Saponins Isolated from *Bupleurum falcatum* L.; Components of Saikosaponin b

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The components of the saikosaponins a and b derived from the root of *Bupleurum falcatum* L. have been re-investigated. Saikosaponin b has been shown to consist of 16α, 23, 28-trihydroxyoleana-11,13(18)-dien-3β-yl D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside (II) (saikosaponin b_2) and 16α,23,28-trihydroxy-11α-methoxy-olean-12-en-3β-yl D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside (V) (saikosaponin b_4), and saikosaponin a contains 16β,23,28-trihydroxy-11α-methoxyolean-12-en-3β-yl D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside (XXI) (saikosaponin b_3) and a small amount of 16β,23,28-trihydroxyoleana-11,13(18)-dien-3β-yl D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside (XXII) (saikosaponin b_1).

THE root of *Bupleurum falcatum* L. (Mishima-saiko in Japanese) is used as an important Chinese drug. Its saponin components were investigated by Kubota [1] and Shibata [2,3] independently. Kubota isolated saikosaponins a, b, c, and d, and assigned them structures (I)—(IV), respectively. Shibata isolated saikosides Ia, Ib, and II, which correspond to saikosaponins a—c, respectively. Kubota [1] suggested that saikosaponin b is an artefact derived from saikosaponin d (IV) during the isolation process. Shibata [3] separated saikoside Ib into saikosides Ib-1 and Ib-2; the latter showed diene u.v. absorption which the former lacked. We have re-investigated the saikosaponin b fraction [1] derived from the methanolic extract of the root of *Bupleurum falcatum* L., and have separated it into saikosaponins b_2 (II) and b_4 (V).

Saikosaponin b_2 (II), $C_{42}H_{68}O_{13}$, m.p. 235—240°, showed a heteroannular diene u.v. absorption and gave saikogenin D (VI),[4] glucose, and fucose on hydrolysis with 2% sulphuric acid in dioxan–water. As saikosaponin b_2 was obtained by treatment of saikosaponin d (IV) with 5% hydrochloric acid in methanol, its structure (II) was confirmed.

Saikosaponin b_4 (V), $C_{43}H_{72}O_{14}$, m.p. 245—250°, had no u.v. absorption band above 210 nm, and appears to be Shibata's saikoside (Ib-1). It gave saikogenin D (VI) on acidic hydrolysis, and a nona-acetate on acetylation. The n.m.r. spectrum of the acetate showed a singlet OMe signal at δ 3.25.

When saikosaponin b_4 was treated with 0.05% toluene-*p*-sulphonic acid in dioxan or with 5% hydrochloric acid in methanol, it gave saikosaponin d (IV) or saikosaponin b_2 (II), respectively (Table 1). These results indicate that saikosaponin b_4 may be represented by formula (V). In order to confirm this, saikogenin G (VII),[5] a genuine sapogenin corresponding to saikosaponin d (IV), was treated with acids under the same conditions

TABLE 1

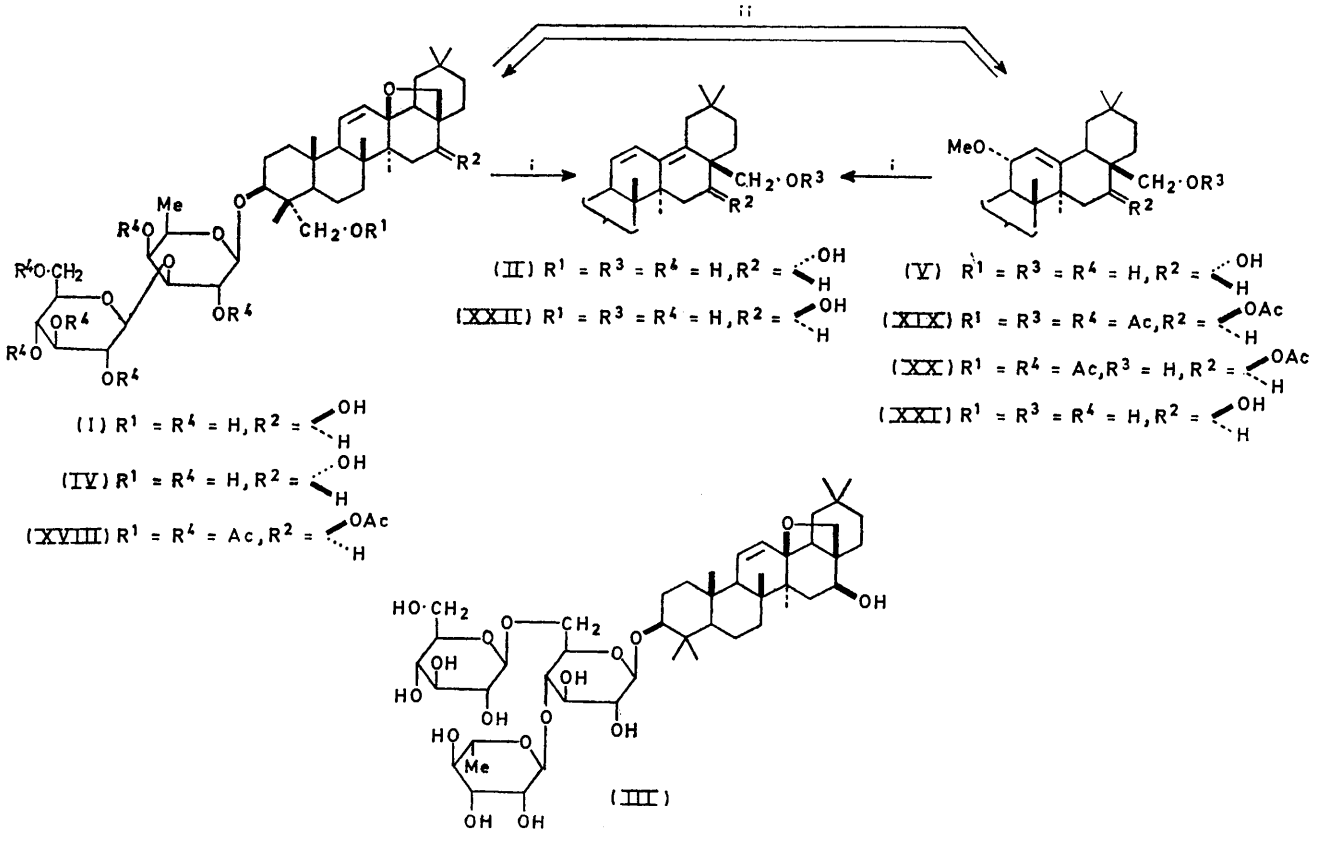
Acidic treatment of compounds (IV) and (V)

Starting material	Conditions	Products (%) (IV)	(II)	(V)
(V)	0.05% TsOH in dioxan, 20 min	*ca.* 100		
(V)	1% TsOH in dioxan, 1 h	50	50	
(V)	5% HCl–MeOH, 20 h		*ca.* 90	
(V)	10% AcOH–MeOH, 6 h	50		50
(IV)	5% HCl–MeOH, 20 h		*ca.* 90	
(IV)	10% AcOH–MeOH, 6 h	50		50
(IV)	10% AcOH–EtOH, 16 h	87		13 *

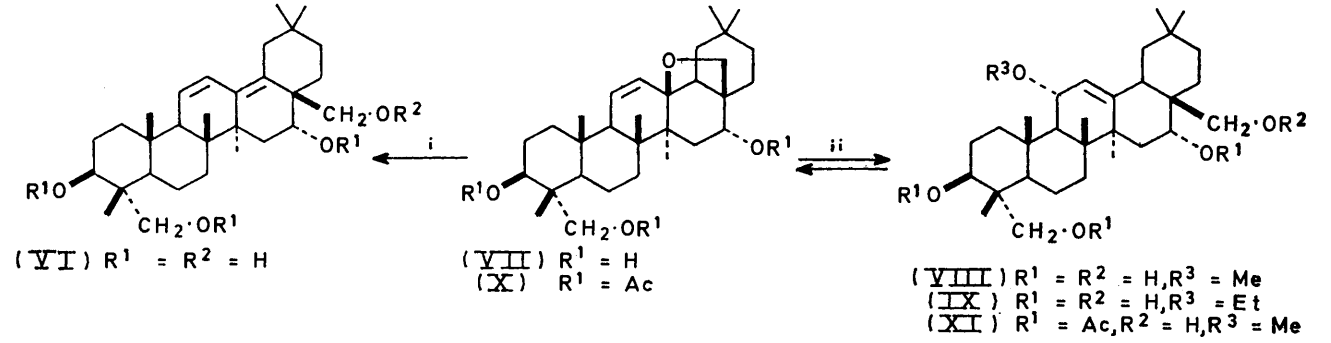
* 11-Ethoxy-derivative.

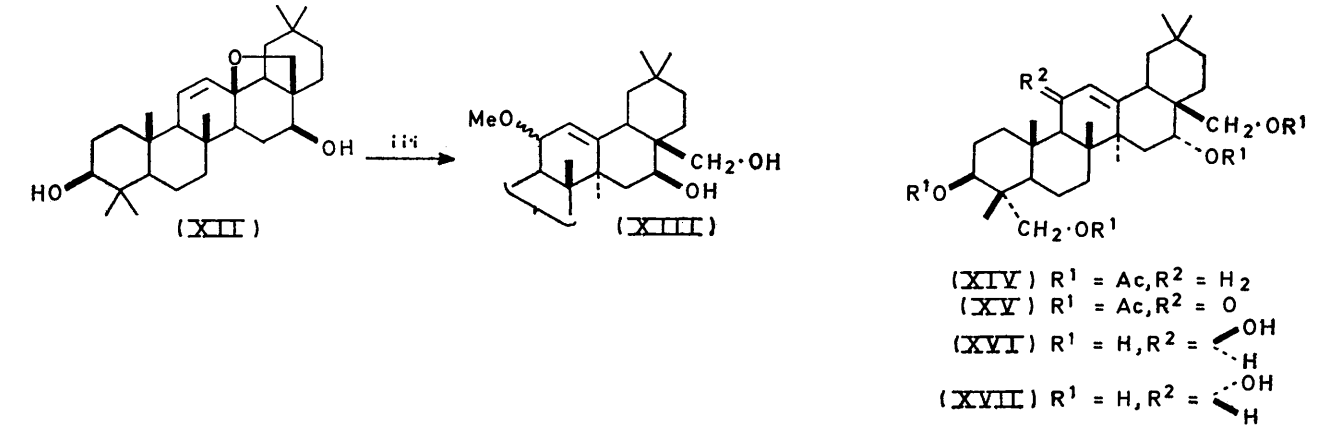
as for saikosaponin d (IV). The results were the same (Table 2). When saikogenin G (VII) was treated with 10% acetic acid in methanol, it afforded a new compound (VIII), $C_{30}H_{49}O_4(OCH_3)$, m.p. 180—184°, δ 3.12 (OMe). We considered that the methoxy-group originated from the methanol used as reaction solvent, and confirmed this by treating saikogenin G (VII) with 10% acetic acid in

[1] T. Kubota and H. Hinoh, *Tetrahedron Letters*, 1968, 303.
[2] S. Shibata, I. Kitagawa, and H. Fujimoto, *Chem. and Pharm. Bull.* (*Japan*), 1966, **14**, 1023.
[3] N. Aimi, H. Fujimoto, and S. Shibata, *Chem. and Pharm. Bull.* (*Japan*), 1968, **16**, 641.
[4] T. Kubota, F. Tonami, and H. Hinoh, *Tetrahedron*, 1967, **23**, 3333.
[5] T. Kubota and H. Hinoh, *Tetrahedron*, 1968, **24**, 675.



Reagents: i, 5% HCl; ii, 10% AcOH–MeOH or 0.05% TsOH–MeOH





Reagents: i, 5% HCl; ii, 10% AcOH–MeOH (or EtOH); iii, TsOH–MeOH

ethanol, giving a product (IX), $C_{30}H_{49}O_4(OC_2H_5)$, m.p. 166—168°, which showed an ethoxy- in place of a methoxy-signal in the n.m.r. spectrum. Saikogenin G triacetate (X) was treated with 10% acetic acid in methanol to give a compound (XI), $C_{37}H_{58}O_8$, m.p. 221—222°, which had three acetoxy-groups and showed hydroxy absorption [produced by cleavage of the ether bridge of (X)] at 3 560 cm^{-1} in the i.r. spectrum. The n.m.r. spectra of compounds (VIII) and (XI) indicated the presence of one olefinic proton [δ 5.30 (d, *J* 3.0 Hz) and 5.46 (d, *J* 3.5 Hz), respectively] and (XI) showed a methoxy-signal at δ 3.24. Although a C*H*·OMe signal could not be identified in the spectrum of (VIII), an appropriate resonance appeared at δ 3.9 (q, *J* 10 and 3.5 Hz) in that of (XI). Moreover, the C-28 methylene protons in each case give rise to an AB quartet (*J* 11 Hz), at δ 3.4 in (VIII) or 3.2 in (XI).

TABLE 2

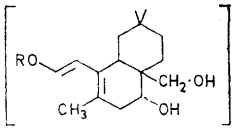
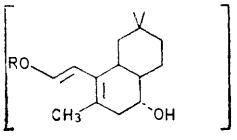
Acidic treatment of compounds (VII) and (VIII)

Starting material	Conditions	Products (%) (VII)	(VI)	(VIII)
(VIII)	0.05% TsOH in dioxan, 20 min	*ca.* 100		
(VIII)	10% AcOH–MeOH, 6 h	50		50
(VII)	5% HCl–MeOH, 20 h		90	
(VII)	10% AcOH–MeOH, 6 h	50		50
(VII)	10% AcOH–EtOH, 16 h	80		20 *

* Compound (IX).

TABLE 3

Mass spectra of compounds (VIII) and (IX)

	Compound (VIII) (R = Me)	(IX) (R = Et)
M^+	504	518
$[M - H_2O]^+$	486	500
$[M - ROH]^+$	472	472
$[M - (ROH + CH_2OH)]^+$	441	441
		
	280	294
	249	263

These results indicate that the product derived from saikogenin G (VII) has structure (VIII), obtained by cleavage of the ether bridge of (VII) with acetic acid, followed by migration of the double bond and attack of methanol at C-11 from the less hindered α-side of the molecule. Mass spectral data of (VIII) and (IX) support this conclusion (Table 3).

Shibata [3] obtained a methoxy-derivative (XIII) on treatment of saikogenin E (XII) with toluene-*p*-sulphonic acid in methanol, and assumed the configuration of the methoxy-group of (XIII) to be β on the basis of the reaction mechanism. However the coupling constants (10 and 3.5 Hz) of the proton on C-11 of (XI) agree well with data for the 11β-proton of an 11α-hydroxy-compound reported by Barton *et al.*[6] C.d. data of (VIII) and (XI) show negative Cotton effects, $[\theta]_{213}$ −18 400 and $[\theta]_{212}$ −23 800, respectively. This result leads us to conclude that the methoxy-group of (VIII) or (IX) has the α-configuration, according to the data of Scott *et al.*[7] On oxidation with *N*-bromosuccinimide,[8] dihydrosaikogenin D tetra-acetate [9] (XIV) afforded an 11-oxo-derivative (XV), which was reduced with lithium aluminium hydride to give two products. Predominant attack of the reagent from the less hindered α-side gives the 11β-hydroxy-derivative (XVI) as the major product and the 11α-hydroxy-derivative (XVII) in minor amount; each gave a penta-acetate on acetylation under drastic conditions. The c.d. data of the 11β-hydroxy-derivative (XVI) and its penta-acetate showed a positive Cotton effect, whereas the 11-epimers [(XVII) and its penta-acetate] showed a negative Cotton effect.

As saikosaponin b_4 (V) and its octa-acetate showed negative Cotton effects, $[\theta]_{213}$ −15 100 and −21 200, respectively, the 11-methoxy-group is α-oriented. We therefore conclude that saikosaponin b_4 is represented by formula (V).

Neither saikosaponin b_2 (II) nor b_4 (V) was obtained by extraction of the plant with methanol containing 2% pyridine or 2% sodium hydroxide, in contrast to extraction with methanol only. Moreover, when saikosaponin b_4 or b_2 was heated under reflux in 2% pyridine–methanol or in 2% sodium hydroxide–methanol, the starting material was recovered quantitatively. Therefore, saikosaponins b_2 (II) and b_4 (V) are both artefacts derived from saikosaponin d (IV) by the action of the acid substance in the plant body upon extraction with methanol.

Saikosaponin a (I) has a β-equatorial hydroxy-group at C-16, whereas saikosaponin d (IV) has an α-axial hydroxy-group. The ether bridge of saikosaponin d may easily be cleaved to give saikosaponins b_2 and b_4 with release of steric strain caused by the 1,3-diaxial relation between the C-16 OH and the C-14 CH_3, the C-18 proton, or the C-22 proton. As these interactions are not present in saikosaponin a (I), its ether bridge might be expected to be more stable than that of saikosaponin d (IV).

However, although saikosaponin a showed one spot on t.l.c., its peracetate showed two spots and was separated into saikosaponin a octa-acetate (XVIII) and a new compound (XIX) (ratio 9 : 1). Compound (XIX), $C_{61}H_{90}O_{23}$, showed an n.m.r. methoxy-signal at δ 3.23. When saikosaponin a octa-acetate (XVIII) was treated with 0.05% toluene-*p*-sulphonic acid in methanol, it gave a methoxy-derivative (XX) and the starting material (XVIII) (ratio 1:1). Compound (XX), $C_{59}H_{88}O_{22}$, showed i.r. absorption due to a newly-produced hydroxy-group and an n.m.r. methoxy-signal (δ 3.32). When (XX) was acetylated with acetic anhydride in pyridine, it gave (XIX). On hydrolysis with 2% sodium hydroxide in methanol,

[6] D. H. R. Barton, E. F. Lier, and J. F. McGhie, *J. Chem. Soc.* (*C*), 1968, 1031.

[7] A. I. Scott and A. D. Wrixon, *Tetrahedron*, 1971, **27**, 4787.

[8] B. W. Finucane and J. B. Thomson, *J.C.S. Perkin I*, 1972, 1856.

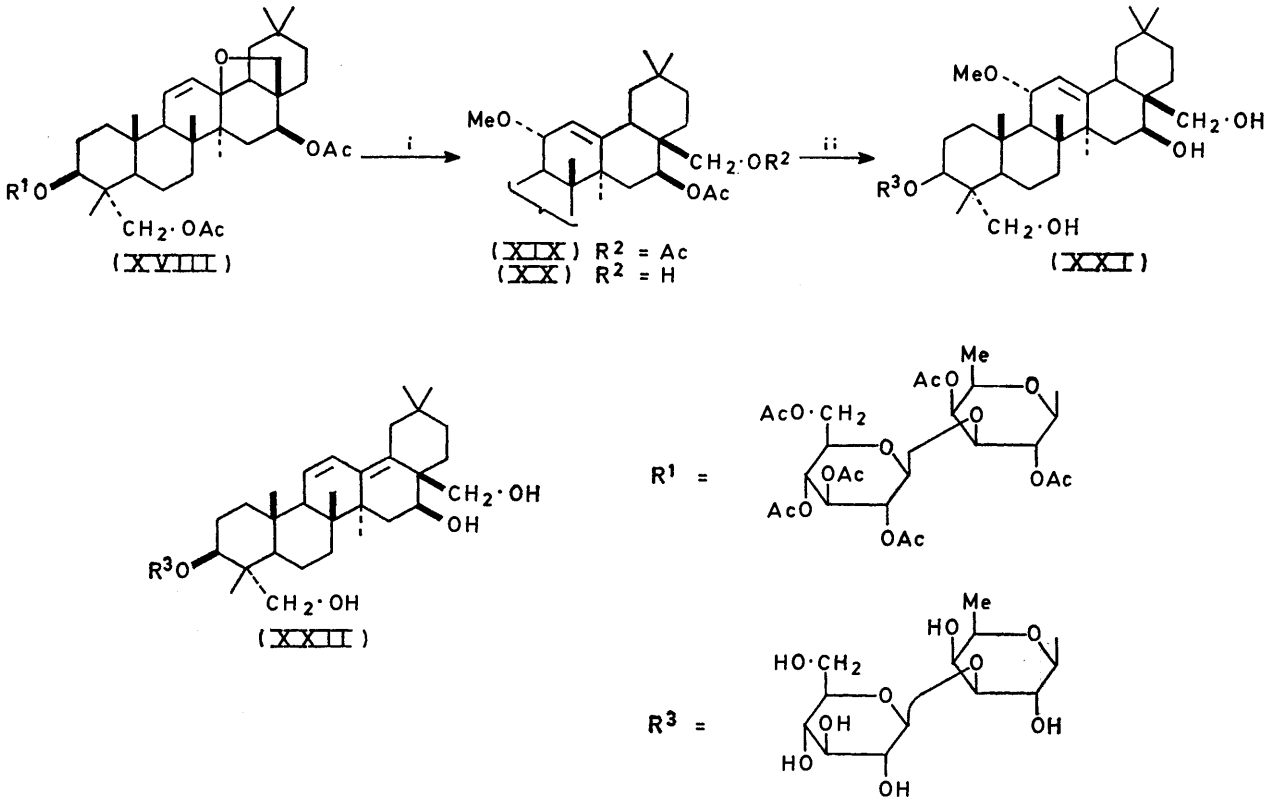
[9] T. Kubota, H. Kitatani, and H. Hinoh, *Tetrahedron Letters*, 1969, 771.

(XIX) afforded compound (XXI), $C_{43}H_{72}O_{14}$. As the c.d. data of compounds (XIX)—(XXI) all showed a negative Cotton effect, $[\theta]_{215}$ −29 000, −28 400, and −14 000, respectively, the methoxy-groups all have the α-orientation.

These results indicated that Kubota's saikosaponin a is a mixture of saikosaponin a (I) and (XXI) in the ratio *ca.* 9 : 1. We wish to name compound (XXI) saikosaponin b_3.

As a crude saikosaponin a fraction shows slight u.v. absorption due to a heteroannular diene system, compound (XXII) may occur in this fraction. However, saikosaponin a (I) no longer showed u.v. absorption after purification. On treatment with 5% hydrochloric acid in methanol at room temperature for 10 h, saikosaponin a (I) afforded the diene (XXII) named saikosaponin b_1, which was purified by precipitation with methanol–ether. Saikosaponin b_1 (XXII) ran concurrently with saikosaponin a (I) on t.l.c.

Although saikosaponin c (III) was not further investigated, it probably contains *ca.* 10% of a methoxy-derivative, because, in common with saikosaponin a, it has a β-equatorial hydroxy-group at C-16.



Reagents: i, 0.05% TsOH–MeOH; ii, 2% NaOH

EXPERIMENTAL

Unless otherwise stated, u.v. spectra were taken for solutions in 95% ethanol, and optical rotations and c.d. data for solutions in methanol. M.p.s were measured with a Kofler hot-stage apparatus. Solvent systems employed were A, $CHCl_3$–CH_3OH (5 : 1); B, $CHCl_3$–MeOH–H O (30 : 10 :1).

Isolation of Saikosaponins b_2 (II) *and b_4* (V).—The dried and sliced root of *Bupleurum falcatum* L. (4 kg) was extracted with ether (2 × 10 l) and then with methanol (3 × 8 l) at room temperature. Material from the methanolic extract (310 g) was dissolved in butan-1-ol (3 l) and the solution was washed with water. The aqueous layer was extracted with butan-1-ol. The combined butan-1-ol layer was washed with sodium chloride solution, dried (Na_2SO_4), and evaporated *in vacuo*. The residue (112 g) was extracted with acetone (2 × 600 ml) to leave crude saponin (56 g). The saponin was chromatographed on silica gel (solvent A) to give saikosaponins a and d [fraction (A)] (20 g), saikosaponin b [fraction (B)] (6.4 g), and saikosaponin c (10 g). Fraction (A) was rechromatographed on silica gel (solvent EtOAc–EtOH–H_2O, 8 : 2 : 1) to give saikosaponins a (I) (12.4 g) and d (IV) (6.6 g). Fraction (B) (1 g) was separated by preparative t.l.c. (silica gel; solvent B) into saikosaponin b_2 (II) R_F 0.58 (830 mg) and saikosaponin b_4 (V), R_F 0.61 (120 mg).

Saikosaponin b_2 (II) was obtained from methanol–ether as a white powder, m.p. 235—240°, $[\alpha]_D^{25}$ −32.1° (*c* 0.518), $\lambda_{max.}$ 244.5 (ε 22 400), 252.5 (25 500), and 262 nm (16 600) (Found: C, 60.65; H, 8.3. $C_{42}H_{68}O_{13},3H_2O$ requires C, 60.4; H, 8.95%).

Saikosaponin b_4 (V) was obtained from methanol–ether as a white powder, m.p. 245—250°, $[\alpha]_D^{25}$ −5.7° (*c* 0.264), c.d. $[\theta]_{213}$ −15 100 (Found: C, 60.25; H, 8.35. $C_{43}H_{72}O_{14}$, $3H_2O$ requires C, 59.7; H, 8.85%).

Saikosaponin b_4 nona-acetate, a white powder, had m.p. 155—160° (from ether–light petroleum), $[\alpha]_D^{23.5}$ −5.7° (*c* 0.26), c.d. $[\theta]_{212}$ −29 000 (Found: C, 61.55; H, 7.65. $C_{61}H_{90}O_{23}$ requires C, 61.5; H, 7.6%).

Acidic Hydrolysis of Saikosaponins b_2 (II) *and b_4* (V).—Saikosaponin b_2 (II) (20 mg) was dissolved in 3% sulphuric acid (2 ml) and water (1 ml) and heated under reflux for 2 h. The solution was diluted with water (5 ml) and extracted with chloroform. The extract was purified by t.l.c. to give crystals (7 mg), identical with saikogenin D[4] (VI) (i.r. spectrum and mixed m.p.). The aqueous layer was filtered through a column of Dowex 1 × 8 resin and evaporated. The residue was confirmed to be a mixture of glucose and fucose by comparison with authentic samples (t.l.c. and g.l.c.).

Saikosaponin b_4 (V) (20 mg) was treated under the same conditions, giving saikogenin D (VI) (6.6 mg), glucose, and fucose.

Treatment of Saikosaponin d (IV) *with 5% Hydrochloric Acid.*—Saikosaponin d (IV) (100 mg) was dissolved in 5% hydrochloric acid in methanol (5 ml) and left for 1 h at room temperature. The solution was neutralized with 10% sodium carbonate and extracted with butan-1-ol. The extract was purified by precipitation with methanol–ether to give saikosaponin b_2 (II) (98 mg).

Acidic Treatment of Saikosaponin b_4 (V).—Saikosaponin b_4 (V) (20 mg) was dissolved in 0.05% toluene-*p*-sulphonic acid in dioxan (2 ml) and left for 20 min at room temperature. The solution was neutralized with 10% sodium carbonate and extracted with butan-1-ol. The extract was purified with methanol–ether to give a white powder (18 mg), identical with saikosaponin d (IV) (t.l.c., i.r. spectrum, and $[\alpha]_D$).

Saikosaponin b_4 (V) (20 mg) was dissolved in 1% toluene-*p*-sulphonic acid in dioxan (4 ml) and left for 1 h at room temperature. The butan-1-ol extract (19 mg) was separated by preparative t.l.c. (silica gel; solvent B) to give saikosaponin d (IV) (9 mg) and saikosaponin b_2 (II) (9 mg).

Saikosaponin b_4 (V) (20 mg) was dissolved in 5% hydrochloric acid in methanol and left for 20 h at room temperature, giving saikosaponin b_2 (II) (17 mg).

Saikosaponin b_4 (V) or saikosaponin d (IV) (20 mg) was dissolved in 10% acetic acid in methanol (4 ml) and left for 6 h at room temperature, giving a mixture of saikosaponin d (IV) (9 mg) and saikosaponin b_4 (8 mg) in each case.

Treatment of Saikogenin G (VII) *with Acetic Acid.*—Saikogenin G[5] (VII) (130 mg) was dissolved in 10% acetic acid in methanol (30 ml) and left for 6 h at room temperature. The solution was neutralized with 10% sodium carbonate and extracted with ethyl acetate. The extract was washed with water, dried (Na_2SO_4), and evaporated. The residue (132 mg) was separated by preparative t.l.c. (silica gel; solvent A) into the starting material (VII) (58 mg) and 3β,16α,23,28-*tetrahydroxy*-11α-*methoxy-olean*-12-*ene* (VIII) (63 mg), a white powder, m.p. 180—184° (from acetone–n-hexane), $[\alpha]_D^{23}$ −22.6° (*c* 0.45), c.d. $[\theta]_{213}$ −18 400, δ [$(CD_3)_2SO$] 5.3 (1H, d, *J* 3 HZ), 3.12 (CH_3, s), 1.42 (CH_3, s), 1.00 (CH_3, s), 0.88 ($2CH_3$, s), 0.85 (CH_3, s), and 0.57 (CH_3, s) (Found: C, 73.75; H, 10.6; CH_3O, 7.1. $C_{31}H_{52}O_5$ requires C, 73.75; H, 10.4; CH_3O, 6.15%).

Saikogenin G (VII) (90 mg) was dissolved in 10% acetic acid in ethanol (20 ml) and left for 20 h at room temperature, giving the starting material (VII) (68 mg) and 11α-*ethoxy*-3β,16α,23,28-*tetrahydroxyolean*-12-*ene* (IX) (20 mg), a white powder, m.p. 166—168° (from acetone–n-hexane), δ [$(CD_3)_2$-SO] 5.28 (1H, d, *J* 3.5 Hz), 1.42 (CH_3, s), 1.03 (CH_3, t, *J* 7 Hz), 1.00 (CH_3, s), 0.88 ($2CH_3$, s), 0.83 (CH_3, s), and 0.56 (CH_3, s).

Acidic Treatment of 3β,16α,23,28-*Tetrahydroxy*-11α-*methoxyolean*-12-*ene* (VIII).—Compound (VIII) (20 mg) was dissolved in 0.05% toluene-*p*-sulphonic acid in dioxan (3 ml) and left for 20 min at room temperature, giving saikogenin G (VII) (18 mg).

Treatment of Saikogenin G Triacetate (X) *with Acetic Acid.*—Saikogenin G triacetate[5] (X) (70 mg) was dissolved in 10% acetic acid in methanol (30 ml) and left for 60 h at room temperature, giving the starting material (X) (30 mg) and 3β,16α,23-*triacetoxy*-28-*hydroxy*-11α-*methoxyolean*-12-*ene* (XI) (35 mg), needles, m.p. 221—222° (from methanol), $[\alpha]_D^{23}$ −46.5° (*c* 0.42), c.d. $[\theta]_{212}$ −23 800, $\nu_{max.}$ (CCl_4) 3 560 cm^{-1}, δ ($CDCl_3$) 5.46 (1H, d, *J* 3.5 Hz), 5.2 (1H, $W_{\frac{1}{2}}$ 6 Hz), 4.78 (1H, q, *J* 19 and 6 Hz), 3.9 (1H, q, *J* 10 and 3.5 Hz), 3.79 (2H, s), 3.08, 3.28, 3.33, and 3.53 (2H, ABq), 3.24 (CH_3, s), 2.01, 2.07, and 2.08 (3 CH_3CO), and 1.37, 1.12, 0.95, and 0.85 (6 CH_3) (Found: C, 70.45; H, 9.3. $C_{37}H_{58}O_8$ requires C, 70.45; H, 9.25%).

3β,16α,23,28-*Tetra-acetoxyolean*-12-*en*-11-*one* (XV).—Dihydrosaikogenin D tetra-acetate[9] (3β,16α,23,28-tetra-acetoxyolean-12-ene) (XIV) (10 g) was dissolved in dioxan (100 ml) and water (8 ml). To this solution were added calcium carbonate (10 g) and *N*-bromosuccinimide (10 g), and the mixture was stirred in an ice-bath for 20 min with irradiation by a 500 W lamp. Triethylamine (2 ml) was added and the mixture was then filtered. The filtrate was evaporated and the residue was dissolved in ethyl acetate; the solution was washed with water, dried (Na_2SO_4), and evaporated. The residue (10.1 g) was crystallised from ethanol to give 3β,16α,23,28-*tetra-acetoxyolean*-12-*en*-11-*one* (XV) as needles (4.5 g), m.p. 207—210°, $[\alpha]_D^{23}$ +6.4°, $\lambda_{max.}$ 249 nm (ε 11 800).

Reduction of the Ketone (XV) *with Lithium Aluminium Hydride.*—Lithium aluminium hydride (700 mg) was added to a solution of (XV) (1 g) in tetrahydrofuran (20 ml) and stirred for 3 h at 90 °C. Ethyl acetate saturated with water was added dropwise and the mixture was filtered. The filtrate was evaporated and the residue (980 mg) was separated by preparative t.l.c. (silica gel; solvent A) into *olean*-12-*ene*-3β,11β,16α,23,28-*pentaol* (XVI), a white powder (850 mg), $[\alpha]_D^{23}$ +62.4° (*c* 0.53), c.d. $[\theta]_{204}$ +21 700, and *olean*-12-*ene*-3β,11α,16α,23,28-*pentaol* (XVII), a white powder (53 mg), $[\alpha]_D^{23}$ +5.2° (*c* 0.52), c.d. $[\theta]_{216}$ −2 400. Compounds (XVI) and (XVII) each gave a penta-acetate, with acetic anhydride in pyridine at 90 °C for 3 h. The 3β,11β,-16α,23,28-*penta-acetate* was obtained as needles, m.p. 223—225° (from methanol–n-hexane), $[\alpha]_D^{23}$ +42.6° (*c* 0.16), c.d. $[0]_{196}$ +46 300 (Found: C, 68.55; H, 8.65. $C_{40}H_{60}O_{10}$ requires C, 68.55; H, 8.5%). The 3β,11α,16α,23,28-*penta-acetate* was obtained as a white powder, m.p. 128—130°, $[\alpha]_D^{23}$ −54.0° (*c* 0.52), c.d. $[\theta]_{210.5}$ −35 400 (Found: C, 68.1; H, 8.85%).

Saikosaponin b_3 Nona-acetate (XIX).—Saikosaponin a (500 mg), obtained from the fraction (A) described in isolation of saikosaponins b_2 and b_4, was dissolved in acetic anhydride (10 ml) and pyridine (20 ml) and heated at 90 °C for 3 h. The mixture was poured onto ice–water and extracted with ethyl acetate to give an acetate mixture (560 mg). The mixture (70 mg) was separated by preparative t.l.c. (silica gel; benzene–acetone, 9 : 1; triple development) into saikosaponin a octa-acetate[1] (XVIII), a white powder (60 mg), R_F 0.18 and *saikosaponin b_3 nona-acetate* (XIX), a white powder (5 mg), m.p. 163—165° (from acetone–n-hexane), R_F 0.14, $[\alpha]_D^{24}$ +29.5° (*c* 0.53), c.d. $[\theta]_{215}$ −29 000 δ ($CDCl_3$) 3.23 (CH_3, s) (Found: C, 61.45; H, 7.6. $C_{61}H_{90}O_{23}$ requires C, 61.45; H, 7.6%).

Saikosaponin b_3 (XXI).—Compound (XIX) (510 mg) was dissolved in 2% sodium hydroxide in methanol (5 ml) and heated under reflux for 1 h. The solution was diluted with water and extracted with butan-1-ol. The residue was purified by precipitation with methanol–ether to give *saikosaponin* b_3 (XXI), a white powder (390 mg), m.p. 260—260.5°, $[\alpha]_D^{25}$ 0° (*c* 0.42), c.d. $[\theta]_{215}$ −14 000 (Found: C, 60.5; H, 8.85. $C_{43}H_{72}O_{14},2H_2O$ requires C, 60.8; H, 9.0%).

Acidic Treatment of Saikosaponin a Octa-acetate (XVIII).—Compound (XVIII) (70 mg) was dissolved in 0.05% toluene-*p*-sulphonic acid in methanol (5 ml) and left for 3 h at room temperature. The solution was neutralized with aqueous 10% sodium carbonate and extracted with ethyl acetate. The extract was washed with water, dried (Na_2SO_4), and evaporated. The residue (72 mg) was separated by preparative t.l.c. (silica gel; benzene-acetone, 7 : 3) into starting material (XVIII) (30 mg), R_F 0.53, and *saikosaponin* b_3 *octa-acetate* (XX) (35 mg), R_F 0.41, a white powder, m.p. 168—171° (from acetone–n-hexane), $[\alpha]_D^{23}$ +30.2° (*c* 0.52), c.d. $[\theta]_{211}$ −28 400, $\nu_{max.}$ (CCl_4) 3 560 cm^{-1}, δ ($CDCl_3$) 3.32 (CH_3, s) (Found: C, 61.45; H, 7.85. $C_{59}H_{88}O_{22}$ requires C, 61.55; H, 7.7%).

Compound (XX) (30 mg) was dissolved in pyridine (4 ml) and acetic anhydride (2 ml) and heated at 90 °C for 3 h. The crude acetate (29 mg) was purified by preparative t.l.c. to give saikosaponin b_3 nona-acetate (XIX).

Saikosaponin b_1 (XXII).—Saikosaponin a (I) (200 mg) was dissolved in 5% hydrochloric acid in methanol (10 ml) and left for 20 h at room temperature. The solution was neutralized with aqueous 10% sodium carbonate and extracted with butan-1-ol. The extract was washed with water, dried (Na_2SO_4), and evaporated; the residue was purified by precipitation with methanol–ether to give *saikosaponin* b_1 (XXII), a white powder (170 mg), m.p. 237—241°, $[\alpha]_D^{24.5}$ −9.1° (*c* 1.03), $\lambda_{max.}$ 242.5 (ε 23 700), 251 (27 600), and 260.5 nm (17 400) (Found: C, 63.4; H, 9.15. $C_{42}H_{68}O_{13}$, H_2O requires C, 63.15; H, 8.85%).

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