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TRITERPENOIDS FROM *BUPLEURUM FALCATUM* L.—I

THE STRUCTURE OF SAIKOGENINS A, C AND D[1,2]

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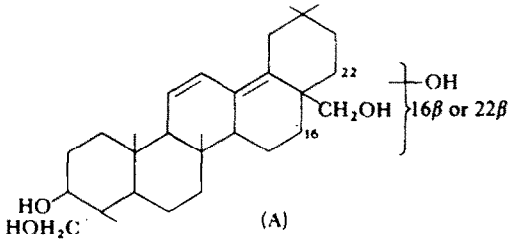
Abstract—The saponin from the roots of *Bupleurum falcatum* L. on acid hydrolysis affords three triterpenes containing a heteroannular diene and an unidentified triterpene fraction which shows a homoannular diene chromophore. The structures of the three heteroannular dienes, saikogenins A, C and D, have been shown to be olean-11,13(18)-diene-3β,16β,23,28-tetrol (Ia), -3β,16β,28-triol (IIa) and -3β,16α,23,28-tetrol (IIIa), respectively.

THE roots of *Bupleurum falcatum* L. (Umbelliferae) provide one of the most important drugs in Chinese medicine. Regarding the components, only some fatty acids[3,4] and adnitol[5] have formerly been identified. Previous papers[6,7] reported the isolation of α-spinasterol, stigmasterol, Δ^7-stigmastenol and Δ^{22}-stigmastenol from the unsaponifiable fraction of the roots. The presence of a saponin has been described[8,9] but no extensive chemical work was done. Shortly before the preliminary communication,[1] Shibata *et al.*[10] reported the isolation of saikogenin A, a major sapogenin of these roots, and proposed the partial structure (A). Independently, we were studying



(A)

sapogenins from the same source and the present paper describes the isolation of two new sapogenins, saikogenins C and D,[2] as well as saikogenin A[10] and elucidation of the complete structure for these three saikogenins.

[1] An outline of this paper was presented in *Tetrahedron Letters* 701 (1966).

[2] Besides the three sapogenins, a triterpenoid fraction showing a homoannular diene chromophore at λ_{max} 282 mμ has been obtained in a somewhat impure state. The name "saikogenin B" is reserved for the purified compound of this fraction, which will form the subject of a succeeding publication.

[3] T. Araki and Y. Miyashita, *J. Taiwan Med. Soc.* **296**, 1 (1929).

[4] Yuoh-Fong Chi and Chi-Ming Ma, *J. Chinese Chem. Soc.* **3**, 78 (1935); *Chem. Abstr.* **29**, 4515 (1935).

[5] F. Wessely and S. Wang, *Monatsh.* **72**, 168 (1938).

[6] K. Takeda, K. Hamamoto and T. Kubota, *J. Pharm. Soc. Japan* **73**, 272 (1953).

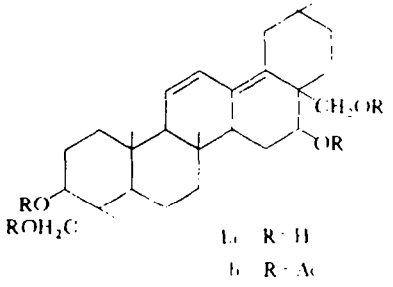
[7] K. Takeda and T. Kubota, *Chem. Pharm. Bull.* **6**, 536 (1958).

[8] T. Ezawa, *Rept. Taiwan Central Res. Lab.* **5**, 179 (1916).

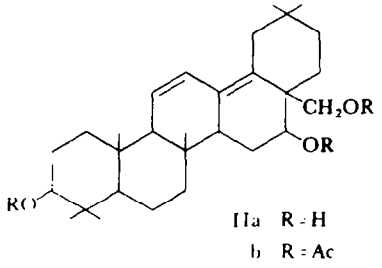
[9] T. H. Tang and C. C. Peng, *J. Pharm. Soc. China* **1**, 17 (1943); *Chem. Abstr.* **39**, 3118 (1945).

[10] S. Shibata, I. Kitagawa and H. Fujimoto, *Tetrahedron Letters* 3783 (1965).

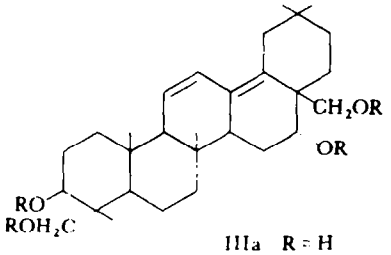
Acid hydrolysis of the crude saponin obtained from the roots of *Bupleurum falcatum* L. yields a mixture of neutral triterpenoid sapogenins, from which saikogenins A and D, as major components, and saikogenin C and another triterpenoid fraction,[2] as minor components, are separated. Saikogenin A (Ia), m.p. 287–290°, $[\alpha]_D$ −43° (EtOH), was identified,[11] through dihydrosaikogenin A tetraaacetate, with a specimen isolated by Shibata *et al.*[10] The new sapogenins are as follows: Saikogenin C (IIa),



Ia R = H
b R = Ac



IIa R = H
b R = Ac



IIIa R = H
b R = Ac

m.p. 291–294°, $[\alpha]_D$ −46°, and saikogenin D (IIIa), m.p. 256–261°, $[\alpha]_D$ −48° (EtOH). Saikogenins A and D have the elementary composition $C_{30}H_{48}O_4$ while saikogenin C is $C_{30}H_{48}O_3$. All three compounds, in their UV spectra, show strong triplet absorptions at 242, 251 and 260 mμ characteristic of a heteroannular diene. The IR spectra of the three saikogenins exhibit OH bands but no CO absorption.

Saikogenins A and C are readily acetylated with acetic anhydride and pyridine at room temperature whereas saikogenin D requires heating with the same reagents for complete acetylation. Saikogenins A and D afford the respective amorphous acetates Ib and IIIb while saikogenin C gives a crystalline triacetate, $C_{36}H_{54}O_6$ (IIb), m.p. 213–215°, $[\alpha]_D$ −70°. They display no OH absorption in their IR spectra. Their NMR spectra indicate that both saikogenins A and D have two primary and two secondary alcohol groups and that saikogenin C lacks one of the two primary alcohol groups in the former two compounds (Table 1). It is, thus, confirmed that all the oxygen functions in the three triterpenes are OH groups. In Ib and IIIb, one of the two CH_2OAc groups can be predicted as equatorial due to higher τ-value of the methylene signal adjacent to the AcO group, and the other and that of IIb as axial from the signal of lower τ-value.[12] The two secondary OH groups in each of

[11] The authors are indebted to Professor S. Shibata for identification of saikogenin A.
[12] A. Gaudemer, J. Polonsky and F. Wenkert, *Bull. Soc. Chim. Fr.* 407 (1964).

saikogenins A and C are probably equatorial (easy acetylation and broad quartet signals for the protons on acetoxy-bearing carbon atoms in the acetates Ib and IIb). On the other hand, one of the two secondary OH groups in saikogenin D (IIIa) must be axial since one OH group resists acetylation under mild conditions and since one of the protons on two carbon atoms bearing secondary AcO groups of the tetraacetate (IIIb) appears as a rather sharp triplet at 4·82τ (J = 3 c/s).

TABLE 1. NMR SIGNALS OF THE ACETATES OF SAIKOGENINS A, C AND D

	$-\overset{\vert}{C}-CH_3$	CH_3COO-	$-\overset{\vert}{C}-CH_2OAc$	$-\overset{OAc}{\overset{\vert}{C}}-H$	Olefinic H
Saikogenin A tetraacetate (Ib)	9·18(3H, s) 9·17(6H, s) 9·02(6H, s) 8·87(3H, s)	7·98(6H, s) 7·95(3H, s) 7·93(3H, s)	6·20(2H, s) 5·89, 5·48 (2H, AB q, J = 12)	5·33–4·72 (2H, m)	4·38, 3·56 (2H, AB q, J = 10)
Saikogenin C triacetate (IIb)	9·18(3H, s) 9·13(6H, s) 9·03(9H, s) 8·88(3H, s)	7·95(9H, s)	5·88, 5·48 (2H, AB q, J = 12)	5·67–4·67 (2H, m)	4·34, 3·56 (2H, AB q, J = 10)
Saikogenin D tetraacetate (IIIb)	9·26(3H, s) 9·17(6H, s) 9·03(6H, s) 8·88(3H, s)	8·00(3H, s) 7·97(3H, s) 7·95(6H, s)	6·21(2H, s) 6·09, 5·71 (2H, AB q, J = 12)	5·20(1H, q, J = 9, 5·5) 4·82(1H, t, J = 3)	4·40, 3·56 (2H, AB q, J = 10)

The spectra were determined in $CDCl_3$ solns at 60 Mc. The chemical shifts are shown in τ-value and the coupling constants (J) in c/s. Signal multiplicities are represented by s (singlet), t (triplet), q (quartet) and m (multiplet).

All three saikogenins on treatment with sodium periodate fail to consume the reagent and starting materials were recovered. On treatment with acetone in the presence of an acidic catalyst, saikogenins A and D afford the corresponding diacetonide, m.p. 200–204°, $[\alpha]_D$ −35° and m.p. 260–265°, $[\alpha]_D$ +237°, while saikogenin C gives the monoacetonide, m.p. 261–264°, $[\alpha]_D$ −34°. These results indicate that the three triterpenes have no vicinal glycol but pairs of 1,3-glycol.

All three saikogenins show strong UV absorptions characteristic of a hetero-annular diene, as already mentioned, and the NMR spectra of these acetates exhibit the signals of two olefinic protons, as an AB quartet, due to a disubstituted double bond. The results, in combination with levorotations observed in the three genins, suggest that they are olean-11,13(18)-diene derivatives. This is supported by the fact that saikogenin C triacetate (IIb) on treatment with selenium dioxide in benzyl acetate yields $C_{36}H_{50}O_8$, m.p. 208–209°, $[\alpha]_D$ −94°, λ_{max} 278 mμ (ε 13,600), which was characterized as triacetoxyolean-9(11),13(18)-diene-12,19-dione (IV). Catalytic hydrogenation of the three triterpenes on platinum oxide in acetic acid affords, as expected, the respective dihydro derivatives, which show no olefinic proton signal in the NMR spectra but give a positive tetranitromethane reaction.

CH2OR
OR
H2
PtO2
IIa R = H
b R = Ac
SrO2
CH2OAc
OAc
O
O
AcO
IV
OH
at 16 or 22
a
OH H+
at 16 or 22
22
16
CH2OH
H+
b
−H+
or
c
RO
RO
ROH2C
Va R = H
b R = Ac
VIa R = H
b R = Ac

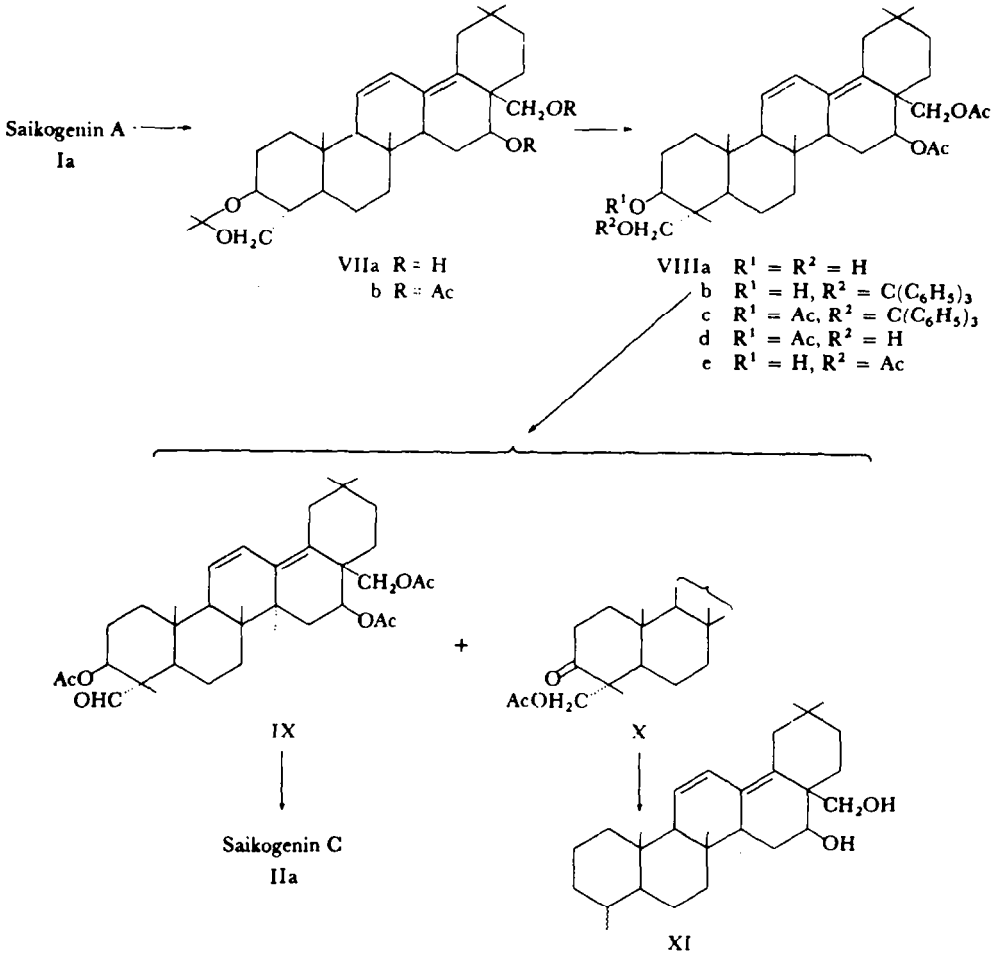
Saikogenin A (Ia) on treatment with conc hydrochloric acid in boiling methanol under nitrogen atmosphere yields a conjugated triene, $C_{29}H_{44}O_2$ (Va), m.p. 246–249° (dec), $[\alpha]_D$ +439°, λ_{max} 319 mμ (ε 19,100). The product Va readily forms the acetonide, m.p. 225–232° and the amorphous diacetate Vb, whose NMR spectrum shows the signals of a singlet at 4·28τ for two olefinic protons, an AB quartet at 6·02 and 6·32τ (J = 11 c/s) due to a methylene bearing a primary acetoxyl group and a broad second-order quartet centered at 5·18τ due to a proton on a carbon atom bearing a secondary acetoxy group. Although the two surviving OH groups of the product Va can be predicted to be located at 3β and 23 from the NMR data of the diacetate Vb, the OH groups at 3β and 23 in saikogenin A have already been confirmed by Shibata *et al.*[10] by chemical correlation with the known hederagenin. The structure of the triene is thus represented as 28-norolean-9(11),12,17-triene-3β,23-diol (Va) arising from (a) elimination of formaldehyde from the hydroxymethyl group at the allylic C_{17}-position *via* the reverse Prins reaction, (b) dehydration of a resultant allylic OH group at C_{16} or C_{22} and (c) double bond migrations. The same acid treatment of saikogenin D (IIIa) affords the identical triene-diol (Va). Consequently, out of the four OH groups existing in each of saikogenins A (Ia) and D (IIIa), three have been proved to occupy the common positions, 3β,23 and 28, and both the sapogenins differ only in the location of the fourth OH group at C_{16} or C_{22}. The same treatment of saikogenin C (IIa) gives rise to the same type of the reaction, giving a monohydroxy-triene, $C_{29}H_{44}O$ (VIa), m.p. 187–191°, $[\alpha]_D$ +457°, λ_{max} 319 mμ (ε 18,000) which seems to lack the C_{23}-OH group in Va and the remaining OH group is supposed to be located at 3β, the usual position in triterpenes.

In view of the acetonide linkage with the C_{28}-OH group in the three saikogenins, the possible location of the secondary OH group in question is limited to 16β and 22β for the equatorial OH group of saikogenins A and C and to 16α for the axial group of saikogenin D. However, of the two possibilities for saikogenin C (IIa), the former is tentatively rejected, because of great discrepancy between the constants, m.p. 213–215°, $[\alpha]_D$ −70°, observed for the triacetate, and m.p. 178–179°, $[\alpha]_D$ −29°, described for olean-11,13(18)-diene-3β,16β,28-triol triacetate (IIb) derived from longispinogenin.[13] Furthermore, it is noticed that saikogenin D (IIIa), $[\alpha]_D$ −48° (EtOH), on acetonide formation shows a striking dextrorotatory change into the diacetonide, $[\alpha]_D$ +237°, which in the ORD measurement exhibits a positive Cotton effect, $[\phi]$ 272 mμ = +55,500, being inconsistent with a negative Cotton predicted for the diacetonide of olean-11,13(18)-diene-3β,16α,23,28-tetrol (IIIa) based on the chirality rule.[14]

From the biogenetic standpoint, it is expected that saikogenin A (Ia) differs from saikogenin C (IIa) only in the presence of the additional C_{23}-OH group. Reductive elimination of the C_{23}-OH group in saikogenin A (Ia) is carried out in order to correlate with saikogenin C (IIa). Treatment of saikogenin A (Ia) with acetone and a catalytic amount of *p*-toluenesulphonic acid yields the mono and diacetonides. The monoacetonide VIIa, m.p. 250–256°, $[\alpha]_D$ −56°, on acetylation readily affords the amorphous acetonide-diacetate VIIb. Treatment in warm aqueous acetic acid results in hydrolysis of the acetonide group, giving the diacetate VIIIa, which on

[13] C. Djerassi, L. E. Geller and A. J. Lemin, *J. Am. Chem. Soc.* **76**, 4089 (1954).
[14] E. Charney, H. Ziffer and U. Weiss, *Tetrahedron* **21**, 3121 (1965).

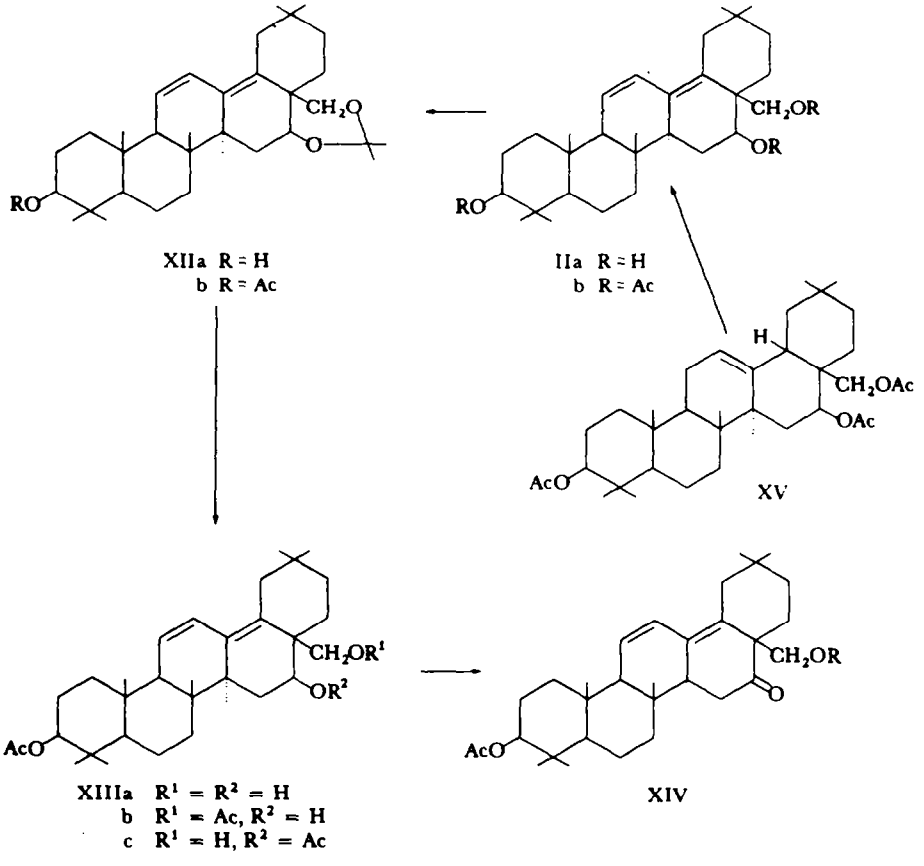
trityl ether formation of the primary alcohol at C_{23} followed by acetylation of the 3β-OH group affords the trityl ether-triacetate (VIIIc), $C_{55}H_{68}O_7$, m.p. 176–181°, $[\alpha]_D$ −12°. Hydrolysis of the trityl ether with aqueous acetic acid gives a mixture of oily triacetates, which are recognized as two spots close together by TLC. It is presumed that the one is the expected 3β,16β (or 22β),28-triacetate (VIIId) and that



the other may be the 16β(or 22β),23,28-triacetate (VIIIe) arising from Ac migration from 3β to 23. Without purification, the mixture was oxidized with Kiliani's reagent in acetone to give a mixture of the carbonyl compounds, which was separated into the two fractions by preparative TLC. One of them, an amorphous triacetate-aldehyde IX, exhibiting the IR band at 2709 cm^{-1} and the NMR signal at 0·72τ characteristic of an aldehyde, was subjected to Huang-Minlon reduction, yielding a triol, $C_{30}H_{48}O_3$, m.p. 291–294°, which is identical with saikogenin C (IIa) in all respects and thus saikogenin A is 23-hydroxylated saikogenin C. The other oxidation product was characterized as the triacetate-ketone X, as expected, by the NMR spectrum indicating the existence of two primary and one secondary AcO groups and on

Huang-Minlon reduction yielding 24(or 23)-norolean-11,13(18)-diene-16β,28-diol (XI), $C_{29}H_{46}O_2$, m.p. 248–250°.

In order to compare with the known 3β,28-dihydroxyolean-11,13(18)-dien-16-one diacetate described by Barton *et al.*,[15] saikogenin C (IIa) was partially acetylated at 3β and 28 and the remaining OH group oxidized. Saikogenin C (IIa) on acetylation through the acetonide XIIa affords the 3-monoacetate XIIIa, which is also obtained by partial saponification of the triacetate IIb. The 3-monoacetate XIIIa on acetylation under controlled conditions yields the two diacetates, besides the recovered XIIIa and the triacetate IIb. Of the two diacetates, the major product, m.p. 163–166°, $[\alpha]_D$ −103° is the expected 3β,28-diacetate XIIIb and on oxidation with Kiliani's reagent in acetone gives a diacetone-ketone, m.p. 247–250°, $[\alpha]_D$ −95°, which differs from the constants, m.p. 210–211°, $[\alpha]_D$ −68°, described for XIV by Barton *et al.*[15]



XIIa R = H
b R = Ac

IIa R = H
b R = Ac

XV

XIIIa $R^1 = R^2 = H$
b R^1 = Ac, R^2 = H
c R^1 = H, R^2 = Ac

XIV

However, our diacetate-ketone exhibits a negative CD band, [θ] 298 mμ = −6,600, as expected for the keto group situated at C_{16} but not at C_{22} based on the octant rule. Thus, it is suspected that both the constants previously described for IIb[13] and XIV[15] may be in error. Oxidation of longispinogenin triacetate (XV)[16] with selenium

[15] D. H. R. Barton, A. Hameed and J. F. McGhie, *J. Chem. Soc.* 5176 (1962).
[16] Longispinogenin triacetate was kindly supplied by Professor C. Djerassi.

Saikogenin D
IIIa
XVI
Saikogenin A
ditrityl ether
XVIIa R = H
b R = Ac
XVIIIa R¹ = R² = H
b R¹ = H, R² = C(C6H5)3
c R¹ = Ac, R² = C(C6H5)3

CH2OAc
OAc
AcO
OHC
XIX
+
AcOH2C
O
XX
CH2OH
OH
XXII
CH2OR1
OR2
R1O
XXIa R1 = R2 = H
b R1 = R2 = Ac
c R1 = Ac, R2 = H
XIV
CH2OR
OR
H
RO
XXIIIa R = H
b R = Ac

dioxide in acetic acid affords, after separation from the unchanged starting material, the dehydrogenated product melting at 213–215°. The m.p. differs from that recorded by Djerassi *et al.*[13] but coincides with that of saikogenin C triacetate (IIb). Identity of the product with saikogenin C triacetate was established by mixed m.p. and IR spectra. The structure of saikogenin C has therefore been elucidated as olean-11,13(18)-diene-3β,16β,28-triol (IIa) and accordingly saikogenin A is olean-11,13(18)-diene-3β,16β,23,28-tetrol (Ia).

Now, it is most probable that the structure of saikogenin D is olean-11,13(18)-diene-3β,16α,23,28-tetrol (IIIa), epimeric at C_{16} with saikogenin A (Ia). Saikogenins A and D were converted into the respective ditrityl ethers (being connected with the primary OH groups) by treatment with trityl chloride in pyridine. Oxidation of both the ditrityl ethers with Kiliani's reagent in acetone affords the same ditrityl ether-diketone (XVI), m.p. 241–245°, $[\alpha]_D$ −58°, and the epimeric relationship of the OH group between saikogenins A (Ia) and D (IIIa) has been established. The above conclusion is further corroborated by correlation of saikogenin D (IIIa) with primulagenin A (XXIIIa).[17] Reductive elimination of the OH group at C_{23} in saikogenin D was achieved by the same sequence as carried out on saikogenin A (Ia). Treatment of saikogenin D (IIIa) with acetone and a catalytic amount of *p*-toluenesulphonic acid yields exclusively the monoacetonide XVIIa, m.p. 259–262°, $[\alpha]_D$ −54°, which on acetylation with heated acetic anhydride and pyridine gives the acetonide-diacetate XVIIb. Acetonide cleavage, trityl ether formation at C_{23} followed by acetylation yields the trityl ether-triacetate (XVIIIc), $C_{55}H_{68}O_7$, m.p. 145–147°, $[\alpha]_D$ −24°. Treatment of XVIIIc with aqueous acetic acid affords a mixture of the two triacetates as observed in the derivative of saikogenin A (Ia). Oxidation with Kiliani's reagent in acetone followed by Huang-Minlon reduction affords olean-11,13(18)-diene-3β,16α,28-triol (XXIa), $C_{30}H_{48}O_3$, m.p. 241–246°, $[\alpha]_D$ −45°, as well as 24(or 23)-norolean-11,13(18)-dien-16α,28-diol (XXII), $C_{29}H_{46}O_2$, m.p. 203–209°. The triol XXIa is identical, as expected, with a specimen prepared by dehydrogenation of authentic primulagenin A triacetate (XXIIIb)[18] with selenium dioxide followed by saponification. Mild acetylation of both specimens affords the same 3,28-diacetate XXIc,[19] m.p. 266–271°, $[\alpha]_D$ −98°, λ_{max} 243, 251 and 261 mμ (ε 27,500, 31,600 and 19,800). Oxidation of XXIc with Kiliani's reagent in acetone yields the diacetateketone (XIV), m.p. 247–251°, which is identical with the above-mentioned specimen obtained by oxidation of saikogenin C diacetate (XIIIb). The structure of saikogenin D has now been established without any doubt as olean-11,13(18)-diene-3β,16α,23,28-tetrol (IIIa). Although three saikogenins show negative Cotton effects as expected from molecular models according to the chirality rule, it should be noted that saikogenin D diacetonide exhibits, as already mentioned, a positive Cotton effect opposite to the prediction. Saikogenin D diacetonide on acid hydrolysis regenerates saikogenin D (IIIa) and no other conformer on account of its rigid structure fixed with acetonide linkage. Therefore, this discrepancy will offer an

[17] B. Bischof, O. Jeger and L. Ruzicka, *Helv. Chim. Acta* **32**, 1911 (1949); **31**, 1760 (1948).

[18] The authors are grateful to Professor D. H. R. Barton for supplying us primulagenin A, which is acetylated by heating with acetic anhydride and pyridine.

[19] For this compound, Barton *et al.*[15] reported m.p. 165–166°, $[\alpha]_D$ −52°, λ_{max} 250 mμ (ε 22,800). We suppose the values might be attributed to contamination with the precursor, olean-12-ene-3β,16α,28-triol 3β,28-diacetate.

exception of the chirality rule[14] for transoid dienes and its explanation is left to further developments in this field of investigation.

EXPERIMENTAL

All m.ps were determined on a Monoscop VS hot plate and are corrected. Unless otherwise stated, specific rotations were measured in $CHCl_3$ solns with a Rudolf Photoelectric Polarimeter Model 200. UV spectra were recorded in 95% EtOH solns on a Hitachi EPS-2 recording spectrometer. IR spectra were determined in Nujol mull with a Nihon Bunko DS-201B spectrometer. ORD and CD curves were recorded on a Nihon Bunko automatic recording spectropolarimeter ORD/UV-5. NMR spectra were determined at 60 Mc in $CDCl_3$ solns containing TMS as an internal standard using a Varian A-60 analytical NMR spectrometer.

Alumina used for chromatography was Al_2O_3 "Woelm" neutral, activity grade II. TLC was performed on Merck Silica gel G. Kiliani's reagent was prepared from $Na_2Cr_2O_7 \cdot 2H_2O$ (6 g) in water (27 ml) by addition of conc H_2SO_4 (8 g).

Isolation of triterpenoids

The dried, cut root (20 kg) of *Bupleurum falcatum* L. was extracted several times with EtOH under reflux and the EtOH soln was evaporated *in vacuo*. The residue (3·9 kg) was extracted with hot pet ether to give the extract (1·02 kg) from which sterols were previously isolated.[6, 7] The insoluble fraction was extracted with ether to remove an oily material (290 g) and then with water to remove a water-soluble portion (1·2 kg).

The dark brown, insoluble portion (1·3 kg) was dissolved in EtOH (3·5 l.) and 2N H_2SO_4 (3·5 l.) was added. The mixture was refluxed for 4 hr. After cooling, an oily ppt was separated by decantation and the supernatant was evaporated *in vacuo* to give an additional ppt. The combined ppts were refluxed with EtOH (2·4 l.) and 20% KOH (600 ml) for 1 hr and evaporated *in vacuo*. The residue was diluted with water and extracted thoroughly with ether. The ethereal soln was washed with dil NaOH and water, dried over Na_2SO_4 and concentrated to separate needles (30 g), which were mostly saikogenin A and purified by recrystallization. The filtrate was evaporated to dryness and the residue (122 g) was dissolved in $CHCl_3$ (1 l.), when insoluble crystals remained. The crystals (22 g) were mostly saikogenin D and purified by recrystallization. The portion dissolved in $CHCl_3$ was diluted with benzene and chromatographed on Al_2O_3 (1·2 kg). Elution with benzene–$CHCl_3$ (5:1 to 2:1) gave a waxy material (6·5 g). The eluates (5·3 g) with benzene-$CHCl_3$ (2:1) on recrystallization from EtOH yielded a mixture of sterols (1·56 g) identified as α-spinasterol and stigmasterol by gas chromatography. The eluates (24·6 g) with benzene-$CHCl_3$ (1:1) and $CHCl_3$, on two recrystallizations from AcOEt, were separated into saikogenin C (2·52 g) and the mother liquors which yielded crude saikogenin B[2] after acetylation and chromatography. Continued elution with $CHCl_3$–MeOH (99:1 to 49:1) afforded solids (13·0 g) which were recrystallized from acetone, giving an additional crop of saikogenin A (2·43 g). The eluates (13·8 g) with $CHCl_3$–MeOH (49:1 to 19:1) were crystallized from $CHCl_3$, giving an additional crop of saikogenin D (7·8 g).

Saikogenin A (1a)

The crude material (30 g), after two recrystallizations from $CHCl_3$–MeOH, gave needles (22·8 g), m.p. 273–280°, which combined with $CHCl_3$ (Found: Cl, 17·96. $C_{30}H_{48}O_4 \cdot CHCl_3$ requires: Cl, 17·97%) and was freed from the $CHCl_3$ on heating *in vacuo* at 110° for 4 hr. The analytical sample was obtained by further recrystallization from MeOH as needles, m.p. 287–290° $[\alpha]_D$ −43·3° (*c* 0·60, EtOH). λ_{max} 242, 250, 260 mμ (ε 26,800, 30,400, 19,400). ν_{max} 3426, 3225, 1051, 1011, 985, 961 cm^{-1}. CD (*c* 0·00922, MeOH): $[\theta]_{258.5}$ −41,000, $[\theta]_{249}$ −65,900, $[\theta]_{241}$ −63,400. (Found: C, 76·18; H, 10·40. Calc. for $C_{30}H_{48}O_4$: C, 76·22; H, 10·24%). This compound was identical with a specimen of saikogenin A isolated by Shibata *et al.*[10] by TLC and identity of the two specimens was further corroborated through dihydrosaikogenin A tetraacetate described below.

Saikogenin A was treated with Ac_2O and pyridine at room temp overnight. After addition of water, the ppt was filtered, washed and dried to give the amorphous *tetraacetate* (1b). ν_{max} 1745, 1246, 1035 cm^{-1}, no OH-band. NMR: τ 9·18, 9·17, 9·02, 8·87 (1, 2, 2, 1 Me, respectively); 7·98, 7·95, 7·93 (2, 1, 1 Me, respectively, of AcO groups); 6·20 (2H at C_{23}, singlet); 5·89, 5·48 (2H at C_{28}, AB quartet, J = 12 c/s); 5·33–4·72 (2H at C_3 and C_{16}, multiplet); 4·38, 3·56 (2H at C_{11} and C_{12}, AB quartet, J = 10 c/s).

Saikogenin C (IIa)

Saikogenin C, after recrystallization from AcOEt, was obtained as prisms, m.p. 291–294°, $[\alpha]_D$ −45·8° *c* 1·06). λ_{max} 242·5, 251, 260 mμ (ε 26,900, 31,000, 19,400). ν_{max} 3339, 1132, 1071, 1045, 1020, 986 cm^{-1}. CD (*c* 0·00622, MeOH): $[\theta]_{259.5}$ −40,000, $[\theta]_{248.5}$ −63,000, $[\theta]_{241}$ −63,000. (Found: C, 79·05; H, 10·58. $C_{30}H_{48}O_3$ requires: C, 78·89; H, 10·59%).

Treatment of saikogenin C with Ac_2O in pyridine at room temp overnight afforded the *triacetate* (IIb), which was obtained after recrystallization from EtOH as needles, m.p. 213–215°, $[\alpha]_D$ −69·9° (*c* 0·83). λ_{max} 241, 249, 259 mμ (ε 27,900, 30,600, 20,800). ν_{max} 1739, 1242, 1078, 1032, 999, 985, 905 cm^{-1}. NMR: τ 9·18, 9·13, 9·03, 8·88 (1, 2, 3, 1 Me, respectively); 7·95 (3 Me of AcO groups); 5·88, 5·48 (2H at C_{28}, AB quartet, J = 12 c/s); 5·67–4·67 (2H at C_3 and C_{16}, multiplet); 4·34, 3·56 (2H at C_{11} and C_{12}, AB quartet, J = 10 c/s).

Saikogenin D (IIIa)

The crude material was recrystallized from $CHCl_3$–MeOH and then from AcOEt, giving prisms, m.p. 256–261°, $[\alpha]_D$ −47·9° (*c* 1·03, EtOH). λ_{max} 242, 252, 261·5 mμ (ε 25,400, 29,300, 19,200). ν_{max} 3405, 1051, 1025, 1008, 955, 820, 793 cm^{-1}. CD (*c* 0·0123, MeOH): $[\theta]_{261.5}$ −31,700, $[\theta]_{251}$ −50,700, $[\theta]_{243}$ −49,400. (Found: C, 76·21; H, 10·29. $C_{30}H_{48}O_4$ requires: C, 76·22; H, 10·24%).

Saikogenin D was acetylated by heating with Ac_2O and pyridine at 100° for 5 hr. After addition of water, the ppt was filtered off, washed and dried to yield the amorphous *tetraacetate* (IIIb). ν_{max} 1743, 1243, 1049, 1028 cm^{-1}, no OH-band. NMR: τ 9·26, 9·17, 9·03, 8·88 (1, 2, 2, 1 Me, respectively); 8·00, 7·97, 7·95 (1, 1, 2 Me, respectively, of AcO groups); 6·21 (2H at C_{23}, singlet); 6·09, 5·71 (2H at C_{28}, AB quartet, J = 12 c/s); 5·20 (1H at C_3, quartet, J = 9, 5·5 c/s); 4·82 (1H at C_{16}, triplet, J = 3 c/s); 4·40, 3·56 (2H at C_{11} and C_{12}, AB quartet, J = 10 c/s).

Isopropylidene derivatives of saikogenin A

(a) Saikogenin A (100 mg) was dissolved in a mixture of acetone (8 ml), ether (33 ml) and conc H_2SO_4 (0·33 ml) and allowed to stand at room temp overnight. The mixture was neutralized with 5% $NaHCO_3$ and evaporated. The residue was extracted with ether and the ethereal soln was washed, dried and evaporated to give a foam (105 mg). Recrystallization from EtOH afforded the *diacetonide* (68 mg) as prisms, m.p. 200–204°, $[\alpha]_D$ −34·8° (*c* 0·71). λ_{max} 242·5, 251, 260·5 mμ (ε 27,700, 31,200, 20,200). ν_{max} 1109, 867, 856, 842 cm^{-1}, no OH-band. CD (*c* 0·0046, cyclohexane): $[\theta]_{260}$ −39,700, $[\theta]_{251}$ −59,500, $[\theta]_{242.5}$ −47,600, $[\theta]_{233}$ −33,300. (Found: C, 78·46; H, 10·33. $C_{36}H_{56}O_4$ requires: C, 78·21; H, 10·21%).

(b) To a soln of *p*-toluenesulphonic acid (125 mg) in acetone (250 ml), saikogenin A (4·0 g) was added and dissolved while stirring for 10 min. The soln was immediately neutralized with 5% $NaHCO_3$ and evaporated *in vacuo*. The residue was washed with water and dried. The crude product (4·67 g) was chromatographed on Al_2O_3. The eluates (2·29 g) with benzene on recrystallization from EtOH gave the diacetonide, m.p. 199–203°, identical with a specimen obtained in (a). The eluates (2·37 g) with benzene–$CHCl_3$ (4:1–1:1) were recrystallized from acetone to give the *monoacetonide* (VIIIa, 1·95 g) as needles, m.p. 250–256°, $[\alpha]_D$ −56·1° (*c* 0·76). λ_{max} 242·5, 251, 260 mμ (ε 28,500, 32,100, 20,600). ν_{max} 3258, 1104, 851 cm^{-1}. CD (*c* 0·0052, cyclohexane): $[\theta]_{261}$ −53,600, $[\theta]_{251}$ −58,500. (Found: C, 77·36; H, 10·06. $C_{33}H_{52}O_4$ requires: C, 77·29; H, 10·22%).

Saikogenin C acetonide (XIIa)

Saikogenin C (500 mg) was dissolved in a mixture of acetone (50 ml) ether (200 ml) and conc H_2SO_4 (1 ml) and allowed to stand at room temp for 2 days. The mixture was neutralized with 5% $NaHCO_3$ and evaporated *in vacuo*. The residue was extracted with ether and the extract was washed, dried and evaporated to dryness. Recrystallizations from AcOEt and from acetone afforded XIIa (332 mg) as needles, m.p. 261–264°, $[\alpha]_D$ −34·3° (*c* 0·82). λ_{max} 242·5, 251, 260 mμ (ε 28,400, 32,200, 20,500). ν_{max} 3515, 1139, 1118, 872, 828, 767 cm^{-1}. (Found: C, 78·55; H, 10·45. $C_{33}H_{52}O_3 \cdot \frac{1}{2} H_2O$ requires: C, 78·38; H, 10·56%).

Isopropylidene derivatives of saikogenin D

(a) Saikogenin D (200 mg) was dissolved in a mixture of acetone (20 ml), dry ether (80 ml) and conc H_2SO_4 (0·4 ml) and allowed to stand at room temp for 24 hr. The mixture was neutralized with 5% $NaHCO_3$ and evaporated *in vacuo*. The resulted ppt was collected, washed and dried. The product (204 mg) was recrystallized from AcOEt to give needles (87 mg) of the *diacetonide*, m.p. 252–261°. Concentration of the

mother liquor gave an additional crop (43 mg), m.p. 252–261°. Further recrystallization from AcOEt–EtOH yielded the analytical sample as needles, m.p. 260–265°, $[\alpha]_D$ +236·8° (*c* 0·50). λ_{max} 246·5, 255, 264 mμ (ε 23,000, 27,300, 19,200). ν_{max} 1623, 1145, 1115, 1075, 1050, 1028, 1019, 901 cm^{-1}, no OH-band. CD (*c* 0·00538, cyclohexane): $[\theta]_{267}$ +66,100, $[\theta]_{259}$ +93,200, $[\theta]_{248}$ +83,100. (Found: C, 78·35; H, 10·20. $C_{36}H_{56}O_4$ requires: C, 78·21; H, 10·21%).

The diacetonide on heating with 70% aq AcOH at 80° for 3 hr regenerated saikogenin D, which after recrystallization from MeOH gave needles, m.p. 256–260°, $[\alpha]_D$ −48·2° (*c* 0·54, EtOH), identical with an authentic specimen.

(b) To a soln of *p*-toluenesulphonic acid (150 mg) in acetone (300 ml), saikogenin D (3·0 g) was added and dissolved while stirring for 10 min. The soln was neutralized with 5% $NaHCO_3$ and evaporated *in vacuo*. The residue was washed with water, dried and recrystallized from AcOEt, giving needles (2·44 g) of the *monoacetonide* (XVIIa), m.p. 258–262°. The analytical sample was obtained after further recrystallization from aq EtOH as needles, m.p. 259–262°, $[\alpha]_D$ −54·1° (*c* 1·05). λ_{max} 244·5, 252·5, 262 mμ (ε 25,500, 28,750, 18,800). ν_{max} 3410, 1109, 863 cm^{-1}. CD (*c* 0·01052, cyclohexane): $[\theta]_{261}$ −38,000, $[\theta]_{252}$ −42,400, $[\theta]_{243}$ −38,400, $[\theta]_{235.5}$ −32,000. (Found: C, 76·69; H, 10·15. $C_{33}H_{52}O_4 \cdot \frac{1}{2}H_2O$ requires: C, 76·64; H, 10·23%).

Catalytic reduction of saikogenin A

A soln of saikogenin A (300 mg) in AcOH (34 ml) was shaken with Adams' catalyst (150 mg) in a H_2 atm for 3·5 hr. The catalyst was filtered off and the filtrate was evaporated *in vacuo*. Recrystallization of the residue from MeOH afforded plates (212 mg) of the dihydro derivative, *olean*-13(18)-*ene*-3β,16β,23,28-*tetrol*, m.p. 277–282°, $[\alpha]_D$ −25·8° (*c* 0·96, EtOH). λ_{max} 207 mμ (ε 10,400). ν_{max} 3443, 3348, 1075, 1063, 1049, 995, 954 cm^{-1}. (Found: C, 76·18; H, 10·71. $C_{30}H_{50}O_4$ requires: C, 75·90; H, 10·71%).

Treatment of dihydrosaikogenin A with Ac_2O in pyridine overnight afforded the *tetraacetate*, which after recrystallization from acetone–n-hexane and then from EtOH was obtained as plates, m.p. 177–179°, $[\alpha]_D$ −33·5 (*c* 1·04). λ_{max} 206 mμ (ε 12,100). ν_{max} 1734, 1250, 1228, 971, 910, 889 cm^{-1}. NMR: τ 9·23, 9·18, 9·05, 8·68 (1, 1, 3, 1 Me respectively); 7·97, 7·93 (3, 1 Me, respectively, of AcO groups); 6·25, 6·16 (2H at C_{23}, AB quartet, J = 5·5 c/s); 5·89, 5·49 (2H at C_{28}, AB quartet, J = 12 c/s); 5·33–4·75 (2H at C_3 and C_{16}, multiplet); no olefinic proton. (Found: C, 70·84; H, 9·22. $C_{38}H_{58}O_8$ requires: C, 70·99; H, 9·09%).

This compound was identified with the respective derivative derived from saikogenin A isolated by Shibata *et al.*,[10] by the mixed m.p. determination, TLC and the IR comparison.[11]

Catalytic reduction of saikogenin C

Saikogenin C (320 mg) in glacial AcOH (30 ml) was shaken with Adams' catalyst (160 mg) in a H_2 atm for 5 hr. The product freed from the catalyst and the solvent was recrystallized from AcOEt, affording needles of the dihydro derivative, *olean*-13(18)-*ene*-3β,16β,28-*triol*, m.p. 279–281°, $[\alpha]_D$ −36·7° (*c* 1·00), which gave yellow colour with tetranitromethane. λ_{max} 207 mμ (ε 11,600). ν_{max} 3480, 3360, 1231, 1062, 1046, 997 cm^{-1}. (Found: C, 78·77; H, 11·13. $C_{30}H_{50}O_3$ requires: C, 78·55; H, 10·99%).

Acetylation with Ac_2O in pyridine at room temp overnight gave the *triacetate*, which was obtained from MeOH as needles, m.p. 225–227°, $[\alpha]_D$ −46·3° (*c* 0·94). λ_{max} 206·5 mμ (ε 11,000). ν_{max} 1742, 1730, 1239, 1026, 1014, 978 cm^{-1}. NMR: τ 9·25, 9·17, 9·14, 9·10, 9·05, 8·68 (1, 1, 1, 1, 2, 1 Me, respectively); 7·97 (3 Me of AcO groups); 5·91, 5·53 (2H at C_{28}, AB quartet, J = 12 c/s); 5·48 (1H at C_3, quartet, J = 6·5, 5 c/s); 5·07 (1H at C_{16}, quartet, J = 12·5, 4 c/s); no olefinic proton. (Found: C, 73·95; H, 9·80. $C_{36}H_{56}O_6$ requires: C, 73·93; H, 9·65%).

Catalytic hydrogenation of saikogenin D

Saikogenin D (300 mg) in glacial AcOH (34 ml) was hydrogenated over Adams' catalyst (150 mg) in a H_2 atm for 4 hr. After removal of the catalyst, the filtrate was evaporated to dryness. The residue on two recrystallizations from AcOEt gave the dihydro derivative, *olean*-13(18)-*ene*-3β,16α,23,28-*tetrol* (153 mg) as needles, m.p. 257–263°, $[\alpha]_D$ +25·7° (*c* 0·74, EtOH). λ_{max} 207 mμ (ε 11,200). ν_{max} 3324, 1576, 1041, 1027, 1000, 722 cm^{-1}. (Found: C, 73·02; H, 10·48. $C_{30}H_{50}O_4 \cdot H_2O$ requires: C, 73·12; H, 10·64%).

Dihydrosaikogenin D was treated with Ac_2O in pyridine at room temp overnight and afforded the amorphous *tetraacetate*. ν_{max} 1734, 1245 cm^{-1}, no OH-band. NMR: τ 9·20, 9·18, 9·05, 8·73 (1, 1, 3, 1 Me, respectively); 7·98, 7·95 (2, 2 Me, respectively, of AcO groups); 6·22 (2H at C_{23}, singlet); 6·00, 5·75 (2H at C_{28}, AB quartet, J = 12 c/s); 5·20 (1H at C_3, quartet, J = 10, 6 c/s); 4·86 (1H at C_{16}, triplet, J = 3 c/s); no olefinic proton.

Oxidation of saikogenin C triacetate (IIb) *with selenium dioxide*

A mixture of IIb (100 mg) and SeO_2 (100 mg) in benzyl acetate (3 ml) was refluxed for 20 hr and filtered. The filtrate was evaporated to dryness *in vacuo*. The residue was purified by preparative TLC developed with toluene–AcOEt (2:1) on silica gel GF_{254} plates and recrystallization of the main fraction (71 mg) from MeOH gave yellow needles (40 mg) of 3β,16β,28-*triacetoxyolean*-9(11),13(18)-*diene*-12,19-*dione* (IV), m.p. 208–209°, $[\alpha]_D$ −93·7° (*c* 1·10). λ_{max} 278μ (ε 13,600). ν_{max} 1734, 1708, 1691, 1669, 1653, 1628, 1614, 1600, 1242, 1030, 980 cm^{-1} (Found: C, 70·94; H, 7·98. $C_{36}H_{50}O_8$ requires: C, 70·79; H, 8·25%).

Treatment of saikogenin A (Ia) *with hydrochloric acid*

A mixture of saikogenin A (100 mg) in MeOH (10 ml) and conc HCl (2 ml) was refluxed in a N_2 stream for 3·5 hr, when the mixture deposited crystals. After cooling, the crystals were filtered, washed with water and dried (75 mg). Two recrystallizations from MeOH gave plates (29 mg) of Va, m.p. 246–249° (dec), $[\alpha]_D$ +439·4° (*c* 0·57). λ_{max} 319 mμ (ε 19,100). ν_{max} 3235, 1634, 1048, 825 cm^{-1}. (Found: C, 82·00; H, 10·55. $C_{29}H_{44}O_2$ requires: C, 82·02; H, 10·44%).

Treatment of Va with Ac_2O and pyridine at room temp overnight afforded the amorphous *diacetate* (Vb), which was isolated through extraction with ether. NMR: τ 9·13, 9·07, 8·95, 8·73 (3, 1, 1, 1 Me, respectively); 7·98 (2 Me of AcO groups); 6·32, 6·02 (2H at C_{23}, AB quartet, J = 11 c/s); 5·18 (1H at C_3, second-order quartet); 4·32, 4·26 (2H at C_{11} and C_{12}, AB quartet, J = 7 c/s).

A soln of Va (60 mg) in acetone (5 ml), dry ether (20 ml) and conc H_2SO_4 (0·1 ml) was allowed to stand in a N_2 atm at room temp overnight, neutralized with 5% $NaHCO_3$ and evaporated *in vacuo*. The residue was extracted with ether and the ethereal soln was washed, dried and evaporated to leave a crystalline solid (65 mg). Recrystallization from MeOH afforded the *acetonide* (35 mg) as scales, m.p. 225–232°, $[\alpha]_D$ +384·4° (*c* 0·69). λ_{max} 319 mμ (ε 19,100). ν_{max} 1631, 1205, 1117, 856, 825 cm^{-1}, no OH-band. (Found: C, 82·96; H, 10·42. $C_{32}H_{48}O_2$ requires: C, 82·70; H, 10·41%).

Treatment of saikogenin C (IIa) *with hydrochloric acid*

A soln of saikogenin C (100 mg) in MeOH (10 ml) and conc HCl (2 ml) was refluxed in a N_2 stream for 4 hr. The mixture was diluted with water and extracted with ether. The extract was washed with 5% $NaHCO_3$ and water, dried and evaporated to give the residue (75 mg). Two recrystallizations from MeOH gave pale yellow plates (41 mg) of 28-*norolean*-9(11),12,17-*trien*-3β-*ol* (VIa), m.p. 187–191°, $[\alpha]_D$ +456·6° (*c* 0·63). λ_{max} 319 mμ (ε 18,000). ν_{max} 3645, 3310, 1632, 1036, 990, 825 cm^{-1}. NMR: τ 9·17, 9·12, 9·05, 8·97, 8·93, 8·77 (1, 2, 1, 1, 1, 1 Me, respectively); 6·75 (1H at C_3, second-order quartet); 4·32, 4·26 (2H at C_{11} and C_{12}, AB quartet, J = 7·5 c/s). (Found: C, 84·40; H, 11·02. $C_{29}H_{44}O \cdot \frac{1}{2}H_2O$ requires: C, 84·34; H, 10·86%).

Treatment of saikogenin D (IIIa) *with hydrochloric acid*

A solution of saikogenin D (100 mg) in MeOH (10 ml) and conc HCl (2 ml) was refluxed for 4 hr in a N_2 stream. The deposited crystals were collected and recrystallized from MeOH to give Va (25 mg), m.p. 244–248° (dec), which was identified with a specimen derived from saikogenin A by a mixed m.p. and IR comparison.

Saikogenin A 3β,23-*acetonide* 16β,28-*diacetate* (VIIb)

The monoacetonide VIIa (1·945 g) was treated with Ac_2O (10 ml) and pyridine (24 ml) at room temp overnight. The mixture was poured into ice-water and the ppt was filtered off, washed and dried to give amorphous VIIb (2·27 g). Attempts to crystallize were unsuccessful. ν_{max} 1734, 1225, 1101, 1021, 859 cm^{-1}, no OH-band.

Saikogenin A 16β,28-*diacetate* (VIIIa)

The foregoing VIIb (2·1 g) in 70% aq AcOH (80 ml) was heated at 80° for 1·5 hr and evaporated *in vacuo*. The residue (2·0 g) was chromatographed on Al_2O_3. The eluates (1·52 g) with benzene–$CHCl_3$ (1:1) to $CHCl_3$–MeOH (100:1) were recrystallized from AcOEt giving prisms (1·389 g), m.p. 214–219°. The analytical sample was obtained after further recrystallization from AcOEt–n-hexane as prisms, m.p. 220–221°, $[\alpha]_D$ −72·1° (*c* 1·03). λ_{max} 241, 249·5, 258·5 (ε 28,500, 32,500, 20,400). ν_{max} 3527, 1741, 1712, 1266, 1255, 1045, 1034 cm^{-1}. (Found: C, 73·34; H, 9·41. $C_{34}H_{52}O_6$ requires: C, 73·47; H, 9·58%).

Saikogenin A 16β,28-*diacetate* 23-*trityl ether* (VIIIb)

A mixture of VIIIa (1·28 g) and trityl chloride (2·56 g) in pyridine (30 ml) was heated at 80–90° for 3 hr. Additional trityl chloride (1·28 g) was added and the heating was continued for 2 hr. The mixture was poured into water and the ppt was filtered off, washed, dried and chromatographed on Al_2O_3. Elution with pet ether–benzene (1:1) and benzene afforded amorphous VIIIb (1·22 g).

Saikogenin A 3β,16β,28-*triacetate* 23-*trityl ether* (VIIIc)

The foregoing VIIIb (1·22 g) in Ac_2O (3 ml) and pyridine (8 ml) was allowed to stand at room temp overnight and then heated at 100° for 4 hr. The mixture was poured into water and the ppt was filtered off, washed and dried. The product (1·21 g) on recrystallization from EtOH gave small prisms (1·05 g), m.p. 160–168°. The analytical sample was obtained after 2 recrystallizations from AcOEt–EtOH as prisms, m.p. 176–181°, $[\alpha]_D$ −12·2° (*c* 1·03). λ_{max} 233·5, 241, 250, 259 mμ (ε 25,300, 30,800, 33,200, 21,400). ν_{max} 3480 (H_2O), 1735, 1598, 1245, 1075, 1027, 889, 769, 753, 711, 702 cm^{-1}. (Found: C, 77·77; H, 8·26. $C_{55}H_{68}O_7 \cdot \frac{1}{2}H_2O$ requires: C, 77·70; H, 8·18%)

Acid hydrolysis of the trityl ether-triacetate (VIIIc)

A mixture of VIIIc (1·0 g) in 80% aq AcOH (45 ml) was heated at 70–75° for 4 hr and poured into water. The ppt was filtered off, washed, dried and chromatographed on Al_2O_3. Elution with benzene–$CHCl_3$ (9:1 and 4:1) afforded a glassy material (515 mg), which consisted of two *isomeric triacetates*, VIIId and VIIIe, as shown by the fact that the TLC exhibited two spots adjacent together. This was subjected to the next step without separation.

Oxidation of the isomeric triacetates of saikogenin A

The foregoing product (255 mg) in acetone (25 ml) was treated with the Kiliani's reagent (1·06 ml) under stirring at 18° for 5 min. After dilution with water, the excess oxidant was destroyed with $NaHSO_3$ soln. The mixture was extracted with ether and the extract was washed with 2·5% $NaHCO_3$ and water, dried and evaporated. The residue (253 mg) was separated into two fractions by preparative TLC developed with n-hexane–AcOEt (3:1) on silica gel GF_{254} plates. The easily mobile fraction afforded an oil (98 mg) of the 23-*aldehyde* (IX). λ_{max} 241·5, 249·5, 259 mμ. ν_{max} 2708, 1738, 1234, 1025 cm^{-1}. NMR: τ 9·20, 9·03, 8·93, 8·87 (2, 2, 1, 1 Me, respectively; 8·04, 8·00, 7·97 (1 Me each of AcO groups); 5·91, 5·48 (2H at C_{28}, AB quartet, J = 11 c/s); 4·98 (2H at C_3 and C_{16}, multiplet); 4·38, 3·53 (2H at C_{11} and C_{12}, AB quartet, J = 10·5 c/s); 0·72 (1H at C_{23}, singlet).

The less mobile fraction afforded the 3-*ketone* (X, 110 mg) as an oil. λ_{max} 241·5, 249·5, 259 mμ. ν_{max} 1737, 1707, 1235, 1033 cm^{-1}. NMR: τ 9·20, 9·13, 9·05, 8·98, 8·85 (1, 1, 1, 2, 1 Me, respectively); 7·98, 7·97, 7·94 (1 Me each of AcO groups); 5·92 (2H at C_{23}, singlet); 5·89, 5·48 (2H at C_{28}, AB quartet, J = 12 c/s); 4·90 (1H at C_{16}, quartet, J = 12, 4 c/s); 4·38, 3·54 (2H at C_{11} and C_{12}, AB quartet, J = 10 c/s).

Huang-Minlon reduction of the 23-*aldehyde* (IX)

A mixture of the foregoing IX (90 mg), hydrazine hydrate (0·8 ml) and ethylene glycol (8 ml) was refluxed for 1 hr. After cooling, KOH (0·8 g) was added to the mixture, which was heated without condenser until temp reached 195°. After refluxing for additional 3 hr, the mixture was poured into water and extracted with ether. The ethereal soln was washed, dried and evaporated to give a solid (66 mg). Two recrystallizations from AcOEt gave saikogenin C (IIa), m.p. 290–293°, identical with a specimen isolated from the plant. Identity between both the specimens was further established through the triacetate IIb.

Huang-Minlon reduction of the 3-*ketone* (X)

The 3-ketone X (86 mg) was reduced with the same manner as described in the preceding experiment. The product (56 mg) obtained through extraction with ether was recrystallized from AcOEt, giving prisms (40 mg) of 24 (or 23)-*norolean*-11,13(18)-*diene*-16β,28-*diol* (XI), m.p. 248–250°, $[\alpha]_D$ −35° (*c* 0·50). λ_{max} 242·5, 251, 260 mμ (ε 24,200, 27,500, 17,400). ν_{max} 3270, 1071, 1046, 1008, 990 cm^{-1}. (Found: C, 81·75; H, 11·11. $C_{29}H_{46}O_2$ requires: C, 81·63; H, 10·87%).

Saikogenin C *acetonide acetate* (XIIb)

The acetonide XIIa (300 mg) was treated with Ac_2O (3 ml) and pyridine (7 ml) at room temp overnight and poured into ice-water. The ppt was filtered off, washed, dried and recrystallized from acetone giving XIIb (304 mg) as prisms, m.p. 279–280°, $[\alpha]_D$ −34·6° (*c* 0·93). λ_{max} 242·5, 251, 260 mμ (ε 28,500, 32,400,

20,600). ν_{max} 1730, 1249, 1140, 764 cm^{-1}. (Found: C, 77·79; H, 10·17. $C_{35}H_{54}O_4$ requires: C, 78·02, H, 10·10%).

Saikogenin C 3-*monoacetate* (XIIIa)

(a) *From saikogenin C acetonide acetate* (XIIb). A mixture of XIIb (270 mg) in 85% aq AcOH (20 ml) was refluxed for 2 hr and evaporated *in vacuo*. Recrystallization of the residue from AcOEt gave XIIIa (108 mg) as needles, m.p. 277–281°, $[\alpha]_D$ −40·2° (*c* 0·90). λ_{max} 242·5, 251, 260 mμ (ε 27,600, 31,000, 19,800). ν_{max} 3300, 1739, 1245, 1028, 986 cm^{-1}. (Found: C, 76·92; H, 10·22. $C_{32}H_{50}O_4$ requires: C, 77·06; H, 10·11%).

(b) *From saikogenin* C *triacetate* (IIb). The triacetate IIb (1·28 g) in MeOH (200 ml) was refluxed with K_2CO_3 (640 mg) in water (10 ml) for 45 min, neutralized with dil AcOH and evaporated *in vacuo*. After addition of water, the separated crystals were filtered off, washed, dried and recrystallized from EtOH and from AcOEt, yielding prisms (542 mg), m.p. 276–279°, which was identical with a specimen of XIIIa obtained in (a).

Partial acetylation of saikogenin C 3-*monoacetate* (XIIIa)

A soln of XIIIa (340 mg) in Ac_2O (1·1 ml), pyridine (5·5 ml) and $CHCl_3$ (28 ml) was allowed to stand in a refrigerator for 18 hr and then at 25° for 4 hr. To the mixture, water and ether were added and the organic layer was washed successively with 5% HCl, 5% $NaHCO_3$ and water, dried and evaporated. The residue (381 mg) was chromatographed on Al_2O_3. Elution with pet ether–benzene (1:1) and benzene gave crystals (84 mg), which on recrystallization from MeOH afforded needles (47 mg) of IIb, m.p. 211–213°. Elution with benzene–$CHCl_3$ (19:1 to 9:1) gave oily fractions (147 mg), which were separated into two fractions by preparative TLC developed with toluene–AcOEt (2:1) on silica gel GF_{254} plates. The more mobile fraction (16 mg) on recrystallization from EtOH gave the 3,16-*diacetate* (XIIIc, 10 mg), m.p. 233–237°, $[\alpha]_D$ +17·7° (*c* 0·35). ν_{max} 3545, 1723, 1720, 1260, 1242, 1025 cm^{-1}. (Found: C, 75·68; H, 9·41. $C_{34}H_{52}O_5$ requires: C, 75·51; H, 9·68%). The less mobile fraction (129 mg) was recrystallized from EtOH to give the 3,28-*diacetate* XIIIb (99 mg) as needles, m.p. 163–166°, $[\alpha]_D$ −103·4° (*c* 0·80). λ_{max} 241·5, 250, 259 mμ (ε 27,800, 31,800, 20,200). ν_{max} 3532, 1728, 1722, 1633, 1275, 1261, 1242, 1025 cm^{-1}. (Found: C, 75·53; H, 9·73. $C_{34}H_{52}O_5$ requires: C, 75·51; H, 9·69%).

Oxidation of saikogenin C 3,28-*diacetate* (XIIIb)

A soln of XIIIb (40 mg) in acetone (4 ml) was treated with the Kiliani's reagent (0·2 ml) under stirring at 20° for 5 min. The excess reagent was destroyed with a $NaHSO_3$ soln and the mixture was diluted with ether, washed with 5% $NaHCO_3$ and water, and dried. Evaporation of the solvent afforded the residue (37 mg), which was recrystallized twice from MeOH to give prisms (21 mg), m.p. 247–250°, $[\alpha]_D$ +94·9° (*c* 0·82), which was identified with a specimen of 3β,28-*diacetoxyolean*-11,13(18)-*dien*-16-*one* (XIV) obtained by oxidation of XXIc. λ_{max} 242, 250, 259 mμ (ε 27,300, 30,000, 18,500). ν_{max} 1741, 1730, 1714, 1248, 1035 cm^{-1}. CD (*c* 0·03916, MeOH): $[\theta]_{298}$ −6,600. (Found: C, 75·70; H, 9·47. $C_{34}H_{50}O_5$ requires: C, 75·80; H, 9·35%).

Oxidation of longispinogenin triacetate (XV) *with selenium dioxide*

A mixture of XV (30 mg)[16] and SeO_2 (30 mg) in glacial AcOH (2 ml) was refluxed for 6 hr. A red ppt was filtered off and the filtrate was evaporated *in vacuo*. The residue was dissolved in ether and washed with 5% $NaHCO_3$ and water. Evaporation of the ether afforded an oily residue (21 mg), which was separated into two fractions by preparative TLC developed with n-hexane–AcOEt (4:1) on silica gel G. The more mobile fraction (7 mg) was identified as the recovered XV. The less mobile one (7 mg) on recrystallization from EtOH gave needles (5 mg), m.p. 213–215°, which was identified with a specimen of saikogenin C triacetate (IIb) by the mixed m.p. determination, TLC and the IR spectra.

Saikogenin A 23,28-*dityrityl ether*

A mixture of saikogenin A (300 mg), trityl chloride (486 mg) and pyridine (6 ml) was heated at 70–80° for 6 hr. Additional trityl chloride (300 mg) was added and the heating was continued for 2 hr. The mixture was diluted with ether, washed with water, dried and evaporated. The residue was chromatographed on Al_2O_3 and the eluates (544 mg) with benzene were recrystallized from acetone-n-hexane to give needles (460 mg) of the 23,28-dityrityl ether, m.p. 167–171°. Further recrystallization from the same solvents afforded the analytical sample, m.p. 169–172°, $[\alpha]_D$ −118·1° (*c* 0·37), which combined with acetone as shown by the IR spectrum and analysis. λ_{max} 234, 242·5, 251·5, 261 mμ (ε 29,000, 29,300, 30,600, 20,200).

ν_{max} 3580, 3490, 1596, 1076, 779, 769, 748, 705 cm^{-1} and 1713 cm^{-1} (acetone). (Found: C, 84·41; H, 8·07. $C_{68}H_{76}O_4 \cdot \frac{1}{2}C_3H_6O$ requires: C, 84·63; H, 8·07%).

The above material on recrystallization from AcOEt afforded an isomorphic form, m.p. 195–199° which contained AcOEt as shown by the IR bands at 1738 and 1252 cm^{-1}. These combined solvents could not be removed on heating *in vacuo* at 110° for 5 hr.

Saikogenin D 23,28-*dityityl ether*

A mixture of saikogenin D (200 mg) and trityl chloride (325 mg) in pyridine (4 ml) was heated at 80° for 6 hr. Additional trityl chloride (325 mg) was added and the heating was continued for 2 hr. The mixture was diluted with $CHCl_3$, washed with water, dried and evaporated to give the oily residue (850 mg); which was purified by chromatography on Al_2O_3. The fractions (369 mg) eluted with benzene afforded, on recrystallization from n-hexane, the 23,28-dityityl ether (300 mg) as needles, m.p. 173–175°, $[\alpha]_D$ −138·7° (*c* 1·10), which contained n-hexane as shown by the analytical result. λ_{max} 235, 244, 253, 262 mμ (ε 27,300, 27,800, 30,600, 20,800). ν_{max} 3600, 1076, 991, 902, 780, 770, 750, 708 cm^{-1}. (Found: C, 85·47; H, 8·53. $C_{68}H_{76}O_4 \cdot \frac{1}{2}C_6H_{14}$ requires: C, 85·25; H, 8·32%).

23,28-*Dityityloxyolean*-11,13(18)-*diene*-3,16-*dione* (XVI)

(a) *From saikogenin* A 23,28-*dityityl ether*. A soln of the 23,28-dityityl ether (250 mg) in acetone (20 ml) was treated with the Kiliani's reagent (1·35 ml) under stirring at 16–18° for 10 min. After dilution with water, the excess reagent was destroyed with $NaHSO_3$ soln. The mixture was extracted with ether and the extract was washed with 5% $NaHCO_3$ and water, dried and evaporated. The residue (231 mg) was recrystallized twice from MeOH–$CHCl_3$ giving XVI (190 mg) as prisms, m.p. 241–245°, $[\alpha]_D$ −58·4° (*c* 1·09). λ_{max} 234, 243, 251·5, 261 mμ (ε 32,700, 32,700, 33,900, 21,900). ν_{max} 1703, 1598, 1073, 1058, 980, 900, 711, 707, 697 cm^{-1}. (Found: C, 85·46; H, 7·56. $C_{68}H_{72}O_4$ requires: C, 85·67; H, 7·61%).

(b) *From saikogenin* D 23,28-*dityityl ether*. The dityityl ether (50 mg) in acetone (4 ml) was treated with the Kiliani's reagent (0·27 ml) at 18° for 10 min. The product was isolated by the same manner as described in (a) and recrystallized twice from MeOH–$CHCl_3$ to give XVI (35 mg), m.p. 241–245°. Identity with the specimen obtained in (a) was established with the mixed m.p. and IR comparison.

Saikogenin D 3β,23-*acetonide* 16α,28-*diacetate* (XVIIb)

The monoacetonide XVIIa (1·90 g) was treated with Ac_2O (10 ml) in pyridine (24 ml) at room temp for 18 hr and then at 100° for 6 hr. The mixture was poured into water and the ppt was filtered off, washed and dried to give amorphous XVIIb (2·10 g). Attempts to crystallize it were failed. ν_{max} 1739, 1236, 1110, 1066, 1028, 866 cm^{-1}, no OH-band.

Saikogenin D 16α,28-*diacetate* (XVIIIa)

A soln of the foregoing XVIIb (2·10 g) in 70% aq AcOH (100 ml) was heated at 80° for 1·5 hr and evaporated *in vacuo*. The residue was chromatographed over Al_2O_3. Elution with benzene–$CHCl_3$ (4:1) to $CHCl_3$–MeOH (100:1) afforded amorphous XVIIIa (1·506 g), which exhibited one spot on TLC.

Saikogenin D 16α,28-*diacetate* 23-*trityl ether* (XVIIIb)

The foregoing XVIIIa (1·506 g) in pyridine (30 ml) was heated with trityl chloride (3·0 g) at 80° for 4 hr. The mixture was diluted with $CHCl_3$, washed with water, dried and evaporated to give an oily residue (4·19 g), from which triphenylmethanol (920 mg) was removed by crystallization from benzene. The mother liquor was evaporated and chromatographed on Al_2O_3. Elution with benzene and benzene–$CHCl_3$ (9:1) gave amorphous XVIIIb (1·49 g). λ_{max} 243, 251·5, 261 mμ. ν_{max} 3470, 1733, 763, 746, 700 cm^{-1}.

Saikogenin D 3β,16α,28-*triacetate* 23-*trityl ether* (XVIIIc)

The foregoing XVIIIb (1·49 g) was treated with Ac_2O (5 ml) in pyridine (12 ml) at room temp overnight and then at 90° for 4·5 hr. The mixture was poured into water and the ppt was filtered off, washed and dried. Recrystallization from EtOH gave XVIIIc (1·08 g) as small crystals, m.p. 145–147°, $[\alpha]_D$ −23·6° (*c* 0·22). λ_{max} 242, 251, 260 mμ (ε 30,400, 33,400, 21,200). ν_{max} 1740, 1597, 1245, 1029, 768, 749, 709 cm^{-1}. (Found: C, 78·67; H, 8·33. $C_{55}H_{68}O_7$ requires: C, 78·53; H, 8·15%).

Acid hydrolysis of the trityl ether-triacetate (XVIIIc)

A mixture of XVIIIc (1·07 g) in 80% aq AcOH (30 ml) was heated at 70–75° for 5·5 hr and poured into

water. The resulted ppt was filtered off, washed, dried and chromatographed on Al_2O_3. Flution with benzene–$CHCl_3$ (9:1 and 4:1) afforded a glassy material (679 mg), which consisted of the two *isomeric triacetates*, XVIIId and XVIIIe, as shown by the TLC exhibiting two spots adjacent together and by the succeeding experiment.

Oxidation of the isomeric triacetates of saikogenin D

The foregoing product (500 mg) in acetone (50 ml) was treated with the Kiliani's reagent (2·3 ml) under stirring at 18° for 5 min. After dilution with water, the excess reagent was destroyed with $NaHSO_3$ soln. The mixture was extracted with ether and the ether soln was washed with 5% $NaHCO_3$ and water, dried and evaporated to give an oil (496 mg), which showed two spots on the TLC. A part (12 mg) of the product was separated, by preparative TLC developed with n-hexane–AcOEt (4:1) on a silica gel GF_{254} plate, into the respective fractions. The more mobile fraction was characterized as the 23-*aldehyde* (XIX) by the IR bands at ν_{max} 2705, 1734, 1724 cm^{-1} and the less mobile one as the 3-*ketone* (XX) by the bands at ν_{max} 1735, 1725, 1705 cm^{-1}. Both the fractions did not crystallize and the remainder of the product was subjected to the next step without separation.

Huang-Minlon reduction of the oxidation product

A mixture of the foregoing oxidation product (484 mg), hydrazine hydrate (5 ml) and ethylene glycol (50 ml) was refluxed for 1 hr. After cooling, KOH (5 g) was added to the mixture, which was heated without condenser until temp reached 195°. After refluxing for additional 3 hr, the mixture was poured into water and extracted with ether. The ethereal soln was washed, dried and evaporated to give a solid (391 mg), which was chromatographed on Al_2O_3. The eluates (138 mg) with benzene–$CHCl_3$ (2:1), on recrystallization from AcOEt, gave needles (72 mg), m.p. 198–203°. Further recrystallization from EtOH gave 24(or 23)-*norolean*-11,13(18)-*diene*-16α,28-*diol* (XXII) as needles, m.p. 203–209°, $[\alpha]_D$ −29·3° (*c* 0·50). λ_{max} 244, 252·5, 262 mμ (ε 22,000, 25,600, 16,400). ν_{max} 3435, 1618, 1040, 1019, 1004, 981 cm^{-1}. (Found: C, 79·79; H, 10·97. $C_{29}H_{46}O_2 \cdot \frac{1}{2}H_2O$ requires: C, 79·97; H, 10·88%).

The next fractions (120 mg) eluted with benzene–$CHCl_3$ (1:1) and $CHCl_3$ were recrystallized from aq EtOH giving needles (68 mg), m.p. 231–238°, which was identified with a specimen of XXIa prepared by SeO_2 oxidation of primulagenin A triacetate (XXIIIb) followed by saponification. Identity of both the specimens was further established through the diacetate (XXIc).

Oxidation of primulagenin A triacetate (XXIIIb) *with selenium dioxide*

A mixture of XXIIIb (90 mg),[18] m.p. 158–161°, $[\alpha]_D$ −9·9°, and SeO_2 (100 mg) in glacial AcOH (5 ml) was refluxed for 1 hr. The filtered soln was diluted with ether, washed with 5% $NaHCO_3$ and water, dried and evaporated. The residue (87 mg) was chromatographed on Al_2O_3 and elution with pet ether–benzene (1:1) gave the amorphous 11,13(18)-*diene triacetate* (XXIb, 68 mg).

The foregoing product was treated with 3% KOH–EtOH (3 ml) under reflux for 2 hr. The mixture was diluted with water and evaporated. The separated crystals were filtered, dried and recrystallized from AcOEt to give needles (47 mg), m.p. 208–233°. Recrystallization from acetone gave *olean*-11,13(18)-*diene*-3β,16α,28-*triol* (XXIa, 44 mg) as needles, m.p. 237–243°, which combined with 1·5 moles of acetone as shown by the IR spectrum and analytical result. $[\alpha]_D$ −37·6° (*c* 0·53, EtOH), (−45° on calculation as acetone-free). λ_{max} 244·5, 252·5, 262 mμ (ε 27,200, 32,000, 20,600). ν_{max} 3446, 1693, 1240, 1021 cm^{-1}. (Found: C, 76·31; H, 10·31. $C_{30}H_{48}O_3 \cdot 1\frac{1}{2}C_3H_6O$ requires: C, 76·23; H, 10·57%).

The above specimen on recrystallization from aq EtOH gave an isomorphic form, needles, m.p. 237–242°, which contained EtOH. ν_{max} 3365, 1051, 1027, 1009, 987 cm^{-1}. (Found: C, 76·63; H, 10·78. $C_{30}H_{48}O_3 \cdot C_2H_5OH$ requires: C, 76·44; H, 10·83%).

Olean-11,13(18)-*diene*-3β,16α,28-*triol* 3,28-*diacetate* (XXIc)[19]

The triol XXIa (26 mg) in Ac_2O (0·4 ml) and pyridine (1 ml) was stored in a refrigerator overnight and poured into ice-water. The resulted ppt was filtered, washed, dried and recrystallized twice from $CHCl_3$–MeOH to give XXIc (16 mg) as plates, m.p. 266–271°, $[\alpha]_D$ −98·2° (*c* 0·44). λ_{max} 243, 251, 261 mμ (ε 27,500, 31,600, 19,800). ν_{max} 3520, 1733, 1710, 1279, 1250, 1026 cm^{-1}. (Found: C, 74·52; H, 9·81. $C_{34}H_{52}O_5 \cdot \frac{1}{2}H_2O$ requires: C, 74·29; H, 9·72%).

Oxidation of olean-11,13(18)-*diene*-3β,16α,28-*triol* 3,28-*diacetate* (XXIc)

A mixture of XXIc (50 mg) and the Kiliani's reagent (0·25 ml) in acetone (5 ml) was stirred at 18° for

5 min. The excess oxidant was destroyed with $NaHSO_3$ soln and the mixture was diluted with ether, washed with 5% $NaHCO_3$ and water, and dried. After evaporation of the solvent, the residue was recrystallized from MeOH, giving prisms (38 mg) of XIV, m.p. 247–251°. This was identified with the above-mentioned specimen derived from oxidation of saikogenin C 3,28-diacetate (XIIIc) by the mixed m.p., TLC and IR comparison.

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