THESIS

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by

JAMES BERNARD THOMSON

Chemistry Department

The Royal College of Science and Technology

Glasgow

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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STUDIES ON STEROIDS AND TRITERPENOIDS

A. M. D. G.

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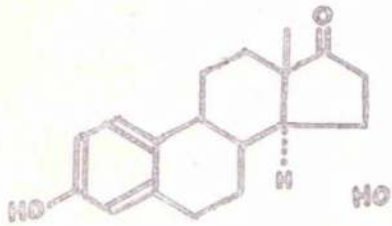
Part I

PARTIAL AROMATISATION OF THE STEROID NUCLEUS

HISTORICAL REVIEW

Introduction

Aromatic steroid-like compounds have been known since 1929 when the sex hormone, oestrone (I), was isolated.[1,2] Since 1929 relatively few naturally occurring aromatic compounds of this type have been discovered, and all of these, with the exception of the steroidal alkaloid veratramine (II),[3] are female sex hormones.



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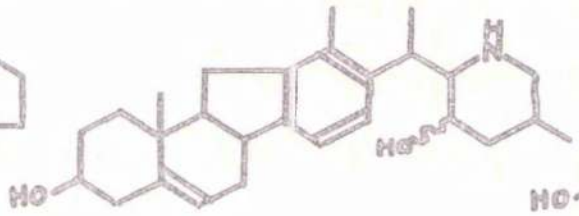

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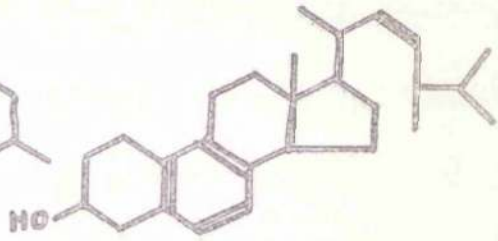

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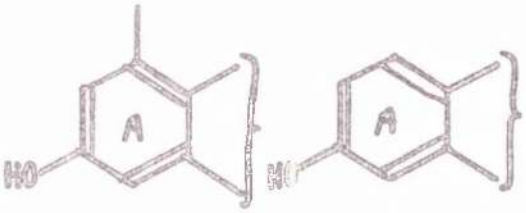

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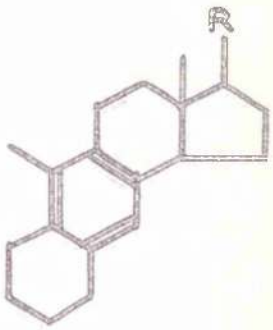
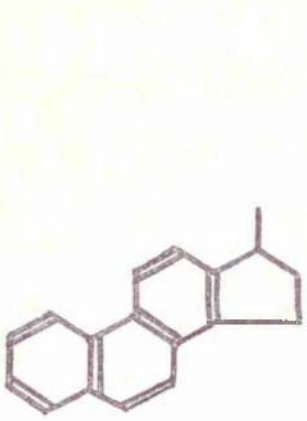


(VII)

However, laboratory preparations of 'aromatic steroids" are more numerous and, indeed, neoergosterol (III) was first prepared[4,5] in 1928 before the isolation of oestrone, although the aromatic nature of this compound was not recognised until 1932.[6] Compounds of the types (IV),[7] (V),[8,9] (VI),[10] and (VII)[11,12] have been prepared by means of the "dienone-phenol rearrangement", and type (VII) has also been prepared[13,14,15] by means of the "anthrasteroid rearrangement".

Steroidal aromatic compounds are known in which the aromatic

system is either ring A, or ring B, or rings A and B. Unless the aromatic hydrocarbons obtained from steroids by selenium dehydrogenation,[16] e.g. Diels' hydrocarbon (VIII), are included, no ring C aromatic compound was known prior to the work described in this thesis. However, ring C aromatic derivatives containing the carbon skeletons (IX)[17,18] and (X)[19] have been described; their nuclear structures differ profoundly from that of the normal steroids.



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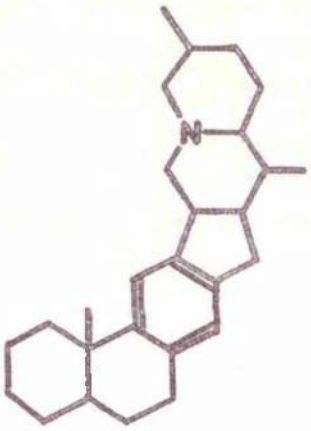

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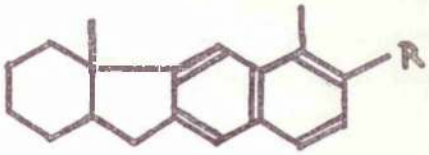


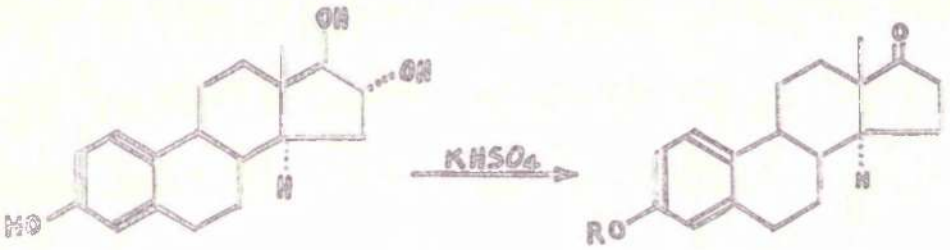
(X)

Rings A and B

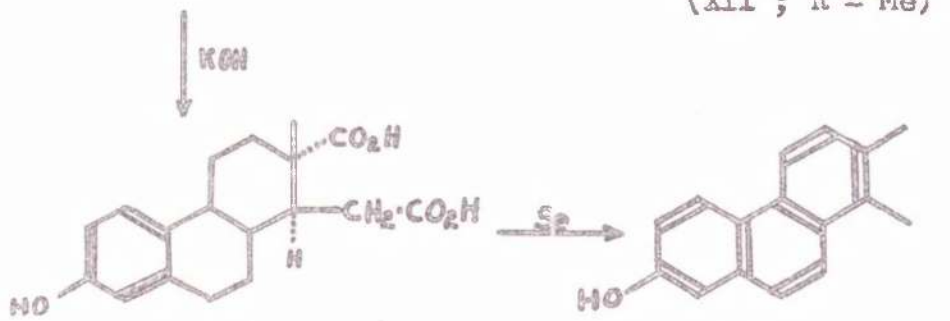
The most important of all aromatic derivatives of steroids is the female sex hormone oestrone (I) which was isolated independently in 1929 by Doisy and his co-workers[1] in the United States, and by Butenandt[2] in Germany, from the urine of pregnant women. The hormone (I) has been isolated from various animal sources[20] and also, surprisingly, in minute quantities from palm kernel extract[21] (18 mg. from 50 kg.) and from female willow flowers[22] (7 mg. from 65 kg.).

Shortly after the isolation of oestrone Marrian[23] obtained a less active oestrogen, oestriol (XI), from human pregnancy urine.

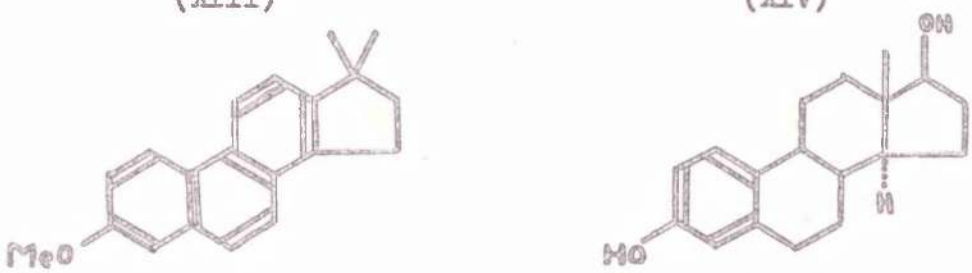
Oestriol (XI) can be dehydrated[24] to the phenolic[24,25,26] ketone[25] oestrone (I), and when it is fused with alkali[27] marrianolic acid (XIII) is obtained. Selenium dehydrogenation of this acid[28] gives the dimethylphenanthrol (XIV) which has been identified by synthesis.[29] When the carbinol prepared from oestrone methyl ether (XII), by treatment with methyl magnesium iodide, is dehydrated and the product dehydrogenated by heating with selenium, 7-methoxy-3':3'-dimethyl-1:2-cyclopenteno-



(XI) (I ; R = H) (XII ; R = Me)



(XIII) (XIV)



(XV) (XVI)

phenanthrene (XV) is formed, and this has been identified by synthesis.[30] These experiments established the structures of oestriol (XI) and oestrone (I), and the latter has been confirmed by synthesis.[31]

Reduction of oestrone by sodium in ethanol,[32] or by hydrogenation in neutral or alkaline solution,[33] gives a diol (XVI) which has been found to possess a much higher oestrogenic potency. This discovery

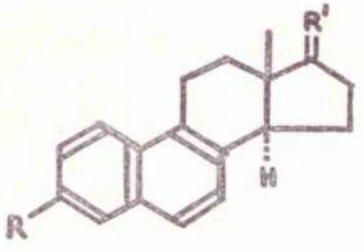
led workers in this field to suspect that the substances secreted in urine may not be primary hormones, and in 1935 Doisy[34] succeeded in isolating 35 mg. of oestradiol (XVI) from 4 tons of sow ovaries. The diol has since been obtained from other animal sources.[35] The configuration at C_{17} is based on optical and chemical evidence.[36]

Oestradiol appears to be a true primary hormone, whereas the related substances are products of metabolic changes. The primary oestrogens control the growth of the reproductive organs and promote the growth of secondary sex characteristics. The oestrogen, produced in the ovary, passes to the uterus and vagina where it causes the changes of oestrus, in which the vaginal lining acquires a characteristic structure differing from that at rest. These changes form the basis of the Allen-Doisy test[37] for oestrogens. A test solution is injected into the ovariectomised test animal; the amount of material required just to produce oestrus response, which is observed by microscopic examination of vaginal smears, gives a measure of oestrogenic potency.

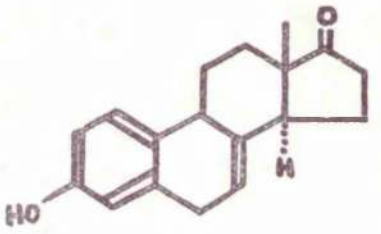
Two new oestrogens of much lower activity than oestrone were isolated[38] in small amounts from mare's pregnancy urine in 1932. These substances, equilenin (XVII) and equilin (XX), have been degraded[30] in the same manner as oestrone, to the cyclopentenophenanthrene (XV). Equilenin shows the optical characteristics of a naphthol and degradation to the ether (XV) establishes its structure as (XVII). Equilin is readily dehydrogenated to equilenin (XVII) and contains a non-conjugated double bond;[39] the structure (XX) was proposed by various workers[39] and later confirmed by Serini.[40] Since the amount of oestrone (I) in mare's urine falls as that

of equilenin (XVII) and equilin (XX) increases, it appears that as pregnancy progresses oestrone is partially dehydrogenated.[38]

Other naturally occurring ring A-B aromatic steroids are "δ-follicular hormone" (XVIII), which has been isolated from mare's pregnancy urine[41] and synthesised[42] from equilenin; 3-desoxyequilenin (XIX), which has been isolated from the same source and related to equilenin;[43] and tetrahydroequilenin (XXI), which has been isolated



(XVII; R = OH; R' = O)
(XVIII; R = OH; R' = H,OH)
(XIX; R = H; R' = O)



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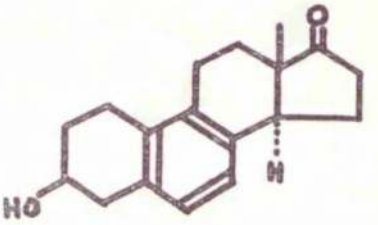
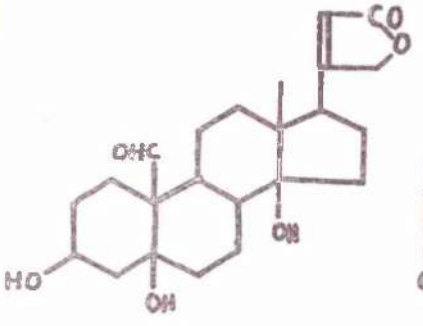


(XXI)



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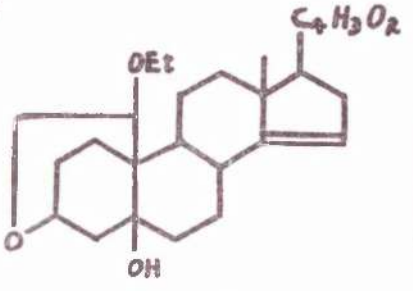

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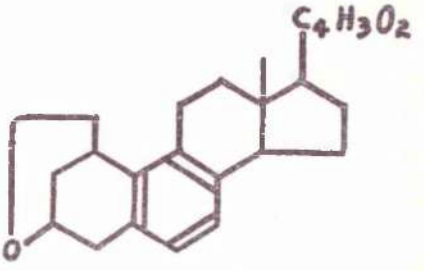


(XXIV)

from equine pregnancy urine.[44]

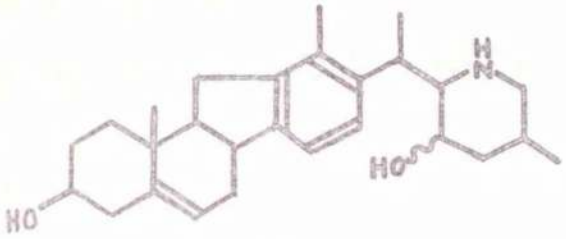
An aromatisation reaction involving ring B was discovered during work on the cardiac glycoside strophanthidin (XXII), which, when treated with ethanolic hydrochloric acid in the cold,[45] gives the anhydroacetal (XXIII). Concentrated hydrochloric acid converts the acetal (XXIII) into a trianhydrostrophanthidin[46] for which the structure (XXIV) has been proposed.[46,47] No mechanism has been suggested for this rearrangement. An aromatisation reaction involving

ring A has been reported in the ōubagenin series.[48]

The formation of neosteroids, the "dienone-phenol rearrangement", and the "anthrasteroid rearrangement" are discussed in separate sections (pp. 9,16 , and 26 resp.).

Rings C and D

Although no example is known of a D-homosteroid in which ring D is aromatic, the steroidal alkaloid veratramine (II)[3] and



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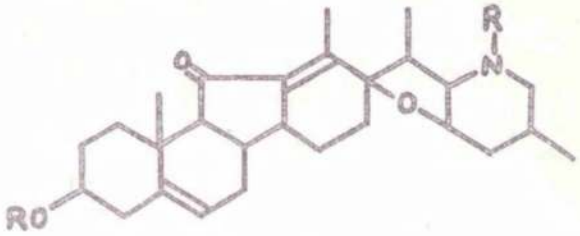
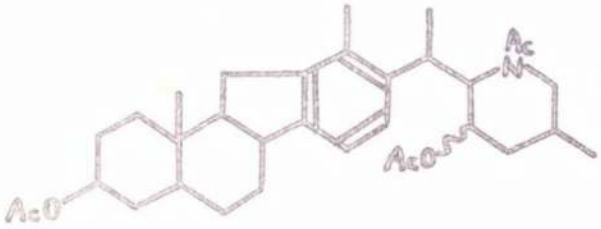
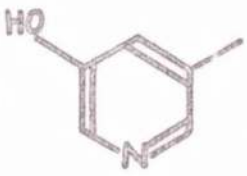


(XXV; R = H)
(XXVI; R = Ac)



(XXVII)



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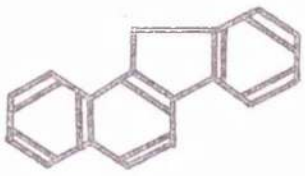

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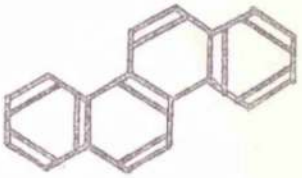
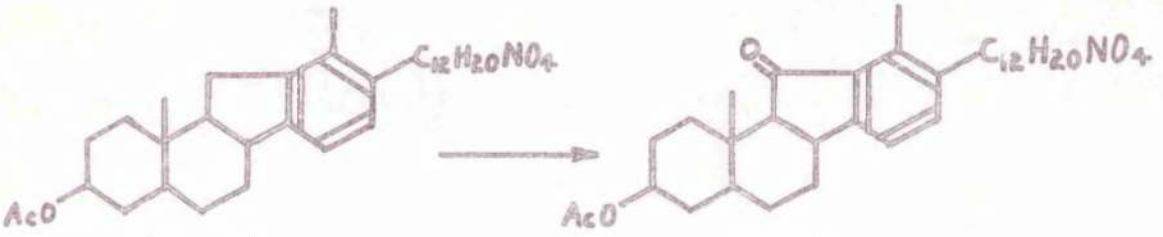


(XXX)

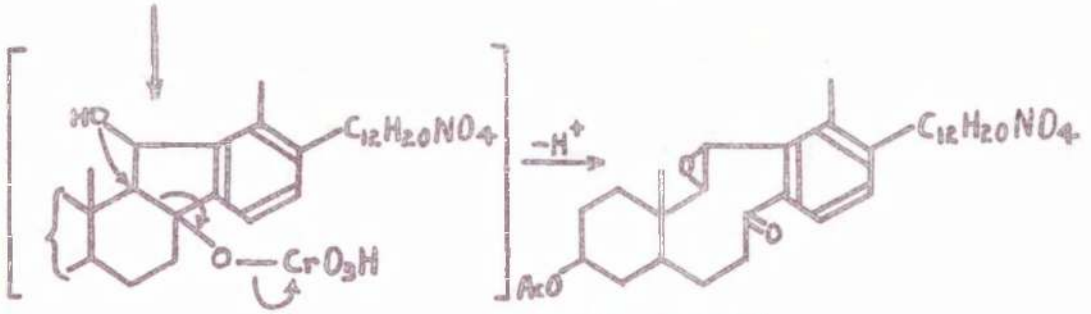
certain derivatives of jervine (XXV) are related to this type of structure. Veratramine and jervine are members of the physiologically active[49] veratrum series of alkaloids. These alkaloids decrease the rate of respiration, the lethal effect of heavy doses being due largely to this action, and they also affect the circulatory system. In small doses the effect is partly due to reflex vasodilation, and in large doses it is partly due to their ability to stimulate adrenalin secretion. The veratrum alkaloids have been used as hypotensives and also as insecticides.

Veratramine (II) was first isolated from Veratrum grandiflorum

Loes. fil.;[50] it contains[3,51] two alcoholic hydroxyl groups, a double bond, and an aromatic ring, the presence of which is shown by its ultraviolet absorption spectrum and by the formation of an aromatic nitro-compound[52] from triacetyldihydroveratramine (XXVII). Veratramine gives a precipitate with digitonin and the presence of a 5:6-double bond and a 3β-hydroxyl group have been demonstrated by conventional methods.[3,51] Selenium dehydrogenation gives 5-hydroxy-3-methylpyridine (XXVIII), identified by synthesis, together with a hydrocarbon $C_{22}H_{20}$.



(XXVII) (XXXI)



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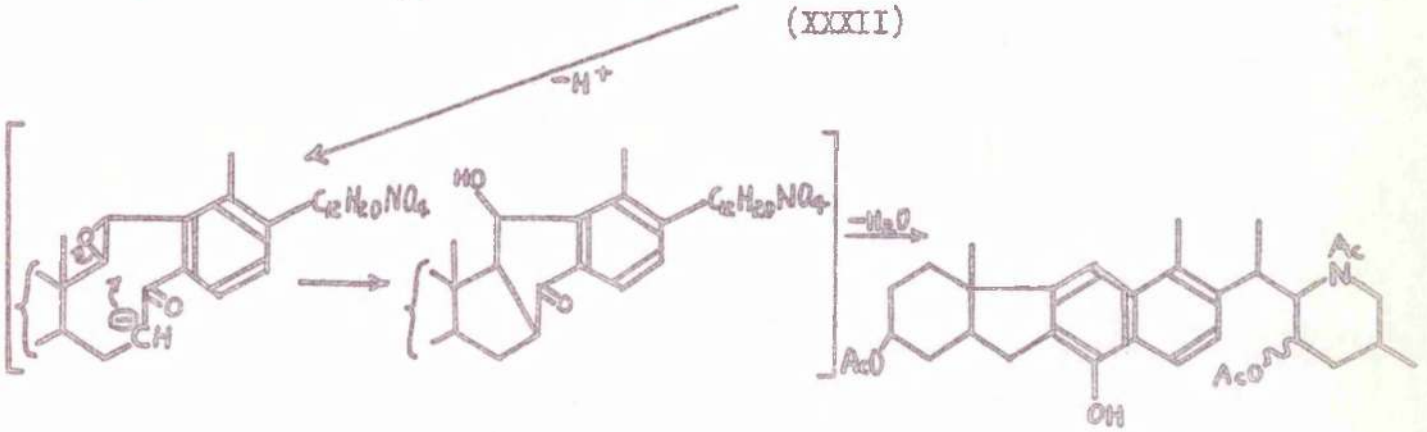
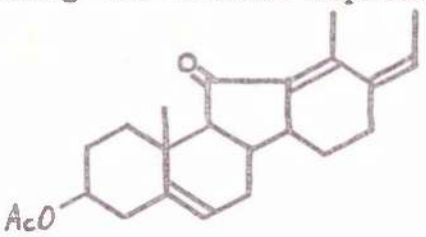


(XXXIII)

The latter is also obtained in the same manner from jervine (XXV) and it is believed to be either a homologue of 1:2-benzofluorene (XXIX)[53] or of chrysene (XXX).[51] Since selenium dehydrogenation of all other veratrum alkaloids gives pyridine derivatives with an ethyl group at position 6, a reaction ascribed to scission of the C_{17}-C_{20} bond, Tamm and

Wintersteiner[52] reason that the side-chain in veratramine is attached to the aromatic ring and that dehydrogenation is accompanied by scission of the C_{20}-C_{22} bond. They therefore propose the structure (II) for veratramine. Support for this structure has been obtained[52] by the oxidation of triacetyldihydroveratramine (XXVII) to the indanone (XXXI) which contains an unreactive carbonyl group and is identical with a compound previously obtained from jervine.[54] The major product of this oxidation[19] is the keto-epoxide (XXXII) which is very unstable to acids, alkalis, and even active surfaces, e.g. chromatographic alumina, thereby forming the unusual naphthol (XXXIII) by the sequence shown on page 7.



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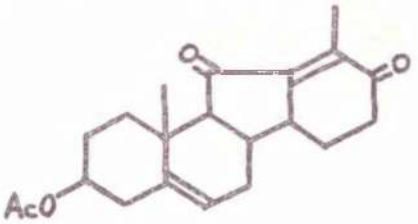

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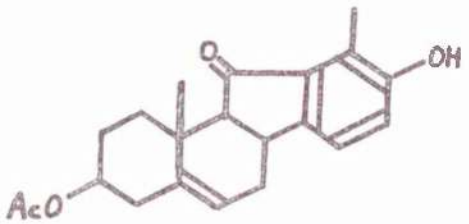

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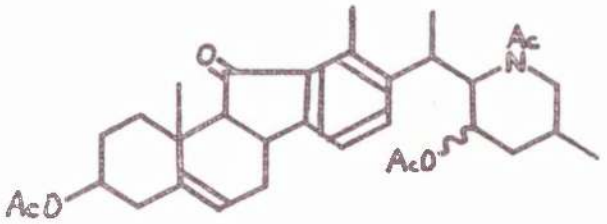
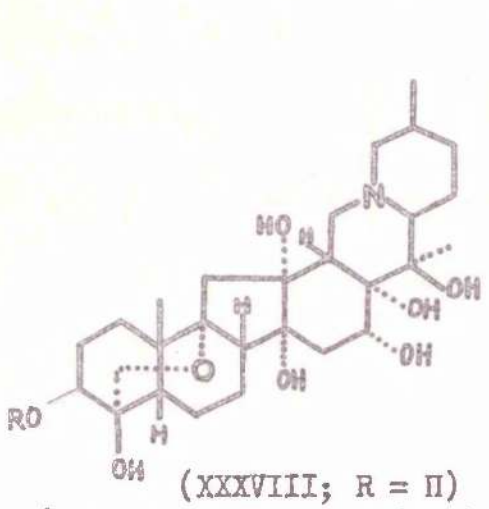


(XXXVII)

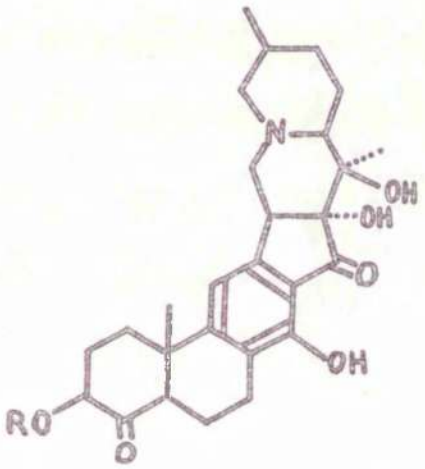
Treatment of the steroidal alkaloid jervine (XXV), a constituent of Veratrum album,[55] with acetic anhydride and anhydrous zinc chloride gives the trienone (XXXIV) which, when oxidised by chromic acid, yields the diendione (XXXV). This diendione (XXXV) readily forms the phenol (XXXVI) on treatment with alkali.[56]

When diacetyljervine (XXVI) is treated with acetic acid and acetic anhydride containing a catalytic amount of sulphuric acid the product is the indanone (XXXVII), catalytic hydrogenation of which gives the dihydrocompound (XXXI), an oxidation product of triacetyl-dihydroveratramine (XXVII).[54]

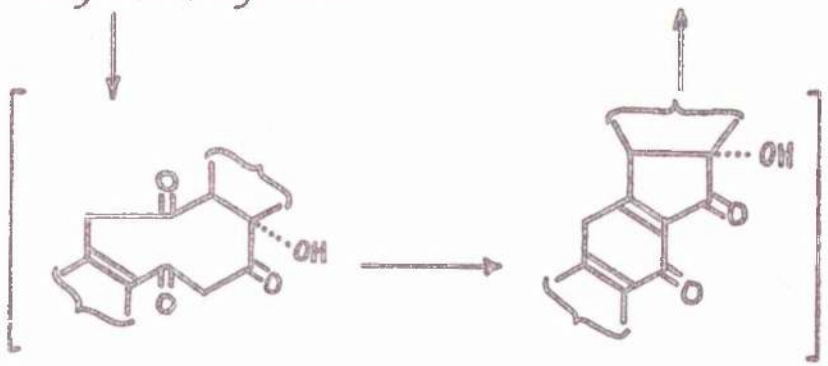
Two examples of ring C aromatic compounds derived from steroidal substances have been described and in both cases the carbon skeleton differs profoundly from that of the normal steroid nucleus. The naphthol (XXXIII), described above, is one of these compounds and the other is the phenol (XL) which is formed[18] by the Kiliani oxidation[57]



(XXXVIII; R = H)
(XXXIX; R = $CH_3 \cdot CH=C(CH_3) \cdot CO-$)



(XL; R = $CH_3 \cdot CH=C(CH_3) \cdot CO-$)

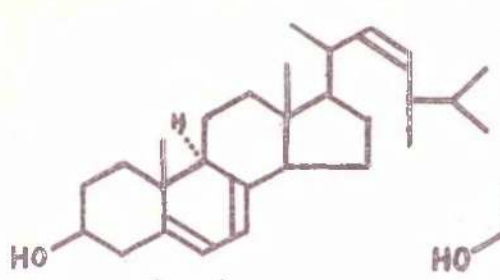


of cevadine (XXXIX), the angelica acid ester of the veratrum alkamine, veracevine (XXXVIII).

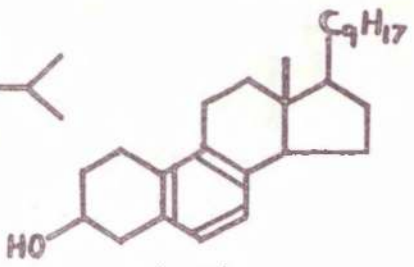
The Neosteroids

Windaus,[4,5] in 1928, studied the action of visible light on ergosterol (XLI), in the presence of eosin and in the absence of oxygen, and obtained the bisergostatrienol (XLII).[58,59] When distilled in high vacuum, the bis-steroid (XLII) loses methane and gives a mixture of neoergosterol (III) and "isoneoergosterol";

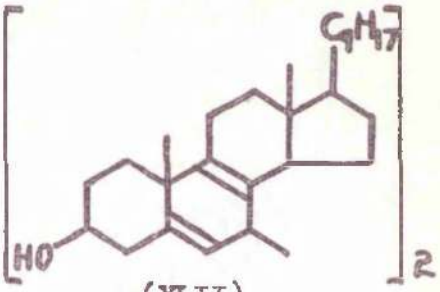
this latter substance is discussed on page 16. Neoergosterol (III) does not give the typical sterol colour reactions and it contains only one reactive double bond[60] which was shown to be in the C_{22}-C_{23} position by the isolation of methylisovaleraldehyde (XLIII) as a product of ozonolysis.[6] Three additional double bonds are indicated by elementary analysis, and that they are present in a benzene ring was inferred[6] from the ultraviolet absorption spectrum. Oxidation of neoergosterol by concentrated nitric acid gives prehnitic acid (XLIV) which was shown to be a fragment of ring B and not ring C by the ready dehydrogenation[61] of neoergosterol, without loss of methane, to the naphthol (XLV),



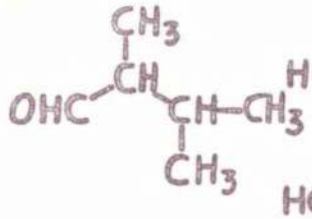
(XLI)



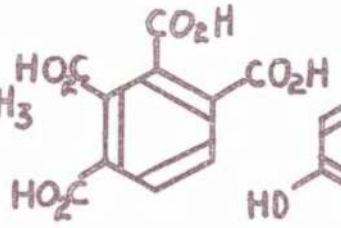
(III)



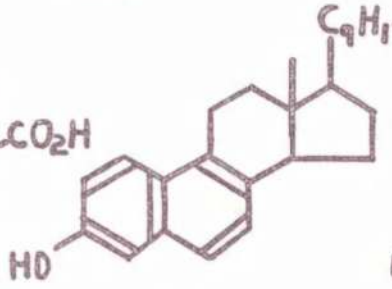
(XLII)



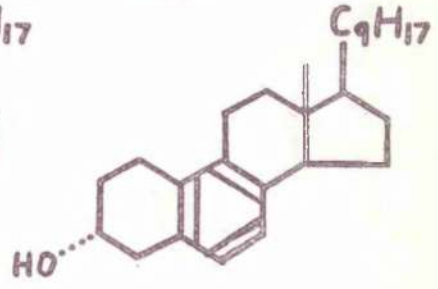
(XLIII)



(XLIV)



(XLV)



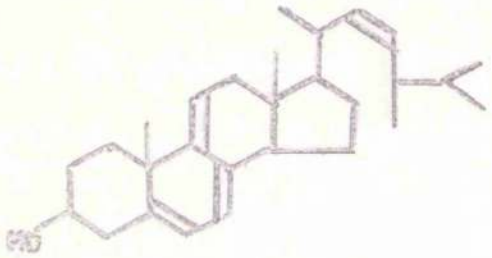
(XLVI)

reducible by means of sodium in amyl alcohol[62] to epineoergosterol (XLVI). The 3β-hydroxyl group[63,64] in neoergosterol is epimerised[62] by refluxing with sodium in amyl alcohol; the hydroxyl group in both epimers is "equatorial".[65]

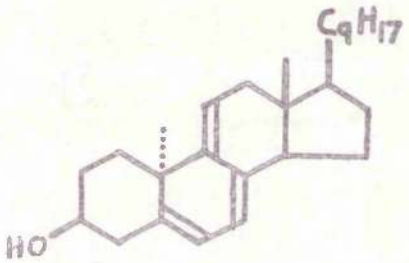
Windaus[5] also prepared a bis-sterol from dehydroergosterol (XLVII) and Dimroth[66] has shown that dehydrolumisterol (XLVIII), but not lumisterol (XLIX), gives a similar dimer. Kennedy and Spring[64] confirmed the observation that lumisterol fails to dimerise, and also showed that whereas isopyrocalciferol (L) does not give a

bis-steroid, pyrocalciferol (LI) readily does so. Pyrolysis of this dimer yields neoergosterol (III). From these experiments it was deduced that photodimerisation takes place only when the C_{10}-methyl group and the C_9-hydrogen atom are trans (but see page 14).

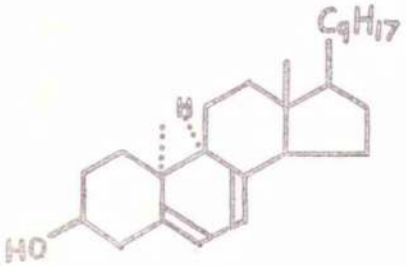
A similar dimerisation has been observed[67] for 7-dehydrocholesterol (LII) and the dimer (LIV) has been pyrolysed to a neosterol (LV), formulated by analogy with the products of the same reaction in the



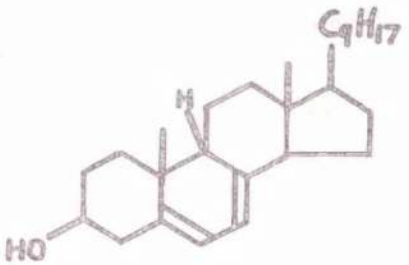
(XLVII)



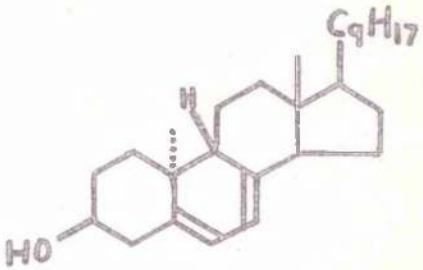
(XLVIII)



(XLIX)



(L)

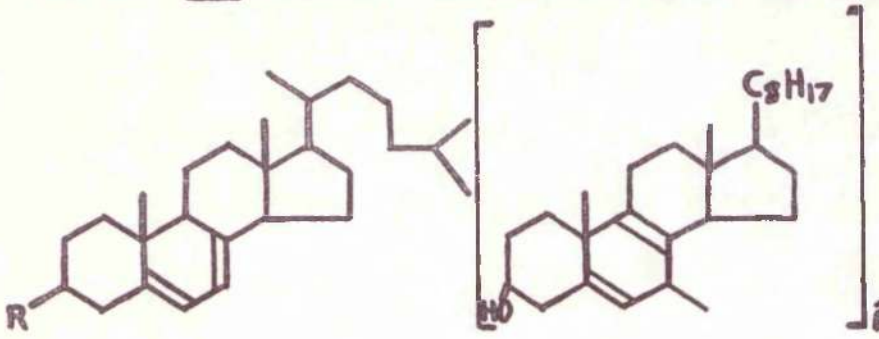


(LI)

ergostoryl series. The same dimer (LIV) and neosterol (LV) have been prepared[68] from cholesta-6:8-dien-3β-ol (LVI) and coprosta-6:8-dien-3β-ol (LVII). Akira Tominaya[69] has shown that the hydroxyl group at position 3 is not necessary for the reaction, by preparing a bis-steroid from cholesta-5:7-diene (LIII). That the nature of the side-chain has no bearing on the course of the reaction is shown by the formation of bis-steroids and neosteroids from the dienes of the ergosterol and cholesterol series mentioned above, and also from methyl 3β-acetoxy-bisnorchola-5:7-dienate (LVIII) and 22-iscspirosta-5:7-dien-3β-yl

acetate (LIX).[70,71]

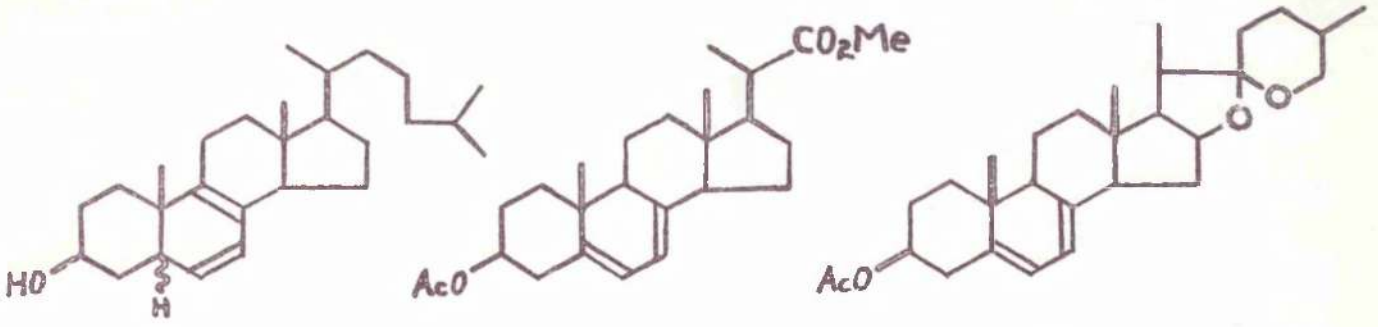
Steroids with unsaturation in ring A have also been subjected to this reaction. Irradiation of cholesta-2:4-diene (LX) with a tungsten lamp causes dimerisation to what Jacobsen[72] suggests is the 3:3'-bis-steroid. An isomeric dimer was obtained by Kosheleva[73]



(LII ; R = OH)
(LIII; R = H)

(LIV)

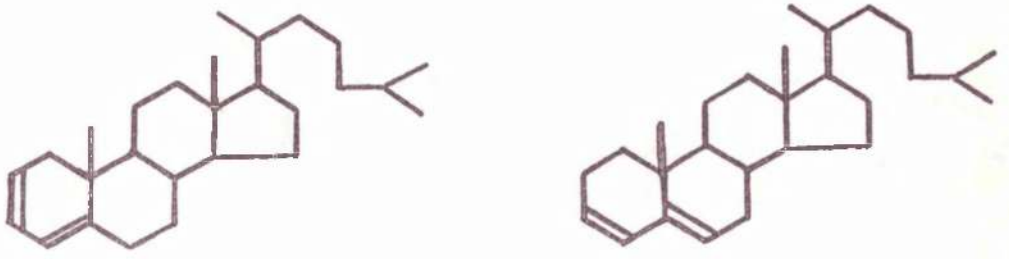
(LV)



(LVI; 5α)
(LVII; 5β)

(LVIII)

(LIX)



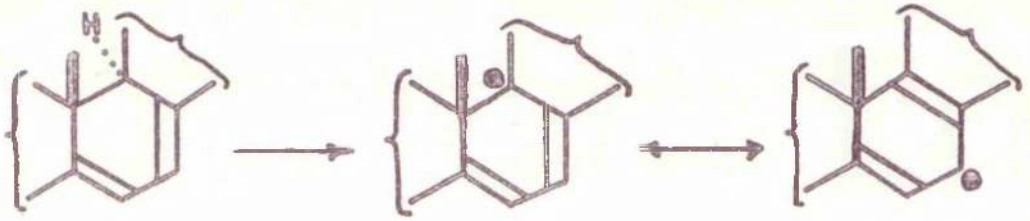
(LX)

(LXI)

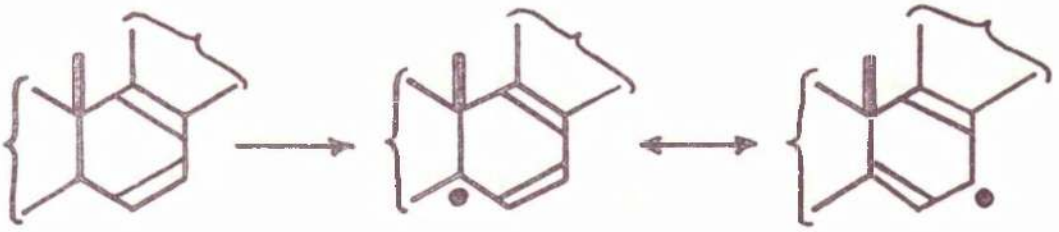
when the same diene (LX) was irradiated with sunlight; this dimer, for which the 4:4'-structure is suggested, was also obtained, in the same manner, from cholesta-3:5-diene (LXI).

Owades[74] has studied the photodimerisation reaction and has proposed

a free radical mechanism. In the case of the 5:7-dienes a hydrogen atom may be ejected, with formation of a radical, from positions 6, 7, or 9.* Of these positions, loss of a hydrogen atom from positions 7 or 9 would give a radical capable of resonance stabilisation, thus permitting coupling at either of these positions. Since position 9 is sterically hindered coupling should take place at 7 to give dimers of the structure proposed by Inhoffen:[58]



The 6:8-dienes can lose a hydrogen atom from positions 5, 6, or 7, of which loss from 5 produces a resonating radical:



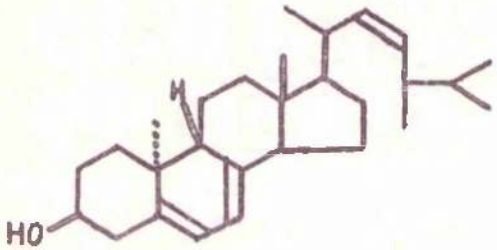
Since position 5 is hindered, coupling should take place at 7 to give the same dimer as the 5:7-dienes (cf. Windaus[68]). The same radical will be formed from 5α and 5β steroids, thus accounting for the formation of the same bis-steroid from cholesta-6:8-dien-3β-ol (LVI) and from coprosta-6:8-dien-3β-ol (LVII).

Owades[74] explains the deduction[64] that ergosterol isomers form photodimers only if the C_{10}-methyl group and the C_9-hydrogen atom are trans, on the grounds that a study of molecular models shows that the C_9-hydrogen atom is sterically hindered when it is cis to

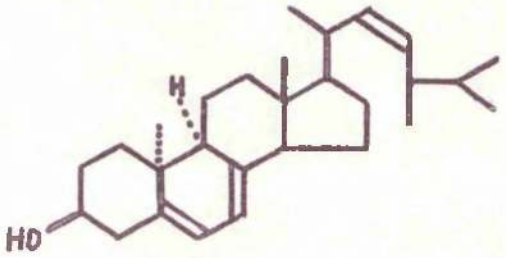
---

* Owades[74] does not consider loss of a hydrogen atom from position 4.

the C_{10}-methyl group. The author of this thesis has constructed molecular models of the wire type, and of the Stuart type, and he is not able to agree with Owades. The C_9-hydrogen atom is not particularly hindered when it is cis to the C_{10}-methyl group, and there appears to be no reason why it should not be expelled to form a radical. That



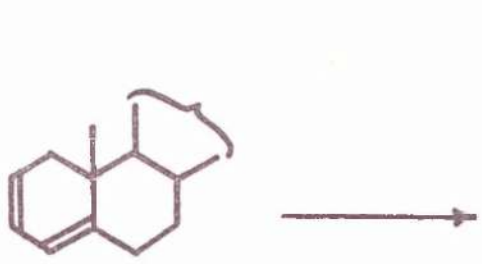
(LI)



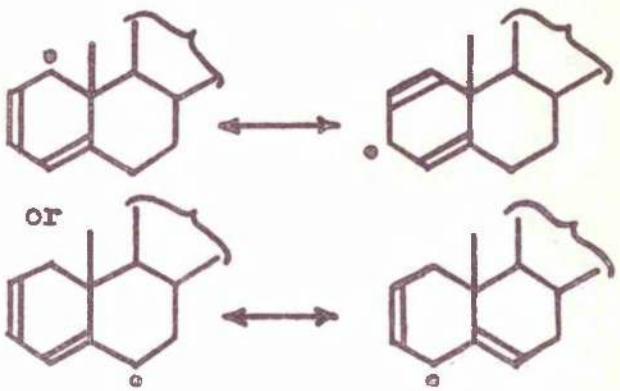
(XLIX)

the deduction is in fact false is shown by Castells, Jones, Meakins, and Williams[75] who, in a recent publication, have presented unambiguous proof that lumisterol is the 9β-steroid (LI) and that pyrocalciferol is 9α-lumisterol (XLIX) (see page 11).

The formation of two isomeric dimers from cholesta-2:4-diene (LX) is explained[74] in the following manner: the 2:4-diene can lose a hydrogen atom from positions 1, 2, 3, 4, or 6, of which only loss from 1 or 6 would produce a resonance stabilised radical:

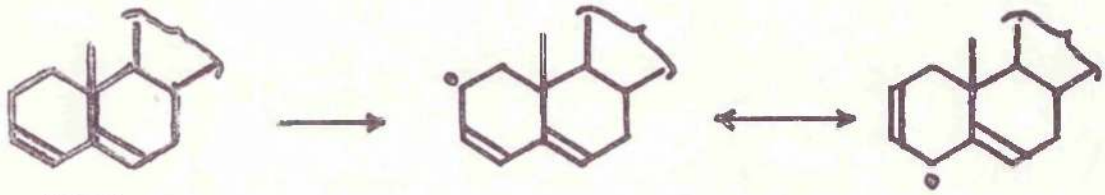


(LX)



None of the four possible positions for coupling can be eliminated for steric reasons but positions 3 and 4 would be favoured because they are α to two double bonds, whereas the other two positions are merely allylic to one double bond. The two isomeric dimers reported in the

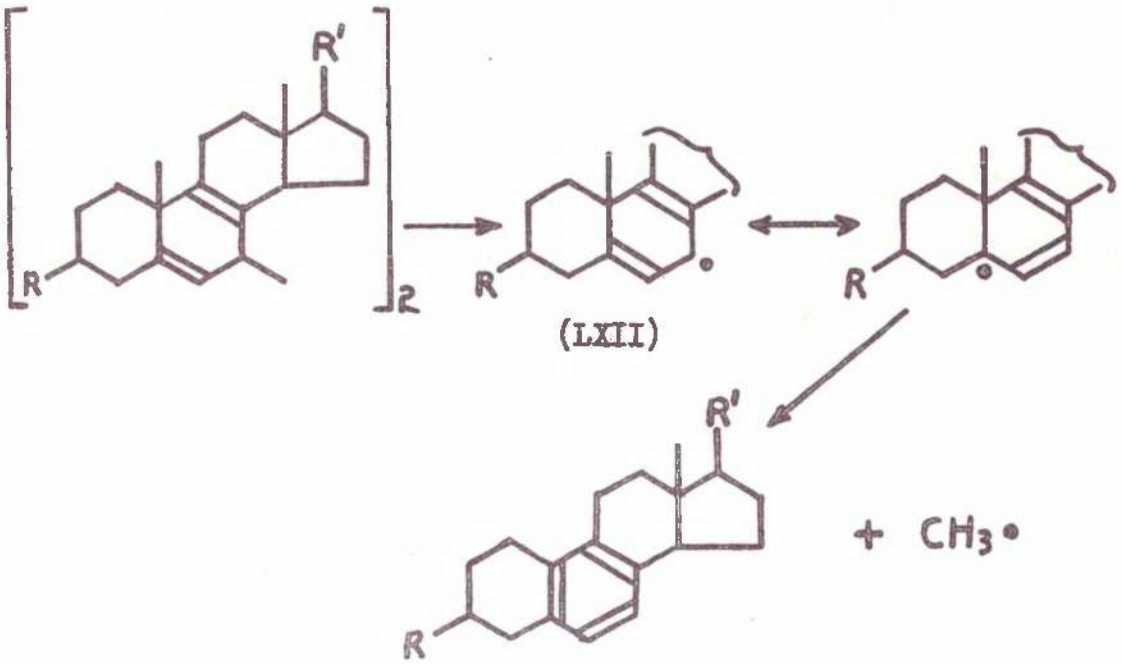
literature[72,73] will be, therefore, the 3:3'- and the 4:4'-bis-steroids. Applying the same reasoning to cholesta-3:5-diene (LXI) it is apparent that coupling would take place best at position 4:



(LXI)

The dimer obtained by Kosheleva[73] from the 2:4- and 3:5-dienes should therefore be the 4:4'-bis-steroid, and that obtained by Jacobsen[72] from the 2:4-diene should be the 3:3'-bis-steroid.

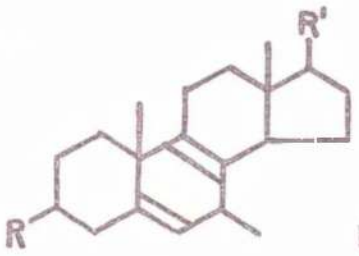
No mechanism has been proposed for the formation of neosteroids from the dimers, but, because of the pyrolytic conditions necessary for the reaction, it is almost certainly a free radical process. Scission of the dimer according to Schmidt's double bond rule[76] - that on pyrolysis a bond once removed from a double bond is preferentially broken - accounts both for the rupture of the 7-7' bond and of the 10-19 bond. A feasible route might be:



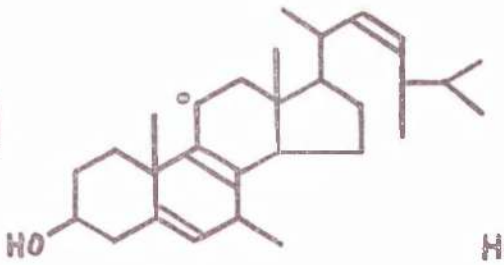
(LXII)

The methyl radical might be expected to either dimerise, to give ethane,

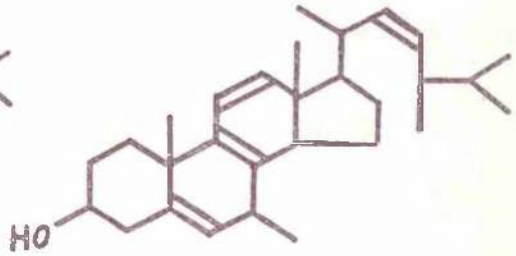
or to combine with the radical (LXII). Inhoffen[6] has identified the gaseous product as methane, but the presence of ethane cannot be excluded. Combination of a methyl radical with the radical (LXII) would lead to the methylsteroid (LXIII). Ando[77] has examined the solid by-product from the pyrolysis of bisergostatrienol (XLII), the so-called "isoneoergosterol," and has shown it to be a molecular compound (1:1) of neoergosterol and the non-aromatic "isodehydroergosterol." The latter compound is considered to be a tetraene with one double bond in the side-chain, two double bonds conjugated in one ring, and one isolated nuclear double bond. The analyses reported in the literature[77] do not distinguish between C_{28} and C_{29}; it is therefore



(LXIII)



(LXIV)



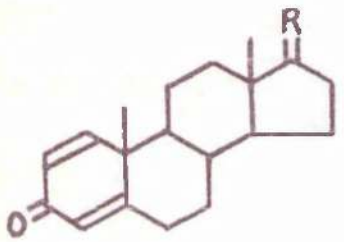
(LXV)

possible that "isodehydroergosterol" might be represented by (LXV) It is well known that the lower alkyl radicals can abstract allylic hydrogen atoms; such a process could lead to the radical (LXIV) which, by disproportionation, would yield the tetraene (LXV) and the triene (LXIII; R = OH; R' = C_9H_{17}). A more careful examination of the by-products might disclose the presence of this latter triene.

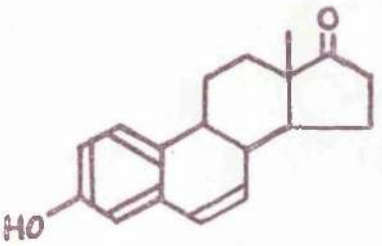
The Dienone-Phenol Rearrangement

cycloHexadienones were first described by von Auwers[78] who showed that they can be converted to phenols by heating with zinc dust in acetic acid. Inhoffen[58] applied a

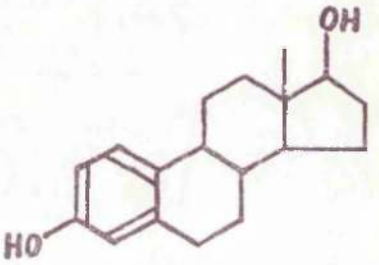
reaction of this type to the steroids and found that on heating the dehydrobromination product from dibromoandrostandione, presumably (LXVI), methane is evolved and phenolic products are formed, one of which is the isoequilin (LXVIII). Oestradiol (XVI) has been prepared[79] in very poor yield by a similar pyrolysis of 3-oxo-androsta-1:4-dien-17β-ol (LXVII).



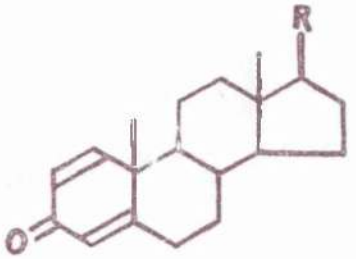
(LXVI; R = O)
(LXVII; R = H,OH)



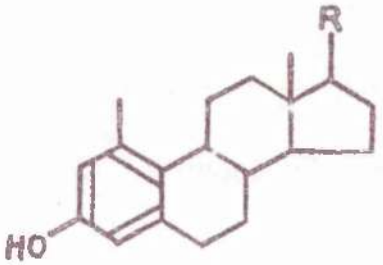
(LXVIII)



(XVI)



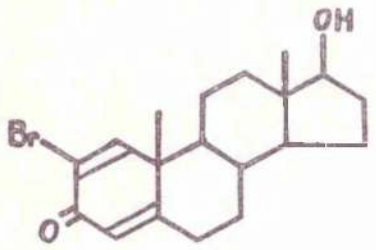
(LXIX; R = C_8H_{17})
(LXX; R = CO_2H)
(LXXI; R = $CHMe \cdot CH_2 \cdot CH_2 \cdot CO_2H$)



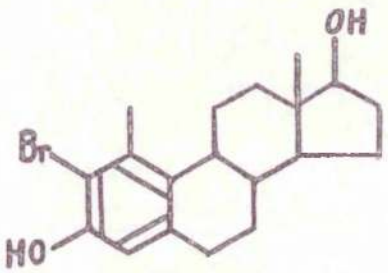
(LXXII; R = C_8H_{17})
(LXXIII; R = OH)
(LXXIV; R = CO_2H)
(LXXV; R = $CHMe \cdot CH_2 \cdot CH_2 \cdot CO_2H$)

Subsequently it was discovered[80,81] that when conditions similar to those used for the santonin aromatisation[82,83] are applied to 3-oxocholesta-1:4-diene (LXIX) the product is isomeric with the starting material but contains a benzene ring; Inhoffen and his co-workers[80,81] formulated this aromatic product as the 1-methyl compound (LXXII). By means of this reaction methyloestradiol, formulated as the phenol (LXXIII),[81] was prepared and it proved to be physiologically inactive. These results were confirmed by Djerassi and his co-workers[10] who also

reported the formation of the phenol (LXXIV)[84] from 3-oxoalloetiocho-la-1:4-dienic acid (LXX), and of the bromophenol (LXXVII)[85] from 2-bromo-3-oxoandrosta-1:4-dien-17β-ol (LXXVI). The phenol (LXXV),



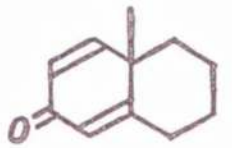
(LXXVI)



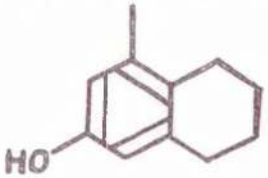
(LXXVII)

prepared from 3-oxochola-1:4-dienic acid (LXXI), has been described.[86]

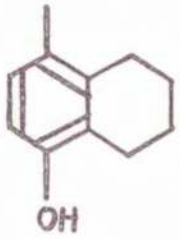
Singh and Woodward[7] studied the action of acetic anhydride in the presence of sulphuric acid on 10-methyl-2-oxo-$\Delta_{1(9):3(4)}$-hexahydronaphthalene (LXXVIII) and were able to show that the product is not



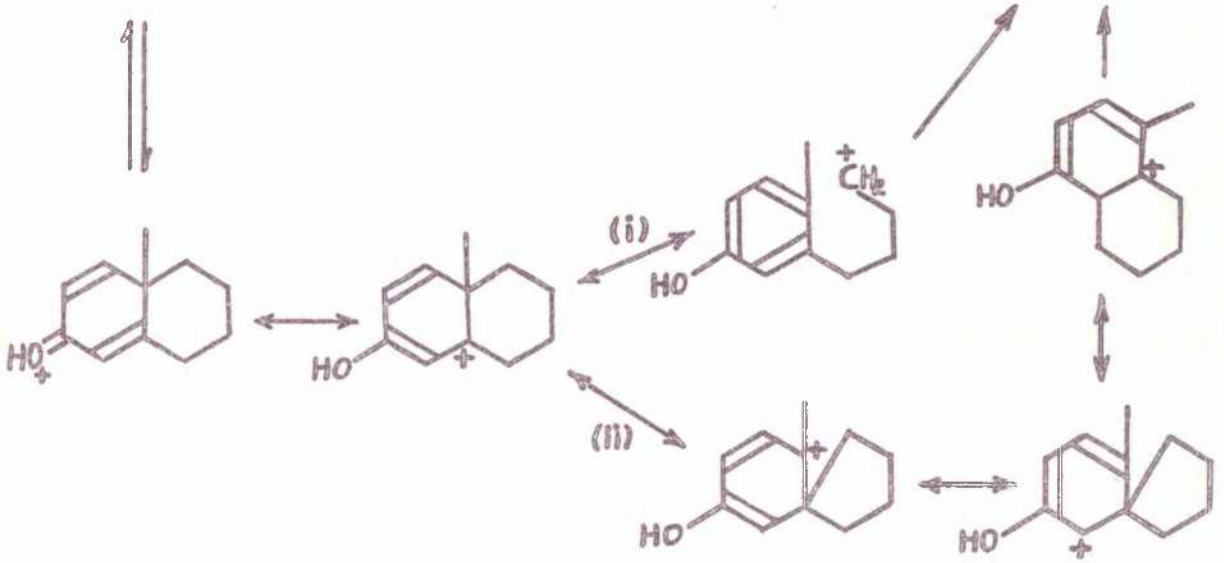
(LXXVIII)



(LXXIX)



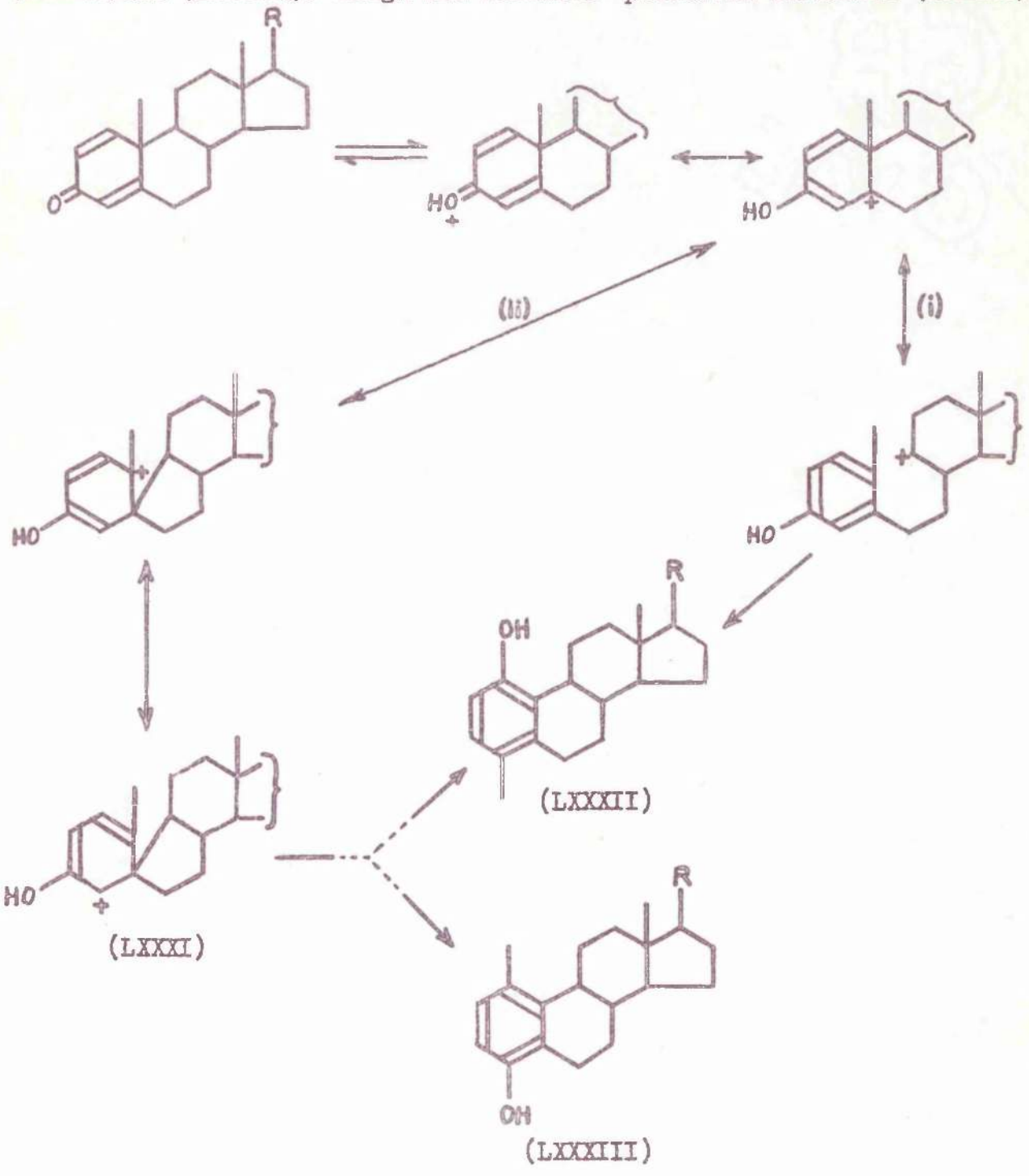
(LXXX)



the expected 3-hydroxy-1-methyl compound (LXXIX) but the 4-hydroxy-1-methyl compound (LXXX). To account for this behaviour these workers[7] proposed an ionic mechanism for the rearrangement. Two possible routes, illustrated above, would be operative unless there are special factors in a particular

case which would favour simple methyl migration.

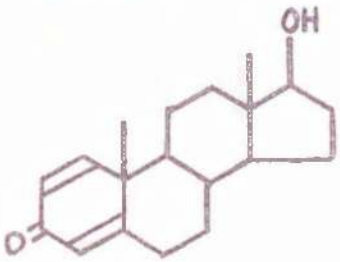
In the case of the steroids there is no such special factor; the rearrangement should therefore lead to one or other of the phenols (LXXXII) and (LXXXIII). Singh and Woodward[7] preferred structure (LXXXII).



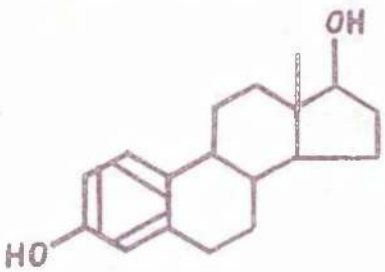
Evidence in favour of the structures (LXXXII) or (LXXXIII) is that this type of compound possesses cryptophenolic properties[87] whereas oestradiol (XVI), of which (LXXIII) is merely the 1-methyl derivative, has normal phenolic properties. Furthermore the phenol

from the dienone (LXVII) is completely devoid of oestrogenic activity,[87] a remarkable fact if the methyloestradiol structure (LXXIII) is accepted, in view of the non-specificity of this type of hormonal action.

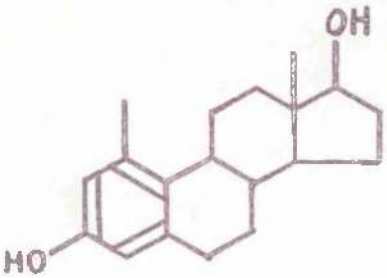
Inhoffen and Zühlsdorff[79] converted the phenol from 3-oxocholesta-1:4-diene (LXIX) into a monobromo derivative and in an attempt to demonstrate that a second position ortho to the phenolic hydroxyl group is open, treated the bromoderivative with sodium nitrophenyldiazotate; while coupling did in fact take place, the bromine atom was displaced. Such



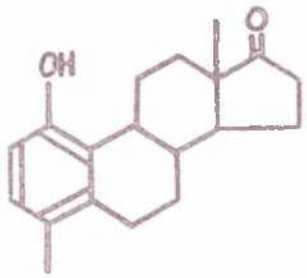
(LXVII)



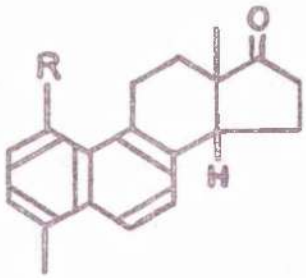
(XVI)



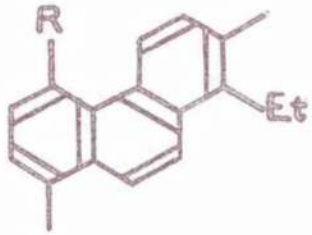
(LXXIII)



(LXXXIV)



(LXXXV; R = OMe)
(LXXXVI; R = H)

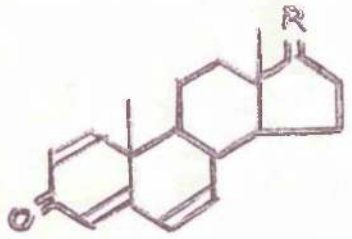


(LXXXVII; R = H)
(LXXXVIII; R = OMe)

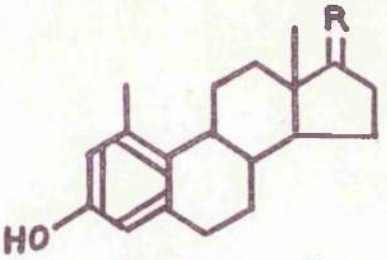
behaviour is characteristic of bromophenols with no free ortho or para positions.[88]

Dreiding and his co-workers[89] have shown that structure (LXXXII) is correct. The methyl ether of the phenol (LXXXIV) has been dehydrogenated by heating with palladium, to yield a mixture of 1-methoxy-4-methyl--3-desoxyisoequilenin (LXXXV), 4-methyl-3-desoxyisoequilenin (LXXXVI), 1-ethyl-2:8-dimethylphenanthrene (LXXXVII), and its 5-methoxy derivative (LXXXVIII); these last two compounds, (LXXXVII) and (LXXXVIII), have been identified by synthesis.

Djerassi and his co-workers[8,90,91] have subjected 1:4:6-trien-3-ones to the dienone-phenol rearrangement and have discovered that in this case simple methyl migration takes place; 3:17-dioxoandrosta-1:4:6-triene



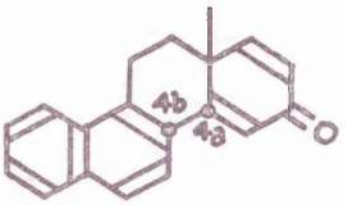
(LXXXIX; R = O)
(XC; R = H,OH)



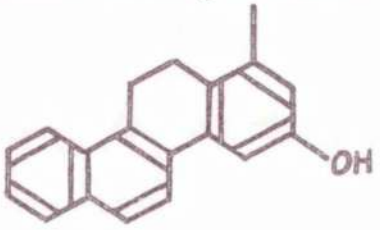
(XCI; R = O)
(LXXIII; R = H,OH)

(LXXXIX) and its 17β-hydroxy derivative gave, after hydrogenation of the products, 1-methyloestrone (XCI) and 1-methyloestradiol (LXXIII) respectively. These phenols, in contrast with the products from the 1:4-dien-3-ones, are readily soluble in aqueous alkalis and are active oestrogens.

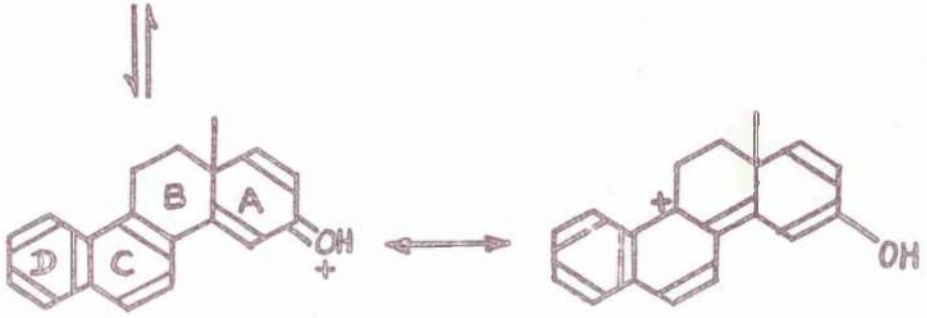
This rearrangement is formally much more closely related to the



(XCII)



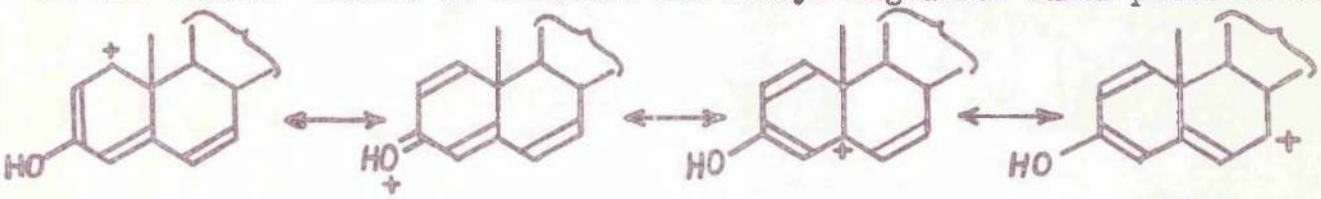
(XCIII)



(XCIIa) (XCIIb)

proven case in the chrysene series[92] in which the product from the dienone (XCII) is the phenol (XCIII). Woodward[7] has explained this "anomaly" in the following manner: the positive charge in the conjugate acid (XCIIa) will be distributed over rings A, C, and D (cf. XCIIb and

four similar forms), thus conferring a certain amount of double bond character to the 4a-4b bond. Since this partial double bond character will provide a considerable barrier to rotation, the rearrangement in the "normal" manner is retarded and methyl migration takes precedence.

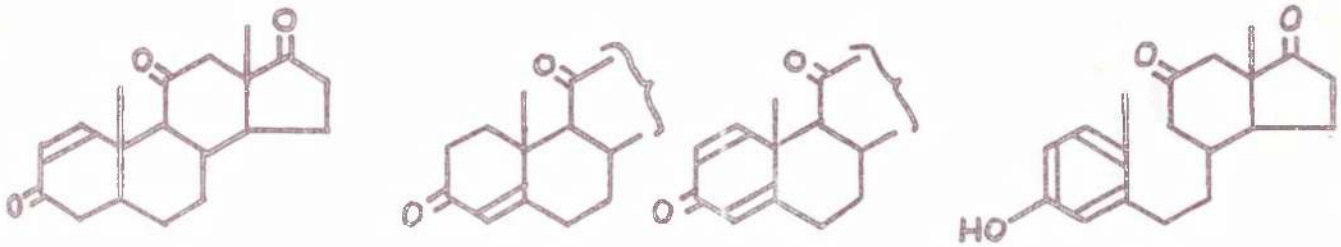


(XCIV)

In a similar manner, because of the presence of the ion (XCIV), there will be a certain amount of double bond character about the 5-6 bond in 1:4:6-trien-3-ones; hence methyl migration takes place in preference to spiran formation.

The pyrolysis of dienones and trienones has been investigated by Djerassi,[91,93] Hershberg,[94] and their co-workers who have confirmed Inhoffen's observations[58] that methane is evolved and that 19-nor compounds are formed:

Magerlein and Hogg[95] have recently shown that pyrolysis of 3:11:17-trioxoandrost-1-ene (XCV) or 4-ene (XCVI), or of 3:11:17-trioxo-

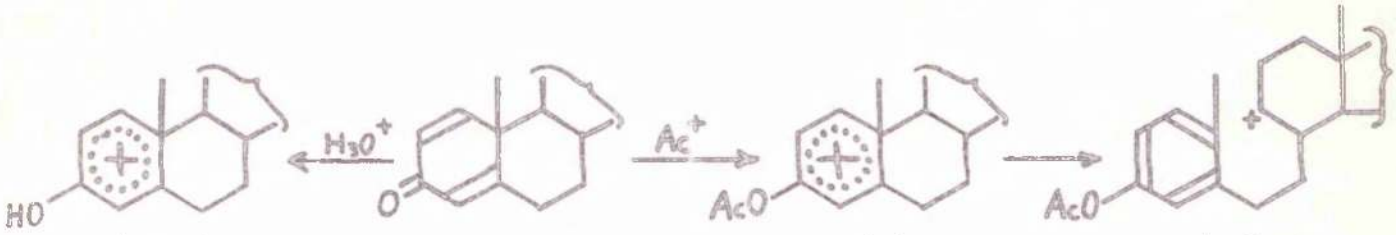


(XCV) (XCVI) (XCVII) (XCVIII)

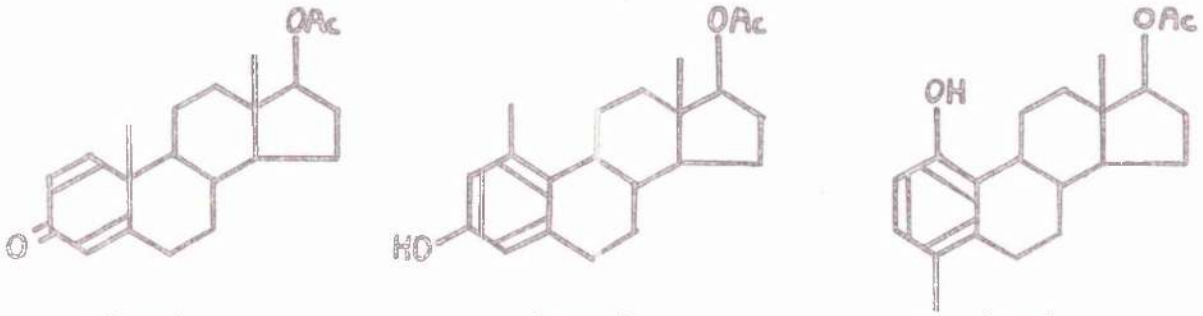
androsta-1:4-diene (XCVII), gives the 9:10-seco compound (XCVIII).

The dienone-phenol rearrangement in the steroid series is generally carried out under anhydrous conditions; Dreiding, Pummer, and

Tomasewsky[96] have studied the reaction in aqueous media and have shown that in this case methyl migration predominates. The difference, they suggest, is probably due to the fact that in aqueous solution the dienone will be attacked by a hydroxonium ion to give the intermediate (XCIX), whereas under anhydrous conditions in acetic anhydride the intermediate is more likely to be (C). The two subsequent aromatisations differ in the number of hydrogen atoms on the migrating carbon atom, in the location of the migrating group with respect to the dienone ring, and in the nature of the shift itself. It is thus possible that



(XCIX) (C) (CI)



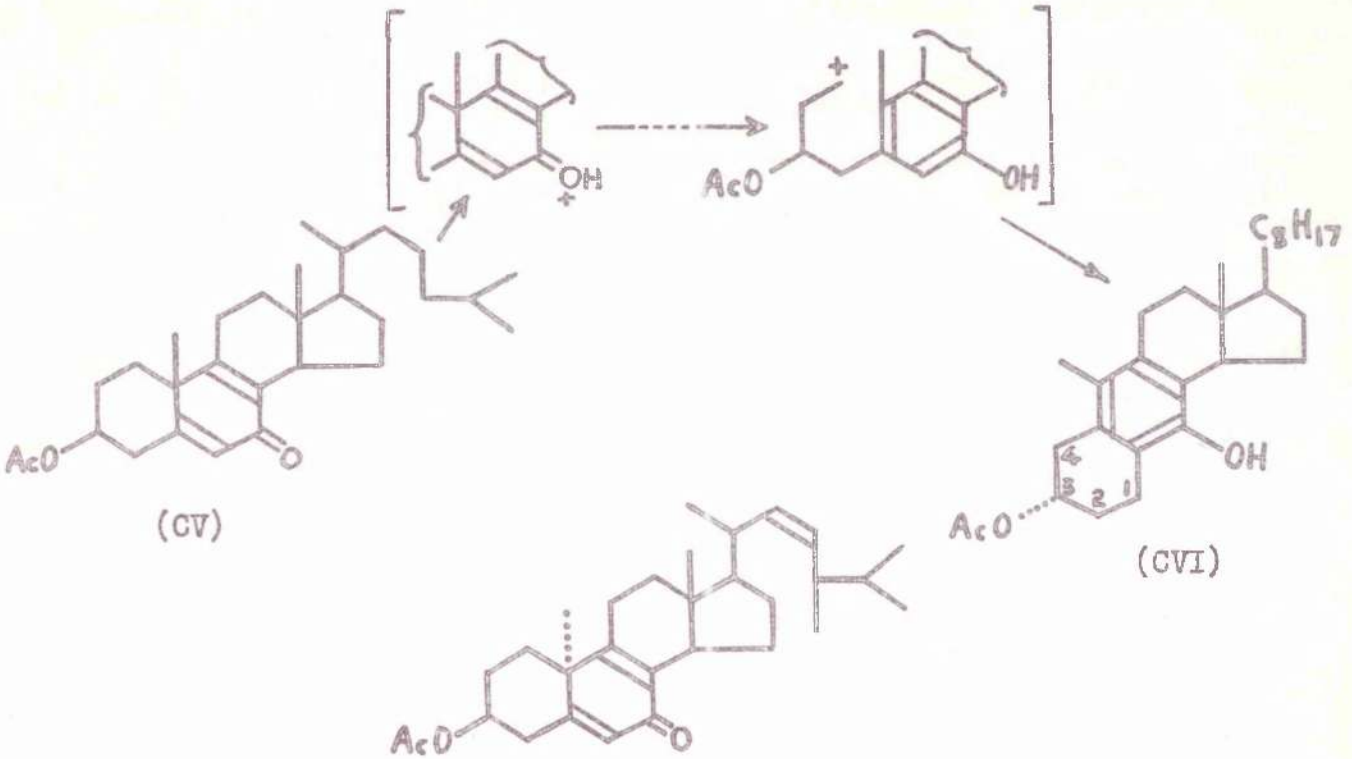
(CII) (CIII) (CIV)

different mechanisms are involved, e.g. the ion (XCIX) might favour a Wagner-Meerwein type shift, by the simplest path, of the methyl group (with three hydrogen atoms); whereas in acetic anhydride the reaction might go through an intermediate of type (CI), as postulated by Woodward,[7] which would favour migration of the secondary carbon atom by a 1:3 shift.

Bosshard, Dutler, and Jeger[97] have recently studied the action of ultraviolet light on 3-oxoandrosta-1:4-dien-17β-yl acetate (CII) and have isolated, together with ketonic products, 1-methyloestradiol 17-acetate (CIII), 1-hydroxy-4-methyloestran-17β-yl acetate (CIV), and two other

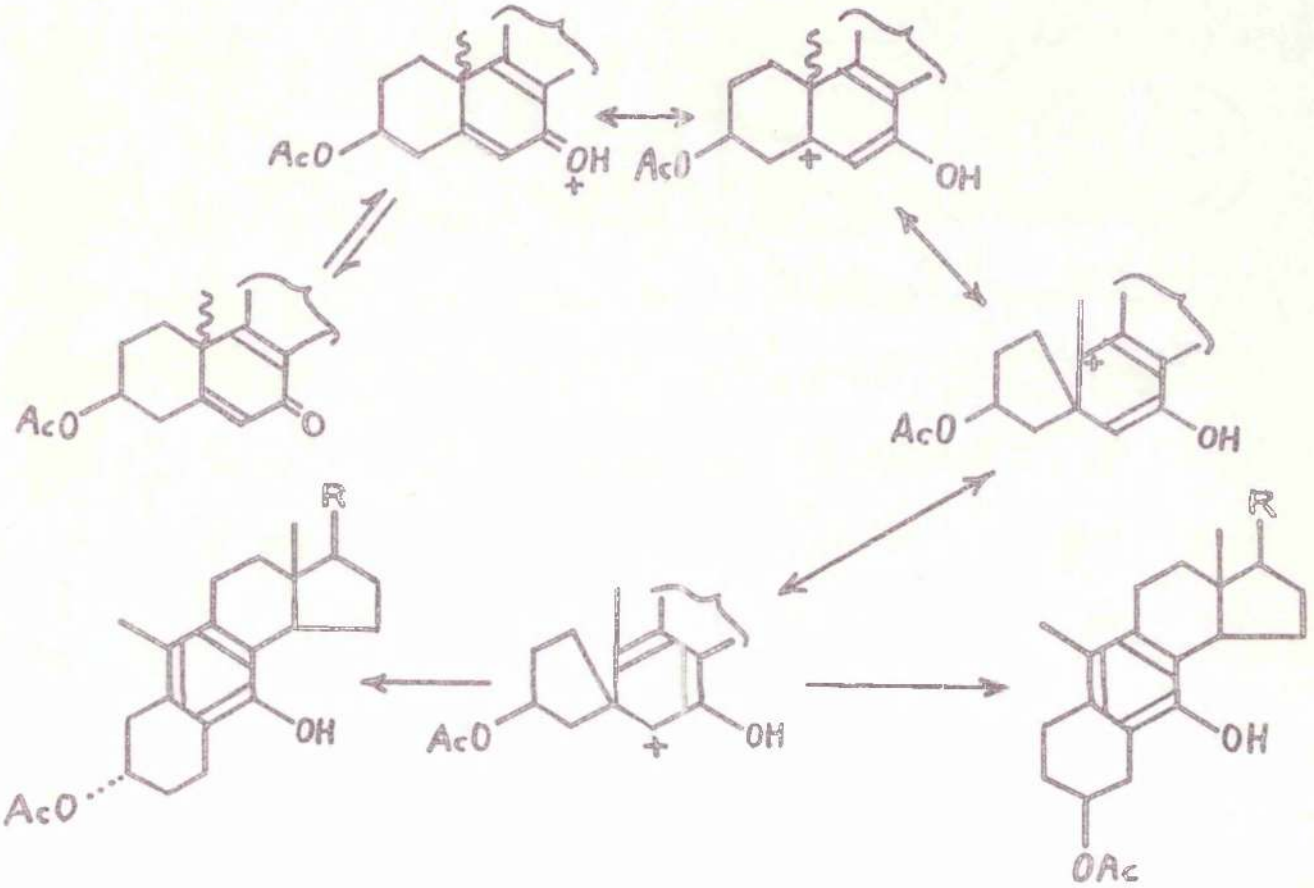
phenols which they suggest might be the C_9 epimers of (CIII) and (CIV).

Only two examples of the dienone-phenol rearrangement of steroid ring B dienones have been described. Kyosuke Tsuda, Ko Arima, and Ryoichi Hayatsu[11] have reported the rearrangement of 7-oxocholesta-5:8--dien-3β-yl acetate (CV) and the subsequent dehydrogenation of the product to a mixture of anthracene derivatives;[98] on the basis of this evidence they have proposed the structure (CVI) for the phenol. Bladon[12] has prepared a similar phenol from 7-oxolumista-5:8:22-trien-3β-yl



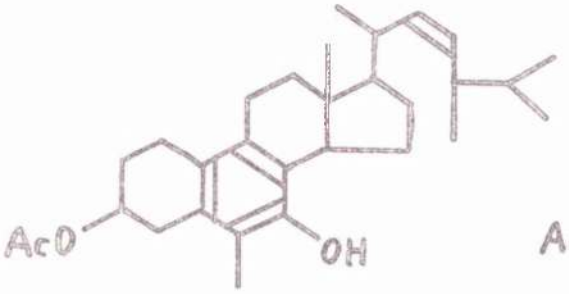
acetate (CVII) and has pointed out that the 2β-acetoxy structure (CVIII) is equally as probable as the 3α-acetoxy structure (CVI) if the mechanism follows the course illustrated on page 25. Bladon could not effect the rearrangement with the conventional reagents but only by means of zinc dust and acetic acid; he therefore points out the possibility that only methyl migration has taken place to give the phenol (CIX). Since the asymmetry at C_{10} is lost in the reaction mechanism proposed overleaf, the same phenol should be

obtained when the reaction is carried out in the ergosterol series;

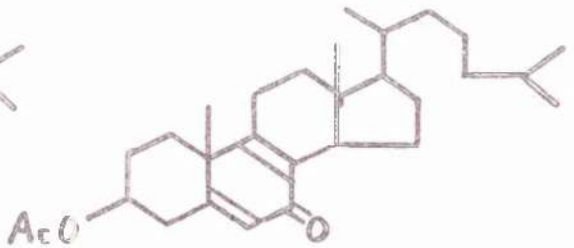


(CVIII)

this has been achieved by the formation of a phenol, from 7-oxo-ergosta-5:8-dien-3β-yl acetate (CX), which is identical with the



(CIX)

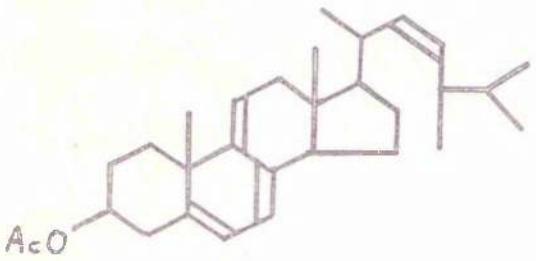


(CX)

hydrogenation product of the phenol from the dienone (CVII).

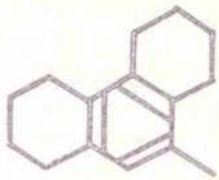
The Anthrasteroid Rearrangement

A novel rearrangement of the steroid nucleus was discovered by Mosettig and Nes[13,14] in 1953. When dehydroergosteryl acetate (CXI) is treated with chloroformic hydrogen chloride the product is a hydrocarbon, anthraergostapentaene, in which the presence of a benzene ring, a double bond conjugated with the benzene ring, and an isolated double bond, have been demonstrated.

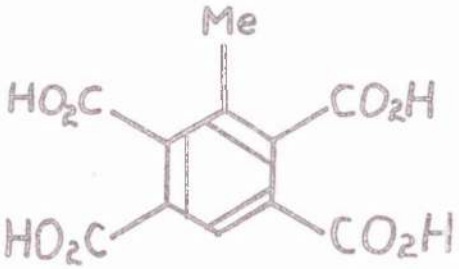


(CXI)

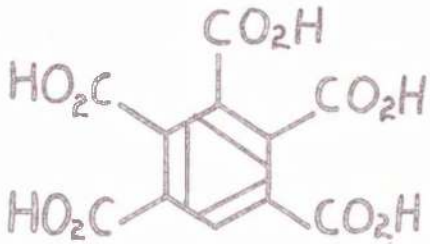
(CXII)



(CXIII)



(CXIV)

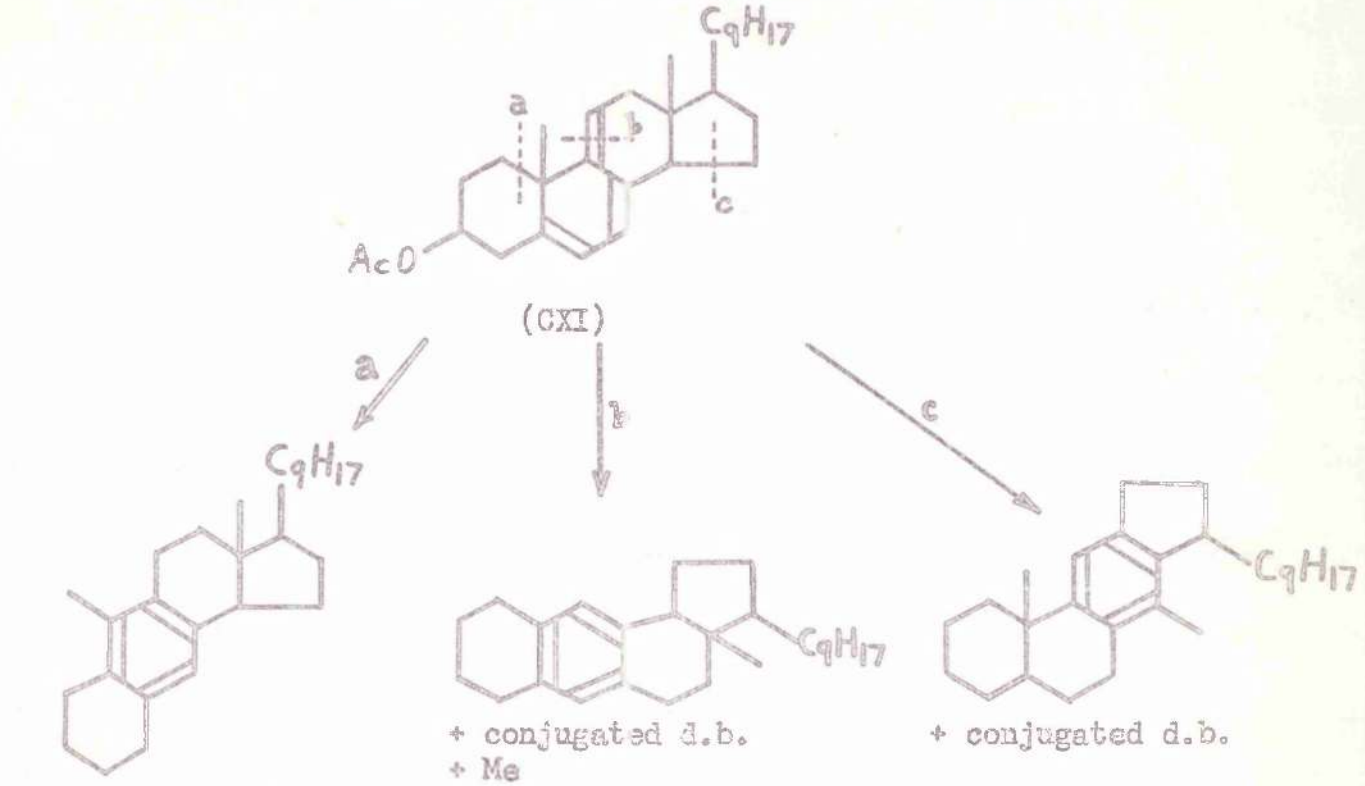


(CXV)

The non-benzenoid double bonds are readily saturated and the ultraviolet absorption spectrum of the tetrahydro derivative is very similar to that of 9-methyl-s-octahydroanthracene (CXII) and different from that of 9-methyl-s-octahydrophenanthrene (CXIII). Further evidence in favour of a reduced anthracene type of carbon skeleton in anthraergostapentaene was obtained by selenium dehydrogenation of the hydrocarbon, when a product was obtained, the ultraviolet absorption spectrum of which resembled an anthracene rather than a phenanthrene derivative. Furthermore, oxidation of the pentaene with concentrated

nitric acid gives a carboxylic acid which was shown[15] to be 1-methyl-2:3:5:6-tetracarboxybenzene (CXIV). It was shown that this acid (CXIV) is also obtained by a similar oxidation of 9-methyl-s-octahydroanthracene (CXII), whereas 9-methyl-s-octahydrophenanthrene (CXIII) gives the pentacarboxylic acid (CXV).

Mosettig and Nes pointed out that a hydrocarbon having a methyloctahydroanthracene skeleton could arise from dehydroergosteryl acetate (CXI) by two types of bond rupture only. Scission of the

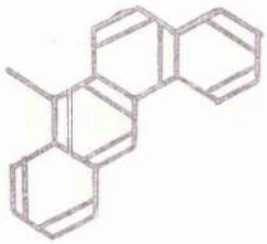


(CXVI) (CXVII) (CXVIII)

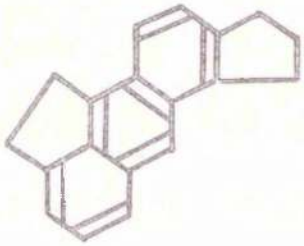
C_1-C_{10} bond (a) would lead to the compound (CXVI) and rupture of the C_9-C_{11} bond (b) would lead to the compound (CXVII). Fracture of the C_{14}-C_{15} bond (c) could give the hydrocarbon (CXVIII) and this structure might satisfy the spectroscopic requirements but it is unlikely that dehydrogenation of (CXVIII) would lead to an anthracene derivative. Since the rearrangement of dehydrolumisteryl acetate (the C_{10} epimer of CXI) also yields anthraergostapentaene formula (CXVIII) cannot

represent the latter hydrocarbon. Of the remaining possible formulae, (CXVI) and (CXVII), Mosettig and Nes favour the former.

Many potent carcinogens are anthracene derivatives with a ring fused in the 1:2-positions [e.g. 10-methylbenzanthracene (CXIX)],

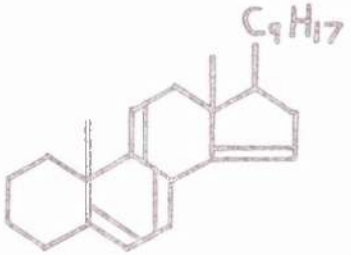


(CXIX)

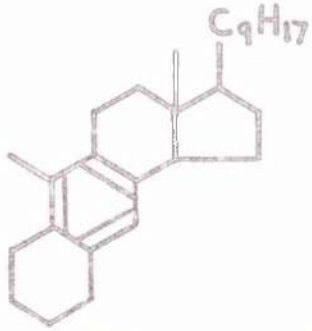


(CXX)

Mosettig and Nes[14] therefore speculate that this type of facile transformation, i.e. steroids into anthracene derivatives, may be part of a biogenetic route to endogenous carcinogens. The hydrocarbon (CXVI) bears a structural resemblance to 1:2-cyclopenteno-5:10-ace-anthracene (CXX) which shows moderate but definite carcinogenic



(CXXI)

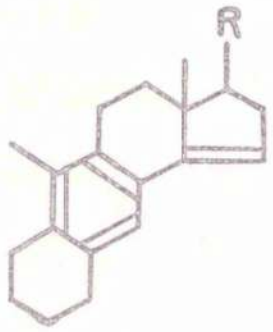


+ conjugated d.b.
(CXVI)

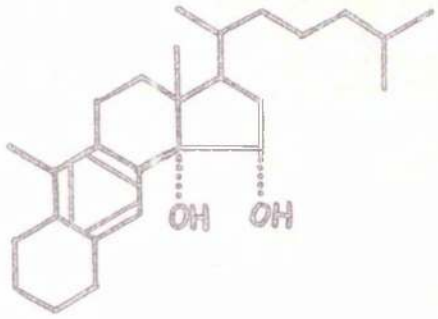
activity.[99]

The first stage in the rearrangement of dehydroergosteryl acetate (CXI) to anthraergostapentaene has been shown [100] to be loss of the oxygen function, by the isolation of the intermediate hydrocarbon (CXXI) which is isomerised to the anthraergostapentaene (CXVI) in high yield by the action of chloroformic hydrogen chloride.

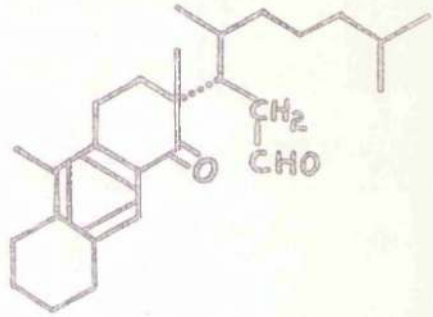
Burgstahler[101] has summarised the available evidence concerning the structure of the anthrasteroids, and has shown that they are represented by the cipher (CXXII) in the following manner: treatment of anthracholestatetraene (CXXII; R = C_8H_{17}) with osmium tetroxide yields the glycol (CXXIII) cleavage of which, by lead tetra-acetate,



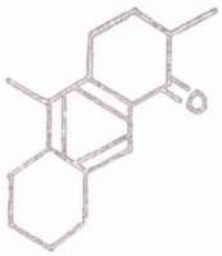
(CXXII)



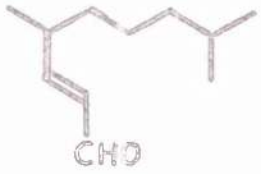
(CXXIII)



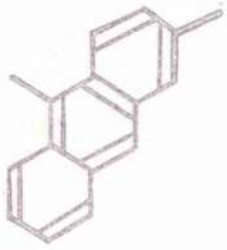
(CXXIV)



(CXXV)



(CXXVI)



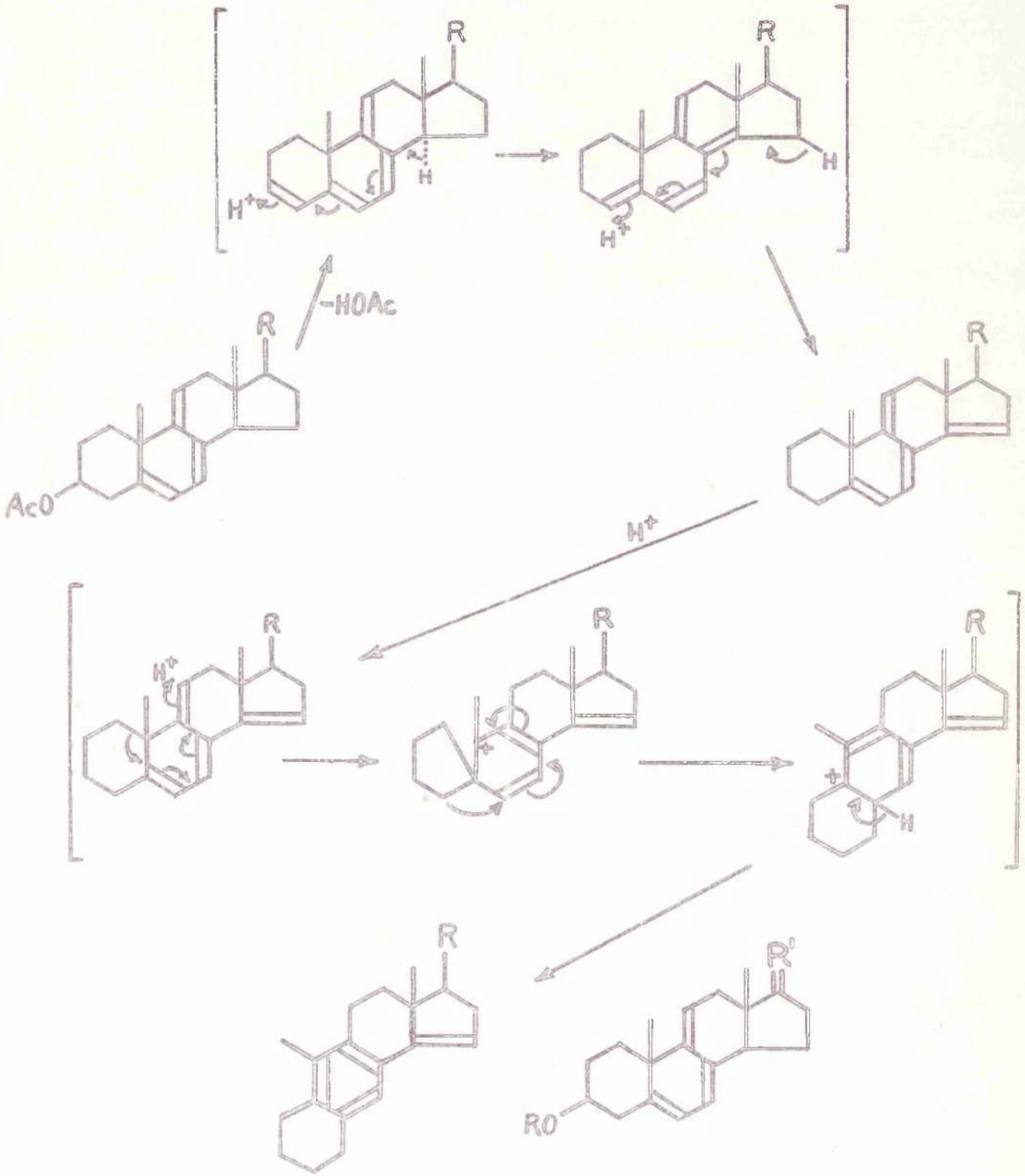
(CXXVII)

gives the ketoaldehyde (CXXIV). Pyrolysis of the latter results in the formation of the volatile aldehyde (CXXVI) and the non-volatile ketone (CXXV) dehydrogenation of which, by heating with palladium--charcoal, gives 3:9-dimethylanthracene (CXXVII), identified by synthesis.

The mechanism outlined on page 30 has been suggested by Burgstahler.[101]

The reaction has been extended[102] by the preparation of the anthrasteroids (CXXVIII) and (CXXIX) from cholesta-5:7:9(11)-trien-3β-yl acetate (CXXX) and 3β-hydroxybisnorchola-5:7:9(11)-trienic acid methyl ester (CXXXI) respectively. The product from 17-oxoandrosta-

-5:7:9(11)-trien-3β-yl isocaproate (CXXXII) is a mixture, a small amount of which is probably the chlorocompound (CXXXIII).

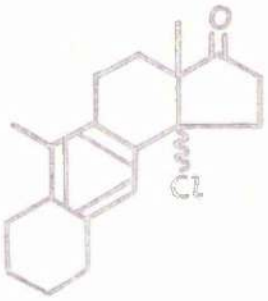


(CXXVIII; R = CHMe·CH_2·CH_2·CH_2·$CHMe_2$) (CXXX; R = Ac; R' = H_2CHMe·CH_2·CH_2·CH_2·CHM

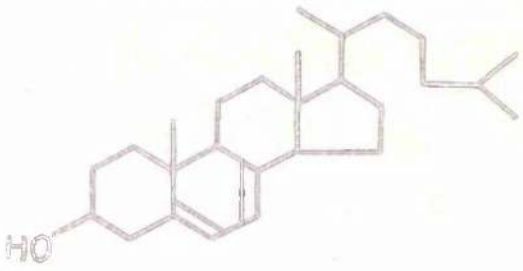
(CXXIX; R = CHMe·CO_2Me) (CXXXI; R = H; R' = H_2CHMe·CO_2Me)

(CXXXII; R = i-$C_6H_{13}O$; R' = O)

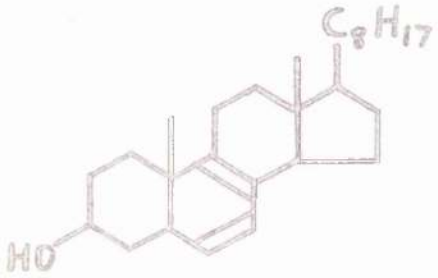
It has been found[103] that irradiation of 7-dehydrocholesterol (LII), isodehydrocholesterol (LVI), or cholesta-5:8-dien-3β-ol (CXXXIV), in the presence of p-toluenesulphonic acid and mercuric acetate, and in the absence of oxygen, gives an aromatic compound. Since an anthracene derivative is formed on selenium dehydrogenation of this aromatic compound, the structure (CXXXV) has been proposed.



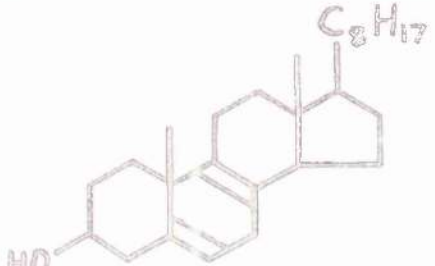
(CXXXIII)



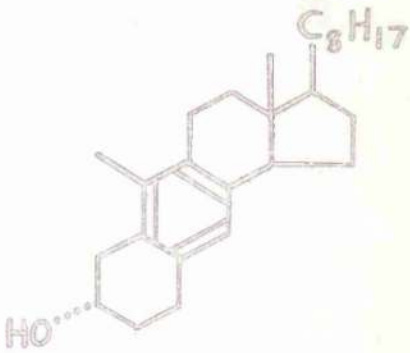
(LII)



(LVI)



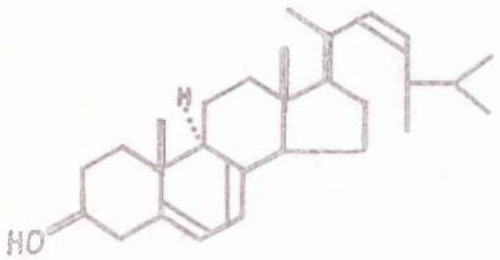
(CXXXIV)



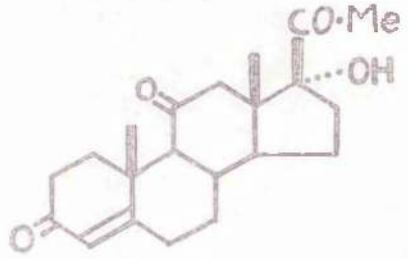
(CXXXV)

DISCUSSION

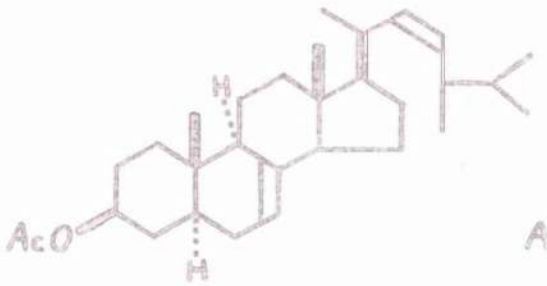
During work on a projected partial synthesis of cortisone (CXXXVI) from ergosterol (XLI), Anderson, Spring, and Stevenson[104] studied the low-temperature bromination[105] of 5α:6-dihydroergosteryl acetate (CXXXVII). The product from this reaction, which has also been obtained by a



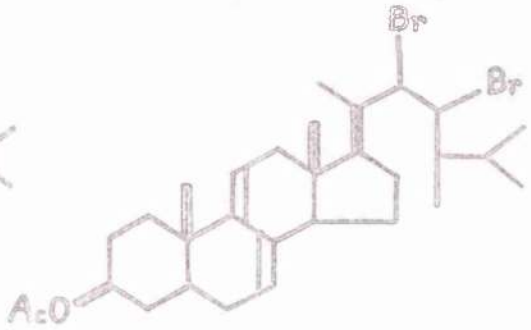
(XLI)



(CXXXVI)



(CXXXVII)

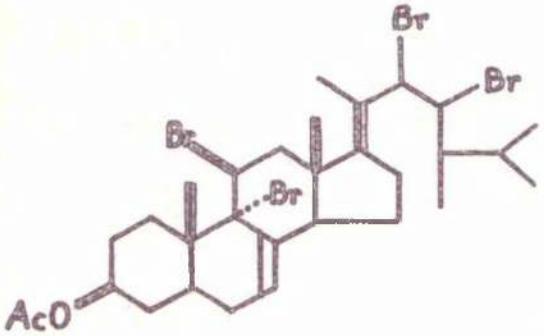


(CXXXVIII)

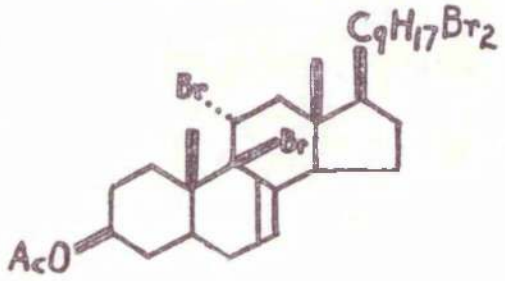
similar bromination of 22:23-dibromoergosteryl-D acetate (CXXXVIII), is a tetrabromide treatment of which, with sodium iodide, yields 22:23-dibromoergosteryl-D acetate (CXXXVIII). It has been suggested[106] that the bromination of dihydroergosteryl acetate (CXXXVII) takes the following course: (i) rapid addition of bromine to the side-chain double bond; (ii) slow oxidation of the 7-ene to a 7:9(11)-diene; (iii) rapid addition of bromine to the 7:9(11)-diene system. There are eight possible structures for the tetrabromide: (CXXXIX), (CXL), or (CXLI, two structures), arising from 1:2-addition of bromine to the 7:9(11)-diene system; or (CXLII, four structures), arising from

1:4-addition. The tetrabromide is unchanged[106] after treatment with concentrated hydrobromic acid.[107] Anderson[106] has described experiments a consideration of which led him to propose the structure (CXXXIX) for the tetrabromocompound, but these experiments are not of a conclusive nature.

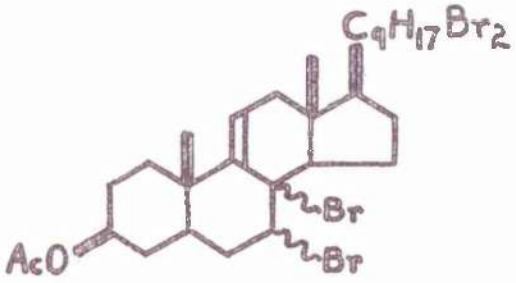
Tetrabromoergostanyl acetate, although reasonably stable in the



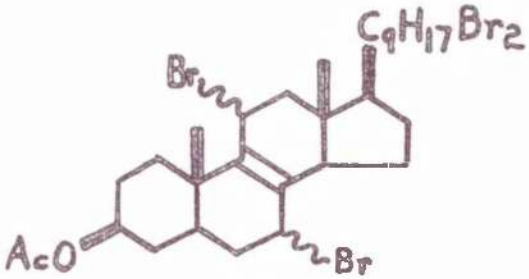
(CXXXIX)



(CXL)



(CXLI)



(CXLII)

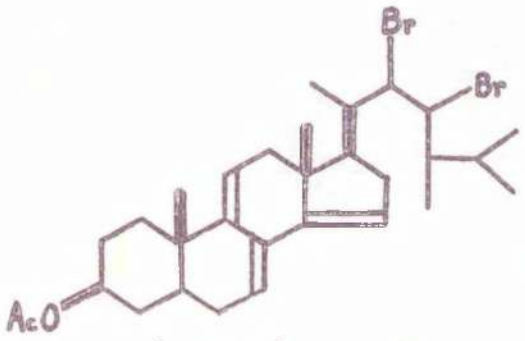
solid state, is unstable in solution. When a benzene solution of the tetrabromide is filtered through a column of alumina a remarkable decomposition occurs.[108] A deep green band, slowly fading through red to brown, is formed at the top of the column and subsequent elution gives three crystalline compounds. Two isomeric substances, $C_{30}H_{44}O_2Br_2$, are eluted with benzene and the third substance, $C_{31}H_{48}O_3Br_2$, is obtained by washing the column with methanol.

The more easily eluted compound, $C_{30}H_{44}O_2Br_2$, m.p. 208-209°, is obtained in greater yield when the benzene solution is filtered through

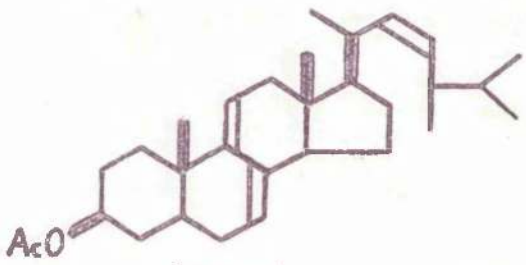
the column quickly. If the solution is kept on the column overnight the second isomer, m.p. 136-137°, is the major product.

The isomer, m.p. 208-209°, has been studied in these laboratories by Mr. D.S.Savage who has identified it as 22:23-dibromoergosta--7:9(11):14-trien-3β-yl acetate (CXLIII).

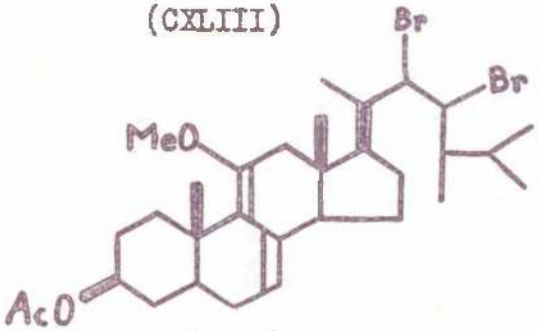
When a solution of the compound, $C_{31}H_{48}O_3Br_2$, obtained by washing



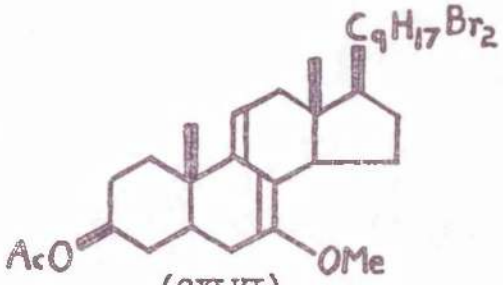
(CXLIII)



(CXLIV)



(CXLV)

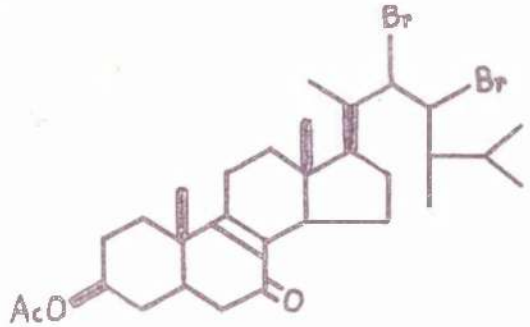


(CXLVI)

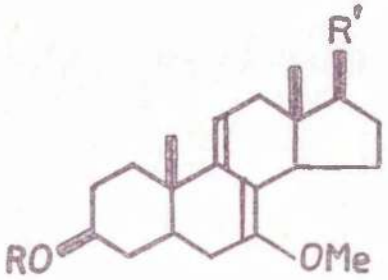
the column with methanol, is chromatographed on alumina it is eluted with petrol-benzene mixtures; it must, therefore, have been formed by the action of the methanol on a complex, possibly an aluminobromide complex. The ultraviolet absorption spectrum of the C_{31} compound, which gives a brown colour with tetranitromethane in chloroform, shows a main maximum at 2460 Å and is very similar in shape and intensity to the spectrum of ergosta-7:9(11):22-trien-3β-yl acetate (ergosteryl-D acetate) (CXLIV). In the infrared there is a moderately strong band at 1068 $cm.^{-1}$ which may be due to symmetrical stretching of the C-O-C system of a vinyl ether. Zeisel estimation shows the presence of one methoxyl group in the C_{31} diene which may therefore be represented by either (CXLV) or (CXLVI). When tetrabromoergostenyl acetate is

dissolved in ether-methanol and filtered through a column of alumina the product is a mixture of 22:23-dibromoergosta-7:9(11):14-trien-3β-yl acetate (CXLIII) and 22:23-dibromo-7-oxo-ergosta-8-en-3β-yl acetate (CXLVII), thus suggesting that the methoxydiene is best represented as the 7-methoxy compound (CXLVI). Alkaline hydrolysis of the methoxydiene (CXLVI) gives 22:23-dibromo-7-methoxyergosta-7:9(11)-dien-3β-ol (CXLVIII), acetylation of which regenerates the original acetate. The dibromomethoxydiene (CXLVI), on treatment with zinc dust in ether-ethanol, yields 7-methoxyergosta-7:9(11):22-trien-3β-yl acetate (CXLIX).

The acetate $C_{30}H_{44}O_2Br_2$, m.p. 136-137°, obtained by the decomposition of tetrabromoergostenyl acetate on alumina, gives a strong yellow



(CXLVII)

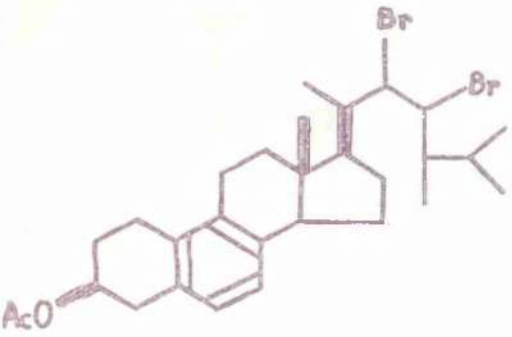


(CXLVI; R = Ac; R' = $C_9H_{17}Br_2$)
(CXLVIII; R = H; R' = $C_9H_{17}Br_2$)
(CXLIX; R = Ac; R' = C_9H_{17})

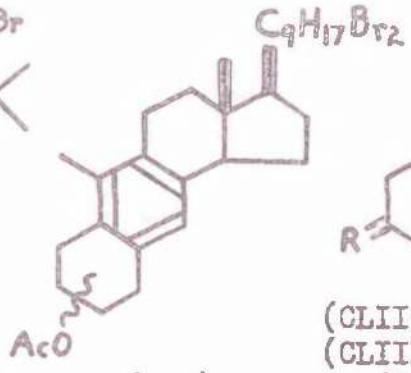
colour with tetranitromethane in chloroform and is unchanged after shaking with hydrogen in the presence of Adams catalyst. Its ultraviolet absorption spectrum shows a very strong band in the 2000-2200 Å region together with a weak composite band in the 2500-3000 Å region, indicative of the presence of a benzene ring.[109] This conclusion is supported by the presence of a moderately strong band in the infrared spectrum at 1592 cm.$^{-1}$ due to aromatic ring vibration.[110] The acetate, m.p. 136-137°, is not a neosteroid since: (i) no gaseous products are observed during its formation; (ii) the elementary analysis of the acetate and its derivatives show that all the carbon atoms of the original ergostane

system are still present; and (iii) it differs from 22:23-dibromoneo-ergosteryl acetate (CL) (m.p. 179-181°).[6] An anthrasteroid structure for the acetate m.p. 136-137°, such as (CLI), is excluded by the following sequence of reactions:

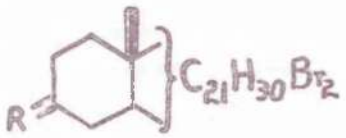
The infrared absorption spectrum of the compound, m.p. 136-137°, (CLII) shows a strong band at 1740 cm.$^{-1}$ attributed to the acetate group. Hydrolysis of the acetate with either methanolic potassium hydroxide or methanolic sulphuric acid gives the corresponding alcohol



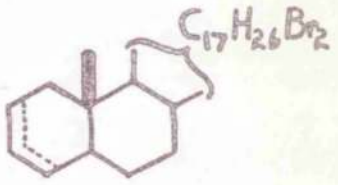
(CL)



(CLI)



(CLII; R = H, β-OAc)
(CLIII; R = H, β-OH)
(CLIV; R = O)
(CLV; R = H, α-Cl)

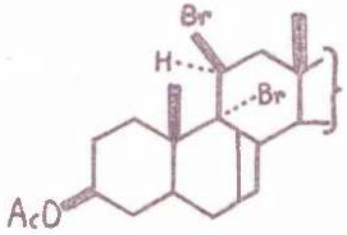


(CLVI)

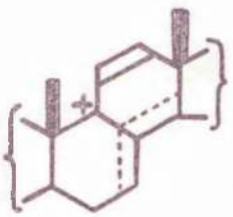
(CLIII), $[\alpha]_D$ + 5°, acetylation of which regenerates the original acetate. Oxidation of the alcohol (CLIII), by means of the chromium trioxide-pyridine complex, yields the ketone (CLIV), $[\alpha]_D$ + 23°, the infrared absorption spectrum of which shows a strong band at 1720 cm.$^{-1}$ indicative of a carbonyl group in a six membered ring. The molecular rotation difference between the alcohol and the ketone (+ 99°) is in fair agreement with the average figure (+ 73° ± 8°)[111] for the oxidation of 3β-hydroxysteroids to 3-oxostercids. Attempted dehydration of the alcohol (CLIII) with phosphorus oxychloride in pyridine gave the 3α-chloro-compound (CLV); the configuration at C_3 is assigned assuming that a Walden inversion has taken place.[112] Dehydration of the alcohol is effected by means of phosphorus pentoxide when the product is a mixture

of double bond isomers (CLVI), separable by fractional crystallisation, neither of which differs from the dibromoaromatic acetate or alcohol in its ultraviolet absorption spectrum. Furthermore, both isomers are recovered unchanged after treatment with strong mineral acid. The double bond of the 3-ene, which must be one of the isomers, is therefore not in conjugation with the benzene ring.

At this point it is pertinent to speculate on the mechanism of the debromination of the tetrabromoergostenyl acetate (CXXXIX) by alumina. Preliminary dehydrobromination involving the tertiary bromine



(CXXXIX)

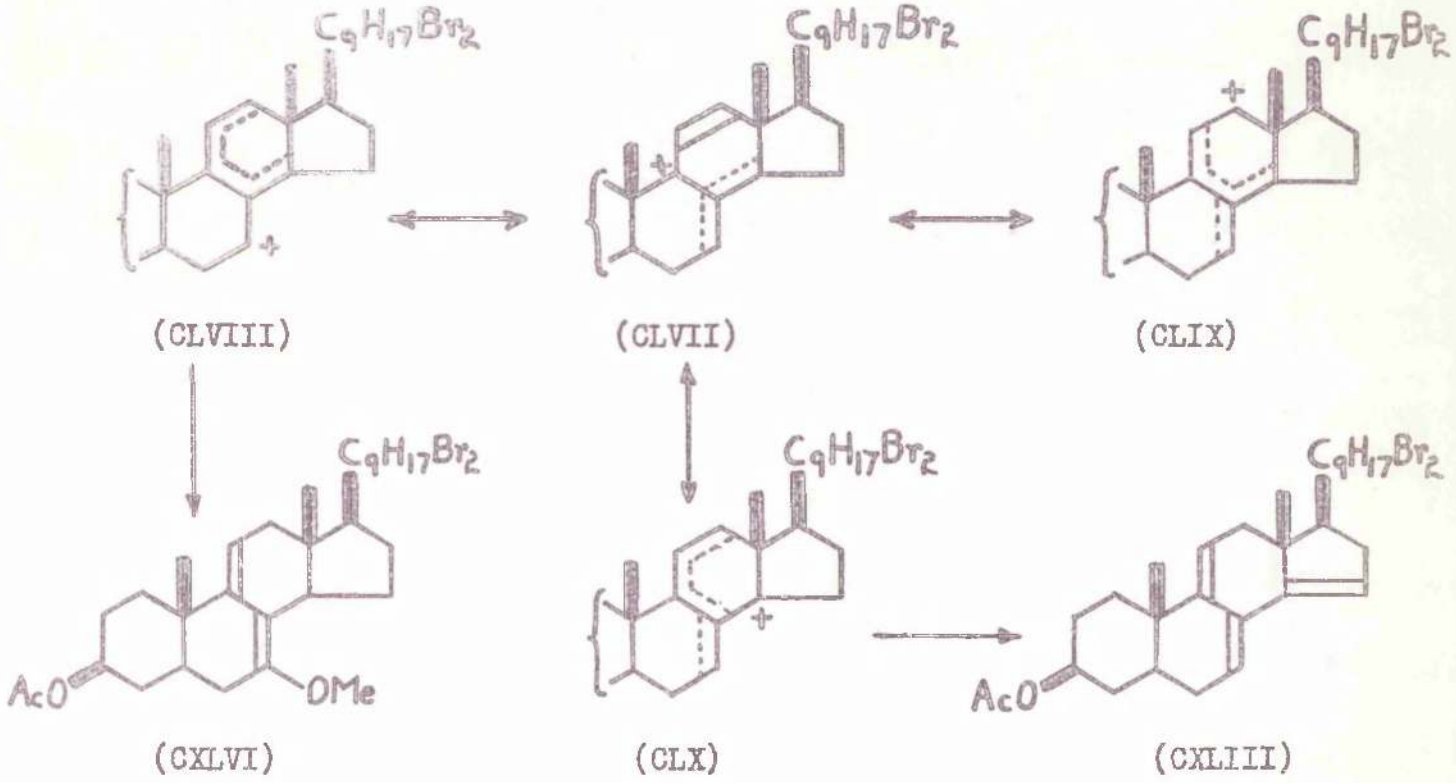


(CLVII)

atom at C_9 is unlikely as this atom is <u>cis</u> to the only hydrogen atom available for elimination, that at C_{11}. The first step cannot be allylic rearrangement followed by elimination of hydrogen bromide, since Anderson[106] has shown that the tetrabromide exists in the acid-stable allylic form. The most likely initial stage, then, would seem to be formation of the carbonium ion (CLVII). Allylic rearrangement of this ion can lead to the formation of three other carbonium ions (CLVIII), (CLIX), and (CLX), which might stabilise themselves to a certain extent by the formation of ionic complexes, perhaps of the type:

Because of the bulky nature of such an anion it may be expected that the complex with the hindered tertiary carbonium ion, if it forms at all, will be the least stable, while that with the least hindered

carbonium ion (CLVIII) will be the most stable. Loss of a proton from the ion (CLX) will give the dibromotriene (CXLIII) and, as will be shown later, the ion (CLIX) will lead to the dibromoaromatic compound. The ion (CLVIII) could give (CXLVI), which is the suggested structure of the methoxydiene. That the complex involving this ion, (CLVIII), is relatively stable is shown by the fact that it is not



decomposed by non-polar solvents; the polar solvent methanol causes decomposition, with substitution, to give the methoxydiene.

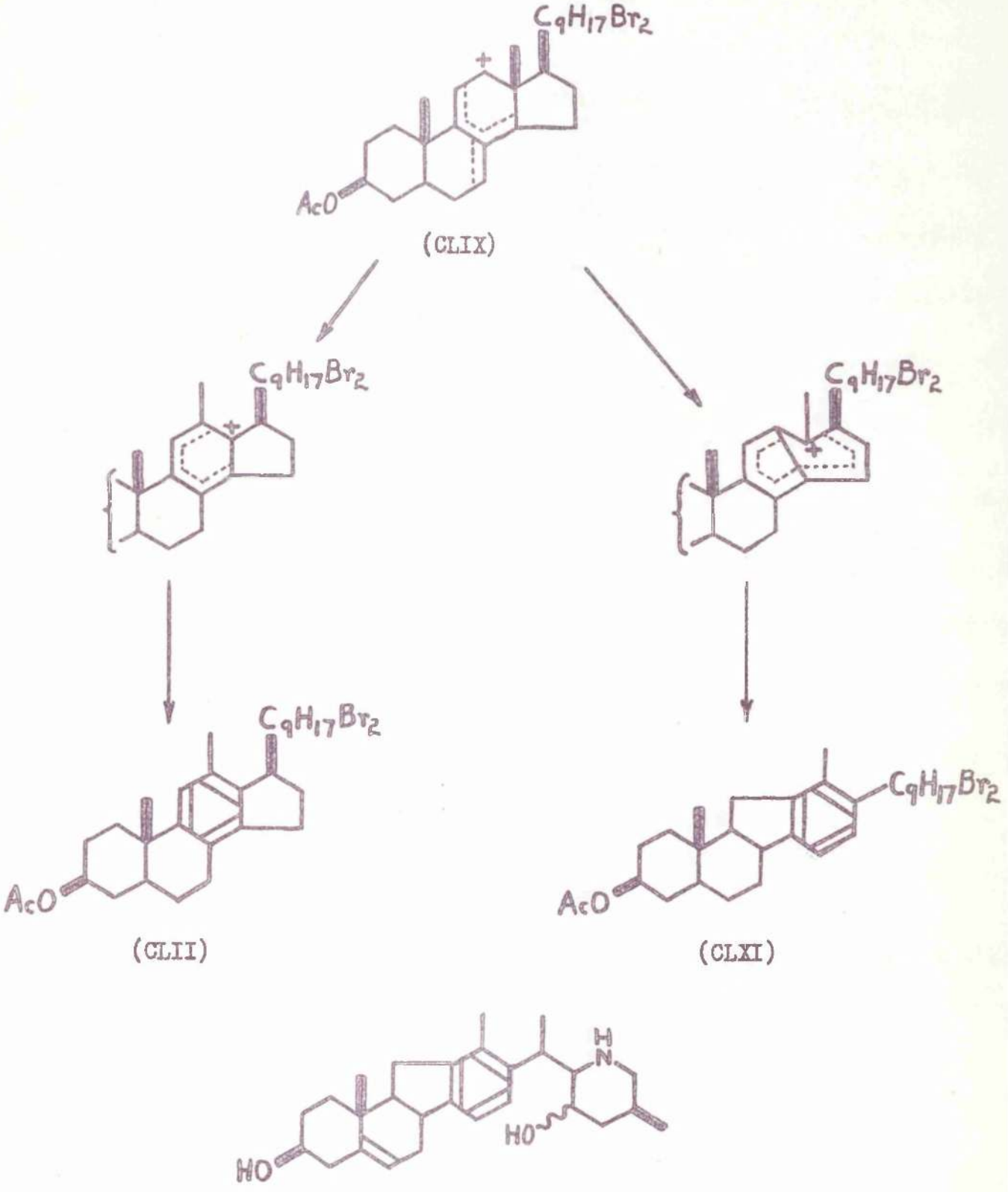
When the reaction mixture from the tetrabromide is quickly eluted from the site of the reaction the crystalline products consist of approximately 90% triene (CXLIII), 8% aromatic compound (CLII), and, after elution of the column with methanol, 2% methoxydiene (CXLVI). When the reaction mixture is left at the site of the reaction overnight the crystalline products consist of approximately 10% triene (CXLIII), 85% aromatic compound (CLII), and 5% methoxydiene (CXLVI). These results can be explained by assuming that the equilibrium mixture of carbonium

ions will consist largely of the relatively stable tertiary ions (CLVII) and (CLX). When the reaction mixture is quickly removed from the reaction site of the alumina column the ion (CLX) will immediately stabilise itself by losing a proton to give the triene (CXLIII), and since this will obviously be a much faster reaction than the rearrangement of the ion (CLIX) to the aromatic compound, this triene will be the major reaction product. Since the ion (CLVIII) is fixed as an ionic complex, and the tertiary carbonium ions (CLVII) and (CLX) are relatively stable, when the reaction mixture is left in the reaction site for some time then the predominating process will be rearrangement of the ion (CLIX) to the aromatic acetate (CLII). The aromatic compound will thus accumulate in the system at the expense of the triene. The fact that the structure of the tetrabromide is not known with certainty does not affect this reasoning since similar reaction schemes are applicable starting from any of the possible structures (CXXXIX) to (CXLII).

According to the scheme suggested above, it might be possible to isomerise the triene (CXLIII) with mineral acid, via the ions (CLX) and (CLVII), to the aromatic compound. However, treatment of the triene with chloroformic hydrogen chloride does not give the aromatic compound. The triene (CXLIII) thus cannot be a direct intermediate in the aromatisation reaction. It is not possible to duplicate the reaction conditions by mixing aluminium bromide and alumina; when the triene (CXLIII) is left for several days in contact with such a mixture, to which a small amount of hydrobromic acid has been added, no colour is produced and the starting material can be recovered quantitatively.

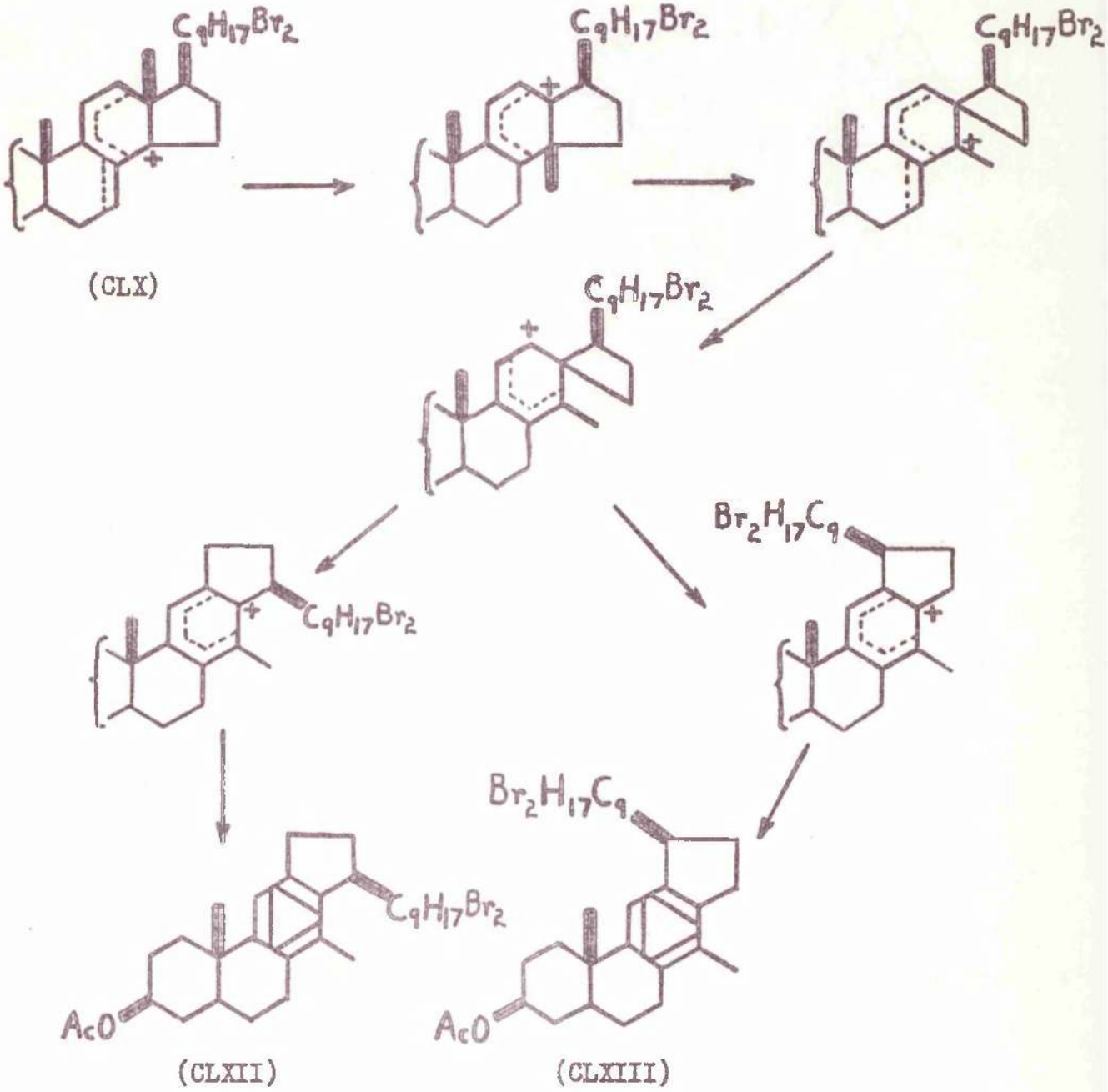
In the case of the ion (CLIX) it is possible for a Wagner-Meerwein

type of rearrangement to take place; this could follow two paths as illustrated below:



Structure (CLXI) is of great interest since it could serve as a starting material for a partial synthesis of veratramine (II).

The possibility that an aromatic structure, (CLXII) or (CLXIII), might arise from the ion (CLX) seems to be excluded by the fact that no aromatic products can be obtained from the triene (CXLIII) under



conditions which would be expected to produce this ion, although the special reaction conditions on the alumina column may be necessary for such a transformation.

A detailed examination of the B-band[109] in the ultraviolet absorption spectrum of the dibromoaromatic acetate (B, Fig. 1) shows

that there is a marked similarity to the spectrum of 9-methyl-s-octahydrophenanthrene (CXIII) (A, Fig. 1)[113] and to the spectrum typical of the neosteroids (CLXIV) (C, Fig. 1).[113] It is to be expected that the spectrum of structures (CLXII) and (CLXIII) would resemble that of 9-methyl-s-octahydroanthracene (CXII) and that of the dihydroanthrasteroids (CLXV) (D, Fig 1);[113] these structures may, therefore,

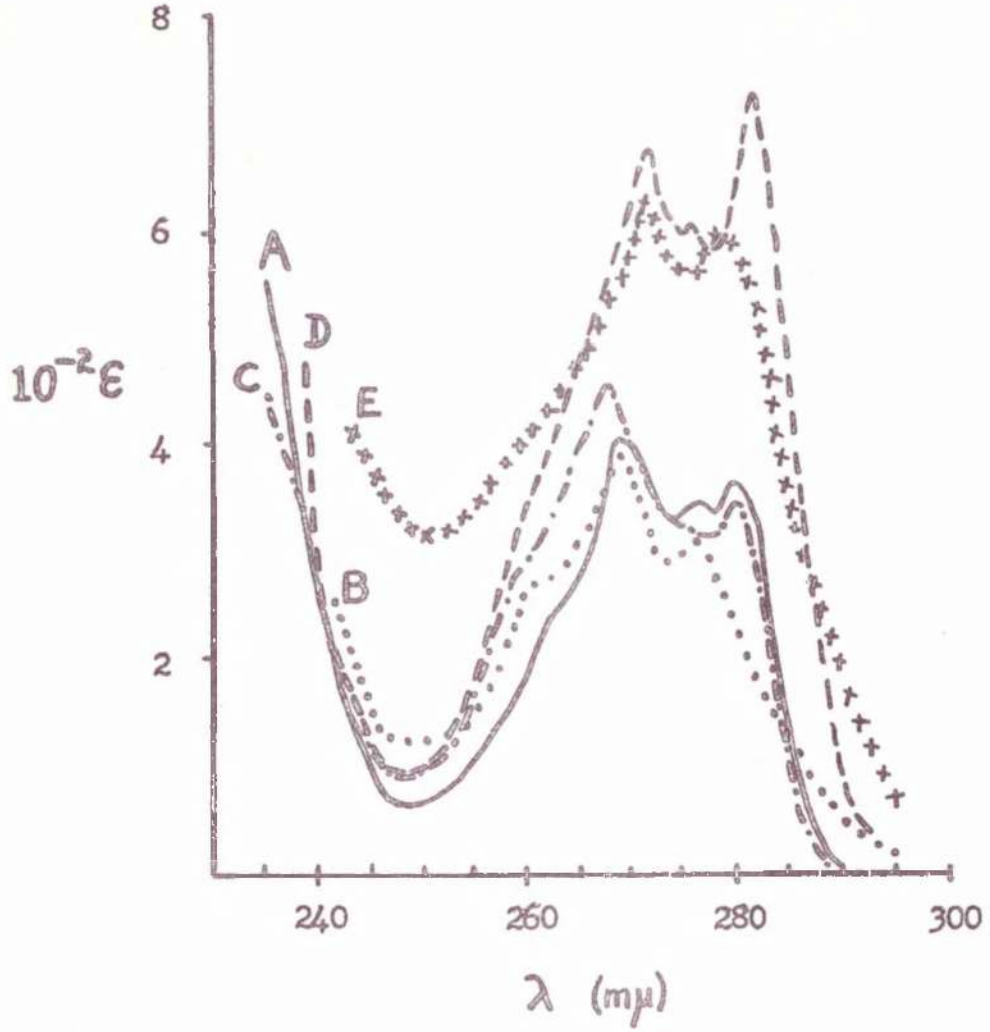
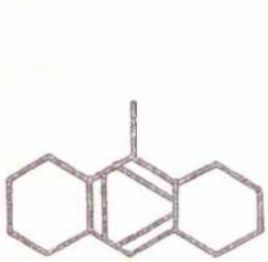


Fig. 1

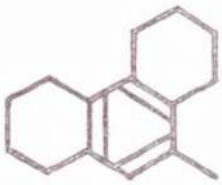
reasonably be excluded as possible representations of the dibromoaromatic acetate. The spectrum of structure (CLXI) should be very similar to that of veratramine (II); since the latter (E, Fig. 1)[3,51] resembles the dihydroanthrasteroids (CLXV) in its spectrum, rather than the neosteroids (CLXIV), structure (CLII) must be favoured for the dibromo-

aromatic acetate. Further evidence in favour of structure (CLII) is obtained from the infrared absorption spectrum which shows a moderately strong band at 862 cm.$^{-1}$ characteristic of a penta-substituted benzene ring, whereas there is complete absence of absorption in the 812-804 cm.$^{-1}$ region which might be attributed to the presence of a tetra-substituted benzene ring,[110,114] as in structure (CLXI).

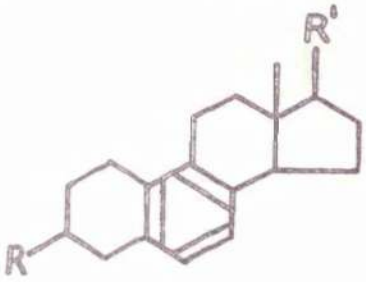
With the object of providing proof of the penta-substituted nature



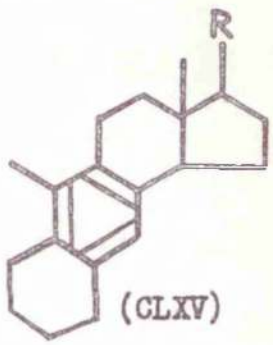
(CXII)



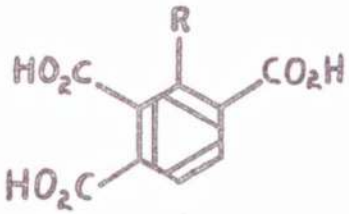
(CXIII)



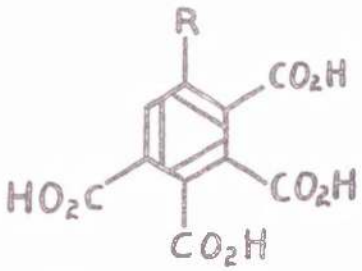
(CLXIV)



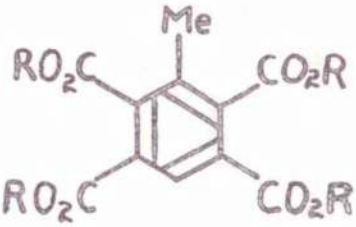
(CLXV)



(CLXVI; R = Me)
(XLIV; R = CO_2H)



(CLXVII; R = Me)
(CXV; R = CO_2H)

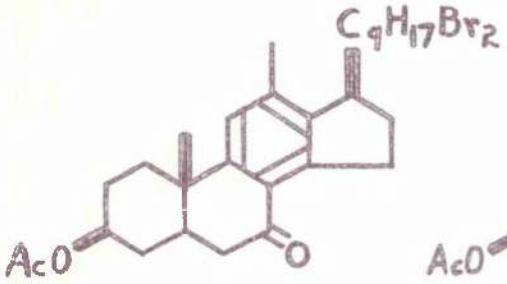


(CXIV; R = H)
(CLXVIII; R = Me)

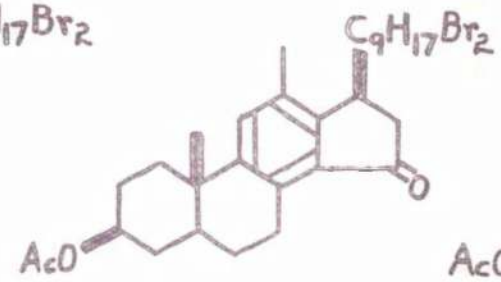
of the benzene ring, the dibromoaromatic acetate (CLII) was oxidised with concentrated nitric acid; using the method previously employed for the neosteroids[6] and the anthrasteroids.[14] A tetra-substituted benzene ring, as in structure (CLXI), would be expected to give either toluene-2:3:6-tricarboxylic acid (CLXVI) or benzene-1:2:3:4-tetracarboxylic acid (prehnitic acid)[115] (XLIV); and a penta-substituted benzene ring, as in structure (CLII), would be expected to give either toluene-2:3:4:5-tetracarboxylic acid (CLXVII) or benzenepentacarboxylic acid (CXV). Despite a careful examination of the oxidation product, however, no crystalline material has been isolated, although a similar oxidation

of ergosterol readily yields the expected product,[14] toluene-2:3:5:6--tetracarboxylic acid (CXIV), characterised as the tetramethyl ester (CLXVIII).

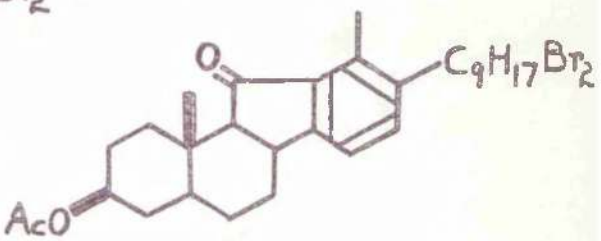
When the dibromoaromatic acetate is treated with chromium trioxide in acetic acid at room temperature the major product is a neutral substance, $C_{30}H_{42}O_3Br_2$, which does not give a 2:4-dinitrophenylhydrazone



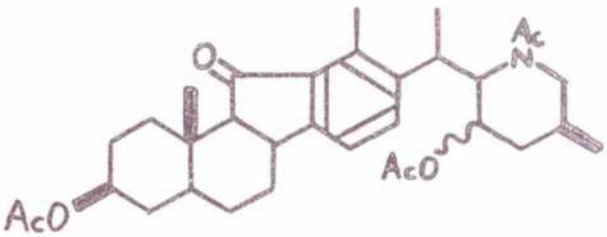
(CLXIX)



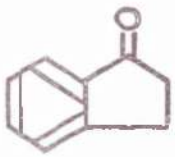
(CLXX)



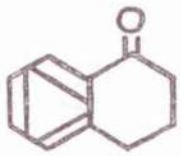
(CLXXI)



(XXXI)



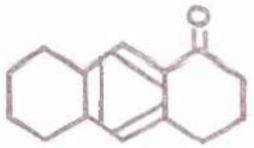
(CLXXII)



(CLXXIII)

when treated with Brady's reagent, but which shows a strong absorption band in the infrared at 1684 cm.$^{-1}$ (aromatic C=O stretching frequency). Triacetyldihydroketoveratramine (XXXI) shows a similar absorption band[52] but no firm conclusions as to whether the carbonyl group is present in a five or six membered ring can be drawn from this observation since, to the author's knowledge, no system comparable with structure (CLXIX) is available for comparison and in the simple cases of 1-oxoindane (CLXXII) and 1-oxotetralin (CLXXIII) the carbonyl stretching frequencies are very close (1709 cm.$^{-1}$ and 1703 cm.$^{-1}$ respectively).[110] The indanone (XXXI) absorbs at 1700 cm.$^{-1}$; it is therefore possible that the

dibromoaromatic keto-acetate , absorbing at a lower frequency, might be the tetralone (CLXIX). The ultraviolet absorption spectrum of the chromic acid oxidation product shows a strong band at 2650 Å together with a weak band at 3100 Å. Indanones and tetralones generally show a main maximum in the 2450-2500 Å region, and a low intensity maximum in the 2800-2900 Å region;[19] and in accord with the fact is the spectrum of the veratramine ketone (XXXI) which shows a strong absorption band at 2510 Å together with a weak band at 3000 Å.[19,52] Structure (CLXXI) cannot, therefore, represent the dibromoaromatic ketone which must be either (CLXIX) or (CLXX). The pronounced bathochromic shift



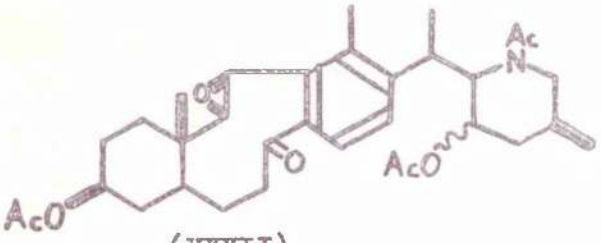
(CLXXIV)

observed in the spectrum of the ketone, (CLXIX) or (CLXX), when compared with the normal benzocyclanone spectrum, must be attributed to the presence of the second ring fused to the benzene nucleus. The same type of effect is observed in the spectrum of the cyclohexeno-tetralone (CLXXIV) which shows a strong band at 2620-2640 Å and a weak band at 3050 Å.[109]

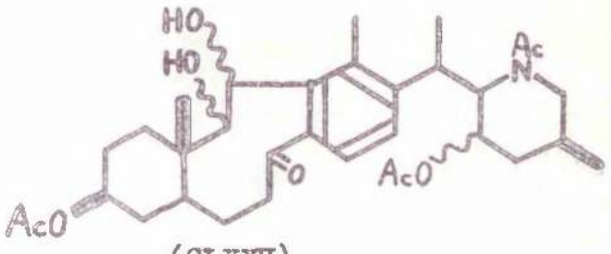
The spectroscopic characteristics of the dibromoaromatic ketone are very similar to those of the ketoglycol (CLXXV) ($\lambda_{max.}$ 2650 Å; 3000 Å; 1660 cm.$^{-1}$) which Hosansky and Wintersteiner[19] prepared from the ketoepoxide (XXXII), the major product of the chromic acid oxidation of triacetyldihydroveratramine (see page 8). The dibromo-aromatic ketone cannot be represented by a similar structure, as is shown by the elementary analysis, by the absence of absorption due

to hydroxyl groups in the infrared, and by its stability to alkali.[116] Thus when refluxed with methanolic potassium hydroxide the corresponding alcohol is obtained, acetylation of which regenerates the original acetate. The aromatic ketone is not reduced by sodium borohydride at room temperature but in refluxing methanol the diol, (CLXXVI) or (CLXXVII), is formed.

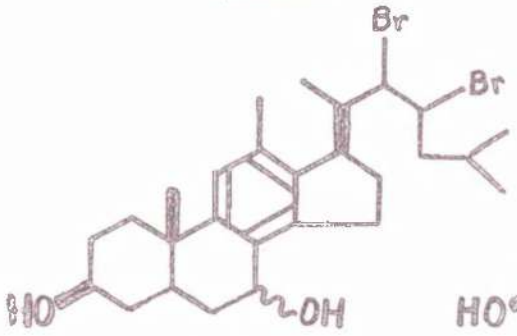
The aromatic ketone, (CLXIX) or (CLXX), fails to condense with



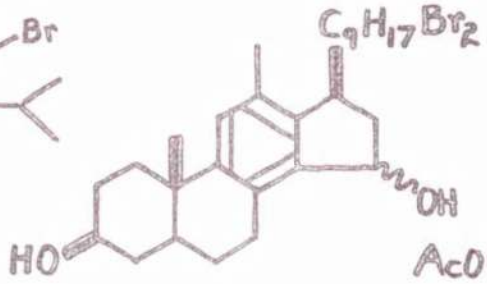
(XXXII)



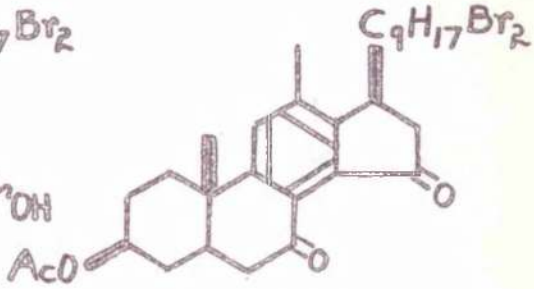
(CLXXV)



(CLXXVI)



(CLXXVII)



(CLXXVIII)

benzaldehyde or acetaldehyde, but this negative result does not permit a firm differentiation between the two possible formulae.

If the aromatic ketone is represented by either (CLXIX) or (CLXX), then it should be possible to prepare the diketone (CLXXVIII). The formation of such a diketone would exclude the possibility of the ring D aromatic structure (CLXXI), since the latter could not give a compound with two carbonyl groups in conjugation with the benzene ring without rupture of the molecule. Treatment of the dibromoaromatic keto-acetate with excess chromium trioxide in acetic acid, however, yields, as the sole product, an acid $C_{30}H_{42}O_6Br_2$ which is also obtained

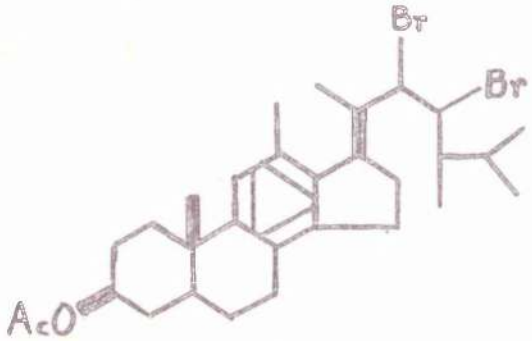
as the minor product of the room temperature oxidation of the dibromoaromatic acetate. This acid shows a broad absorption band in the 3000 cm.$^{-1}$ region and a broad band with a maximum at 1720 cm.$^{-1}$, indicative of the presence of one or more carboxyl groups. The ultraviolet absorption spectrum shows a strong band at 2570 Å and a weak band at 3000 Å [cf. 5:6:7:8-tetrahydro-2-naphthoic acid (CLXXIX)[109]]. The acid is obtained crystalline only with great difficulty; the anhydride, methyl ester, ethyl ester, p-nitrobenzyl ester, and anilide, of the 3β-acetate all fail to crystallise. Alkaline hydrolysis of the acid-acetate, followed by acidification, yields the corresponding

(CLXXIX)

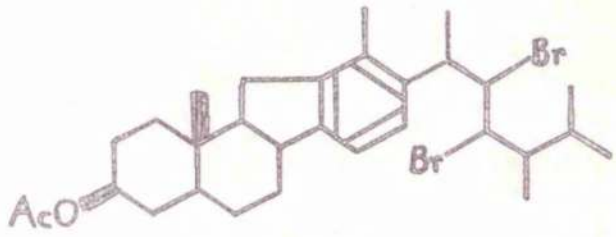
acid-alcohol as an amorphous solid; the corresponding benzoate and 3:5-dinitrobenzoate both failed to crystallise. The elementary analysis of the acid-acetate, $C_{30}H_{42}O_6Br_2$, and the acid-alcohol, $C_{28}H_{40}O_5Br_2$, show that no carbon atoms have been lost in the oxidation, and that the acid contains two carboxyl groups the presence of which is confirmed by determination of the equivalent weight. The acid does not give a precipitate with 2:4-dinitrophenylhydrazine sulphate in ethanol, even after prolonged refluxing, nor is the elementary analysis consistent with the presence of a carbonyl group. The dibromoaromatic-dicarboxylic acid must therefore be represented by either (CLXXX) or (CLXXXI), derived from 22:23-dibromo-12-methyl-18-norergosta--8(14):9(11):12-trien-3β-yl acetate (CLII), since the structure (CLXI) could only give the ketocarboxylic acid (CLXXXII) or the ketodicarboxylic

acid (CLXXXIII) without loss of carbon atoms.

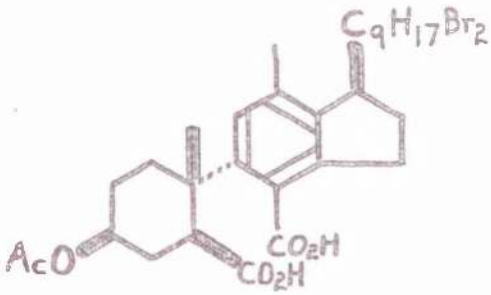
Further evidence concerning the position of the aromatic ring is obtained by degradation of the side-chain, but experiments in this direction are hindered by the lack of crystalline compounds. Satisfactory



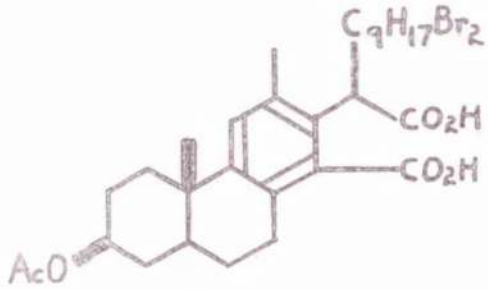
(CLII)



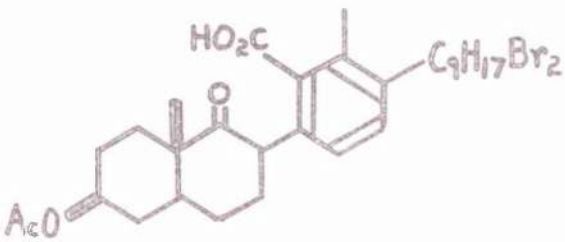
(CLXI)



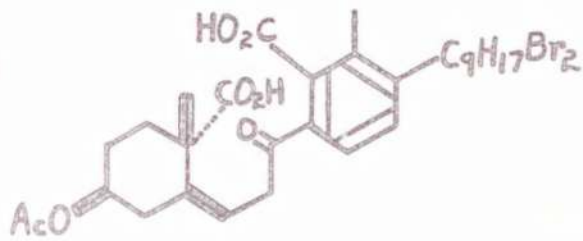
(CLXXX)



(CLXXXI)



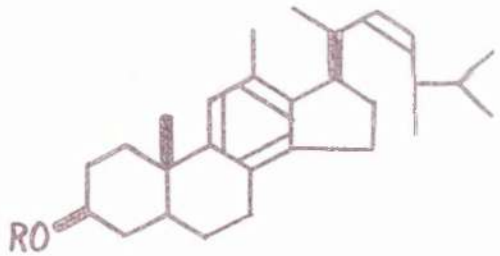
(CLXXXII)



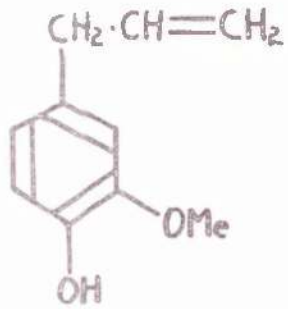
(CLXXXIII)

results are obtained, however, by adopting the following procedure for the purification of the non-crystalline products: the non-crystalline material is chromatographed on alumina; the required product, identified by its infrared and ultraviolet absorption spectra, is then rechromatographed and a middle cut of the eluted material is used for the next stage in the degradation.

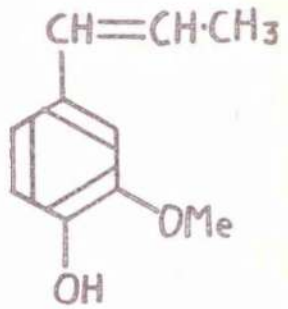
When the dibromoaromatic acetate (CLII) is refluxed with zinc dust in acetic acid, activated zinc dust in ethanol, or Raney nickel sludge in benzene, the non-crystalline tetraene acetate (CLXXXIV) is obtained. Alkaline hydrolysis of this acetate yields the corresponding alcohol (CLXXXV), also non-crystalline, which is characterised as its crystalline 3:5-dinitrobenzoate (CLXXXVI). Hydrolysis of the 3:5-dinitrobenzoate, by refluxing with methanolic potassium hydroxide or by filtering its solution in benzene through a column of alkaline alumina,[117] regenerates the non-crystalline alcohol (CLXXXV). Only intractible gums are obtained when the tetraene acetate (CLXXXIV) is treated with



(CLXXXIV; R = Ac)
(CLXXXV; R = H)
(CLXXXVI; R = 3:5-$(NO_2)_2 \cdot C_6H_3 \cdot CO$-)



(CLXXXVII)



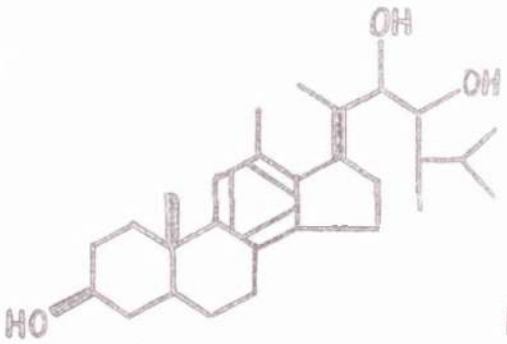
(CLXXXVIII)

bromine, N-bromosuccinimide, or 2:4-dinitrobenzenesulphenyl chloride (cf. Kharasch et al.[118]).

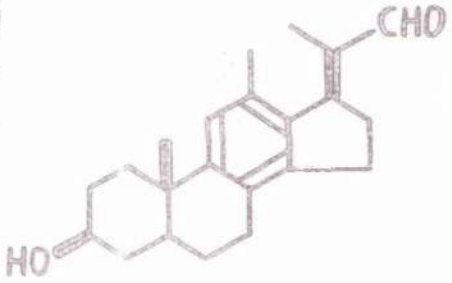
The alcohol (CLXXXV) is recovered unchanged, as indicated by its infrared and ultraviolet absorption spectra, after long refluxing with 50% potassium tert.-butoxide in anhydrous tert.-butanol or with 50% aqueous potassium hydroxide - conditions which effect the eugenol (CLXXXVII) to iso-eugenol (CLXXXVIII) rearrangement.[119] No change in the ultraviolet absorption spectrum of the acetate (CLXXXIV) is observed after prolonged refluxing with hydrochloric acid and acetic acid, but refluxing boron trifluoride-acetic acid complex causes the introduction of a strong band at 2660 Å; this is most probably due to some deep seated

change in the molecule since it is unlikely that such vigorous treatment should be necessary to bring a double bond, β:γ- to the benzene ring, into conjugation.

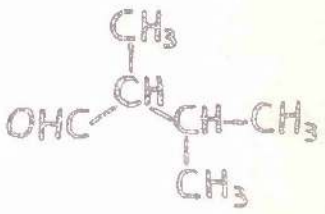
Hydroxylation of the tetraene acetate (CLXXXIV), by means of osmium tetroxide in ether, followed by decomposition of the osmium complex with lithium aluminium hydride, gives the non-crystalline triol (CLXXXIX). When this triol is treated with periodic acid and the reaction mixture is steam distilled, a volatile fraction is obtained



(CLXXXIX)



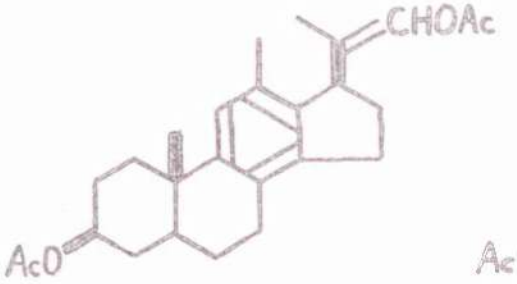
(CXC)



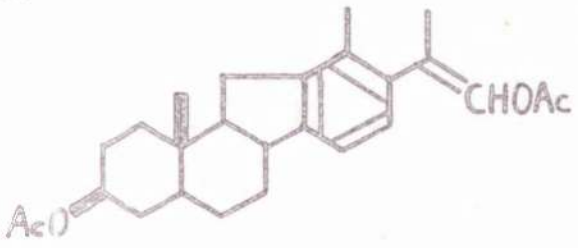
(XLIII)

which is shown to be (-)-methylisovaleraldehyde (XLIII) by comparison of its 2:4-dinitrophenylhydrazone with an authentic specimen, kindly supplied by Dr. P.Bladon. The assumption made in the previous discussion, that the bromine atoms in the dibromoaromatic acetate are at positions 22 and 23 in a normal ergosterol type side-chain, is thus verified. The non-volatile fraction, from the steam distillation, did not crystallise but it is characterised as the aldehyde (CXC) by the preparation of a crystalline 2:4-dinitrophenylhydrazone. The ultra-violet absorption spectrum of the aldehyde (CXC), which shows no high intensity absorption above 2300 Å, is not changed after prolonged refluxing with potassium acetate and acetic anhydride. The product from this reaction is non-crystalline but shows strong absorption bands in the infrared at 1740 $cm.^{-1}$ (due to the presence of the O-acetyl

group at C_3) and at 1765 cm.$^{-1}$ (due to the presence of an enol-acetate group[110]). The substance does not give a precipitate with 2:4-dinitrophenylhydrazine sulphate in ethanol and is readily hydrolysed, by refluxing with methanolic potassium hydroxide, to the aldehyde (CXC), the identity of the latter being established by its infrared absorption spectrum and by melting point, mixed melting point, and infrared comparison of the 2:4-dinitrophenylhydrazone with the same derivative previously obtained from the aldehyde (CXC). Since there is no change in the ultraviolet absorption spectrum, the enol-acetate must be



(CXCI)



(CXCII)

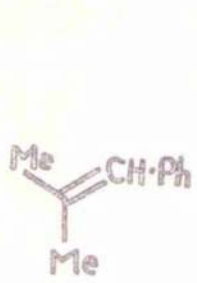
represented by (CXCI) and not by (CXCII).

Further degradation of the side-chain does not lead to any crystalline substances, nor are any crystalline derivatives obtained, but the ultraviolet absorption spectra of the products are in accord with the structure (CLII) for the dibromoaromatic acetate.

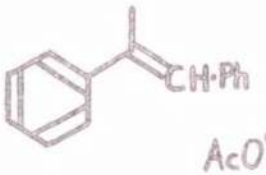
When the aldehyde (CXC) is treated with phenylmagnesium bromide and the product is refluxed with acetic anhydride, a non-crystalline substance is obtained the ultraviolet spectrum of which is very similar to that of β:β-dimethylstyrene (CXCIII) ($\lambda_{max.}$ 2520 Å; ϵ = 10,000)[109] and quite different from that of α-methylstilbene (CXCIV) (broad band from ca. 2700 Å to ca. 3000 Å; ϵ = 15,000).[109] This substance must therefore be represented by (CXCV) and not by (CXCVI).

The 3β-acetate of the aldehyde (CXC) is unchanged after treatment

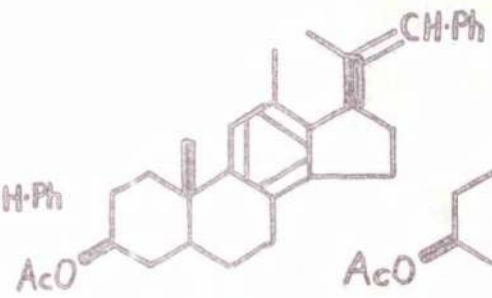
with hydrogen peroxide in acetic acid but is oxidised, by chromium trioxide in acetic acid, to an amorphous acid the methyl ester of which, also non-crystalline, when treated with phenylmagnesium bromide and then refluxed with acetic anhydride, yields a non-crystalline product showing a marked similarity in its ultraviolet absorption spectrum to α-phenylstyrene (CXCVII) ($\lambda_{max.}$ 2500 Å; ε = 15,000) [109] and differing from α-phenylstilbene (CXCVIII) ($\lambda_{max.}$ 3000 Å; ε = 15,000; high



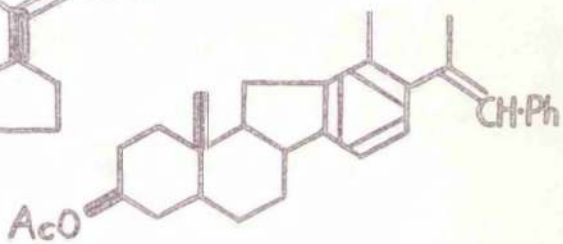
(CXCIII)



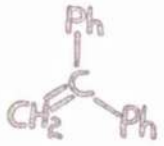
(CXCIV)



(CXCV)



(CXCVI)



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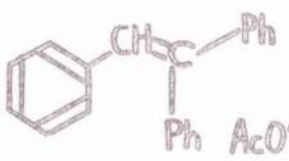

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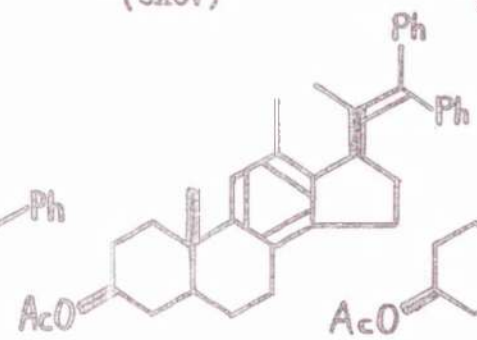

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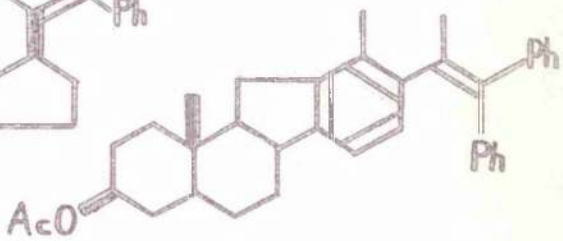


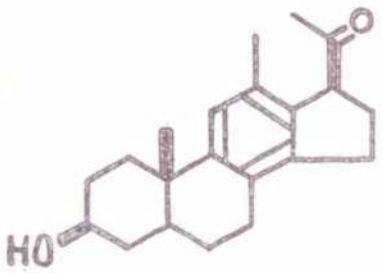
(CC)

intensity maximum at 2650 Å).[109] The spectrum is thus in agreement with structure (CXCIX) and not with (CC).

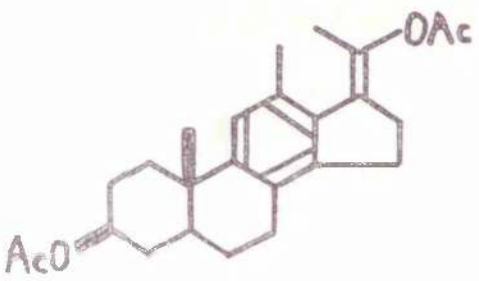
Treatment of the compound (CXCIX) with osmium tetroxide and then with periodic acid gives a non-crystalline product showing no high intensity absorption in the ultraviolet above 2400 Å. This substance, which gives an oily precipitate with 2:4-dinitrophenylhydrazine sulphate in ethanol, shows a strong band at 1710 cm.$^{-1}$ (due to a ketonic carbonyl group) in the infrared and must be the methyl ketone (CCI). When this ketone is refluxed with potassium acetate and acetic

anhydride the product, again non-crystalline, shows a strong absorption band at 2460 Å in the ultraviolet spectrum; it does not give a precipitate with Brady's reagent and it shows a strong band in the infrared at 1740 cm.$^{-1}$ (due to the O-acetyl group at C_3) with a high shoulder at 1750 cm.$^{-1}$ (due to an enol-acetate group[110]) - the enol-acetate must, then, be represented by (CCII).

In these latter experiments on the side chain, little weight can



(CCI)



(CCII)

be placed on the results since the products having the required spectroscopic characteristics are accompanied by much non-crystalline material of unknown constitution. In all cases, however, no absorption due to compounds derived from the structure (CLXI) is detected.

EXPERIMENTAL

Specific rotations were measured in chloroform solution, unless otherwise stated, at room temperature in a 1 dm. tube. Ultraviolet absorption spectra were measured in ethanol solution, unless otherwise stated, with a Unicam S.P. 500 spectrophotometer. Infrared absorption spectra were measured in Nujol mulls, unless otherwise stated, with a Grubb Parsons S. 4 double beam spectrophotometer with sodium chloride optics. Grade II alumina and light petroleum (b.p. 60-80°) were used for chromatography.

The author is indebted to Dr. A.C.Syme, Mr. W.McCorkindale, and Miss P.Adams for microanalyses, to Miss D.Adams for the determination of ultraviolet spectra, and to Miss N.Caramando for the determination of infrared spectra.

Tetrabromoergostenyl Acetate.- A solution of 5α:6-dihydroergosteryl acetate (20 g.) in dry ether (2 l.) was cooled to -30°. Bromine (32 g.; 10.32 ml.) in glacial acetic acid (100 ml.) was added in one portion, the solution cooled rapidly to -75° in acetone-"drikold", and then removed from the bath and allowed to warm slowly to room temperature (2-3 hrs.) with occasional swirling, avoiding direct sunlight. The precipitated solid was collected and washed free from colour with dry ether then dried *in vacuo* at room temperature (1 hr.). This crude product (12 g.) was used for the next stage. A specimen recrystallised from benzene-petrol gave the tetrabromide as colourless matted needles, m.p. 126-127° (decomp.); $[\alpha]_D$ + 260° (c, 1.2 in benzene). (Found: C, 47.7; H, 6.4; Br, 42.5. Calc. for $C_{30}H_{46}O_2Br_4$: C, 47.5; H, 6.1; Br, 42.2%).

The Action of Alumina on Tetrabromoergosteryl Acetate.- a) With immediate elution. A solution of the tetrabromide (20 g.) in benzene (500 ml.) was chromatographed on alumina (500 g.; 60 cm. x 4 cm.). Immediately the solution came into contact with the alumina a deep blue-green colour was formed; this colour gradually faded through green and red to brown. Elution with benzene (1700 ml.) gave a fraction (11.9 g.) which crustallised from acetone-methanol in pale yellow leaflets (5.5 g.) of 22:23-dibromoergosta-7:9(11):14-trien-3β-yl acetate, m.p. 208-209°; $[\alpha]_D$ - 56° (c, 1.3). Repeated crystallisation did not remove the yellow colour, but filtration through a short column of alumina gave colourless leaflets, m.p. 200-201°; $[\alpha]_D$ - 53° (c, 1.2), $\lambda_{max.}$ 2280 Å (ε = 12,200), 2340 Å (ε = 11,900), 2650 Å (ε = 10,700). The triene gives an orange-red colour, fading to strong yellow, with tetranitromethane in chloroform. (Found: C, 60.1; H, 7.65. $C_{30}H_{44}O_2Br_2$ requires: C, 60.4; H, 7.4%).

Continued elution with benzene (1100 ml.) yielded a yellow gum (3.1 g.) which when dissolved in acetone-methanol slowly deposited colourless needles (500 mg.) of 22:23-dibromo-12-methyl-18-norergosta--8(14):9(11):12-trien-3β-yl acetate, m.p. 136-137°; $[\alpha]_D$ - 3° ± 1° (c, 1.1), $\lambda_{max.}$ in n-hexane 2080 Å (ε = 31,000), 2690 Å (ε = 330), 2760 Å (ε = 254), inflexion at 2620 Å (ε = 286); 1740 cm.$^{-1}$ and 1241 cm.$^{-1}$ (O-acetyl), 1592 cm.$^{-1}$ (aromatic ring vibration), 862 cm.$^{-1}$ (penta-substituted benzene ring). The substance gives a deep yellow colour with tetranitromethane in chloroform. (Found: C, 60.4; H, 7.5. $C_{30}H_{44}O_2Br_2$ requires: C, 60.4; H, 7.4%).

Elution with benzene-ether mixtures (1000 ml.) and ether (1000 ml.) gave only intractible gums (1.5 g.). When the column was washed with ether-methanol mixtures (1000 ml.) and methanol (500 ml.), a dark brown

gum (2.5 g.) was obtained which, when dissolved in methanol and allowed to stand for some weeks, slowly deposited a crystalline solid. Recrystallisation of this solid from ether-methanol gave 22:23-dibromo--7-methoxyergosta-7:9(11)-dien-3β-yl acetate as large colourless blades (125 mg.), m.p. 180-182°; $[\alpha]_D$ + 92° (c, 0.9), giving a brown colour with tetranitromethane in chloroform; $\lambda_{max.}$ 2460 Å (ε = 18,000); 1740 cm.$^{-1}$ and 1240 cm.$^{-1}$ (O-acetyl), 1068 cm.$^{-1}$ (C=C-O-C ?). (Found: C, 59.15; H, 7.9; OMe, 5.5. $C_{30}H_{45}O_2Br_2 \cdot OMe$ requires: C, 59.2; H, 7.7; OMe, 4.9%).

When the methoxydiene (50 mg.) was dissolved in petrol-benzene (1:3, 20 ml.) and chromatographed on alumina (2 g.) it was recovered unchanged on elution with the same solvent mixture (150 ml.).

b) With delayed elution. A solution of tetrabromoergostenyl acetate (18 g.) in benzene (500 ml.) was brought into contact with a column of alumina (500 g.; 60 cm. x 4 cm.) and left overnight before eluting with benzene (600 ml.). The first fraction (1.9 g.) crystallised from acetone-methanol in pale yellow leaflets (650 mg.) of 22:23-dibromo-ergosta-7:9(11):14-trien-3β-yl acetate, m.p. 204-206° (decomp.); $[\alpha]_D$ - 54° (c, 1.2). Continued elution with benzene (2 l.) gave a second fraction (15.1 g.) which crystallised slowly from acetone-methanol in colourless needles (5.2 g.) of 22:23-dibromo-12-methyl-18-nor-ergosta-8(14):9(11):12-trien-3β-yl acetate, m.p. 136-137°; $[\alpha]_D$ - 3° (c, 1.3). Washing the column with ether-methanol mixtures (500 ml.) and methanol (200 ml.) gave a dark brown gum (750 mg.) which crystallised very slowly from methanol. Recrystallisation from ether-methanol gave 22:23-dibromo--7-methoxyergosta-7:9(11)-dien-3β-yl acetate as colourless blades (200 mg.), m.p. 181-182°; $[\alpha]_D$ + 90° ± 2° (c, 1.0).

c) In ether-methanol solution. A saturated solution of tetrabromo-ergostenyl acetate (2 g.) in ether-methanol was filtered through a

column of alumina (17 g.; 6 cm. x 2 cm.). Elution with methanol, and crystallisation from ether-methanol gave a mixture of yellow crystals (150 mg.), m.p. 198-202° and 217-220°, which was dissolved in petrol (10 ml.) and rechromatographed on alumina (5 g.). Elution with petrol-benzene mixtures (250 ml.) gave a fraction (60 mg.) which crystallised from chloroform-methanol in colourless leaflets of 22:23-dibromoergosta-7:9(11):14-trien-3β-yl acetate, m.p. 198-200°. Elution with benzene (150 ml.) and ether (50 ml.) gave a fraction (100 mg.) which crystallised from chloroform-methanol in colourless needles, m.p. 244-245°; $[\alpha]_D$ - 27° (c, 0.9), $\lambda_{max.}$ 2530 Å (ε = 10,000); identical (m.p., mixed m.p., and infrared comparison) with 22:23-dibromo--7-oxoergosta-8-en-3β-yl acetate. (Found: C, 58.95; H, 7.85. Calc. for $C_{30}H_{46}O_3Br_2$: C, 58.6; H, 7.55%). Further elution of the column yielded only intractible gums.

Action of Aluminium Bromide-Alumina on 22:23-Dibromoergosta--7:9(11):14-trien-3β-yl Acetate.- The pale yellow crystals of the triene (250 mg.) were dissolved in benzene (30 ml.) and the solution was filtered through a column containing an intimate mixture of alumina (9 g.) and anhydrous aluminium bromide (1 g.), to which a few drops of concentrated hydrobromic acid had been added. After three days at room temperature the column was eluted with benzene (1000 ml.) to give a fraction which crystallised from chloroform-methanol in colourless leaflets of the triene (200 mg.), m.p. 198-200°; $[\alpha]_D$ -54° (c, 2.5). No colour developed on the column but the pale yellow colour was removed from the triene.

Action of Chloroformic Hydrogen Chloride on 22:23-Dibromoergosta-7:9(11):14-trien-3β-yl Acetate.- The triene (800 mg.) was dissolved in dry chloroform (40 ml.) and chloroformic hydrogen chloride (10 ml.; 0.26N) was added; a green colour formed immediately. After 12 hours at room temperature the solution was washed with saturated aqueous sodium bicarbonate, water, then dried and evaporated to dryness to give a brown gum (800 mg.) the ultraviolet absorption spectrum of which shows no high intensity absorption. The gum was dissolved in benzene (50 ml.) and chromatographed on alumina (25 g.; 8 cm. x 2 cm.). Elution with benzene (300 ml.) gave a fraction which crystallised from chloroform-methanol in colourless leaflets identical, m.p. and mixed m.p., with the starting material (30 mg.). Elution with benzene-ether mixtures (425 ml.) gave a fraction (170 mg.) from which no crystalline material was obtained. The ultraviolet absorption spectrum of this gum showed no high end absorption. Elution with ether (700 ml.) yielded a fraction which crystallised from chloroform-methanol in colourless needles (90 mg.), m.p. 225-226°; $[\alpha]_D$ - 20° (c, 1.1), $\lambda_{max.}$ 2500 Å (ε = 19,000). (Found: C, 60.5; H, 8.1. $C_{30}H_{46}O_2Br_2$ requires: C, 60.2; H, 7.75%). These constants are identical with those of 22:23-dibromoergosta-8:14-dien-3β-yl acetate (22:23-dibromoergosteryl-B_1 acetate) but no direct comparison has been made. It is difficult to see how such a diene could be formed from the triene under the conditions of the experiment, it is probably formed by the action of the acid on the impurity present in the pale yellow crystals of the triene. Further elution of the column with ether (200 ml.), ether-methanol (500 ml.), and methanol (500 ml.) gave only intractible gums which showed no high intensity absorption in the 2000-2200 Å region which might be attributed to the presence of an aromatic substance.

Hydrolysis of the Methoxydiene Acetate.- The methoxydiene acetate (150 mg.) was refluxed (1 hr.) with 5% methanolic potassium hydroxide (20 ml.). The product was worked up in the usual manner, through ether, and crystallised from chloroform-methanol in colourless needles of the dibromomethoxydiene alcohol, m.p. 169-170°; $\lambda_{max.}$ 2450 Å (ε = 16,000). (Found: C, 59.6; H, 7.9. $C_{29}H_{46}O_2Br_2$ requires: C, 59.4; H, 7.9%). Acetylation of the alcohol regenerated the original acetate (m.p., mixed m.p., and infrared comparison).

Debromination of the Methoxydiene Acetate.- The dibromomethoxydiene acetate (250 mg.) was refluxed (2.5 hr.) with activated zinc dust (5 g.) in ether-ethanol (1:1, 50 ml.). After working up through ether in the usual manner the product was a clear gum (160 mg.) which crystallised from ether-methanol as large colourless blades of the methoxytriene acetate, m.p. 101-102°; $[\alpha]_D$ + 135° (c, 1.5), $\lambda_{max.}$ 2460 Å (ε = 16,000). (Found: C, 79.7; H, 10.3. $C_{31}H_{48}O_3$ requires: C, 79.4; H, 10.3%).

Attempted Catalytic Hydrogenation of the Dibromoaromatic Acetate.- A solution of the dibromoaromatic acetate (250 mg.) in glacial acetic acid (50 ml.) was shaken (24 hrs) with hydrogen at atmospheric pressure, in the presence of a platinum catalyst (prepared by pre-reducing 250 mg. of platinum oxide). No uptake of hydrogen was observed and, after working up the acetic acid solution in the normal manner, the starting material was recovered quantitatively.

Hydrolysis of the Dibromoaromatic Acetate.- a) The dibromoaromatic acetate (250 mg.) was refluxed (1 hr.) with 2% methanolic potassium

hydroxide (25 ml.). After working up in the usual manner through ether, the product crystallised from n-hexane in colourless felted needles (90 mg.) of 22:23-dibromo-12-methyl-18-norergosta-8(14):9(11):12-trien-3β-ol, m.p. 110-111°; $[\alpha]_D$ + 5° ± 2° (c, 1.1), $\lambda_{max.}$ 2080 Å (ϵ = 30,000), 2680 Å (ϵ = 400), 2760 Å (ϵ = 260); 3390 cm.$^{-1}$ (hydroxyl group), 1598 cm.$^{-1}$ (aromatic ring vibration), 865 cm.$^{-1}$ (penta-substituted benzene ring). (Found: C, 60.2; H, 7.6. $C_{28}H_{42}OBr_2$ requires: C, 60.6; H, 7.6%).

b) The dibromoaromatic acetate (250 mg.) was refluxed (1 hr.) with 18% methanolic sulphuric acid (50 ml.) and then allowed to cool overnight when colourless felted needles (180 mg.), m.p. 74-84°, separated. One recrystallisation from aqueous methanol gave the dibromoaromatic alcohol (150 mg.), m.p. 110-111°; $[\alpha]_D$ + 5° ± 2° (c, 1.6), identical (m.p., mixed m.p., ultraviolet, and infrared spectra) with the alkaline hydrolysis product. Acetylation of the alcohol regenerated the dibromoaromatic acetate, m.p. 136-137°; $[\alpha]_D$ - 3° (c, 2.0).

Dibromoaromatic 3-Ketone.- The dibromoaromatic alcohol (100 mg.) was dissolved in pyridine (1 ml.) and a paste of chromium trioxide (100 mg.) in pyridine (2 ml.) was added; the mixture was allowed to stand (24 hrs.) at room temperature, with occasional aggitation. Methanol (5 ml.) was added to the reaction mixture, followed by 2% aqueous sodium hydroxide (20 ml.). The product was then worked up in the usual manner through ether. The resulting pale yellow gum crystallised slowly from methanol in rosettes of colourless needles, m.p. 102-105° (80 mg.). Several recrystallisations from chloroform-methanol gave 3-oxo-22:23-dibromo--12-methyl-18-norergosta-8(14):9(11):12-triene, m.p. 139-140°; $[\alpha]_D$ + 23°

(c, 1.1), $\lambda_{max.}$ 2080 Å (ε = 30,000), 2680 Å (ε = 500), 2760 Å (ε = 260); 1721 cm.$^{-1}$ (six-ring ketone), 1597 cm.$^{-1}$ (aromatic ring vibration), 872 cm.$^{-1}$ (penta-substituted benzene ring). (Found: C, 60.7; H, 7.2. $C_{28}H_{40}OBr_2$ requires: C, 60.9; H, 7.3%).

Action of Phosphorus Oxychloride and Pyridine on the Dibromo-aromatic Alcohol.- A solution of the dibromoaromatic alcohol (85 mg.) in pyridine (10 ml.) was warmed on the steam-bath (1 hr.) with phosphorus oxychloride (1 ml.) and then allowed to stand overnight at room temperature before working up in the usual manner through ether. The product was a white solid (60 mg.), m.p. 190-193°, which was recrystallised from chloroform-methanol to give colourless needles of 22:23-dibromo--3α-chloro-12-methyl-18-norergosta-8(14):9(11):12-triene, m.p. 195-197°; $[\alpha]_D$ + 13° (c, 1.7), giving a deep yellow colour with tetranitromethane in chloroform; $\lambda_{max.}$ 2150 Å (ε = 25,000), 2580 Å (ε = 467), 2670 Å (ε = 476), 2760 Å (ε = 400). (Found: C, 58.65; H, 7.3; Br, 27.2; Cl, 6.05. $C_{28}H_{41}Br_2Cl$ requires: C, 58.7; H, 7.2; Br, 27.9; Cl, 6.21%). Increasing the reaction scale lowers the yield; e.g. 336 mg. of the alcohol yielded only 95 mg. of the chloro-compound.

Action of Phosphorus Pentoxide on the Dibromoaromatic Alcohol.- A solution of the dibromoaromatic alcohol (160 mg.) in dry benzene (5 ml.) was shaken (22 hrs.) at room temperature with phosphorus pentoxide (160 mg.). After addition of water the reaction mixture was worked up in the normal manner to give a pale yellow gum (140 mg.), $\lambda_{max.}$ 2080 Å (ε = 27,000), 2690 Å (ε = 370), which slowly crystallised from acetone in colourless, stout, glistening blades (70 mg.), m.p.124-125°; $[\alpha]_D$ + 53° (c, 0.7), $\lambda_{max.}$ 2080 Å (ε = 30,000), 2690 Å (ε = 370).

The compound gives a strong yellow colour with tetranitromethane in chloroform. (Found: C, 62.65; H, 7.7. $C_{28}H_{40}Br_2$ requires: C, 62.7; H, 7.5%). This compound must be the 2-ene or the 3-ene. A second isomer, or a mixed crystal of this isomer with the first, was obtained from the mother liquors which yielded a second crop (30 mg.), m.p. 123-128°. Recrystallisation from chloroform-methanol gave colourless needles, m.p. 128-131°, not improved by repeated crystallisation, $[\alpha]_D$ + 41° ± 2° (c, 0.6), $\lambda_{max.}$ 2080 Å (ε = 30,000), 2690 Å (ε = 370); gives a strong yellow colour with tetranitromethane in chloroform. (Found: C, 62.5; H, 7.5. $C_{28}H_{40}Br_2$ requires: C, 62.7; H, 7.5%). A mixture of the two isomers, which show only insignificant differences in their infrared spectra, melts at 124-125°. The ultraviolet absorption spectra of both isomers, in n-hexane, is not changed after refluxing (1 hr.) with concentrated hydrochloric acid and acetic acid (1:5).

Action of Nitric Acid on Ergosterol.- Concentrated nitric acid (s.g. 1.42; 45 ml.) was carefully added to ergosterol (2 g.); a vigorous reaction took place, after which the solution was gently warmed. When further vigorous evolution of nitrous fumes had ceased the solution was refluxed gently (17 hrs.) and then concentrated until 35 ml. of distillate had been collected. The cooled solution was filtered and the crystalline precipitate was washed with a little cold ether and recrystallised from water to give toluene-2:3:5:6-tetracarboxylic acid (130 mg.), m.p. 238-241°, changing from prisms to blades at 210°.

Methylation of the acid with excess diazomethane in ether yielded the tetramethyl ester, m.p. 118-122°, from benzene-n-hexane (Inhoffen[14] gives m.p. 121-123°).

Action of Nitric Acid on the Dibromoaromatic Acetate.- The dibromoaromatic compound (2 g.) was warmed with concentrated nitric acid (s.g. 1.42; 45 ml.); a vigorous reaction took place with copius evolution of nitrous fumes. When the reaction ceased the solution was gently refluxed (16 hrs.) and then concentrated until 35 ml. of distillate had been collected, but on cooling no solid material separated. Addition of water caused the precipitation of a gum which could not be crystallised. The aqueous phase was extracted with ether and the gum obtained from this extract was combined with the precipitated gum and treated with excess diazomethane in ether. The methylated material was carefully chromatographed on alumina but only intractible gums were obtained.

Action of Chromium Trioxide in Acetic Acid on the Dibromoaromatic Acetate.- A solution of the dibromoaromatic acetate (1 g.) in stabilised acetic acid (80 ml.) was mixed with a solution of chromium trioxide (222 mg.; 2 atoms oxygen per mole) in stabilised acetic acid (5 ml.). After 2½ hours, when most of the oxidant had been consumed, a further quantity of chromium trioxide (333 mg.; 3 atoms oxygen per mole) in stabilised acetic acid (10 ml.) was added and the reaction mixture maintained at room temperature (19 hrs.). The product was precipitated by the addition of water (ca. 200 ml.), filtered off, and washed free from acid with water. A solution of the crude material in acetone--methanol slowly deposited colourless rods of the dibromoaromatic keto-acetate (600 mg.), m.p. 133-8°. Recrystallisation from methanol gave long matted needles, m.p. 135-138°; $[\alpha]_D$ - 10° (c, 1.9), $\lambda_{max.}$ 2190 Å (ϵ = 31,000), 2650 Å (ϵ = 15,000), 3.00 Å (ϵ = 3,000); 1736 cm.$^{-1}$ (O-acetyl), 1684 cm.$^{-1}$ (carbonyl group adjacent to benzene ring),

1592 cm. $^{-1}$ (aromatic ring vibration). (Found: C, 59.2; H, 7.2. $C_{30}H_{42}O_3Br_2$ requires: C, 59.0; H, 6.9%).

The ketone did not give a 2:4-dinitrophenylhydrazone even after prolonged refluxing with Brady's reagent.

Alkaline hydrolysis of the ketone acetate (200 mg.) yielded the corresponding alcohol (150 mg.), m.p. 199-201°; $[\alpha]_D$ - 5° (c, 0.9). (Found: C, 59.3; H, 7.4; Br, 28.8. $C_{28}H_{40}O_2Br_2$ requires: C, 59.2; H, 7.1; Br, 28.1%). Acetylation of the alcohol regenerated the original acetate (m.p., mixed m.p., and infrared comparison).

The ketone acetate was recovered unchanged after treatment with sodium borohydride in methanol at room temperature, but after refluxing (2.5 hrs.) the product, which crystallised slowly from the reaction mixture, was the dibromoaromatic diol, m.p. 115-117°; $[\alpha]_D$ + 11° (c, 1.2), $\lambda_{max.}$ 2080 Å (ε = 35,000), 2690 Å (ε = 400); 3236 cm.$^{-1}$ (alcoholic hydroxyl groups). (Found: C, 58.7; H, 7.2. $C_{28}H_{42}O_2Br_2$ requires: C, 58.95; H, 7.4%).

Action of Benzaldehyde on the Dibromoaromatic Keto-Acetate.- (cf. Barton, Head, and May[120]). The ketone (200 mg.) was dissolved in 0.1M potassium hydroxide in 99% methanol (30 ml.) and treated with redistilled benzaldehyde (350 mg.; 0.334 ml.) in a stoppered flask, overnight, in the dark, at room temperature. The product was worked up through ether in the usual manner and crystallised from methanol in colourless needles, m.p. 199-201°; $[\alpha]_D$ - 5° (c, 1.1), identical (m.p. and mixed m.p.) with the dibromoaromatic ketone alcohol. Acetylation gave the dibromoaromatic ketone acetate (m.p. and mixed m.p.).

Action of Acetaldehyde on the Dibromoaromatic Keto-Acetate.- (cf. Barton, Head, and May[120]). The ketone (200 mg.) was dissolved in ether (30 ml.); acetaldehyde (190 mg.; 0.25 ml.) and concentrated sulphuric acid (5 drops) were then added, the mixture shaken, and left overnight at room temperature in the dark. After working up in the usual manner the product was identical, m.p. and mixed m.p., with the starting material.

Further Action of Chromium Trioxide in Acetic Acid on the Dibromoaromatic Acetate.- a) A solution of the dibromoaromatic (900 mg.) in stabilised acetic acid (50 ml.) was mixed with a solution of chromium trioxide (1.05 g.; 12 atoms oxygen per mole) in stabilised acetic acid (15 ml.) and left standing (2 days) at room temperature. The reaction mixture was worked up through ether in the usual way and separated into acid and neutral fractions. The neutral fraction crystallised from acetone-methanol in colourless rods (200 mg.), m.p. 135-138°; $[\alpha]_D$ - 10° (c, 1.0), identical (m.p., mixed m.p., and infrared) with the dibromoaromatic ketone acetate.

The acid fraction was an amorphous solid (0.5 g.) which failed to crystallise from the normal solvents or solvent mixtures; it was purified by repeated precipitation, with water, from its solution in glacial acetic acid and finally obtained as a crystalline film on allowing the concentrated solution in acetic acid to evaporate slowly to dryness at room temperature, m.p. 150-170° (decomp.); $\lambda_{max.}$ 2570 Å (ϵ = 11,000), 3000 Å (ϵ = 3000); broad bands at 3300-2850 cm.$^{-1}$ and at 1720-1710 cm.$^{-1}$ (carboxyl groups). (Found: C, 54.6; H, 6.7. $C_{30}H_{42}O_6Br_2$ requires: C, 54.7; H, 6.4%) (Equivalent weight; found: 357. $C_{28}H_{40}O_2Br_2(CO_2H)_2$ requires: 329.)

The dicarboxylic acid did not give a 2:4-dinitrophenylhydrazone

even after prolonged refluxing with Brady's reagent, nor did it yield a crystalline anhydride after refluxing (20 hrs.) with acetic anhydride. Only intractible gums were obtained when the acid was treated with diazomethane or diazoethane in ether, p-nitrobenzyl bromide, or thionyl chloride followed by aniline. Alkaline hydrolysis of the dicarboxylic acid acetate followed by acidification yielded the amorphous dicarboxylic acid alcohol, m.p. 140-170° (decomp.) (Found: C, 54.9; H, 6.8. $C_{28}H_{40}O_5Br_2$ requires: C, 54.6; H, 6.5%), which was partially purified by repeated precipitation from acetic acid solution, with water. Treatment of the acid alcohol with benzoyl chloride, or 3:5-dinitrobenzoyl chloride, and pyridine yielded only intractible gums.

b) A similar experiment, but at reflux temperature (30 mins.), gave the amorphous dicarboxylic acid acetate with only traces of neutral material.

c) Treatment of the dibromoaromatic ketone acetate with excess chromium trioxide in acetic acid at room temperature, in the manner described above, yielded approximately 50% of the amorphous dicarboxylic acid together with approximately 50% starting material.

Debromination of the Dibromoaromatic Acetate.- a) The dibromoaromatic acetate (300 mg.) was dissolved in stabilised acetic acid (30 ml.) and heated on the steam-bath, with vigorous stirring. Zinc dust (2.7 g.) (activated by warming with 10% aqueous ammonium chloride) was added in portions over a period of 2 hours; heating and stirring was continued for a further 2 hours and then the zinc dust was filtered off. The filtrate was poured into water and worked up through ether in the normal manner to give 12-methyl-18-norergosta-8(14):9(11):12:22-tetraen-3β-yl acetate (205 mg.), $[\alpha]_D$ + 60° (c, 1.3), as a clear gum which defied

all attempts at crystallisation. Alkaline hydrolysis gave the corresponding alcohol, $[\alpha]_D$ + 77° (c, 1.1), also as a clear gum, $\lambda_{max.}$ 2080 Å (ε = 31,000), 2680 Å (ε = 490); 862 cm.$^{-1}$ (penta-substituted benzene ring).

Treatment of the alcohol with 3:5-dinitrobenzoyl chloride in pyridine, at room temperature, overnight, gave the 3:5-dinitrobenzoate as stout yellow blades, m.p. 156-157°; $[\alpha]_D$ - 52° (c, 2.5), from chloroform-methanol. (Found: C, 71.4; H, 7.2; N, 4.6. $C_{35}H_{44}N_2O_6$ requires: C, 71.4; H, 7.5; N, 4.8%).

Hydrolysis of the 3:5-dinitrobenzoate by refluxing with methanolic potassium hydroxide or by filtering its solution in benzene through a column of alkaline alumina[117] gave the non-crystalline alcohol, $[\alpha]_D$ + 77°, (infrared comparison).

b) Refluxing the dibromoaromatic acetate (3.65 g.) with activated zinc dust (30 g.) in ethanol (250 ml.) for 5 hours yielded the debromo acetate (2.7 g.), $[\alpha]_D$ + 60°, again characterised as the corresponding 3:5-dinitrobenzoate.

c) The same debromo acetate was obtained by refluxing the dibromo-aromatic acetate (300 mg.) with Raney nickel sludge (ca. 6 ml.; 3.6 g.) in dry benzene (50 ml.) and ethanol (50 ml.) for 3 hours. The product (205 mg.), $[\alpha]_D$ + 60°, was identified by infrared comparison and by the preparation of the 3:5-dinitrobenzoate, m.p. and mixed m.p. 156-157°; $[\alpha]_D$ - 53° (c, 1.7).

Action of Bromine on the Debrominated Aromatic Acetate.- The debrominated acetate (300 mg.), carefully chromatographed, was dissolved in dry carbon tetrachloride (10 ml.). Bromine (150 mg.; 0.05 ml.) in dry carbon tetrachloride (10 ml.) was titrated into the solution of the acetate over a period of one hour. The solution was then washed

with 10% aqueous sodium thiosulphate, water, and dried. Evaporation of the carbon tetrachloride solution to dryness gave a dark red gum (425 mg.) from which no crystalline material could be isolated, even after careful chromatography.

Action of N-Bromosuccinimide on the Debrominated Aromatic Acetate.- The debrominated acetate (90 mg.), purified by careful chromatography, was refluxed (30 mins.) with N-bromosuccinimide (36 mg.; 1 mole) in dry carbon tetrachloride (10 ml.). The cooled reaction mixture was filtered free from succinimide and the filtrate was washed with 10% aqueous sodium thiosulphate, 2% aqueous sodium hydroxide, and water, then dried and evaporated to dryness to give a dark gum (70 mg.) from which no crystalline material could be isolated, even after careful chromatography.

Action of 2:4-Dinitrobenzenesulphenyl Chloride on the Debrominated Aromatic Acetate.- The debrominated acetate (80 mg.), purified by chromatography, was dissolved in glacial acetic acid (2 ml.) together with 2:4-dinitrobenzenesulphenyl chloride (80 mg.) and the mixture warmed on the steam-bath. After 15 minutes a drop of the reaction mixture gave no colour with acidified starch-potassium iodide solution;[118] the reaction mixture was heated for a further 15 minutes, allowed to cool, and, since no crystalline material separated, poured into water. The precipitated gum was crystallised from aqueous dimethylformamide to give yellow micro-prisms of bis- 2:4-dinitrophenylsulphide), m.p. > 360°. (Found: N, 13.5. Calc. for $C_{12}H_6O_8N_4S_2$: N, 13.7%). No more crystalline material could be isolated.

Action of Alkali on the Debrominated Aromatic Acetate.- a) The debrominated acetate (150 mg.) was refluxed (20 hrs.) with a solution of potassium (1.5 g.) in tert-butanol (50 ml.). The product was worked up through ether in the normal manner to give a pale yellow gum (130 mg.), $\lambda_{max.}$ 2080 Å (ϵ = 31,000), 2680 Å (ϵ = 450), identical (infrared comparison) with the debrominated aromatic alcohol.

b) The debrominated acetate (300 mg.) was refluxed (17 hrs.) with 50% potassium hydroxide in 50% aqueous methanol (50 ml.) in a quartz flask. The reaction mixture was worked up through ether in the normal manner to give a clear gum (250 mg.), $\lambda_{max.}$ 2080 Å (ϵ = 31,000), 2680 Å (ϵ = 470), identical (infrared comparison) with the debrominated aromatic alcohol.

Action of Acid on the Debrominated Aromatic Acetate.- a) The debrominated acetate (250 mg.), purified by chromatography, was refluxed (17 hrs) with concentrated hydrochloric acid (2 ml.) in glacial acetic acid (50 ml.). The reaction mixture was worked up through ether in the normal manner to give a yellow gum (250 mg.), $\lambda_{max.}$ 2080 Å (ϵ = 32,000), 2680 Å (ϵ = 500), identical (infrared comparison) with the starting material.

b) The debrominated acetate (250 mg.), purified by chromatography, was refluxed (3 hrs.) with boron trifluoride-acetic acid complex (30 ml.), then worked up in the usual manner through ether to give a dark brown gum (215 mg.), $\lambda_{max.}$ 2660 Å ($\epsilon \sim 10,000$), from which no crystalline material could be isolated even after careful chromatography.

Hydroxylation of the Debrominated Aromatic Acetate.- The debrominated acetate (940 mg.) was treated with osmium tetroxide in dry ether (75 ml.)

(1 g. osmium tetroxide) at room temperature (6 days). Lithium aluminium hydride (3 g.) was then added and the mixture refluxed (3 hrs.), cooled, and dilute sulphuric acid (100 ml.) carefully added. The black sludge was filtered off, with difficulty, and the ether layer, after drying, was evaporated to dryness to give a colourless solid froth (999 mg.), $[\alpha]_D$ + 60° (c, 1.0) which must be the 3:22:23-triol. No crystalline materials were obtained after treatment with acetic anhydride in pyridine, benzoyl chloride in pyridine, or 3:5-dinitrobenzoyl chloride in pyridine.

Periodic Acid Scission of the Aromatic Triol.- The triol (912 mg.) was dissolved in ethanol (100 ml.) and treated with periodic acid ($HIO_4, 2H_2O$) (950 mg.) in water (3 ml.) and allowed to stand (30 hrs.) at room temperature in the dark. The reaction mixture was then neutralised (to litmus) by sodium bicarbonate; 10% aqueous sodium thiosulphate (100 ml.) was added and the mixture steam distilled.

To the distillate an aqueous ethanolic solution of 2:4-dinitrophenylhydrazine sulphate was added and the solution left overnight when an orange coloured crystalline precipitate was formed (180 mg.), m.p. 95-110°. The crystalline solid was dissolved in petrol-benzene (2:1, 15 ml.) and chromatographed on alumina (6 g.; 10 cm. x 1 cm.). The same solvent mixture (75 ml.) eluted a fraction (97 mg.) which crystallised from n-hexane in orange plates, m.p. 100-104°. Further recrystallisation from ethanol gave the 2:4-dinitrophenylhydrazone of (-)-methyliscvaleraldehyde, m.p. 110-114°; $[\alpha]_D$ - 20° (c, 0.8), identical with an authentic specimen (m.p., mixed m.p., and infrared comparison) kindly supplied by Dr. P.Bladon. A second fraction (43 mg.), eluted with the same solvent mixture (100 ml.), crystallised from

ethanol in orange needles, m.p. 164-166°, identical (m.p., mixed m.p., and infrared comparison) with acetaldehyde 2:4-dinitrophenylhydrazone, presumably formed by oxidation of the solvent.

The non-volatile residue was obtained by working up through ether which yielded the hexanor-aldehyde (630 mg.) as a colourless solid froth, $[\alpha]_D$ - 18° ± 1° (c, 1.0), $\lambda_{max.}$ 2080 Å (ϵ = 30,000), 2680 Å (ϵ = 530); 3378 cm.$^{-1}$ (alcoholic hydroxyl), 1724 cm.$^{-1}$ (aliphatic aldehyde). Treatment of the aldehyde 3β-ol with Brady's reagent gave the 2:4-dinitrophenylhydrazone as lemon yellow micro-blades from ethanol (after filtering through a short column of alumina, in benzene solution), m.p. 194-197°; $[\alpha]_D$ + 51° (c, 0.9). (Found: C, 66.3; H, 6.6; N, 11.1. $C_{28}H_{34}O_5N_4$ requires: C, C, 66.4; H, 6.8; N, 11.1%).

The aldehyde 3β-acetate did not crystallise and its 2:4-dinitrophenylhydrazone forms orange-red spheres from ethanol, m.p. 231-233°; $[\alpha]_D$ ± 0° (c, 0.6). (Found: N, 10.11. $C_{30}H_{36}O_6N_4$ requires: N, 10.2%).

Enol-Acetate of the Hexanoraldehyde.- The aldehyde (530 mg.) was heated (6 hrs.) at 120-130° (bath temperature) with acetic anhydride (3 ml.) and freshly fused potassium acetate (200 mg.). The deep red solution was poured into water and worked up through ether to give a dark gum (560 mg.), $\lambda_{max.}$ 2070 Å (ϵ = 37,000), 2500-2700 Å (ϵ = 1000). The gum was dissolved in petrol-benzene (7:1, 32 ml.) and chromatographed on alumina (15 g.: 7 cm. x 1.5 cm.). Elution with petrol-benzene mixtures (750 ml.) yielded a fraction 300 mg.), as a colourless gum which must be the enol-acetate, $\lambda_{max.}$ 2080 Å (ϵ = 31,000), 2680 Å (ϵ = 400); 1740 cm.$^{-1}$ (O-acetyl), 1765 cm.$^{-1}$ (enol-acetate). The substance does not give a 2:4-dinitrophenylhydrazone even after refluxing with Brady's reagent. Refluxing

the enol-acetate (400 mg.) with 2% methanolic potassium hydroxide (30 ml.) for 30 minutes yields the hexanoraldehyde (infrared comparison), which was readily identified by the preparation of its 2:4-dinitrophenylhydrazone (the 2:4-dinitrophenylhydrazone is precipitated immediately a solution of the aldehyde is mixed with Brady's reagent).

Action of Phenylmagnesium Bromide on the Hexanoraldehyde.- The hexanoraldehyde (270 mg.; 0.73 m.mole) in dry benzene (15 ml.) was added to a solution of phenylmagnesium bromide (prepared from 390 mg., 16 mg. atoms, magnesium turnings and 2.52 g., 1.68 ml., 16 m.moles, dry redistilled bromobenzene in 20 ml. dry ether) and the mixture refluxed (3 hrs.) then poured into ice (ca. 60 ml.) and concentrated hydrochloric acid (ca. 20 ml.) and worked up through ether in the normal manner. The resulting oil was steam distilled to remove excess bromobenzene and some diphenyl. The residue was dried, via its solution in ether, and refluxed (1 hr.) with acetic anhydride (20 ml.) and acetic acid (40 ml.). The solution was concentrated to approximately 10 ml. and then worked up through ether to yield an intractible gum (340 mg.) from which no crystalline material was isolated, even after careful chromatography. The ultraviolet absorption spectrum of the gum ($\lambda_{max.}$ 2520 Å; ε = 6,000) is very similar to that of β:β-dimethylstyrene ($\lambda_{max.}$ 2520 Å; ε = 10,000).[109]

Action of Hydrogen Peroxide on the Hexanoraldehyde.- The aldehyde (100 mg.) in glacial acetic acid (35 ml.) was treated with 30% hydrogen peroxide (6 ml.) at room temperature overnight. After working up in the usual manner through ether the product was identical with the starting material (infrared comparison).

Action of Chromium Trioxide in Acetic Acid on the Hexanoraldehyde Acetate.- The hexanoraldehyde acetate (900 mg.), purified by careful chromatography, was dissolved in stabilised acetic acid (20 ml.) and titrated with a solution of chromium trioxide (800 mg.) in stabilised acetic acid (50 ml.) until a faint permanent red colour persisted (ca. 20 ml. of the oxidising solution). The reaction mixture was then poured into water (ca. 200 ml.) and worked up through ether to give an acid product (750 mg.) which could not be crystallised. The acid was treated with diazomethane in ether and the non-crystalline methyl ester, isolated in the normal manner, was purified by careful chromatography, $\lambda_{max.}$ 1740 cm.$^{-1}$ (O-acetyl), 1730 cm.$^{-1}$ shoulder (ester carbonyl).

Action of Phenylmagnesium Bromide on the Hexanoracid Methyl Ester.- Magnesium turnings (1 g.) were washed with dry ether and covered with a solution of dry, redistilled bromobenzene (6.5 g.; 4.35 ml.) in dry ether (50 ml.). A crystal of iodine was added to start the reaction and when the vigorous refluxing had ceased a solution of the hexanoracid methyl ester (615 mg.) in dry benzene (20 ml.) was added. The reaction mixture was refluxed (3 hrs.) and then worked up in the normal manner through ether, with steam distillation to remove diphenyl and excess bromobenzene, to give a yellow gum (690 mg.) which was refluxed (2 hrs.) with acetic anhydride (60 ml.) and acetic acid (60 ml.). The product, isolated through ether, was a dark gum (700 mg.) which was filtered through a short column of alumina to remove the colour. The product could not be crystallised. Its ultraviolet absorption spectrum ($\lambda_{max.}$ 2460 Å; ε = 12,500) is similar to that of α-phenylstyrene ($\lambda_{max.}$ 2500 Å; ε = 15,000).

Hydroxylation and Periodic Acid Scission of the Grignard Product.- The product from the above experiment (500 mg.), purified to some extent by careful chromatography, was dissolved in ether (30 ml.) and treated with osmium tetroxide (1 g.) in ether (30 ml.) at room temperature for 5 days. The reaction mixture was then refluxed (3 hrs) with lithium aluminium hydride (1.5 g.) and the product isolated in the usual manner. The resultant gum was dissolved in ethanol (40 ml.) and treated with periodic acid ($HIO_4, 2H_2O$) (500 mg.) in water (0.25 ml.) at room temperature (2 days) in the dark. The reaction mixture was poured into water (200 ml.), extracted with ether, and the ether extract washed with saturated aqueous sodium bicarbonate, 10% aqueous sodium thiosulphate, and water, then dried and evaporated to dryness. The residue could not be crystallised. It gave an oily precipitate on mixing its solution in ethanol with Brady's reagent and showed a strong band at 1710 $cm.^{-1}$ (ketonic carbonyl) in the infrared. The ketone, after careful chromatography, showed no high intensity absorption above 2300 Å in the ultraviolet.

The ketone (350 mg.) was refluxed (70 hrs.) with acetic anhydride (50 ml.) and potassium acetate (1 g.). The reaction mixture was worked up in the normal manner to yield a dark brown gum (350 mg.) which was dissolved in petrol-benzene (5:7, 12 ml.) and chromatographed on alumina (35 g.). The first fraction (70 mg.), a yellow gum eluted with the same solvent mixture (300 ml.), could not be crystallised but it did not give a precipitate with Brady's reagent and showed the following spectroscopic properties: $\lambda_{max.}$ 2060 Å (ε = 33,500), 2460 Å (ε = 10,300); 1740 $cm.^{-1}$ (C-acetyl), 1750 $cm.^{-1}$ shoulder (enol--acetate). Alkaline hydrolysis of the enol-acetate regenerated the non-crystalline ketone; the identity of the substances was established by infrared comparison.

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105. cf. Eck, and Hollingsworth, J.Amer.Chem.Soc., 1942, 64, 140.

106. Anderson, Ph.D. thesis, Glasgow, 1952, pp. 75-95.

107. cf. Lane, and Young, J.Amer.Chem.Soc., 1938, 60, 847.

108. Stevenson, Ph.D. thesis, Glasgow, 1952, p. 84. (For examples of the instability of allylic bromides on alumina v. Sutton, and Dutta, J., 1949, 939; Fukushima, Lieberman, and Praetz, J.Amer.Chem.Soc., 1950, 72, 5205; Keverling Buisman, Stevens, and van der Vliet, Rec.Trav.chim., 1947, 66, 83; and for other changes caused by alumina v. Lederer, and Lederer, "Chromatography," Elsevier, 2nd English Edition, Amsterdam, 1957, pp. 61-65.

109. Information concerning ultraviolet absorption spectra was obtained from: Gillam, and Stern, "An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry," 2nd Edition, Edward Arnold Ltd., London, 1958; Friedel, and Orchin, "The Ultraviolet Absorption Spectra of Aromatic Compounds," John Wiley and Sons, Inc., New York, 1951; and from: Harshenson, "Ultraviolet and Visible Absorption Spectra,

Index for 1930-1954," Academic Press, New York, 1956.

110. Information concerning infrared absorption spectra was obtained from: Jones, and Sandorfy, "Technique of Organic Chemistry;" Vol. IX, "Chemical Applications of Spectroscopy;" Chap. IV, "The Application of Infrared and Raman Spectrometry to the Elucidation of Molecular Structure," Interscience, New York, 1956, pp. 247-563; and from: Randall, Fowler, Fuson, and Dangl, "Infrared Determination of Organic Structures," van Nostrand, New York, 1949.

111. Barton, J., 1945, 813.

112. cf. Shoppee, et al., J., 1952, 1790; and many subsequent papers.

113. Nes, Scheer, and Smeltzer, J.Amer.Chem.Soc., 1955, 77, 3300.

114. Colthup, J.Optical Soc., 1950, 40, 397.

115. cf. Hosenski, Moore, and Wintersteiner, J.Amer.Chem.Soc., 1953, 75, 2781.

116. cf. Ref. 19.

117. Castells, and Fletcher, J., 1956, 3245.

118. Kharasch, Tigerman, and Wehrmeister, J.Amer.Chem.Soc., 1947, 69, 1612; Buess, and Kharasch, ibid., 1949, 71, 2724.

119. Priester, Riechstoffind, 1930, 5, 83, 108 (Chem.Abs., 1931, 25, 1632).

120. Barton, Head, and May, J., 1957, 935.

Part II

THE CHEMISTRY OF AESCIGENIN

HISTORICAL REVIEW

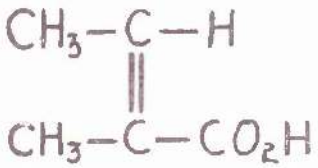
The earliest recorded chemical study of the seeds of the horse chestnut (Aesculus hippocastanum L.) is that of Rochleder[1] who, in 1867, isolated a saponin, aescin, from which he obtained the sapogenin, aescigenin. Rochleder assigned the formula $C_{28}H_{40}O_4$ to aescigenin. Later workers,[2] who studied both aescin and aescigenin, produced conflicting results which van der Haar[3] attributed to the fact that aescin can be fully hydrolysed only with difficulty. van der Haar described a bromo-substitution product, m.p. 167-175°, and expressed the opinion that aescigenin is a triol, $C_{21}H_{31}O(OH)_3$, m.p. 311°; $[\alpha]_D$ + 35.28° (in acetic acid).

In 1931 Winterstein[4] described the isolation of pure aescin as a white crystalline powder from which, by a partial hydrolysis in aqueous mineral acid, he prepared a mixture of prosapogenins A and B. By repeated precipitation of this mixture, with aqueous sodium hydroxide, from its solution in dilute alcoholic sulphuric acid, followed by charcoal treatment, he obtained a white powder which when dissolved in alcohol and precipitated with ether, in five cases out of one hundred yielded crystalline prosapogenin B, m.p. 220-230° (decomp.). Like aescin, the prosapogenins are sternutatory. Winterstein prepared aescigenin by prolonged acid hydrolysis of the prosapogenins and prepared a bromo-derivative, m.p. 196-197°. He proposed the formula $C_{35}H_{58}O_7$ for aescigenin, m.p. 309°; $[\alpha]_D$ + 27° (in alcohol), which, he stated, is a pentahydroxy alcohol and yields tiglic acid (I) after prolonged digestion with alcoholic potassium hydroxide. Winterstein therefore represented aescigenin

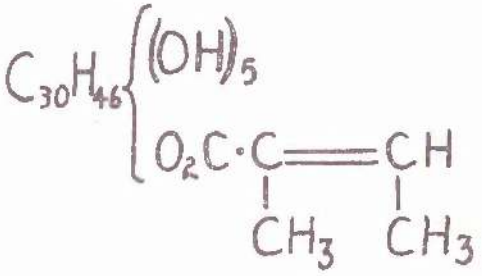
by the partial formula (II).

Further confusion arose when Bures and Volak[5] reported that aescigenin forms a potassium salt, a tetra-acetate, and a tris-phenyl-hydrazone. These workers preferred the formula $C_{32}H_{54}(CO)_3(OH)_4$ for aescigenin.

Janett, Rey, and Ruzicka[6] prepared what they described as aescigenin penta-acetate, $C_{45}H_{66}O_{11}$, and, on the basis of their analyses, they proposed the formula $C_{35}H_{56-58}O_6$ for aescigenin. Since the ultraviolet absorption spectra of aescigenin and of its acetate show a weak band at 2800 Å the Swiss workers suggested that the inert oxygen atom is present in a hindered carbonyl group,



(I)



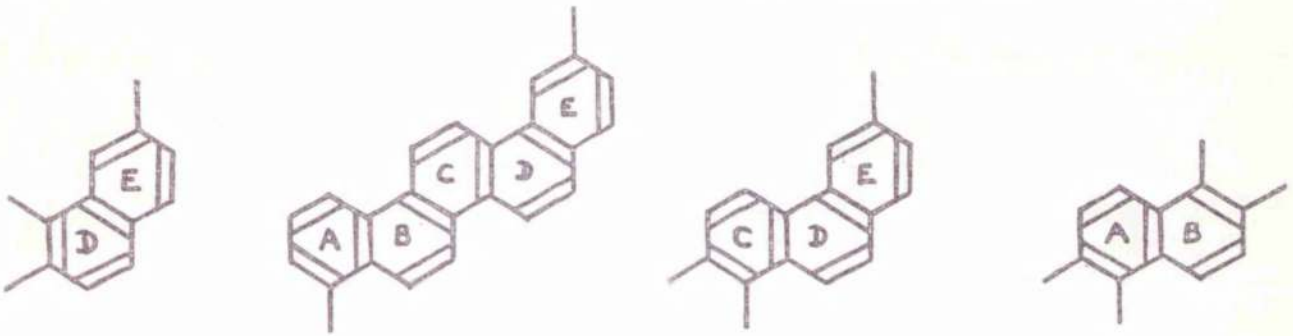
(II)

but it was also pointed out that it might be an ether oxygen. The formation of a yellow colour with tetranitromethane indicated the presence of a double bond in aescigenin. Confirmation of this point was obtained by oxidising aescigenin acetate, with chromium trioxide in acetic acid, to an α:β-unsaturated ketone, $C_{45}H_{64}O_{12}$.

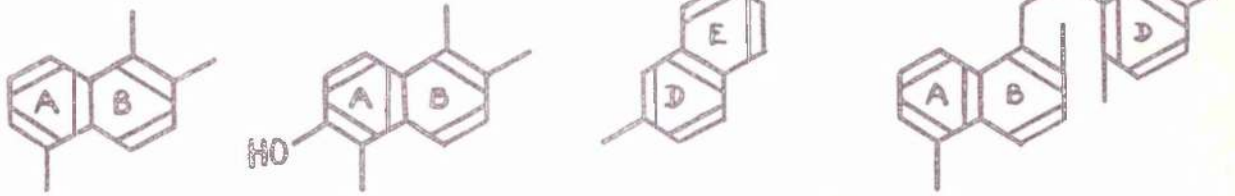
Prolonged refluxing of aescigenin with ethanolic hydrochloric acid was shown, by Hofer and Janett,[7] to give a substance $C_{30}H_{46}O_5$ (later shown to be isoaescigenin[8]) which forms a penta-acetate, $C_{40}H_{58}O_{10}$. Baumgartner, Prelog, and Ruzicka[9] reported that, despite careful examination of the products from this reaction, no other substance could be isolated. These workers also noted that their

analytical data for the C_{35} formula agreed equally well with the formula $C_{30}H_{48-50}O_5$ for aescigenin. Careful ebullioscopic determination of the molecular weight of aescigenin acetate then showed that it is in fact $C_{38}H_{56-58}O_9$, the tetra-acetate of aescigenin, $C_{30}H_{48-50}O_5$.

The first direct evidence concerning the triterpenoid[10] nature of aescigenin was provided by Ruzicka and van Veen[11] who showed that selenium dehydrogenation of this substance leads to the formation



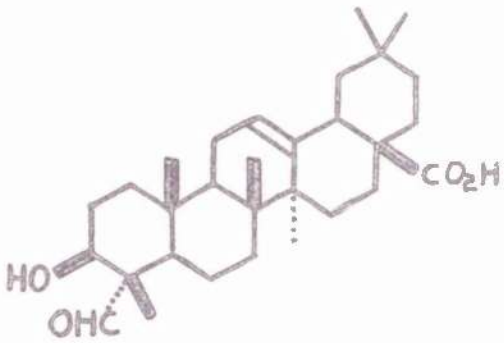
(III) (IV) (V) (VI)



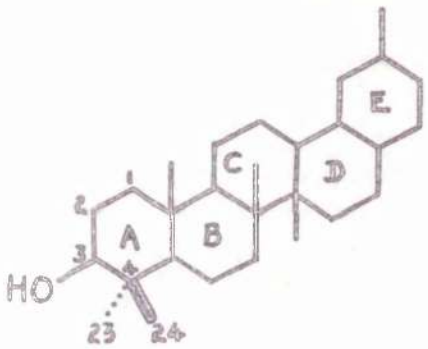
(VII) (VIII) (IX) (X)

of sapotalin (1:2:7-trimethylnaphthalene) (III), a characteristic dehydrogenation product of the pentacyclic triterpenes.[12] Baumgartner, Prelog, and Ruzicka[9] later studied this experiment in more detail and although they were unable to confirm the formation of sapotalin (III), they did isolate and characterise the following compounds which are also characteristic dehydrogenation products of the pentacyclic triterpenes: 1:8-dimethylpicene (IV), 1:2:6-trimethylphenanthrene (V), 1:2:5:6-tetramethylnaphthalene (VI), 1:2:5-tri-

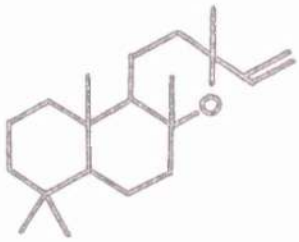
methylnaphthalene (VII), 1:5:6-trimethyl-β-naphthol (VIII), and 2:7-dimethylnaphthalene (IX). They were also able to isolate a hydrocarbon, $C_{26}H_{26}$, which has since been identified[13] as the tetramethyldinaphthylethane (X). This latter compound is not a typical product of the reaction but a probably identical hydrocarbon has been obtained by dehydrogenation of gypsogenin (XI).[13,14] On the basis of these experiments it was concluded[9] that aescigenin most probably incorporates the partial structure (XII), together



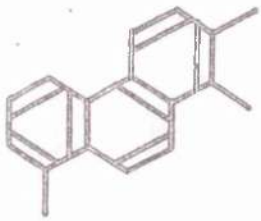
(XI)



(XII)



(XIII)



(XIV)

with two methyl groups, four oxygen functions, and an ethylenic linkage. A tetracarbocyclic skeleton, although less probable, was not excluded since dehydrogenation of terpene rings can sometimes cause ring closure at a new position, e.g. manoyl oxide (XIII) on dehydrogenation with selenium gives 1:2:8-trimethylphenanthrene (XIV).[15]

The presence of an ethylenic linkage in aescigenin, which had been indicated by the formation of a yellow colour with tetra-

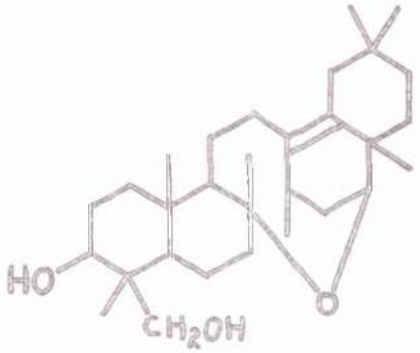
nitromethane, by the preparation of an α:β-unsaturated ketone from aescigenin tetra-acetate, and by end absorption in the ultra-violet,[6] was confirmed by the preparation of an oxide, $C_{38}H_{56}O_{10}$, which is saturated to tetranitromethane, by the action of monoperphthalic acid on aescigenin tetra-acetate.

Ruzicka and his co-workers[9] deduced that aescigenin contains four hydroxyl groups, which are either primary or secondary, since four of its five oxygen functions are readily esterified and since a Zerewitinoff estimation indicated the presence of four active hydrogen atoms. Furthermore, since aescigenin forms a bis-ethylidene derivative, $C_{32}H_{54}O_5$, and a bis-benzylidene derivative, $C_{44}H_{56}O_5$, neither of which possesses active hydrogen, it was assumed that the hydroxyl functions are present in two groups with each pair forming a 1:2- or a 1:3-glycol system. The formation of the naphthol (VIII) on selenium dehydrogenation of aescigenin indicated that one hydroxyl group is at C_3 and that another will be at either C_1, C_2, C_{23}, or C_{24}.

The elucidation of the function of the fifth oxygen atom in aescigenin presented some difficulty, but a decision was eventually made[8,9] with the aid of both infrared and chemical methods. The infrared spectrum of aescigenin in a Nujol mull shows no carbonyl absorption but in this spectrum, and in that of aescigenin tetra-acetate in carbon tetrachloride solution, there is a moderately strong band in the 1110 cm.$^{-1}$ region indicative of the presence of the group C-O-C. In the course of the examination described below[8] it was shown that this ether oxygen atom is situated in an oxide ring. Aescigenin, $C_{30}H_{48}O_5$, is therefore an unsaturated, pentacarbocyclic, tetrahydroxy triterpene with an oxide ring. From the formula $C_{30}H_{50}O_5$,

which could not be excluded, it would follow that aescigenin is a tetracarbocyclic compound. The only other naturally occurring triterpene ether known is soyasapogenol-D which Jeger, Meyer, and Ruzicka[16] have suggested is represented by (XV).

A Zeisel estimation on aescigenin gave a negative result, thus excluding the possibility that an alkoxyl group with a small alkyl group, such as methyl or ethyl, might be present. In order to decide whether two large fragments are linked through the ether oxygen atom, or whether an oxide ring is present, aescigenin tetra--acetate was treated with a series of ether splitting reagents.



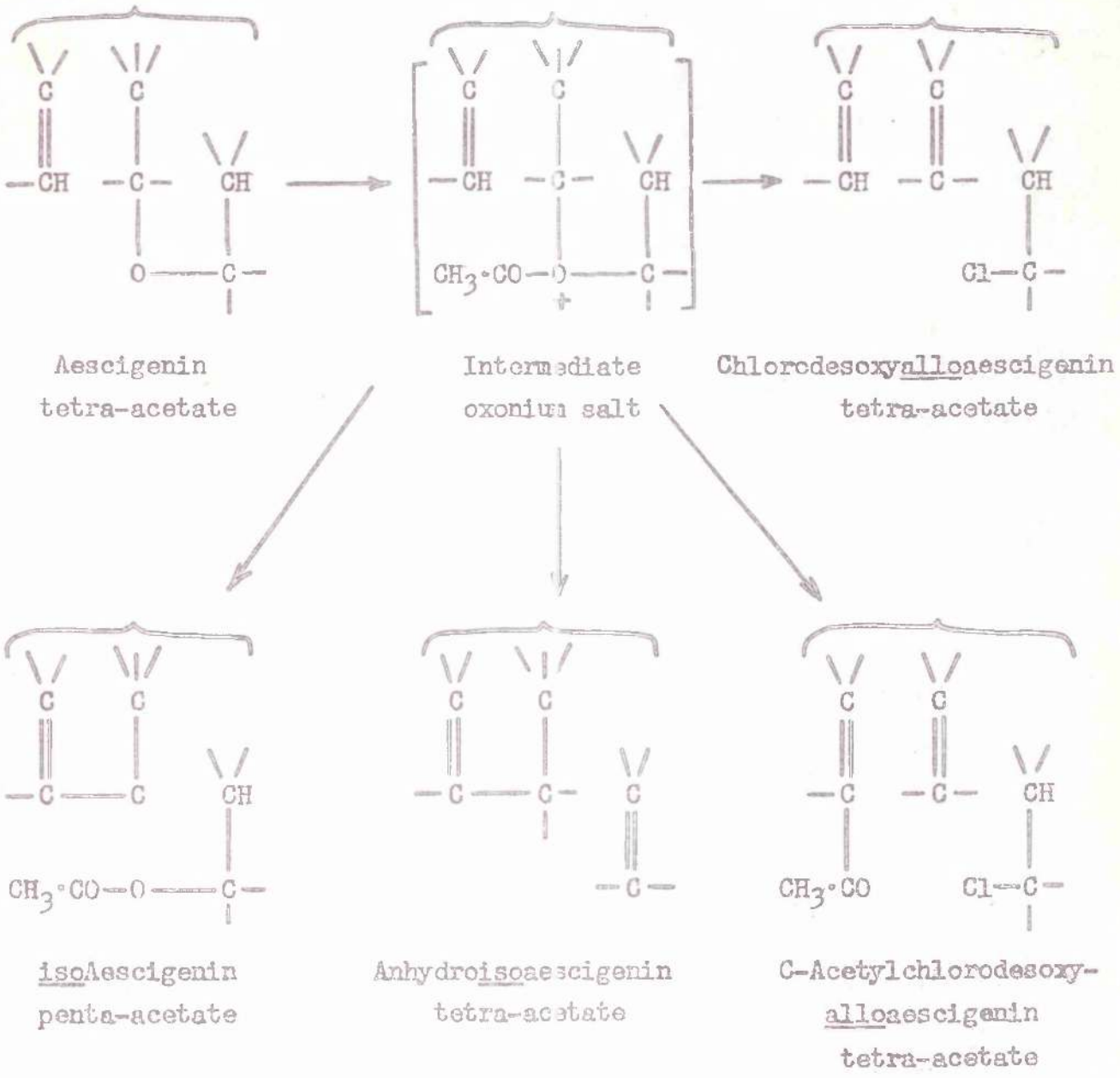
(XV)

After treatment with acetyl chloride and zinc chloride, acetyl chloride and aluminium chloride, acetic anhydride and boron trifluoride, or with acetic anhydride and p-toluenesulphonic acid, several reaction products were obtained which no longer possessed an ether link, as was shown by their infrared absorption spectra. These substances were classified, on the basis of their properties, in two groups which were named the iso-series and the allo-series. isoAescigenin contains one double bond and was assumed to be hexacarbocyclic, while alloaescigenin is pentacarbocyclic with two conjugated double bonds. Since all of the original thirty carbon atoms are present in both these series of compounds, the ether oxygen atom of aescigenin must be present in an oxide ring.

Two members of the iso-series were prepared from aescigenin tetra-acetate, isoaescigenin penta-acetate, $C_{40}H_{58}O_{10}$, and a tetra--acetate, $C_{38}H_{54}O_8$, which differs from isoaescigenin penta-acetate in that the derived alcohol has formally lost the elements of water, and which Ruzicka and his co-workers[8] named anhydroisoaescigenin tetra-acetate. This anhydro-compound was obtained in poor yield and was not examined in detail. isoAescigenin penta-acetate was the major product from all the ether splitting reactions, and isoaescigenin itself was obtained in low yield by prolonged refluxing of aescigenin with ethanolic hydrochloric acid. isoAescigenin contains five hydroxyl groups and its penta-acetate gives a monoxide, $C_{40}H_{58}O_{11}$, which is saturated to tetranitromethane, on treatment with monoperphthalic acid. There is thus only one double bond in isoaescigenin which must therefore be hexacarbocyclic. The double bond in isoaescigenin cannot be reduced catalytically. When isoaescigenin penta-acetate is oxidised by chromium trioxide in acetic acid the product is an $\alpha:\beta$-unsaturated ketone, $C_{40}H_{56}O_{11}$, m.p. 324-326°; $[\alpha]_D$ - 8° ± 2°, which the Swiss workers claim to be identical with the product, m.p. 337°; $[\alpha]_D$ - 9° ± 2°, obtained by treatment of ketoaescigenin tetra-acetate with acetyl chloride and zinc chloride. They therefore concluded that the ethylenic linkage in isoaescigenin is in the same position as that in aescigenin.

The simplest member of the allo-series was obtained in low yield by the action of acetyl chloride and zinc chloride on aescigenin tetra-acetate. It is a chloro-compound, $C_{38}H_{55}O_8Cl$, which was named chlorodesoxyalloaescigenin tetra-acetate, and since its ultraviolet absorption spectrum shows a high intensity maximum at 2480 Å it was assumed to be a conjugated diene. Two other

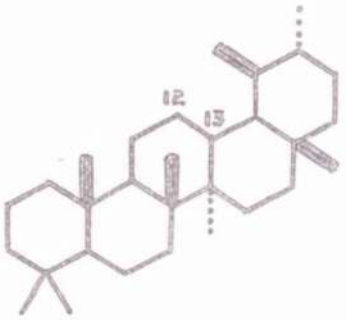
members of the allo-series were obtained from aescigenin tetra-acetate; C-acetylchlorodesoxyalloaescigenin tetra-acetate, $C_{40}H_{57}O_9Cl$, was prepared by means of acetyl chloride and aluminium chloride, and C-acetylalloaescigenin penta-acetate, $C_{42}H_{60}O_{11}$, was prepared by means of either acetyl chloride and zinc chloride or acetic anhydride and boron trifluoride. Both compounds give a positive Legal test for the methyl ketone group and show strong absorption in the ultraviolet at 2900 Å which the Swiss workers attribute to the presence of the



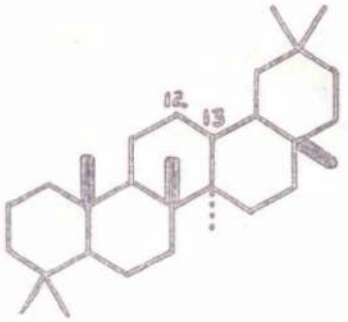
group $CH_3 \cdot CO-C=C-C=C$. These compounds apparently differ only by replacement of the chlorine atom of the first by the acetoxyl group

of the second. On the assumption that no double bond migration or retropinacol rearrangements take place in the ether splitting reactions, Ruzicka and his co-workers[8] represented these reactions as shown in the scheme on page 94.

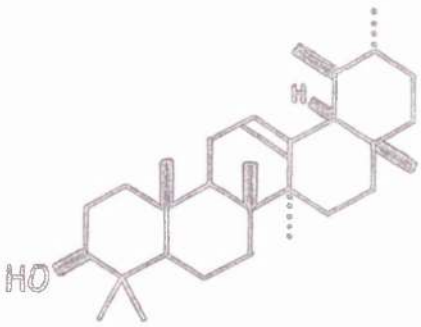
C-Acetylchlorodesoxyalloaescigenin tetra-acetate could be hydrogenolysed to give a product showing a strong absorption maximum at 2520 Å and giving a negative Legal test and in which



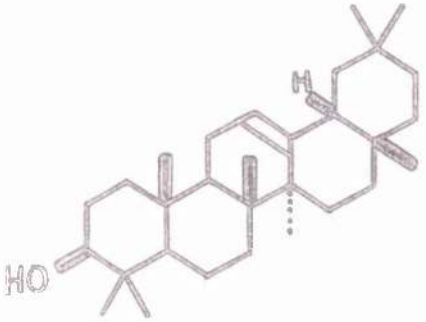
(XVI)



(XVII)



(XVIII)

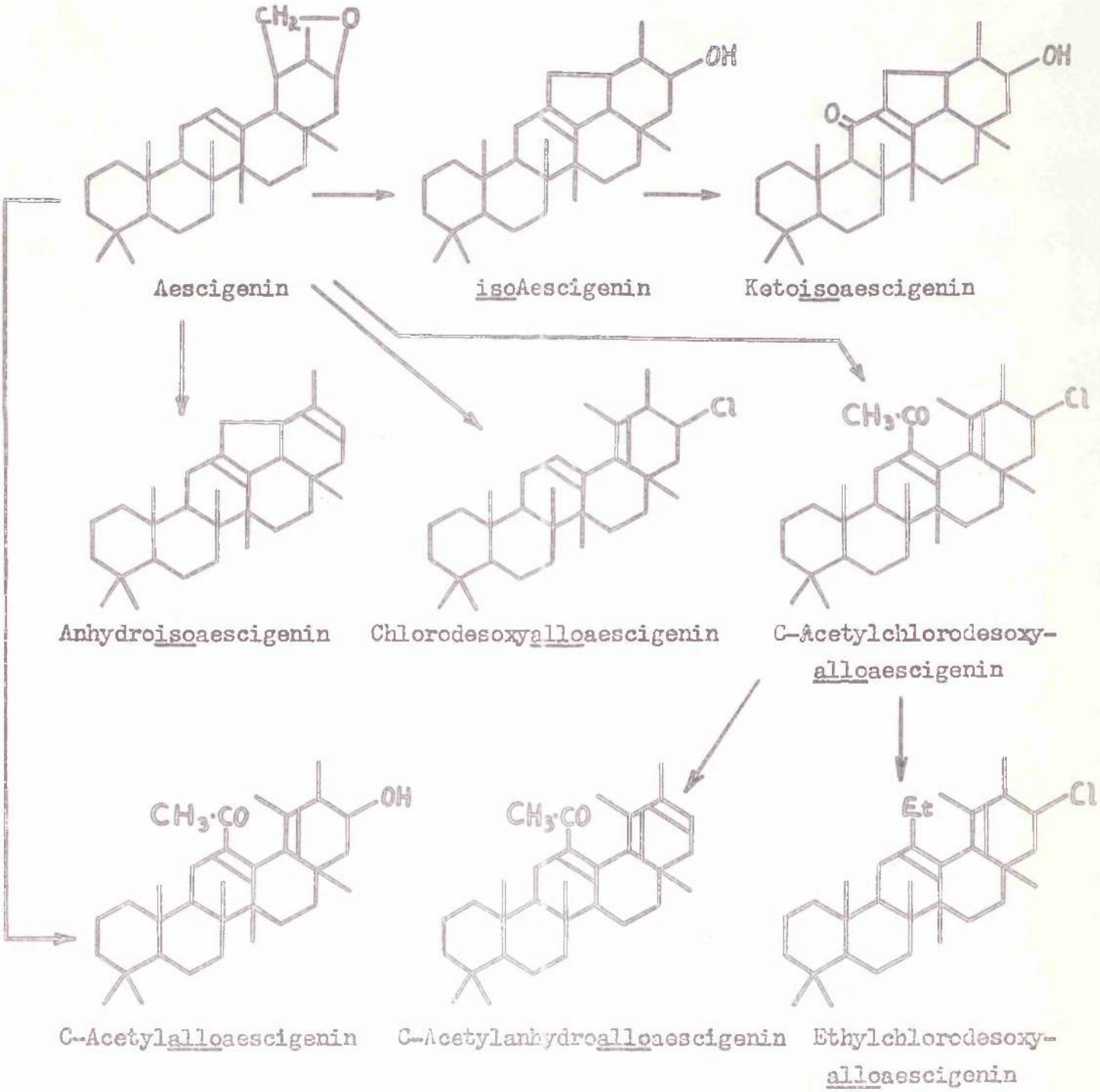


(XIX)

the diene system was retained. When C-acetylchlorodesoxyalloaescigenin tetra-acetate was refluxed with methanolic potassium hydroxide and the product was reacetylated, C-acetylanhydroalloaescigenin tetra-acetate, $C_{40}H_{56}O_9$, was obtained. This compound gave a positive Legal test and showed a high intensity absorption maximum in its ultraviolet spectrum at 3100 Å. The system $CH_3 \cdot CO-C=C-C=C-C=C$ had apparently been formed by elimination of the elements of hydrogen chloride.

If the ether splitting reactions are to be represented as in the

scheme shown on page 94, then the double bond in aescigenin must be in spatial proximity to the ether link. The Swiss workers[8] assumed that the double bond in aescigenin is in the same position (between C_{12} and C_{13} of the pentacyclic skeleton) as the relatively



Aescigenin — isoAescigenin — Ketoisoaescigenin

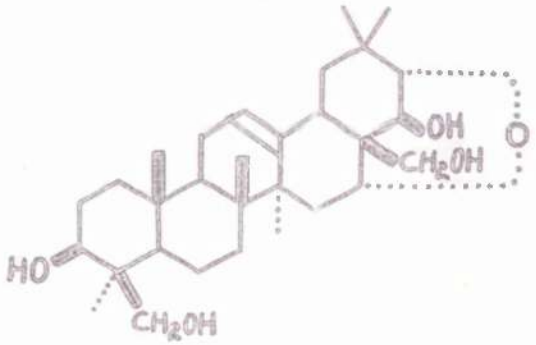
Anhydroisoaescigenin — Chlorodesoxyalloaescigenin — C-Acetylchlorodesoxy-alloaescigenin

C-Acetylalloaescigenin — C-Acetylanhydroalloaescigenin — Ethylchlorodesoxy-alloaescigenin

unreactive double bond of the triterpenes of the ursane (XVI) and oleanane (XVII) series. Assuming that aescigenin is a derivative of either α-amyrin (XVIII) or β-amyrin (XIX), it is not possible to write

a satisfactory structure unless it is supposed that in the formation of the aforementioned products double bond migrations and/or retropinacol rearrangements have taken place. If this is so then the number of possible structures is very large, but as a working hypothesis Ruzicka and his co-workers suggested the partial structures shown on page 96 for aescigenin and its reaction products.

During the course of the work described in this thesis, Arigoni, Cainelli, Melera, and Jeger[17] published the continuation of the studies of the Swiss school, which are described below, and in which they established the structure of aescigenin as 16α:21α-epoxy-

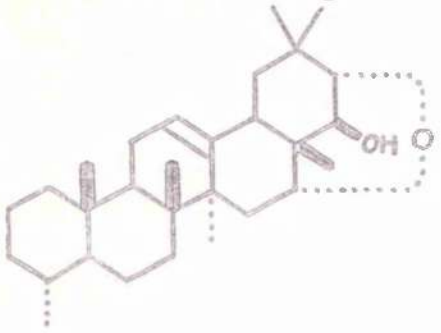


(XX)

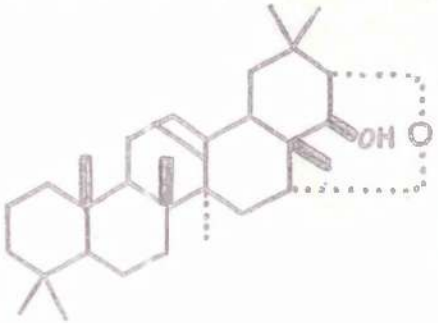
olean-12-ene-3β:22β:24:28-tetrol (XX).

When aescigenin (XX) was oxidised by means of the chromium trioxide-pyridine complex and the crude product was reduced by the Wolff-Kishner method, a mixture of the C_{29} alcohol (XXI) and the C_{30} alcohol (XXII) was obtained. The hydroxyl group in both of these alcohols is unusually stable to oxidising agents but the ketones (XXIII) and (XXIV) were obtained in low yield from their respective alcohols by treatment with chromium trioxide in acetone and sulphuric acid. The infrared spectra of these ketones show an anomalous absorption band at 1765 $cm.^{-1}$. That this absorption is not due to the presence of a γ-lactone grouping was shown by reducing the ketone (XXIII), with lithium aluminium hydride, to the monohydric

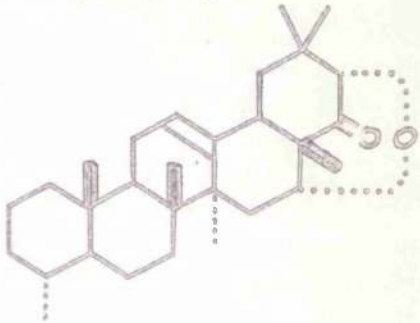
alcohol (XXV) which was reoxidised to the ketone (XXIII). Jeger and his co-workers[17] attribute this absorption to the presence of a carbonyl group in a five membered oxide ring, as in the partial formula (XXVI). In agreement with this hypothesis,[18] it was found that the ether ring in the ketones (XXIII) and (XXIV) was readily



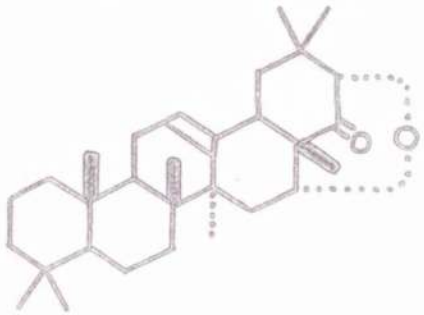
(XXI)



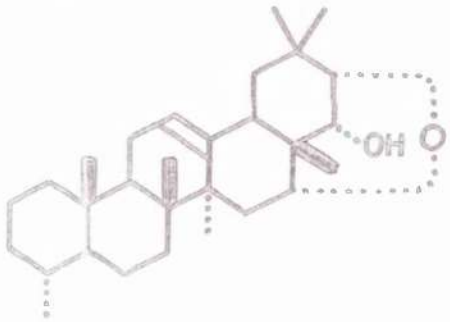
(XXII)



(XXIII)

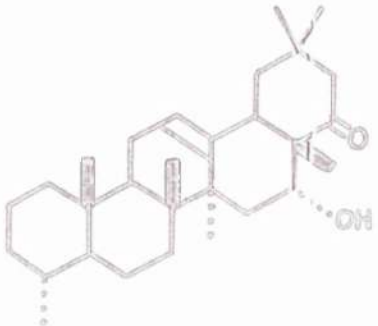


(XXIV)

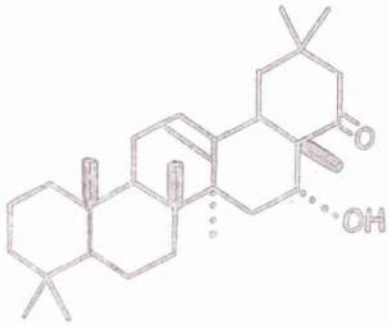


(XXV)

(XXVI)



(XXVII)



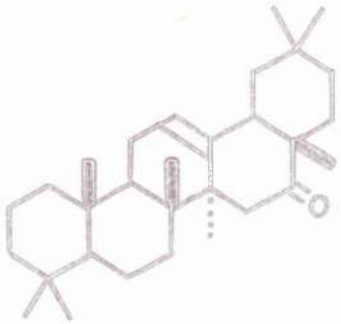
(XXVIII)

opened by means of aluminium amalgam in dry ether to yield the keto-alcohols (XXVII) and (XXVIII) respectively, the infrared absorption spectra of which show a strong band at 1695 cm.$^{-1}$ due to the presence of a carbonyl group in a six membered ring.

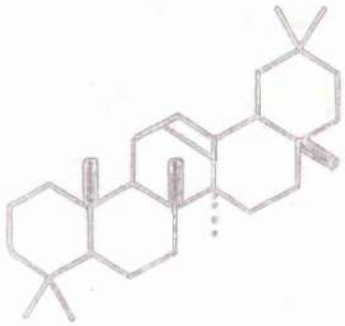
When the keto-alcohol (XXVIII) was reduced by the Wolff-Kishner method, and the crude product was oxidised by the chromium trioxide-

-pyridine complex, the ketone (XXIX) was obtained. Wolff-Kishner reduction of this ketone gave olean-12-ene (XXX), thus establishing the nature of the carbon skeleton of aescigenin and the position of the double bond.

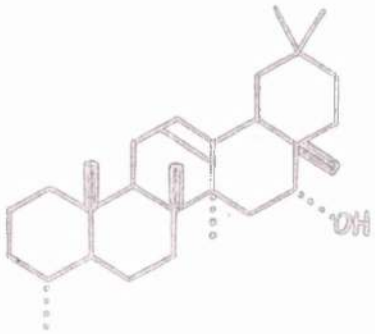
Wolff-Kishner reduction of the C_{29} keto-alcohol (XXVII) gave the amorphous alcohol (XXXI) which was oxidised by the chromium trioxide-pyridine complex to the ketone (XXXII). Since this ketone



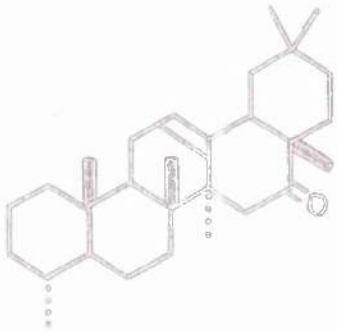
(XXIX)



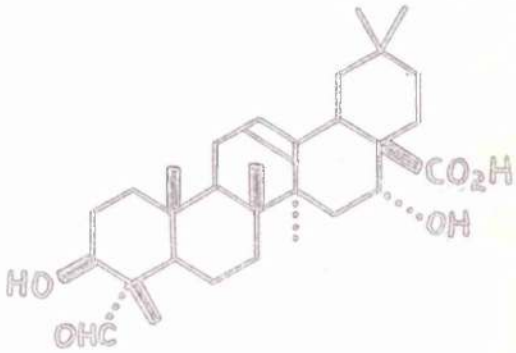
(XXX)



(XXXI)



(XXXII)

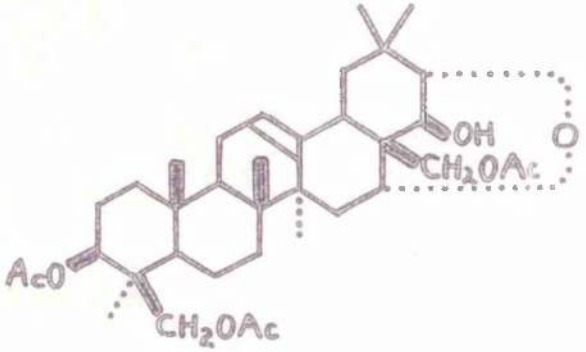


(XXXIII)

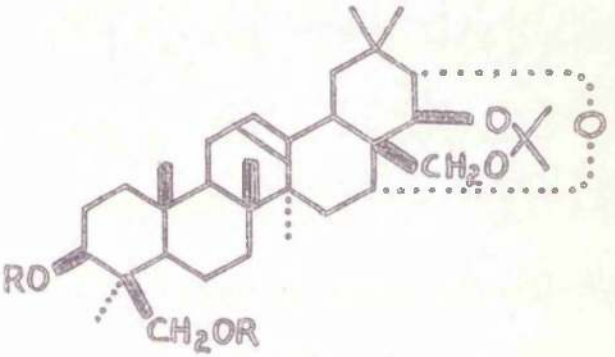
is identical with a known derivative[19] of quillaic acid (XXXIII), one end of the oxide link must be at C_{16} in aescigenin.

Partial hydrolysis of aescigenin tetra-acetate gave the tetrol-triacetate (XXXIV), treatment of which with acetone in the presence of sulphuric acid yielded the monoacetonide-diacetate (XXXV). Alkaline hydrolysis of this diacetate gave the monoacetonide (XXXVI) which was also obtained, together with the diacetonide (XXXVII), directly from aescigenin. The tetrol-monoacetonide (XXXVI) was

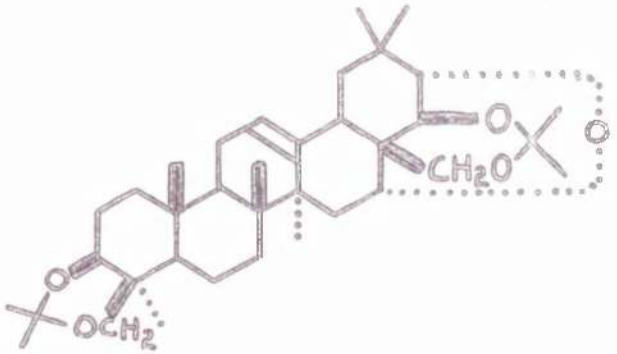
oxidised by means of the chromium trioxide-pyridine complex to a mixture of the keto-aldehyde (XXXVIII) and the keto-acid (XXXIX). Both of these compounds gave the nor-ketone (XL) on heating; indicating the presence of a β-keto-aldehyde group, and a β-keto-acid group, respectively.



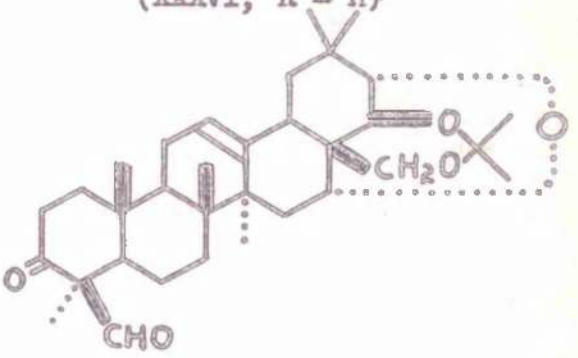
(XXXIV)



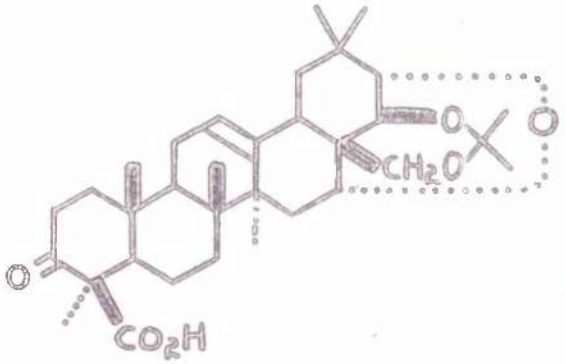
(XXXV; R = Ac)
(XXXVI; R = H)



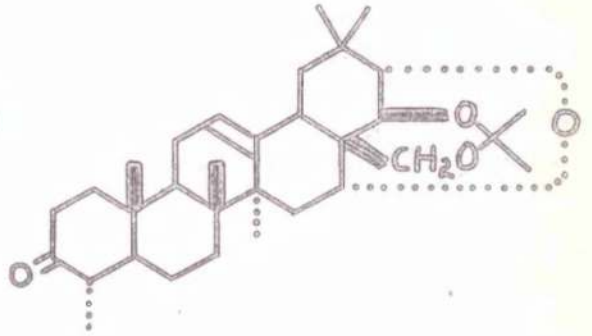
(XXXVII)



(XXXVIII)



(XXXIX)

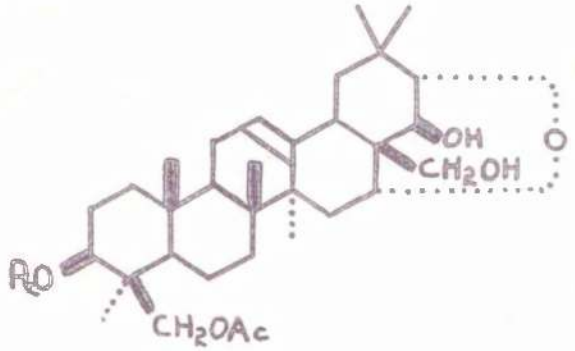


(XL)

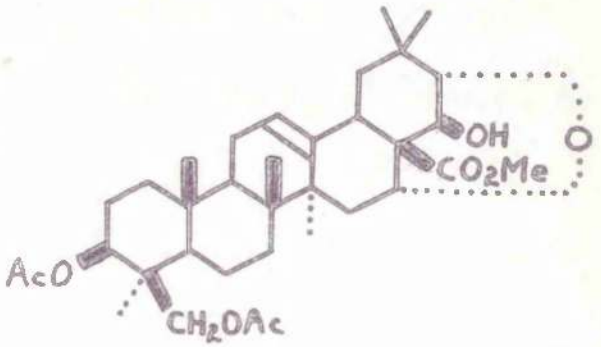
Mild acid hydrolysis of the monoacetonide-diacetate (XXXV) gave the tetrol-diacetate (XLI) which, after oxidation by means of chromium trioxide in acetone and sulphuric acid followed by esterification, yielded a mixture of the hydroxy-ester (XLII) and

the non-enolic β-keto-ester (XLIII). That the compound (XLIII) is a β-keto-ester was shown by refluxing with alkali, then esterifying and acetylating the product, to give the diacetate-dimethylester (XLIV).

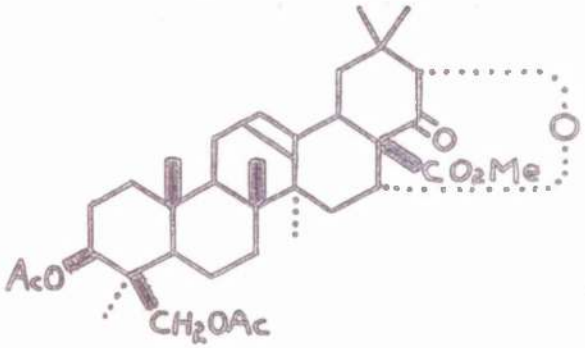
Both pairs of hydroxyl groups in aescigenin must thus be present as 1:3-, primary-secondary, glycol systems, and since the naphthol (VIII) was isolated from the selenium dehydrogenation products,[9] one of the hydroxyl groups must be at C_3 and another at



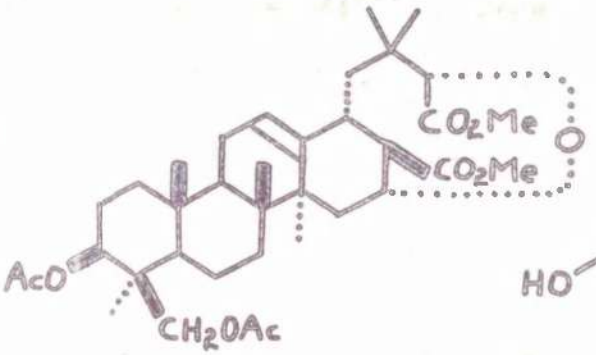
(XLI)



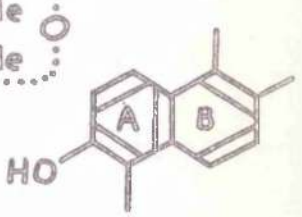
(XLII)



(XLIII)



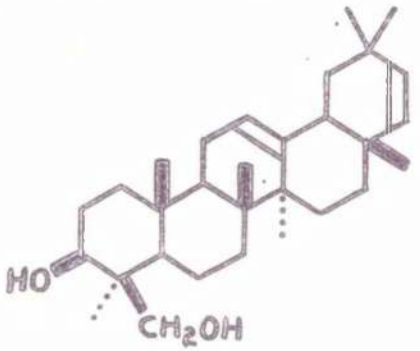
(XLIV)



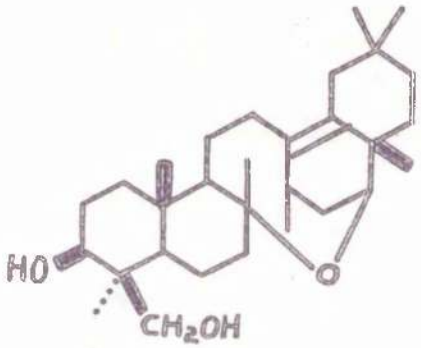
(VIII)

C_{23} or C_{24}. Since the secondary hydroxyl group of the remaining glycol system is in the α-position to the ether link, and since one end of the ether link is at C_{16}, a hydroxyl group was placed at C_{22} and another at C_{28} with the oxide bridge from C_{16} to C_{21}. Lithium aluminium hydride reduction of the keto-aldehyde (XXXVIII) regenerated the monoacetonide (XXXVI), having the natural configuration at C_3. The C_3 hydroxyl group is, therefore, β-orientated. The

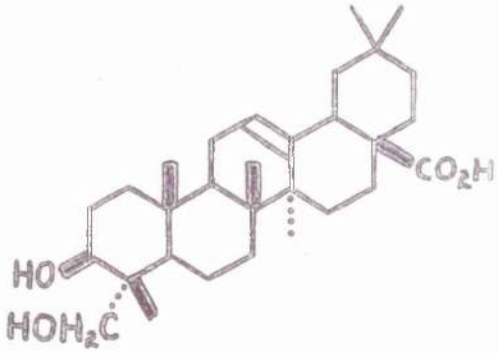
ring A hydroxymethyl group was shown to be at C_{24} by comparison of the molecular rotation difference between the monoacetonide-diacetate (XXXV) and the corresponding diol (XXXVI) with that of known ring A diols. This molecular rotation difference for aescigenin is similar to those for the soyasapogenols C, (XLV),[20] and D, (XV),[21] (3β:24-diols), but differs from those of hederagenin (XLVI)[22] (a 3β:23-diol) and of β-boswellendiol (XLVII)[23] (a 3α:24-diol).



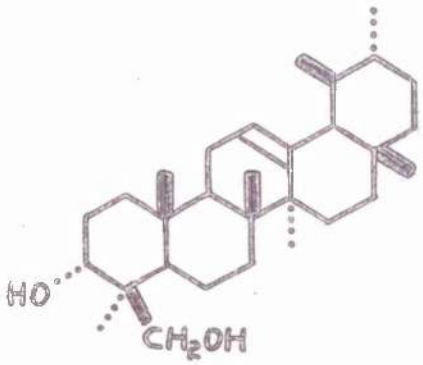
(XLV)



(XV)



(XLVI)

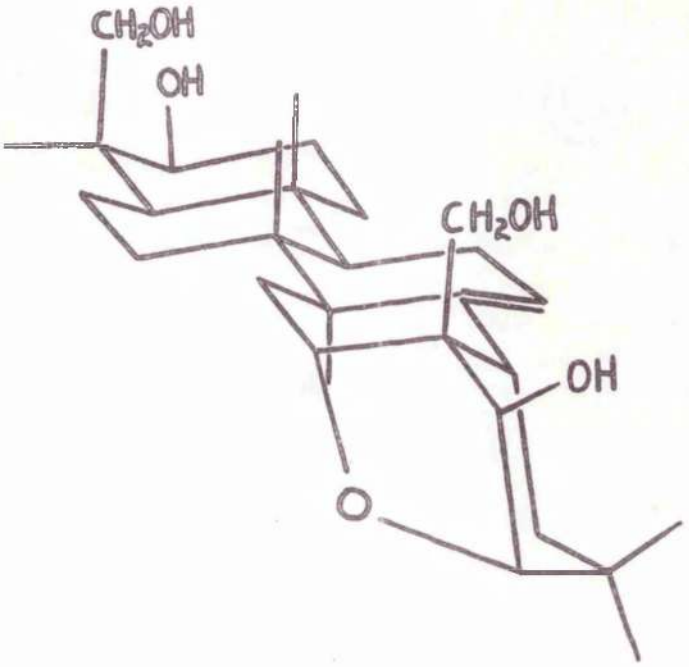


(XLVII)

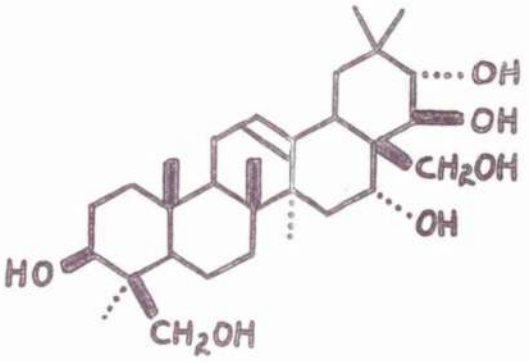
The C_{22} hydroxyl group must have the β-configuration since if it were α it would not be able to participate in ketal or acetal formation with the C_{28} hydroxyl group. The α-configuration for the oxide bridge is the only one which is sterically possible. Aescigenin must, therefore, be fully represented by the structure (XX).

Jeger and his co-workers[17] pointed out that aescigenin may

not be the true aglycone of aescin, because of the vigorous acid treatment which is necessary to hydrolyse the glycoside. It was



(XX)



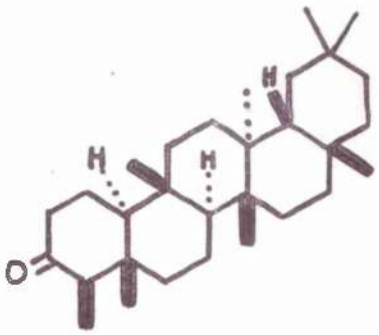
(XLVIII)

suggested that a possible precursor might be the hypothetical olean-12-en-3β:16α:21α:22β:24:28-hexol (XLVIII).

DISCUSSION

The Non-Saponifiable Fraction of Horse Chestnut Seed Oil

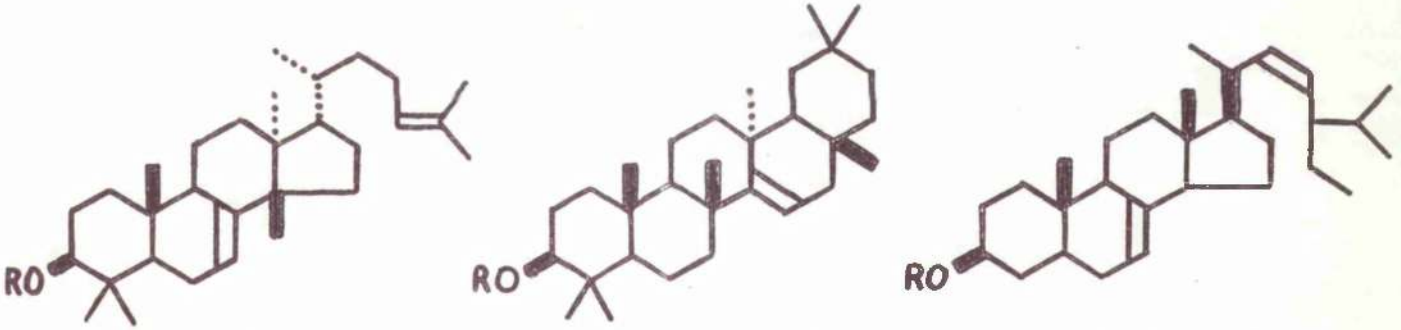
Although the advantage of defatting horse chestnut seeds with petrol, before extracting the glycoside, has been noted by van der Haar[3] and by Winterstein,[4] there is no record of an examination of the constituents of the non-saponifiable fraction of this fat. The material extracted from the seeds by petrol is a dark green mobile oil (2-3% of dry seeds) which when refluxed with methanolic potassium hydroxide



(XLIX)

yielded a non-saponifiable fraction (5% of oil) as an orange, waxy solid. Chromatography of a petrol solution of the non-saponifiable material, on alumina, gave three main fractions. The first of these fractions was readily eluted with petrol and crystallised from ethyl acetate in colourless waxy plates, $C_{30}H_{62}$, the infrared absorption spectrum of which was identical with that of n-triacontane, m.p. and mixed m.p. 60-62°. The second fraction was eluted from the column with benzene-ether mixtures and crystallised from methanol in colourless needles, $C_{30}H_{50}O$, the infrared absorption spectrum of which was identical with that of friedelin (XLIX),[24] m.p. and mixed m.p. 260-262°. Corks were not used at any time during this work.

The third fraction, which consisted of all the material eluted with ether and methanol, was acetylated and a petrol-benzene solution of the mixed acetates was chromatographed on alumina. Three main fractions were obtained, all of which were eluted from the column with petrol-benzene mixtures. The first of these fractions crystallised from chloroform-methanol in colourless needles, $C_{32}H_{52}O_2$, the infrared absorption spectrum of which was identical with that of butyrospermyl acetate (L),[25] m.p. and mixed m.p. 140-142°. Alkaline hydrolysis of the acetate gave butyrospermol (LI), m.p. and mixed m.p. 109-110°. The second fraction was crystallised



(L; R = Ac)
(LI; R = H)

(LII; R = Ac)
(LIII; R = H)

(LIV; R = Ac)
(LV; R = H)
(LVI; R = Ph·CO)

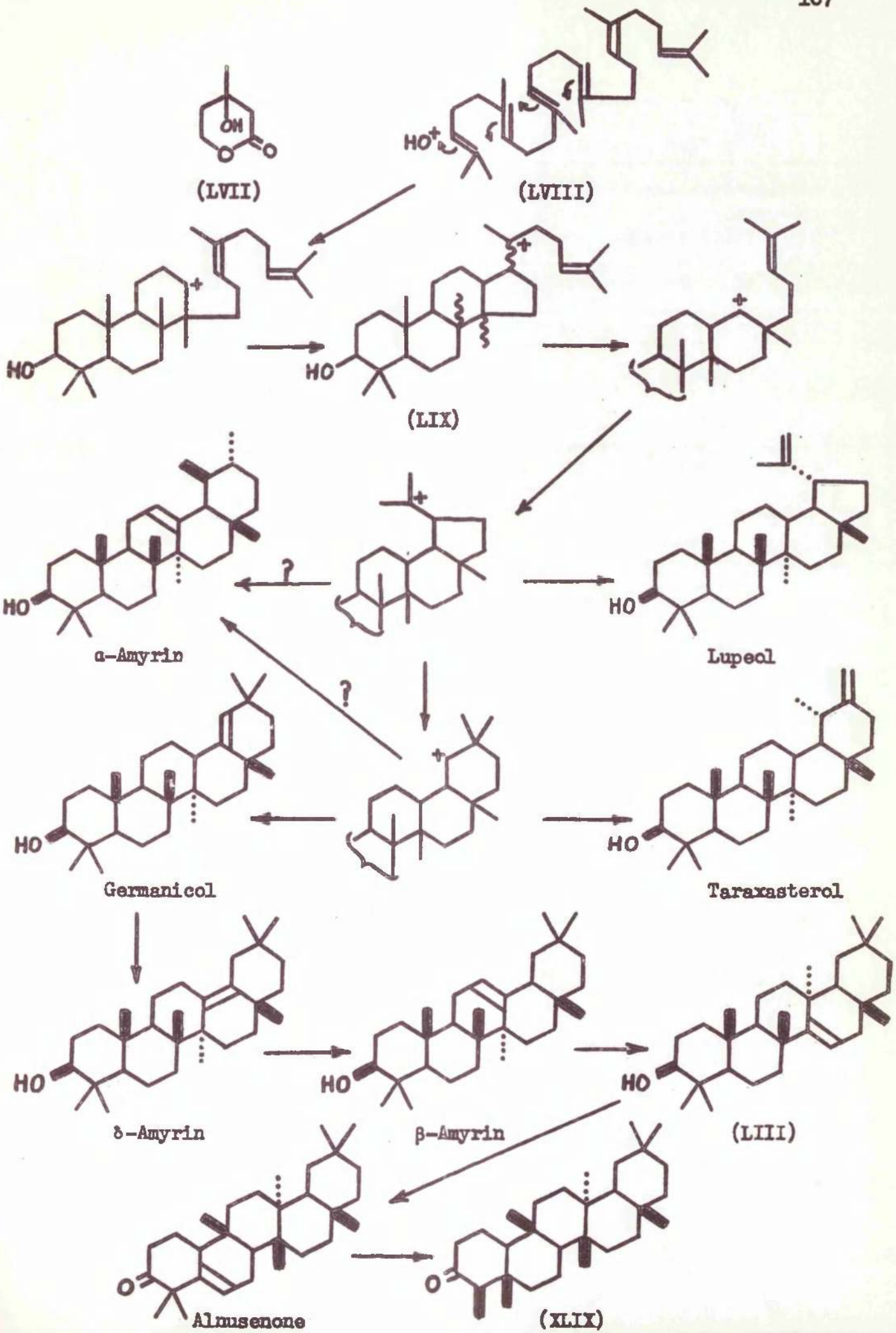
from chloroform-methanol in colourless matted needles, $C_{32}H_{52}O_2$, and was shown to be taraxeryl acetate (LII)[26] by the identity of its infrared absorption spectrum with that of an authentic specimen, m.p. and mixed m.p. 300-302°. Alkaline hydrolysis of the acetate yielded taraxerol (LIII), m.p. and mixed m.p. 270-271°. The third fraction was crystallised from chloroform-methanol in colourless plates, $C_{31}H_{50}O_2$, the infrared absorption spectrum of which was identical with that of α-spinasteryl acetate (LIV),[27] m.p. and mixed m.p. 175-177°. Alkaline hydrolysis of the acetate gave α-spinasterol (LV), m.p. and mixed m.p. 174°, benzoylation of

which yielded α-spinasteryl benzoate (LVI), m.p. and mixed m.p. 200°.

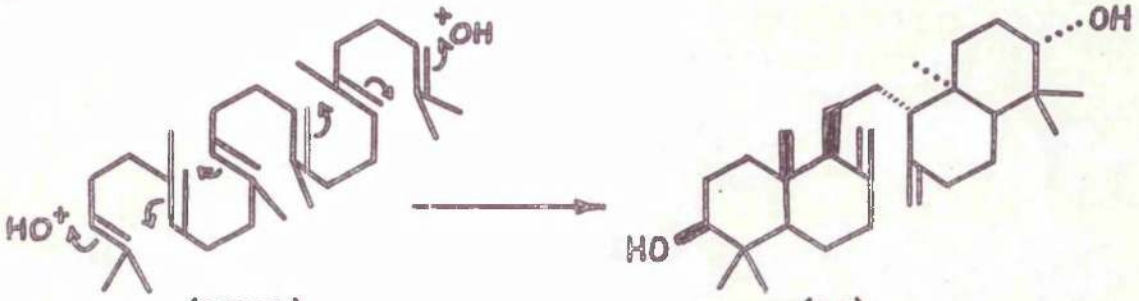
Rechromatography of the residue from the combined mother liquors of the acetate fractions, followed by fractional crystallisation, yielded more butyrospermyl acetate (L), taraxeryl acetate (LII), and α-spinasteryl acetate (LIV), together with a small quantity of an acetate, $C_{32}H_{52}O_2$, which crystallised from chloroform-methanol in large colourless blades, m.p. 122-123°; $[\alpha]_D$ - 30°. Alkaline hydrolysis of the acetate gave the corresponding alcohol, $C_{30}H_{50}O$, m.p. 85-87°; $[\alpha]_D$ -45°. The alcohol and acetate gave a red colour with a strong green fluorescence in the Liebermann-Burchardt test and a strong yellow colour with tetranitromethane in chloroform solution. Their ultraviolet absorption spectra showed only end absorption ($\lambda_{max.}$ 2040 Å; ε = 8,500) suggestive of the presence of one or more double bonds. This substance was not obtained in sufficient quantity for further investigation.

Biogenesis

It is interesting that taraxerol (LIII) and friedelin (XLIX) should occur together in horse chestnuts, since the former is regarded as a stabilised intermediate in the biogenesis of the latter.[28] Recent work[29] has shown that acetic acid (or its biological equivalent) is utilised in forming mevalonolactone (LVII), which in turn is incorporated into squalene (LVIII). The cyclisation of squalene (LVIII) to form, under the appropriate biological conditions, all known basic representatives of the pentacyclic triterpenes has been envisaged as taking place according to the scheme shown on page 107:[28]

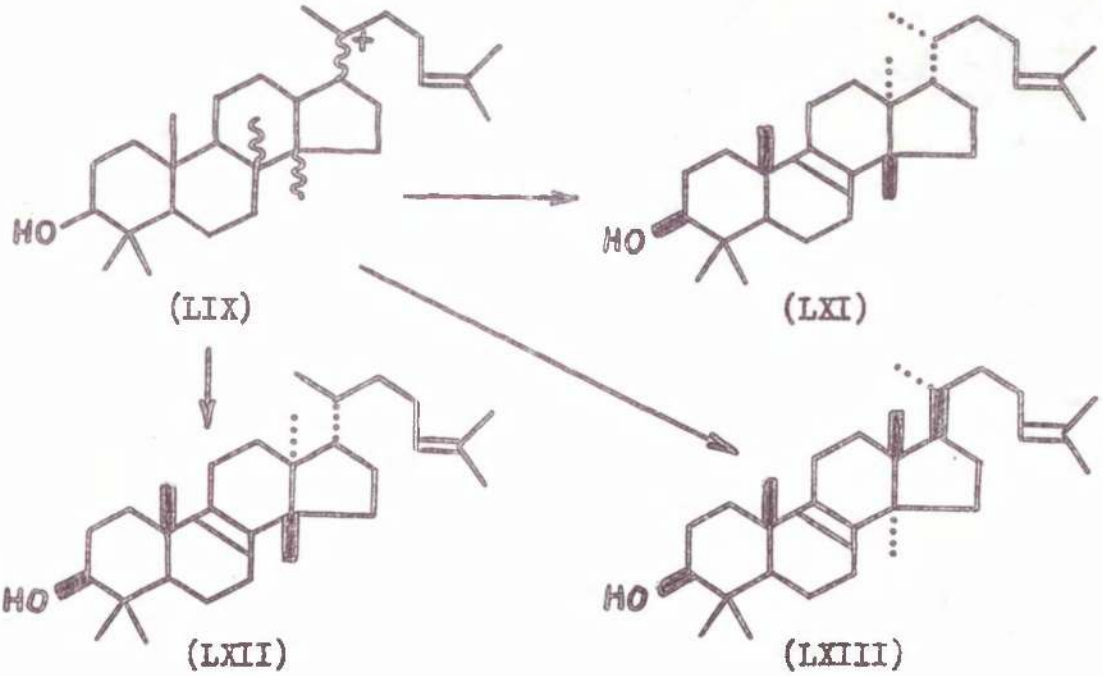

(LVII)
(LVIII)
(LIX)
α-Amyrin
Lupeol
Germanicol
Taraxasterol
δ-Amyrin
β-Amyrin
(LIII)
Almusenone
(XLIX)

The unusual tetracyclic triterpenoid onocerin (LX)[30] would appear to be formed by simultaneous attack at both ends of the squalene (LVIII) chain.



(LVIII) (LX)

The intermediate ion (LIX) is regarded as the precursor of the tetracyclic triterpenes typified by euphol (LXI),[31] tirucallol (LXII),[32]

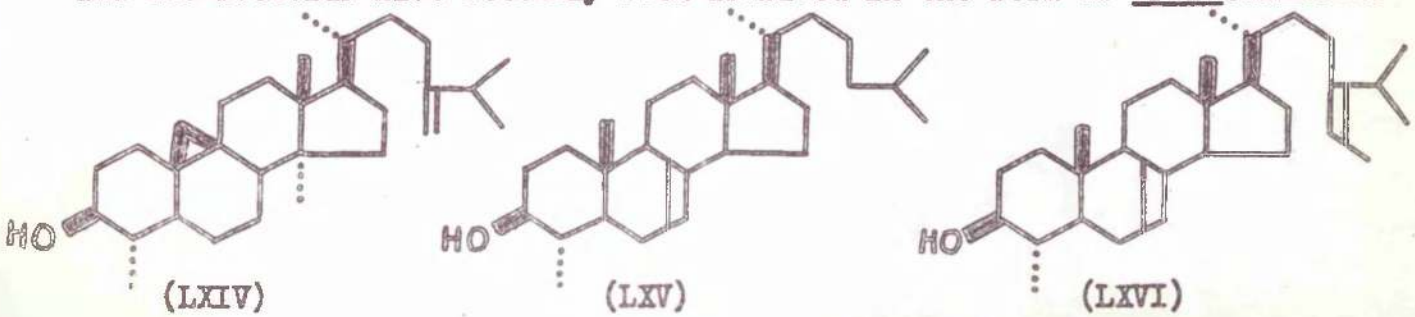


(LIX) (LXI)

(LXII) (LXIII)

and lanosterol (LXIII),[33] and of the steroids through lanosterol (LXIII), by demethylation.[29,34]

Representatives of the intermediates between the lanosterol series and the steroids have recently been isolated in the form of cycloeucalenol



(LXIV) (LXV) (LXVI)

(LXIV),[35] lophenol (LXV),[36] and citrostadienol (LXVI).[37]

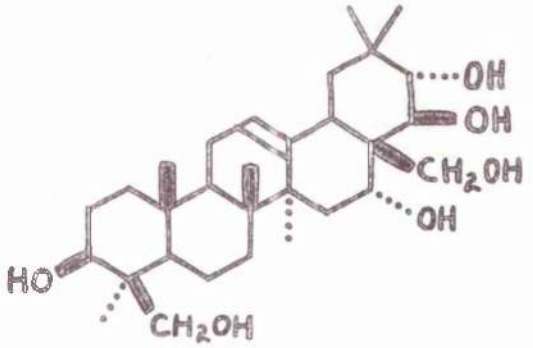
The Glycoside and Aglycone of Horse Chestnut Seeds

The procedure used for the isolation of aescigenin during the work described in this thesis was essentially that of Winterstein,[4] but difficulty was experienced in obtaining this substance in reasonable amounts until it was realised that aescigenin is not the true aglycone of aescin.

Evaporation of an aqueous ethanolic extract from defatted horse chestnut seeds gave a dark, sticky mass of crude aescin which was partially hydrolysed, by aqueous mineral acid, to a mixture of the water insoluble prosapogenins. Refluxing the crude prosapogenins, with aqueous ethanolic hydrochloric acid for three days, gave a mixture of alcohols which was acetylated and crystallised from methanol. The first crop consisted of aescigenin tetra-acetate and was characterised by hydrolysis to the alcohol, aescigenin (ca. 20% of the mixed alcohols). The second crop consisted of isoaescigenin penta-acetate and was characterised by hydrolysis to the alcohol, isoaescigenin (ca. 3% of the mixed alcohols). No more crystalline material could be obtained from the acetate mother liquors, even after careful chromatography, but alkaline hydrolysis of the resinous acetate followed by crystallisation from aqueous ethanol gave an alcohol, $C_{30}H_{50}O_6$, m.p. 300-302°; $[\alpha]_D$ + 31° (ca. 60% of the mixed alcohols). The benzoate, methanesulphonate, p-toluenesulphonate, and ethylidene derivative of the compound $C_{30}H_{50}O_6$ all failed to crystallise but the original alcohol could be regenerated readily from these amorphous derivatives.

When the crude prosapogenins were refluxed with aqueous ethanolic hydrochloric acid for 12 hours the only isolable product was the compound $C_{30}H_{50}O_6$ which must, therefore, be the true aglycone of aescin. When the aglycone was refluxed with aqueous ethanolic hydrochloric acid for three days the same mixture of products was obtained as that from the prosapogenins under the same conditions.

The compound $C_{30}H_{50}O_6$ may well be the hexahydroxyoleanene (XLVIII) which Jeger and his co-workers[17] suggested as the hypothetical precursor of aescigenin. In agreement with this structure are the following properties:



(XLVIII)

i) The substance $C_{30}H_{50}O_6$ is unsaturated to tetranitromethane and shows end absorption in the ultraviolet consistent with the presence of a single double bond, probably trisubstituted ($\lambda_{max.}$ 2040 Å; ϵ = 5,000). The infrared absorption spectrum of the compound shows a moderately strong band at 855 cm.$^{-1}$ indicative of the presence of a trisubstituted double bond; the ether band at 1110 cm.$^{-1}$ is absent.

ii) When the compound $C_{30}H_{50}O_6$ was heated with copper bronze, formaldehyde was evolved and characterised as its dimedone derivative, thus indicating the presence of one or more primary-secondary, 1:3-glycol systems.[38]

iii) The compound $C_{30}H_{50}O_6$ consumed one mole of sodium metaperiodate,

thus indicating the presence of a single 1:2-glycol group.

Acetylation of the aglycone, $C_{30}H_{50}O_6$, gave an amorphous penta-acetate, $C_{40}H_{60}O_{11}$, the infrared absorption spectrum of which showed a band at 3333 cm.$^{-1}$ indicative of the presence of a free hydroxyl group. Treatment of the amorphous acetate with phosphorus oxychloride and pyridine gave a substance, $C_{40}H_{58}O_{10}$, isomeric with but differing from isoaescigenin penta-acetate, and which was unchanged after refluxing with hydrochloric acid in acetic acid. This substance was obtained in very poor yield and has not been investigated further. The amorphous aglycone acetate, $C_{40}H_{60}O_{11}$, was unchanged after refluxing with acetic anhydride and p-toluenesulphonic acid but with hydrochloric acid in acetic acid a mixture of aescigenin tetra-acetate and isoaescigenin penta-acetate was formed in low yield.

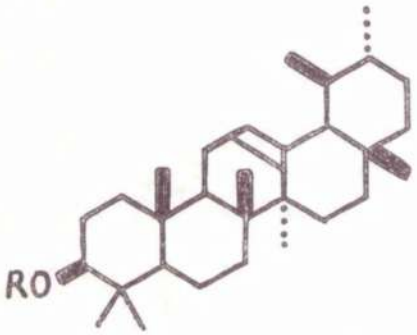
Aescigenin

The benzoate, methanesulphonate, p-toluenesulphonate, and trityl ether of aescigenin all failed to crystallise but the alcohol has been further characterised as the bisethylidene derivative, $C_{34}H_{52}O_5$, and the bisbenzylidene derivative, $C_{44}H_{56}O_5$, previously reported by Ruzicka and his co-workers.[9]

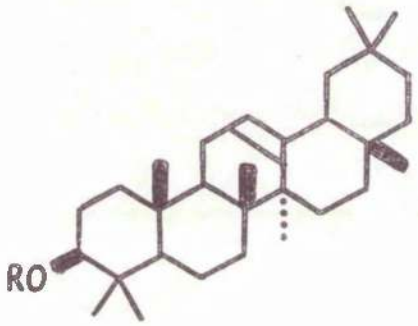
Aescigenin could not be classified as a derivative of either α-amyrin (XVIII) or β-amyrin (XIX) by the usual oxidation experiments, viz. oxidation by N-bromosuccinimide and by selenium dioxide. When α-amyrin acetate (LXVII) is refluxed with N-bromosuccinimide in carbon tetrachloride the homoannular diene (LXIX) ($\lambda_{max.}$ 2800 Å) is obtained,[39] whereas, under similar conditions, β-amyrin acetate (LXVIII) yields the triene (LXX) ($\lambda_{max.}$ 3080 Å).[39,40] When aescigenin

tetra-acetate was refluxed with N-bromosuccinimide in carbon tetrachloride the crude product showed only general absorption in the ultraviolet with no distinct maxima, and the only isolable compound was starting material (70% recovery).

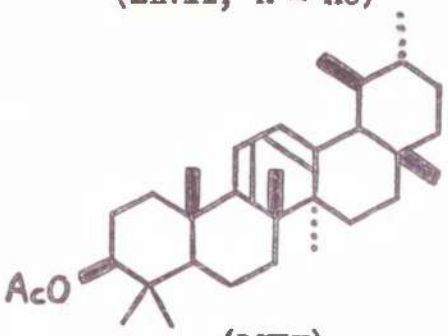
β-Amyrin acetate (LXVIII) is readily oxidised to the heteroannular



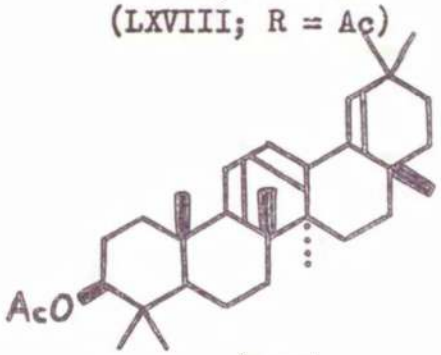
(XVIII; R = H)
(LXVII; R = Ac)



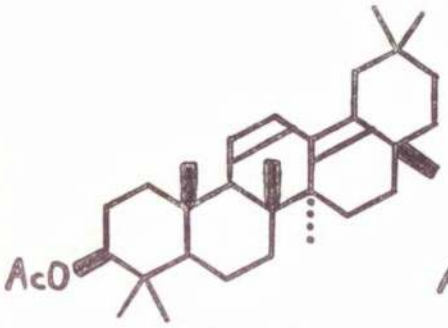
(XIX; R = H)
(LXVIII; R = Ac)



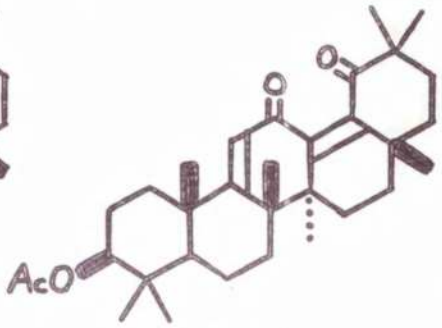
(LXIX)



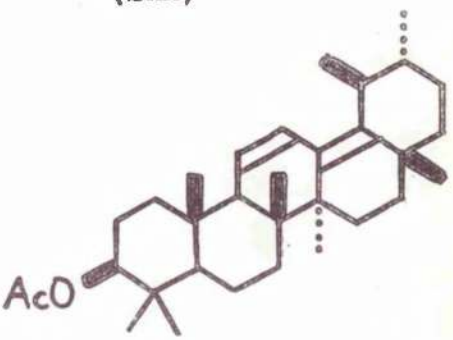
(LXX)



(LXXI)



(LXXII)

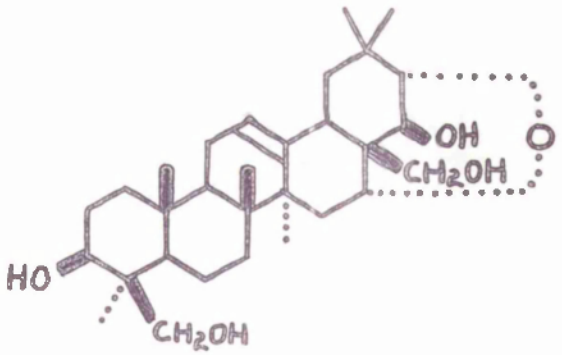


(LXXIII)

diene (LXXI) ($\lambda_{max.}$ 2420 Å; 2500 Å; 2600 Å) by selenium dioxide in refluxing acetic acid,[41] and to the dienedione (LXXII) ($\lambda_{max.}$ 2800 Å) by selenium dioxide in refluxing benzyl acetate.[42] α-Amyrin acetate (LXVII) is unchanged after refluxing with selenium dioxide in acetic acid, and gives the heteroannular diene (LXXIII), ($\lambda_{max.}$ 2440 Å; 2510 Å;

2600 Å) in low yield, when it is refluxed overnight with selenium dioxide in benzyl acetate.[43] When aescigenin tetra-acetate was refluxed with selenium dioxide in acetic acid the crude product showed a very weak absorption band in the 2500 A region of the ultraviolet, but the only isolable compound was starting material (80% recovery). After refluxing with selenium dioxide in benzyl acetate the crude product showed general absorption in the ultraviolet with no distinct maxima; the only crystalline material isolable was unchanged aescigenin tetra-acetate (50% recovery).

Although aescigenin has now been shown to be the β-amyrin



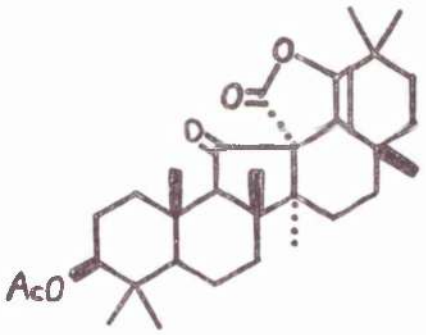
(XX)

derivative (XX),[17] its failure to give the expected oxidation products may be explained by the fact that the oxide bridge will prevent the flattening of rings C, D, and E, which is necessary for the formation of the heteroannular diene system and the dienedione system.

Chromic acid oxidation of aescigenin tetra-acetate, at room temperature, gave the keto-tetra-acetate, $C_{38}H_{54}O_{10}$, m.p. 233-234°, previously prepared by Ruzicka and his co-workers.[9] Alkaline hydrolysis of this compound yielded the corresponding keto-tetrol, $C_{30}H_{46}O_6$, acetylation of which regenerated the keto-tetra-acetate. Further oxidation of the keto-tetra-acetate, with chromium trioxide

in refluxing acetic acid, gave a neutral compound, $C_{38}H_{50}O_{12}$, which was also obtained directly from aescigenin tetra-acetate under similar conditions. This "O_{12}-tetra-acetate" does not give a colour with tetranitromethane in chloroform or with ethanolic ferric chloride, but its properties do not parallel those of the "O_5-acetate" (LXXIV) ($\lambda_{max.}$ 2300 Å; inflexion at 3000 Å; 1781 cm.$^{-1}$; 1740 cm.$^{-1}$; 1745 cm.$^{-1}$; 1692 cm.$^{-1}$) which is formed by oxidation of several β-amyrin derivatives.[44]

The ultraviolet absorption spectrum of the "O_{12}-tetra-acetate" shows a strong band at 2400 Å, suggesting the presence of an α:β-unsaturated ketone grouping, and a moderately high inflexion

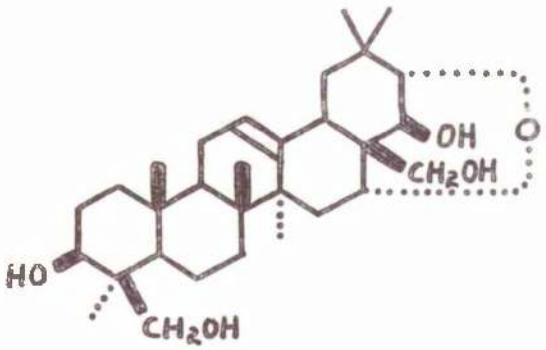


(LXXIV)

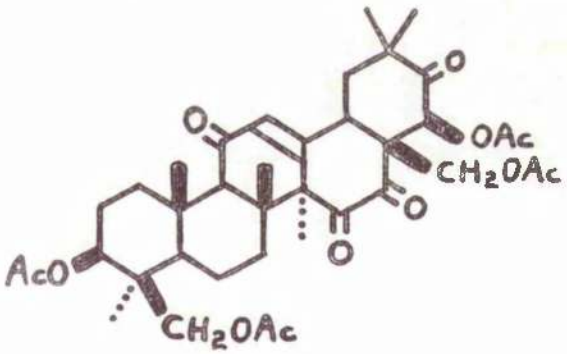
at 2960 Å which may be due to the presence of one or more isolated carbonyl groups. The presence of an α:β-unsaturated ketone group was confirmed by the infrared spectrum which shows a strong band at 1667 cm.$^{-1}$, but the 1740 cm.$^{-1}$ band, although rather broad, is not resolved into acetate and ketonic carbonyl absorption. Another feature of the infrared spectrum is a moderately strong band at 1802 cm.$^{-1}$ which has not been allocated. This band cannot be due to the presence of a five membered ring enol-lactone,[45] or of a cyclic carboxylic acid anhydride,[45] since catalytic hydrogenation of the "O_{12}-tetra-acetate" gave a compound, $C_{38}H_{56}O_{12}$, which showed only two bands in the carbonyl region of the infrared, at 1669 cm.$^{-1}$

(α:β-unsaturated ketone) and at 1740 cm.$^{-1}$ (acetate), together with a moderately strong band at 3333 cm.$^{-1}$ (hydroxyl). It is extremely unlikely that the carbonyl group of a carboxylic acid derivative would be reduced while the unsaturated carbonyl function and the acetate carbonyl functions remain untouched. Further hydrogenation of the compound $C_{38}H_{56}O_{12}$ gave an amorphous product which showed no selective absorption above 2200 Å in the ultraviolet and only one band in the carbonyl region of the infrared, at 1740 cm.$^{-1}$ (acetate).

A deep purple colour was formed when the "O_{12}-tetra-acetate"



(XX)



(LXXV)

was dissolved in methanolic potassium hydroxide at room temperature, and even when the reaction mixture was worked up immediately the only crystalline product was a trace of starting material. Acetylation of the non-crystalline product did not regenerate the starting material. No colour was produced when the "O_{12}-tetra-acetate" was refluxed with methanolic hydrochloric acid but the product was again non-crystalline and did not regenerate the starting material on acetylation.

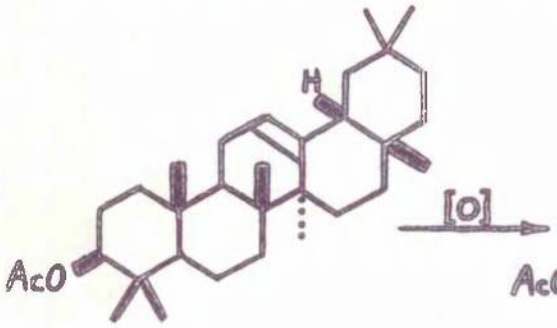
On the basis of structure (XX) for aescigenin,[17] the carbonyl group of the α:β-unsaturated ketone system in the "O_{12}-tetra-acetate" will be at C_{11}. The presence of two unhindered carbonyl groups, which must be ketonic since an aldehyde group would not survive the vigorous

oxidising conditions used in preparing the "O_{12}-tetra-acetate", was demonstrated by the preparation of a dioxime, $C_{38}H_{52}O_{12}N_2$. The ether band, in the 1110 cm.$^{-1}$ region of the infrared, is absent from the spectrum of the "O_{12}-tetra-acetate". If the oxide bridge has been opened oxidatively, then one would expect a carbonyl group at C_{16} and another at C_{21}. Since there is no absorption band in the 3300 cm.$^{-1}$ region of the infrared spectrum, characteristic of a hydroxyl group, it is tentatively suggested that the remaining oxygen function is present as a hindered carbonyl group and structure (LXXV) is proposed for the "O_{12}-tetra-acetate."

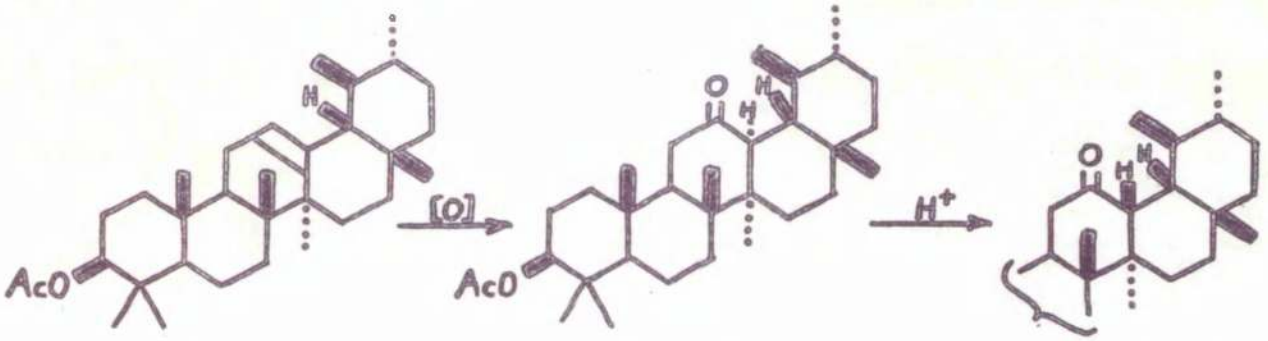
When aescigenin tetra-acetate was treated with peracetic acid, or better, performic acid, a compound, $C_{38}H_{56}O_{10}$, (LXXXIV) which is saturated to tetranitromethane, was obtained and characterised by hydrolysis to the corresponding alcohol, $C_{30}H_{48}O_6$. Acetylation of the alcohol regenerated the compound $C_{38}H_{56}O_{10}$. The spectra of both the alcohol and the acetate show no high intensity absorption in the ultraviolet and a strong band at 1710 cm.$^{-1}$ in the infrared, indicative of the presence of a carbonyl group in a six membered ring. The double bond in aescigenin must, therefore, be trisubstituted. The compound $C_{38}H_{56}O_{10}$ was unchanged after treatment with strong mineral acid. This behaviour recalls that of β-amyrin acetate (LXVIII) which, under the same oxidative conditions, yields the acid stable ketone (LXXVI),[46] whereas α-amyrin acetate (LXVII) yields the unstable 13α ketone (LXXVII) which with mineral acid gives the stable 13β isomer (LXXVIII).[47]

Bromination of the saturated ketone, $C_{38}H_{56}O_{10}$, gave a bromo-ketone, $C_{38}H_{55}O_{10}Br$, the ultraviolet absorption spectrum of which shows a weak band at 3100 Å (ε = 120). Barton and Cookson[48] have

shown that substitution of an axial bromine atom in the α-position to the carbonyl group of a six membered ring ketone causes a batho-



(LXVIII) (LXXVI)



(LXVII) (LXXVII) (LXXVIII)

chromic shift of 290 Å, together with a threefold increase in absorption intensity. Since the ultraviolet absorption spectrum of the saturated ketone, $C_{38}H_{56}O_{10}$, shows a weak band at 2810 Å ($\varepsilon = 40$) these conditions are realised for the aescigenin derivative,

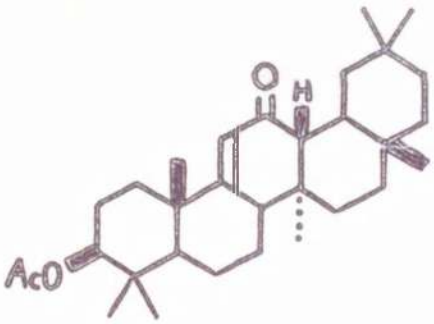
(LXXIX)

$C_{38}H_{55}O_{10}Br$, which, if the ethylenic linkage in aescigenin is assumed to be at position 12(13), must be the 11β-bromo-12-oxo-compound (LXXIX).

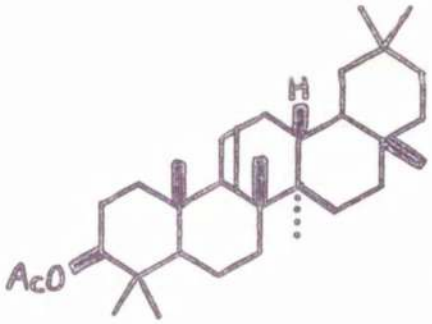
Brief refluxing of the bromoketone (LXXIX) with collidine gave a compound, $C_{38}H_{54}O_{10}$, (LXXXVI), which was characterised by hydrolysis to the corresponding alcohol, $C_{30}H_{46}O_6$. Acetylation

of this alcohol regenerated the compound $C_{38}H_{54}O_{10}$. Both the alcohol and the acetate failed to give a colour with tetranitromethane in chloroform, and their absorption spectra showed a strong band at 2470 Å in the ultraviolet and a strong band at 1681 cm.$^{-1}$ in the infrared, indicative of the presence of an α:β-unsaturated ketone grouping.[45]

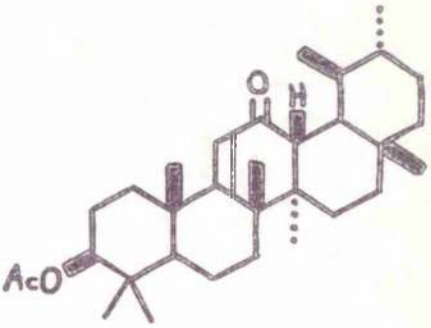
In one experiment the saturated ketone, $C_{38}H_{56}O_{10}$, was brominated and the crude product was refluxed with collidine to yield, in addition to the α:β-unsaturated ketone, $C_{38}H_{54}O_{10}$, a small quantity of a pale yellow compound, $C_{38}H_{53}O_{10}Br$. The infrared absorption



(LXXX)



(LXXXI)



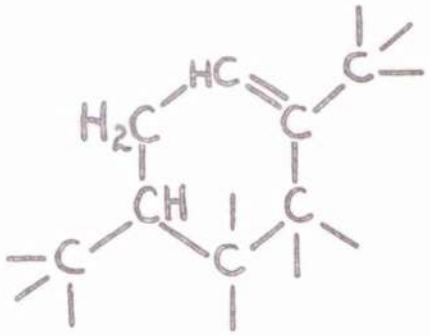
(LXXXII)

spectrum of this compound shows a strong band at 1670 cm.$^{-1}$ attributable to an α:β-unsaturated ketone system, but the main maximum in the ultraviolet is at 2920 Å. Braude and his co-workers[49] have shown that substitution of a halogen atom for the α-hydrogen atom of an α:β-unsaturated ketone causes a bathochromic shift of from 400 Å to 500 Å. The bromo-compound, $C_{38}H_{53}O_{10}Br$, is therefore most probably the α-bromo-α:β-unsaturated ketone, (LXXXVII), which would be formed by dehydrobromination of the α:α-dibromo-derivative of the saturated ketone, $C_{38}H_{56}O_{10}$.

The α:β-unsaturated ketone, $C_{38}H_{54}O_{10}$, was smoothly hydrogenolysed in the presence of a platinum catalyst to the compound $C_{38}H_{56}O_9$, (LXXXVIII),

isomeric with aescigenin tetra-acetate. This compound gave a pale yellow colour with tetranitromethane in chloroform and was characterised by hydrolysis to the corresponding alcohol, acetylation of which regenerated the original acetate. This reaction may be compared with the hydrogenolysis of 12-oxo-olean-9(11)-en-3β-yl acetate (LXXX) to olean-9(11)-en-3β-yl acetate (ε-amyrin acetate) (LXXXI).[50] Under similar conditions 12-oxours-9(11)-en-3β-yl acetate (LXXXII) resists catalytic reduction.[51]

Lithium aluminium hydride reduction of the α:β-unsaturated ketone, $C_{38}H_{54}O_{10}$, followed by refluxing the crude product with acetic anhydride,



(LXXXIII)

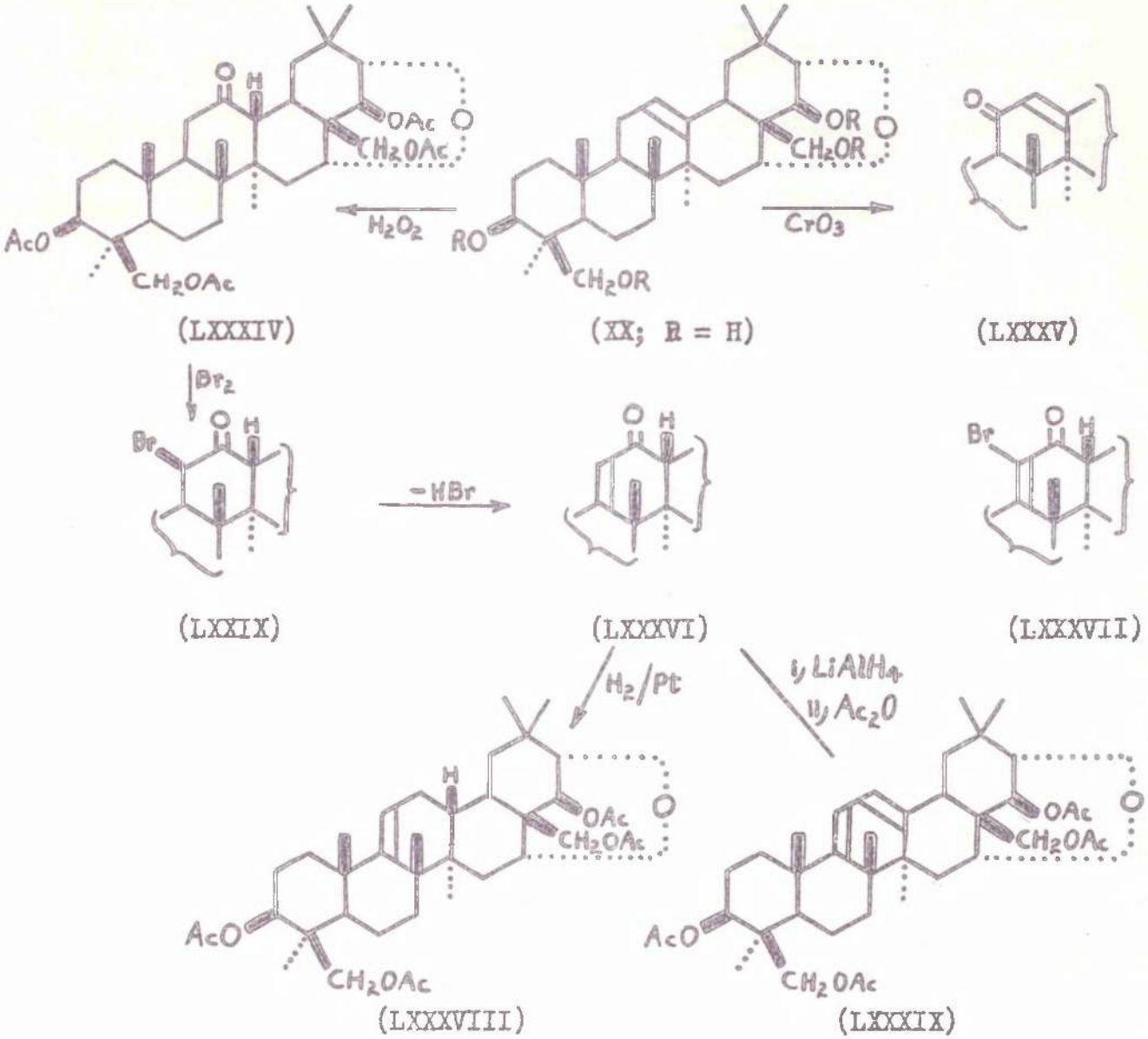
gave the conjugated diene, $C_{38}H_{54}O_9$, (LXXXIX), ($\lambda_{max.}$ 2800 Å) which, like the 9(11):12-dienes of the ursane and oleanane series,[52] is strongly dextrorotatory. When the conjugated diene was treated with mineral acid the only isolable product was starting material (40% recovery). The ultraviolet spectrum of the residue showed only general, high intensity, absorption.

Refluxing the α:β-unsaturated ketone, $C_{38}H_{54}O_{10}$, with selenium dioxide in acetic acid led to extensive decomposition; the only isolable product was starting material (30% recovery).

The results of these experiments in the region of the double bond show that aescigenin possesses the partial structure (LXXXIII), with

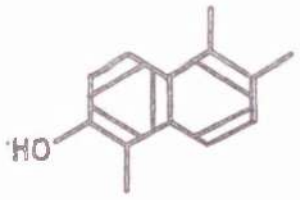
the unsaturated centre most probably at position 12(13). On the basis of structure (XX) for aescigenin[17] the aforementioned reaction products must be formulated as shown in the scheme below.

That aescigenin does not possess a 1:2-glycol system was shown by its stability to sodium metaperiodate and to periodic acid. Two 1:3-glycol systems must, therefore, be present in aescigenin since

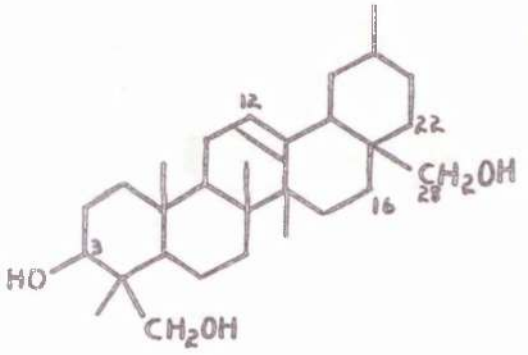


Ruzicka and his co-workers[9] have shown that it forms a bis-ethylidene derivative and a bis-benzylidene derivative. When aescigenin was heated with copper bronze, formaldehyde was evolved and characterised as its dimedone derivative. This behaviour is characteristic of a primary-secondary, 1:3-glycol system,[38] and since 1:5:6-trimethyl-

-β-naphthol (VIII) is a product of selenium dehydrogenation of aescigenin,[9] two of the hydroxyl groups of the latter must be at positions 3 and 23 (or 24) respectively. Only ring A of a normal pentacyclic triterpenoid nucleus could accomodate a disecondary 1:3-glycol system, but since this would involve position 3 the second glycol system of aescigenin must also be primary-secondary. Many naturally occurring triterpenoids are oxygenated at one or more of positions 16, 22, and 28;[10] it is therefore reasonable to assume, as a first approximation, that this will be true also



(VIII)



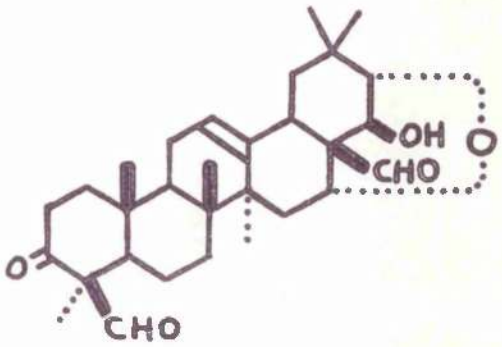
(XC)

of aescigenin. Taking account of the selenium dehydrogenation products[9] aescigenin may be represented by the partial formula (XC), together with a hydroxyl group at C_{16} or at C_{22} and an ether ring.

As a first step towards preparing the parent hydrocarbon from aescigenin, the latter was oxidised by the Kiliani reagent to give a compound, $C_{30}H_{42}O_5$. The infrared absorption spectrum of this compound showed a strong band at 1715 $cm.^{-1}$ indicative of the presence of a carbonyl group in a six membered ring, a very strong band at 1730 $cm.^{-1}$ indicative of the presence of one or more aldehyde groups, and a moderately strong band at 3400 $cm.^{-1}$ indicative of the presence of a hydroxyl group.

At this stage Jeger and his co-workers[17] published their

work on the structure of aescigenin. The Swiss workers prepared but did not characterise the aforementioned oxidation product.



(XCI)

From their work it is obvious that this compound must be the ketol--dialdehyde (XCI).

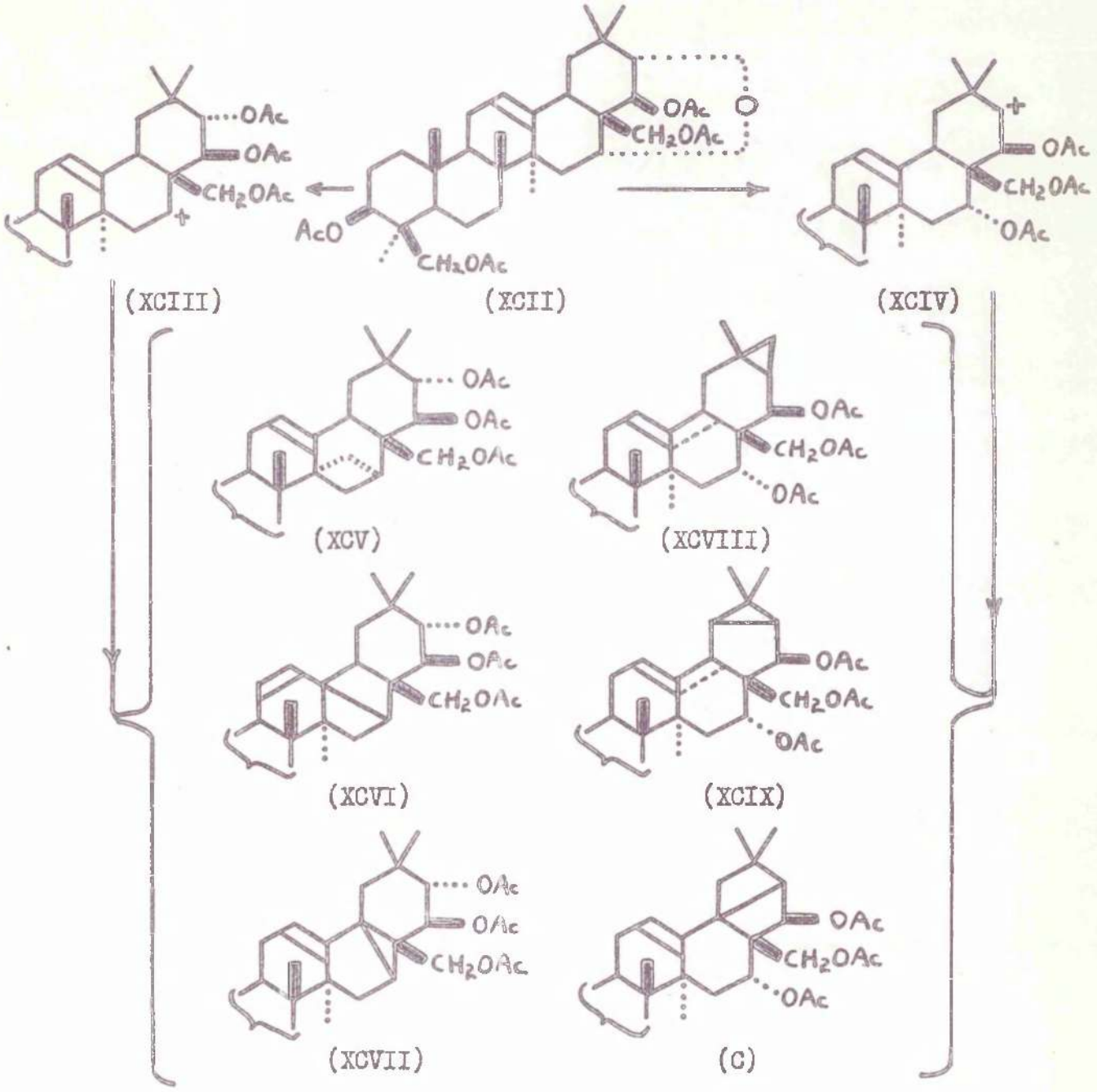
isoAescigenin

isoAescigenin penta-acetate was prepared from aescigenin tetra-acetate by the action of acetic anhydride and p-toluenesulphonic acid,[8] of boron trifluoride-acetic acid,[8] and of acetic anhydride and hydrobromic acid. The benzoate, methanesulphonate, and p-toluenesulphonate of isoaescigenin failed to crystallise.

isoAescigenin penta-acetate contains only one double bond since it forms a saturated monoxide,[8] and must, therefore, be hexacarbocyclic. The oxide ring in aescigenin tetra-acetate (XCII) can open in one of two ways to form the intermediate carbonium ion (XCIII) or (XCIV). isoAescigenin penta-acetate must then be represented by one of the six structures (XCV) to (C). Of these structures (XCV), (XCVI), and (XCVII), need not be considered since isoaescigenin does not react with sodium metaperiodate or with periodic acid.

When isoaescigenin penta-acetate was refluxed with selenium dioxide in benzyl acetate a non-crystalline product was obtained,

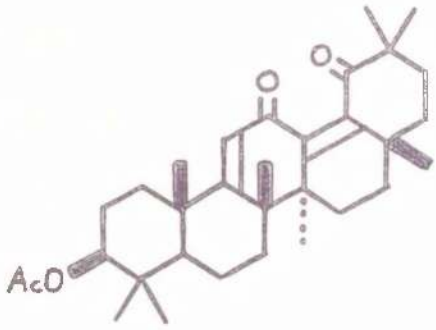
the infrared absorption spectrum of which showed a strong band at 1740 cm.$^{-1}$ (acetate) together with a high intensity shoulder at 1690 cm.$^{-1}$ and moderate to strong bands at 1661 cm.$^{-1}$, 1637 cm.$^{-1}$, 1595 cm.$^{-1}$, and 1316 cm.$^{-1}$, consistent with the presence of a 12:19-dioxo-9(11):13(18)-diene system.[53] If this system has been



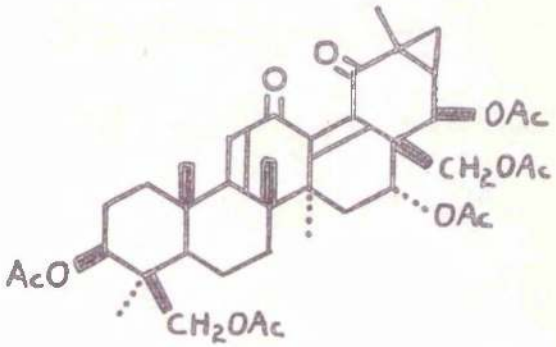
formed, then structures (XCIX) and (C) may also be eliminated. The ultraviolet absorption spectrum of the amorphous dienedione showed a strong band at 2900 Å, whereas 12:19-dioxo-9(11):13(18)-dienes,

e.g. the β-amyrin derivative (LXXII), generally absorb at 2800 Å.[20,42] This bathochromic shift might be accounted for by the presence of extended conjugation, in the isoaescigenin derivative, in the form of a cyclopropane ring, as shown in structure (CI), leading to structure (XCVIIIa or XCVIIIb) for isoaescigenin penta-acetate.

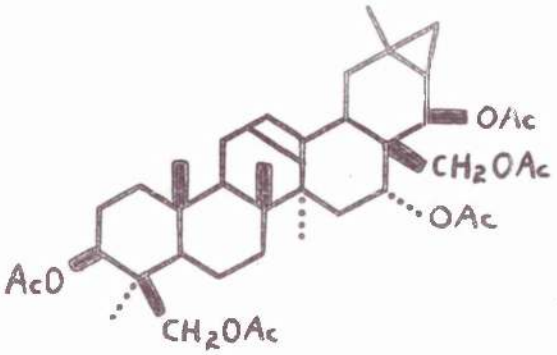
The intensity of the end absorption in the ultraviolet spectrum of isoaescigenin penta-acetate ($\lambda_{max.}$ 2040 Å; ε = 8000), as compared



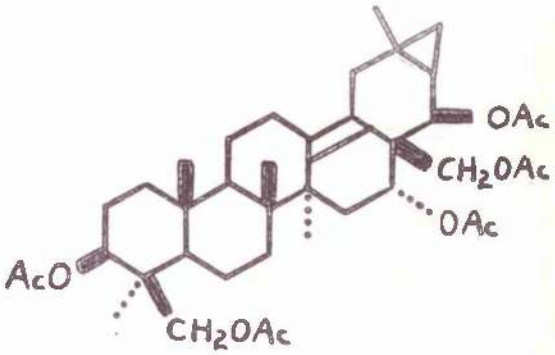
(LXXII)



(CI)



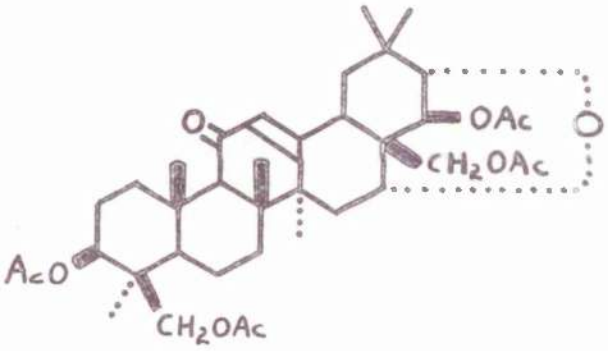
(XCVIIIa)



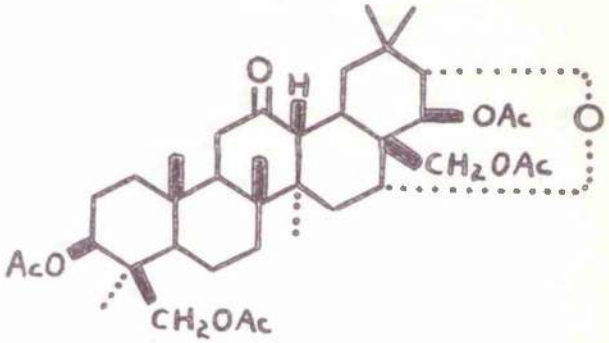
(XCVIIIb)

with that of aescigenin tetra-acetate ($\lambda_{max.}$ 2040 Å; ε = 5,000), is more in accord with a tetrasubstituted double bond than a trisubstituted double bond. Ruzicka and his co-workers[8] consider that the unsaturated centre in isoaescigenin is in the same position as that in aescigenin since they were able to prepare an α:β-unsaturated ketone (m.p. 337°; $[\alpha]_D$ - 9°), by scission of the ether link in ketoaescigenin tetra-acetate (LXXXV), which, they claim, is identical with the compound (m.p. 324-326°; $[\alpha]_D$ - 8° ± 2°) obtained by oxidation of isoaescigenin penta-acetate.

Since the Swiss workers made no mention of the method used to compare these compounds, it seemed desirable that these experiments should be repeated. Treatment of 11-oxoaescigenin tetra-acetate (LXXXV) with acetyl chloride and zinc chloride gave, in poor yield, a compound, m.p. 333-334°; $[\alpha]_D$ - 9°. Oxidation of isoaescigenin penta-acetate with chromium trioxide in acetic acid gave, also in poor yield, a compound, m.p. 321-324°; $[\alpha]_D$ - 9°. A mixture of the two compounds melted at 320-325°; further comparison was not possible due to lack of material.



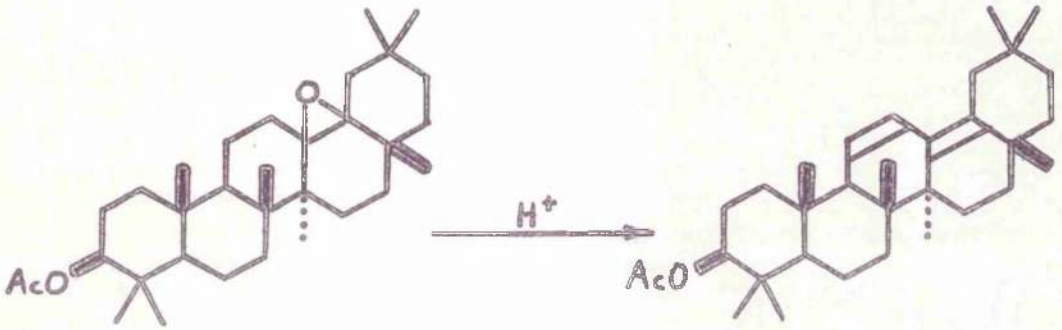
(LXXXV)



(LXXXIV)

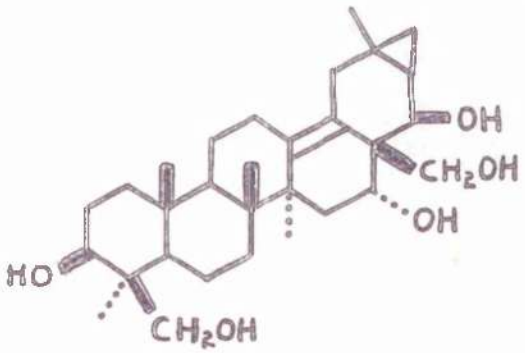
Treatment of the saturated ketone (LXXXIV) with acetic anhydride and p-toluenesulphonic acid gave the corresponding saturated ketone of the isoaescigenin series. Performic acid oxidation of isoaescigenin penta-acetate yielded, surprisingly, an epoxide, m.p. 330-332°; $[\alpha]_D$ - 2°, apparently identical with the compound, m.p. 330-331°; $[\alpha]_D$ - 2° ± 2°, previously prepared by the Swiss workers[8] by oxidation of isoaescigenin penta-acetate eith monoperphthalic acid. This result would seem to suggest that the double bond in isoaescigenin is tetrasubstituted, i.e. at position 13(18). However, treatment of the oxide with mineral acid did not lead to the formation of a conjugated diene [cf. δ-amyrin acetate oxide (CII)[42]],

but caused extensive decomposition. The only isolable product was a trace of a colourless compound, m.p. 318-320°, which showed



(CII) (LXXI)

no absorption in the ultraviolet and which gave a strongly positive Beilstein test for halogen.



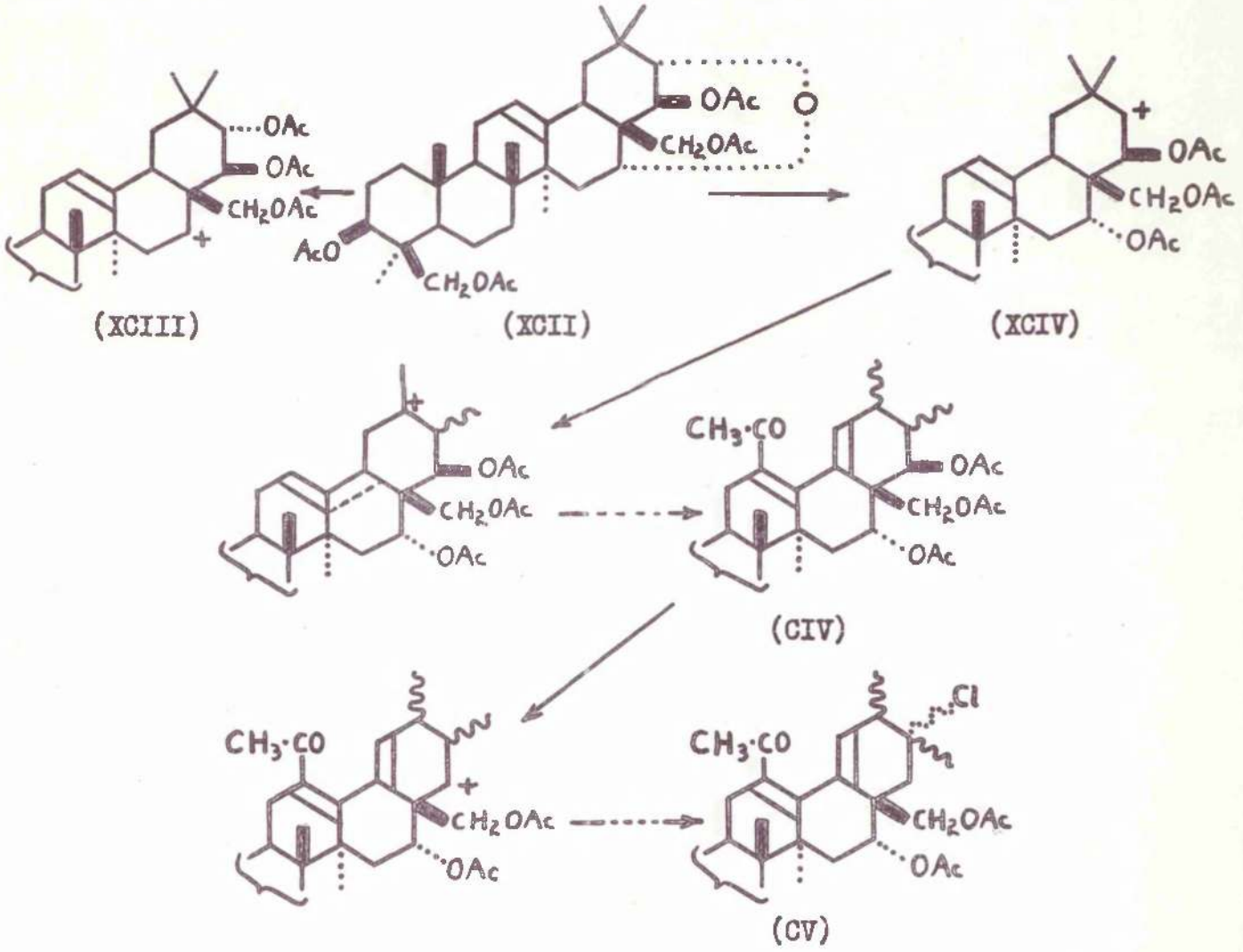
(CIII)

Although the evidence at present available is not sufficient for the precise location of the double bond in isoaescigenin, it does seem probable that it is in the 13(18) position and structure (CIII) is tentatively proposed for isoaescigenin.

alloAescigenin

It is not easy to account for the formation of, or to propose structures for, the members of the alloaescigenin series. This is especially true if the ion (XCIV), assumed above to be the precursor of the isoaescigenin series, is considered as the first, transient, intermediate. One hypothetical reaction scheme which

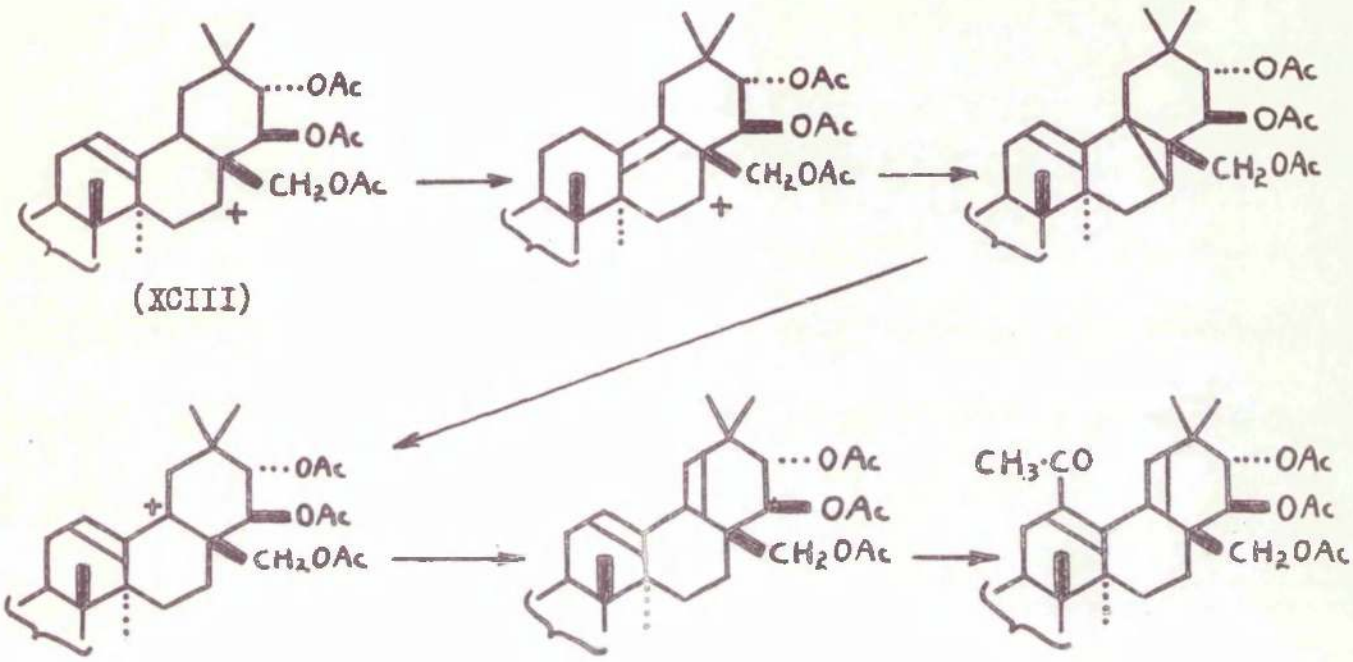
might lead to the formation of compounds having the properties described by Ruzicka and his co-workers[8] is illustrated below. It is not intended that the ciphers shown here will represent the actual intermediates, or that the various steps will take place in the order shown. From the ion (XCIV) this route would result in structure (CIV) for C-acetylalloaescigenin penta-acetate,



from which C-acetylchlorodesoxyalloaescigenin tetra-acetate may be envisaged as (CV), or a 20-chloro-isomer.

If it is assumed that scission of the oxide ring takes place in the opposite sense, via the ion (XCIII), it is possible to write down the following hypothetical reaction scheme, leading to structure (CVI) for C-acetylalloaescigenin penta-acetate.

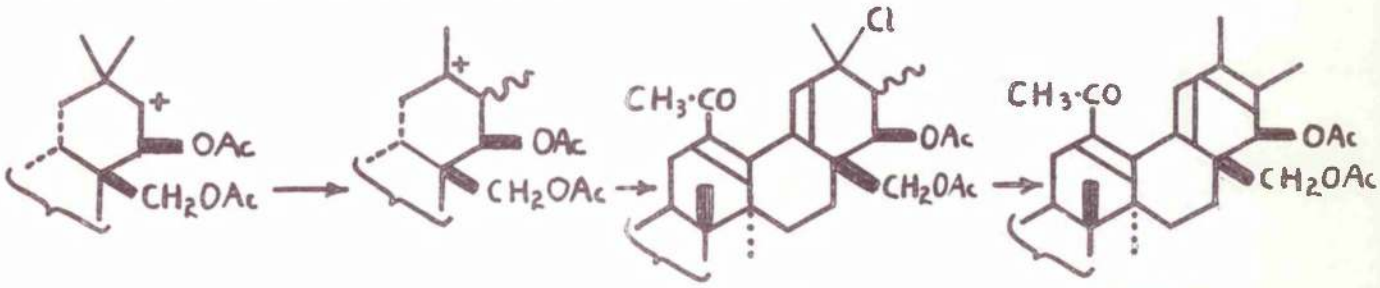
The facile formation of a conjugated trienone (CVIII), by dehydrochlorination of C-acetylchlorodesoxyalloaescigenin tetra-acetate



(XCIII)

(CVI)

(CVII),[8] may then be explained by assuming that methyl migration has taken place, at some stage after scission of the oxide ring, to give the chloro-compound (CVII) in the manner shown below:



(CVII) (CVIII)

Much work remains to be done on these compounds, but their inaccessibility has proved to be a major obstacle.

EXPERIMENTAL

Specific rotations were measured in chloroform solution, unless otherwise stated, at room temperature in a 1 dm. tube. Ultraviolet absorption spectra were measured in ethanol solution with a Unicam S.P. 500 spectrophotometer. Infrared absorption spectra were measured in Nujol mulls, unless otherwise stated, with a Grubb Parsons S. 4 double beam spectrophotometer with sodium chloride optics, unless otherwise stated. Grade II alumina and light petroleum (b.p. 60–80°) were used for chromatography.

The author is indebted to Dr. A.C.Syme, Mr. W.McCorkindale, and Miss P.Adams for microanalyses, to Miss D.Adams and Miss I.O'Hagen for the determination of ultraviolet spectra, and to Miss N.Caramando for the determination of infrared spectra.

Samples for analysis were dried for 3 days at 130°/0.05 m.m., and melting points were determined in vacuum sealed tubes.

The Non-Saponifiable Fraction of Horse Chestnut Seed Oil.- Dry, crushed, horse chestnut seeds (54 kg.) were extracted (12 hrs.) with petrol (12 l.) in a Soxhlet apparatus. Evaporation of the extract to dryness gave a dark green mobile oil (1.25 kg.) which was dissolved in benzene (100 ml.) and methanol (5 l.), and refluxed (4 hrs.) with potassium hydroxide (500 g.) in water (1000 ml.). The hydrolysate was diluted with water (5 l.), concentrated (to ca. 2 l.), and worked up through ether to give an orange gum (70 g.).

Isolation of n-Triacontane and Friedelin.- The non-saponifiable material (70 g.) was dissolved in petrol (500 ml.) and chromatographed on alumina (3 kg.). After development with petrol (1000 ml.), elution

with the same solvent (2 l.) gave a clear oil (1.4 g.) which crystallised from ethyl acetate in waxy plates, m.p. 60-62°. The compound was optically inactive and did not depress the melting point of an authentic specimen of n-triacontane. Its infrared absorption spectrum was identical with that of the latter hydrocarbon (as a solid film from the melt). (Found: C, 84.95; H, 14.6; M.W. [Rast], 440. Calc. for $C_{30}H_{62}$: C, 85.2; H, 14.8%; M.W. 422).

Elution with petrol-benzene mixtures (3 l.), with benzene (3 l.), and with benzene-ether (4:1, 1.5 l.) gave an intractible gum (1.6 g.). Continued elution with benzene-ether (1:1, 2 l.) gave a fraction (1.8 g.) which crystallised readily from chloroform-methanol in colourless needles, m.p. 260-262°; $[\alpha]_D$ - 25° (c, 1.3), identical (m.p., mixed m.p., and infrared) with friedelin. Corks were not used at any stage of the extraction. (Found: C, 84.65; H, 11.5. Calc. for $C_{30}H_{50}O$: C, 84.4; H, 11.8%).

Further elution with benzene-ether mixtures (500 ml.), with ether (7 l.), and with ether-methanol (49:1, 3.5 l.) gave an intractible oil (700 mg.). Elution with ether-methanol (19:1, 3.5 l.) gave a yellow solid (35 g.), m.p. 120-60°.

Isolation of Butyrospermol, Taraxerol, and α-Spinasterol.- The fraction, m.p. 120-160°, (35 g.) was acetylated (2 hrs.) on the steam-bath with pyridine (100 ml.) and acetic anhydride (60 ml.). After working up in the normal manner through ether, a mixture of acetates (36 g.) was obtained. A solution of the mixed acetates in petrol-benzene (9:1, 1000 ml.) was chromatographed on alumina (1.5 kg.). Elution with petrol-benzene mixtures (10 l.) gave only a trace of oily material. Elution with petrol-benzene (13:7, 1000 ml.)

gave a fraction (2.4 g.) which crystallised from chloroform-methanol in colourless needles, m.p. 140-142°; $[\alpha]_D$ + 12° (c, 1.5), identical with butyrospermyl acetate (m.p., mixed m.p., and infrared). (Found: C, 81.8; H, 11.1. Calc. for $C_{32}H_{52}O_2$: C, 82.0; H, 11.2%). Refluxing the acetate (100 mg.) with 5% potassium hydroxide in methanol (20 ml.) gave butyrospermol (60 mg.), m.p. and mixed m.p. 109-110°; $[\alpha]_D$ - 12° (c, 1.0). (Found: C, 84.25; H, 11.7. Calc. for $C_{30}H_{50}O$: C, 84.4; H, 11.8%).

Further elution with petrol-benzene (13:7, 2 l.) gave a fraction (12 g.) which crystallised from chloroform-methanol in colourless needles, m.p. 300-302°; $[\alpha]_D$ + 10° (c, 0.9), identical (m.p., mixed m.p., and infrared) with taraxeryl acetate. (Found: C, 81.9; H, 11.0. Calc. for $C_{32}H_{52}O_2$: C, 82.0; H, 11.2%). Alkaline hydrolysis of the acetate (100 mg.) gave taraxerol (85 mg.), m.p. and mixed m.p. 270-271°; $[\alpha]_D$ + 5° (c, 0.8). (Found: C, 84.15; H, 11.75. Calc. for $C_{30}H_{50}O$: C, 84.4; H, 11.8%).

Elution with petrol-benzene (1:19, 2.5 l.) gave a fraction (2.5 g.) which crystallised from chloroform-methanol in colourless plates, m.p. 175-177°; $[\alpha]_D$ - 3° (c, 1.1), identical (m.p., mixed m.p., and infrared) with α-spinasteryl acetate. (Found: C, 81.75; H, 10.9. Calc. for $C_{31}H_{50}O_2$: C, 81.9; H, 11.1%). Hydrolysis of the acetate (200 mg.) with 10% methanolic potassium hydroxide gave α-spinasterol (170 mg.), m.p. and mixed m.p. 174°; $[\alpha]_D$ - 3° (c, 0.9). (Found: C, 84.4; H, 11.8. Calc. for $C_{29}H_{48}O$: C, 84.4; H, 11.7%). Heating the alcohol (100 mg.) with benzoyl chloride (1 ml.) in pyridine (5 ml.) on the steam-bath (2 hrs.) gave α-spinasteryl benzoate (95 mg.), m.p. and mixed m.p. 200°; $[\alpha]_D$ + 4° (c, 1.4). (Found: C, 83.4; H, 10.0. Calc. for $C_{36}H_{52}O_2$: C, 83.7; H, 10.1%).

Elution with benzene (4.5 l.), benzene-ether mixtures (4.5 l.), and ether (1000 ml.) gave an intractible gum (2.7 g.). Elution with ether-methanol mixtures (2.5 l.) gave a fraction (10 g.) which crystallised from methanol in ill-defined crystalline masses, m.p. 150-160°. Acetylation of this material, with acetic anhydride (25 ml.) in pyridine (50 ml.) on the steam-bath (1 hr.), and crystallisation of the product (10.8 g.) from chloroform-methanol, gave α-spinasteryl acetate, m.p. and mixed m.p. 174-175°; $[\alpha]_D$ - 4° (c, 1.1).

The mother liquors from the crystallisations of the acetate fractions were combined and evaporated to dryness to give a clear gum (13 g.) which was dissolved in petrol-benzene (19:1, 250 ml.) and chromatographed on alumina (550 g.). Elution of the column with petrol-benzene mixtures gave three fractions which on crystallisation gave butyrospermyl acetate, taraxeryl acetate, and α-spinasteryl acetate respectivaly. Fractional crystallisation of the mother liquor residues from these acetates gave, besides a further small quantity of the aforementioned compounds, an acetate, m.p. 122-123°; $[\alpha]_D$ - 30° (c, 1.1) as large colourless blades (250 mg.) from chloroform-methanol. (Found: C, 81.8; H, 10.9. $C_{32}H_{52}O_2$ requires: C, 82.0; H, 11.2%). Hydrolysis of the acetate (150 mg.) by refluxing (2 hrs.) with 5% methanolic potassium hydroxide (25 ml.) gave the corresponding alcohol (100 mg.), m.p. 85-87°; $[\alpha]_D$ - 45° (c, 1.3). (Found: C, 84.1; H, 11.6. $C_{30}H_{50}O$ requires: C, 84.4; H, 11.8%). Both the alcohol and its acetate gave a red colour with a green fluorescence in the Liebermann-Burchardt test, and a strong yellow colour with tetranitromethane in chloroform. Both substances show a band at 2040 Å (ϵ = 8,500) in the ultraviolet.

Isolation of Aescin and the Prosapogenin Mixture.- Dry, crushed, defatted horse chestnuts (6 kg.) were extracted (3 x 8 hrs.) with boiling 75% (v/v) methylated spirits (3 x 12 l.). Evaporation of the combined extracts to dryness gave a dark, sticky, honey-like mass of crude aescin. The crude glycoside was dissolved in hot water and the volume of the solution was made up to 4 litres with hydrochloric acid of such a strength as to bring the total acid concentration to 5%. Almost immediately a heavy, dark brown precipitate began to form. The mixture was warmed on the steam-bath (1 hr.) with occasional stirring, diluted slowly with hot water (4 hrs.) while stirring vigorously, and then allowed to cool slowly overnight. The supernatant liquor was decanted and the soil-like residue filtered off. After washing with boiling water (12 l.), and drying at 110°, the yield of dark brown prosapogenins was 300 g.

Acid Hydrolysis of the Prosapogenin Mixture.- a) The crude prosapogenin mixture (300 g.) was refluxed (3 days) with 5% alcoholic hydrochloric acid (318 ml. conc. hydrochloric acid and 2.5 l. methylated spirits). The hot solution was filtered free from a dark mauve coloured amorphous solid (93 g.) and the deep red filtrate was stirred into warm 10% aqueous potassium hydroxide (4 l.). After adding warm water (2 l.), the mixture was left overnight and then filtered. The precipitate was washed with near boiling water (2 l.), sucked dry, and then refluxed (3 x 2 hrs.) with methanol (1.5 l.), potassium hydroxide (64 g.; 5%), water (25 ml.) and charcoal (3 x 10 g.). After filtering off the charcoal, hot water was added to the clear red solution until a faint permanent cloudiness was produced. On cooling a yellow crystalline solid (35 g.)

separated. Further dilution of the mother liquors (to ca. 4 l.) caused precipitation of a second crop of yellow solid (26 g.) which was filtered off with difficulty. The combined, dry, precipitates (61 g.) were dissolved in pyridine (400 ml.) and heated on the steam-bath (1 hr.) with acetic anhydride (200 ml.), then worked up in the normal manner, through ether, and the product dissolved in hot methanol (250 ml.). On cooling, a hard crystalline mass of crude aescigenin tetra-acetate (15 g.), m.p. 195-198°, separated. Concentrating the mother liquors (to ca. 75 ml.) gave a second crop (3 g.) of crystals consisting of crude isoaescigenin penta-acetate, m.p. 280-300°. No further crystalline material was obtained by concentrating the acetate mother liquor.

Evaporation of the acetate mother liquor to dryness gave a dark red resin (52 g.) which was dissolved in petrol-benzene (5:2, 250 ml.) and chromatographed on alumina (1 kg.). Elution with petrol-benzene mixtures (5 l.) gave only coloured, intractible, gums (4 g.). Elution with benzene (3 l.) and benzene-ether mixtures (8 l.) gave a yellow resinous solid (43 g.) which failed to crystallise. Further elution, with ether, methanol, and their mixtures, gave only highly coloured intractible gums (3 g.). The yellow resin (43 g.) was refluxed (3 hrs.) with 10% methanolic potassium hydroxide (500 ml.). Addition of water to the hydrolysate caused precipitation of a pale yellow amorphous solid which was filtered off and crystallised from aqueous ethanol to give colourless prisms of the true aglycone (36 g.), m.p. 300-302°; $[\alpha]_D$ + 31° (c, 0.8 in ethanol). ($\lambda_{max.}$ 2040 Å; ε = 5,000). The infrared absorption spectrum of the aglycone shows a strong band at 3380 cm.$^{-1}$ (hydroxyl) and a moderately strong band at 855 cm.$^{-1}$ (trisubstituted double bond). There is no absorption in the carbonyl

region, nor at 1110 cm.$^{-1}$ (ether link). (Found: C, 71.3; H, 10.0. $C_{30}H_{50}O_6$ requires: C, 71.1; H, 9.95%). The aglycone is moderately soluble in methanol and ethanol but is insoluble in almost all other common solvents. Acetylation of the pure aglycone gave an amorphous penta-acetate, $[\alpha]_D$ + 41° (c, 1.3), the infrared absorption spectrum of which showed a moderately strong band at 3333 cm.$^{-1}$ (hydroxyl). (Found: C, 67.2; H, 8.3. $C_{40}H_{60}O_{11}$ requires: C, 67.0; H, 8.4%). The acetate was recovered unchanged after refluxing with acetic anhydride, with acetic anhydride and pyridine, and with p-toluene-sulphonic acid and acetic anhydride. The benzoate, methanesulphonate, and p-toluenesulphonate of the aglycone failed to crystallise but were readily hydrolysed to starting material by refluxing with methanolic potassium hydroxide. Treatment of the aglycone with acetaldehyde in the presence of a trace of concentrated sulphuric acid gave a resinous material from which the aglycone was recovered by refluxing with 5% methanolic sulphuric acid.

Several recrystallisations of the crude aescigenin tetra-acetate, from chloroform-methanol, gave colourless platelets (10 g.), m.p. 204-205°; $[\alpha]_D$ + 60° (c, 1.5). Ruzicka and his co-workers[9] reported m.p. 207-208°; $[\alpha]_D$ + 58° ± 1° for this compound. (Found: C, 69.4; H, 8.8. Calc. for $C_{38}H_{56}O_9$: C, 69.5; H, 8.6%).

Recrystallisation of the crude isoaescigenin penta-acetate from chloroform-methanol gave colourless prisms (1.2 g.), m.p. 317-318°; $[\alpha]_D$ - 9° ± 1° (c, 1.1). Ruzicka and his co-workers[8] reported m.p. 321°; $[\alpha]_D$ - 6° ± 5° for this compound. (Found: C, 68.4; H, 8.5. Calc. for $C_{40}H_{58}O_{10}$: C, 68.7; H, 8.4%).

b) The crude prosapogenin mixture (300 g.) was refluxed (12 hrs.) with 5% hydrochloric acid in methylated spirits (3 l.). The hot

solution was filtered from a deep red amorphous solid (88 g.) and the filtrate was stirred into warm 10% aqueous potassium hydroxide (6 l.). The mixture was allowed to stand overnight at room temperature and then filtered. The precipitate was washed with near boiling water (2 l.) and then refluxed (3 x 2 hrs.) with 5% methanolic potassium hydroxide (1.5 l.) and charcoal (3 x 10 g.). The charcoal was filtered off and hot water was added to the boiling filtrate until a faint permanent cloudiness was produced. On cooling, a yellow crystalline solid (32 g.) separated. A second crop (25 g.) was obtained by addition of a further quantity of water (1.5 l.) to the mother liquor. The combined precipitates (57 g.) were dissolved in pyridine (300 ml.) and heated on the steam-bath (1 hr.) with acetic anhydride (150 ml.). After working up in the normal manner a dark red resin was obtained. No crystalline material could be isolated from this resin which was chromatographed on alumina as in a) above (p. 134). The bulk of the eluted material was a yellow resin (54 g.) which was hydrolysed by refluxing (3 hrs.) with 10% methanolic potassium hydroxide (500 ml.). Addition of water (2 l.) to the hydrolysate gave a yellow amorphous solid which was crystallised from aqueous ethanol to give colourless prisms of the aglycone (48 g.), m.p. and mixed m.p. 300-302°; $[\alpha]_D$ + 30° (c, 1.0 in ethanol).

Action of Mineral Acid on the Aglycone.- a) The aglycone (10 g.) was refluxed (24 hrs.) with 5% hydrochloric acid in ethanol (300 ml.). Water (1000 ml.) was added to the reaction mixture and the precipitate was filtered off, washed free from acid with water, and dried (9.5 g.). The precipitate was acetylated on the steam-bath (1 hr.) with acetic anhydride (50 ml.) and pyridine (100 ml.), then worked up in the

normal manner to give a colourless resin from which no crystalline material could be isolated. The resin was refluxed (3 hrs.) with 5% methanolic potassium hydroxide (200 ml.) and the product was crystallised from the hydrolysate by adding water until a faint permanent cloudiness formed. Recrystallisation from aqueous ethanol gave the aglycone (8.3 g.), m.p. and mixed m.p. 300-302°; $[\alpha]_D$ + 30° (c, 0.9 in ethanol).

b) The aglycone (10 g.) was refluxed with ethanolic mineral acid as in a) above, for three days. The acetylated product was dissolved in methanol (150 ml.) and on standing, a crystalline solid slowly separated. After three days the solid was filtered off and recrystallised from chloroform-methanol to give colourless platelets of aescigenin tetra-acetate (2 g.), m.p. 205°; $[\alpha]_D$ + 60° (c, 1.3). Concentration of the crude acetate mother liquor (to ca. 50 ml.) gave a second crop, recrystallisation of which, from chloroform-methanol, gave isoaescigenin penta-acetate (250 mg.), m.p. 317-318°; $[\alpha]_D$ - 9° (c, 0.8).

Hydrolysis of the crude acetate mother liquor, by refluxing with the addition of 10% methanolic potassium hydroxide (50 ml.), followed by working up in the normal manner (by precipitation), gave a cream coloured solid (3.5 g.), m.p. 250-290°. This solid was repeatedly crystallised from aqueous ethanol to give the aglycone (1.2 g.), m.p. 300-302°; $[\alpha]_D$ + 30° (c, 0.8 in ethanol).

c) The aglycone (10 g.) was refluxed with ethanolic mineral acid, as in a) above, for seven days. The acetylated product slowly deposited a crystalline solid, from its solution in methanol. Recrystallisation from chloroform-methanol gave colourless prisms of isoaescigenin penta-acetate (95 mg.), m.p. 316-318°; $[\alpha]_D$ - 8° (c, 0.9).

No further crystalline material was obtained from the acetate mother liquor. Alkaline hydrolysis gave a dark gum from which no crystalline material could be isolated.

d) A solution of the amorphous aglycone penta-acetate (1 g.) in acetic acid (50 ml.) was refluxed (48 hrs.) with concentrated hydrochloric acid (5 ml.), then evaporated to dryness in vacuo. The residue was dissolved in pyridine (15 ml.) and heated on the steam-bath (1 hr.) with acetic anhydride (5 ml.). After working up in the usual manner the product was dissolved in methanol and stored in the refridgerator (7 days). The crystalline deposit (200 mg.), m.p. 180-230°, was fractionally crystallised from chloroform-methanol to yield aescigenin tetra-acetate (55 mg.), m.p. 204-205°; $[\alpha]_D$ + 59° (c, 1.5), and isoaescigenin penta-acetate (40 mg.), m.p. 316-318°; $[\alpha]_D$ - 9° (c, 0.8). No further crystalline material was obtained from the crude acetate mother liquors, even after hydrolysis of the solute.

Pyrolysis of the Aglycone with Copper Bronze.- An intimate mixture of the aglycone (1 g.) and copper bronze (5 g.) was heated (90 mins.) at 300-315° (metal-bath temperature). The evolved gases were bubbled through a saturated aqueous solution of dimedone (10 ml.) which was then heated on the steam-bath (2½ hrs.). After standing overnight at room temperature the solution was filtered and the residue (8 mg.) was recrystallised from ethanol to give colourless needles of formaldehyde-dimedone, m.p. and mixed m.p. 190-191°. Extraction of the copper bronze residue with ether gave a solid froth (890 mg.) from which no crystalline material could be obtained, even after careful chromatography.

Action of Sodium Metaperiodate on the Aglycone.-[54] A standard (0.03M) solution of sodium metaperiodate was prepared by by dissolving sodium metaperiodate trihydrate (803 mg.) (prepared according to Hill[55]) in water (42 ml.) and making the volume up to 100 ml. by the addition of ethanol. The aglycone (30.4 mg.) was dissolved in ethanol (10 ml.) and water (7 ml.) was added. The optical density of an aliquot of this solution, diluted 500 times with water, was then measured, against water as blank, at 2230 Å. The optical density of an aliquot of the standard sodium metaperiodate, diluted 500 times with water, was also measured at 2230 Å. To the aglycone solution was added 17 ml. of the standard sodium metaperiodate and the reaction mixture was then stored in the dark at room temperature. At intervals, aliquots of the reaction mixture were removed, diluted 250 times with water, and the optical density was measured at 2230 Å. The consumption of sodium metaperiodate was calculated according to Dixon and Lipkin.[54]

The consumption of sodium metaperiodate by the aglycone was: after 24 hours, 0.74 mole; after 2 days, 1.02 mole; after 3 days, 1.15 mole; after 14 days, 1.25 mole.

Action of Phosphorus Oxychloride and Pyridine on the Aglycone Penta-acetate.- The amorphous aglycone penta-acetate (1.27 g.) was dissolved in pyridine (13 ml.) and refluxed (2 hrs.) with redistilled phosphorus oxychloride (13 ml.). After cooling to room temperature and carefully adding crushed ice (ca. 80 ml.), the reaction mixture was worked up in the normal manner to give a solid froth (1.04 g.) which was dissolved in petrol-benzene (1:1, 12 ml.) and chromatographed on alumina (35 g.). Elution of the column with petrol-benzene mixtures (450 ml.) and with benzene (200 ml.) removed a pale yellow intractible

gum (140 mg.). Elution with benzene-ether mixtures (250 ml.) gave a fraction (360 mg.) which crystallised from chloroform-methanol in colourless hexagonal plates of a compound (115 mg.), m.p. 253-254°; $[\alpha]_D$ + 11° ± 1° (c, 1.1). (Found: C, 68.4; H, 8.2. $C_{40}H_{58}O_{10}$ requires: C, 68.7; H, 8.4%). The compound gave a yellow colour with tetranitromethane in chloroform and showed an absorption band at 2040 Å (ε = 7,500).

The compound (50 mg.) was unchanged (45 mg. recovered) after refluxing (20 hrs.) with hydrochloric acid (2 ml.) in acetic acid (20 ml.).

Aescigenin (XX).- Aescigenin tetra-acetate (6.5 g.) was refluxed (1 hr.) with 5% potassium hydroxide in methanol (200 ml.). After concentrating to ca. 75 ml., water was added until a faint permanent cloudiness was formed. On cooling, the solution set to a microcrystalline mass which was filtered off, washed with water (100 ml.) and recrystallised from aqueous ethanol to give colourless microneedles of aescigenin (5 g.), m.p. 320-321°; $[\alpha]_D$ + 60° (c, 0.9 in ethanol). The melting point varied considerably according to the pressure; in an open tube the melting point was 310-311° (decomp.); sealed at 0.1 m.m., m.p. 317-318°; sealed at 0.05 m.m., m.p. 320-321°. Ruzicka and his co-workers[9] reported m.p. 317-318°; $[\alpha]_D$ + 46° ± 2° (in ethanol). (Found: C, 73.4; H, 10.1. Calc. for $C_{30}H_{48}O_5$: C, 73.7; H, 9.9%). Aescigenin shows a moderately strong band at 1110 $cm.^{-1}$ in the infrared.

The benzoate, methanesulphonate, and p-toluenesulphonate of aescigenin failed to crystallise but the parent alcohol was readily regenerated by alkaline hydrolysis of the amorphous esters.

Aescigenin Ditrityl Ether Diacetate.- A solution of aescigenin (250 mg.) and triphenylmethylchloride (750 mg.) in dioxane (10 ml.)

and pyridine (10 ml.), heated on the steam-bath (8 hrs.) and worked up through chloroform, gave an amorphous solid, m.p. 120-140°. Chromatography of a benzen solution of this solid on alumina (30 g.) gave two fractions. Elution with benzene (700 ml.) and benzene-ether mixtures (500 ml.) gave a fraction (400 mg.) which crystallised from methanol in colourless blades of triphenylmethanol (370 mg.), m.p. and mixed m.p. 162-163°. Elution of the column with chloroform--benzene (1:1, 200 ml.) gave an intractible gum (220 mg.) which was acetylated and dissolved in hexane. The hexane solution slowly deposited aescigenin ditrityl ether diacetate as a white amorphous solid, m.p. 130-145°; $[\alpha]_D$ + 48° (c, 1.3). (Found: C, 81.6; H, 7.8. $C_{72}H_{82}O_7$ requires: C, 81.6; H, 7.8%).

Refluxing a solution of the trityl ether acetate (100 mg.) in 5% methanolic hydrochloric acid (50 ml.) for 5 hours followed by precipitation of the product with water and crystallisation from ethanol, gave aescigenin (75 mg.), m.p. and mixed m.p. 320-321°.

bis-Ethylidene Aescigenin.- Aescigenin (250 mg.) was suspended in dry ether (25 ml.) and shaken (4 hrs.) at room temperature with acetaldehyde (4 ml.) and concentrated sulphuric acid (5 drops). The then clear solution was washed with saturated aqueous sodium bicarbonate, with water, and then dried over anhydrous sodium sulphate. Evaporation of the ether solution to dryness gave a solid froth which slowly crystallised from methanol in colourless needles of bis-ethylidene aescigenin (190 mg.), m.p. 269-270°; $[\alpha]_D$ + 23° (c, 1.3). Ruzicka and his co-workers[9] reported m.p. 270-271°; $[\alpha]_D$ + 21° ± 1°. (Found: C, 75.4; H, 9.7. Calc. for $C_{34}H_{52}O_5$: C, 75.5; H, 9.7%).

bis-Benzylidene Aescigenin.- Aescigenin (250 mg.) was shaken (4 hrs.) at room temperature with redistilled benzaldehyde (10 ml.) and concentrated sulphuric acid (10 drops). The reaction mixture was worked up through ether in the normal manner to give a clear gum which crystallised from methanol in colourless needles (220 mg.) of bis-benzylidene aescigenin, m.p. 260-261°; $[\alpha]_D$ + 34° (c, 1.1). Ruzicka and his co-workers[9] reported m.p. 260-262°; $[\alpha]_D$ + 35° ± 2°. (Found: C, 79.3; H, 8.2. Calc. for $C_{44}H_{56}O_5$: C, 79.5; H, 8.5%).

Action of N-Bromosuccinimide on Aescigenin Tetra-acetate.- Aescigenin tetra-acetate (100 mg.) was refluxed (8 hrs.) with N-bromosuccinimide (60 mg.) in dry carbon tetrachloride (20 ml.). The cooled reaction mixture was filtered free from succinimide then washed with water (40 ml.), 5% aqueous potassium hydroxide (40 ml.), and with 10% aqueous sodium thiosulphate (40 ml.). Evaporation of the dried solution to dryness gave a yellow solid, the ultraviolet absorption spectrum of which showed a broad band from 2000 Å to 3000 Å. Chromatography of a benzene solution of the product gave a single crystallisable fraction (70 mg.) identical with aescigenin tetra-acetate (m.p., mixed m.p., and infrared).

Action of Selenium Dioxide on Aescigenin Tetra-acetate.- a) A solution of aescigenin tetra-acetate (100 mg.) in acetic acid (20 ml.) was refluxed (16 hrs.) with selenium dioxide (100 mg.). The crude product, obtained by working up the reaction mixture through ether in the normal manner, showed general absorption, from 2200 Å to 3000 Å, in the ultraviolet, of low intensity with a maximum at ca. 2500 Å (ε = 1,200). Chromatography of a benzene solution of the crude product on alumina gave a single crystallisable fraction (80 mg.) identical

with aescigenin tetra-acetate (m.p., mixed m.p., and infrared).

b) Aescigenin tetra-acetate (100 mg.) and selenium dioxide (100 mg.) were refluxed (24 hrs.) in benzyl acetate (25 ml.). The solution was evaporated to dryness in vacuo at 190° to give a dark gum which showed only general absorption in the ultraviolet. Chromatography of a solution of the crude product, on alumina, gave only one crystallisable fraction (50 mg.) identical with aescigenin tetra-acetate (m.p., mixed m.p., and infrared).

11-Oxoaescigenin Tetra-acetate (LXXXV).- A solution of chromium trioxide (100 mg.) in acetic acid (5 ml.) and water (1 ml.) was added over a period of 10 minutes to a solution of aescigenin tetra-acetate (250 mg.) in acetic acid (10 ml.). After 24 hours at room temperature the neutral product was worked up in the usual manner, dissolved in benzene (10 ml.) and chromatographed on alumina (8 g.). One major fraction was obtained and crystallised from chloroform-methanol to give colourless needles (150 mg.) of 11-oxoaescigenin tetra-acetate, m.p. 233-234°; $[\alpha]_D$ + 54° (c, 1.4). Ruzicka and his co-workers[9] reported m.p. 233-234°; $[\alpha]_D$ + 55.5°. (Found: C, 67.9; H, 7.9. Calc. for $C_{38}H_{54}O_{10}$: C, 68.0; H, 8.1%). ($\lambda_{max.}$ 2480 Å; ϵ = 11,700).

11-Oxoaescigenin.- 11-Oxoaescigenin tetra-acetate (100 mg.) was refluxed (30 mins.) with 5% methanolic potassium hydroxide (25 ml.). Addition of water (50 ml.) caused precipitation of a white solid which was crystallised from aqueous ethanol to give colourless needles (30 mg.) of 11-oxoaescigenin, m.p. 337-338°; $[\alpha]_D$ + 37° ± 2° (c, 0.8 in ethanol), $\lambda_{max.}$ 2480 Å (ϵ = 11,000). (Found: C, 71.35; H, 9.1. $C_{30}H_{46}O_6$ requires: C, 71.7; H, 9.2%). Acetylation of the alcohol regenerated

11-oxoaescigenin tetra-acetate.

Aescigenin "O_{12}-Tetra-acetate" (LXXV).- a) 11-Oxoaescigenin tetra-acetate (500 mg.) was refluxed (15 mins.) with chromium trioxide (650 mg.) in stabilised acetic acid (50 ml.). The neutral product was isolated through ether in the normal manner and crystallised from chloroform-methanol in colourless needles (220 mg.) of the "O_{12}-tetra-acetate", m.p. 245-246°; $[\alpha]_D$ + 34° (c, 2.0), $\lambda_{max.}$ 2400 Å (ε = 11,000); inflexion 2960 Å (ε = 500); 1802 cm.$^{-1}$; 1740 cm.$^{-1}$; 1667 cm.$^{-1}$ (Found: C, 65.15; H, 7.1. $C_{38}H_{50}O_{12}$ requires: C, 65.3; H, 7.2%). The compound is chromatographically homogeneous, it does not give a colour with tetranitromethane in chloroform or with ethanolic ferric chloride but gives a deep purple colour with methanolic potassium hydroxide. Acidification of the alkaline solution destroys the colour but the product, isolated through ether in the normal manner, is an intractible gum together with a trace of the "O_{12}-tetra-acetate" which separated from a solution of the gum in methanol. Acetylation of this gum gave an intractible product. Refluxing the "O_{12}-tetra-acetate" with 10% methanolic hydrochloric acid gave an uncrystallisable gum which was not improved after acetylation.

b) A solution of aescigenin tetra-acetate (500 mg.) in stabilised acetic acid (50 ml.) was refluxed (15 mins.) with chromium trioxide (650 mg.). After working up through ether in the normal manner the neutral product crystallised from methanol in colourless needles of the "O_{12}-tetra-acetate", m.p. and mixed m.p. 245-246°; $[\alpha]_D$ + 34° (c, 1.3). (200 mg.).

Catalytic Hydrogenation of the "O_{12}-Tetra-acetate".- A solution of the "O_{12}-tetra-acetate" (250 mg.) in acetic acid (60 ml.) was

shaken (24 hrs.) with hydrogen, in the presence of a platinum catalyst (from 250 mg. platinum oxide), at room temperature and at atmospheric pressure. After filtering off the catalyst, the solution was evaporated to dryness under reduced pressure and the residue crystallised from chloroform-methanol to give a compound, m.p. 256-259°; $[\alpha]_D$ - 46° (c, 1.4), as colourless plates (190 mg.), $\lambda_{max.}$ 2450 Å (ε = 12,000); 3333 cm.$^{-1}$; 1740 cm.$^{-1}$; 1669 cm.$^{-1}$. (Found: C, 64.4; H, 7.8. $C_{38}H_{56}O_{12}$ requires: C, 64.75; H, 8.0%). This compound does not give a colour with tetra-nitromethane in chloroform.

A similar experiment, for 7 days, with the compound $C_{38}H_{56}O_{12}$ (100 mg.) and platinum (from 100 mg. platinum oxide) in acetic acid (30 ml.) gave an intractible gum which was chromatographically homogeneous and which gave a yellow colour with tetranitromethane in chloroform; $\lambda_{max.}$ 2040 Å (ε = 5,000); 1740 cm.$^{-1}$

Aescigenin "O_{12}-Tetra-acetate" Dioxime.- The "O_{12}-tetra-acetate (100 mg.) was heated (3 hrs.) on the steam-bath with hydroxylamine hydrochloride (50 mg.) and pyridine (4 ml.). The product was worked up through ether in the normal manner and crystallised from methanol to give the dioxime, m.p. 195-196°; $[\alpha]_D$ + 51° (c, 1.2), as colourless feathery needles (105 mg.); $\lambda_{max.}$ 2440 Å (ε = 11,000); 3390 cm.$^{-1}$; 1739 cm.$^{-1}$; 1667 cm.$^{-1}$ (Found: C, 62.45; H, 7.2. $C_{38}H_{52}O_{12}N_2$ requires: C, 62.6; H, 7.1%) (N, found: 3.4; requires: 3.8%).

16α:21α-Epoxy-12-oxo-13β-olean-3β:22β:24:28-tetrol Tetra-acetate (LXXXIV).- a) Hydrogen peroxide (100 vols.; 3 ml.) in acetic acid (3 ml.) was added over a period of 10 minutes, with stirring, to a solution of aescigenin tetra-acetate (500 mg.) in acetic acid (25 ml.)

at 100°. After 2 hours at that temperature a further quantity of hydrogen peroxide (100 vols.; 2 ml.) in acetic acid (2 ml.) was added and heating and stirring was continued for another hour. The reaction mixture was then poured into water and worked up through chloroform, in the normal manner, to give the ketone (160 mg.), m.p. 265-267°; $[\alpha]_D$ + 33° ± 1° (c, 1.5), as colourless platelets from chloroform-methanol. The compound does not give a colour with tetranitromethane in chloroform; $\lambda_{max.}$ 2810 Å (ϵ = 40); 1740 cm^{-1}; 1710 $cm.^{-1}$ (CaF_2 optics). (Found: C, 67.5; H, 8.4; O, 23.4. $C_{38}H_{56}O_{10}$ requires: C, 67.8; H, 8.4; O, 23.8%).

A solution of the ketone (100 mg.) in acetic acid (15 ml.) was heated (22 hrs.) on the steam-bath with concentrated hydrochloric acid (3 ml.). The reaction mixture was evaporated to dryness under reduced pressure and the residue left overnight at room temperature with pyridine (6 ml.) and acetic anhydride (3 ml.). After working up through ether in the normal manner, and crystallisation from chloroform-methanol, the product was the saturated ketone (85 mg.), m.p. and mixed m.p. 265-267°; $[\alpha]_D$ + 32° (c, 1.1).

b) Hydrogen peroxide (100 vols.; 3.25 ml.) in formic acid (17 ml.) was added, over a period of 90 minutes, to a stirred solution of aescigenin tetra-acetate (500 mg.) in ethyl acetate (20 ml.) at 45°. After 3 hours with stirring at 45°, the solution was poured into water and worked up through chloroform in the normal manner. Crystallisation of the resinous product, from methanol, gave the saturated ketone (250 mg.), m.p. 265-267°; $[\alpha]_D$ + 33° (c, 1.6).

16α:21α-Epoxy-12-oxo-13β-olean-3β:22β:24:28-tetrol.- The saturated ketone tetra-acetate (LXXXIV) (80 mg.) was refluxed (45 mins.) with 5% methanolic potassium hydroxide (25 ml.). Water (50 ml.) was added to the reaction mixture and the product was isolated by filtration. Crystallisation from aqueous methanol gave the keto-tetrol (45 mg.) as colourless needles, m.p. 338-340°; $[\alpha]_D$ + 24° (c, 0.7 in ethanol); $\lambda_{max.}$ 2800 Å (ϵ = 45); 1710 cm.$^{-1}$ (Found: C, 71.05; H, 9.5. $C_{30}H_{48}O_6$ requires: C, 71.4; H, 9.6%). Acetylation of the alcohol regenerated the keto-tetra-acetate, m.p. 265-267°; $[\alpha]_D$ + 33° (c, 1.1).

11β-Bromo-16α:21α-epoxy-12-oxo-13β-olean-3β:22β:24:28-tetrol Tetra-acetate (LXXIX).- A solution of bromine (131.5 mg.; 1 mole) in acetic acid (25 ml.) was added dropwise, with swirling, at room temperature to a solution of the saturated ketone tetra-acetate (LXXXIV) (500 mg.) in acetic acid (25 ml.) containing concentrated hydrobromic acid (2 drops). The pale yellow solution was then poured into water (100 ml.) and worked up through ether in the normal manner to give a solid froth which crystallised from methanol in colourless platelets (430 mg.) of the bromoketone, m.p. 242-243°; $[\alpha]_D$ + 11° (c, 1.2), $\lambda_{max.}$ 3100 Å (ϵ = 120). (Found: C, 60.95; H, 7.55; Br, 10.6. $C_{38}H_{55}O_{10}Br$ requires: C, 60.7; H, 7.4; Br, 10.6%).

Dehydrobromination of the Bromoketone (LXXIX).- a) A solution of the bromoketone (250 mg.) in collidine (20 ml.) was refluxed (30 mins.). On cooling, collidine hydrobromide separated and was removed by filtration. The filtrate was mixed with ether (50 ml.) and the ether solution was washed with 2N hydrochloric acid (3 x 50 ml.), saturated sodium bicarbonate solution (50 ml.), and water (50 ml.). Evaporation of

the dried ether solution to dryness, followed by crystallisation of the product from methanol, gave 16α:21α-epoxy-12-oxo-13β-olean--9(11)-en-3β:22β:24:28-tetrol tetra-acetate (LXXXVI) (160 mg.), m.p. 248-250°; $[\alpha]_D$ + 65° ± 2° (c, 1.3), $\lambda_{max.}$ 2470 Å (ε = 10,600); 1681 cm.$^{-1}$; 1740 cm.$^{-1}$ The compound does not give a colour with tetranitromethane in chloroform. (Found: C, 67.8; H, 8.4. $C_{38}H_{54}O_{10}$ requires: C, 68.0; H, 8.1%).

Refluxing the tetra-acetate (LXXXVI) (100 mg.) with 5% methanolic potassium hydroxide (20 ml.) for 45 minutes and isolation of the product by precipitation with water gave a solid, recrystallisation of which, from aqueous ethanol, gave 16α:21α-epoxy-12-oxo-13β-olean--9(11)-en-3β:22β:24:28-tetrol (65 mg.), m.p. 317-318°; $[\alpha]_D$ + 41° (c, 0.8 in ethanol), as colourless needles; $\lambda_{max.}$ 2470 Å (ε = 11,000); 1681 cm.$^{-1}$ The alcohol does not give a colour with tetranitromethane. (Found: C, 71.55; H, 9.1. $C_{30}H_{46}O_6$ requires: C, 71.7; H, 9.2%). Acetylation of the alcohol regenerated the tetra-acetate (LXXXVI), m.p. 248-250°; $[\alpha]_D$ + 66° (c, 1.8).

b) The crude bromoketone (2.2 g.) was refluxed (30 mins.) in collidine (50 ml.) and the product was isolated as in a) above. A solution of the product in methanol (ca. 40 ml.) deposited colourless plates (1.3 g.) of the α:β-unsaturated ketone (LXXXVI), m.p. 248-250°; $[\alpha]_D$ + 65° (c, 0.9). Concentrating the mother liquor (to ca. 10 ml.) gave pale yellow needles (55 mg.) of 11-bromo-16α:21α-epoxy-12-oxo--13β-olean-9(11)-en-3β:22β:24:28-tetrol tetra-acetate (LXXXVII), m.p. 232°; $[\alpha]_D$ + 162° (c, 1.3). The compound does not give a colour with tetranitromethane in chloroform; $\lambda_{max.}$ 2080 Å (ε = 6,000); 2920 Å (ε = 8,000); 1740 cm.$^{-1}$; 1670 cm.$^{-1}$ (Found: C, 60.8; H, 7.15; O, 20.9; Br, 11.0. $C_{38}H_{53}O_{10}Br$ requires: C, 60.9; H, 7.1; O, 21.3; Br, 10.7%).

Opening of the Oxide Ring in the α:β-Unsaturated Ketone (LXXXVI).- A solution of the α:β-unsaturated ketone (LXXXVI) (100 mg.) and p-toluenesulphonic acid (60 mg.) in acetic anhydride (5 ml.) was heated (30 mins.) at 117-120°. The product was isolated through chloroform, in the usual manner, and crystallised from chloroform--methanol to give colourless prisms (15 mg.), m.p. 310-312°; $[\alpha]_D$ + 35° ± 1° (c, 0.9), of the isoaescigenin analogue of the α:β-unsaturated ketone (LXXXVI); $\lambda_{max.}$ 2480 Å (ε = 12,000). (Found: C, 65.5; H, 7.6. $C_{40}H_{56}O_{12}$ requires: C, 65.9; H, 7.7%).

16α:21α-Epoxy-13β-olean-9(11)-en-3β:22β:24:28-tetrol Tetra-acetate (LXXXVIII).- A solution of the α:β-unsaturated ketone (LXXXVI) (100 mg.) in acetic acid (30 ml.) was shaken with platinum (from 100 mg. platinum oxide) at atmospheric pressure and at room temperature, for 48 hours. The catalyst was removed by filtration, the filtrate was evaporated to dryness under reduced pressure, and the residue crystallised from chloroform-methanol to give colourless leaflets (75 mg.) of the $\Delta_{9(11)}$-compound, m.p. 214-216°; $[\alpha]_D$ + 69° (c, 1.2), $\lambda_{max.}$ 2040 Å (ε = 5,000). The compound gives a pale yellow colour with tetranitromethane in chloroform. (Found: C, 69.5; H, 8.8. $C_{38}H_{56}O_9$ requires: C, 69.5; H, 8.6%).

The $\Delta_{9(11)}$-tetra-acetate (50 mg.) was refluxed (1 hr.) with 5% methanolic potassium hydroxide (25 ml.). The solution was poured into water (50 ml.), the precipitate was filtered off, washed with water (50 ml.) and crystallised from ethanol to give colourless needles of 16α:21α-epoxy-13β-olean-9(11)-en-3β:22β:24:28-tetrol (30 mg.), m.p. 327-329°; $[\alpha]_D$ + 51° (c, 1.0 in ethanol). (Found: C, 73.5; H, 9.9. $C_{30}H_{48}O_5$ requires: C, 73.7; H, 9.9%).

Acetylation of the alcohol regenerated the original acetate, m.p. 215-216°; $[\alpha]_D$ + 69° (c, 1.1).

16α:21α-Epoxyolean-9(11):12-dien-3β:22β:24:28-tetrol Tetra-acetate LXXXIX).- A solution of the α:β-unsaturated ketone (LXXXVI) (250 mg.) in dry ether (75 ml.) was refluxed (24 hrs.) with lithium aluminium hydride (500 mg.). The cooled reaction mixture was carefully treated with iced dilute hydrochloric acid, the ether layer removed, and the aqueous layer extracted with ethyl acetate (2 x 50 ml.). The combined extracts were washed with saturated aqueous sodium bicarbonate then dried and evaporated to dryness. The residue (130 mg.) was refluxed (2 hrs.) with sodium acetate (120 mg.) in acetic anhydride (50 ml.), then poured into water (100 ml.) and worked up through ether in the normal manner. The product was recrystallised several times from chloroform-methanol to give colourless leaflets of the homoannular diene (40 mg.), m.p. 203-205°; $[\alpha]_D$ + 240° (c, 1.3), $\lambda_{max.}$ 2800 Å (ε = 9,000). The compound gives a brown colour with tetranitromethane in chloroform. (Found: C, 69.6; H, 8.1. $C_{38}H_{54}O_9$ requires: C, 69.7; H, 8.3%).

A solution of the homoannular diene (25 mg.) in acetic acid (10 ml.) was refluxed (6 hrs.) with concentrated hydrochloric acid (2 ml.). The product, isolated in the normal manner through ether, showed general high intensity absorption in the ultraviolet between 2000 Å and 3000 Å. A methanol solution of the crude product slowly deposited colourless crystals of the starting material (10 mg.), m.p. 202-205°; $[\alpha]_D$ + 236° (c, 0.6). No crystalline material could be isolated from the mother liquor, even after careful chromatography.

Action of Selenium Dioxide on the α:β-Unsaturated Ketone (LXXXVI).- A solution of the α:β-unsaturated ketone (LXXXVI) (130 mg.) in acetic acid (30 ml.) was refluxed (22 hrs.) with selenium dioxide (150 mg.). The reaction mixture was cooled, filtered, and the filtrate poured into water (100 ml.) then worked up through ether in the normal manner. A solution of the dark coloured product in methanol slowly deposited colourless leaflets (45 mg.) which on recrystallisation gave the α:β-unsaturated ketone (LXXXVI), m.p. and mixed m.p. 248-250°; $[\alpha]_D$ + 66° (c, 1.1). The residue from the mother liquor (100 mg.) did not yield any crystalline material after careful chromatography.

Action of Periodates on Aescigenin.- a) A solution of aescigenin (30.0 mg.) in ethanol (10 ml.) and water (7 ml.) was treated with sodium metaperiodate as described on page 139 (cf. Dixon and Lipkin[54]). After 30 days no consumption of periodate was observed. The reaction mixture was boiled and water added until a faint permanent coludiness appeared; on cooling, colourless needles of aescigenin (25 mg.) separated, m.p. and mixed m.p. 320-321°.

b) A solution of periodic acid (100 mg.) in water (5 ml.) and ethanol (5 ml.) was added to a solution of aescigenin (25 mg.) in the same mixture of solvents. After 2 weeks, at room temperature in the dark, the reaction mixture was poured into water (25 ml.) and the precipitate was filtered off. Crystallisation from ethanol gave colourless needles (20 mg.) identical (m.p., mixed m.p., and infrared) with aescigenin.

Pyrolysis of Aescigenin with Copper Bronze.- An intimate mixture of aescigenin (0.5 g.) and copper bronze (2 g.) was heated (60 mins.)

at 300-315° (metal-bath temperature). The evolved gases were bubbled through a saturated aqueous solution of dimedone (5 ml.) which was afterwards heated on the steam-bath (3 hrs.). After standing overnight at room temperature the solution deposited colourless feathery needles (3 mg.) recrystallisation of which, from ethanol, gave formaldehyde-dimedone, m.p. and mixed m.p. 189-190°. The copper bronze residue was extracted with boiling ether and the extract evaporated to dryness to give a dark gum (430 mg.). No crystalline material could be obtained from this gum, even after careful chromatography. The infrared absorption spectrum of the non-volatile fraction showed a strong band at 1715 cm.$^{-1}$ indicative of the presence of a carbonyl group in a six membered ring. The ether absorption band at 1110 cm.$^{-1}$ was absent.

16α:21α-Epoxy-3:24:28-trioxo-olean-12-en-22β-ol (XCI).- The Kiliani reagent was made by dissolving sodium dichromate dihydrate (17.6240 g.) in water, adding dilute sulphuric acid (1:6, 7 ml.), and making the volume up to 100 ml. with water.

Aescigenin (250 mg.) was dissolved in stabilised acetic acid (20 ml.). The Kiliani reagent (1.127 ml.; 4 equivalents) was then added dropwise, with swirling. After 10 minutes at room temperature, methanol (5 ml.) was added and the reaction mixture was worked up through ether in the normal manner. The neutral product (150 mg.), which gave an oily precipitate with Brady's reagent, crystallised slowly from methanol to give colourless needles of the ketodialdehyde, m.p. 264-265°; $[\alpha]_D$ + 15° ± 3° (c, 1.2), $\lambda_{max.}$ 3400 cm.$^{-1}$; 1730 cm.$^{-1}$; 1715 cm.$^{-1}$ (Found: C, 74.5; H, 8.6. $C_{30}H_{42}O_5$ requires: C, 74.65; H, 8.8%).

isoAescigenin Penta-acetate.- a) Aescigenin tetra-acetate (2.5 g.) in acetic anhydride (75 ml.) was heated at 117-120° (oil-bath temperature) for 30 minutes with p-toluenesulphonic acid (1.25 g.). The reaction mixture was worked up in the normal manner through chloroform and the product crystallised from chloroform-methanol to give isoaescigenin penta-acetate (650 mg.) as colourless prisms, m.p. 317-318°; $[\alpha]_D$ - 9° (c, 1.2), $\lambda_{max.}$ 2040 Å ($\varepsilon \simeq 8{,}000$). (Found: C, 68.55; H, 8.45. Calc. for $C_{40}H_{58}O_{10}$: C, 68.7; H, 8.4%).

b) A solution of aescigenin tetra-acetate (500 mg.) in acetic acid (10 ml.) was mixed with boron trifluoride-acetic acid (5 ml.). After 3 weeks at room temperature the reaction mixture was worked up through chloroform in the normal manner and the product dissolved in chloroform-methanol. On cooling, colourless prisms of isoaescigenin penta-acetate (75 mg.) separated, m.p. 317-318°; $[\alpha]_D$ - 8° (c, 1.1). (Found: C, 68.6; H, 8.2%).

c) A solution of aescigenin tetra-acetate (100 mg.) in acetic anhydride (5 ml.) and concentrated hydrobromic acid (1 ml.) was stored at room temperature for 4 weeks, after which time large colourless prisms (10 mg.) had separated. The crystals were removed by filtration, combined with the material obtained by working up the reaction mixture through chloroform, and recrystallised from chloroform-methanol to give isoaescigehin penta-acetate (20 mg.), m.p. 317-318°; $[\alpha]_D$ - 9° (c, 1.2). (Found: C, 68.4; H, 8.4%).

isoAescigenin.- isoAescigenin penta-acetate (250 mg.) was refluxed (90 mins.) with 10% potassium hydroxide in methanol (15 ml.) and pyridine (25 ml.). The reaction mixture was diluted with water until a faint permanent cloudiness was observed. On cooling, colourless

needles of isoaescigenin (200 mg.) separated. Recrystallisation from ethanol gave isoaescigenin, m.p. 322-333°; $[\alpha]_D$ + 23° (c, 1.0 in ethanol). Ruzicka and his co-workers[8] reported m.p. 317-318°; $[\alpha]_D$ + 45° ± 2° (in pyridine). (Found: C, 73.4; H, 9.8. Calc. for $C_{30}H_{48}O_5$: C, 73.7; H, 9.9%).

The benzoate, methanesulphonate, and p-toluenesulphonate failed to crystallise but were readily hydrolysed by methanolic potassium hydroxide to regenerate isoaescigenin.

Action of Periodates on isoAescigenin.- isoAescigenin was treated with sodium metaperiodate and with periodic acid as described on page 151 for aescigenin. No consumption of periodate was observed over a period of 4 weeks.

Action of Selenium Dioxide on isoAescigenin Penta-acetate.- A solution of isoaescigenin penta-acetate (500 mg.) in benzyl acetate (20 ml.) was refluxed (18 hrs.) with selenium dioxide (500 mg.). The reaction mixture was evaporated to dryness under reduced pressure (oil pump) at 190°, the residue was dissolved in petrol (30 ml.)and chromatographed on alumina (30 g.). Elution with petrol (100 ml.) and petrol-benzene mixtures (800 ml.) gave dark coloured gums. Elution with benzene (400 ml.) and benzene-ether (19:1, 200 ml.) gave a fraction (200 mg.) which could not be crystallised. Further elution of the column gave only dark coloured gums.

The benzene and benzene-ether eluates were evaporated to dryness and the residue, a solid froth (200 mg.), was dissolved in petrol-benzene (3:2, 5 ml.) and rechromatographed on alumina (6 g.). A single fraction (190 mg.) was obtained as a white solid froth, $\lambda_{max.}$ 2900 Å (ϵ = 11,000);

1740 cm.$^{-1}$; shoulder 1690 cm.$^{-1}$; 1661 cm.$^{-1}$; 1637 cm.$^{-1}$; 1595 cm.$^{-1}$; 1316 cm.$^{-1}$.

Opening of the Oxide Ring in 11-Oxoaescigenin Tetra-acetate.- A solution of 11-oxoaescigenin tetra-acetate (200 mg.) in dry carbon tetrachloride (20 ml.) was refluxed (9 hrs.) with acetyl chloride (0.6 ml.) and a trace of anhydrous zinc chloride. On cooling, some crystalline material separated and was brought into solution by adding chloroform (25 ml.). The solution was washed with water (5 x 25 ml.), saturated aqueous sodium bicarbonate (2 x 25 ml.) and water (25 ml.), then dried and concentrated with the addition of methanol until crystallisation began. When cool, colourless prisms (25 mg.) of the isoaescigenin analogue were obtained, m.p. 333-334°; $[\alpha]_D$ - 9° (c, 0.9). Ruzicka and his co-workers[8] reported m.p. 337°; $[\alpha]_D$ - 9° ± 2°. (Found: C, 67.4; H, 8.1. Calc. for $C_{40}H_{56}O_{11}$: C, 67.4; H, 7.9%).

Oxidation of isoAescigenin Penta-acetate.- isoAescigenin penta-acetate (120 mg.) in stabilised acetic acid (10 ml.) was treated with a solution of chromium trioxide (80 mg.) in stabilised acetic acid (5 ml.). After 3 days at room temperature, the neutral product was isolated in the normal manner through chloroform and crystallised from chloroform-methanol in colourless prisms (20 mg.) of ketoisoaescigenin penta-acetate, m.p. 321-324°; $[\alpha]_D$ - 9° (c, 1.2). Ruzicka and his co-workers[8] reported m.p. 324-326°; $[\alpha]_D$ - 8° ± 2°. (Found: C, 67.1; H, 7.7. Calc. for $C_{40}H_{56}O_{11}$: C, 67.4; H, 7.9%).

A mixture of this compound and the isoaescigenin analogue of 11-oxoaescigenin tetra-acetate melted at 320-325°.

Opening of the Oxide Ring of the Saturated Ketone (LXXXIV).- A solution of the saturated ketone (LXXXIV) (100 mg.) in acetic anhydride (5 ml.) was heated (30 mins.) at 117-120° with p-toluene-sulphonic acid (50 mg.). After working up through chloroform in the normal manner, the product was crystallised from chloroform-methanol to give colourless prisms (20 mg.) of the isoaescigenin analogue of the saturated ketone, m.p. 322-325°; $[\alpha]_D$ + 7° ± 1° (c, 1.1). (Found: C, 67.05; H, 8.1. $C_{40}H_{58}O_{11}$ requires: C, 67.2; H, 8.2%).

isoAescigenin Penta-acetate Epoxide.- A solution of isoaescigenin penta-acetate (500 mg.) in formic acid (50 ml.) was heated to 45° and treated, with stirring, with a mixture of hydrogen peroxide (100 vols.; 10 ml.) in formic acid (25 ml.) added over a period of 1 hour. Heating and stirring were continued for a further 3 hours; the solution was then evaporated to dryness under reduced pressure and the residue dissolved in methanol. The filtered solution slowly deposited colourless prismatic needles (200 mg.) of the epoxide, m.p. 330-332°; $[\alpha]_D$ - 2° (c, 1.8), $\lambda_{max.}$ 1740 cm.$^{-1}$; 1087 cm.$^{-1}$; 928 cm.$^{-1}$; 840 cm.$^{-1}$. Ruzicka and his co-workers[8] reported m.p. 330-331°; $[\alpha]_D$ - 2° ± 2°. (Found: C, 66.9; H, 8.1. Calc. for $C_{40}H_{58}O_{11}$: C, 67.2; H, 8.2%).

Action of Mineral Acid on isoAescigenin Penta-acetate Epoxide.- isoAescigenin penta-acetate epoxide (65 mg.) was heated on the steam-bath (5 hrs.) with concentrated hydrochloric acid (1 ml.) and acetic acid (15 ml.). After working up in the normal manner through chloroform, starting material was recovered quantitatively. The epoxide was redissolved in acetic acid (5 ml.) and refluxed (3 hrs.)

with concentrated hydrochloric acid (2 ml.). The solution was evaporated to dryness under reduced pressure and the residue was acetylated by heating (1 hr.) on the steam-bath with acetic anhydride (1 ml.) and pyridine (2 ml.). After working up through chloroform the product was dissolved in methanol from which colourless crystals (2 mg.), m.p. 318-320° (decomp.), separated. This substance gave a strong positive test for halogen (Beilstein's copper foil test). No further crystalline material could be isolated from the reaction mixture, the ultraviolet spectrum of which showed only general, low intensity absorption.

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