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TRITERPENOIDS—I

THE STRUCTURE OF ISOAESCIGENIN. SOME DERIVATIVES OF AESCIGENIN

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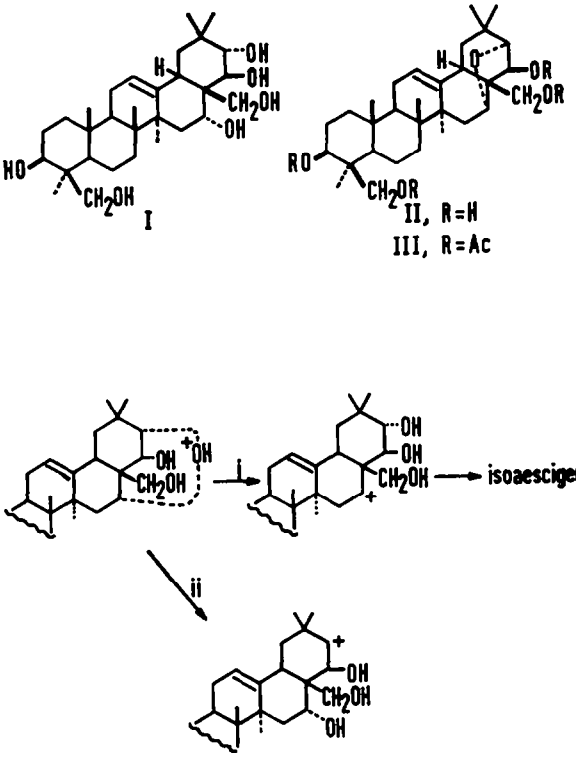
Abstract—Isoaescigenin is shown to be olean-12,15-diene-3β,21α,22β,24,28-pentol (V). Isoaescigenin oxide is the 12β,13β-epoxide (VII). Selenium dioxide oxidation of isoaescigenin penta-acetate gives 12,19-diketo-olean-9(11),13(18),15-triene-3β,21α,22β,24,28-pentol penta-acetate (X) and olean-9(11), 12,15,18-tetraene-3β,21α,22β,24,28-pentol penta-acetate (XI). A number of aescigenin derivatives, involving changes in ring C, are reported.

ACID hydrolysis of aescin,[1] a saponin from the seeds of the horse chestnut (*Aesculus hippocastanum* L.), gives protoaescigenin[2,3] (I) and, on prolonged heating, the artifact aescigenin[4] (II). Also present[5] in the hydrolysate, and formed when either protoaescigenin[2,5] or aescigenin[2,3,6] is refluxed with aqueous acoholic hydrochloric acid, is isoaescigenin, $C_{30}H_{48}O_5$. Isoaescigenin penta-acetate, $C_{40}H_{58}O_{10}$, is the major product when aescigenin tetra-acetate (III) is treated with a variety of ether-splitting reagents, *viz.* zinc chloride or aluminium chloride in acetyl chloride,[7] boron trifluoride in acetic anhydride[7] or in acetic acid,[2] hydrobromic acid in acetic anhydride,[2] or *p*-toluenesulphonic acid in acetic anhydride.[7] Isoaescigenin is most conveniently obtained by prolonged hydrolysis of protoaescigenin, being readily separated from the mixture* of products as the difficultly soluble penta-acetate.

Isoaescigenin penta-acetate gives a yellow colour with tetranitromethane but, on treatment with monoperphthalic acid, forms an oxide, $C_{40}H_{58}O_{11}$, which does not give a colour with this reagent. It was concluded,[7] therefore, that isoaescigenin is hexacyclic and contains one double bond.

* This series of compounds recalls the barringtogenols C (aescinidin[5]) (1) [A. K. Barua and P. Chakrabarti, *Tetrahedron* **21**, 381 (1965); R. Tschesche and G. Wulff, *Tetrahedron Letters* No. 21, 1569 (1965)] and D (2) [S. K. Chakraborti and A. K. Barua, *Tetrahedron* **19**, 1727 (1963)], and isobarringtogenol D (S. K. Chakraborti and A. K. Barua, *loc. cit.*). In view of the evidence presented here for the structure of isoaescigenin, isobarringtogenol D must be the diene (**3a**). The 100 Mc NMR spectrum[8] of isobarringtogenol D tetra-acetate (mol. wt. 640 by mass spectrometry[8]) is consistent with structure **3b**, *viz.* an AB quartet with doublets centred at τ 4·29 and 4·49 (J = 10·5 c/s) for H-15,H-16; an AB quartet with doublets centred at τ 4·62 and 5·05 (J = 2·5 c/s) for H-21β,H-22α; a multiplet centred at τ 4·61 for H-12; a triplet of multiplets at τ 5·49 (J_{app} = 8 c/s) for H-3α; and an A_2 singlet at τ 6·18 ($W_{1/2}$ = 3 c/s) for the 17β-acetoxy-methyl group.

[1] R. Tschesche, U. Axen and G. Snatzke, *Liebig's Ann.* **669**, 171 (1963) and Refs therein cited.
[2] J. B. Thomson, Ph.D. Thesis, Glasgow (1958).
[3] R. Kuhn and I. Löw, *Liebig's Ann.* **669**, 183 (1963).
[4] G. Cainelli, A. Melera, D. Arigoni and O. Jeger, *Helv. Chim. Acta* **40**, 2390 (1957).
[5] R. Kuhn and I. Löw, *Tetrahedron Letters* No. 15, 891 (1964).
[6] W. Hofer, Dissertation, Zürich (1948).
[7] L. Ruzicka, W. Baumgartner and V. Prelog, *Helv. Chim. Acta* **32**, 2069 (1949).
[8] R. Kuhn and I. Löw, private communication.



Cleavage of the ether ring in aescigenin may take place in two ways (paths *i* or *ii*). A decision in favour of path *i* was made by detection of a 1,2-glycol system in isoaescigenin. Although isoaescigenin reacts only slowly with sodium metaperiodate (0·2 mole consumed after 20 hr at room temp) in aqueous ethanol, it is rapidly cleaved[8] by periodic acid in pyridine–methanol.[9] The NMR spectra (see Table) of isoaescigenin penta-acetate and its derivatives show the expected[10] AB quartet in the τ5 region. The magnitude of the coupling constant ($J \sim 3$ c/s) does not enable a distinction to be made between H-21α and H-21β but the slow rate of reaction of isoaescigenin with aqueous alcoholic periodate indicates that the hydroxyl groups at C-21 and C-22 are *trans*-diaxial.

The NMR spectrum of isoaescigenin penta-acetate is highly informative and, indeed, leads directly to the proposed[11] structure (IV). In the low-field region the spectrum consists[12] of three AB quartets, a multiplet (H, τ 4·58) characteristic[10] of H-12 in pentacyclic Δ^{12}-triterpenes, a triplet of multiplets[13] (H, τ 5·39, $W_{1/2} = 17$ c/s)[10] for

[9] L. Cagliotti, G. Cainelli and F. Minutilli, *Gazzetta Chim. Ital.* **91,** 1387 (1961).

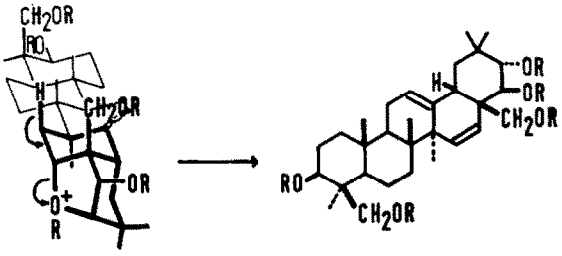
[10] M. Shamma, R. E. Glick and R. O. Mumma, *J. Org. Chem.* **27,** 4512 (1962).

[11] J. B. Thomson, *Tetrahedron Letters* No. 26, 2229 (1965).

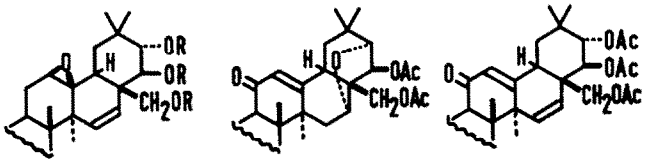
[12] Interpretation was facilitated by comparison of 60 and 80 Mc spectra and by calculation, for the AB quartets, of D (separation of the mid-points of the A and B doublets). See J. Parello, *Bull. Soc. Chim. Fr.* 2033 (1964).

[13] The multiplicity of this signal is due to virtual coupling with the C–1 protons. J. I. Musher and E. J. Corey, *Tetrahedron* **18,** 791 (1962).

the axial[14] H-3α and an A_2 singlet (τ 6·18) for the 17β-acetoxymethyl[15] group. The allylic H-11α and H-18β appear as a doublet of multiplets (τ 7·35) and a quartet (τ 7·72) respectivley. The six methyl signals are sharp singlets. The three AB quartets are: that discussed above for the protons on C-21 and C-22, that expected[15,16] for the 4β-



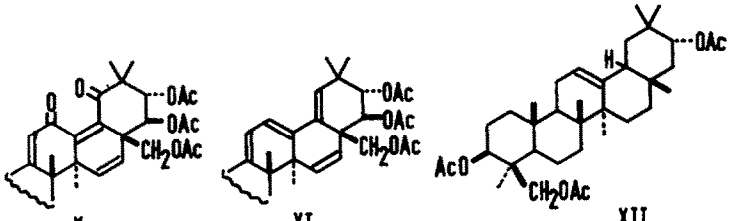
IV, R = Ac
V, R = H



VI, R = Ac
VII, R = H

VIII

IX



X

XI

XII

acetoxymethyl group (centred at τ 5·74), and another (centred at τ 4·4, J = 10·8 c/s) that must be attributed[17] to the vinylic protons of a disubstituted double bond in a six-membered ring. These structural features can be accommodated only in the pentacyclic formula (V) for isoaescigenin, formed by loss of the favourably positioned 15β-proton of aescigenin (II).

The "saturated" oxide,[7] prepared by treatment of isoaescigenin penta-acetate (IV) with monoperphthalic acid or, better, *m*-chloroperbenzoic acid,[18] is shown to be an epoxide (VI) and not a ketone by the absence of carbonyl absorption in the IR spectrum

[14] A. Hassner and C. Heathcock, *J. Org. Chem.* **29**, 1350 (1964).

[15] These assignments are based on the insensitivity of the downfield acetoxymethyl signal to changes in rings C, D and E while the upfield signal varies from one derivative to another (Table). A 4β-acetoxymethyl group usually appears as an AB quartet.[16]

[16] J. Polonsky, A. Gaudemer and E. Wenkert, *Bull. Soc. Chim. Fr.* 407 (1964).

[17] N. S. Bhacca and D. H. Williams, *Applications of NMR Spectroscopy in Organic Chemistry* p. 54. Holden-Day, San Francisco (1964).

[18] I thank FMC Corporation, New York, for a generous gift of this reagent.

of the corresponding alcohol (VII). Surprisingly, the same epoxide (VI) is obtained on treatment of isoaescigenin penta-acetate with warm performic acid. Under these conditions aescigenin tetra-acetate (III) readily forms the expected[19] 12-keto-12,13-dihydro derivative (XIII) (see below). That the oxide (VI) is a 12,13-epoxide is shown by the shift of the H-12 signal in the NMR spectrum from τ 4·58, for isoaescigenin penta-acetate, to τ 7·01 and by the absence of the H-11 and H-18 signals from the τ 7·3–7·8 region while the disubstituted double bond is retained (Table). The 12β, 13β configuration is assigned on the grounds that the change in the 17β-acetoxymethyl signal from an A_2 singlet, for the diene (IV), to an AB quartet, for the epoxide, suggests that the oxide ring is on the same side of the molecule as the acetoxymethyl group. The IR spectrum of the epoxide shows vinylic C—H stretching vibrations at 3001 and 3009 cm^{-1} and C=C stretching at 1645 cm^{-1} for the *cis*-disubstituted double bond.[20] These bands are also present in the spectrum of isoaescigenin itself together with a third C—H stretching vibration at 3027 cm^{-1} for the 12(13) double bond.[20] The epoxide (VII) absorbs in the UV at 204 mμ (ε 2,500) and isoaescigenin at 204 mμ (ε 8,000), clearly indicating that the latter is a non-conjugated diene.

Isoaescigenin cannot be hydrogenated under forcing conditions[7] nor can it be induced to consume a second mole of peracid (Experimental). Examination of models shows that the remarkable inertness of the 15(16) double bond is due to steric hinderance by (on the α-face) ring E, the 21α-hydroxyl (or acetoxyl) and the 14α-methyl groups, and (on the β-face) by the 8β-methyl and the 17β-hydroxymethyl (or acetoxymethyl) groups.

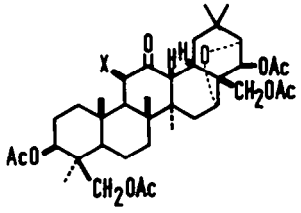
The presence of a 12(13) double bond in isoaescigenin has been demonstrated[7] from the identity of the ketone prepared by scission of the ether ring in 11-keto-aescigenin tetra-acetate (VIII) with that (IX) obtained on oxidation of isoaescigenin penta-acetate. In the NMR spectrum of 11-ketoisoaescigenin penta-acetate (IX) the H-12 signal is a singlet[21] at τ 4·33.

With the intention of studying the environment of the 12(13) double bond, isoaescigenin penta-acetate was treated with selenium dioxide in refluxing benzyl acetate.[19] The product is a complex mixture from which the 12,19-diketo-9(11),13(18),15-triene (X) and the 9(11),12,15,18,-tetraene (XI) are readily isolated by chromatography on alumina. The UV spectrum of the crude product showed the characteristic[22] triplet (244, 251 and 262 mμ) of an 11,13(18)-diene system, superimposed on general high-intensity background, but none of this product could be isolated. Due to the strong non-bonding interactions between the substituents on ring E, the chromophores in the trienedione (X) and the tetraene (XI) are considerably distorted from planar. This is reflected in the UV spectra where the absorption maxima (271 mμ for X and 301 mμ for XI) occur at somewhat shorter than usual[23] wavelengths. Confirmation of the structures of these oxidation products is found in their NMR spectra where the trienedione (X) shows three vinylic protons, the H-15,H-16 AB quartet and the H-11 singlet (τ 4·00), and the tetraene (XI) shows five vinylic protons, the H-15,H-16 AB

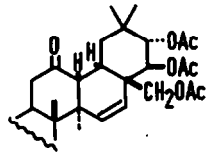
[19] H. M. Smith, J. M. Smith and F. S. Spring, *Tetrahedron* **4**, 111 (1958).
[20] A. R. H. Cole and D. W. Thornton, *J. Chem. Soc.* 1332 (1957).
[21] In a preliminary communication[11] this signal was erroneously reported as τ 4·17.
[22] J. Simonsen and W. C. J. Ross, *The Terpenes* Vol. IV, p. 196; Vol. V, p. 239. The University Press, Cambridge (1957).

quartet, the H-11,H-12 AB quartet (centred at τ 4·44, J = 12 c/s) and the H-19 singlet (τ 4·43). The tetraene (XI) has the expected[23] high (+345°) optical rotation and gives a brown colour with tetranitromethane.

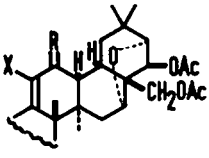
Olean-9(11),12,18-trienes may be prepared by dehydrogenation of olean-12-enes,[24] olean-9(11),12-dienes or olean-11,13(18)-dienes[25] with N-bromosuccinimide but do not



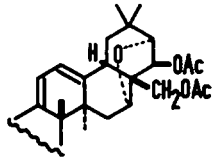
XIII, X = H
XV, X = Br



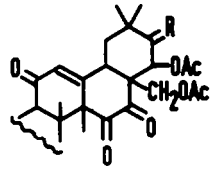
XIV



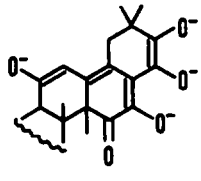
XVI, R = O, X = H
XVII, R = O, X = Br
XVIII, R = H_2, X = H



XIX



XX, R = O
XXI, R = H, OH



XXII

appear to have been detected previously as products of the oxidation of olean-12-enes by selenium dioxide, although it is known[25] that they are readily oxidised by the latter reagent to 9(11),13(18)-diene-12,19-diones. The formation of the trienedione (X) and the tetraene (XI), together with the tertiary nature of the methyl and acetoxymethyl groups in isoaescigenin penta-acetate (as shown by the NMR spectrum), excludes the possibility of any rearrangement having occurred on opening the ether ring of aescigenin.

Assignment of the methyl signals in the NMR spectra is not obvious (see Table, footnote *d*) with the possible exception of C-23. The 4α-methyl group is sufficiently isolated to be but little affected by changes in rings C, D and E and should be found near[26] τ 8·9. Of the possible signals, τ 8·86 $\pm$ 0·03 and $8{\cdot}95_5 \pm 0{\cdot}03_5$, the former is

[23] Ref. 22, Vol. IV, pp. 198 and 222.
[24] L. Ruzicka, O. Jeger and J. Redel, *Helv. Chim. Acta* **26**, 1235 (1943).
[25] G. T. Newbold and F. S. Spring, *J. Chem. Soc.* 532 (1944).
[26] Cf. the 4β(eq)-methyl group in beyerol derivatives. P. R. Jefferies, R. S. Rosich and D. E. White, *Tetrahedron Letters* No. 26, 1793 (1963).

TABLE 1. CHEMICAL SHIFTS[a] (τ-VALUES)[b] FOR

Compound	IV	VI
H-3	5·39(t of ms) J_{app} = 8 c/s; $W_{1/2}$ = 17 c/s	5·41(t of ms) J_{app} = 7·7 c/s; $W_{1/2}$ = 17 c/s
H-11	7·35(d of ms) J_{app} = 16·4 c/s	
H-12	4·58(m)	7·01(t) J_{app} = 2·5 c/s; $W_{1/2}$ = 5 c/s
H-15,H-16	4·51(d) 4·30(d) J = 10·8 c/s	4·45(d) 4·28(d) J = 10·5 c/s
H-18	7·72(q) J_1 = 9 c/s J_2 = 3 c/s	
H-19		
H-21,H-22[d]	5·04(d) 4·68(d) J = 2·8 c/s	5·03(d) 4·70(d) J = 3·3 c/s
CH_2 C-24	5·86(d) 5·62(d) J = 11·8 c/s	5·88(d) 5·67(d) J = 12·7 c/s
CH_2 C-28	6·18 $W_{1/2}$ = 3 c/s	6·09(d) 5·88(d) J = 12 c/s
Me[e] C-23	8·89	8·89
Me[e] C-25, 26, 27, 29 and 30	8·72 9·06 8·96 9·19 9·15	8·80 9·08 8·99 9·13 9·13
Me[e] Acetate	8·03(3H) 7·95(12H)	8·00(3H) 7·97(3H) 7·95(6H) 7·94(3H)

prefered as there is no shift on introduction of the epoxide ring. This signal (τ 8·87) also occurs in the spectrum[10] of soyasapogenol-B triacetate (XII).

The mass spectra of isoaescigenin[8] (V), its penta-acetate (IV), the epoxide[8] (VII) and protoaescigenin[8] (I) show molecular ions at *m/e* 488, 698, 504 and 506, respectively, together with species corresponding to loss of water or acetic acid from the molecular ion, e.g. *m/e* 638 $(M\text{-AcOH})^+$ and 578 $(M\text{-2AcOH})^+$ for the penta-acetate (IV). As expected,[27] retro-Diels-Alder fragments[28] are abundant at *m/e* 282 (*a'*) for protoaescigenin, at *m/e* 264 (*a*, R = H) for isoaescigenin and at *m/e* 390 (*a*, R = Ac) for the penta-acetate (IV). Fragment *a* is also formed by loss of water from *a'*. Loss of the C-17 substituent, *m/e* 233 (*a*-CH_2OH) and 317 (*a*-CH_2OAc), and of H-18 leads to the fully aromatic species, *m/e* 232 (*b*, R = H) and 316 (*b*, R = Ac). Very intense peaks at *m/e* 215, from I and V, and 257, from IV (loss of hydroxyl or acetoxyl radicals from species *b*) are probably the stable even-electron tropylium ions (*c*). A peak at *m/e* 128·5, for the doubly charged fragment (*c'* or a tropylium ion equivalent), is observed in the spectrum of the penta-acetate (IV). A moderately abundant fragment at *m/e* 197 is apparently formed from *c* (R = H or Ac) in a one-step process [metastable ions at *m/e* 180·6 (calc. for 215 → 197, 180·5) and 151·1 (calc. for 257 → 197, 151·0)] and may be the rearranged species (*d*). Fragments containing rings A and B

[27] H. Budzikiewicz, J. M. Wilson and C. Djerassi, *J. Amer. Chem. Soc.* **85**, 3688 (1963); H. Budzikiewicz, C. Djerassi and D. H. Williams, *Structure Elucidation of Natural Products by Mass Spectrometry* Vol. 2, pp. 121 *et seq.* Holden-Day, San Francisco (1964).

[28] In the absence of the appropriate labelling experiments the interpretation which follows is, of course, largely speculative. However, the structures and mechanisms proposed are plausible in the light of previous experience.[27] See also H. Budzikiewicz, C. Djerassi and D. H. Williams, *Interpretation of Mass Spectra of Organic Compounds.* Holden-Day, San Francisco (1964).

ISOAESCIGENIN PENTA-ACETATE DERIVATIVES

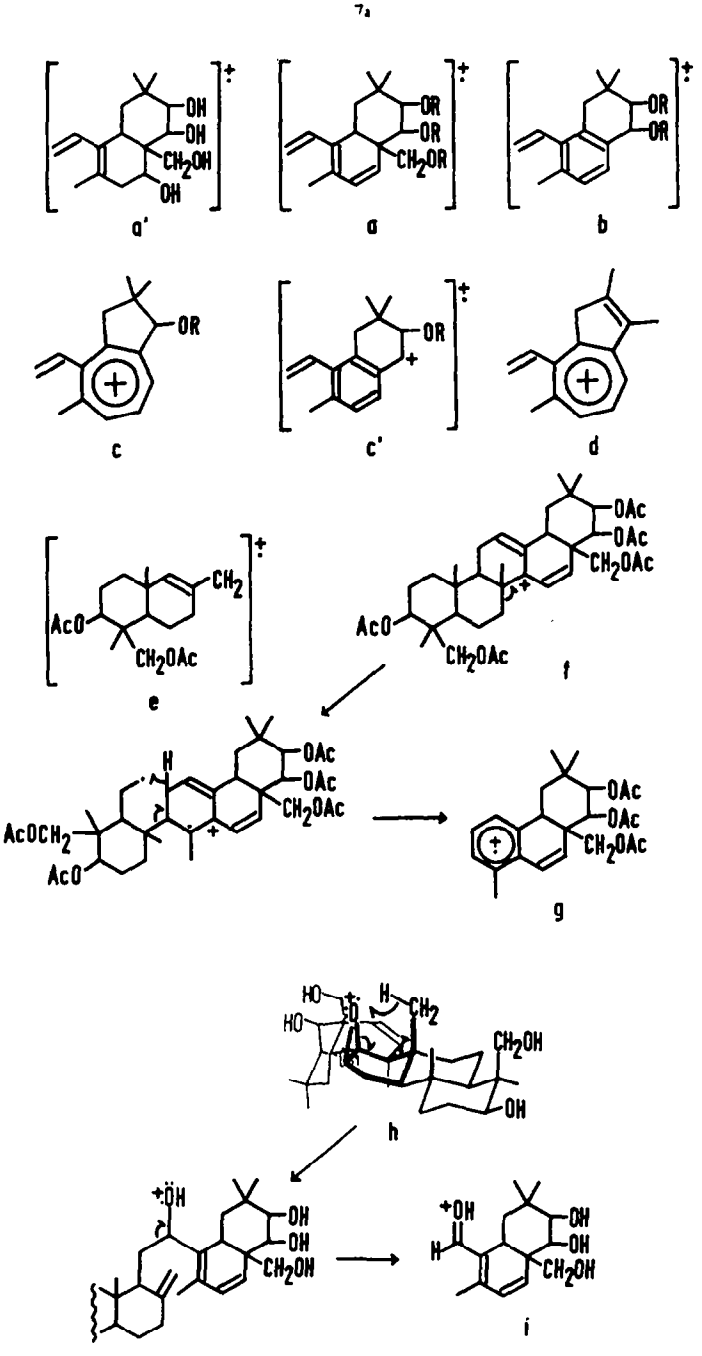
IX[c]	X	XI
5·4(m)	5·40(t of ms) J_{app} = 8·5 c/s; $W_{1/2}$ = 17 c/s	5·37(t of ms) J_{app} = 7·8 c/s; $W_{1/2}$ = 17 c/s
—	4·00	
4·33	—	4·51(d) 4·37(d) J = 12 c/s
4·6(d) 4·2(d) J ~ 10 c/s	4·47(d) 4·26(d) J = 10·2 c/s	4·25(d) 4·14(d) J = 10·2 c/s
7·7(m)	—	—
	—	4·43
5·0(d) 4·7(d) J ~ 3 c/s	4·90(d) 4·47(d) J = 3·9 c/s	4·75(d) 4·40(d) J = 2·5 c/s
5·8(d) 5·6(d) J ~ 12 c/s	5·80(d) 5·64(d) J = 11·8 c/s	5·82(d) 5·62(d) J = 12 c/s
6·1(m)	6·12(d) 5·85(d) J = 11·7 c/s	6·06(d) 5·97(d) J = 12 c/s
8·86	8·83	8·84
8·81 9·06 8·98 9·13 9·13 7·99(3H) 7·94(12H)	8·53 8·62 8·76 8·72 8·94 7·96(12H) 7·92(3H)	8·78 8·92 8·95 8·95 9·12 8·00(3H) 7·95(6H) 7·94(3H) 7·92(3H)

[a] Determined for $CDCl_3$ solutions with TMS as internal standard on Varian spectrometers. Multiplicities: d (Doublet), t (triplet), q (quartet), m (multiplet). All other signals indicated here are singlets. [b] True τ_A and τ_B are given for the AB quartets. [c] The low solubility of the ketone (IX) renders difficult the observation of signals other than Me and only approximate τ-values are given. [d] In all derivatives H-22α is closer to the double bonds, and to the planes of these double bonds, than H-21β and is, therefore, probably the more highly deshielded of the two. [e] The information at present available is not sufficient for precise assignment of the Me signals, with the exception of C-23 (see Text). The assignments (the signals are listed in the order C-25 . . . C-30) given here are tentative proposals based on a consideration of bond anisotropies. Because of the uncertainties involved in such considerations no discussion is warranted at the present time.

are observed at *m/e* 307 (*e*) and 247 (*e*-AcOH) in the spectrum of isoaescigenin penta-acetate. A feature of the spectrum of the penta-acetate (IV) is a moderately abundant fragment at *m/e* 414 which is formed in a one step process (metastable ion at *m/e* 251·4; calc. for 683 → 414, 250·95) from a species of low abundance at *m/e* 683 $(M\text{-Me})^+$. As the most labile methyl group is probably the doubly allylic C-27, the *m/e* 683 fragment may be represented as *f* and the *m/e* 414 fragment as *g*. In the spectrum of the epoxide (VII), the most abundant fragment occurs at *m/e* 267. This, assuming *h* to be the molecular ion, may be envisaged as the resonance stabilized species (*i*).

In the early stages of this work[2] a number of new derivatives of aescigenin (II) were prepared. Although not required for the determination of the structure of isoaescigenin, this opportunity is taken to report these compounds.

Treatment of aescigenin tetra-acetate with warm peracetic acid or, better, performic acid yields the ketone (XIII), ν_{max} 1710 cm^{-1}, λ_{max} 281 mμ (ε 40), which is unchanged


7a
OH
OH
CH2OH
OH
a'
OR
OR
CH2OR
a
OR
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c
OR
c'
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CH2
AcO
CH2OAc
e
OAc
OAc
CH2OAc
AcO
CH2OAc
f
H
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g
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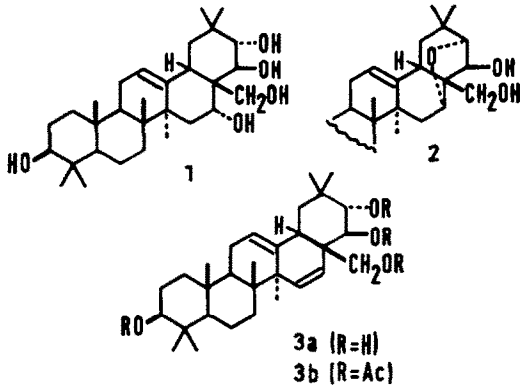
after treatment with acid or base followed by reacetylation.[29] The isoaescigenin analogue (XIV) of this ketone is obtained on scission of the ether ring with *p*-toluenesulphonic acid in acetic anhydride. Bromination of the ketone (XIII) furnishes the 11β-bromo derivatives (XV), λ_{max} 310 mμ (ε 120) ($\Delta\lambda = +29$ mμ, $\Delta\varepsilon = +80$),[30] which, with refluxing *s*-collidine, affords the α,β-unsaturated ketone (XVI), λ_{max} 247 mμ (ε 10,000), ν_{max} 1681 cm^{-1}. In some experiments, when the crude bromination product is treated with *s*-collidine, a small amount of the α-bromo-α,β-unsaturated ketone (XVII), λ_{max} 292 mμ (ε 8,000), ν_{max} 1670 cm^{-1}, is obtained. Hydrogenolysis of the ketone (XVI) gives the $\Delta^{9(11)}$-isomer (XVIII) of aescigenin tetra-acetate. Lithium aluminium hydride reduction of the α,β-unsaturated ketone (XVI), followed by treatment of the crude product with sodium acetate in refluxing acetic anhydride, yields the homoannular diene (XIX), λ_{max} 280 mμ (ε 9,000).



When aescigenin tetra-acetate (III) or 11-ketoaescigenin tetra-acetate (VIII) is heated briefly with chromium trioxide in acetic acid the product is an O_{12} tetra-acetate, $C_{38}H_{50}O_{12}$, which does not give a colour with tetranitromethane or alcoholic ferric chloride but which forms a purple solution with methanolic potassium hydroxide. This purple colour is discharged on acidification but only non-crystalline material can be isolated either before or after reacetylation. The O_{12} tetra-acetate is an α,β-unsaturated ketone, λ_{max} 240 mμ (ε 11,000), and shows strong IR absorption at 1667 (cyclohexenone), 1709 (cyclohexanone), 1740 (acetate) and 1802 cm^{-1}. Despite the remarkably high frequency,[31] the 1802 cm^{-1} band may be attributable to a non-enolisable cyclohexan-1,2-dione since it is absent in the spectrum of the dioxime, $C_{38}H_{52}N_2O_{12}$, which retains the cyclohexenone (λ_{max} 244 mμ, ε 11,000; ν_{max} 1667 cm^{-1}) and cyclohexanone (ν_{max} 1709 cm^{-1}) groupings. Catalytic reduction of the O_{12} tetra-acetate yields a dihydro compound, $C_{38}H_{52}O_{12}$, λ_{max} 245 mμ (ε 12,000), ν_{max} 1669 (cyclohexenone), 1740 (acetate), 1779 (cyclohexan-1,2-dione?), 3300 cm^{-1} (broad; bonded

[29] Cf. D. H. R. Barton and N. J. Holness, *J. Chem. Soc.* 78 (1952); G. G. Allan, F. S. Spring and R. Stevenson, *Ibid.* 3072 (1955).

[30] D. H. R. Barton and R. C. Cookson, *Quarterly Revs.* 10, 64 (1956).

[31] L. J. Bellamy, *The Infra-red Spectra of Complex Molecules* (2nd Edition) p. 141. Methuen, London (1958); see K. Alder, H. K. Schäfer, H. Esser, H. Krieger and R. Reubke, *Liebig's Ann.* 593, 23 (1955).

hydroxyl). The O_{12} tetra-acetate is probably the tetraketone (XX) and its dihydro derivative the triketone (XXI). The purple colour with alkali would then be due to the formation of a chromophore such as XXII.

EXPERIMENTAL

Specific rotations were determined in $CHCl_3$, unless otherwise stated, at room temp (16–22°). M.ps, which vary considerably according to the conditions, were determined in vacuum sealed capillaries in an electrically heated aluminium block. The samples were inserted ca. 30° below the m.p. and heated at ca. 4° per min. Analytical samples were dried for 60 hr at 78°/10^{-3} mm.

Isoaescigenin penta-acetate (IV)

(a) *From protoaescigenin.* A solution of protoaescigenin (10 g) in EtOH (300 ml) containing conc. HCl (50 ml) was refluxed (3 days) and poured into water (ca. 1 l). The precipitated solid was washed well with water, dried (vacuum desiccator) and heated (1 hr) on the steam-bath with acetic anhydride–pyridine. The product was isolated through ether in the usual way and dissolved in MeOH (150 ml). After 3 days at 0° a first crop of crystals was removed and recrystallized from $CHCl_3$–MeOH to yield III (2 g), m.p. 205°, $[\alpha]_D$ +60° (*c*, 1·5) (lit.[32] m.p. 207–208°, $[\alpha]_D$ +58 ± 1°). (Found: C, 69·4; H, 8·8. Calc. for $C_{35}H_{56}O_9$: C, 69·5; H, 8·6%.) Concentration of the crude acetate mother liquor (to ca. 50 ml) furnished a second crop recrystallization of which from $CHCl_3$–MeOH gave prisms (250 mg) of isoaescigenin penta-acetate, m.p. 318–319°, $[\alpha]_D$ −8° (*c*, 0·8) (lit.[7] m.p. 321°, $[\alpha]_D$ −6 ± 5°), λ_{max} (EtOH) 204 mμ (ε 8,000), ν_{max} (Nujol) 3027 (w), 3009 (w), 3001 (w) cm^{-1}, ν_{max} (KBr) 1731 (vs), 1655–1645 (w), 1235 (vs), 841 (w), 828 (w), 819 (w), 808 (w), 789 (w), 773 (w), 761 (m), 729 (w), 715 (m) cm^{-1}. (Found: C, 68·55; H, 8·45. Calc. for $C_{40}H_{58}O_{10}$: C, 68·7; H, 8·4%.) The remaining mother liquor was saponified with dil. methanolic KOH to yield, after crystallization from aqueous EtOH, protoaescigenin (1·2 g).

(b) *From aescigenin tetra-acetate using boron trifluoride in acetic acid.* Aescigenin tetra-acetate (500 mg) in acetic acid (10 ml) containing BF_3-acetic acid (5 ml) was left (3 weeks) at room temp, the solution was diluted with water, and the product isolated through $CHCl_3$ in the usual way. Crystallization from $CHCl_3$–MeOH gave isoaescigenin penta-acetate (75 mg), m.p. 317–318°, $[\alpha]_D$ −8° (*c*, 0·65).

(c) *From aescigenin tetra-acetate using hydrobromic acid in acetic anhydride.* A solution of aescigenin tetra-acetate (100 mg) in acetic anhydride (5 ml) containing conc. HBr (1 ml) was left at room temp for 4 weeks after which time large colourless prisms (10 mg) had separated. The crystals were collected, combined with the material obtained by working up the reaction mixture through $CHCl_3$, and recrystallized from $CHCl_3$–MeOH to yield isoaescigenin penta-acetate (20 mg), m.p. 317–319°, $[\alpha]_D$ −9° (*c*, 0·72).

Isoaescigenin (V)

Isoaescigenin penta-acetate (3·5 g) in dioxan (50 ml) or pyridine (50 ml) was refluxed (2 hr) with 10% methanolic KOH (50 ml) and water then added dropwise until the solution became faintly cloudy. After 24 hr the product was collected and recrystallized from EtOH to yield isoaescigenin (2·2 g), m.p. 330–332° (dec) (lit.[7] m.p. 317–318°), $[\alpha]_D$ +23° (*c*, 1·0 in EtOH), λ_{max} (EtOH) 204 mμ (ε 8,000), ν_{max} (Nujol) 3028 (w), 3009 (w), 3001 (w) cm^{-1}, ν_{max} (KBr) 3367 (s), 1655–1642 (w), 848 (w), 828 (w), 822 (w), 807 (w), 782 (m), 762 (m), 726 (m) cm^{-1}. (Found: C, 73·4; H, 9·8. Calc. for $C_{30}H_{48}O_5$: C, 73·7; H, 9·9%.) The analytical sample was dried for 10 days at 140°/10^{-3} mm.

Action of sodium metaperiodate on isoaescigenin

A solution of isoaescigenin (30·4 mg) in 55% (w/w) aqueous EtOH was mixed with an equal volume of 0·03M sodium metaperiodate[33] in the same solvent and stored in the dark at room temp. The consumption of periodate was followed spectrophotometrically.[34] After 20 hr 0·23 mole

[32] L. Ruzicka, W. Baumgartner and V. Prelog, *Helv. Chim. Acta* **32**, 2057 (1949).
[33] A. E. Hill, *J. Amer. Chem. Soc.* **50**, 2678 (1928).
[34] J. S. Dixon and D. Lipkin, *Analyt. Chem.* **26**, 1092 (1954); G. O. Aspinall and R. J. Ferrier, *Chem. & Ind.* 1216 (1957).

periodate had been consumed. Dilution of the reaction mixture with water and crystallization of the precipitate from EtOH gave isoaescigenin (18 mg). Under the same conditions, protoaescigenin and aescigenin consumed 0·74 and 0·01 mole periodate, respectively.

Isoaescigenin penta-acetate epoxide (VI)

(a) To isoaescigenin penta-acetate (150 mg) in $CHCl_3$ (10 ml) was added, with swirling, *m*-chloroperbenzoic acid[18] (108 mg) in $CHCl_3$ (5 ml). TLC showed that most of the starting material was consumed in the first few min. The solution was stored (5 hr) at room temp and then washed successively with 10% Na_2SO_3aq, 10% $NaHCO_3$aq and water. Concentration of the wet solution with slow addition of MeOH gave colourless needles (150 mg) of the epoxide, m.p. 331–332° (dec), $[\alpha]_D$ $-8 \pm 3°$ (*c*, 0·5) (lit.[7] m.p. 330–331°, $[\alpha]_D$ $-2 \pm 2°$), ν_{max} (Nujol) 3009 (w), 3001 (w) cm^{-1}, ν_{max} (KBr) 1740 (vs), 1647 (w), 1242 (vs), 1087 (s), 928 (m), 840 (w), 816 (w), 779 (m) cm^{-1}. (Found: C, 66·9; H, 8·3. Calc. for $C_{40}H_{58}O_{11}$: C, 67·2; H, 8·2%.) A sample heated (5 hr) with conc. HCl (1 ml) in acetic acid (15 ml) was unchanged (m.p. and IR).

(b) A mixture of isoaescigenin penta-acetate (50 mg) and *m*-chloroperbenzoic acid (100 mg) in $CHCl_3$ (20 ml) was refluxed for 10 days during which time fresh portions (4 × 50 mg) of peracid were added at 48 hr intervals. The product, isolated as above, was isoaescigenin penta-acetate monoepoxide (36 mg), m.p. 330–332°, $[\alpha]_D$ $-9°$ (*c*, 0·65), identical (IR) with the product described above. Careful chromatography (column and thin-layer) of the mother liquors yielded a further 7 mg monoepoxide but revealed no trace of a second epoxide.

(c) To a stirred solution of isoaescigenin penta-acetate (100 mg) in formic acid (10 ml) at 45° was added, over a period of 30 min, a mixture of H_2O_2 (100 vol, 2 ml) and formic acid (5 ml). Heating and stirring were continued for a further 3 hr, the solution was evaporated to dryness under red. press., and the residue was crystallized from MeOH to give the monoepoxide (20 mg), m.p. 330–332°, $[\alpha]_D$ $-5°$ (*c*, 0·58).

Isoaescigenin epoxide (VII)

Isoaescigenin penta-acetate epoxide (50 mg) in dioxan (3 ml) was refluxed (3 hr) with 5% methanolic KOH (5 ml) and water was then added until incipient crystallization. On cooling, the solution gave fine needles recrystallization of which from aqueous EtOH furnished *isoaescigenin epoxide* (30 mg), m.p. 328–330° (dec), $[\alpha]_D$ $+11°$ (*c*, 1·1 in EtOH), λ_{max} (EtOH) 204 mμ (ε 2,500), ν_{max} (Nujol) 3009 (w), 3001 (w) cm^{-1}, ν_{max} (KBr) 3534 sh, 3425 (vs), 3322 sh, 1645 (w), 1090 (s), 930 (m), 846 (w), 815 (w), 772 (m) cm^{-1}. (Found: C, 71·1; H, 9·5. Calc. for $C_{30}H_{48}O_6$: C, 71·4; H, 9·6%.) The analytical sample was dried for 6 days at 115°/10^{-3} mm. Acetylation of the alcohol regenerated isoaescigenin penta-acetate epoxide.

11-*Ketoisoaescigenin penta-acetate* (IX)

The ketone, prepared as described previously,[7] was obtained in 28% yield and had m.p. 324–326°, $[\alpha]_D$ $-9°$ (*c*, 0·6) (lit.[7] m.p. 324–326°, $[\alpha]_D$ $-8 \pm 2°$). (Found: C, 67·1; H, 7·7. Calc. for $C_{40}H_{56}O_{11}$: C, 67·4; H, 7·9%.)

Selenium dioxide oxidation of isoaescigenin penta-acetate

(a) A solution of isoaescigenin penta-acetate (117 mg) in acetic acid (20 ml) was refluxed (18 hr) with SeO_2 (200 mg) in water (0·5 ml). After work up through $CHCl_3$ in the usual way the product was crystallized from $CHCl_3$–MeOH to give material (100 mg), m.p. 315–318°, identical (m.p. and IR) with starting material.

(b) A mixture of isoaescigenin penta-acetate (2·02 g) and SeO_2 (2 g) in benzyl acetate (40 ml) was refluxed (24 hr), cooled and filtered. The residue was washed with $CHCl_3$ (ca. 20 ml) and the combined filtrate and washings evaporated to dryness under red. press. to yield a dark gum (2·78 g) which was dissolved in CH_2Cl_2 and chromatographed on alumina (Merck; 100 g). Elution with CH_2Cl_2, with TLC control (alumina G and CH_2Cl_2—for which system the R_f values are given below), gave gummy crystals (2 mg), R_f 0·92, that were not examined further, benzyl acetate (248 mg), R_f 0·85, isoaescigenin penta-acetate (22 mg), R_f 0·50, a resin (534 mg), R_f of major component ca. 0·4, and a gum (958 mg), R_f of major component ca. 0·15.

Rechromatography of the resin (R_f 0·4) and crystallization of the fractions from MeOH yielded material, m.p. 185–189°, which was sublimed at 180°/10^{-7} mm to give an amorphous film, m.p. ca. 160° then resolidifies and melts at 192–193°. Crystallization from aqueous MeOH furnished feathery needles (150 mg) of *olean*-9(11),12,15,18-*tetraene*-3β,21α,22β,24,28-*pentol penta-acetate* (XI), m.p. 192–192·5°, $[\alpha]_D$ +345° (*c*, 1·2), λ_{max} (EtOH) 301 mμ (ε 13,500), ν_{max} (KBr) 1735 (vs), 1647 (w), 1637 (w), 1235 (vs), 840 (m), 828 (m), 773 (m), 743 (w), 724 (w) cm^{-1}. (Found: C, 69·1; H, 7·8. Calc. for $C_{40}H_{54}O_{10}$: C, 69·1; H, 7·8%.)

Rechromatography of the gum (R_f 0·15), crystallization of the fractions from MeOH, and sublimation of the combined crystalline fractions at 220°/10^{-7} mm gave an amorphous solid, m.p. ca. 170° then resolidifies and melts at 221·5–222°. Crystallization from MeOH yielded either stout prisms or feathery needles (475 mg) of 12,19-*diketo-olean*-9(11),13(18),15-*triene*-3β,21α,22β,24,28-*pentol penta-acetate* (X), m.p. 221·5–222°, $[\alpha]_D$ +22° (*c*, 1·3), λ_{max} (EtOH) 271 mμ (ε 9,700), ν_{max} (KBr) 1747 sh, 1735 (vs), 1717 sh, 1688 (m), 1661 (vs), 1645 (m), 1596 (m), 1244 (vs), 1217 (vs), 786 (s), 703 (m) cm^{-1}. (Found: C, 66·2; H, 7·2. Calc. for $C_{40}H_{58}O_{12}$: C, 66·3; H, 7·2%.)

16α,21α-*Epoxy*-12-*keto*-13β-*oleanan*-3β,22β,24,28-*tetrol tetra-acetate* (XIII)

(a) To a stirred solution of aescigenin tetra-acetate (500 mg) in acetic acid (25 ml) at 100° was added, over a period of 10 min, H_2O_2 (100 vol, 3 ml) in acetic acid (3 ml). After 2 hr at 100° a further quantity of H_2O_2 (100 vol, 2 ml) in acetic acid (2 ml) was added, heating and stirring were continued for 1 hr, and the mixture was poured into water. Isolation of the product through $CHCl_3$ in the usual way and crystallization from $CHCl_3$–MeOH gave platelets (160 mg) of the *ketone* (XIII), m.p. 265–267°, $[\alpha]_D$ +33 ± 1° (*c*, 1·5), λ_{max} (EtOH) 281 mμ (ε 40), ν_{max} (Nujol) 1740 (vs), 1710 (s) cm^{-1}. (Found: C, 67·5; H, 8·4; O, 23·4. Calc. for $C_{38}H_{56}O_{10}$: C, 67·8; H, 8·4; O, 23·8%.) The compound did not give a colour with tetranitromethane in $CHCl_3$ and was unchanged after treatment with HCl in acetic acid (3 hr at 100°) or with KOH in refluxing MeOH followed by reacetylation.

(b) Formic acid (17 ml) containing H_2O_2 (100 vol, 3·25 ml) was added, over a period of 90 min, to a stirred solution of aescigenin tetra-acetate (500 mg) in ethyl acetate (20 ml) at 45°. After 3 hr at 45° the solution was poured into water and worked up through $CHCl_3$ to yield the ketone (XIII; 250 mg), m.p. 265–267°, $[\alpha]_D$ +33° (*c*, 1·6), after crystallization from MeOH.

12-*Keto*-13β-*olean*-15-*ene*-3β,21α,22β,24,28-*pentol penta-acetate* (XIV)

The ketone (XIII; 100 mg) in acetic anhydride (5 ml) was heated (30 min) at 117–120° with *p*-toluenesulphonic acid (50 mg). The reaction mixture was diluted with water, the product isolated in the usual way through $CHCl_3$ and crystallized from $CHCl_3$–MeOH to yield prisms (20 mg) of the 15-*ene*-12-*one* (XIV), m.p. 322–325°, $[\alpha]_D$ +7 ± 1° (*c*, 0·9). (Found: C, 67·05; H, 8·1. Calc. for $C_{40}H_{58}O_{11}$: C, 67·2; H, 8·2%.)

11β-*Bromo*-16α,21α-*epoxy*-12-*keto*-13β-*oleanan*-3β,22β,24,28-*tetrol tetra-acetate* (XV)

Bromine (131·5 mg) in acetic acid (25 ml) was added dropwise, with swirling, to a solution of XIII (500 mg) in acetic acid (25 ml) containing conc. HBr (2 drops). The pale yellow solution was diluted with water, worked up through ether in the usual way, and the product crystallized from MeOH to give platelets (430 mg) of the *bromo ketone* (XV), m.p. 242–243°, $[\alpha]_D$ +11° (*c*, 1·2), λ_{max} (EtOH) 310 mμ (ε 120). (Found: C, 60·95; H, 7·55; Br, 10·6. Calc. for $C_{38}H_{55}O_{10}Br$: C, 60·7; H, 7·4; Br, 10·6%.)

16α,21α-*Epoxy*-12-*keto*-13β-*olean*-9(11)-*ene*-3β,22β,24,28-*tetrol tetra-acetate* (XVI)

A solution of XV (250 mg) in *s*-collidine (20 ml) was refluxed (30 min), cooled, filtered and the residue washed with ether (ca. 50 ml). The combined filtrate and washings were washed successively with 2N HCl, saturated $NaHCO_3$aq and water. The dried (Na_2SO_4) ether solution was evaporated to dryness and the residue crystallized from MeOH to give plates (160 mg) of the α,β-*unsaturated ketone* (XVI), m.p. 248–250°, $[\alpha]_D$ +65 ± 2° (*c*, 1·3), λ_{max} (EtOH) 247 mμ (ε 10,600), ν_{max} (Nujol) 1740 (vs), 1681 (s) cm^{-1}. (Found: C, 67·8; H, 8·4. Calc. for $C_{38}H_{54}O_{10}$: C, 68·0; H, 8·1%.) The compound did not give a colour with tetranitromethane in $CHCl_3$.

11-*Bromo*-16α,21α-*epoxy*-12-*keto*-13β-*olean*-9(11)-*ene*-3β,22β,24,28-*tetrol tetra-acetate* (XVII)

The crude XV (2·2 g) in *s*-collidine (50 ml) was refluxed (30 min) and the product isolated as described above. Crystallization from MeOH (40 ml) gave XVI (1·3 g), m.p. 247–249°, $[\alpha]_D$ +63° (*c*, 0·9). The concentrated (to ca. 10 ml) mother liquor slowly deposited pale yellow needles (55 mg) of the α-*bromo*-α,β-*unsaturated ketone* (XVII), m.p. 232°, $[\alpha]_D$ +162° (*c*, 1·3), λ_{max} (EtOH) 208 mμ (ε 6,000), 292 mμ (ε 8,000), ν_{max} (Nujol) 1740 (vs), 1670 (s) cm^{-1}. (Found: C, 60·8; H, 7·15; O, 20·9; Br, 11·0. Calc. for $C_{38}H_{53}O_{10}Br$: C, 60·9; H, 7·1; O, 21·3; Br, 10·7%.)

16α,21α-*Epoxy*-13β-*olean*-9(11)-*ene*-3β,22β,24,28-*tetrol tetra-acetate* (XVIII)

The compound XVI (100 mg) in acetic acid (30 ml) was agitated (48 hr) with Pt (from 100 mg PtO_2) under H_2 at atm. press. The filtered solution was evaporated to dryness under red. press. and the residue crystallized from $CHCl_3$–MeOH to yield leaflets (75 mg) of the 9(11)-*ene* (XVIII), m.p. 214–216°, $[\alpha]_D$ +69° (*c*, 1·2), λ_{max} (EtOH) 204 mμ (ε 5,000). (Found: C, 69·5; H, 8·8. Calc. for $C_{38}H_{56}O_9$: C, 69·5; H, 8·6%.) The compound gives a pale yellow colour with tetranitromethane in $CHCl_3$.

16α,21α-*Epoxyolean*-9(11),12-*diene*-3β,22β,24,28-*tetrol tetra-acetate* (XIX)

A solution of XVI (250 mg) in dry ether (75 ml) was refluxed (24 hr) with LAH (500 mg). The product, isolated in the usual way, was dissolved in acetic anhydride (50 ml) and the solution refluxed (2 hr) with anhydrous sodium acetate (120 mg). Dilution of the reaction mixture with water, isolation of the product through ether, and repeated crystallization from $CHCl_3$–MeOH yielded the *diene* (XIX) as leaflets (40 mg), m.p. 203–205°, $[\alpha]_D$ +140° (*c*, 1·3), λ_{max} (EtOH) 280 mμ (ε 9,000). (Found: C, 69·6; H, 8·1. Calc. for $C_{38}H_{54}O_9$: C, 69·7; H, 8·3%.)

11-*Ketoaescigenin tetra-acetate* (VIII)

The compound, prepared as described previously,[21] was obtained in 60% yield and had m.p. 230–232°, $[\alpha]_D$ +47° (*c*, 1·4) (lit.[21] m.p. 233–234°, $[\alpha]_D$ +55·5°), λ_{max} (EtOH) 248 mμ (ε 11,000). (Found: C, 67·7; H, 8·4. Calc. for $C_{38}H_{54}O_{10}$: C, 68·0; H, 8·1%.)

Aescigenin O_{12} *tetra-acetate* (XX)

(a) 11-Ketoaescigenin tetra-acetate (500 mg) in stabilized acetic acid (50 ml) was refluxed (15 min) with CrO_3 (650 mg), the neutral product isolated in the usual way, and crystallized from $CHCl_3$–MeOH to give the O_{12} *tetra-acetate* (220 mg) as needles, m.p. 245–246°, $[\alpha]_D$ +34° (*c*, 1·5), λ_{max} (EtOH) 240 mμ (ε 11,000), 296 mμ inflexion (ε 500), ν_{max} (Nujol) 1802 (s), 1740 (vs), 1709 (s), 1667 (s) cm^{-1}. (Found: C, 65·15; H, 7·1. Calc. for $C_{38}H_{50}O_{12}$: C, 65·3; H, 7·2%.) The compound does not give a colour with tetranitromethane in $CHCl_3$ or with alcoholic $FeCl_3$ but dissolves in methanolic KOH to form a deep purple solution. Immediate acidification of this solution discharges the colour but the product, before and after reacetylation, is an intractible gum. Attempted hydrolysis with aqueous methanolic HCl gives a similar gum.

(b) A solution of aescigenin tetra-acetate (500 mg) in stabilized acetic acid (50 ml) refluxed (15 min) with CrO_3 (650 mg) furnished, after isolation of the neutral product through ether, the O_{12} tetra-acetate (200 mg), m.p. 245–246°, identical (IR) with the product described above.

Aescigenin O_{12} *tetra-acetate dioxime*

The O_{12} tetra-acetate (100 mg) in pyridine (4 ml) was heated (3 hr) on a steam-bath with hydroxylamine hydrochloride (50 mg). The product, isolated through ether in the usual way, crystallized from MeOH as feathery needles (102 mg) of the *dioxime*, m.p. 195–196°, $[\alpha]_D$ +51° (*c*, 1·2), λ_{max} (EtOH) 244 mμ (ε 11,000), ν_{max} (Nujol) 3390 (s), 1740 (vs), 1709 shoulder, 1667 (s) cm^{-1}. (Found: C, 62·3; H, 7·2; N, 3·4. Calc. for $C_{38}H_{52}O_{12}N_2$: C, 62·6; H, 7·1; N, 3·8%.)

Hydrogenation of the O_{12} *tetra-acetate*

A solution of the O_{12} tetra-acetate (250 mg) in acetic acid (60 ml) at room temp was shaken (24 hr) with H_2, at atm. press. over Pt (from 250 mg PtO_2). The filtered solution was evaporated to dryness under red. press. and the residue crystallized from $CHCl_3$–MeOH to give colourless plates (190 mg) of the *dihydro*-O_{12} *tetra-acetate* (XXI), m.p. 256–259°, $[\alpha]_D$ −46° (*c*, 0·95), λ_{max} (EtOH)

245 mμ (ε 12,000), ν_{max} (Nujol) 3333 (m), 1779 (s), 1740 (vs), 1669 (s) cm^{-1}. (Found: C, 64·8; H, 7·1. Calc. for $C_{26}H_{32}O_{12}$: C, 65·1; H, 7·5%.) The compound does not give a colour with tetranitromethane in $CHCl_3$.

Prolonged hydrogenation (7 days) furnished an intractible gum, λ_{max} 204 mμ (ε 5000), ν_{max} (Nujol) 1740 (vs) cm^{-1}, which was chromatographically homogeneous and which gave a yellow colour with tetranitromethane in $CHCl_3$.

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