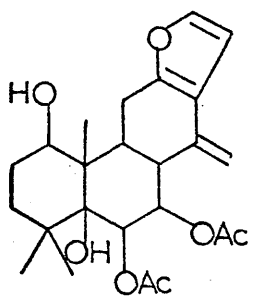
Scheme 5

accord with the trans-antiparallel relationship of the $C_{(10)}$ - $C_{(5)}$ and $C_{(6)}$ - O bonds. This would yield initially the cis-fused A-homo, B - nor ketone 90.[60] The infra-red spectrum of this compound does not show any absorption in the carbonyl region, and it must therefore exist mainly in the hemi-ketal form 91. Inspection of models indicates that the 1 α -hydroxyl is suitably oriented for participation in transannular hemi-ketal formation[cf.64] (scheme (5)). The A/B cis-fusion is demanded by the nature of the rearrangement, which is known to proceed with greater facility if the four reacting centres lie in one plane.[61,62] This requirement, together with a concerted electron shift, results in the β -configuration of the bridgehead proton.

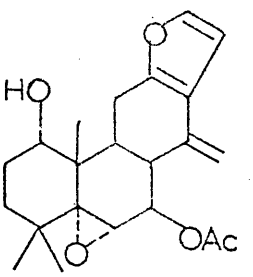
Thus the hemi-ketal 91 was the true transformation product of the tosylate, and not the "red herring" epoxide 84. This is supported in retrospect by the n.m.r. spectrum, since the $C_{(1)}$- H signal (diffuse doublet, J = 5 Hz) at τ 5.76 is more in agreement with the hemi-ketal formulation. Lithium aluminium hydride reduction of 91 presumably occurred via the equilibrium amount of ketone 90, ultimately furnishing the diol 92. A model of this compound, which displays considerable conformational mobility, allows solution of the case of the 'missing' proton (see page 54) if the $C_{(5)}$ hydroxyl has the β-configuration. The model indicated that this molecule should be able to exist comfortably in a conformation in which the $C_{(5)}$ α - hydrogen penetrates the diamagnetic shielding cone of the benzene ring. Consequently in

the n.m.r. spectrum this particular proton appeared at considerably higher field than would normally be expected, the signal in fact being one of the complex system of multiplets originally ascribed only to the benzylic hydrogens. Even the extremely large downfield shift (ca. 2 τ) of this resonance on acetylation to the diacetate 93 could be explained, since it is known that changes in the substituents of cycloheptane rings can cause drastic changes in conformation.[60] This is also in accord with the flexibility of substituted cycloheptanes observed in perhydroazulenes.[63]

The foregoing argument raised doubts as to the validity of the epoxide structure 69 assigned by Canonica[2e] to one of the products of the Serini reaction on the δ - caesalpin derivative 68 (see page 43).



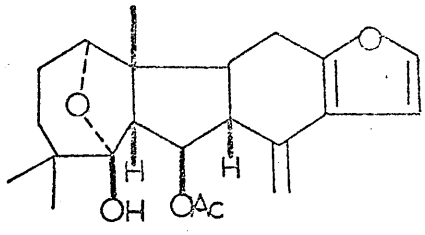
68



69

A careful examination of the n.m.r. spectrum* of the supposed epoxide indicated that a more likely structure was the hemi-ketal 94. Double irradiation studies had shown that the >CHOAc proton, which resonated as a pair of doublets (J = 8, 4 Hz). at τ 3.89, was coupled

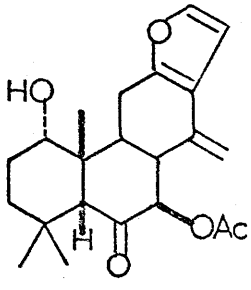
* Supplied by Dr. E. Ghisalberti, University of Milan.



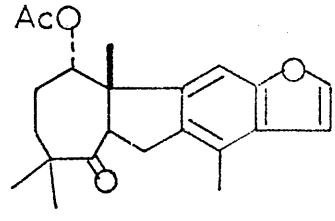
94

with a partially obscured doublet at τ 7.50. This high field signal was originally assigned to the $C_{(6)}$ proton of 69, but on the basis of structure 94 is attributable to the $C_{(6)}$ bridgehead hydrogen. The observed coupling constants (J = 8, 4 Hz.) for the >CHOAc proton can be admirably correlated with the required dihedral angles of 94 according to the Karplus equation.[71]

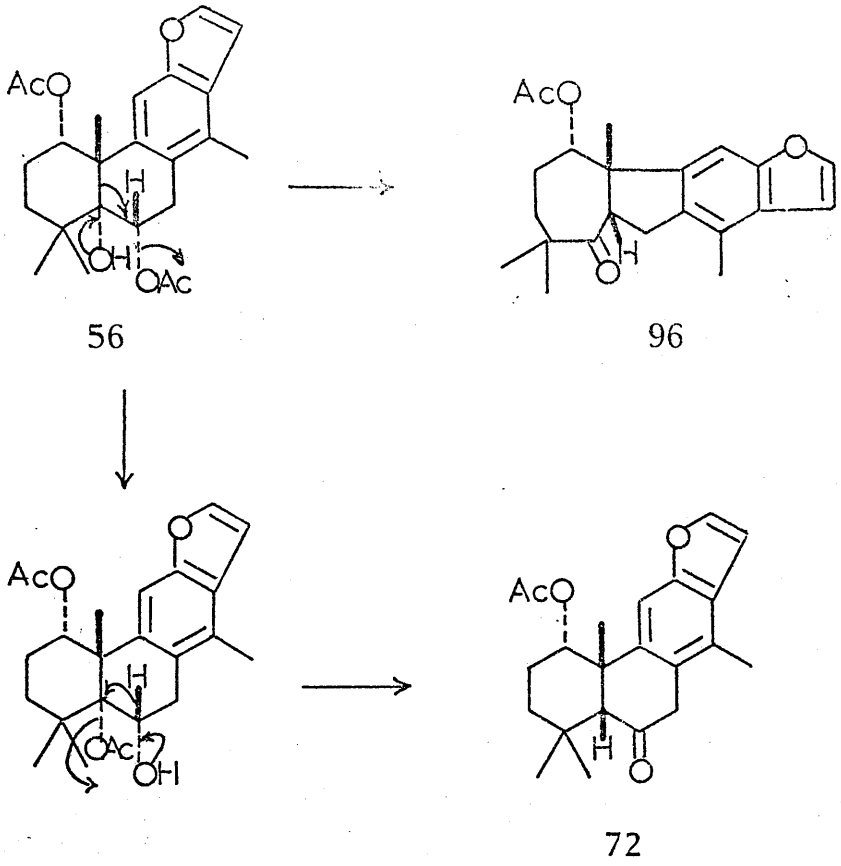
From mechanistic considerations it might have been anticipated that the main product of a Serini reaction on 68 would be, by analogy with the results in the aromatic series, the cis-fused ketone 95. In this respect it should be noted that the yield quoted for the only



95



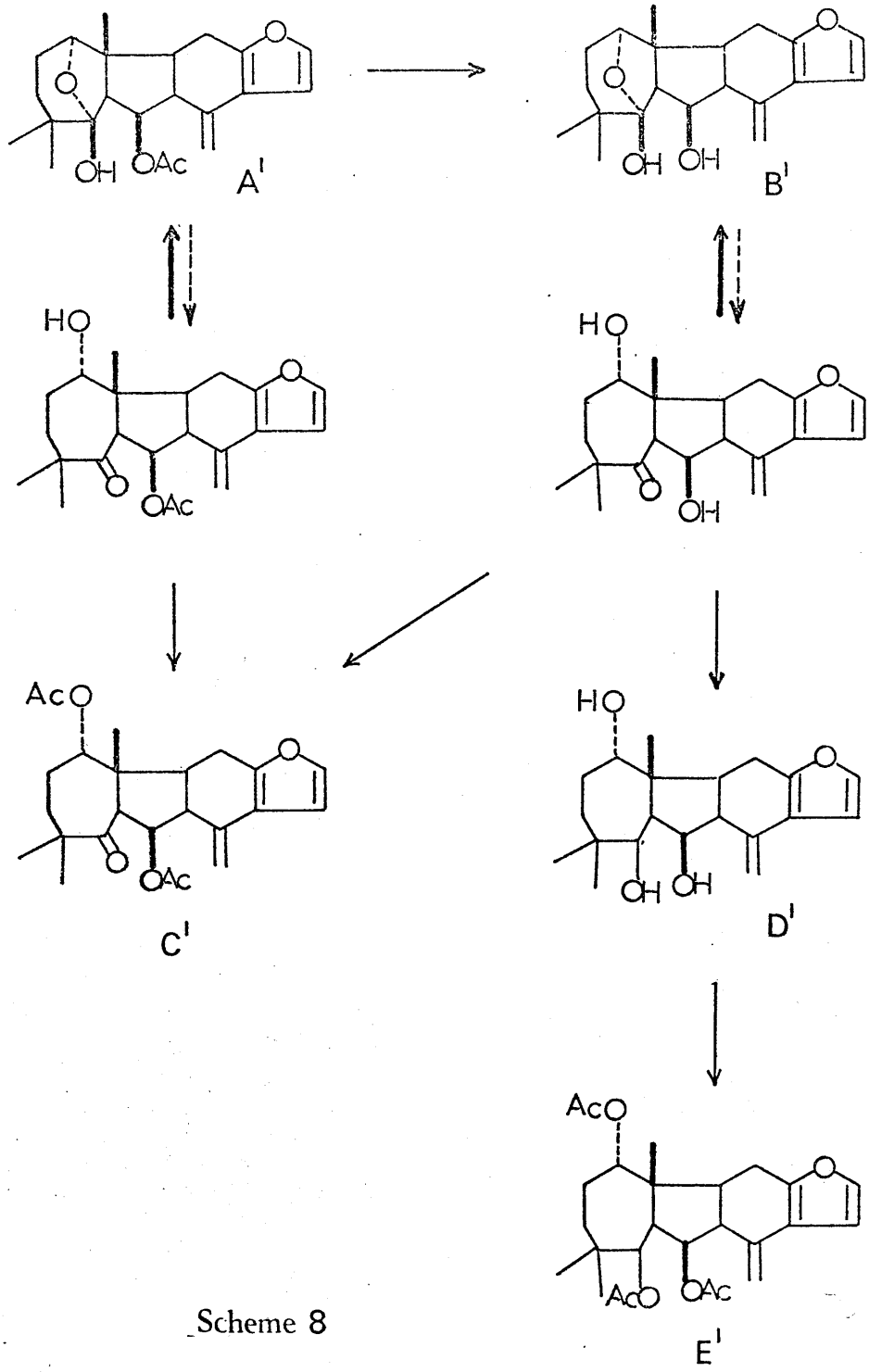
96



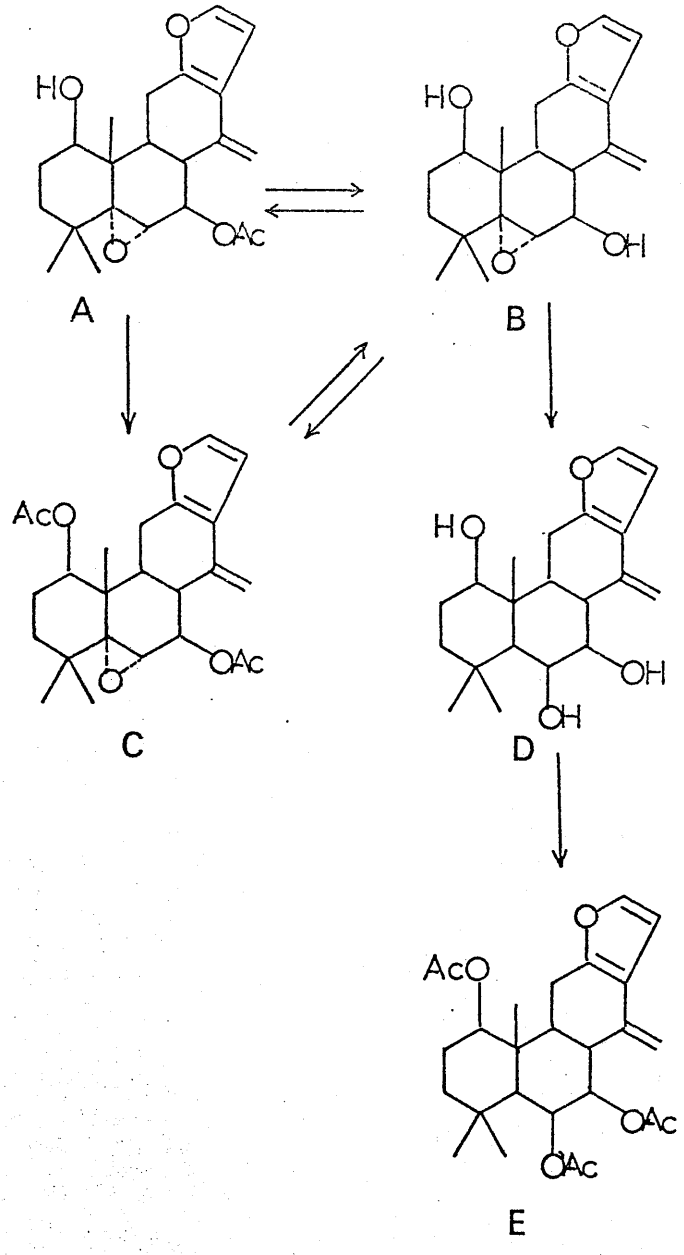
Scheme 6

isolated product is low (ca. 20%), and it is distinctly possible that 95 was produced but never isolated from the reaction mixture. The logical extension of this argument is that a ring expanded product analogous to 94 should have been formed during the Serini reaction on the benzofuran 56. Accordingly, the reaction was repeated, and careful chromatographic separation of the products yielded a small amount of a compound having spectral characteristics which would be expected of the A - homo, B - nor ketone 96 : $\nu_{max}^{CCl_4}$ 1740, 1230 cm.$^{-1}$ (acetate), 1723 cm.$^{-1}$ (ketone) ; τ = 8.87, 8.78, 8.62 (singlets, tertiary methyls), 8.21 (singlet $\underline{CH}_3COO-$), 7.68 (singlet, Ar - $\underline{CH}_3$), 4.50 (diffuse doublet, J = 4 Hz., $>\underline{CH}$ OAc), 2.85 (singlet, Ar - $\underline{H}$). Lack of time precluded complete characterisation of this product. The conclusion may be reached, therefore, that the Serini reaction can occur by a pinacol-type rearrangement both before and after acyl transfer (scheme (6)).

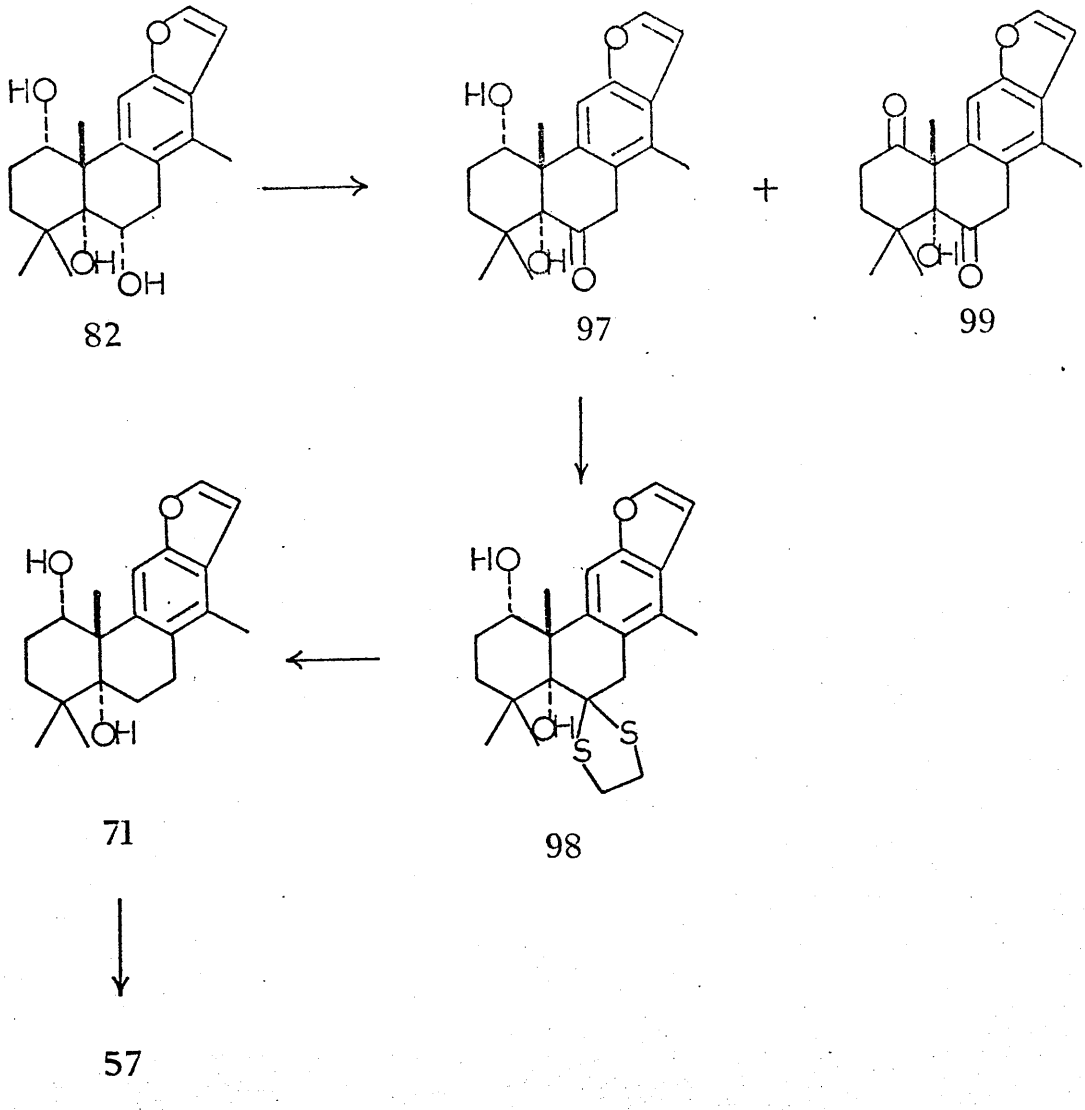
The assignment of structure 94 to Canonica's "epoxide" (69) necessitates the re-interpretation of a number of reactions, since he used this material as the starting point for the reaction sequence shown in scheme (7). The physical constants reported for these compounds agree much better with their counterparts in scheme (8), e.g. the infra-red spectrum of compound C has bands at 1739 and 1724 cm.$^{-1}$, which are obviously more in agreement with the alternative formulation C^1.



Scheme 8



Scheme 7

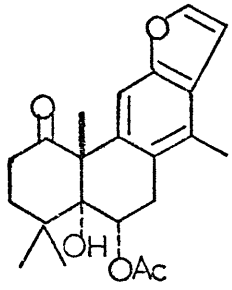


Scheme 9

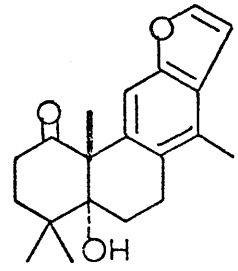
All of these diversions, interesting as they may be, were not contributing towards the realisation of our original objective, viz. the chemical inter-relation of α - and ε - caesalpin. One last despairing effort was made to relate the two compounds by a route which envisaged removal of the $C_{(6)}$ oxygen function from the triol 82 by oxidation to the 6 - ketone 97, followed by Raney nickel hydrogenolysis of the corresponding thioketal 98 (scheme (9)).

Jones oxidation of the triol 82 afforded a 40 : 60 ratio of the 6 - ketone 97 and the 1, 6 diketone 99. The yield of the desired monoketone was subsequently increased by employing the Sarett reagent, which appeared to oxidise selectively the 6 - hydroxyl. Preliminary experiments directed towards formation of the thioketal 98 in ethane · dithiol/BF_3 mixtures were extremely discouraging, since no pure product could be isolated.

An attempt to determine the absolute configuration of α - caesalpin despite the catalectic chemical inter-relation described above was made by comparing the O.R.D. curve of the benzofuran 55 with that of the intermediate 57 derived from ε - caesalpin. (Fig. 3)



55



57

However this comparison proved inconclusive, and as yet no definite assignment of absolute configuration can be made.

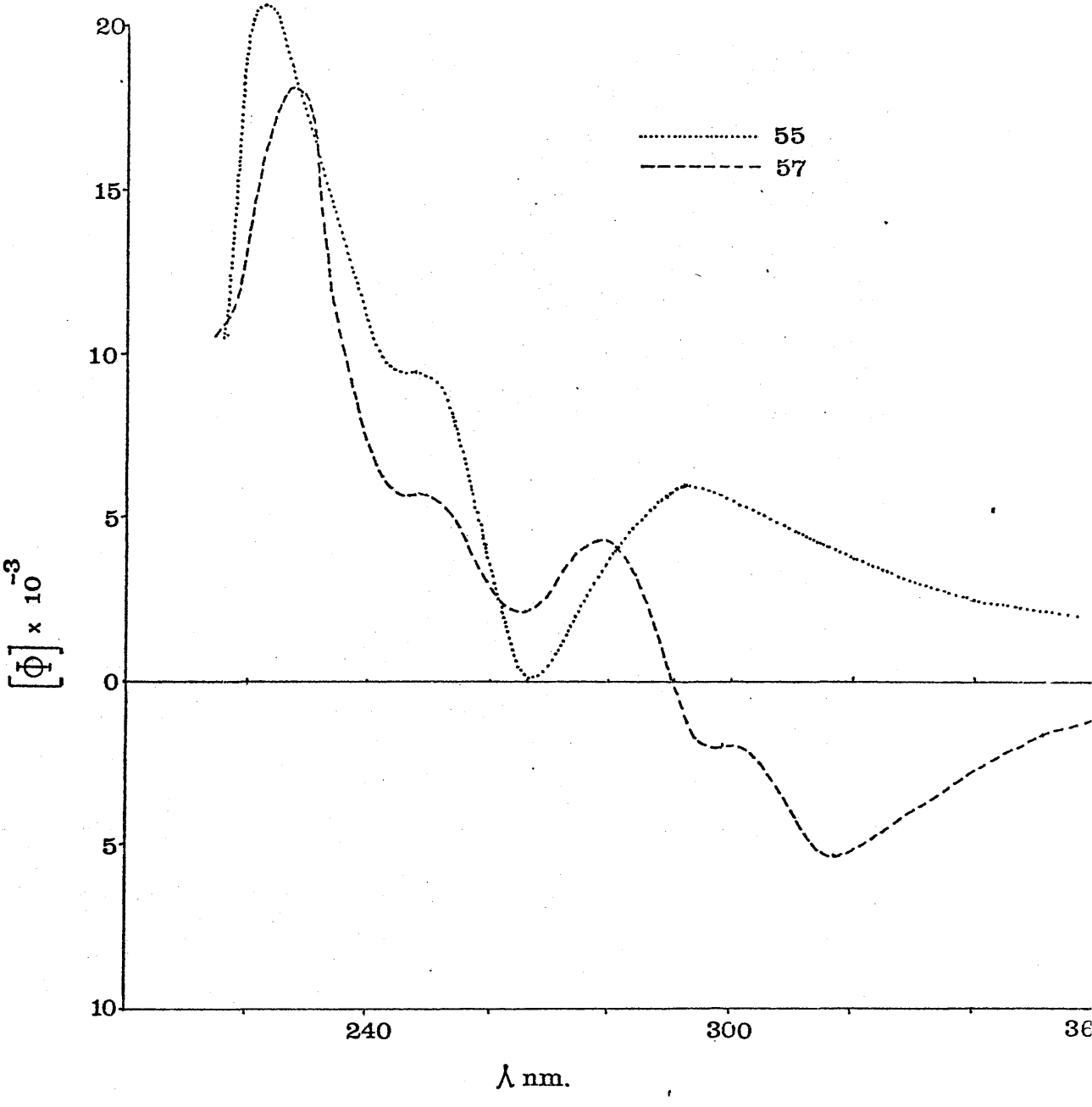
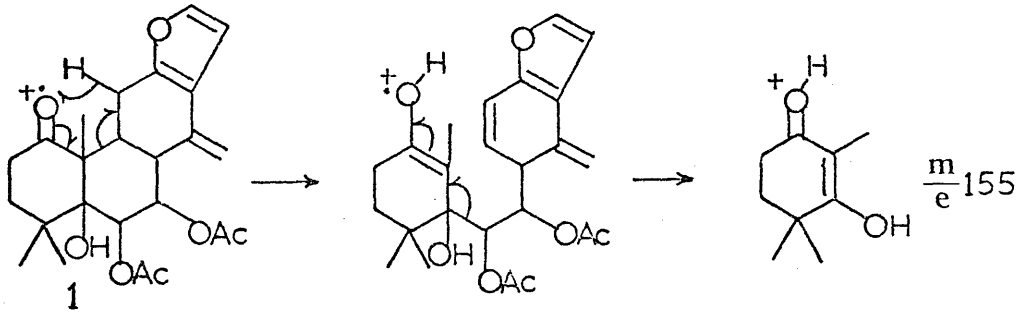


FIG 3

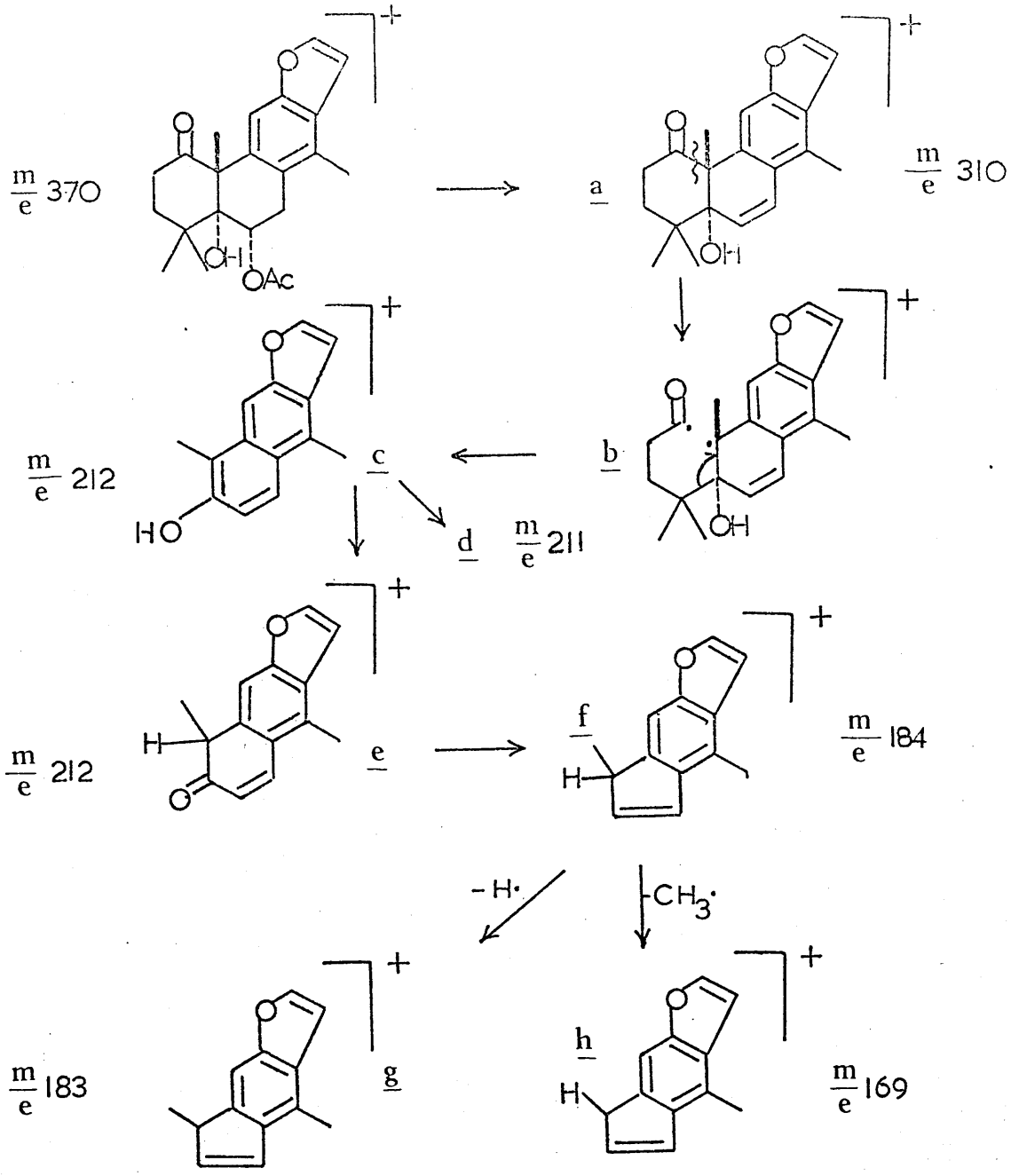
THE MINOR CONSTITUENTS OF CAESALPINIA BONDUCELLA.

(1) α - caesalpin was isolated in low yield from the Nigerian seeds of Caesalpinia bonducella, and its identity established by comparison of its physical data with those published by Canonica et al.[2e] (See experimental section). Some additional support for the promulgated structure (excluding stereochemistry) can be gleaned from the mass spectrum, the base peak of which occurs at $\frac{m}{e}$ 155. This peak is not observed to any significant extent in the spectra of the other compounds of this series, and can be attributed to McLafferty rearrangement involving the $C_{(1)}$ carbonyl and the $C_{(11)}$ equatorial hydrogen, followed by cleavage of the allylic $C_{(5)} - C_{(6)}$ bond.



1

It has already been mentioned that treatment of α - caesalpin with a solution of hydrochloric acid gas in chloroform resulted in formation of the benzofuran 55, m.p. 176-178°; λ_{max} 251 nm. (ε 7800), 280 nm. (ε 2800), 290 nm. (ε 2850). The n.m.r. spectrum revealed the loss of the $C_{(7)}$- acetate, since the benzylic methylene could now be seen as the AB part of an ABX system at τ 6.98,

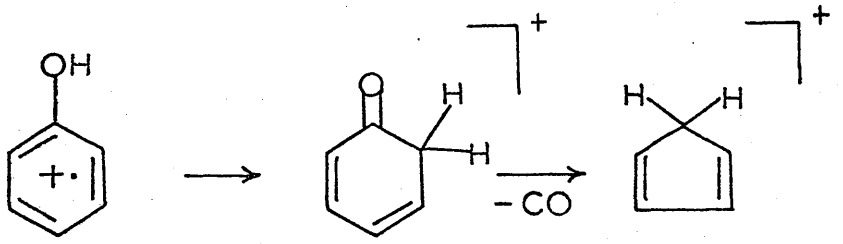

m/e 370
OH
OAc
a
m/e 310
OH
b
OH
m/e 212
c
HO
d
m/e 211
m/e 212
H
e
f
H
m/e 184
-H·
CH3·
m/e 183
g
h
H
m/e 169

Scheme 10

with the X part resonating as a quartet at τ 4.40 ($J_{obs.}$ = 9, 7 Hz., >C<u>H</u>OAc).

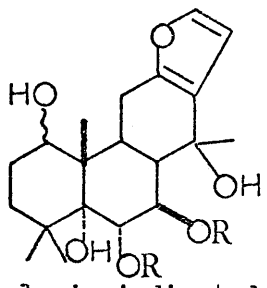
Confirmatory evidence of acetic acid loss was derived from the mass spectrum, which displayed a parent peak at $\frac{m}{e}$ 370 ($C_{22}H_{26}O_5$ requires M^+ = 370). Prominent ions were also observed at $\frac{m}{e}$ 310, 237, 212, 211 (base peak), 184, 183 and 169. Most of these may be rationalised by the breakdown pattern shown in scheme (10).

The postulated loss of carbon monoxide from the fragment ion <u>e</u> has precedent in the work of Aczel and Lumpkin on the mass spectra of phenols.[65] Their proposal that the main fragmentation was associated with loss of 28 mass units was later vindicated by exact mass measurements[66] as well as deuterium labelling studies.[67] The expulsion of carbon monoxide from phenol itself has been demonstrated[68] to proceed through a cyclohexadienone intermediate <u>j</u> to give the cyclopentadienyl cation <u>k</u>, i.e. a process similar to <u>e</u> → <u>f</u>

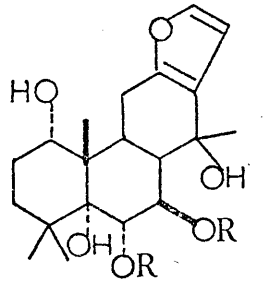


<u>j</u> <u>k</u>

(2) A hydroxy-diacetate, m.p. 178-179°, which was eluted directly after α-caesalpin has been formulated as a mixture of the $C_{(1)}$ epimers of structure 100 on the following evidence.



100, R=Ac
101, R=H



4, R = H
89, R = Ac

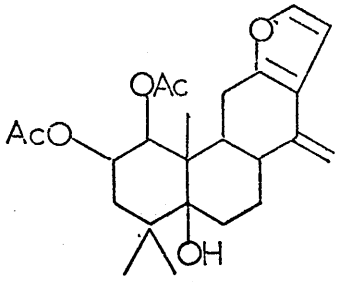
Analysis indicated the molecular formula $C_{24}H_{34}O_8$, and this together with the infra-red spectrum ($\nu_{max}^{CCl_4}$ 3603, 3549 cm.$^{-1}$ (hydroxyl), 1754 cm.$^{-1}$ (acetate)) was initially suggestive of a dihydro-derivative of α - caesalpin. The n.m.r. spectrum was almost directly superposable on that of the diacetate 89, m.p. 148-150°, which had previously been obtained by acetylation of δ - caesalpin 4. There was a slight difference, however, in the peak width at half-height of the $\geq$ CHOH signal ($W_{\frac{1}{2}}$ = 10 Hz for 100, as opposed to 7 Hz for 89). The work of Canonica et al[2e] has shown that the diacetate 89 has a trans- A/B ring junction, and in addition that the $C_{(1)}$ hydroxyl has the α - configuration. Thus the original supposition was made that the naturally occurring diacetate 100 probably had the 1 β - orientation, which would explain the observed broadening of the >CH OH signal. However, an attempt to obtain this compound by sodium borohydride reduction of α - caesalpin resulted in the formation of another

hydroxy-diacetate, m.p. 198-199°, whose spectral characteristics coincided exactly with those of 100. This difference of 20° in the melting points of two otherwise identical compounds may be rationalised if both are mixtures of $C_{(1)}$ epimers. All attempts to distinguish between these epimers by chromatographic methods (including G.L.C. on two columns) met with failure.

The above borohydride reduction product (m.p. 198-199°) was subjected to further reduction with lithium aluminium hydride. The pentaol produced (101), although homogeneous by t.l.c., melted over the range 207-211° (cf. δ - caesalpin 4, which melts sharply at 251°).

(3) The structural elucidation of 7 - hydroxy - ε - caesalpin 102 m.p. 186-188°, was accomplished fairly readily by the combined techniques of n.m.r. and mass spectroscopy. The likelihood that the compound was a derivative of ε - caesalpin was first seen from the acetate pattern in the n.m.r. spectrum (τ = 4.74 (double quartet, J = 12, 5, 2 Hz., >CHOAc), 4.80 (doublet, J = 2 Hz., >CHOAc)). A >CHOH signal at τ 5.91 (double quartet, J = 10, 5, 2 Hz) was ascribed to the proton attached to $C_{(7)}$, since this is the only carbon having the three adjacent hydrogens required for such a multiplicity.

The mass spectral breakdown pattern of 102 was in general very similar to that of ε - caesalpin, but the peaks above $\frac{m}{e}$ 200 occurred at two mass units less for the former compound, owing to the additional



M−18 $\frac{m}{e}$ 416

$\downarrow -H_2O$

$\frac{m}{e}$ 398

$\downarrow -CH_3CO_2H$

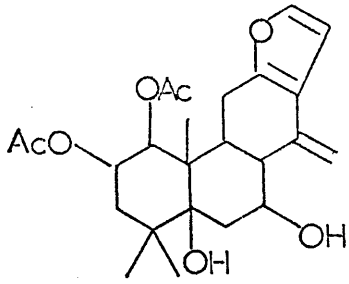
$\frac{m}{e}$ 338

$\downarrow -CH_3CO_2H$

$\frac{m}{e}$ 278

$\downarrow -CH_3\cdot$

$\frac{m}{e}$ 263



M−18 $\frac{m}{e}$ 432

$\downarrow -2H_2O$

$\frac{m}{e}$ 396

$\downarrow -CH_3CO_2H$

$\frac{m}{e}$ 336

$\downarrow -CH_3CO_2H$

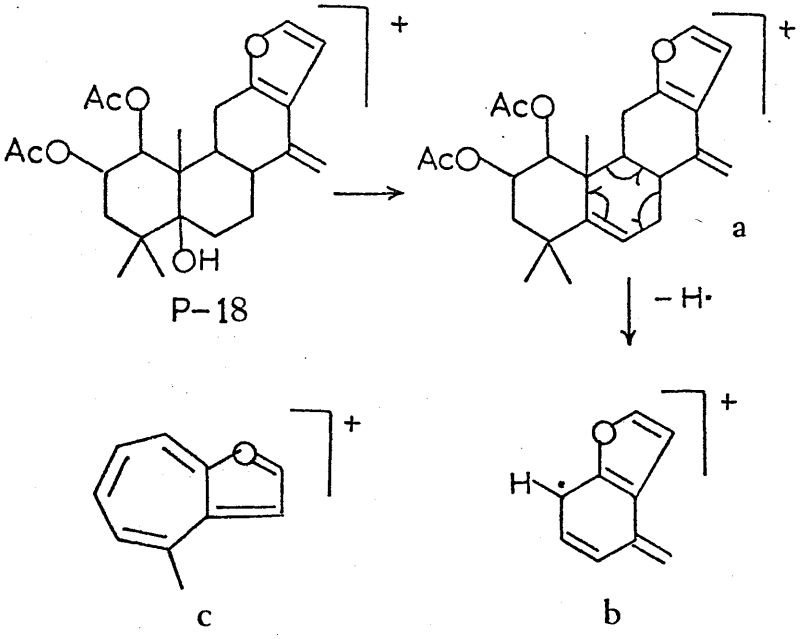
$\frac{m}{e}$ 276

$\downarrow -CH_3\cdot$

$\frac{m}{e}$ 261

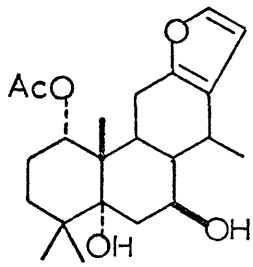
Scheme 11

loss of water from the parent ion (scheme (11)). The lower mass region was exactly the same for both compounds, the only noteworthy ions occurring at $\frac{m}{e}$ 145, 131, 91 and 43. The ion at $\frac{m}{e}$ 131 (b) may be attributed to retro Diels Alder cleavage of the P - 36 ion (a).



The fragment at $\frac{m}{e}$ 145 is common to the mass spectra of all compounds in this series which have a tertiary hydroxyl at $C_{(14)}$, and is probably due to the highly stabilised tropylium derivative [69] (c).

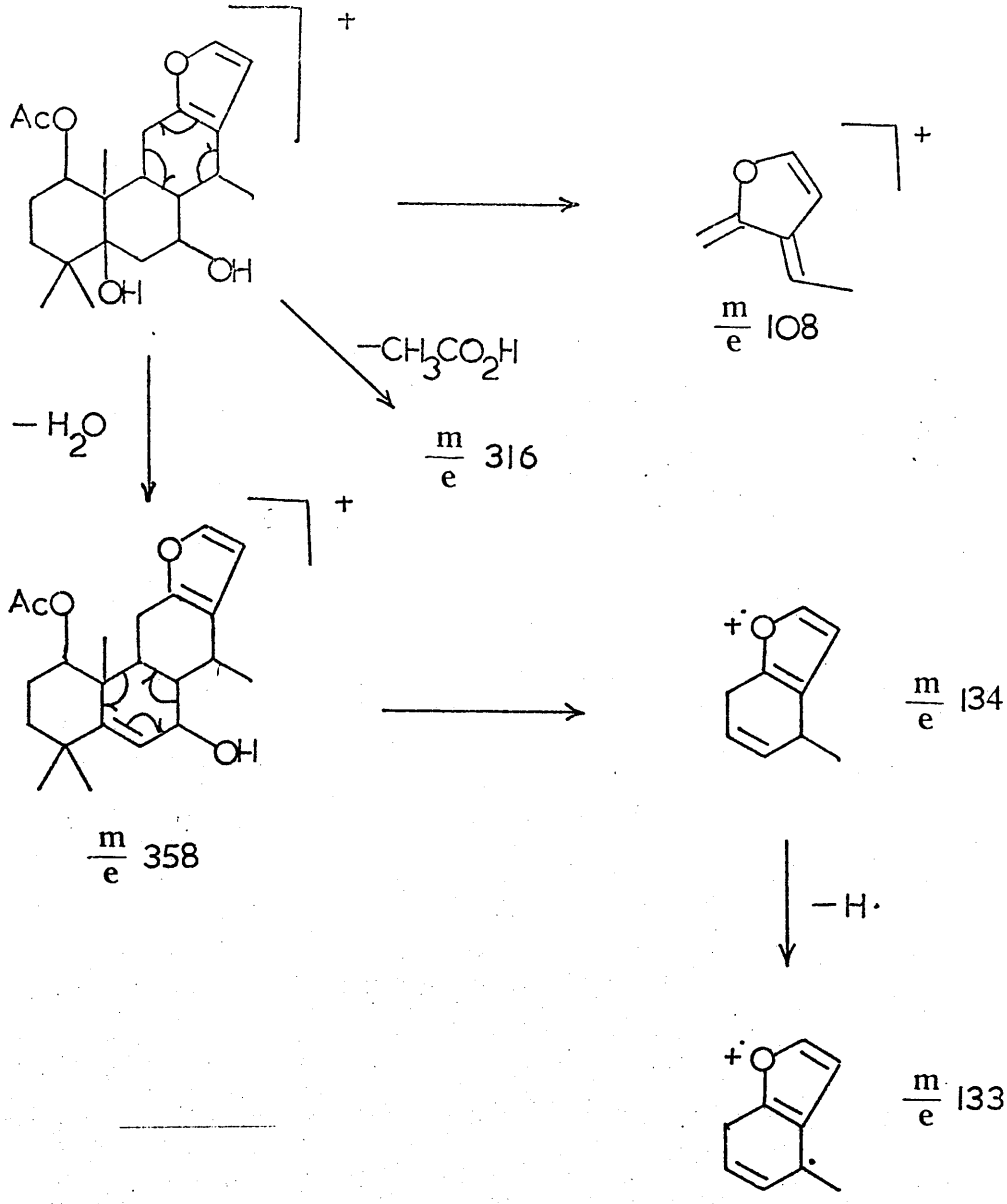
(4) The monoacetate 103, $C_{22}H_{32}O_5$, m.p. 167°, was eluted from the column using 10% chloroform/benzene. The infra-red spectrum of this compound showed acetate and hydroxyl absorption ($\nu_{max}^{CHCl_3}$ 3591, 3579, 1739 $cm.^{-1}$). The n.m.r. spectrum confirmed the presence of an acetate (τ = 7.97 (singlet, CH_3COO-), 5.14 (broad singlet, >CHOAc)) and in addition showed that one of the hydroxyl groups must be secondary (τ = 5.85 (double triplet, J = 11, 6 Hz., >CHOH)). The appearance of only three tertiary methyl singlets (τ = 8.97, 8.93, 8.90) suggested that a secondary methyl group was present at $C_{(14)}$ as in most of the naturally occurring cassanes,[72] with the remaining tertiary hydroxyl at $C_{(5)}$. This was supported by the stability of this compound



103

to treatment with mild acid under conditions which would have effected dehydration of any tertiary hydroxyl at $C_{(14)}$.

The positioning of the secondary acetate at $C_{(1)}$ is based solely on analogy with the n.m.r. spectra of the other $C_{(1)}$ acetates of this series, all of which display broad singlets around τ 5.15. Assignment of the secondary hydroxyl to the $C_{(7)}$ position follows from the multiplicity of the >CHOH signal, which requires three adjacent hydrogens. In addition, the observed coupling constants of this

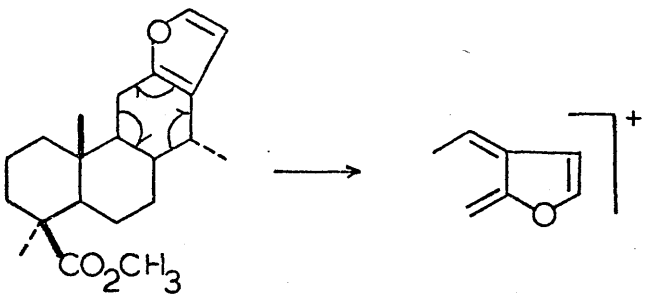


Scheme 12

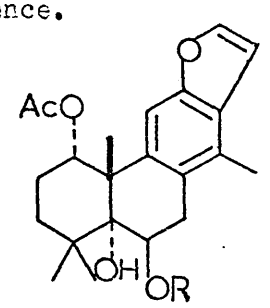
resonance can best be correlated with the appropriate dihedral angles of a chair ring B in which the $C_{(7)}$ hydroxyl is β - oriented.

The most significant ions in the mass spectrum of 103 are at $\frac{m}{e}$ 316, 134, 133 and 108, and are presumed to arise as shown in scheme (12). The retro-Diels Alder cleavage leading to the ion at $\frac{m}{e}$ 108 has previously been observed by Fetizon[50] in the spectrum of methyl voucapenate 104.

104



(5) Also isolated from the extract was a benzofuran monoacetate, $C_{22}H_{28}O_5$, m.p. 209-211°, which is assigned structure 105 on spectroscopic evidence.

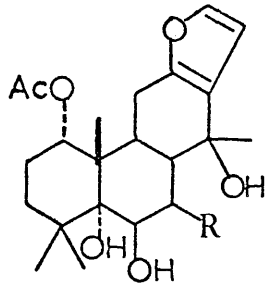


105 R=H
56 R=Ac

The ultraviolet spectrum displayed the typical benzofuran absorption bands at 250 mm. (ε 7,500), 281 mm. (ε 2700) and 291 nm. (ε 2900). This was confirmed by the n.m.r. spectrum (τ = 3.27, 2.45 (doublets, J = 2 Hz, furan protons), 7.64 (singlet, Ar - $C\underline{H}_3$), 2.95 (singlet, Ar - $\underline{H}$)), which also revealed the presence

of one secondary acetate (τ = 8.08 (singlet, $\underline{CH}_3COO$ -), 4.35 broad singlet, $\geq \underline{CH}OAc$)) and a secondary hydroxyl (τ = 5.50 (triplet, J = 8 Hz, $\geq \underline{CH}OH$)). The chemical shift of the $\geq \underline{CH}OAc$ proton compares favourably with that of τ 4.32 observed for the $C_{(1)}$ acetate proton of the benzofuran 56, suggesting that the acetate of 105 is also situated at the 1 - position. Double irradiation studies showed that the $\geq \underline{CH}OH$ proton resonated as the X-part of an ABX system involving the benzylic $C_{(7)}$ hydrogens, thus confirming the placing of the secondary hydroxyl at $C_{(6)}$.

It is most probable that this compound is an artefact arising from a non-aromatic precursor such as 106.



106, R=H, OH or OAc

(6) From one of the more polar fractions of the extract was obtained a very small amount of a compound, m.p. 193-195°, which appeared to contain a cyclopropane ring. Analysis and mass spectroscopy indicated the molecular formula $C_{20}H_{34}O_2$, while the n.m.r. spectrum showed the presence of four tertiary methyl groups (τ = 9.28, 9.22, 9.17 and 8.82 (singlets, each 3H)) two secondary hydroxyls (τ = 6.80 (triplet, J = 10 Hz.), 6.22 (double triplet, J = 10, 5, 2 Hz, 2>$\underline{CH}OH$)

and a cyclopropane ring (τ = 9.74, 9.47 (multiplets, each 1H)). As yet the structure of this compound has not been established.

EXPERIMENTAL

All melting points were recorded on a Kofler hot-stage apparatus and are uncorrected. Routine infra-red spectra (carbon tetrachloride or chloroform solutions) were recorded on a Perkin-Elmer 257 instrument, and high resolution spectra were obtained using a Unicam S.P.100 double beam spectrometer equipped with an S.P. 130 sodium chloride prism grating monochromator operated under vacuum.

Ultraviolet absorption spectra were measured in ethanol solution using a Unicam S.P. 800 Spectrometer.

Nuclear magnetic resonance spectra were obtained on Perkin Elmer R - 10, Varian T - 60 or Varian HA 100 instruments, using tetramethylsilane as an internal reference in deuterochloroform unless otherwise stated.

Mass spectra were routinely determined on an A.E.I. - M.S. 12 spectrometer high resolution, spectra being obtained on a G.E.C. - A.E.I. M.S. 9 instrument.

Chromatographic separations were effected using commercial 'Woelm' alumina for column separations and Merck's 'Kieselgel G', deactivated according to the Brockman scale,[26] for thin and thick layer chromatoplates.

Light petroleum refers to the fraction boiling between 60-80°.

The seed kernels of Caesalpinia bonducella (Fleming) (2.47 kg.) were ground to a fine powder and left to stand in light petroleum (5l.) for 3 days at 20°. The slurry was filtered, washed with light petroleum, and the defatted seed kernels extracted with ethyl acetate (5l.) for 50 hours. Filtration, followed by evaporation of the solvents in vacuo gave a dark-brown viscous oil (51g.), which gradually solidified.

The total extract was dissolved in a minimum volume of benzene and chromatographed on neutral 'Woelm' alumina (grade V, 1.5 kg.) using the technique of gradient elution. Light petroleum was used initially to remove any residual fats, and the polarity of the eluting solvent was increased gradually through benzene and chloroform to 5% methanol/ chloroform for the most polar fractions.

After characterisation of each fraction by analytical t.l.c., the appropriate fractions were combined, and a sequence of preparative t.l.c. separations was carried out to purify the individual components of the extract.

ε- caesalpin 5

ε- caesalpin crystallised from ether as very fine needles m.p. 191-194°; $(\alpha)_D$ + 2° (chloroform); $\nu_{max}^{CCl_4}$ 3596 (hydroxyl), 1758, 1745 (acetates) $cm.^{-1}$; λ_{max} 215 nm (ε 7,800) ;

(Found : C, 66.60 ; H, 7.90% ; $C_{24}H_{34}O_7$ requires C, 66.35 ; H, 7.90%)

α- caesalpin 1

Recrystallisation from ethyl acetate/ether afforded α - caesalpin as white needles, m.p. 159° (reported[2e] 160°) ; $(\alpha)_D$ = + 34° (ethanol) (reported[2e] + 35°); $\nu_{max}^{CCl_4}$ 3597, (hydroxyl), 1758 (acetate) 1720, (ketone) cm.$^{-1}$; λ_{max} 216 nm. (ε 8600); τ = 8.83, 8.66, 8.51, 8.48 (singlets 4 CH_3 - $\overset{|}{C}$ -), 8.02, 7.93 (singlets, 2 CH_3COO -), 2.84, 3.70 (doublets, each 1H, furan protons) 4.50, 4.51 (multiplets, each 1H, 2 >CHOAc)

(Found : C, 64.05 ; H, 7.35% ; $C_{24}H_{32}O_8$ requires C, 64.30 ; H, 7.20%)

1, 6, 7 triacetoxy δ - caesalpin 54.

The amorphous triacetate of δ - caesalpin was purified by preparative t.l.c. followed by precipitation from ether solution by dropwise addition of light petroleum. The powdery compound thus obtained had $\nu_{max}^{CHCl_3}$ 3587 (hydroxyl), 1748, 1742, 1250 (acetates), cm.$^{-1}$, λ_{max} 218 nm. (ε 6000); τ = 8.51, 8.74, 8.85, 8.85 (singlets, 4 CH_3 - $\overset{|}{C}$ -), 7.97, 7.97, 8.07 (singlets, 3 CH_3 - COO -), 5.21 (broad singlet, > $C_{(1)}$HOAc), 4.41 (2H multiplet, 2>CHOAc)

(Found ; C, 63.40 ; H, 7.60% ; $C_{26}H_{36}O_9$ requires C, 63.40 ; H, 7.40%)

Hydroxy - diacetate 100

Two recrystallisations from ethyl acetate/light petroleum afforded pure white needles of the diacetate, m.p. 178-179°, $\nu_{max}^{CCl_4}$ 3603, 3549 (hydroxyl), 1754 (acetate), cm.$^{-1}$ $(\alpha)_D$ = + 35° (chloroform) ; τ = 8.87, 8.82, 8.80, 8.47 (singlets, $4CH_3 - \overset{|}{C} -$), 8.01, 7.93 (singlets $2\underline{CH}_3COO$ -) 4.48, 4.50 (multiplets, 2 >$\underline{CH}$OAc), 6.35 (broad singlet, >$\underline{CH}$OH).

(Found : C, 63.90 ; H, 7.70% ; $C_{24}H_{34}O_8$ requires
C, 64.00 ; H, 7.60%)

Monoacetate 103

White prisms m.p. 167°, containing water of crystallisation were obtained on recrystallisation of the monoacetate 103 from aqueous methanol. $\nu_{max}^{CHCl_3}$ 3591, 3579 (hydroxyl), 1739 (acetate), cm.$^{-1}$

(Found : C, 68.50 ; H, 8.55% ; $C_{22}H_{32}O_5 \cdot \frac{1}{2} H_2O$ requires
C, 68.60 ; H, 8.65%)

7 - hydroxy - ε - caesalpin 102

This compound was obtained as colourless needles m.p. 186-187°, from ethyl acetate/ether. $\nu_{max}^{CCl_4}$ 3585 (hydroxyl), 1758, 1746 (acetates) cm.$^{-1}$

(Found : C, 64.30 ; H, 7.50% ; $C_{24}H_{34}O_8$ requires
C, 64.00 ; H, 7.60%)

Benzofuran monoacetate 105

The Benzofuran monoacetate 105 was obtained as prisms, m.p. 209-211° from ether/light petroleum and was identified from the n.m.r. spectrum (page 77). $\nu_{max}^{CCl_4}$ 3590, 3512 (hydroxyl), 1742 (acetate) $cm.^{-1}$
(Found : C, 71.10 ; H, 7.80% ; $C_{22}H_{28}O_5$ requires
C, 70.95 ; H, 7.60%)

Lithium Aliminium Hydride Reduction of ε - caesalpin.

A solution of ε - caesalpin (330 mg.) in anhydrous ether (15 ml.) was heated under reflux for two hours with lithium aluminium hydride (150 mg.). A saturated solution of sodium sulphate was added dropwise to the cooled reaction mixture, until evolution of hydrogen had ceased and a white precipitate had formed. Filtration through celite 535 and removal of the solvent gave a crystalline mixture of two products which were separated by preparative t.l.c. using 5% methanol in chloroform as eluting solvent.

The main component (198 mg.) was the tetraol 6 m.p. 194-196° (ethyl acetate).

(Found : C, 68.30 ; H, 8.20% ; $C_{20}H_{30}O_5$ requires
C, 68.55 ; H, 8.54%)

The minor product from the reaction was the exomethylene compound 7 (75 mg.), recrystallised from ether/ethyl acetate as needles m.p. 183-185°. λ_{max} 232 nm. (ε 8600) 213 nm (ε 9400); $\nu_{max}^{CHCl_3}$ 3553, 3470 (hydroxyl), 1643 (furan) 1600, 902 (exomethylene) cm.$^{-1}$
(Found : C, 72.40 ; H, 8.75% ; $C_{20}H_{28}O_4$ requires
C, 72.25 ; H, 8.50%).

Acetylation of tetraol 6

A solution of the tetraol 6 (50 mg.) in pyridine (1 ml.) and acetic anhydride (0.5 ml.) was allowed to stand at room temperature overnight. Addition of methanol and removal of the solvent after about ten minutes yielded the monoacetate 9 as a gum (56 mg.), which crystallised from aqueous methanol as prisms, m.p. 195-197° ; $\nu_{max}^{CHCl_3}$ 3590, 3478 (hydroxyl), 1737 (acetate), 1642 cm.$^{-1}$;
(Found : C, 67.50 ; H, 8.30% ; $C_{22}H_{32}O_6$ requires
C, 67.30 ; H, 8.20%)

Acetylation of exomethylene triol 7

The exomethylene compound 7 (47 mg.) was acetylated as above and the resultant monoacetate 8 was recrystallised from ether/light

petroleum as needles m.p. 203-205° ; λ_{max} 232 nm. (ε 8600) ;
$\nu_{max}^{CCl_4}$ 3598, 3515 (hydroxyl), 1746 (acetate), cm.$^{-1}$
(Found : C, 70.90 ; H, 8.25% ; $C_{22}H_{30}O_5$ requires
C, 70.55 ; H, 8.10%).

Sodium meta-periodate cleavage of tetraol 6

To a solution of the tetraol 6 (100 mg.) in methanol (5 ml.) was added sodium meta-periodate (85 mg.) in water (1.5 ml.) and the mixture left at 20° for one hour. The reaction mixture was diluted with water, thoroughly extracted with chloroform, the combined extracts being washed several times with brine, dried and evaporated to give a mixture of the epimeric hemiacetal aldehydes 12. Preparative t.l.c. gave one epimer as needles (m.p. 158-159°, 24 mg.) and the other as a gum (16 mg.).
$\nu_{max}^{CHCl_3}$ (both epimers) 3604, 3468 (hydroxyl), 1711 (aldehyde) cm.$^{-1}$
τ = 9.07, 8.89, 8.85, 8.65 (singlets, 4 $CH_3-\overset{|}{\underset{|}{C}}-$), 4.50 (multiplet, $\simeq$ C<u>H</u>-OH), - 0.1 (singlet, - C<u>H</u>O).

Attempted oxidation of hemiacetal 12

A solution of the mixture of epimers 12 (5 mg.) in acetone at 0° was treated with Jones reagent (2 drops). Normal work-up procedure afforded only acidic products, resulting probably by oxidative cleavage

of the furan ring. Further small-scale attempts to obtain the desired lactone acid 14 using methods described by Sarett,[20] Snatzke,[21] and Filler[22] were similarly unsuccessful.

Acid Treatment of ε - caesalpin.

A solution of hydrochloric acid in chloroform was prepared by bubbling HCl gas from a generator through chloroform (10 ml.) for three minutes. The resulting solution was used in each of the following reactions.

(1) ε - caesalpin 5 (181 mg.) in chloroform (5 ml.) was treated with ten drops of the above solution and the mixture left at 20° for thirty minutes. Following the reaction by t.l.c. at five minute intervals showed that the initial product was the exomethylene compound 20, which on standing formed the benzofuran 19 and the dihydrobenzofuran 18. Evaporation of the chloroform and preparative t.l.c. of the product (20% light petroleum/chloroform) gave the benzofuran 19 (98 mg.), which crystallised from ether/light petroleum as needles, m.p. 191-192°;

λ_{max} 251 nm. (ε 7,500), 282 nm. (ε 2,700), 292 nm. (ε 2800)[5];

$\nu_{max}^{CCl_4}$ 3591 (hydroxyl), 1755, 1750 (acetates), 1605 (aromatic ring) $cm.^{-1}$

(Found : C, 69.30 ; H, 7.20% ; $C_{24}H_{30}O_6$ requires

C, 69.55 ; H, 7.30%)

The dihydrobenzofuran 18 (47 mg.) was obtained as colourless needles from ethyl acetate/ether, m.p. 210-211° ; λ_{max} 225 nm. (ε 7000), 287 nm. (ε 4800), 291 nm (ε 5000)[5]; $\nu_{max}^{CCl_4}$ 3591 (hydroxyl), 1755, 1748 (acetates) cm.$^{-1}$

(Found : C, 69.00 ; H, 8.00% ; $C_{24}H_{32}O_6$ requires C, 69.2 ; H, 7.75%).

(2) The acid reagent (3 ml.) was added rapidly to a solution of ε-caesalpin (41 mg.) in chloroform (1 ml.). Removal of the solvent *in vacuo* after ten minutes afforded, virtually in quantitative yield, the dihydrobenzofuran 18, which was decolourised and recrystallised to give a pure sample identical (i.r., m.p.) with that prepared in (1) above.

(3) The acid treatment of ε - caesalpin was carried out as in (1), except that the reaction mixture was maintained under an oxygen atmosphere while bubbling oxygen through the solution. All solvents were flushed with oxygen before use. After removal of solvent, the yields of the two products were found to be exactly the same as in part (1). An analogous procedure using nitrogen rather than oxygen caused no alteration in the product distribution.

D.D.Q. Oxidation of dihydrobenzofuran 18.

The dihydrobenzofuran 18 (75 mg.) was dissolved in benzene (5 ml.) and heated under reflux for 60 hours with 2, 3 - dichloro - 5, 6 - dicyano - p - benzoquinone (70 mg.). The cooled reaction mixture was filtered through celite 535, evaporated and chromatographed in 20% light petroleum/chloroform, furnishing the benzofuran 19 (18 mg., 24%) as well as starting material (22 mg.) and other unidentified products. The benzofuran thus prepared was identical (t.l.c., i.r., n.m.r.) with an authentic specimen.

Lithium aluminium hydride reduction of dihydrobenzofuran.

A solution of the dihydrobenzofuran 18, (25 mg.) in anhydrous ether was stirred at 20° for one hour with lithium aluminium hydride (20 mg.). Excess reagent was destroyed by dropwise addition of sodium sulphate solution, the resultant precipitate was removed by filtration and the ethereal solvent was evaporated. This gave the triol 24 (21 mg.) which crystallised from ethyl acetate as small prisms, m.p. 263-265°, $\nu_{max}^{CCl_4}$ 3580, 3500 (hydroxyl) cm.$^{-1}$; τ = 8.73, 8.85, 8.88 (singlets, 3 CH_3 - C -), 7.86 (singlet, Ar - CH_3), 6.86, 5.45 (triplets, J = 9 Hz., dihydrofuran methylenes), 5.62 (doublet, J = 2 Hz $>C_{(1)}$ HOH), 5.82 (double quartet, J = 12, 5, 2 Hz., $>C_{(2)}$ HOH).

(Found : C, 71.95 ; H, 8.20 ; $C_{20}H_{28}O_4$ requires
C, 72.25 ; H, 8.50).

Sodium meta-periodate cleavage of triol 24.

The triol 24 (72 mg.) in methanol (4 ml.) was allowed to stand for three hours with sodium meta-periodate (65 mg.) in water (1.5 ml.). The reaction mixture was diluted with water and the products extracted into ethyl acetate. The extracts were washed, dried and evaporated in the usual way to leave a residual gum (59 mg.) which contained the two epimers of the hemiacetal 25. Chromatographic separation of these epimers proved extremely difficult, and consequently the recorded physical data are for a mixture of the two components: m.p. 197-199° (ether) ; $\nu_{max}^{CCl_4}$ 3605 (hydroxyl), 2710, 1719 (aldehyde) $cm.^{-1}$;
τ = 8.82, 8.60, 8.99 (singlets, 3 CH_3 - C -), 7.84 (singlet, Ar-CH_3), 6.93, 5.51 (triplets, J = 9 Hz., dihydrofuran methylenes), 3.89 (singlet, Ar-H), -0.03 (singlet, -CHO), 4.49 (triplet, J = 6 Hz, $\geq$ CH-OH).
(Found : C, 72.65 ; H, 7.75% ; $C_{20}H_{26}O_4$ requires
C, 72.70 ; H, 7.95%).

Jones Oxidation of hemiacetal 25.

8N. Jones reagent was added dropwise to a stirred ice cold solution

of the mixture of hemiacetal epimers 25 (44 mg.) in acetone (4 ml.) until a permanent orange coloured solution was obtained. Water was added and the mixture extracted with ethyl acetate, the extracts being washed with brine, dried and the solvent removed to furnish the crystalline γ - lactone 26 (37 mg.), m.p. 289-292° decomp. (ethylacetate/ether) ; $\nu_{max}^{CCl_4}$ 1778 (lactone), 1717 (aldehyde) cm.$^{-1}$; τ = 8.52, 8.74, 8.88 (singlets, 3 CH_3 - C -), 7.84 (singlet, Ar-CH_3), 6.89, 5.46 (triplets, J = 9 Hz., dihydrofuran methylenes) 3.88 (singlet, Ar-H), 0.08 (singlet, -CHO).

(Found : C, 73.05 ; H, 7.55% ; $C_{20}H_{24}O_4$ requires C, 73.15 ; H, 7.4%).

Dehydration of ε - caesalpin.

A solution of ε - caesalpin (50 mg.) in dry Analar acetone (10 ml.) was stirred at room temperature for 35 minutes with anhydrous copper sulphate (220 mg.). Filtration and evaporation of the solvent under reduced pressure at 20° afforded a gum, comprising mainly the exomethylene compound 20, λ_{max} 232 nm. (ε 8500), 212 nm. (ε 9000), which refused to crystallise and could not be purified by normal chromatographic procedures. Analytical t.l.c. indicated the tendency of this compound to undergo rapid aerobic dehydrogenation to the

benzofuran 18. Accordingly, the physical data presented are only representative of a crude sample.

$\nu_{max}^{CHCl_3}$ 3590 (hydroxyl), 1758, 1745 (acetates), 895 (exomethylene) cm.$^{-1}$; mass spectrum : $M^+ = 428$ ($C_{24}H_{32}O_6$ requires $M^+ = 428$).

p-Bromobenzoate 27 of ε - caesalpin.

To a solution of the tetraol 6 (35 mg.) in dry pyridine (1.5 ml.) was added recrystallised p -bromobenzoyl chloride (40 mg.) in pyridine (1 ml.) and the mixture set aside overnight. The reaction was diluted with water, extracted with ethyl acetate and the combined extracts thoroughly washed with dilute mineral acid, bicarbonate, and brine. Drying and evaporation of the solvent gave a non crystalline solid containing two t.l.c. spots in the ratio 70 : 30. The minor spot corresponded in R_f value to the unreacted tetraol 6, while the major product was shown, after preparative t.l.c. in 5% methanol/ chloroform, to be the p -bromobenzoate 27, obtained as colourless needles, m.p. 169-170°, from ethyl acetate/ether.

τ = 8.90, 8.84, 8.78, 8.65 (singlets, 4 CH_3 - C -), 6.06 (doublet, J = 2 Hz, >CHOH), 4.48 (double quartet, J = 12, 5, 2 Hz >CHOCO.Ph.Br), 2.20 (A_2B_2 quartet, aromatic protons).

(Found : C, 61.05 ; H, 6.50% ; $C_{27}H_{33}O_6Br$ requires C, 60.80 ; H, 6.25%).

In order to obtain crystals which would be amenable to X-ray analysis, a pure specimen of the bromobenzoate was crystallised in a flat bottomed vessel by controlled evaporation of solvent (acetone: ether, 50 : 50). By this method the derivative was deposited as crystals belonging to the monoclinic space group $P2_1$.

Tosylation of tetraol 6.

Recrystallised p - toluenesulphonyl chloride (29 mg. 0.016 m.moles) was added to a solution of the tetraol 6 (43 mg., 0.012 m.moles) in anhydrous pyridine (1.5 ml.) and the reaction mixture was set aside for five days at room temperature. Ethyl acetate and water were added and the organic layer was washed with dilute mineral acid, bicarbonate, brine then dried and the solvent removed to give a gummy residue which after chromotography in 1% methanol/chloroform yielded the desired tosylate 28 (44 mg.). Crystallisation from ether/light petroleum provided needles, m.p. 126-128° : $\nu_{max}^{CHCl_3}$ 3580, 3485 (hydroxyl), 1189, 1177 (S = O) cm.$^{-1}$

(Found : C, 64.05 ; H, 7.25% ; $C_{27}H_{36}O_7S$ requires C, 64.25 ; H, 7.2%).

Lithium aluminium hydride reduction of tosylate 28.

The tosylate 28 (27 mg.) in anhydrous ether (4 ml.) was heated

under reflux with excess lithium aluminium hydride (21 mg.) for 2 hours. The cooled reaction mixture was worked up in the usual way to give a mixture of products which could not be separated by preparative t.l.c. The ultraviolet spectrum of the crude mixture had bands at 232, 250, 280 and 290 nm., showing that most of the products had resulted from aromatisation of ring C.

Hydrogenation of exomethylene triol 7.

The exomethylene triol 7 (55 mg.) was hydrogenated in ethanol solution with a trace of triethylamine by shaking with 10% palladium/charcoal (40 mg.) under hydrogen at one atmosphere pressure. After twenty minutes, uptake of hydrogen had ceased, and the solution was filtered through celite 535 and evaporated *in vacuo* affording a quantitative yield of the triol 31. Colourless needles, m.p. 196°, were obtained from ethyl acetate/light petroleum.

$\nu_{max}^{CHCl_3}$ 3550, 3470 (hydroxyl) $cm.^{-1}$; λ_{max} 214 nm. (ε 7500) ; τ = 9.02 (6H), 8.94(3H) (singlets, 3 CH_3 - C -), 5.40 (diffuse doublet, J = 2 Hz, >CHOH) 5.04 (double quartet, J = 12, 5, 2 Hz., >CHOH), 2.80 (doublet, J = 2 Hz, $C_{(16)}$-H), 3.83 (pair of doublets, each J = 2Hz, $C_{(15)}$- H).

(Found : C, 71.80 ; H, 8.90% ; $C_{20}H_{30}O_4$ requires C, 71.8 ; H, 9.0%).

Tosylate of triol 31.

To a solution of the triol 31 (40 mg.) in dry pyridine (1 ml.) was added recrystallised p-toluenesulphonyl chloride (34 mg.) in pyridine (0.5 ml.) and the mixture allowed to stand at 20° for three days. Normal work-up procedure afforded the highly crystalline tosylate 32 (51 mg., 86%) which crystallised as fine needles, m.p. 168-170° from ethyl acetate/light petroleum.

$\nu_{max}^{CHCl_3}$ 3580, 3480 (hydroxyl), 1170 (S = O) cm.$^{-1}$; τ = 2.42 (A_2B_2 quartet, J_{AB} = 8 Hz, aromatic protons), 5.13 (double quartet, J = 12, 5, 2 Hz., $\geq$ C<u>H</u>OTos), 6.32 (diffuse doublet, J = 2 Hz, $\geq$C<u>H</u>OH).

(Found : C, 66.55 ; H, 7.50% ; $C_{27}H_{36}O_6S$ requires C, 66.4 ; H, 7.45%).

Attempted Hydrogenolysis of tosylate 32.

Excess lithium aluminium hydride (25 mg.) was added to a stirred solution of the tosylate 32, (26 mg.) in anhydrous ether (5 ml.) and the stirring continued for a further two hours. The usual work up for hydride reductions afforded a crude product (19 mg.) which on t.l.c. analysis contained three compounds in the ratio 3 : 2 : 1. Preparative scale chromatography (0.25 mm. plate) in 2% methanol/ chloroform gave returned starting material (8 mg.), the triol 31 (3 mg.), and the ring contracted primary alcohol 34 (5 mg.).

Crystallisation of the latter compound from ether gave colourless prisms, m.p. 158-160°.

τ = 8.97 (3H), 8.87 (6H) (singlets, 3 $C\underline{H}_3$ - C -), 6.37 (AB quartet, J_{AB} = 12 Hz, $>C\underline{H}_2OH$).

(Found : C, 75.55 ; H, 9.55% ; $C_{20}H_{30}O_3$ requires C, 75.45 ; H, 9.50%).

Acetylation of 34 in acetic anhydride/pyridine gave the primary acetate 35 as a gum : $\nu_{max}^{CHCl_3}$ 3589 (hydroxyl), 1742 (acetate) cm.$^{-1}$; τ= 8.03 (singlet $C\underline{H}_3COO$-), 5.68 (AB quartet, J_{AB} = 12. Hz, $>C\underline{H}_2OAc$) ; mass spectrum : M^+ = 360 ($C_{22}H_{32}O_4$ requires M^+ = 360).

Tetrahydropyranyl ether[23] of tosylate 32

A solution of the tosylate 32 (15 mg.) in freshly distilled dihydropyran (0.5 ml.) was allowed to stand overnight with one small drop of $POCl_3$. Ether and water were added and the organic layer was washed several times with water, dried and evaporated. The product was shown by t.l.c. and ir. spectrum to contain mainly returned starting material.

Benzyl Thioether[73] of tosylate 32.

Sodium metal (8 mg.) was added to benzyl mercaptan (0.12 ml.) and the mixture allowed to stand until the sodium had dissolved.

The solution was taken up in dry dimethylformamide (D.M.F., 0.5 ml.) and added to a solution of the tosylate 32 (15 mg.) in D.M.F. (1 ml.). The reaction mixture was heated under dry nitrogen in an oil bath at 90° for six hours, water was added and the products extracted into ether. The combined extracts were washed repeatedly with water, dried and the solvent removed, furnishing an oily product. The only component which could be isolated by preparative t.l.c. was a gum (5 mg.); $\nu_{max}^{CCl_4}$ 3590 (hydroxyl), 1740 (carbonyl), presumed to be the ring contracted aldehyde 43.

Carbonate[27] of triol 32.

The triol 32 (14 mg.) in pyridine (1 ml.) was treated with ethyl chloroformate (0.2 ml.) and the mixture allowed to stand overnight. Normal work-up followed by preparative t.l.c. gave a product (7 mg.) which had infra-red bands corresponding to the expected carbonate 44. ($\nu_{max}^{CHCl_3}$ 3590, 3480 (hydroxyl), 1740 (C = O) $cm.^{-1}$) Without further purification this sample was vacuum sealed into a pyrex tube and heated to 250-260° in a sublimation block for one hour. The brownish pyrolysis product, which was predominantly one compound by t.l.c., had a strong absorption at 1797 $cm.^{-1}$, and was thought to be the cyclic carbonate 47.

Lithium Aluminium hydride reduction of benzofuran 19.

The benzofuran 19 (135 mg.) in anhydrous ether (12 ml.) was stirred with lithium aluminium hydride (80 mg.) for twenty minutes at room temperature. Normal work-up for metal hydride reductions gave the triol 58 (102 mg.), which crystallised from di-isopropyl ether as needles, m.p. 227-230° ; $\nu_{max}^{CHCl_3}$ 3584, 3476 (hydroxyl) $cm.^{-1}$ Mass spectrum : $M^+ = 330$ ($C_{20}H_{26}O_4$ requires $M^+ = 330$).

A sample of the triol 58 (68 mg.) was acetylated overnight in acetic anhydride/pyridine. Preparative t.l.c. of the product after work-up gave 67 mg. of the monoacetate 59, obtained as small prisms, m.p. 210-211°, from ether/light petroleum : $\nu_{max}^{CHCl_3}$ 3595, 3503 (hydroxyl), 1741 (acetate) $cm.^{-1}$; τ = 7.93 (singlet, $C\underline{H}_3COO$ -), 4.59 (double quartet, J = 12, 5, 2 Hz., >C<u>H</u>OAc), 5.45 (diffuse doublet, J = 2 Hz., >C<u>H</u>OH).

(Found : C, 70.50 ; H, 7.75% ; $C_{22}H_{28}O_5$ requires C, 70.95 ; H, 7.6%).

Sarett oxidation[20] of monoacetate 59.

The Sarett reagent was prepared by dissolving chromium trioxide (30 mg.) in dry pyridine (1 ml.) at 0°. To this was added a solution of the monoacetate 59 (10 mg.) in pyridine (0.5 ml.) and the mixture was stirred at 0° for thirty minutes, then allowed to stand for

sixteen hours at room temperature. Addition of ice and normal ethyl acetate/water work-up gave only returned starting material (8 mg.), identified by t.l.c. and i.r. spectrum.

Jones oxidation of monoacetate 59.

The benzofuran monoacetate 59 (45 mg.) in acetone (3 ml.) at 0° was oxidised by dropwise addition of 8N Jones reagent until a permanent orange colouration was observed. The reaction was worked up as described previously and furnished an amorphous solid (37 mg.) which contained several components by t.l.c. Preparative scale chromatography gave the desired keto-acetate 60 (12 mg.) as well as starting material 59 (16 mg.). The keto-acetate 60 crystallised from di-isopropyl ether as needles, m.p. 174-178° ; $\nu_{max}^{CHCl_3}$ 3590, 3470 (hydroxyl) 1740-1730 (acetate, ketone) $cm.^{-1}$.

(Found : C, 71.05 ; H, 7.05% ; $C_{22}H_{26}O_5$ requires
C, 71.35 ; H, 7.1%).

Attempted reductive cleavage of the keto-acetate 60.

(1) With zinc and acetic acid.

To a stirred solution of the keto-acetate 60 (3 mg.) in acetic acid (2 ml.) was added zinc dust (80 mg.), and the mixture was heated

under reflux for 19 hours. The reaction mixture was diluted with ethyl acetate and water, filtered and the organic layer washed with bicarbonate, brine, then dried and evaporated. Analytical t.l.c. of the gummy residue showed that an array of about nine products had been formed, with none present in a major amount.

(2) With calcium in liquid ammonia.[37,38]

A solution of the keto-acetate 60 (13 mg.) in dry toluene (2 ml.) was added dropwise with vigorous stirring to calcium turnings (4 mg.) in freshly distilled liquid ammonia (5 ml.). The addition was made in two minutes, the mixture was stirred for a further three minutes, and excess reagent was destroyed by addition of bromobenzene (5 drops). Water (10 ml.) was added and the products were extracted into ethyl acetate. Washing with brine, drying and removal of solvent gave a crude product which was a mixture of five compounds.

(3) With chromous chloride.

A modification of the procedure of Rosencranz and Djerassi[39] was employed.

Amalgamated zinc dust was prepared by shaking vigorously 10g. of zinc dust, 0.8g. of mercuric chloride, 10 ml. of water and 0.5 ml. concentrated HCl for five minutes. The supernatant liquid was decanted, and a further 20 ml. water and 2 ml. conc. HCl were added to

the solid residue. Chromic chloride (5g.) was then added portionwise with stirring under a nitrogen atmosphere. The resultant dark blue solution of chromous chloride was kept under nitrogen until ready for use.

The keto-acetate 60 (5 mg.) in acetone (5 ml.) was treated with the above solution of chromous chloride (2 ml.), the addition being carried out with stirring under a nitrogen atmosphere. The mixture was then heated under reflux for 14 hours, brine was added to the cooled solution, and normal extraction/washing procedure produced a semi-crystalline solid (4 mg.). t.l.c. indicated two components, one of which corresponded in R_f value to the starting keto-acetate 60, and the other was subsequently shown to be the α- ketol 61. The i.r. spectra of these two compounds were identical with those of authentic samples.

Base hydrolysis of keto-acetate 60.

To a solution of the keto-acetate 60 (3 mg.) in ethanol (2 ml.) was added 10 drops of 1N potassium hydroxide, and the mixture heated gently on a steam bath for 1½ hours. The reaction was diluted with water, extracted with chloroform and the products worked up to give a gum (2 mg.): $\nu_{max}^{CHCl_3}$ 3560 (hydroxyl), 1784 (carbonyl) $cm.^{-1}$ Thus none of the desired ketol 62 was produced.

Acid hydrolysis of keto-acetate 60.

The keto-acetate 60 (34 mg.) in methanol (3 ml.) was treated with 5N hydrochloric acid solution (10 drops), and the mixture was set aside at 20° for three days. Ethyl acetate and water were added, the organic layer was separated, washed with brine, and the dried solvent removed to provide the crystalline α-ketol 61 (28 mg.). Crystallisation from di-isopropyl ether gave colourless needles, m.p. 175-177° : $\nu_{max}^{CCl_4}$ 3575, 3495 (hydroxyl), 1720 (ketone) cm.$^{-1}$; τ = 8.85, 8.59, 8.21 (singlets, 3CH_3 - C -), 7.61 (singlet, Ar - CH_3), 5.04 (quartet, J = 7,11 Hz, >C<u>H</u>OH)

Mass spectrum : M^+ = 328 ($C_{20}H_{24}O_4$ requires M^+ = 328).

Keto-Tosylate 64.

Recrystallised p-toluene sulphonyl chloride (21 mg.) was added to a solution of the ketol 61 (27 mg.) in dry pyridine (1 ml.) and the reaction mixture was allowed to stand at 20° for three days. Water was added and the usual ethyl acetate/water work-up gave an oily product (30 mg.), homogeneous by t.l.c., which proved to be the keto-tosylate 64 $\nu_{max}^{CCl_4}$ 3568, 3480 (hydroxyl), 1188, 1178 (S = O) cm.$^{-1}$.

The crude sample thus prepared was dissolved in acetone (4 ml.) and refluxed under a nitrogen atmosphere for 20 hours with chromous chloride solution (prepared as previously described). The cooled blue-green reaction mixture was diluted with water and extracted with

chloroform, the combined extracts being washed with brine, dried and the solvent evaporated to furnish a viscous gum (19 mg.). t.l.c. examination showed two constituents, one of which corresponded to the keto-tosylate 64. The other more polar component, isolated as a crystalline solid (9 mg.) after preparative t.l.c., was identified as the desired ring A-desoxy caesalpin 57. This compound crystallised as clusters of fine white needles, m.p. 172-175°, from di-isopropyl ether: $(\alpha)_D$ -2.8° (chloroform) ; $\nu_{max}^{CCl_4}$ 3629 (hydroxyl), 1712 (ketone) cm.$^{-1}$.

(Found : C, 76.65 ; H, 7.80% ; $C_{20}H_{24}O_3$ requires C, 76.9 ; H, 7.75%).

Tosylate 65 of benzofuran triol 58.

Tosylation of the benzofuran triol 58 (75 mg.) was carried out in the usual way and afforded the hydroxy-tosylate 65 (82 mg.). Two recrystallisations from di-isopropyl ether provided small colourless prisms, m.p. 171-172°; $\nu_{max}^{CHCl_3}$ 3580, 3480 (hydroxyl), 1170 (S = O)cm.$^{-1}$

(Found : C, 67.20 ; H, 6.70% ; $C_{27}H_{32}O_6S$ requires C, 66.9 ; H, 6.65%).

A sample of the above hydroxy-tosylate 65 (60 mg.) in acetone (4 ml.) was oxidised with Jones reagent according to the method previously described, in the hope of obtaining directly the keto tosylate

64. However the only products obtained were starting material (16 mg.), and several more polar compounds which were not identified.

Acid treatment of 1, 6, 7 - triacetoxy - δ - caesalpin 54.

The triacetate 54 (320 mg.) in chloroform (5 ml.) was treated with 0.5 ml. of a solution of HCl in chloroform. The resulting deep pink solution was evaporated under reduced pressure after five minutes, providing a semi-crystalline mass which was purified by t.l.c. (20% light petroleum/chloroform). Crystallisation from di-isopropyl ether gave the benzofuran 56 (192 mg.), as prisms, m.p. 220°; $\nu_{max}^{CCl_4}$ 3588 (hydroxyl), 1742 (acetates) cm.$^{-1}$; λ_{max} 251 nm (ε 7800), 281 nm (ε 2700), 291 nm (ε 2800).

τ = 7.68 (singlet, Ar - CH_3), 2.94 (singlet, Ar - H), 7.86, 8.10 (singlets, 2 CH_3COO -).

(Found : C, 69.50 ; H, 7.30% ; $C_{24}H_{30}O_6$ requires C, 69.55 ; H, 7.3%).

Exomethylene triacetate 67.

A solution of 1, 6, 7 - triacetoxy - δ - caesalpin 54 (140 mg.) in dry acetone (8 ml.) was stirred for 20 minutes with anhydrous copper sulphate (1g.). The mixture was filtered through a celite pad and evaporated to dryness at room temperature. This afforded

the amorphous exomethylene compound 67 (129 mg.), which was unstable at 20° and tended to form the benzofuran 56 on standing. A freshly prepared sample of 67 had $\nu_{max}^{CHCl_3}$ 3587 (hydroxyl), 1740-1735 (acetates) ; λ_{max} 232 nm (ε 9600), 210 nm.(ε 10,000).

τ = 7.94, 7.96, 8.06 (singlets, 3 CH_3COO -), 5.16 (broad singlet, >CHOAc), 4.49 (multiplet, 2H, 2 >CHOAc), 4.98, 5.11 (singlets, CH_2 = C<).

Mass spectrum : M^+ = 474 ($C_{26}H_{34}O_8$ requires M^+ = 474).

Serini reaction[43-45] on benzofuran diacetate 56.

A mixture of powdered zinc (400 mg.) and the benzofuran 56 (40 mg.) was placed in a sublimation tube and the temperature increased to 220°, the pressure being maintained at 0.3 mm. After one hour the sublimed product was taken up in chloroform and chromatographed on a 0.5 mm. plate run in 30% light petroleum/chloroform. The major product was the cis-fused 6 - ketone 72 (13 mg.), crystallised from ether as small prisms, m.p. 192-194°; $\nu_{max}^{CCl_4}$ 1747, 1235 (acetate), 1714 (ketone) $cm.^{-1}$.

(Found : C, 74.65 ; H, 7.40% ; $C_{22}H_{26}O_4$ requires C, 74.55 ; H, 7.4%).

Also isolated from the plate was the 6, 7 - diketone 75 (13 mg.). Recrystallisation from di-isopropyl ether afforded long yellow needles,

m.p. 184-190°(decomp.) ; $\nu_{max}^{CCl_4}$ 1750, 1228 (acetate), 1730 ($C_{(6)}$ ketone), 1689 ($C_{(7)}$ ketone) cm.$^{-1}$; λ_{max} 215 nm. (ε 17,000), 251 nm. (ε 13000), 311 nm. (ε 4000).

(Found : C, 71.75 ; H, 6.65% ; $C_{22}H_{24}O_5$ requires
C, 71.75 ; H, 6.55%).

The third product obtained from this reaction was a gum (4 mg.), which is presumed to be the A - homo- B - nor ketone 96 : $\nu_{max}^{CCl_4}$ 1740, 1230 (acetate), 1723 (ketone) cm.$^{-1}$.

Lithium aluminium hydride reduction of benzofuran 56.

The benzofuran 56 (50 mg.) in anhydrous ether (8 ml.) was stirred at 20° with excess (35 mg.) lithium aluminium hydride for 20 minutes. Normal work-up for metal-hydride reductions gave, as the sole product, the crystalline triol 82 (39 mg.), m.p. 201-203° (ether/light petroleum); $\nu_{max}^{CHCl_3}$ 3575, 3480 (hydroxyl) cm.$^{-1}$; τ = 5.56 (broad singlet, >C<u>H</u>OH), 5.46 (multiplet, >C<u>H</u>OH).

(Found : C, 72.70 ; H, 7.90% ; $C_{20}H_{26}O_4$ requires
C, 72.7 ; H, 7.9%).

Tosylate of triol 82.

Tosylation of the triol 82 (35 mg.) was carried out in the usual manner with excess p-toluenesulphonyl chloride in pyridine for two days. Work-up afforded an oily product containing two components which

were separated on a 0.5 mm. chromatoplate run in 10% light petroleum/chloroform. This gave the tosylate 83 (20 mg.) as a gum : $\nu_{max}^{CCl_4}$ 3570, 3480 (hydroxyl), 1177, 1187 (S = O) ; τ = 5.53 (broad singlet, >CHOH), 4.46 (triplet, J = 8 Hz., >CHOTos), 2.34 (A_2B_2 quartet, J_{AB} = 8 Hz., aromatic protons).

The tosylate was unstable at room temperature and eliminated p-toluenesulphonic acid to form the hemiketal 91. This compound (13 mg.) was also isolated as the minor product of the above tosylation. Crystallisation from anhydrous ether gave colourless prisms, m.p. 181-183°; $\nu_{max}^{CCl_4}$ 3594 (hydroxyl), 1044, 1144 (C-O) cm.$^{-1}$.
Mass spectrum : M^+ = 312 ($C_{20}H_{24}O_3$ requires M^+ = 312).

Conversion of the tosylate into the hemiketal was facilitated by treatment of 83, in ethanol solution, with a few drops of 1% ethanolic KOH.

Lithium aluminium hydride reduction of hemiketal 91.

Lithium aluminium hydride (8 mg.) was added to a stirred solution of the hemiketal 91 (10 mg.) in dry ether (3 ml.). After two hours, excess reagent was destroyed and the filtered solution was evaporated to yield the diol 92 (8 mg.), m.p. 201-203°(ether) ; $\nu_{max}^{CCl_4}$ 3566, 3429 (hydroxyl) cm.$^{-1}$; τ = 8.96, 8.95, 8.71 (singlets, $3CH_3 - \underset{|}{C} -$), 7.62 (singlet, Ar - CH_3), 5.76 (diffuse doublet, J = 5 Hz., >CH-OH).

Mass spectrum : M^+ = 314 ($C_{20}H_{26}O_3$ requires M^+ = 314).

(Found : C, 76.35 ; H, 8.35% ; $C_{20}H_{26}O_3$ requires C, 76.40 ; H, 8.35%).

Acetylation of diol 92.

A solution of the diol 92 (13 mg.) in acetic anhydride (2 ml.) was heated under reflux for 3 hours with anhydrous sodium acetate (10 mg.). The reaction was worked up by extraction with ethyl acetate, the acetic anhydride being removed by thorough washing with 5% sodium bicarbonate solution and brine. Removal of the dried solvent gave substantially one product - the diacetate 93 (11 mg.) which crystallised from di-isopropyl ether as fine needles, m.p. 174-178°; $\nu_{max}^{CCl_4}$ 1738, 1229 (acetates) $cm.^{-1}$ - no hydroxyl absorption.

Mass spectrum : M^+ = 398 ($C_{24}H_{30}O_5$ requires M^+ = 398).

Treatment of the diacetate 93 with lithium aluminium hydride in ether gave a product which was identical (t.l.c., i.r., n.m.r.) with the parent diol 92.

Oxidation of benzofuran triol 82.

A stirred, ice cold solution of the triol 82 (38 mg.) in acetone (3 ml.) was treated with 8N Jones reagent dropwise until a permanent orange colouration was observed. The reaction mixture was diluted

with water and worked up, affording a 60 : 40 mixture of two compounds (by t.l.c.). Preparative scale chromatography in 5% light petroleum/chloroform gave the main product, the 1, 6 di-ketone 99 (17 mg.) as a gum : $\nu_{max}^{CCl_4}$ 3498 (hydroxyl), 1730-1720 cm.$^{-1}$ (carbonyls) cm.$^{-1}$

τ = 9.20, 9.10, 8.80 (singlets $3CH_3 - C -$), 7.60 (singlet Ar-CH_3), 6.20 (sharp singlet, 2H, $C_{(7)}$ methylene), 3.17 (singlet Ar-H).

The other component was the 6 - ketone 97 (8 mg.); $\nu_{max}^{CCl_4}$ 3570, 3560 (hydroxyl), 1720 (ketone) cm.$^{-1}$; τ = 8.68 (6H), 8.41 (3H) (singlets, 3 $CH_3 - C -$), 7.56 (singlet Ar - CH_3), 6.24 (AB quartet, J_{AB} = 19 Hz., $C_{(7)}$ methylene), 5.24 (broad singlet, $>CHOH$).

Oxidation of the benzofuran triol 82 in CrO_3/pyridine[20] afforded only the mono-ketone 97, (58% yield).

Attempted formation of thioketal 98.

A solution of the 6 - ketone 97 (15 mg.) in ethane dithiol (0.5 ml.) with one drop of acetic acid added, was allowed to stand at 20° for four days with freshly distilled BF_3 etherate (2 drops). The solution was diluted with ether, washed repeatedly with saturated sodium bicarbonate solution and brine, then dried and evaporated. The

oily residue was chromatographed on a 0.25 mm. plate run in 25% light petroleum/chloroform. The only isolable material was a compound (5 mg.) of doubtful purity which was less polar than the starting ketone but still showed carbonyl absorption in the i.r. spectrum ($\nu_{max}^{CCl_4}$ 1720 cm.$^{-1}$).

δ- caesalpin 4.

A solution of the naturally occurring 1, 6, 7 - triacetoxy - δ - caesalpin 54 (1.3g) in ethanol (25 ml.) was heated under reflux with 5% ethanolic potassium hydroxide (10 ml.) for fifteen minutes. Most of the solvent was evaporated under reduced pressure and the precipitate formed on addition of water (10 ml.) to the residue was filtered, washed and recrystallised from aqueous methanol, affording δ - caesalpin (450 mg.) as very fine needles, m.p. 251°(sharp), (reported[2e] 251°). Extraction of the filtrate several times with ethyl acetate gave, after work-up, a further 380 mg. of δ - caesalpin. (Found : C, 65.25 ; H, 8.20% ; $C_{20}H_{30}O_6$ requires C, 65.55 ; H, 8.2%).

Acetylation of δ- caesalpin.

Under normal acetylation conditions, viz. acetic anhydride/ pyridine overnight, δ - caesalpin (50 mg.) yielded a 50 : 50 mixture of two hydroxy-acetates which were separated by preparative t.l.c. (3% methanol/chloroform). The more polar component was the monoacetate 88 (24 mg.), obtained as a gum which tended to form several compounds on standing. A freshly prepared sample had τ = 8.92 (6H), 8.90, 8.56 (singlets, 4 CH_3 - C -), 7.88 (singlet, CH_3COO-) 6.37 (broad singlet, $\succ C_{(1)}\underline{H}OH$) 5.70 (triplet, J = 10 Hz., $\succ C_{(7)}\underline{H}OH$), 4.63 (doublet, J = 10 Hz., $\succ \underline{CH}$ OAc).

The faster running component, the diacetate 89 (21 mg.) crystallised from ether as needles, m.p. 148-150°(reported[2e] 148-150°); $(\alpha)_D$ + 30° (chloroform) ; $\nu_{max}^{CCl_4}$ 3608, 3548 (hydroxyl), 1756 (acetates) cm.$^{-1}$; τ = 8.04, 7.97 (singlets, 2 CH_3COO-), 4.44, 4.48 (multiplets, 2 $\succ$ CHOAc), 6.34 (broad singlet, $\succ$CHOH).

(Found: C, 64.10 ; H, 7.75% ; $C_{24}H_{34}O_8$ requires C, 64.00 ; H, 7.6%).

Reduction of α- caesalpin 1.

To a stirred solution of α - caesalpin 1 (100 mg.) in aqueous methanol (1 : 3, 12 ml.) was added portionwise sodium borohydride (70 mg.). The mixture was stirred for one hour, diluted with water

and worked up without acidification to give a crude product (87 mg.) which had m.p. 192-198°, increasing to 198-199° on recrystallisation from ether. The analytical and spectral data of this compound (i.r., n.m.r.) were identical with those of the naturally occurring hydroxy-diacetate 100, m.p. 178-179° (see discussion).

The product from the above borohydride reduction was dissolved in dry T.H.F. and stirred for 30 minutes with lithium aluminium hydride (40 mg.). Normal ethyl acetate/water work up gave the pentaol 101 (55 mg.), which after two recrystallisations from aqueous methanol had m.p. 207-211°. (reported[2e] for pure δ- caesalpin 251° (sharp)).

Acid treatment of α- caesalpin 1.

α-caesalpin 1 (28 mg.) in chloroform (5 ml.) was smoothly converted to the benzofuran 55 by treatment with a solution of HCl in chloroform. The product (17 mg. after t.l.c.) crystallised from di-iospropyl ether as prisms, m.p. 176-178°; λ_{max} 251 nm. (ε 7800), 280 nm. (ε 2800), 290 nm. (ε 2850) ; $\nu_{max}^{CCl_4}$ 3587 (hydroxyl), 1720 (ketone), 1745 (acetate) cm.$^{-1}$.

(Found : C, 71.05 ; H, 6.90% ; $C_{22}H_{26}O_5$ requires C, 71.35 ; H, 7.1 %).

Acid treatment of monoacetate 103.

The monoacetate 103 (3 mg.) in chloroform (1 ml.) was treated with acid as above, and the solvent removed after 30 minutes to leave a crystalline residue which was identical in all respects with the starting material.

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SECTION 2

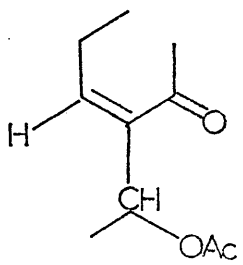
CONSTITUENTS OF ANDROGRAPHIS PANICULATA

Introduction

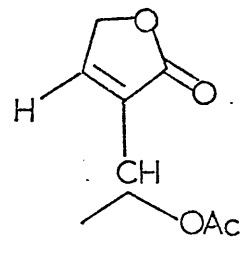
The plant Andrographis paniculata (Nees) is indigenous to the plains of India and the West Indies. Its extremely bitter leaves have been credited with prophylactic qualities against a plethora of diseases, including such diverse afflictions as snake bites and infantile diarrhoea.[1]

The main crystalline bitter constituent of the plant, andrographolide, was first isolated by Gorter,[2] and has been the subject of numerous chemical investigations.[3-7] Some of the pertinent early work showed that andrographolide was a bicyclic diterpenoid containing three hydroxyl groups and an $\alpha\beta$- unsaturated lactone function. However it was not until the advent of n.m.r. spectroscopy that the first definitive structural assignment was attempted.[27]

Acetylation of andrographolide with acetic anhydride and zinc chloride afforded the corresponding triacetate, the n.m.r. spectrum of which provided evidence of the substitution and immediate environment of the $\alpha\beta$-unsaturated lactone function. A signal at τ 4.09 was presumed to be characteristic of an allylic $>$CHOAc proton, while a triplet (J = 6Hz) at τ 3.03 was ascribed to the β- proton of the unsaturated lactone. This interpretation led to the part structure 1.



1

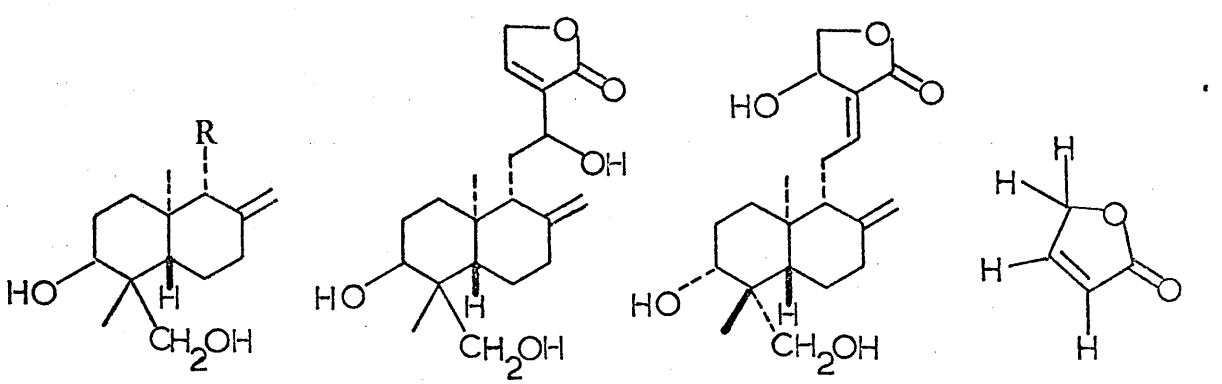


2

The infra-red spectrum of andrographolide in Nujol and KBr shows carbonyl absorption around 1727 cm.$^{-1}$, and this was taken by Kleipool as proof of an $\alpha\beta$ - unsaturated γ - lactone. Cava et al[27] found that the spectrum in acetonitrile solution had a corresponding band at 1754 cm.$^{-1}$, which was claimed to be in better agreement with such a system. This, together with the above n.m.r. data, led to part structure 2 for the lactone moiety.

Chemical degradation studies had elucidated the nature of the bicarbocyclic nucleus as 3, and consequently led to the promulgation of structure 4 for andrographolide.

3 4 5 6



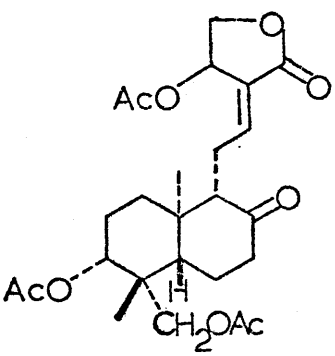
This was later amended to 5 on the basis of a re-interpretation of the n,m.r. spectra.[11]

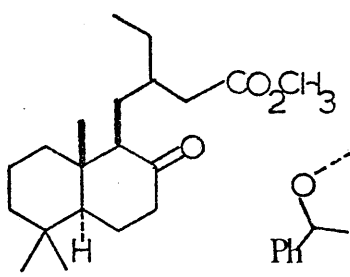
Studies in the model compound 2 - butenolide 6 showed that the coupling constant between the β - proton (τ 2.37) and the adjacent methylene group (τ 5.08) was 1.7 Hz. In the spectrum of triacetylandrographolide, the signal representing the β - proton of the lactone system (τ 3.03, triplet) has a coupling constant of 6.5Hz.

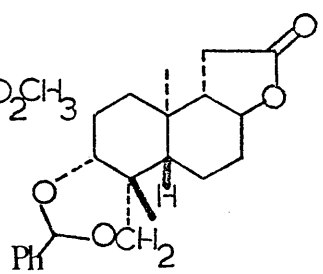
This is more in agreement with the exocyclic structure 5.

The stereochemical assignments were made on the following evidence. Hydroxylation of triacetyl andrographolide followed by periodate cleavage of the resulting diol furnished the nor-ketone 7, whose O.R.D. curve was characterised by a strong, positive Cotton effect. This curve was an exact mirror image of that shown by the keto ester 8 derived from labdanolic acid, indicating that the two compounds belong to enantiomeric series.

7 8 9





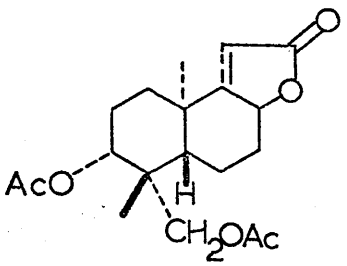


In later publications,[16,28] Cava and his collaborators presented conclusive evidence of a cis-relationship between the hydroxyl and hydroxy-methyl functions at $C_{(3)}$ and $C_{(4)}$, since the benzylidine derivative 9 could be formed from a transformation product of andrographolide. Assignment of the α- orientation to both these groups followed from a report by Wenkert[15] that the protons of oxygenated methylenes display n.m.r. signals at significantly lower field when the function has the axial rather than the equatorial configuration. Thus dehydroabietyl acetate has an AB quartet at 6.16 τ (equatorial methylene) while a value of τ 5.84 is recorded for C-methyl

podocarpyl acetate (axial methylene). The corresponding quartet in the n.m.r. spectra of seven andrographolide derivatives appears in the range τ5.65-5.76, consequently confirming the axial (α-) configuration for the $C_{(4)}$ acetoxymethyl group.

The above stereochemical assignments were subsequently confirmed by stereoselective synthesis[29] of the $\alpha\beta$- unsaturated lactone 10 which had previously been obtained by degradation of andrographolide.[16]

10



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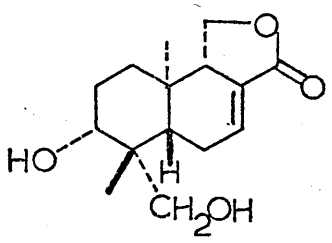
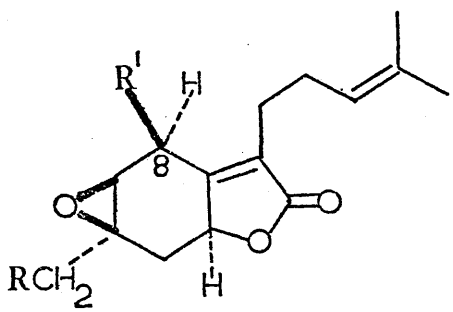


Andrographolide is thus a diterpene analogue of the sesquiterpene iresin 11.

DISCUSSION

The tissue cultures of Andrographis paniculata have been found to synthesise the three sesquiterpenoid lactones, paniculides A, B and C.[9] An investigation of the whole plant was deemed appropriate, therefore, to determine whether these sesquiterpenes, or related compounds, were present among the minor constituents.



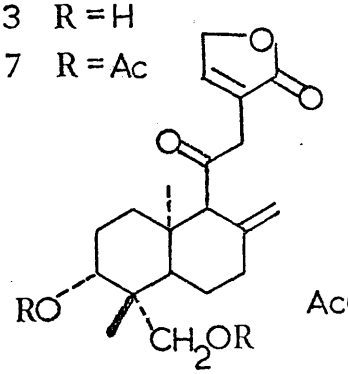
	R	R'
A	H	OH
B	OH	OH
C	OH	8-Ketone

After extraction of the powdered whole plant and chromatography of the extract, the only compounds obtained were andrographolide 5, neoandrographolide 12, (see later) and three crystalline substances which were shown by combustion analysis and mass spectroscopy to be diterpenoids. The three do not appear to have been previously isolated from the plant, but from spectral data are closely related to andrographolide.

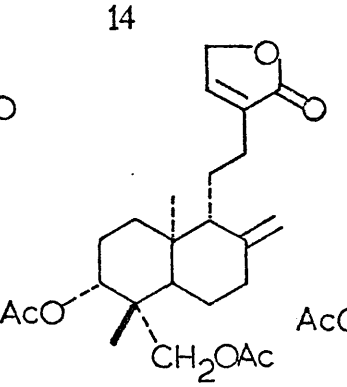
The first compound, $C_{20}H_{28}O_5$, m.p. 98-100°, $(\alpha)_D$-13.1°, was shown to be 11 - keto - deoxyandrographolide 13.

The i.r. spectrum (CCl_4 solution) displayed bands which were readily assigned to hydroxyl (ν_{max} 3608, 3500 cm^{-1}), lactone (1766 cm^{-1}) and exomethylene groups (1647, 902 cm^{-1}). A strong absorption at 1721 cm^{-1} was attributed to a cyclohexanone or an acyclic ketone. That the carbonyl frequency at 1766 cm^{-1} represents an $\alpha\beta$ - unsaturated γ - lactone function can be deduced empirically by comparison with the spectra of the known andrographolide transformation products 14 and 15. This is further substantiated by the ultraviolet absorption of 13 at 227 nm. (ε 9000) - a value which has been shown by Dorfman[10] to be characteristic of unsaturated γ - lactones substituted in the α-position.

13 R = H
17 R = Ac



14



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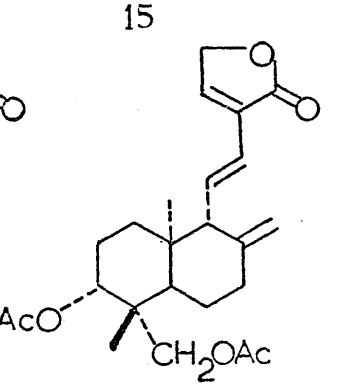


The n.m.r. spectrum of 13 showed, inter alia, signals for two tertiary methyls (τ 9.02, 8.80 singlets, 3H), one primary and one secondary hydroxyl (τ 6.28 (AB quartet, J_{AB} = 12Hz - $C\underline{H}_2OH$), 6.50 (multiplet, >C<u>H</u>OH)). Another AB quartet centred at τ 6.57 (J_{AB} = 18Hz) was subsequently ascribed to the $C_{(12)}$ methylene protons. It has been demonstrated by Cava et al[11] that two mutually coupled doublets at τ 2.49 (1H) and 5.15 (2H, J = 1.8Hz) are consistent with the presence of an endocyclic double bond conjugated to the lactone

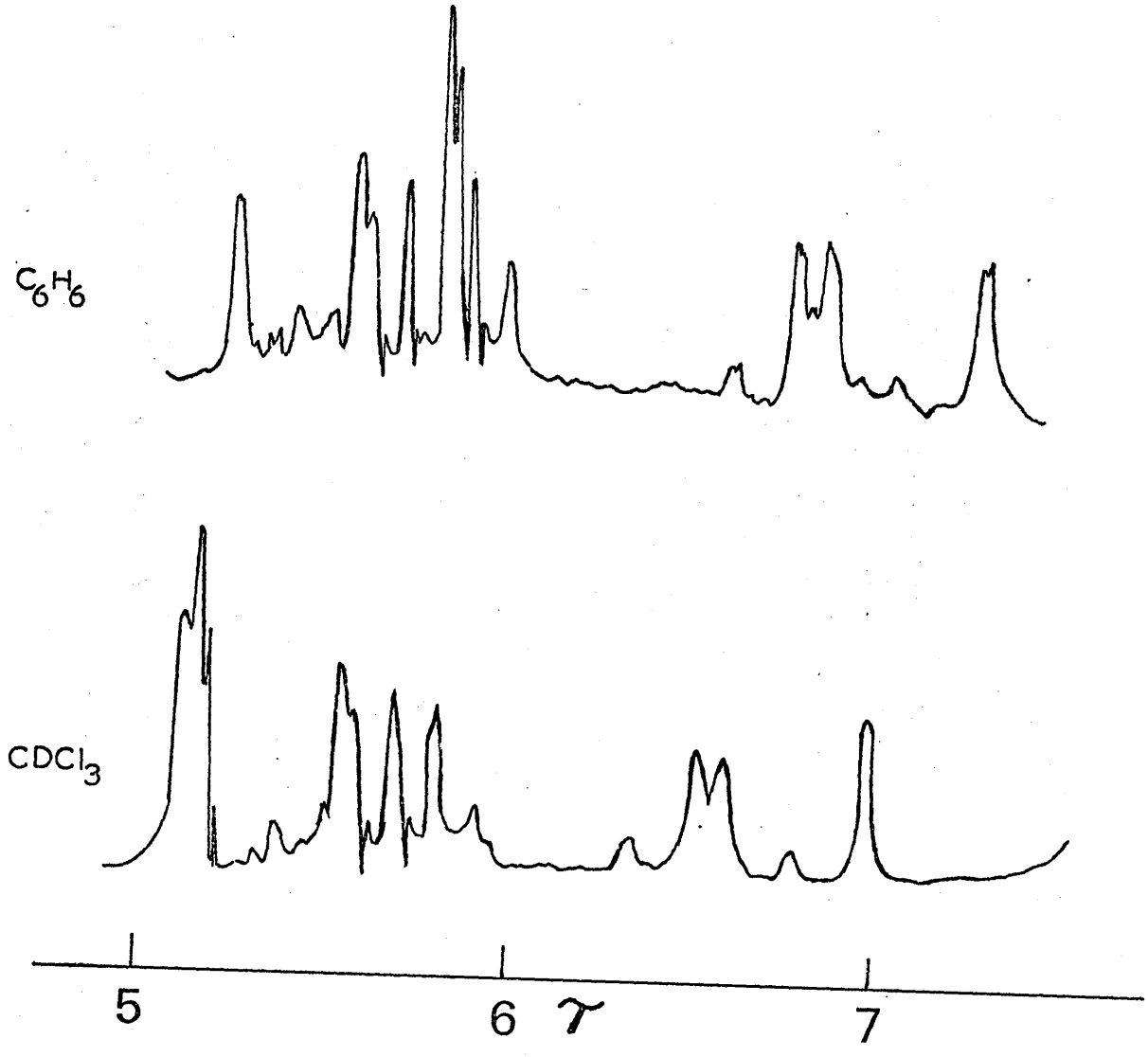
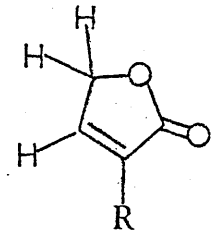


FIG 1

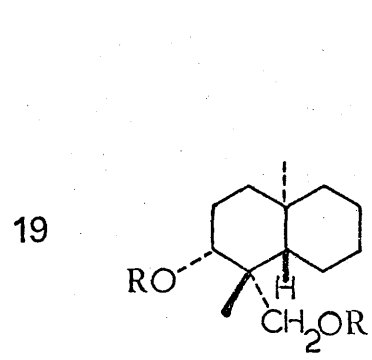
carbonyl, as in part structure 16.



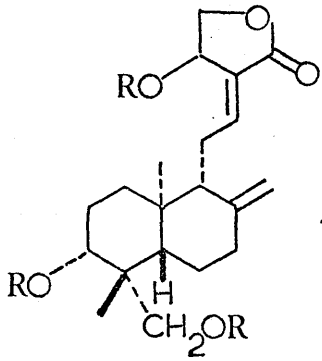
16

Further evidence in support of part structure 16 is obtained from solvent shift studies on the amorphous diacetate 17. The spectrum of 17 in $CDCl_3$ shows considerable complexity between 5-6 τ. However, dropwise addition of benzene to the n.m.r. tube makes this region more amenable to analysis (Fig.1.) by causing incremental upfield shifts of a doublet (J = 1.8Hz) at 5.15 τ. Consideration of the empirical rules[13,14] normally used to rationalise solvent shifts supports the assignment of this doublet to the lactone terminus methylene. This spectrum also allows confirmation of the presence of a primary and a secondary hydroxyl in the isolated compound, since the $-CH_2OH$ and $>CHOH$ resonances of 13 at τ 6.28 and 6.50 have suffered downfield shifts of 0.54 and 1.07 p.p.m. respectively on formation of the acetate 17.

The positional and stereochemical assignment of these two oxygen functions rests on the following evidence. First, the n.m.r. spectra of andrographolide 5 and its acetate 18 exhibit extremely similar characteristics to those of 13 and 17. This, together with the co-occurrence of the compounds is intuitively suggestive that they contain the same part structure 19.



19

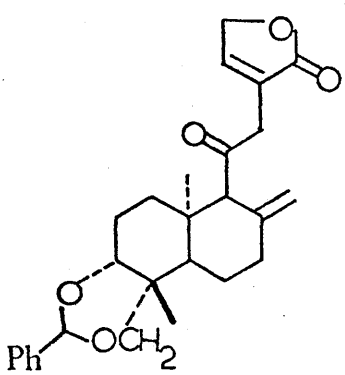


5 R = H

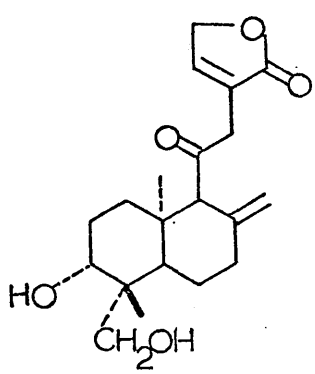
18 R = Ac

Secondly, it is known that the methylene protons of $C_{(4)}$ acetoxy-methyl groups resonate at a lower field (~τ 5.7) when the function has the axial rather than the equatorial orientation.[15] The corresponding protons in 17 appear as an AB quartet centred at τ 5.74, consistent with the presence of an axial $-CH_2OAc$

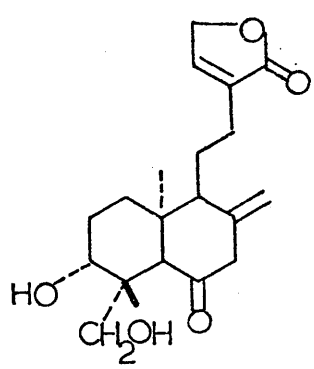
Finally, treatment of 13 with benzaldehyde in the presence of zinc chloride[17] resulted in the formation of the benzylidine derivative 20.



20



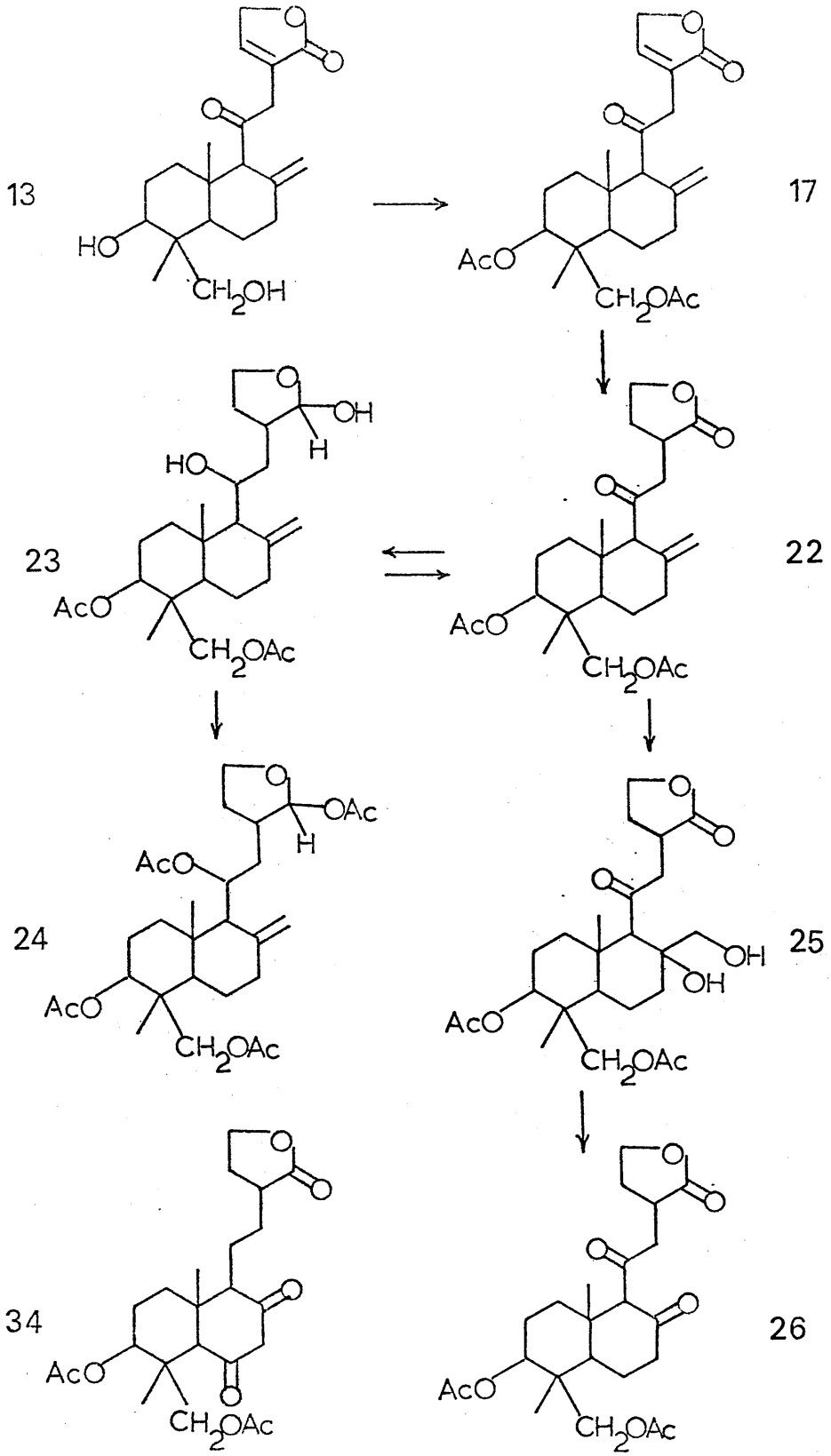
13



21

Consequently the two hydroxyls must have a cis-relationship, with the $C_{(3)}$-CH also in the α- orientation.

The above evidence eliminates all but two possible structures, 13 or 21, for the isolated compound. A decision between these in


13
HO
CH2OH
17
AcO
CH2OAc
23
HO
OH
H
AcO
CH2OAc
22
AcO
CH2OAc
24
AcO
OAc
H
AcO
CH2OAc
25
AcO
OH
OH
CH2OAc
34
AcO
CH2OAc
26
AcO
CH2OAc

Scheme 1

favour of 13 was reached by a detailed analysis of the n.m.r. and mass spectra, and confirmed chemically by formation of the β - diketone 26. (Scheme (1)).

In the n.m.r. spectra of both 13 and 17 the $C_{(14)}$ olefinic proton resonates as a doublet (J = 1.8Hz) around τ 2.50. The other compounds of this series which contain an αβ - unsaturated γ - lactone have the equivalent resonance occurring within the range τ 2.80 - 2.98. This deshielding of >0.3τ could be accounted for by placing the carbonyl group in the 11 - position, where it would be suitably disposed to influence the chemical shift of the $C_{(14)}$ hydrogen. In the alternative formulation 11, inspection of models indicates that a $C_{(6)}$ carbonyl would be too far away to have any appreciable deshielding effect.

Additional evidence in support of this can be adduced in the form of double irradiation experiments performed on the lactone diacetate 17. From these it is evident that the AB quartet at τ 6.56 - attributable to the methylene protons adjacent to the ketone - shows a small allylic coupling with the proton attached to $C_{(14)}$. This coupling would only be feasible with structure 13 for the isolated product.

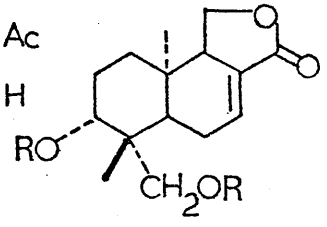
Treatment of the acetate 17 with sodium borohydride in aqueous methanol afforded a 1 : 1 ratio of two products, assigned structures 22 and 23. The faster running product was the saturated lactone 22. $C_{24}H_{34}O_7$; $\nu_{max}^{CCl_4}$ 1783 cm.$^{-1}$ (γ -lactone), 1742 cm.$^{-1}$ (acetate). 1720 cm.$^{-1}$ (ketone), 900 cm.$^{-1}$ (exomethylene). This suggested that only reduction of the double bond of the unsaturated lactone moiety had occurred, without affecting the remainder of the gross structure.

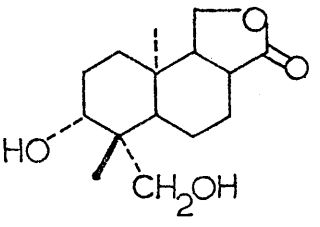
Confirmation of this was obtained from the n.m.r. spectrum, which lacked the characteristic signals for the butenolide system.

The more polar product was the saturated lactol 23, m.p. 120–123°, $\nu_{max}^{CHCl_3}$ 3610, 3440 cm.$^{-1}$ (hydroxyl), 1730 cm.$^{-1}$ (acetate). Its formulation as 23 was supported by analytical and mass spectral data, and by acetylation in acetic anhydride/pyridine to the gummy tetra-acetate 24 (τ = 8.02 (singlet, 12H, 4 CH_3COO-); M^+ = 522 : $C_{28}H_{42}O_9$ requires M^+ = 522). In addition, 13 could be transformed by Jones oxidation into the saturated keto-lactone 22.

The production of 22 and 23 as the only isolable compounds from this borohydride reduction raises one or two interesting mechanistic points about the reaction. It has been observed[18] that the exocyclic double bond of the $\alpha\beta$- unsaturated γ-lactone iresin 28 is reduced by borohydride ion giving the corresponding saturated lactone dihydroiresin 29. On the other hand the endocyclic system of the naturally occurring butenolides is unaffected by borohydride under normal conditions.[19]

27 R = Ac
28 R = H

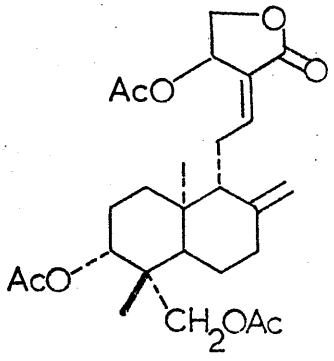




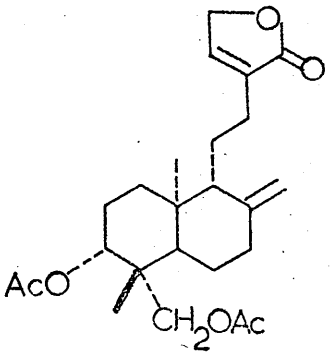
29

Cava and his collaborators have noted the analogous transformation of androgrepholide triacetate 18 to deoxyandrographolide diacetate 14

which is stable to sodium borohydride under the conditions of its formation.[16]

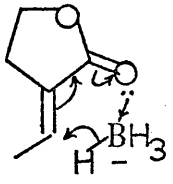


18



14

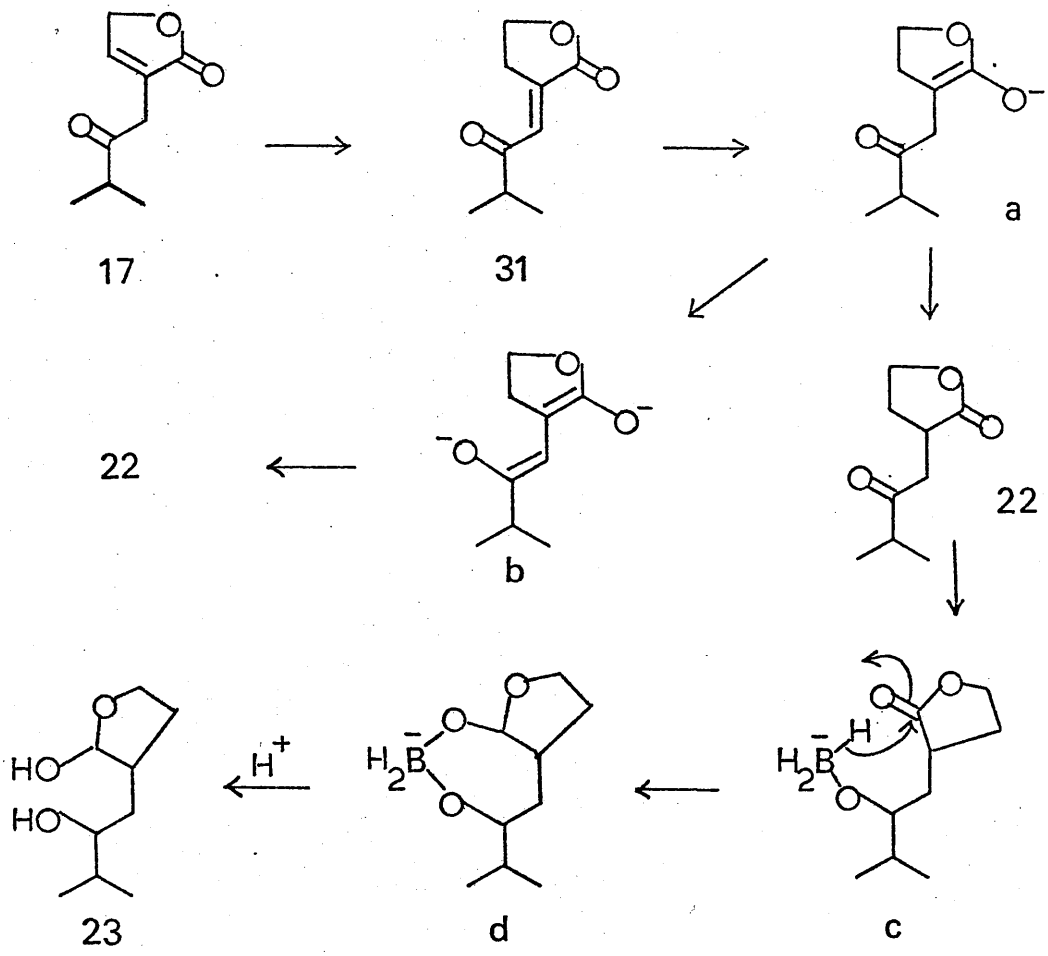
The mechanism postulated to rationalise these observations[18] is one involving a cyclic intermediate favoured by the cisoid unsaturated carbonyl system 30. This has precedent in the mechanism proposed by Lutz and Gillespie[20] to explain the results of their



30

investigations into the reduction of unsaturated 1, 4 diketones with lithium aluminium hydride.

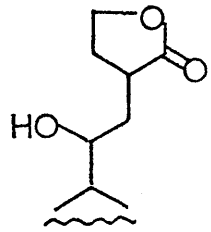
In view of the stability of endocyclic double bonds to reduction (vide supra), the formation of 22 and 23 must involve, as a first step, the isomerisation of 17 to the exocyclic structure 31 (Scheme (2)). Normal reduction could then proceed as postulated above to give the



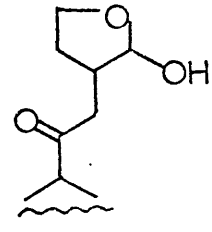
Scheme 2

enolate (a), which would lead to the saturated keto-lactone 22, probably via the diene - diolate (b). This route explains the isolation of 22 even after the reaction has been carried out with a large excess of borohydride and stirring continued for one hour.

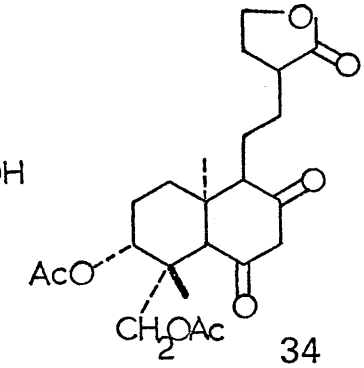
The mode of formation of the lactol 23 must invoke hydrolysis of the initially formed enolate (a) by the aqueous methanol medium before working up the reaction. The expected rapid reduction of the $C_{(11)}$ carbonyl could then be followed by an intra-molecular hydride transfer to the lactone (c). This would furnish an intermediate of type (d) which on acid hydrolysis leads to the observed lactol 23. The mechanism postulated is the one which best explains the apparently anomalous result that no 'cross-over' products, i.e. 32 or 33 could be isolated from the reaction.



32



33

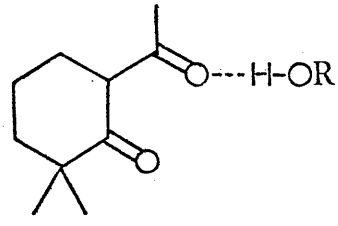


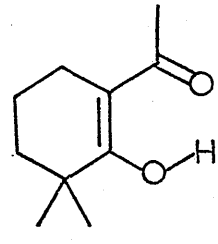
34

Hydroxylation of the keto-lactone 22 with osmium tetroxide in benzene afforded the diol 25 as an oil which was cleaved without further purification by sodium meta-periodate to the β-diketone 26; $\nu_{max}^{CCl_4}$ 1778 cm^{-1} (lactone), 1743 cm^{-1} (acetates), 1712 cm^{-1} (ketones). High resolution mass spectroscopy confirmed the molecular

formula $C_{23}H_{32}O_8$ and in addition revealed the main fragmentation pattern (see page 142).

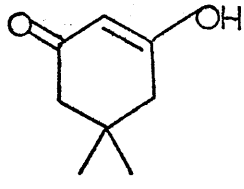
Our original assignment, on n.m.r. evidence, of structure 13 to the naturally occurring diol is reinforced by the u.v. spectrum of 26. The alternative formulation 34 can be discounted on the basis of the well documented u.v. spectra of 'flexible' and 'trans-fixed' β- diketones.[21] For alicyclic diketones, the situations obtaining in different solvents can be summarised as follows.[22]

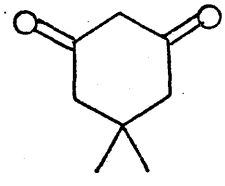




"Flexible" case

Hydroxylic Solvent hydrocarbon solvent





"Trans-fixed" case

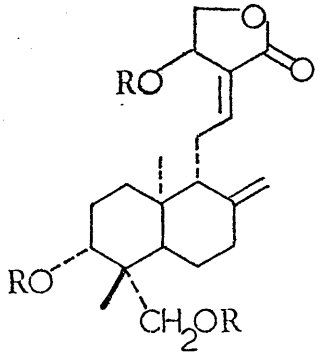
Hydroxylic Solvent hydrocarbon solvent

In ethanol solution, 26 exists wholly in the diketone - form, suggesting that it is in the category of 'flexible' β- diketones. This argument confirms the placing of the ketonic carbonyl group at $C_{(11)}$.

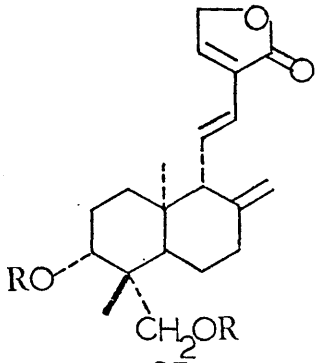
The other two new crystalline components obtained from the extract were eluted from the column by the same solvent system. Fractional crystallisation failed to promote any effective separation and recourse was eventually made to repeated chromatography on plates impregnated with silver nitrate.[23]

The major component of the mixture, $C_{20}H_{28}O_4$, m.p. 203.5 - 204.5°, had strong ultraviolet absorption at 248nm. (ε 11,000) indicating the presence of a new conjugated double bond. The n.m.r. spectrum revealed the typical bicyclic nucleus with two tertiary methyls, one primary and one secondary hydroxyl, an exomethylene group, and the familiar endocyclic unsaturated lactone system. It also showed that the new double bond was disubstituted, since two additional vinyl protons could be observed at τ 3.28 (quartet, J = 16, 10Hz, 1H) and τ 4.07 (doublet, J = 16, 1H). The appropriate decoupling experiments suggested that this compound was anhydroandrographolide 35.

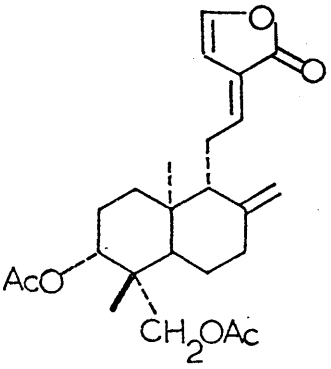
An obvious method of confirming this structure lay in the report by Cava and co-workers[16] that acetylation of andrographolide 5 proceeded with loss of acetic acid to give the diacetate 15. In our



R H 5
R Ac 18



35
15

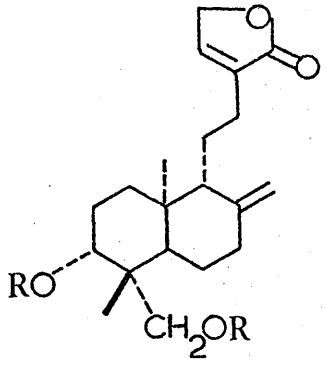


36

hands, acetylation of 5 under normal conditions gave mainly the enol lactone 36, m.p. 139-142° ($\nu_{max}^{CHCl_3}$ 1780 cm^{-1} (lactone carbonyl)), with the desired diacetate 26 constituting only a small amount (Ca 5%) of the total yield. Presumably the stronger conditions used by the previous workers, viz refluxing sodium acetate/acetic anhydride, had isomerised the initially formed enol lactone 36 to the thermodynamically more stable transoid enone system of 15. This isomerisation was shown to occur by repeating the acetylation of andrographolide using refluxing acetic anhydride and pyridine. This resulted in the formation only of the diacetate 15, which was shown to be identical in all respects with the acetate derived from natural anhydroandrographolide 35.

Analytical and mass spectral data on the third new compound obtained from the extract indicated the molecular formula $C_{20}H_{30}O_4$. Its n.m.r. spectrum displayed similar features to those of its congener 35, with the exception of the vinyl signals representing the disubstituted double bond. This evidence together with the lack of any appreciable ultraviolet absorption, strongly favoured assignment of structure 37 to this compound.

Diacetyldeoxyandrographolide 14 has been reportedly obtained from triacetylandrographolide 18 by treatment with sodium borohydride in aqueous methanol.[16] A sample of 14 from this source was identical by melting point and spectral comparison with the acetate derived from 37, thus confirming that the isolated compound is deoxyandrographolide.

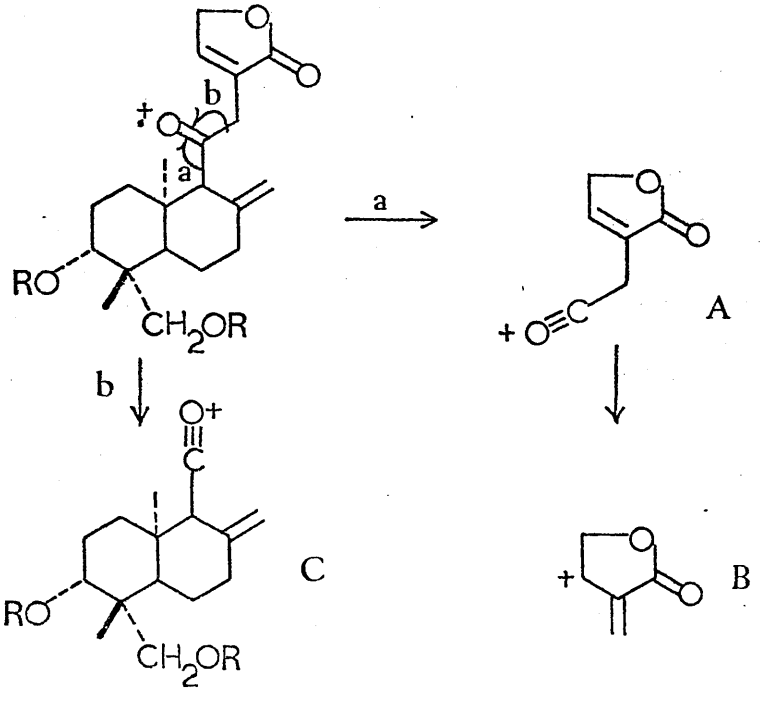


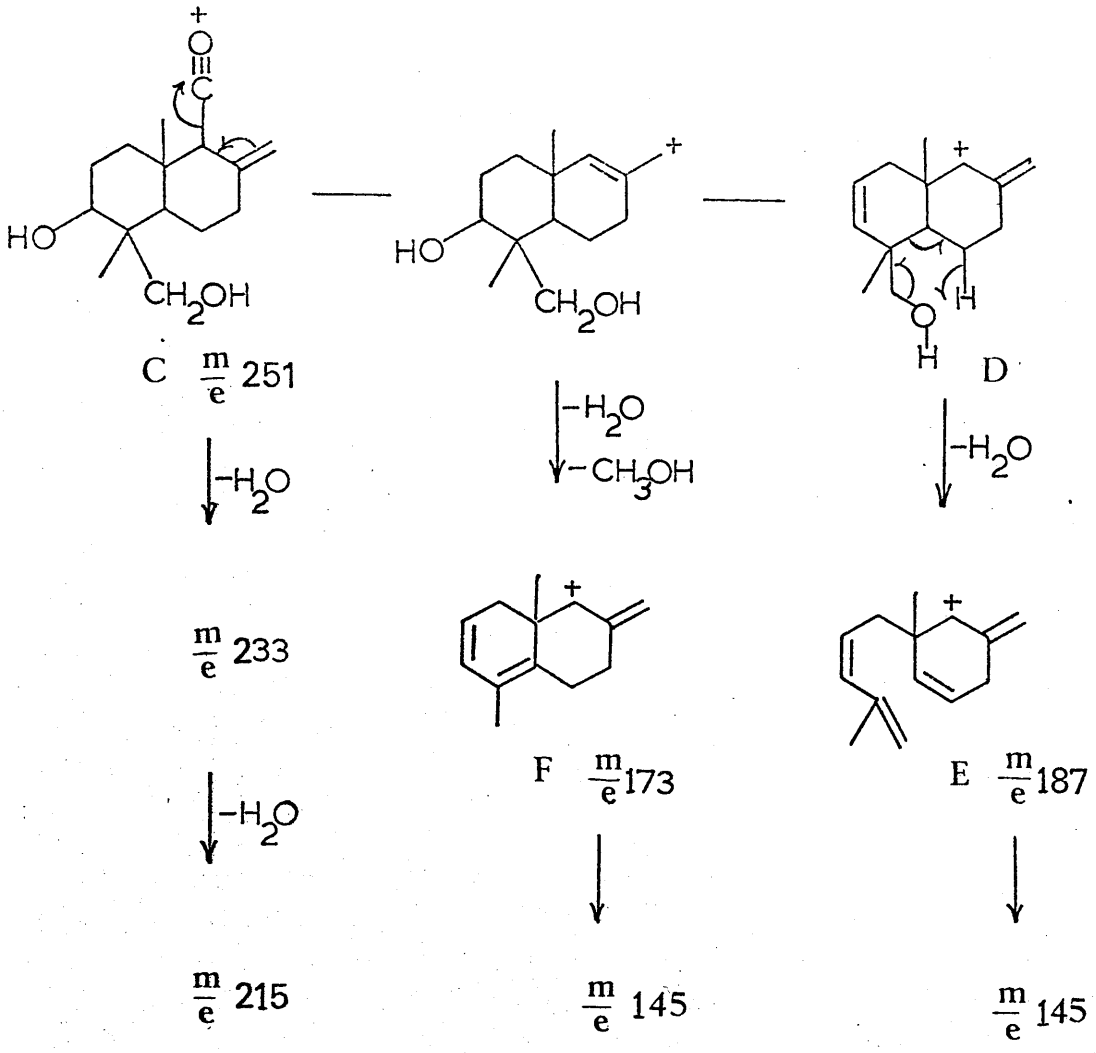
37 R = H

14 R = Ac

Mass Spectra of Andrographolide Derivatives.

Complete interpretation of the mass spectra of the compounds 13 - 37 is hindered in some cases by a pattern of oxygenation and unsaturation which can allow several equally probable breakdown sequences. This can lead to simultaneous production from one compound of ions of the same molecular weight but of different structures, or, alternatively, ions of the same structure but arising by different mechanisms. However, some order can be seen in the chaos by observing the peak displacements caused by altering the substitution pattern, and by careful consideration of previous work in this field.[31-35] The ion assignments which result from this are obviously open to question since definitive labelling studies were not carried out to verify the fragmentation patterns. Nevertheless, useful and




HO
CH2OH
C m/e 251
-H2O
m/e 233
-H2O
m/e 215
HO
CH2OH
-H2O
-CH3OH
F m/e 173
m/e 145
D
-H2O
E m/e 187
m/e 145

Scheme 3

significant results can be obtained which, together with the other physical data, provide conclusive evidence for the structures of the compounds investigated.

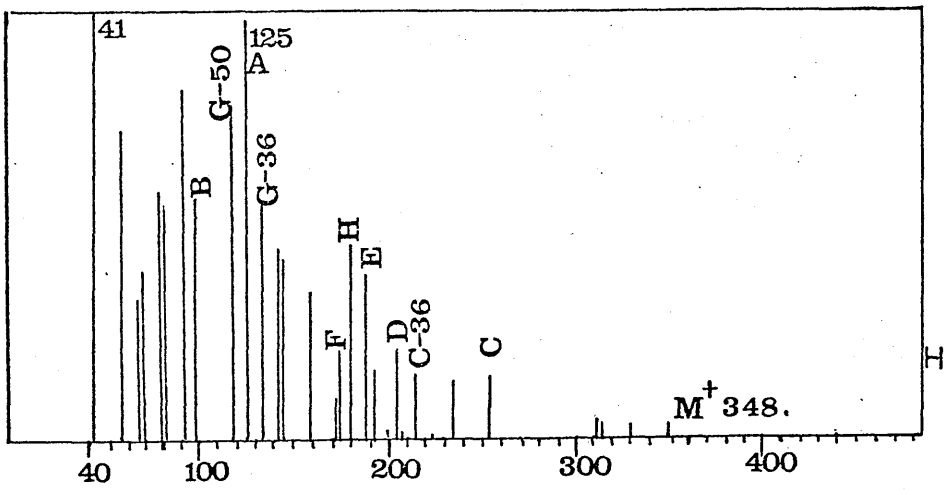
The mass spectra of 11- ketodeoxyandrographolide and its derivatives are shown in Figs. 2-4. The primary fragmentation of these compounds is the facile α- cleavage initiated by the carbonyl group.[34]

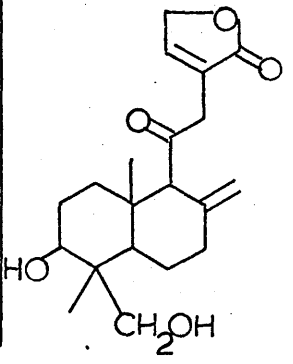
Thus path a gives rise to the base peak at $\frac{m}{e}$ 125 in the spectrum of the benzylidine compound 20, and also furnishes a prominent ion in the breakdown of 13, 17, 22 and 26. When the lactone is unsaturated, the ion A can lose carbon monoxide to form the fragment B at $\frac{m}{e}$ 97, a transition which is supported by a metastable peak at $\frac{m}{e}$ 75.3.

The ion C, formed by cleavage of the $C_{(11)} - C_{(12)}$ bond, may undergo further fissions, the products depending on the substituents present. Thus, in the spectrum of 11- ketodeoxyandrographolide 13, C can react by elimination of water, methanol, carbon monoxide, or any combination of these to give the fragments shown in scheme (3).

The frequent occurrence of a peak at $\frac{m}{e}$ 145 in these spectra may be due to elimination of ethylene from F, or to removal of a three carbon fragment from the ion E at $\frac{m}{e}$ 187. The appearance of a metastable peak at $\frac{m}{e}$ 112.5 favours the latter route. It is noteworthy that the ion at $\frac{m}{e}$ 189 in the spectrum of the β - diketone 26 is markedly less abundant than the corresponding ion E in Scheme (3). This is an accord with the expected destabilising influence of a carbonyl group α to the carbonium ion.

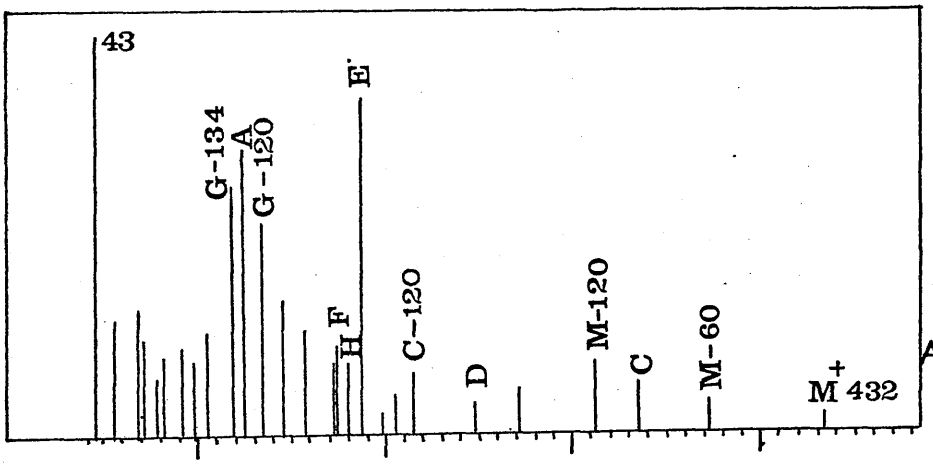
Fig 2

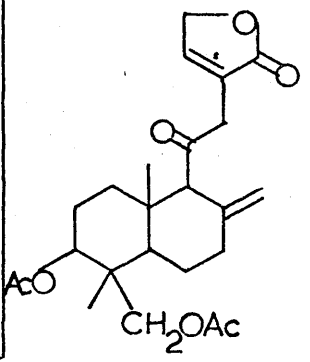




13

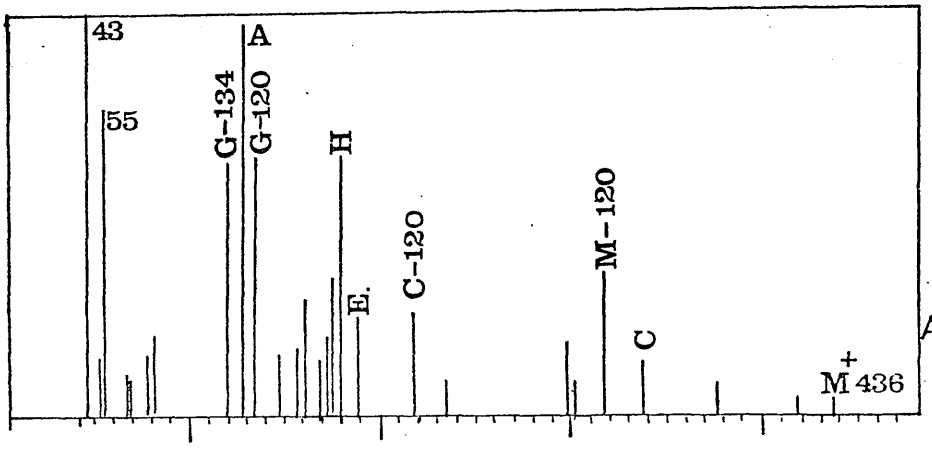
Fig 3

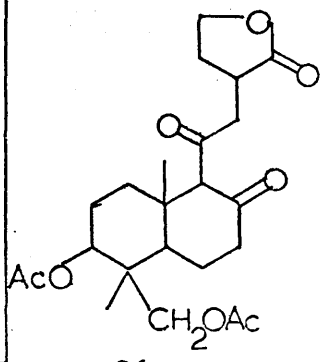




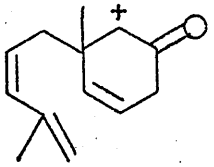
17

Fig 4

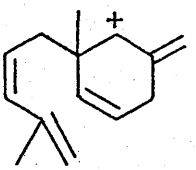




26

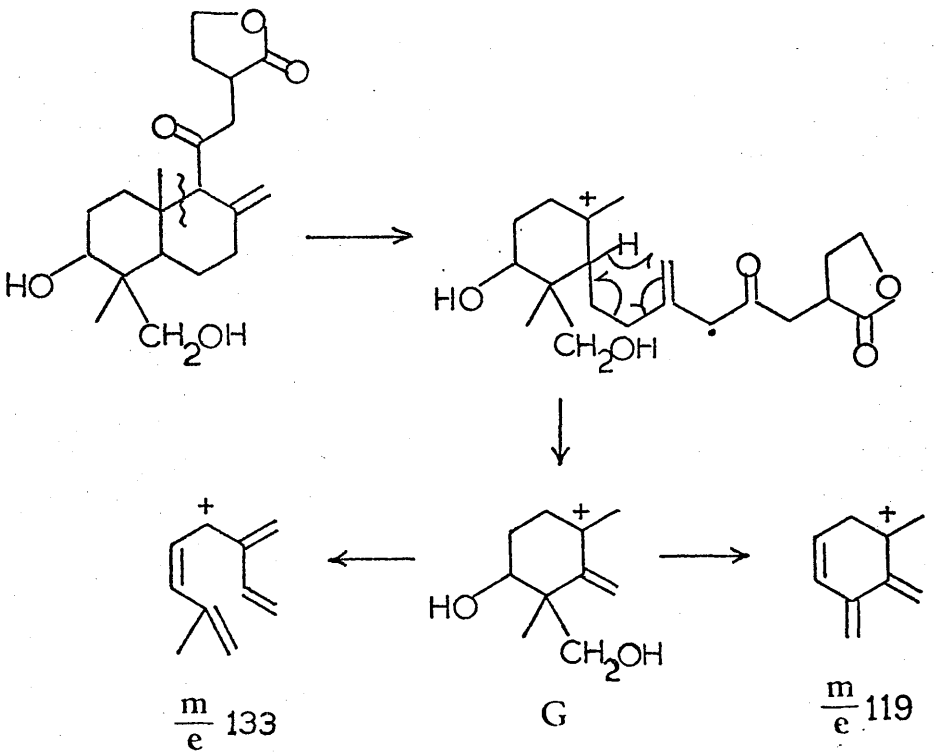


$\frac{m}{e}$ 189



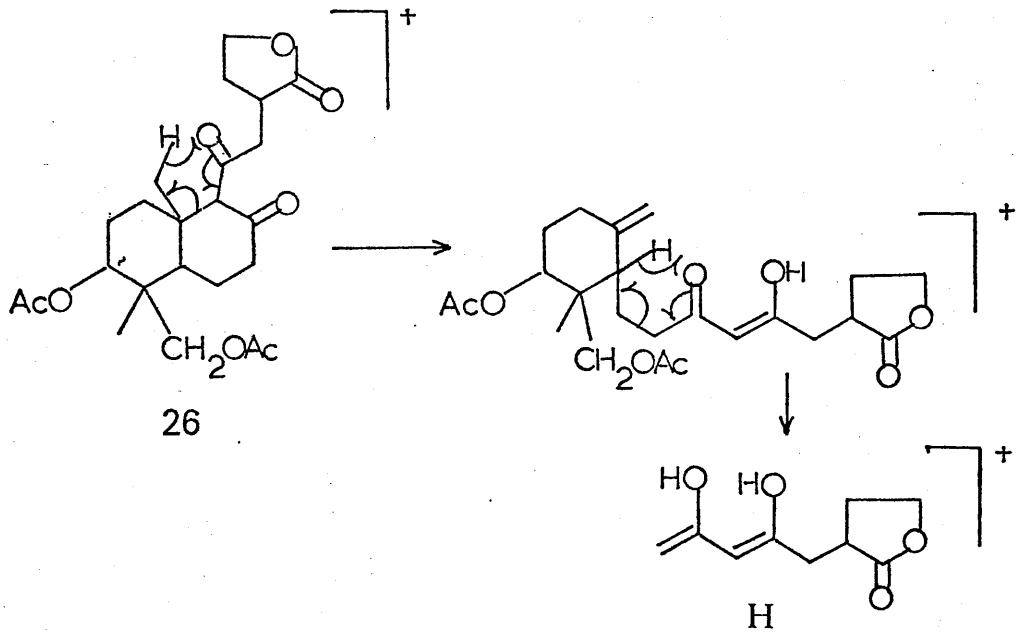
$\frac{m}{e}$ 187

The work of Biemann[31] has shown that a favoured reaction for bicyclic diterpenoids of this type involves allylic cleavage of the $C_{(9)} - C_{(10)}$ and $C_{(6)} - C_{(7)}$ bonds with formation of an ion of type G. This ion is also characteristic of tricarbocyclic diterpenes with a $C_{(8)} - C_{(14)}$ double bond,[32] and can eliminate water or methanol to give hydrocarbon fragments of mass 133 and 119.



$\frac{m}{e}$ 133 G $\frac{m}{e}$ 119

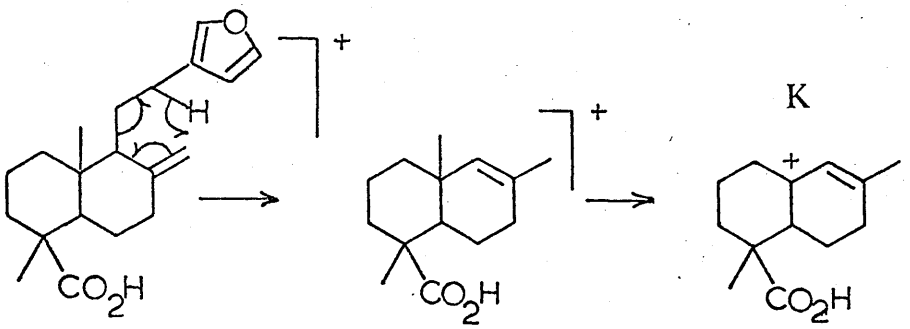
A similar fragmentation can proceed by McLafferty rearrangement involving the $C_{(11)}$ carbonyl, resulting in retention of charge by the larger fragment. Substantial support for this lies in the presence of the ion H at $\frac{m}{e}$ 180 in the spectra of 13, 17 and 20, while the corresponding fragments of the keto-lactone 22 and the β-diketone 26 occur at $\frac{m}{e}$ 182 and 184 respectively. The enhanced intensity of this peak in the latter spectrum is no doubt due to the presence of the $C_{(8)}$ ketone, which can facilitate production of the ion H by a double McLafferty rearrangement.



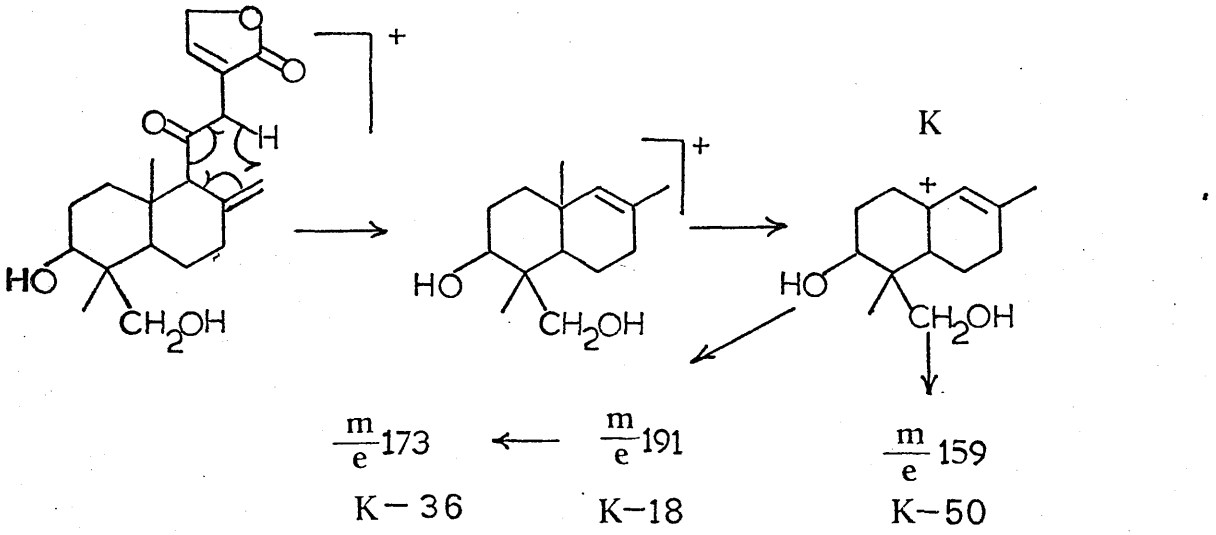
Confirmation of this is derived from high resolution mass analysis of the ion H, which indicates a molecular weight of 184.0734. ($C_9H_{12}O_4$ requires 184.0735).

The parent ion of the $C_{(11)}$- keto derivatives may also fragment at the $C_{(9)}$ - $C_{(11)}$ bond with concerted transfer of an activated $C_{(12)}$

hydrogen to the exomethylene group. A sequence of this type is known to result in formation of the ion K during the breakdown of Daniellic acid.[33]



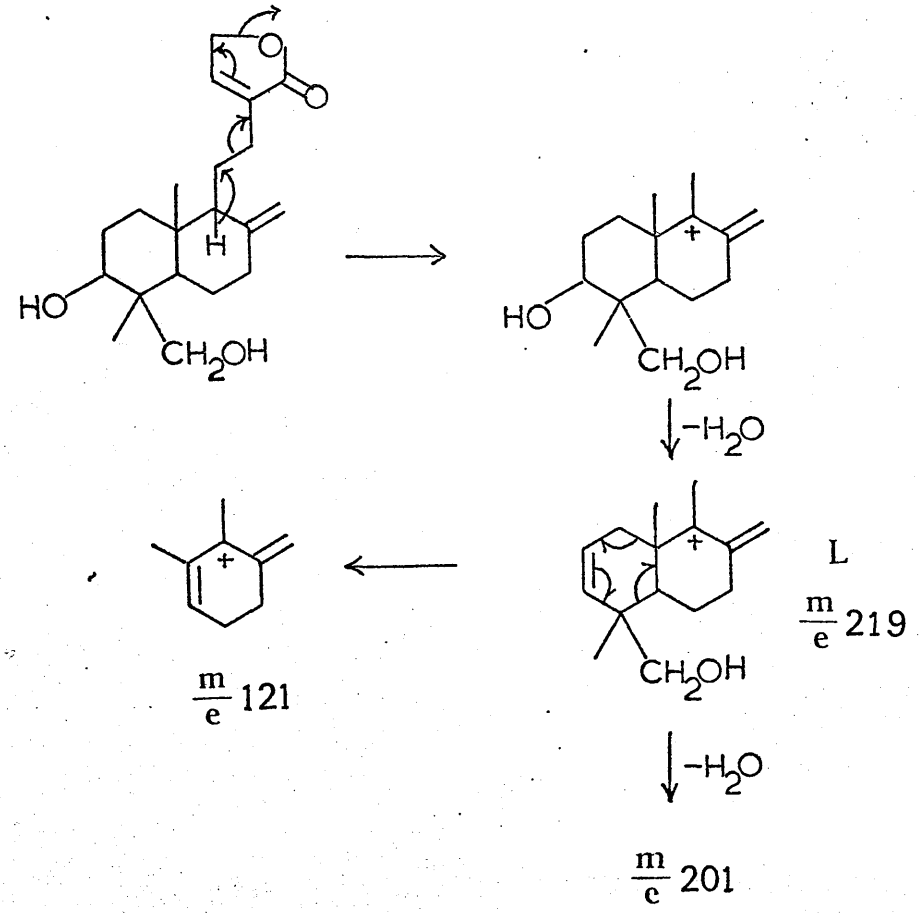
A similar cleavage in 11 - ketodeoxyandrographolide could give rise, after elimination of the substituents, to fragments at $\frac{m}{e}$ 191, 173 and 159.



The fragmentation of deoxyandrographolide 37 does not have any major directing influence, and consequently its mass spectrum has a more random distribution of ions than those previously discussed. There are, however, indications that most of the above rearrangements are taking place to some extent. The base peak occurs at $\frac{m}{e}$ 121, an ion which is not greatly significant in the other spectra, and can

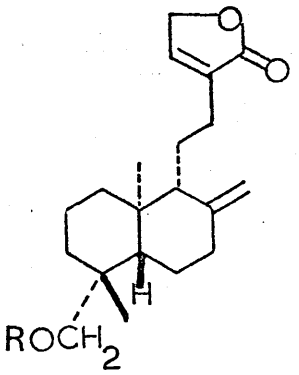
probably be attributed in part to retro Diels-Alder cleavage of the ion L.

Formation of L from the parent ion involves a 1, 2 hydrogen shift, since this would increase the stability of the product.



Neoandrographolide.

Neoandrographolide, a diterpeneglucoside, was first isolated from Andrographis paniculata by Kleipool in 1952.[6] He advanced the molecular formula $C_{23}H_{38}O_8$, and deduced the presence of an $\alpha\beta$-unsaturated γ- lactone from solubility experiments and a positive Legal test.[24] In a recent re-examination of this problem by Chan et al,[8] chemical and spectroscopic evidence is adduced in support of structure 12 for neoandrographolide. This formulation has now been confirmed by results obtained independently in this laboratory.



12 R = Glu

38 R = $GluAc_4$

The glucoside 12, m.p. 167-168 (reported[8] 167-168°) was isolated in low yield from the methanol extract of the powdered whole plant by column chromatography on silica gel, followed by preparative t.l.c. in 10% methanol/chloroform. Because of its extremely low solubility in most organic solvents, the structural determination of the compound was facilitated by formation of the tetra-acetate 38

$C_{34}H_{48}O_{12}$, m.p. 156-157° (reported[8] 155-157°).

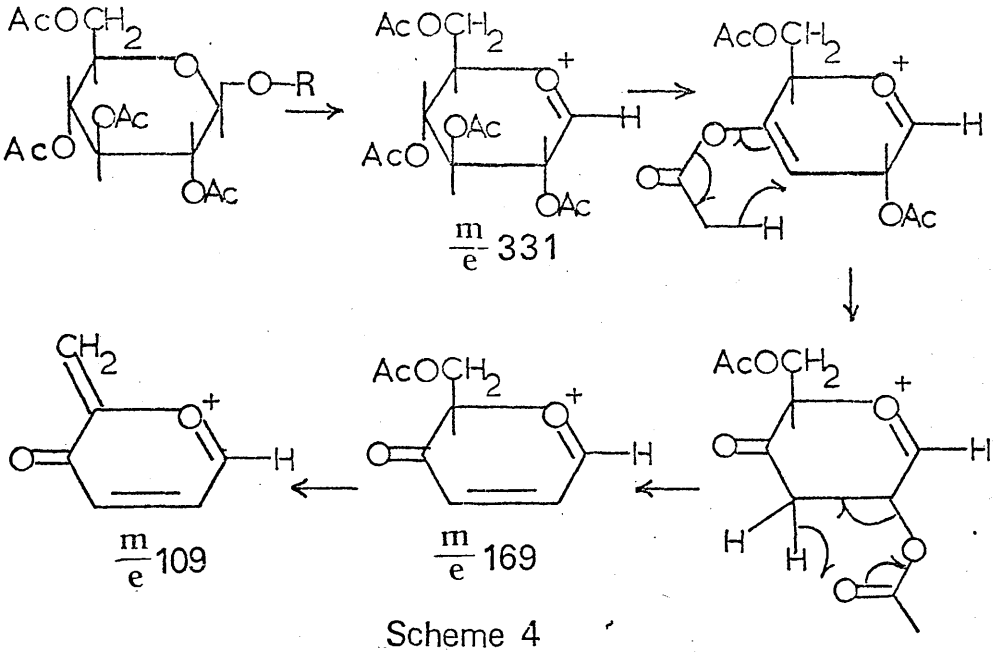
Subtraction of the glucose residue from the molecular formula leaves an aglucone which has one oxygen less than deoxyandrographolide 37. It is evident from the n.m.r. spectrum that the molecule contains an endocyclic $\alpha\beta$- unsaturated γ - lactone system:

τ = 2.88 (narrow multiplet, $W_{\frac{1}{2}}$ = 4 Hz, $C_{(14)}$ - H);

5.26 (doublet, J = 1.8Hz, $C_{(15)}$ methylene).

The 'missing' oxygen of neoandrographolide must therefore have been removed from either the $C_{(3)}$ or $C_{(19)}$ position. The remainder of the n.m.r. spectrum allowed unequivocal distinction between these two possibilities, since only two tertiary methyls were observed (τ 9.34, 9.08 (singlets, 3H)), along with an AB quartet (τ 6.44, J_{AB} = 9 Hz) attributable to the protons of the $C_{(19)}$ methylene. Other features of the spectrum which are compatible with the defined structure 38 for neoandrographolide acetate are a pair of singlets at τ 5.15 and 5.41 (each 1H) which can be ascribed to the exomethylene group, and a doublet (J = 7Hz) at τ 5.61 characteristic of the anomeric proton of a β - glucoside.[25]

The main peaks in the mass spectrum of 38 occur at $\frac{m}{e}$ 331, 169, 109 and 43 (base peak), a sequence which is well documented in the carbohydrate field[30] and represents the breakdown of a tetra-acetyl glucoside as shown in Scheme (4).



Scheme 4

Neoandrographolide itself does not exhibit a parent ion in the mass spectrum (Fig. 5) owing to the very facile cleavage of the glycosidic bond to give the aglucone a at $\frac{m}{e}$ 318. The presence of a primary hydroxyl grouping in a is shown by loss of 18 (H_2O) and 31 ($-CH_2-OH$) mass units.[35]

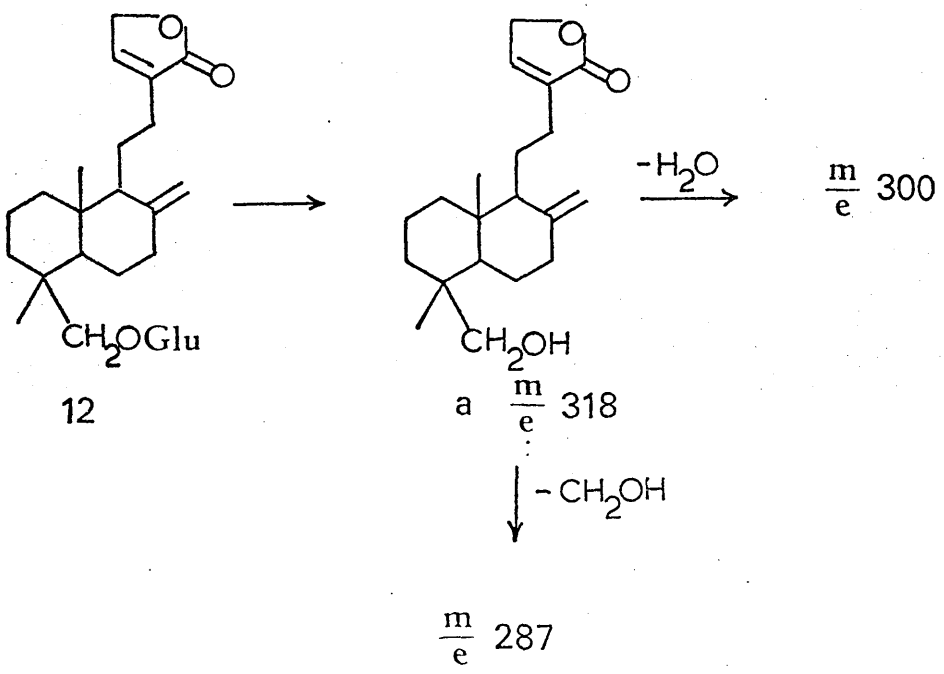
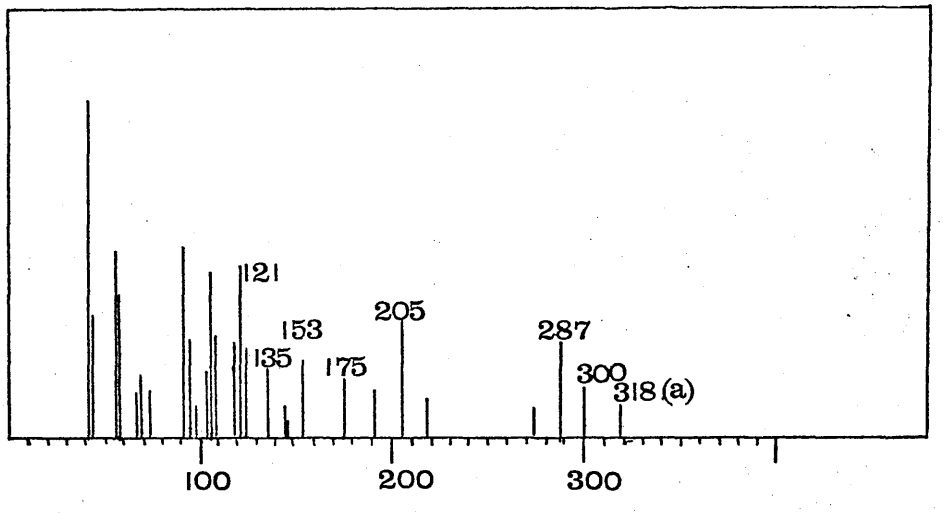
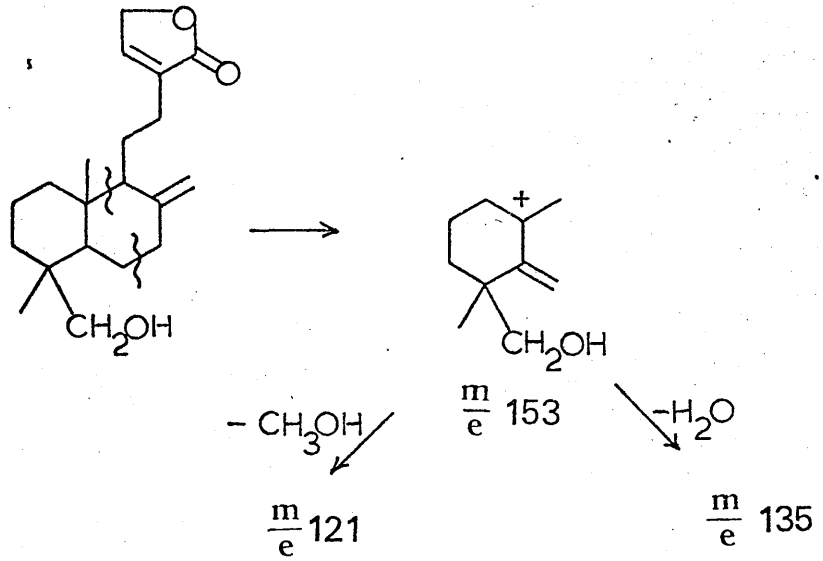


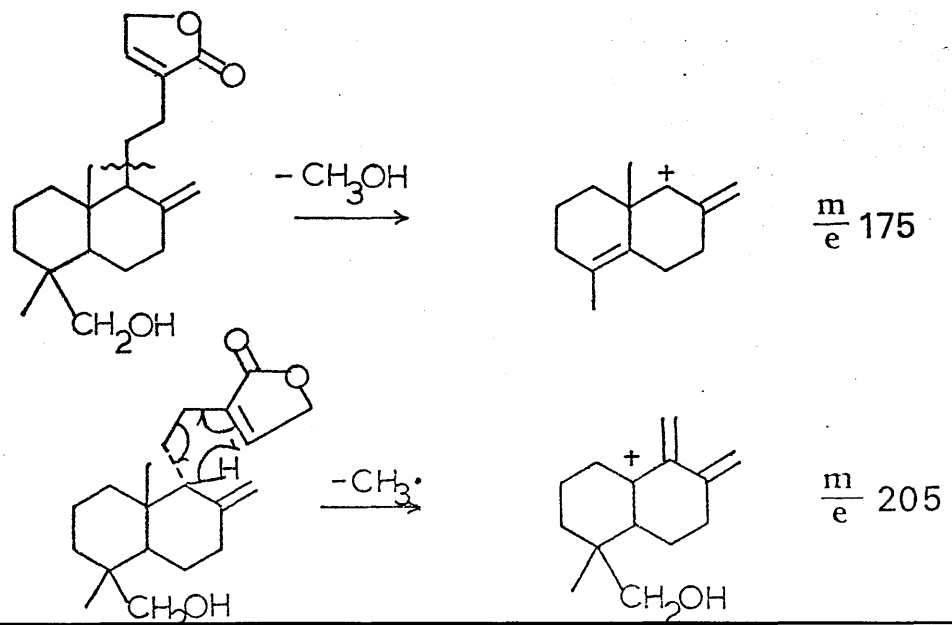
Fig 5 Mass Spectrum of Neoandrographolide



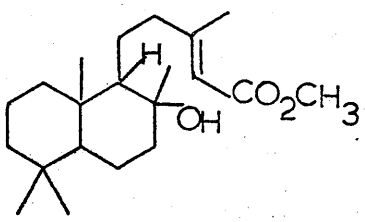
Many of the other significant ions can be assigned structures by analogy with the previously discussed breakdown of andrographolide derivatives. Thus, the peaks at $\frac{m}{e}$ 153, 135 and 121 can arise after cleavage of the allylic $C_{(9)} - C_{(10)}$ and $C_{(6)} - C_{(7)}$ bonds



Fragmentation may also occur at the other allylic bonds in the molecule, i.e. $C_{(9)} - C_{(11)}$ or $C_{(11)} - C_{(12)}$, with the formation of the ions at $\frac{m}{e}$ 175 and 205.



The hydrogen transfer from $C_{(9)}$ to $C_{(14)}$ by a six-membered ring mechanism has previously been observed in the spectrum of the unsaturated ester 39.[35,36]



39

EXPERIMENTAL

The material required for this investigation was obtained from the dried powdered whole plant Andrographis paniculata (Nees) by extraction first with ethyl acetate in a Soxhlet apparatus, then with hot methanol, to obtain the more polar constituents of the plant.

The two extracts were chromatographed separately on neutral alumina (grade IV) using the 'gradient elution' technique. The solvent used initially was benzene, the polarity being gradually increased through chloroform to 20% methanol/chloroform for the most polar fractions. The appropriate fractions (as determined from analytical t.l.c. plates) were combined and, where necessary, re-chromatographed on thick layer (0.5 mm.) plates to give pure samples of the compounds shown in Table 1.

COMPOUND		R_f*
andrographolide	5	0.22
neoandrographolide	12	0.09
11-keto-deoxyandrographolide	13	0.41
anhydroandrographolide	35	0.47
deoxyandrographolide	37	0.47

TABLE I

* R_f values were measured in 5% methanol/chloroform.

TABLE 2. N.M.R. SPECTRA OF ANDROGRAPHOLIDE DERIVATIVES.

COMPOUND	H-3	H-11	H-12	H-14	H-15	H-17	H-19	Me	CH_3COO
13	6.50m	-	6.57a	2.49b	5.15b	5.57s 5.16s	6.28c	9.02s 8.80s	
14	5.35m	-		2.90b	5.25b	5.10s 5.37s	5.77c	9.30s 9.00s	8.89s (6H)
15	5.3m	3.02d	3.84e	2.80b	5.21b	5.20s 5.37s	5.75c	9.12s 8.99s	7.98 (6H)
17	5.43m		6.56a	2.50b	5.15b	5.54s 5.07s	5.74c	9.00s 8.90s	7.98 (6H)
18	5.40m		2.92f	4.02m	5.6m	5.06s 5.44s	5.72c	9.21s 8.94s	7.94(6.H) 7.87
22	5.40m					5.60s 5.19s	5.76c	9.05s 8.95s	8.01 (6H)
23	5.51m	6.40m				5.60s 5.17s	5.77c	9.04s 8.94s	8.02 (6H)
24	5.42m	5.8m				5.70s 5.17s	5.78m	9.0s 8.91s	8.00 (12H)

COMPOUND	H-3	H-11	H-12	H-14	H-15	H-17	H-19	Me	CH_3COO
26	5.30m						5.68	8.88s 8.77s	7.94 (6H)
36	5.38m		3.32h	3.86i	3.02j	5.10s 5.52s	5.72c	9.15s 8.91s	7.92 (6H)
35	6.82m	3.28d	4.07e	2.98b	5.38b	5.40s 5.66s	6.28c	9.36s 8.93s	-
37	6.5m			2.87b	5.25b	5.11s 5.40s	6.26c	9.34 8.76	-

a : AB quartet, J_{AB} = 18Hz.

b : doublet, J = 1.8Hz.

c : AB quartet, J_{AB} = 12Hz.

d : quartet, J = 16, 10 Hz.

e : doublet, J = 16 Hz.

f : double triplet J = 8, 2 Hz.

g : AB quartet, J_{AB} = 9 Hz.

h : triplet, J = 8 Hz.

i : doublet, J = 4 Hz.

j : doublet, $W_{\frac{1}{2}}$ = 6 Hz.

m : multiplet

s : singlet

Anhydroandrographolide 35 and deoxyandrographolide 37 have the same R_f value and were eluted in the same fractions. Separation of these two compounds was only achieved by repeated chromatography on plates impregnated with silver nitrate.[23]

Andrographolide 5 was recrystallised from ethanol as plates, m.p. 230-231° (reported[4] 227.5°); λ_{max} 223 nm (ε 12,300): ν_{max}^{Nujol} 3448, 3390-3280 (hydroxyl), 1727 (lactone), 1647, 906 (exomethylene) cm.$^{-1}$

Mass spectrum: M^+ = 350; $C_{20}H_{30}O_5$ requires M^+ = 350.

11-keto-deoxyandrographolide 13 was obtained as needles, m.p. 98-100° from chloroform/ether: $(\alpha)_D$ - 13.1° ; λ_{max} 227 nm. (ε 9000) ; $\nu_{max}^{CCl_4}$ 3608, 3500 (hydroxyl), 1766 (lactone), 1720 (ketone), 1642. 902 (exomethylene) cm.$^{-1}$

Mass spectrum: M^+ = 348 ; $C_{20}H_{28}O_5$ requires M^+ = 348.

(Found: C, 68.65 ; H, 8.30% ; $C_{20}H_{28}O_5$ requires C, 68.95 ; H, 8.10%)

Anhydroandrographolide 35 crystallised from ether as fine needles. m.p. 203.5 - 204.5° ; $\nu_{max}^{CHCl_3}$ 3608, 3500 (hydroxyl), 1758 (lactone), 1643, 880 (exomethylene) cm.$^{-1}$

Mass spectrum: M^+ = 332 ; $C_{20}H_{28}O_4$ requires M^+ = 332

(Found : C, 72.50 ; H, 8.65% ; $C_{20}H_{28}O_4$ requires C, 72.25 ; H, 8.50%)

Deoxyandrographolide 37 recrystallised as colourless needles, m.p.175° (reported[27,16] 170-171°) from ether/light petroleum: λ_{max} 214nm. (ε 9, 300) ; $\nu_{max}^{CHCl_3}$ 3608, 3500 (hydroxyl), 1759 (lactone), 1645, 902 (exomethylene) cm.$^{-1}$

Mass spectrum : M^+ = 334 ; $C_{20}H_{30}O_4$ requires M^+ = 334

(Found : C, 71.90 ; H, 9.00% ; $C_{20}H_{30}O_4$ requires C, 71.80 ; H, 9.05%).

Acetylation of Andrographolide

(i) A solution of andrographolide 5 (190 mg.) in pyridine (2ml.) and acetic anhydride (1 ml.) was left to stand at 20° for sixteen hours. Methanol was added, and after ten minutes the solvent was removed in vacuo to give a gum which was shown by t.l.c. to contain two products in the approximate ratio 3:1. Preparative scale chromatography in 5% light petroleum/chloroform afforded the faster running component - the enol lactone 36 - as a gum (126 mg., 57%) and the slower anhydroandrographolide diacetate 15 as white needles (40 mg., 18%).

The enol lactone 36 was recrystallised from chloroform/ether as needles, m.p. 139-142°; λ_{max} 299nm. (ε 7300), 224 nm. (ε 7000) ; $\nu_{max}^{CHCl_3}$ 1780(lactone), 1730, 1245 (acetate), 1648, 900 (exomethylene) cm.$^{-1}$

(Found : C, 69.00 ; H, 7.85% ; $C_{24}H_{32}O_6$ requires C, 69.20 ; H, 7.75%)

Anhydroandrographolide diacetate 15 was recrystallised from chloroform/ether as fine needles, m.p. 134-135°(reported[16] 136.5-137.5°), λ_{max} 246 nm. (ε 13,200), and identified by t.l.c. and spectral comparison with an authentic sample prepared by acetylation of the diol. 35.

(ii) A solution of andrographolide (60 mg.) in pyridine (2 ml.) and acetic anhydride was heated under reflux for 2½ hours. The reaction mixture was left at room temperature for a further fifteen hours, diluted with water and extracted thoroughly with ethyl acetate. The combined extracts were washed with 4N hydrochloric acid, saturated sodium bicarbonate solution, brine and then dried over anhydrous sodium sulphate.

A t.l.c. examination of the crude products (58 mg.) showed that only a trace of the enol lactone 36 was present, the major component being the anhydroandrographolide diacetate 15.

Triacetylandrographolide[7] 18.

A mixture of andrographolide 5 (160 mg.) acetic anhydride (1 ml.) and freshly fused, powdered zinc chloride was heated gently for five minutes until the solution became homogeneous. The reaction mixture was diluted with water, extracted with chloroform, and the extracts washed with bicarbonate, brine and dried over sodium sulphate. Removal of solvent under reduced pressure furnished triacetyl andrographolide 18

(198 mg., 90%) recrystallised from ethanol as silky needles, m.p. 128-129° (reported[7] 128°); $\nu_{max}^{CCl_4}$ 1764 (lactone), 1742 (acetate) 900 (exomethylene) cm.$^{-1}$

(Found : C, 65.45 ; H, 7.60% ; $C_{26}H_{36}O_8$ requires C, 65.55 ; H, 7.60%)

Anhydroandrographolide diacetate from 18

A solution of triacetylandrographolide 18 (15 mg.) in anhydrous pyridine (1.5 ml.) was heated under reflux for fourteen hours. Water was added, and the resulting solution extracted thoroughly with ethyl acetate. The combined extracts were washed successively with 4 N hydrochloric acid, bicarbonate, brine, then dried and evaporated to give anhydroandrographolide diacetate 15 (12 mg.), identified with an authentic sample by melting point, mixed melting point and infra-red spectrum.

Sodium borohydride reduction of 18.

The reduction was carried out according to the method of Cava et al,[16] by which a solution of triacetylandrographolide 18 (55 mg.) in methanol (5 ml.) was treated with an excess (40 mg.) of sodium borohydride and the mixture stirred at 20° for thirty minutes. The reaction mixture was diluted with water, acidified with dilute hydrochloric acid and extracted repeatedly with chloroform. The organic layer was washed in the usual way, dried and evaporated to give crude deoxyandrographolide

acetate 14 (47mg.).

Two recrystallisations from ether gave a pure sample as needles, m.p. 118-119° (reported[16] 120°). There was no depression of the melting point on admixture with an authentic sample prepared by acetylation of deoxyandrographolide.

Benzylidine derivative[17] of 11-ketodeoxyandrographolide.

A solution of 11- ketodeoxyandrographolide 13 (40mg.) in freshly distilled benzaldehyde (3 ml.) was shaken for fourteen hours at 20° with freshly fused, powdered zinc chloride (60 mg.). After addition of ethyl acetate (20 ml.) the excess benzaldehyde was removed by extracting several times with 20% sodium bisulphite solution. Washing with water, drying and evaporation of solvent in vacuo yielded the desired benzylidine derivative 20 (38 mg.) as a gum which failed to crystallise after preparative t.l.c. (5% light petroleum/chloroform as eluting solvent). An analytical sample was prepared by sublimation at 180°/0.02 mm.

$\nu_{max}^{CCl_4}$ 1767 (lactone), 1720 (ketone) cm.$^{-1}$

Mass Spectrum : $M^+ = 436$; $C_{27}H_{32}O_5$ requires $M^+ = 436$

(Found = C, 74.05 ; H, 7.40% ; $C_{27}H_{32}O_5$ requires C, 74.30 ; H, 7.40%)

11- ketodeoxyandrographolide acetate 17.

To a solution of 11-ketodeoxyandrographolide 13 (50 mg.) in pyridine

(1 ml.) was added acetic anhydride (1 ml.) and the mixture was allowed to stand overnight at 20°. Methanol was added, the solvent removed under reduced pressure and the product chromatographed in 1% methanol/chloroform, affording the diacetate 17 (51mg., 89%). This compound resisted all attempts at crystallisation and was purified by sublimation at 180°/0.1 mm.

$\nu_{max}^{CCl_4}$ 1766 (lactone), 1740-1720 (acetate, ketone). 900 (exomethylene) cm.$^{-1}$

Mass Spectrum : $M^+ = 432$; $C_{24}H_{32}O_7$ requires $M^+ = 432$

(Found : C, 66.05 ; H, 7.45% ; $C_{24}H_{32}O_7$ requires C, 66.65 ; H, 7.45%)

Borohydride reduction of diacetate 17.

11-ketodeoxyandrographolide acetate 17 (91 mg.) in aqueous methanol (1:4, 6 ml.) was stirred for 30 minutes with excess sodium borohydride (65 mg.). Water was added and the solution extracted several times with chloroform. Washing of the extracts with water, followed by drying and evaporation of solvent, furnished an amorphous product (84 mg.), which was shown to contain two components in the ratio 70:30 by t.l.c. on silver nitrate impregnated plates.

The less polar constituent of the mixture (major product) proved to be the saturated keto-lactone 22 obtained as a glass after preparative scale chromotography in 5% light petroleum/chloroform.

$\nu_{max}^{CCl_4}$ 1783 (lactone), 1740, 1225 (acetate), 1720 (ketone) cm.$^{-1}$

Mass Spectrum : M^+ = 434; $C_{24}H_{34}O_7$ requires M^+ = 434.

The minor product was the lactol 23, recrystallised from ether as needles, m.p. 120-123° ;

$\nu_{max}^{CHCl_3}$ 3610, 3440 (hydroxyl), 1730 (acetate) 900 (exomethylene) cm.$^{-1}$

Mass Spectrum : M^+ = 438 ; $C_{24}H_{38}O_7$ requires M^+ = 438

(Found : C, 65.50 ; H, 8.70% ; $C_{24}H_{38}O_7$ requires
C, 65.75 ; H, 8.75%)

Jones Oxidation of lactol 23

To a stirred, ice-cold solution of the lactol 23 (10 mg.) in acetone (2 ml.) were added two drops of Jones reagent. After fifteen minutes methanol was added (0.5 ml.) followed by water (10 ml.) and the reaction mixture extracted three times with ethyl acetate. Normal work-up procedure yielded a product, homogeneous by t.l.c., which was identical with the keto-lactone 22 (t.l.c., i.r. spectrum).

Lactol Acetate 24

The lactol 23 (15 mg.) was acetylated in the usual way by allowing to stand overnight in pyridine (0.5 ml.) and acetic anhydride (0.5 ml.).

This provided the crude tetra-acetate 24 (18 mg.) which was purified by preparative t.l.c. and sublimation at 190°/0.2 mm.

$\nu_{max}^{CCl_4}$ 1745, 1230 (acetate), 1650, 902 (exomethylene) cm.$^{-1}$

Mass Spectrum : M^+ = 522 ; $C_{28}H_{42}O_9$ requires M^+ = 522

Anhydroandrographolide Diacetate 15

A solution of annydroandrographolide 35 (20 mg.) in pyridine (0.5 ml.) and acetic anhydride (0.5 ml.) was left at 20° overnight. Working up the reaction in the usual way afforded the crystalline diacetate 15 (21 mg.), m.p. 134-135° (reported[16] 136.5-137.5°); λ max 246 nm. (ε 13,000) ; $\nu_{max}^{CHCl_3}$ 1754(lactone), 1730 (acetate) 902 (exomethylene) cm.$^{-1}$

(Found : C, 69.25 ; H, 7.80% : $C_{24}H_{32}O_6$ requires
C, 69.20 ; H, 7.75%)

Deoxyandrographolide acetate 14

Deoxyandrographolide 37 (15 mg.) was acetylated in acetic anhydride/ pyridine as above and worked up to give the diacetate 14 (16 mg.) recrystallised from ether as needles, m.p. 118-120° (reported[27] 118°, 120°);

λmax 214 nm (ε 9,300), 220 nm. (ε 8,500), 230 nm (ε 4,400).

$\nu_{max}^{CHCl_3}$ 1754 (lactone), 1730 (acetate), 1647, 907 (exomethylene) cm.$^{-1}$

Mass Spectrum : M^+ = 418 ; $C_{24}H_{34}O_6$ requires M^+ = 418

(Found : C, 69.00 ; H, 8.30% ; $C_{24}H_{34}O_6$ requires
C, 68.90 ; H, 8.20%)

Osmium Tetroxide/Sodium Meta-periodate Cleavage of 22

Osmium tetroxide (30 mg.) in benzene (1 ml.) was added dropwise to a solution of the keto-lactone 22 (54 mg.) in benzene (3 ml.) and the mixture was left to stand at room temperature overnight with ten drops of pyridine. The osmate was decomposed by bubbling hydrogen sulphide through the dark-brown solution for fifteen minutes. Filtration through Celite 535 followed by removal of solvent furnished the diol 25 (50 mg.) as a viscous oil which was used for the cleavage reaction without further purification.

A solution of the above diol 25 in methanol (3 ml.) was left for sixteen hours with sodium meta periodate (40 mg) in water (2 ml.).

The reaction mixture was diluted with water, extracted with chloroform and worked up to give as the main product the β- diketone 26 (36 mg.) as a glass which was purified by preparative t.l.c. and sublimation at 190°/0.2mm. $\nu_{max}^{CCl_4}$ 1778 (lactone) 1743, 1233 (acetate), 1712 (ketones) cm.$^{-1}$

λ_{max} (with one drop NaCH) 309 nm. (ε 7500).

Mass Analysis : $M^+ = 436.2095$; $C_{23}H_{32}O_8$ requires $M^+ = 436.2097$.

Neoandrographolide 12

Neoandrographolide[6,8] 12, m.p. 167-168° (reported[8] 167-168°) was

isolated in low yield from the methanol extract of Andrographis paniculata by column chromatography on Silica gel, using 10% methanol/chloroform as eluting solvent. The compound was characterised as the tetra-acetate 38 m.p. 156-157°(reported[8] 155-157°), prepared by allowing a solution of neoandrographolide in pyridine and acetic anhydride to stand at room temperature overnight. Addition of methanol and removal of solvent in vacuo afforded the desired acetate 38 virtually in quantitative yield.

λ_{max} 205 nm (ε 10,500) ;

$\nu_{max}^{CHCl_3}$ 1750-1740 (lactone, acetate) 1629, 909 (exomethylene) $cm.^{-1}$

Mass Spectrum : $M^+ = 648$; $C_{34}H_{48}O_{12}$ requires $M^+ = 648$

(Found : C, 62.85 ; H, 7.40% ; $C_{34}H_{48}O_{12}$ requires
C, 62.95 ; H, 7.45%)

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