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TRITERPENOIDS FROM *BUPLEURUM FALCATUM* L.—II[1]

ISOLATION OF SAIKOGENIN B AND LONGISPINOGENIN[2]

TOKUO KUBOTA and F. TONAMI

Shionogi Research Laboratory, Shionogi & Co. Ltd., Fukushima-ku, Osaka, Japan

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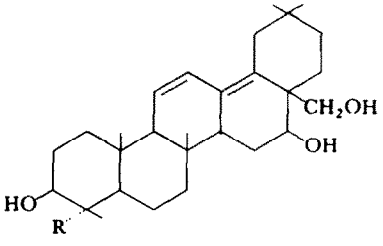
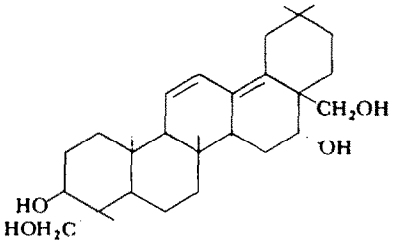
Abstract—A new triterpenoid triol having a homoannular diene together with the known longispinogenin have been isolated, as minor components, by acid hydrolysis of the saponin from the roots of *Bupleurum falcatum* L. The new triol, saikogenin B, has been shown to have the structure olean-9(11),12-diene-3β,16β,28-triol. In the course of the structure elucidation, the epimeric 11-OH derivatives of longispinogenin were prepared and the results compared with those described for β-amyrin.

IN THE preceding paper,[1] it was shown that acid hydrolysis of the saponin fraction from the roots of *Bupleurum falcatum* L. yielded a mixture of triterpenoid sapogenins, including saikogenins A, C and D which were 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. In addition to these three triterpenes possessing a heteroannular diene, a fraction exhibiting an UV absorption at 282 mμ, characteristic of a homoannular diene, was obtained. This paper deals with the separation of the homoannular diene fraction into the known longispinogenin[3] (IVa) and a new substance named saikogenin B, the structure of which is elucidated as olean-9(11),12-diene-3β,16β,28-triol (Va).



Ia R = CH_2OH; saikogenin A
IIa R = CH_3; saikogenin C



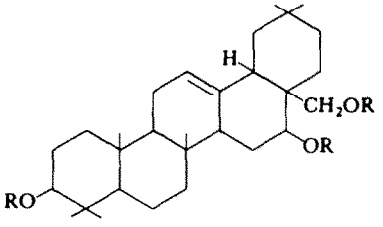
IIIa saikogenin D

The fraction showing a homoannular diene chromophore was initially obtained as the acetate, m.p. 211–214°, $[\alpha]_D$ +177°, λ_{max} 282 mμ (ε 6400), which on saponification gave the alcohol, m.p. 270–272°. Although the fraction gave no indication of being a mixture even by TLC, the intensity of the UV absorption was considerably lower than the value expected for a homoannular diene. Gas chromatography of its trimethylsilyl derivative showed two peaks, indicating a mixture.

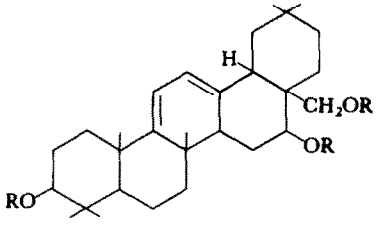
The crude acetate was subjected to TLC on Merck Silica Gel GF_{254} and after development with n-hexane–ethyl acetate–water (40:10:1), a fluorescent spot detectable under UV light was merely the lower two-thirds of an area visible by spraying

[1] Part I: T. Kubota, F. Tonami and H. Hinoh, *Tetrahedron* **23**, 2433 (1967).
[2] A part of this paper was outlined in *Tetrahedron Letters* 701 (1966).
[3] C. Djerassi, L. F. Geller and A. J. Lemin, *J. Am. Chem. Soc.* **76**, 4089 (1954).

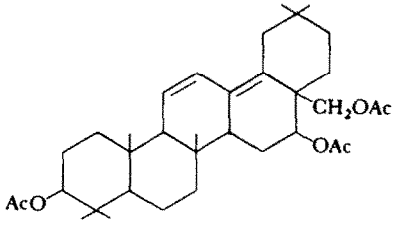
with conc sulphuric acid followed by heating. This suggested that the TLC gave only an imperfect separation of the crude acetate into its components. The method was applied on a preparative scale and the crude acetate divided into a lower fraction, ε 8700 at λ_{max} 282 mμ and an upper one, ε 4560. The lower fraction on sapnonification yielded pure saikogenin B (Va), λ_{max} 282 mμ (ε 9000), which exhibits only one peak on gas chromatography. The upper fraction was again divided by TLC into 3 fractions, of which the upper one, m.p. 221–222°, is identical with authentic longispinogenin triacetate (IVb)[3] by the mixed m.p. and IR spectra but still displays a weak absorption at 282 mμ (ε 1540). Isolation of pure longispinogenin showing no UV absorption is described below.



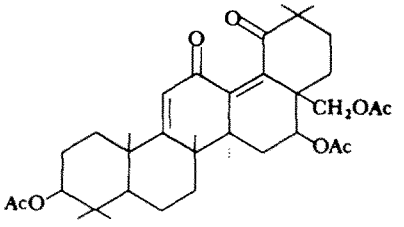
IVa R = H
b R = Ac



Va R = H
b R = Ac



IIb



VI

Saikogenin B (Va), $C_{30}H_{48}O_3$, m.p. 267–269°, $[\alpha]_D$ +286°, is readily acetylated, giving the triacetate, $C_{36}H_{54}O_6$, m.p. 209–211°, $[\alpha]_D$ +224°, which shows no OH absorption in the IR spectrum. The NMR spectrum indicates the existence of two olefinic protons (4·42τ, singlet) and three AcO groups (6H at 7·97τ and 3H at 7·93τ), one of which is primary (2H at 5·97 and 5·80τ, AB quartet) and two are secondary (1H each at 5·48 and 4·48τ, multiplets). From these data, saikogenin B is expected to be an olean-9(11),12-dienetriol, in which the three OH groups are probably located at the same positions, 3β,16β and 28, as in saikogenin C (IIa) and longispinogenin (IVa). Oxidation of saikogenin B triacetate (Vb) with selenium dioxide in glacial acetic acid gives 3β,16β,28-triacetoxyolean-9(11),13(18)-diene-12,19-dione (VI), m.p. 208–209°, λ_{max} 278 mμ (ε 13,600), identical with the product previously obtained by oxidation of saikogenin C triacetate (IIb) with selenium dioxide in benzyl acetate.[1] The structure of saikogenin B has now been elucidated as olean-9(11),12-diene-3β,16β,28-triol (Va) with reservation of the configuration at C_{18}. Since saikogenin B

triacetate (Vb) is readily oxidized with selenium dioxide to the diene-dione VI, pure longispinogenin triacetate (IVb) showing no absorption at 282 mμ can be isolated as the unchanged material after selenium dioxide oxidation of the crude saikogenin B triacetate containing IVb.

The configuration at C_{18} in saikogenin B (Va) is likely to be β from the biogenetical point of view. However, catalytic hydrogenation of saikogenin B triacetate (Vb) gave a complex mixture, which could not be identified with longispinogenin triacetate (IVb). On the other hand, in view of the fact that the Δ^{12}- (IVa), $\Delta^{9(11),12}$- (Va) and $\Delta^{11,13(18)}$- (IIa) derivatives of oleanane-3β,16β,28-triol are found in this plant, it was considered that saikogenin B (Va) and/or C (IIa) may be artifacts produced during acid hydrolysis of the saponin, an acid-labile component, e.g. 11-hydroxylated longispinogenins. Therefore, it was decided to prepare the 11-hydroxy derivatives of longispinogenin[4] as reference samples in search for the true sapogenin and at the same time to establish the C_{18}-configuration in saikogenin B (Va) by dehydration following the partial synthesis of olean-$\Delta^{9(11),12}$-dien-3β-ol from β-amyrin.[5,6]

Oxidation of longispinogenin triacetate (IVb) with sodium dichromate in acetic acid gives 3β,16β,28-triacetoxyolean-12-en-11-one (VII), m.p. 186–188°, λ_{max} 247 mμ (ε 12,400). Reduction of the ketone VII with LAH affords almost exclusively a tetrol, m.p. 243–256°, $[\alpha]_D$ +97°, probably olean-12-ene-3β,11β,16β,28-tetrol (VIIIa), together with a very small amount of the 11α-epimer IXa described below. The main product VIIIa on prolonged treatment with acetic anhydride and pyridine at room temperature yielded the corresponding tetraacetate VIIIb, m.p. 220–229°, $[\alpha]_D$ +150°. In order to obtain the 11α-epimer, the unsaturated ketone VII was subjected to reduction with sodium and isoamyl alcohol,[5,7] yielding a mixture of two tetrols, neither of which is identical with the products obtained by LAH reduction. The major product, m.p. 200–248°, $[\alpha]_D$ −172°, could be an isomer X in which the double bond has migrated to the tetrasubstituted $\Delta^{13(18)}$ position, because of the high intensity (ε 11,500) of UV absorption at 205·5 mμ[8] and the stability to acid treatment. The minor component, m.p. 294–308°, could be a saturated tetrol XI because of its negligible absorption at 207 mμ and lack of colouration with tetranitromethane. Although this result is inconsistent with that reported previously,[5,7] the compounds are not quite the same, and an attempt to prepare the 11α-epimer IXa by this method was unsuccessful. However, the compound IXa was conveniently obtained as described below.

In order to dehydrate the allylic OH group, the 3β,11β,16β,28-tetrol (VIIIa) was heated with anhydrous sodium acetate in acetic anhydride.[5,6] The treatment yielded only a small amount of the expected conjugated diene, saikogenin B triacetate (Vb) and mainly a tetraacetate, m.p. 211–219°, $[\alpha]_D$ −9°, which differs from the tetraacetate VIIIb obtained by acetylation with acetic anhydride and pyridine. The same result was obtained from a similar treatment of the 3β,11β,16β,28-tetraacetate (VIIIb). Saponification of the newly obtained tetraacetate gave the corresponding tetrol,

[4] The authors are grateful to Professor Carl Djerassi for a gift of longispinogenin.

[5] C. W. Picard and F. S. Spring, *J. Chem. Soc.* 1198 (1940).

[6] G. G. Allan, J. D. Johnston and F. S. Spring, *J. Chem. Soc.* 1546 (1954).

[7] I. Agata, E. J. Corey, A. G. Hortmann, J. Klein, S. Proskow and J. J. Ursprung, *J. Org. Chem.* **30**, 1698 (1965).

[8] P. Bladon, H. B. Henbest and G. W. Wood, *J. Chem. Soc.* 2737 (1952).

CH2OH
OH
HO
HO
X
CH2OH
OH
HO
HO
XI
CH2OAc
OAc
H
O
AcO
VII
CH2OAc
OAc
H
AcO
IVb
CH2OR
OR
H
RO
RO
IXa R = H
b R = Ac
CH2OR
OR
H
RO
RO
VIIIa R = H
b R = Ac
CH2OAc
OAc
H
AcO
IIb
CH2OAc
OAc
H
AcO
Vb

m.p. 257–265°, $[\alpha]_D$ +8°, which was identical with the epimeric tetrol IXa isolated in a very low yield from reduction of the unsaturated ketone VII with LAH. The result suggested that treatment with sodium acetate and acetic anhydride resulted in epimerization of the original quasi-axial 11β-OH group at the allylic position, to the 3β,11α,16β,28-tetraacetate (IXb), which on saponification gave the 3β,11α,16β, 28-tetrol (IXa). Assignment of the configurations of the two epimeric tetrols was further supported by comparison of the molecular rotations and the NMR spectra. According to Mills' rule for allylic epimeric alcohols,[9] the more dextrorotatory epimer should be the Δ^{12}-11β-ol (VIIIa) and the $\Delta[M]_D$ values of the epimeric 11-OH compounds, VIIIa and IXa, from longispinogenin (IVa) (Table 1) are in good

TABLE 1

	$[\alpha]_D$	$[M]_D$	$\Delta[M]_D$
Longispinogenin (IVa)	+53·1	+243	—
Olean-12-ene-3β,11β,16β,28-tetrol (VIIIa)	+97·1 (EtOH)	+461	+218
Olean-12-ene-3β,11α,16β,28-tetrol (IXa)	+8·0 (EtOH)	+38	−205
Longispinogenin triacetate (IVb)	+68·0	+397	—
3β,11β,16β,28-tetraacetoxyolean-12-ene (VIIIb)	+149·6	+963	+566
3β,11α,16β,28-tetraacetoxyolean-12-ene (IXb)	−8·6	−55	−452

agreement with the values ±210° calculated by Brewster's quantitative treatment.[10] In both the NMR spectra of the epimeric tetraacetates, VIIIb and IXb, one olefinic proton at C_{12} and two protons on C_{11} and C_{16} bearing the AcO group are crowded in a region of 4·32–4·75τ, in which the olefinic proton in the 11β isomer VIIIb is buried in the multiplet signals by coupling with the 11α-proton, but the olefinic proton in the 11α epimer IXb stands out as a sharp, singlet signal at 4·70τ against the multiplet signals, as expected from Karplus' correlation.[11]

Treatment of the newly obtained 3β,11α,16β,28-tetrol (IXa) with sodium acetate and acetic anhydride again gave only a small amount of the conjugated diene Vb and mainly regenerated the corresponding tetraacetate IXb. Although these results disagree with the examples described earlier,[5,6] sodium acetate is only slightly soluble in pure acetic anhydride even on boiling and it was suspected that acetic anhydride used in the cited experiments may have contained some acetic acid. Acetic anhydride containing 10% glacial acetic acid dissolves sodium acetate and treatment of the tetrol VIIIa with this solution increased the production of the conjugated diene. Treatment of the tetraacetate VIIIb with sodium acetate in glacial acetic acid resulted in complete elimination of the allylic AcO groups and gave a mixture of conjugated dienes consisting of saikogenin B and C triacetates, (Vb and IIb), in the ratio of 3:1. Since a role of sodium acetate in the elimination reaction is questionable, the tetraacetate VIIIb was refluxed in aqueous acetic acid, and yielded a mixture of Vb and IIb in the ratio of 1:1. Saikogenin B triacetate (Vb) obtained in these experiments was

[9] J. A. Mills, *J. Chem. Soc.* 4976 (1952).
[10] J. H. Brewster, *J. Am. Chem. Soc.* **81**, 5475, 5483, 5493 (1959).
[11] M. Karplus, *J. Phys. Chem.* **31**, 11 (1959); M. Karplus, *J. Am. Chem. Soc.* **85**, 2870 (1963).

identified with a specimen isolated from the plant. Since treatment with sodium acetate in glacial acetic acid or with aqueous acetic acid could not bring about interconversion of saikogenin B and C triacetates, saikogenin B must be olean-9(11),12-diene-3β,16β,28-triol (Va) having the usual 18β-configuration.

Thus, the structures of the four new triterpenoids which were isolated by acid hydrolysis of the saponin fraction of *Bupleurum falcatum* L. have been clarified. Since the crude saponin exhibited the strong UV absorptions at 242, 251 and 260 mμ, characteristic of a heteroannular diene system, saikogenins A (Ia), C (IIa) and D (IIIa)[1] were not believed to be artifacts formed during the acid hydrolysis of the saponin. However, in view of the fact that an ordinary Δ^{12}-oleanene derivative, longispinogenin (IVa), is found in addition to saikogenins B (Va) and C (IIa), it is supposed that some of the four saikogenins having the conjugated dienes may be produced from acid-labile compounds during the acid hydrolysis. The search for genuine sapogenins corresponding to the four saikogenins has recently been reported in preliminary communications[12, 13] and will be described in detail shortly.

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 internal standard using a Varian A-60 analytical NMR spectrometer. Alumina used for chromatography was Al_2O_3 "Woelm" neutral, activity grade II.

Gas chromatography of triterpenoids

A Barber-Colman Model 10 Gas Chromatography Ionization Detection System was used. The conditions were as follows: An U-shape glass column of 9 ft packed with 1·5% SE-30 on Gas Chrom P (80–100 mesh); column temp 240°, flash heater temp 270°, cell temp 210°; argon flow rate 95 ml/min. The triterpenes (1–2 mg) in anhyd pyridine (0·2 ml) were shaken with hexamethyldisilazane (0·1 ml) and trimethylchlorosilane (0·1 ml) for 3 min. After standing for 10 min, the supernatants were injected into the chromatograph using a microsyringe.

The relative retention times of the trimethylsilyl derivatives of the triterpenoids isolated from *Bupleurum falcatum* L. to cholestane were as follows: Cholestane 1·00 (7·3 min), saikogenin B (Va) 4·33, longispinogenin (IVa) 5·27, saikogenin C (IIa) 5·75, saikogenin A (Ia) 6·58, and saikogenin D (IIIa) 7·59.

Isolation of crude saikogenin B triacetate

The mother liquors which were previously obtained in the isolation of saikogenin C[1] were evaporated and the residue (22 g) was treated with Ac_2O (50 ml) in pyridine (100 ml) at room temp overnight. The acetate isolated through extraction with ether was chromatographed on Al_2O_3 (300 g). The eluates (14·0 g) with pet ether–benzene (4:1 and 1:1) were crystallized from EtOH to give crystals (3·94 g), which was again chromatographed on Al_2O_3. The eluates (1·669 g) with pet ether–benzene (4:1 and 2:1) on recrystallization from MeOH afforded crude saikogenin B triacetate (1·360 g), m.p. 211–214°, showing λ_{max} 282 mμ (ε 6400). Continued elution with pet ether–benzene (2:1) gave fractions (670 mg), which were shown to be a mixture of crude saikogenin B and saikogenin C by the TLC and UV spectra. Further elution with pet ether–benzene (1:1) to benzene–$CHCl_3$ (4:1) gave fractions (1·044 g), which on 2 recrystallizations from MeOH afforded an additional crop of IIb (716 mg), as plates, m.p. 214–216°.

Separation of saikogenin B (Va) *and longispinogenin* (IVa) *by preparative thin-layer chromatography*

The foregoing crude saikogenin B triacetate (110 mg), λ_{max} 282 mμ (ε 6400), in $CHCl_3$ was spotted in a line on 5 chromatoplates (20 × 20 cm) spread with Merck Silica Gel GF_{254} in thickness of 0·5 mm. After

[12] T. Kubota and H. Hinoh, *Tetrahedron Letters* 4725, 5045 (1966).
[13] N. Aimi and S. Shibata, *Tetrahedron Letters* 4721 (1966).

development with n-hexane–AcOEt–water (40:10:1) by ascending method, a region detected under an UV light was divided into 2 portions, in a ratio of the upper to lower part of 2:3 by width. The respective portions were scraped from the plates and extracted with $CHCl_3$ and the extracts were evaporated.

The lower fraction (65 mg), on recrystallization from EtOH afforded pure *saikogenin* B *triacetate* (Vb, 50 mg), m.p. 209–211°, $[\alpha]_D$ +223·6° (*c* 1·02). λ_{max} 282 mμ (ε 8700). ν_{max} 1742, 1245, 1236, 1027, 978, 835 cm^{-1}. NMR: τ 9·12, 9·08, 8·87, 8·78 (2, 2, 1, 2 Me, respectively); 7·97, 7·93 (2, 1 Me, respectively, of acetoxy groups); 5·97, 5·80 (2H at C_{28}, AB quartet, J = 11 c/s); 5·48, 4·48 (2H at C_3 and C_{16}, two multiplets); 4·42 (2H at C_{11} and C_{12}, singlet). (Found: C, 74·24; H, 9·55. $C_{36}H_{54}O_6$ requires: C, 74·19; H, 9·34%).

The triacetate Vb was treated with 2% KOH–MeOH under reflux in a N_2 atm for 1 hr. The product isolated through extraction with ether–$CHCl_3$ (4:1) was recrystallized from AcOEt, giving saikogenin B (Va) as needles, m.p. 267–269°, $[\alpha]_D$ +285·5° (*c* 1·00). λ_{max} 282 mμ (ε 9000). ν_{max} 3270, 3190, 1633, 1058, 1038, 987, 828 cm^{-1}. CD (*c* 0·01694, MeOH): $[\theta]_{278}$ +68,900°. (Found: C, 78·87; H, 10·57. $C_{30}H_{48}O_3$ requires: C, 78·89; H, 10·59%).

The upper fraction (44 mg) on recrystallization from MeOH gave needles (37 mg), m.p. 219°, λ_{max} 282 mμ (ε 4560). This fraction was again subjected to preparative TLC in the same manner as described above and divided into the following 3 fractions; upper (14 mg), λ_{max} 282 mμ (ε 1540), middle (15 mg), λ_{max} 282 mμ (ε 4970), and lower fraction (10 mg), λ_{max} 282 mμ (ε 7830). The upper fraction on recrystallization from MeOH afforded needles (10 mg), m.p. 221–222°, which showed no depression on admixture with an authentic sample of IVb. The IR spectra of the two specimens were superimposable. However, the separated material still exhibited the weak UV absorption at 282 mμ (ε 1540).

Isolation of longispinogenin (IVa)

The crude saikogenin B triacetate (180 mg), λ_{max} 282 mμ (ε 5600), in glacial AcOH (12 ml) was treated with SeO_2 (180 mg) under reflux for 30 min. The mixture was filtered and the filtrate was diluted with ether, washed with 5% $NaHCO_3$ and water and dried. Evaporation of the solvent gave the residue (193 mg), which was chromatographed on Al_2O_3 (8 g). Elution with pet ether–benzene (2:1) afforded crystalline fractions (49 mg), which on recrystallization from MeOH gave pale pink needles (40 mg), m.p. 221–223°. Further recrystallization from EtOH using Norit gave IVb, m.p. 222–223°, $[\alpha]_D$ +68·0° (*c* 0·96). (lit[3] m.p. 219–221°, $[\alpha]_D$ +70°). λ_{max} 204 mμ (ε 5300). Identity with an authentic sample was confirmed by the mixed m.p., TLC and IR spectra.

The triacetate IVb was heated with 2% KOH–EtOH under reflux for 45 min. The product separated by addition of water was recrystallized from AcOEt, giving IVa, m.p. 246–249°, $[\alpha]_D$ +53·1° (*c* 0·93), (lit.[3] m.p. 247–249°, $[\alpha]_D$ +53°). Identity with an authentic sample was established by the mixed m.p., TLC, GC and IR comparison.

The continued elution of the above-mentioned chromatography with pet ether–benzene (1:1) and benzene afforded fractions (12 mg) showing the UV absorptions at 242, 251, and 260 mμ due to formation of IIb by oxidation of IVb.

The elution with benzene–$CHCl_3$ (9:1) to $CHCl_3$ gave a yellow oil (80 mg), which on crystallization from acetone–n-hexane afforded pale yellow prisms (36 mg), m.p. 207–209°, identical with VI obtained by oxidation of pure Vb with SeO_2.

Oxidation of saikogenin B triacetate (Vb) *with selenium dioxide*

A mixture of Vb (30 mg) and SeO_2 (30 mg) in glacial AcOH (3 ml) was refluxed for 1 hr. The filtered soln was diluted with ether, washed with water, dried and evaporated to give the residue (32 mg). Purification by preparative TLC developed with toluene–AcOEt (2:1) on a silica gel G plate afforded the major fraction (22 mg), which on crystallization from acetone–n-hexane gave yellow needles (14 mg), m.p. 204–209°. Further recrystallization from MeOH afforded pure VI, as pale yellow needles, m.p. 208–209°. Identity with the specimen derived from saikogenin C triacetate (IIb)[1] was confirmed by the mixed m.p., TLC and IR spectra.

Attempted hydrogenation of saikogenin B triacetate (Vb)

A soln of Vb (50 mg) in glacial AcOH (10 ml) was shaken with rhodium–platinum oxide catalyst[14] in a H_2 atm for 2·5 hr. The catalyst was filtered and the filtrate was evaporated. Recrystallization of the residue from EtOH afforded crystalline material (40 mg), m.p. 221–223°, λ_{max} 282 mμ (ε 1280). Comparison of its NMR and IR spectra with those of IVb and Vb suggested that the product may be a complex mixture consisting of IVb, Vb and other compounds.

[14] S. Nishimura, *Bull. Chem. Soc. Japan* **33**, 566 (1960).

3β,16β,28-*Triacetoxyolean*-12-*en*-11-*one* (VII)

Compound IVb (800 mg)[4] in dry benzene (5·6 ml) was heated at 80° with stirring and a soln of $Na_2Cr_2O_7 \cdot 2H_2O$ (912 mg) in glacial AcOH (24 ml) was added dropwise during 2 hr. The mixture was stirred at 80° for additional 20 hr. The excess oxidant was destroyed by addition of EtOH (3·2 ml) and the mixture was diluted with water and extracted with benzene. The extract was washed with 5% $NaHCO_3$ and water, dried and evaporated to give the residue (840 mg). Recrystallization from EtOH afforded VII (614 mg), m.p. 183–188°. Further recrystallization from EtOH gave the analytical sample as prisms combined with ½ mole of EtOH, m.p. 186–188°, $[\alpha]_D$ +79·7° (*c* 0·86). λ_{max} 247 mμ (ε 12,400). ν_{max} 3644, 3460 (EtOH), 1729, 1665, 1652, 1622, 1254, 1226, 1049, 1025 cm^{-1}. NMR: τ 9·12, 9·07, 8·83, 8·48 (2, 2, 2, 1 Me, respectively); 7·97, 7·95, 7·93 (1 Me each of AcO groups); 6·11, 5·87 (2H at C_{28}, AB quartet, J = 11 c/s); 5·50 (1H at C_3, second-order quartet); 4·43 (1H at C_{16}, second-order quartet); 4·38 (1H at C_{12}, singlet). (Found: C, 71·93; H, 9·35. $C_{36}H_{54}O_7 \cdot \frac{1}{2}C_2H_5OH$ requires: C, 71·83; H, 9·17%).

Reduction of 3β,16β,28-*triacetoxyolean*-12-*en*-11-*one* (VII) *with lithium aluminum hydride*

To a suspension of LAH (100 mg) in refluxing dry ether (5 ml), a soln of VII (200 mg) in dry benzene (2 ml) and ether (1 ml) was added dropwise during 30 min with stirring. The mixture was stirred for additional 2·5 hr at reflux. To decompose the complex, water was added carefully and the mixture was extracted with $CHCl_3$. The organic layer was washed, dried and evaporated to give the residue (170 mg). Two recrystallizations from AcOEt afforded *olean*-12-*ene*-3β,11β,16β,28-*tetrol* (VIIIa, 62 mg) as small plates, m.p. 243–256°. $[\alpha]_D$ +97·1° (*c* 0·76, EtOH), $[M]_D$ +461°. λ_{max} 206·5 mμ (ε 7400). ν_{max} 3500, 3304, 1054, 1039, 992, 973, 944, 920 cm^{-1}. (Found: C, 75·86; H, 10·64. $C_{30}H_{50}O_4$ requires: C, 75·90; H, 10·62%).

The combined mother liquors were evaporated and the residue (103 mg) was subjected to preparative TLC developed with benzene–AcOEt (1:1) on silica gel G plates. The less-mobile fraction (77 mg) on recrystallization from AcOEt afforded additional VIIIa (48 mg), m.p. 237–255°.

The slightly more-mobile fraction (9 mg) on recrystallization from EtOH afforded needles (5 mg), m.p. 255–263°, which was identical with a specimen of 3β,11α,16β,28-*tetrol* (IXa) described below.

The 3β,11β,16β,28-tetrol VIIIa (20 mg) was treated with Ac_2O (0·2 ml) in pyridine (0·5 ml) at room temp for 4 days. The product separated by addition of water was recrystallized from EtOH, giving the *tetraacetate* (VIIIb) as plates, m.p. 220–229°, $[\alpha]_D$ +149·6° (*c* 0·94). ν_{max} 1731, 1248, 1227, 1116, 1045, 1020 cm^{-1}, no OH-band. NMR: τ 9·12, 8·75 (4, 3 Me, respectively); 7·97 (4 Me of AcO groups); 6·04, 5·82 (2H at C_{28}, AB quartet, J = 11 c/s); 5·50 (1H at C_3, second-order quartet); 4·75–4·33 (3H at C_{11}, C_{12} and C_{16}, multiplet). (Found: C, 71·09; H, 9·36. $C_{38}H_{58}O_8$ requires: C, 70·99; H, 9·09%).

The tetraacetate VIIIb on saponification with refluxing 5% KOH–EtOH regenerated the original tetrol VIIIa

Treatment of 3β,16β,28-*triacetoxyolean*-12-*en*-11-*one* (VII) *with sodium and isoamyl alcohol*

A solution of VII (200 mg) in isoamyl alcohol (8 ml) was refluxed with stirring and Na (400 mg) was added during 10 min. After refluxing for additional 1 hr, the mixture was washed with water and evaporated *in vacuo*. The residue was taken with ether and the ethereal soln was washed, dried and evaporated. The product was separated into two fractions by preparative TLC developed with benzene–AcOEt (1:1) on silica gel G plates. The more-mobile fraction (73 mg) on two recrystallizations from AcOEt afforded *olean*-13(18)-*ene*-3β,11ξ,16β,28-*tetrol* (X, 48 mg), as needles, m.p. 200–248°, $[\alpha]_D$ −172·3° (*c* 0·54, EtOH), λ_{max} 205·5 mμ (ε 11,500). ν_{max} 3313, 1078, 1044, 992 cm^{-1}. (Found: C, 74·25; H, 10·62. $C_{30}H_{50}O_4 \cdot \frac{1}{2}H_2O$ requires: C, 74·51; H, 10·63%). This tetrol gave yellow colour with tetranitromethane and on treatment with dil H_2SO_4 in EtOH showed no change in the TLC and UV spectrum.

The less mobile fraction (28 mg) on two recrystallizations from AcOEt gave 13ξ,18ξ-*oleanane*-3β,11ξ,16β,28-*tetrol* (XI, 15 mg) as plates, m.p. 294–308°. λ_{max} 207 mμ (ε 1030). ν_{max} 3375, 1044, 1022, 1002, 993 cm^{-1}. This compound gave no colour with tetranitromethane and on treatment with dil H_2SO_4 in EtOH showed no change in the TLC and UV spectrum.

Treatment of olean-12-*ene*-3β,11β,16β,28-*tetrol* (VIIIa) *with sodium acetate in acetic anhydride*

A mixture of VIIIa (40 mg) and anhyd AcONa (20 mg) in Ac_2O (2·5 ml) was refluxed for 1·5 hr. After addition of water, the mixture was extracted with benzene. The solvent layer was washed with 5% $NaHCO_3$ and water, dried and evaporated. The residue (48 mg) was separated into 2 fractions by preparative TLC developed with n-hexane–AcOEt (4:1) on silica gel G plates. The more-mobile fraction (7 mg) on recrystallization from EtOH afforded needles (4 mg), m.p. 208–210°, λ_{max} 282 mμ (ε 8600), which was identified with a specimen of Vb by the mixed m.p., TLC and IR comparison.

The less-mobile fraction (36 mg) on recrystallization from EtOH gave 3β,11α,16β,28-*tetraacetoxyolean*-12-*ene* (IXb, 28 mg), m.p. 207–218°. The analytical sample was obtained by further recrystallization from EtOH as prisms, m.p. 211–219°, $[\alpha]_D$ −8·6° (*c* 1·06). λ_{max} 206 mμ (ε 10,200). ν_{max} 1746, 1736, 1722, 1253, 1238, 1227, 1016, 972 cm^{-1} NMR: τ 9·12, 9·08, 8·93, 8·62 (2, 2, 2, 1 Me, respectively); 8·02, 7·97, 7·93 (1, 2, 1 Me, respectively, of AcO groups); 6·07, 5·87 (2H at C_{28}, AB quartet, J = 11 c/s); 5·52 (1H at C_3, second-order quartet); 4·70 (1H at C_{12}, singlet); 4·75–4·32 (2H at C_{11} and C_{16}, multiplet). (Found: C, 71·22; H, 9·30. $C_{38}H_{58}O_8$ requires: C, 70·99; H, 9·09%).

The tetraacetate IXb (30 mg) was treated with refluxing 5% KOH–EtOH (6 ml) for 1 hr under a N_2 atm. The product (27 mg) isolated through extraction with ether was recrystallized from EtOH to give the *tetrol* IXa (15 mg), as needles, m.p. 257–265°, identical with the minor product obtained from LAH reduction of VII. $[\alpha]_D$ +8·0 (*c* 0·86, EtOH). $[M]_D$ +38. λ_{max} 206·5 mμ (ε 7300). ν_{max} 3300, 1115, 1075, 1056, 1040, 996, 989, 978 cm^{-1}. (Found: C, 75·91; H, 10·72. $C_{30}H_{50}O_4$ requires: C, 75·90; H, 10·62%).

Treatment of 3β,11β,16β,28-*tetraacetoxyolean*-12-*ene* (VIIIb) *with sodium acetate in acetic anhydride*

A mixture of VIIIb (35 mg), anhyd AcONa (20 mg) and Ac_2O (5 ml) was refluxed for 3 hr. After addition of water, the mixture was extracted with ether. The ethereal soln was washed with 5% $NaHCO_3$ and water, dried and evaporated. The residue (35 mg) was separated into 2 fractions by preparative TLC developed with n-hexane–AcOEt (4:1) on silica gel G plates. The more-mobile fraction (5 mg) on recrystallization from EtOH gave prisms, m.p. 208–210°, λ_{max} 282 mμ (ε 8600), identical with Vb. The less-mobile fraction (28 mg) on crystallization from EtOH gave prisms (22 mg), m.p. 211–219°, identical with IXb obtained from the same treatment of VIIIa.

Treatment of olean-12-*ene*-3β,11α,16β,28-*tetrol* (IXa) *with sodium acetate and acetic anhydride*

A mixture of IXa (5 mg) and anhyd AcONa (10 mg) in Ac_2O (2·5 ml) was refluxed for 3 hr. The product isolated by the usual way showed mainly a spot of IXb along with a weak spot of Vb on the TLC.

Treatment of olean-12-*ene*-3β,11β,16β,28-*tetrol* (VIIIa) *with sodium acetate in acetic anhydride containing acetic acid*

A mixture of VIIIa (10 mg) and anhyd AcONa (20 mg) in Ac_2O (1·8 ml) and glacial AcOH (0·2 ml) was refluxed for 3 hr. The Ac_2O was destroyed with addition of dil NaOH soln under cooling and the mixture was extracted with ether. The ether layer was washed, dried and evaporated to give the crude product (12 mg), which on the spectral determination showed λ_{max} 208, 242, 251, 260 and 282 mμ (relative extinction 0·38, 0·45, 0·55, 0·51 and 0·49, respectively). On the basis of the UV intensities of the respective pure compounds, the crude product was estimated to consist of IXb, IIb and Vb in the ratio of 2:1:3. Preparative TLC on silica gel GF_{254} using n-hexane–AcOEt (4:1) was effective to divide into 3 components. The more-mobile fraction (5·5 mg) on recrystallization from EtOH gave Vb (3 mg), m.p. 208–211°. The slightly less-mobile fraction (1·2 mg) was characterized as saikogenin C triacetate by the TLC and UV spectrum. The less-mobile fraction (4·7 mg) on crystallization from EtOH gave IXb (2 mg), m.p. 211–218°.

Treatment of 3β,11β,16β,28-*tetraacetoxyolean*-12-*ene* (VIIIb) *with sodium acetate in glacial acetic acid*

The compound VIIIb (10 mg) was treated with AcONa (10 mg) in refluxing glacial AcOH (2 ml) for 2 hr. The mixture was diluted with water and extracted with ether. The ethereal soln was washed with 5% $NaHCO_3$ and water, dried and evaporated. The product (9 mg), by preparative TLC over silica gel GF_{254} developed with n-hexane–AcOEt (4:1), was divided into its 2 components. The more-mobile fraction (6 mg) on crystallization from EtOH gave Vb (4 mg), m.p. 208–210°. The less-mobile fraction (2 mg) was characterized as IIb by the TLC and UV spectrum.

Treatment of VIIIb *with aqueous acetic acid*

The compound VIIIb (10 mg) in 50% aq AcOH (2 ml) was heated at 95° for 3 hr, when the mixture deposited crystals. After addition of water, the crystals were filtered, washed and dried. The product (8 mg) on the UV determination in an EtOH soln showed the absorption max at 242, 251, 260, and 285 mμ (relative extinction 0·87, 1·00, 0·85, and 0·37, respectively) and was estimated to consist of IIb and Vb in 4:5 ratio. The TLC over silica gel G developed with n-hexane–AcOEt (4:1) exhibited mainly 2 spots of equal degree corresponding to IIb and Vb.

Attempted treatment of saikogenin C triacetate (IIb) *with sodium acetate in glacial acetic acid*

When IIb (40 mg) was treated with AcONa (20 mg) in refluxing glacial AcOH (4 ml) for 5 hr, it gave the recovered IIb (36 mg) showing no change on the m.p., TLC, and the UV and IR spectra.

Attempted treatment of saikogenin B triacetate (Vb)

(a) *With sodium acetate in glacial acetic acid.* A mixture of Vb (5 mg) and AcONa (5 mg) in glacial AcOH (1 ml) was refluxed for 3 hr and extraction with ether gave the recovered Vb, which showed no change on the m.p., TLC and UV spectrum.

(b) *With aqueous acetic acid.* After Vb (5 mg) was heated with 50% aq AcOH (2 ml) for 3 hr, it was recovered without any change in the m.p., TLC and UV spectrum.

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