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TRITERPENOIDS FROM *BUPLEURUM FALCATUM* L.—III
ISOLATION OF GENUINE SAPOGENINS, SAIKOGENINS E, F AND G[1]

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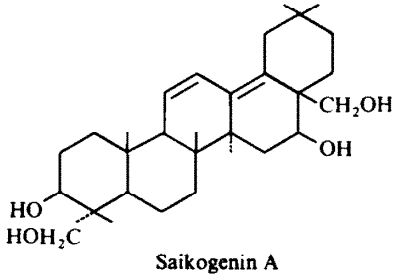
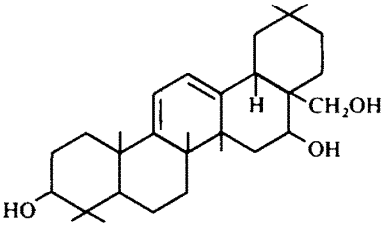
Abstract—The crude saponins from the root of *Bupleurum falcatum* L. previously yielded, on acid hydrolysis, saikogenins A, B, C and D (triterpenoids having conjugated dienes) together with longispinogenin. Examination of the saponins demonstrates that saikogenins A, B and C are artifacts formed during the acid hydrolysis and that the saponin leading to saikogenin D has already suffered transformation during the functionation of the saponins by the effects of acidic components. Extraction of the plant with alcohol containing pyridine gives the saponins showing no UV absorption due to a heteroannular diene. The newly obtained saponins, on repeated degradations by sodium periodate oxidation and alkaline treatment, afford three new triterpenoids, saikogenins E, F and G, which are shown to be genuine sapogenins corresponding to the previously obtained saikogenins A, B, C and D. The structure of saikogenins E, F and G has been elucidated as 13β,28-epoxyloean-11-ene-3β,16β-diol (Ia), -3β,16β,23-triol (IIa) and -3β,16α,23-triol (IIIa), respectively. Some modifications for the degradation of the saponins are also investigated.

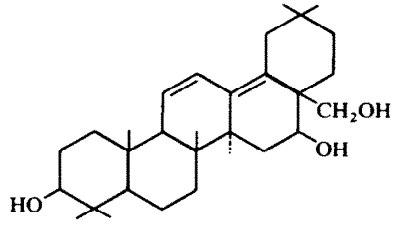
RECENTLY we have reported[2,3] that acid hydrolysis of the crude saponins from the root of *Bupleurum falcatum* L. yielded saikogenins A,* B, C and D as well as longispinogenin. The structure of these four saikogenins has been established as triterpenoids having the conjugated diene structure shown:



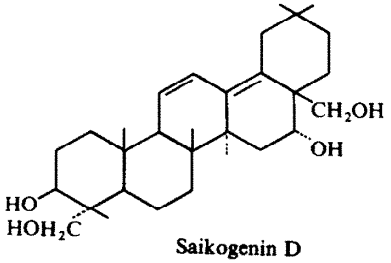
Saikogenin A



Saikogenin B



Saikogenin C



Saikogenin D

* The isolation of which was first described by Shibata *et al.*[4]

Since the crude saponins exhibit a strong UV absorption at 242, 251 and 260 mμ characteristic of a heteroannular diene system,[2,4] saikogenins A, C and D were not recognized as artifacts formed during acid hydrolysis of the saponins. However, as an ordinary Δ^{12}-oleanene derivative, longispinogenin, was found[3] in addition to saikogenins B and C, it was considered that some of the four saikogenins possessing the conjugated dienes could be produced from acid-labile compounds during the acid hydrolysis. Thus, our investigation was directed to the search for genuine sapogenins corresponding to the four saikogenins.

The crude saponin, on TLC using silica gel and $CHCl_3$–MeOH–H_2O (30:10:1 v/v), was shown to consist of three main fractions, in order of chromatographic mobility, *a*, *b* and *c*, which seemed to be respectively equivalent to the saikosides Ia, Ib and II designated by Shibata *et al.*[4] Among these three fractions, only *b* was detectable as a fluorescent spot under a UV light on TLC using Merck silica gel GF_{254}. This suggested that the other two fractions have no conjugated diene chromophore. The respective fractions were isolated by preparative TLC. As expected, the middle fraction *b* showed intensive UV absorptions at 242, 251 and 260 mμ characteristic of a heteroannular diene, but the fractions *a* and *c* exhibited only a very weak absorption in these regions in comparison with an end absorption at 205 mμ due to an unconjugated double bond.* These separate fractions on acid hydrolysis were proved to be the precursors of the previously isolated saikogenins as follows: *a*—Saikogenin A, *b*—saikogenin D† and *c*—saikogenins B and C and longispinogenin. Therefore, setting saikogenin D aside, saikogenins A, B and C must be artifacts formed during the acid hydrolysis.

Recently, Dugan and de Mayo[5] have achieved the isolation of pre-senegenin, an acid-labile genuine sapogenin, by a combination of oxidative and hydrolytic cleavage of the saponin from *Polygala senega*. The method seems to be applicable for isolation of genuine sapogenins of *Bupleurum falcatum* L. In preliminary experiments, the saponins were oxidized with sodium metaperiodate and the intermediates were heated with an ethanolic potassium hydroxide under nitrogen. The saponin *c* on the treatment gave a new aglycone named saikogenin E.‡ On the other hand, the saponin *a* yielded a product which was shown to be much more polar than saikogenin A on TLC. The product, m.p. 246–250°, derived from the saponin *a* agreed with an empirical formula $C_{36}H_{58}O_8 \cdot H_2O$,§ which indicated the presence of a sugar in addition to an expected aglycone through partial degradation of the saponin. The sugar moiety in the prosapogenin was identified as fucose, on acid hydrolyses followed by TLCs and GLC[7] of the hydrolysates. This result suggested that the fucose moiety linking with an aglycone had no vicinal glycol in the saponin *a* owing to linkage with additional sugar molecules. The remaining sugar moiety in the prosapogenin should now be cleaved with periodate, through release

* Shibata *et al.* have described[4] that both the saikosides Ia and Ib exhibited strong UV absorption characteristic of a heteroannular diene.

† Although Shibata *et al.* described[4] that saikogenin A was obtained by acid hydrolysis of both the saikosides Ia and Ib, in a private communication Prof. Shibata later reported that saikoside Ia gave saikogenin A but that saikoside Ib afforded saikogenin D.

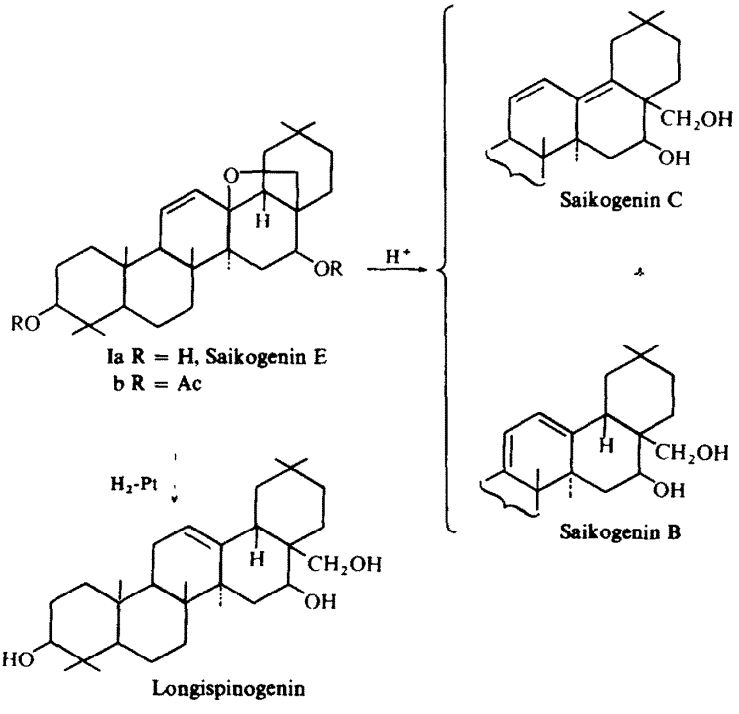
‡ At the same time as we isolated saikogenin E, Shibata *et al.* obtained the same compound.[6] We are indebted to Prof. Shibata for identification of the two specimens.

§The analytical result was interpreted, in the preliminary communication,[1] as $C_{36}H_{58}O_9$ containing a hexose, but has now been amended as $C_{36}H_{58}O_8 \cdot H_2O$ based on the identification of the sugar moiety.

from the linkages with the adjoining sugar molecules. The prosapogenin was again treated with sodium metaperiodate and then with alkali to give a new aglycone named saikogenin F. The saponin *b* on the one step degradation with periodate-alkali yielded the corresponding prosapogenin conjugated with fucose, which on the repeated degradation afforded saikogenin D as obtained previously by the acid hydrolysis.

For preparation of saikogenins E and F on a larger scale, the crude saponins were purified to give a mixture of the saponins *a* and *b* and a fairly pure saponin *c* by column chromatography on Florisil. The former mixture was treated twice with the periodate-alkali sequence to give saikogenins F and D. The latter fraction on the degradation with periodate-alkali afforded saikogenin E and longispinogenin. Otherwise, the crude saponins on repeated treatment with periodate-alkali yielded a mixture of the sapogenins, which was separated by chromatography on alumina.

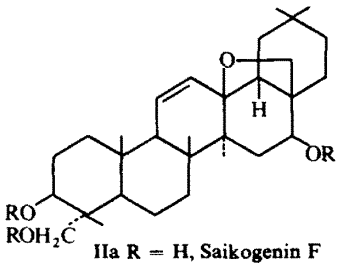
Saikogenin E (Ia), $C_{30}H_{48}O_3$, m.p. 283°, $[\alpha]_D$ + 108°, shows no UV absorption except for an end absorption at 206 mμ. The IR spectrum exhibits OH bands and many sharp bands between 1189 and 883 cm^{-1} suggesting the presence of an ether linkage. Mild acetylation of saikogenin E gave the diacetate Ib, $C_{34}H_{52}O_5$, m.p. 207–211°, $[\alpha]_D$ +111° which shows no OH band in the IR spectrum. Its NMR spectrum shows signals at τ 9·15–8·90 (7 Me), 7·97 (2 AcO groups), 6·83 and 6·02 (2H on C_{28} being involved in the ether bridge, AB quartet, J = 7 c/s), of which the bands at τ 6·83 are split with J = 1·5 c/s by the long-range coupling with 16α-H, 5·52 and 4·58 (1H each on C_3 and C_{16} bearing the AcO groups, two quartets), and 4·62 and 4·13 (2H of the di-substituted double bond at Δ^{11}; AB quartet, J = 10·5 c/s, with small splits in each of the signals).



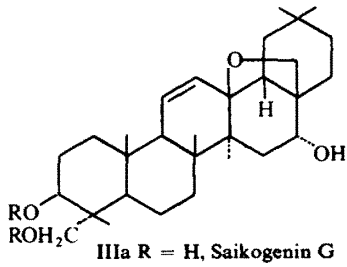
These data suggested the structure Ia having the ether linkage between C_{28} and C_{13} for saikogenin E, in connection with the structure of saikogenins B and C. When saikogenin E was heated with sulphuric acid in aqueous ethanol, it was readily converted into a mixture, which was shown, on examination with GLC[3] of its trimethylsilyl derivative, to be saikogenin C contaminated with a small amount of saikogenin B. After recrystallization, saikogenin C was identified with an authentic specimen. Catalytic hydrogenation of saikogenin E on Adams' catalyst in acetic acid resulted in the hydrogenolysis of the allylic ether linkage as well as the double bond migration, giving longispinogenin. From the above results, the structure of saikogenin E has been established as 13β,28-epoxyolean-11-ene-3β,16β-diol (Ia) and this new aglycone is the genuine sapogenin corresponding to saikogenins B and C.

Saikogenin F (IIa) on recrystallization from chloroform was obtained as plates combined with chloroform, $C_{30}H_{48}O_4 \cdot \frac{1}{2}CHCl_3$, m.p. 265–273°, $[\alpha]_D$ +96° (+108° on calculation as $CHCl_3$-free). Its UV spectrum shows no absorption except at 204 mμ and the IR spectrum exhibits a broad OH absorption and sharp bands at 977, 904 and 884 cm^{-1} indicative of an oxide linkage and at 763 and 754 cm^{-1} due to $CHCl_3$. Mild acetylation afforded the triacetate IIb, $C_{36}H_{54}O_7$, m.p. 205–208·5°, $[\alpha]_D$ +110°, which displays no OH band in the IR spectrum. Its NMR spectrum (Table 1) indicates that saikogenin F triacetate (IIb) has the same skeleton as saikogenin E diacetate (Ib) except for the presence of the additional AcO group at C_{23}.

Since saikogenin F (IIa) on heating with sulphuric acid in aqueous ethanol readily afforded saikogenin A, the former IIa was shown to be the genuine sapogenin corresponding to saikogenin A, which has been obtained as a major sapogenin of this plant.[2,4] From the above-mentioned results, the structure of saikogenin F has now been established as 13β,28-epoxyolean-11-ene-3β,16β,23-triol (IIa), in connection with the structure of saikogenin A and with the correlation between saikogenins C and E.



IIa R = H, Saikogenin F
b R = Ac



IIIa R = H, Saikogenin G
b R = Ac

Saikogenins A, B and C have now been proved to be artifacts produced during the acid hydrolysis of the saponins. On the other hand, as already mentioned, the saponin *b* fraction exhibits a strong UV absorption due to the conjugated diene and even on treatment with the same procedure which yielded saikogenin F (IIa) afforded saikogenin D. These facts may suggest that saikogenin D itself exists as a genuine sapogenin in the plant. However, it should be noted that saikogenin D has the axial 16α-OH group different from saikogenins E and F and, therefore, a hypothetical genuine sapogenin corresponding to saikogenin D may be so unstable as to be transformed during extraction of the saponins by effects of acidic components which would coexist in the plant.

TABLE 1. NMR signals (in $CDCl_3$ at 60 Mc)[a]

	Saikogenin E diacetate (Ib)	Saikogenin F triacetate (IIb)	Saikogenin G diacetate (IIIb)
$-\overset{\vert}{C}-CH_3$	9·15 (6H, s) 9·11 (3H, s) 9·07 (3H, s) 9·02 (3H, s) 8·96 (3H, s) 8·90 (3H, s)	9·17 (3H, s) 9·10 (3H, s) 9·02 (6H, s) 8·94 (3H, s) 8·88 (3H, s)	9·17 (3H, s) 9·08 (3H, s) 9·03 (6H, s) 8·94 (3H, s) 8·73 (3H, s)
$-OCOCH_3$	7·97 (6H, s)	7·98 (3H, s) 7·95 (6H, s)	7·99 (3H, s) 7·95 (3H, s)
$-\overset{\vert}{C}-O-CH_2-\overset{\vert}{C}-$	6·83[b], 6·02 (2H, ABq, J = 7)	6·80[b], 6·00 (2H, ABq, J = 8)	6·83, 6·55[b] (2H, ABq, J = 7·5)
$-\overset{\vert}{C}-CH_2-OAc$	—	6·20 (2H, s)	6·23, 6·20 (2H, ABq)[c]
$-\overset{OAc}{\overset{\vert}{C}_{(3)}}-H$	5·52 (1H, q, J = 9, 7)	5·22 (1H, q, J = 10, 6)	5·22 (1H, q, J = 9, 6)
$-\overset{OAc\ (or\ OH)}{\overset{\vert}{C}_{(16)}}-H$	4·58 (1H, m)	4·55 (1H, m)	6·00 (1H, broad d, J = 5)
Olefinic H	4·62, 4·13 (2H, ABq, J = 10·5)[d]	4·57, 4·13 (2H, ABq, J = 10)[d]	4·57, 4·13 (2H, ABq, J = 10)[d]

[a] The chemical shifts in τ-value and the coupling constants (J) in c/s. Signal multiplicities are represented by s (singlet), d (doublet), q (quartet) and m (multiplet).

[b] The signals split with J = 1 c/s by a long range coupling.

[c] Since the satelites of the AB quartet are not detectable by overlapping with the other signals, the positions of the inner main signals are described.

[d] The higher signals further split with J = 3 c/s and the lower signals with J = 1·5 c/s.

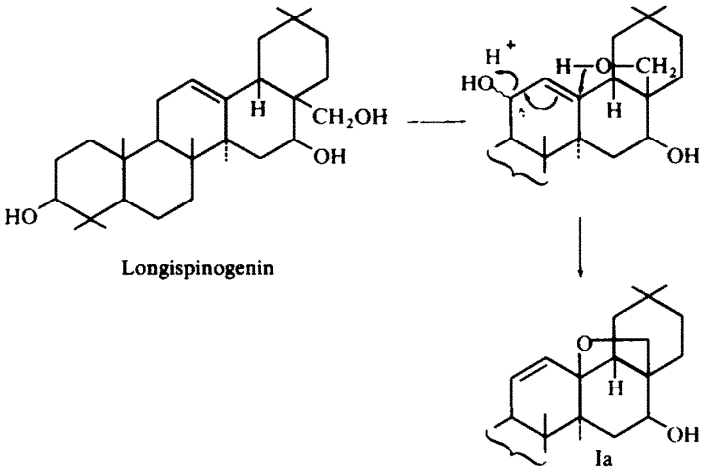
The root of *Bupleurum falcatum* L. was newly extracted with alcohol containing pyridine, to prevent effects of acidic components. Fractionation of the extract yielded a saponin mixture, which shows no UV absorption due to a heteroannular diene. The TLC with the above-mentioned solvent system exhibited only the two spots corresponding to the fractions *a* and *c* but lacked that of the fraction *b*. An intact saponin corresponding to the saponin *b* was assumed to be superimposed on the spot of the saponin *a*. On TLC using another solvent system, AcOEt–EtOH–H_2O (40:10:5 v/v), the spot of the new saponin *d* was detected at slightly higher position than that of the saponin *a*. The newly obtained mixture of the saponins *a* and *d* was treated twice with the periodate-alkali sequence and chromatographic separation of the product gave a new aglycone named saikogenin G* along with saikogenin F.

Saikogenin G (IIIa), $C_{30}H_{48}O_4$, m.p. 238–245°, $[\alpha]_D$ +83° (in $CHCl_3$–MeOH 4:1) shows no UV absorption due to a conjugated diene. Mild acetylation gave the non-crystalline diacetate IIIb, which in the IR spectrum shows OH absorptions at 3615 and 3450 cm^{-1} along with the AcO bands. Its NMR spectrum (Table 1) indicates that saikogenin G diacetate (IIIb) has the axial unacetylated 16α-OH group instead of the 16β-OAc group in saikogenin F triacetate (IIb). Treatment of saikogenin G (IIIa) with sulphuric acid in aqueous ethanol gave saikogenin D. From the above results, the structure of saikogenin G has been established as 13β,28-epoxyolean-11-ene-3β,16α,23-triol (IIIa) and this new aglycone is the genuine sapogenin corresponding to saikogenin D.

Thus, all of the previously isolated saikogenins A,[2,4] B,[3] C[2] and D,[2] which possess conjugated dienes, have been proved to be artifacts produced during the isolation process. All the genuine sapogenins, saikogenins E, F and G, isolated from *Bupleurum falcatum* L. belong to the type of uncommon 13β,28-epoxyolean-11-ene and differ from the initial expectation[3] that a genuine sapogenin corresponding to saikogenins B and C might be 11-hydroxylated derivatives of longispinogenin. Recently, some saturated triterpenoids having the 13β,28-oxide bridge have been isolated[8–10] and this has raised the question whether all the isolated olean-12-ene derivatives possessing the OH group at C_{28} are artifacts formed during acid hydrolyses.[9] It is, however, believed that longispinogenin itself exists as a genuine sapogenin in *Bupleurum falcatum* L. because the new degradation method of the saponins afforded again longispinogenin. From the biosynthetical point of view, it seems unlikely that longispinogenin has been converted directly into saikogenin E by oxidative ether formation accompanied with migration of the double bond, but another course including hydroxylation of longispinogenin at C_{11} followed by allylic dehydration and simultaneous ether formation seems preferable. Although attempts to isolate the 11-hydroxylongispinogenins were unsuccessful, the above assumption is supported by the fact that the 11-hydroxylongispinogenins[3] are readily converted into saikogenin E under a very mild acidic condition.

Although as mentioned the repeated periodate-alkali treatments of the saponins resulted in the isolation of the genuine sapogenins, the yields of saikogenins F and G on this degradation were inferior to those of saikogenins A and D obtained on the acid hydrolysis.[2] The alkali treatment of the aldehyde intermediates obtained by the first cleavage of the saponins with periodate gave a large quantity of acidic

* After we succeeded in the isolation of saikogenin G, it was informed from Prof. Shibata that his group also obtained the same compound.



Longispinogenin

Ia

products which would be produced by undesired side reactions. The acidic by-products on acid hydrolysis gave saikogenins A and D, but on further treatment with periodate-alkali did not release saikogenins F and G. Attempts were, thus, made to modify the alkali degradation of the aldehyde intermediates. The procedure of Goldstein,[11] which includes reduction with sodium borohydride and treatment with a mild acid, brought about smooth degradation into prosapogenins F and G, though the latter was partly transformed into prosapogenin D. However, the same procedure for the aldehydes obtained by the second periodate cleavage of the prosapogenins gave a complicated mixture, which might arise from an interaction of the OH group in the cleaved sugar moiety with the neighbouring C_{23}-OH group. Nevertheless, from the mixture, saikogenin F could be isolated in a better yield but unstable saikogenin G was suffered by the repeated treatment with mild acid. Thus, the Barry degradation using phenylhydrazine[12] was applied as another method for fission of glycoside linkages in the aldehyde intermediates. Repetition of this treatment stained the material but gave a better yield of saikogenins F and G. From the above results, it is expected that the best procedure for the isolation of saikogenins E, F and G may probably be a combination of periodate oxidation, sodium borohydride reduction, mild acid treatment, the second periodate oxidation and treatment with phenylhydrazine.

EXPERIMENTAL

All m.ps were determined on a Monoscop VS hot plate and are corrected. Unless otherwise stated, specific rotations were recorded in $CHCl_3$ solns with a Perkin–Elmer Polarimeter type 141, UV spectra in 95% EtOH solns with a Hitachi EPS-2 recording spectrometer and IR spectra in Nujol mulls with a Nihon Bunko DS-201B spectrometer. 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.

Identities of two specimens were established, unless otherwise stated, by mixed m.p., IR spectra and TLC. Alumina used for chromatography was Al_2O_3 "Woelm" neutral, activity grade II. Qualitative TLCs were performed on Merck Silica Gel G according to Stahl and coloured by spraying with conc H_2SO_4 followed by heating. GLC of the triterpenoids was carried out in the manner described previously.[3]

Isolation of the saponins a, b *and* c

The crude saponin mixture (4·7 g) which was the same quality as previously used for the acid hydrolysis[2]

was washed several times with hot AcOEt to remove an oily material. The resulting amorphous saponins (3·8 g) were purified by column chromatography on Florisil (80 g). Elutions with AcOEt–MeOH (9:1) containing pyridine (0–2%) gave a saponin mixture (1·89 g), which was subjected to prep TLC.

A soln of the above mixture in $CHCl_3$–MeOH (2:1) was spotted in a line on 60 chromatoplates (20 × 20 cm) spread with Merck Silica Gel GF_{254} in thickness of 750 μ. After development with $CHCl_3$–MeOH–H_2O (30:10:1 v/v), zone *b* detected under an UV light, its higher side *a* and lower side *c* were separately scraped from the plates and extracted with $CHCl_3$–MeOH–pyridine (75:25:2). Evaporation of the respective extracts yielded the saponins *a* (596 mg), *b* (340 mg) and *c* (280 mg), which on TLC developed with $CHCl_3$–MeOH–H_2O (30:10:1 v/v) showed each one spot at R_f described below.

The saponin *a* (R_f 0·40) shows UV absorption of a relative extinction (E) 0·78 and 0·26 at 204 and 251 mμ, respectively. After hydrolysis by refluxing with a mixture of 10% H_2SO_4 aq (0·5 ml) and 95% EtOH (1 ml) for 1 hr, the product extracted with ether was identified with saikogenin A by TLC and GLC.

The saponin *b* (R_f 0·31) shows UV absorption of E 0·40 and 0·71 at 202 and 252 mμ, respectively. After acid hydrolysis in the same manner as described for the saponin *a*, the product was identified as saikogenin D by TLC and GLC.

The saponin *c* (R_f 0·19) shows UV absorption of E 0·85 and 0·35 at 204 and 251 mμ, respectively. After acid hydrolysis, the product was shown to consist of saikogenins C and B and longispinogenin by TLC and GLC.

Degradation of the saponin a *with periodate-alkali*

To the saponin *a* (500 mg) in 95% EtOH (10 ml), a soln of $NaIO_4$ (500 mg) in water (5 ml) was added dropwise with stirring at 15°. The mixture was allowed to stand at room temp overnight, diluted with water and extracted with AcOEt. The AcOEt soln was washed with water, dried over Na_2SO_4 and evaporated to give a residue (470 mg). The intermediate was refluxed with 3% KOH in 85% EtOH aq (17 ml) under a N_2 atm for 1 hr. The mixture was diluted with water, acidified to pH 3 with dil HCl under cooling and extracted with AcOEt. The extract was washed with 5% $NaHCO_3$ aq and water, dried over Na_2SO_4 and evaporated to give a neutral product (190 mg).

A half amount (95 mg) of the product was purified by prep TLC using silica gel G and $CHCl_3$–MeOH–H_2O (50:10:1 v/v) to isolate the major product (47 mg), which on crystallization from AcOEt afforded crystals (19 mg), m.p. 235–240°. Further recrystallization from acetone gave an analytical sample of *prosaikogenin* F, m.p. 246–250°. ν_{max} 3360, 1650, 1049, 1021, 1002, 980, 912, 900 cm^{-1}. (Found: C, 67·76; H, 9·52. $C_{36}H_{58}O_8 \cdot H_2O$ requires:[9] C, 67·89; H, 9·50%.) The sugar moiety was identified as fucose by the two methods described in the next section.

The remainder (95 mg) of the crude prosaikogenin F was treated with $NaIO_4$ (100 mg) in a similar way to the first oxidation, giving an aldehyde (80 mg). Refluxing with KOH–EtOH for 1 hr and extraction of the mixture with AcOEt yielded a crude product (41 mg), which on separation by prep TLC using silica gel G and $CHCl_3$-MeOH (5:1) afforded *saikogenin* F (3 mg).

Identification of the sugar moiety in prosaikogenin F

(a) *By TLC.* A mixture of prosaikogenin F (4 mg) in a 2N HCl aq (1 ml) was heated in a sealed tube fulling with N_2 at 100° overnight. The mixture was washed with $CHCl_3$ and the water layer was evaporated *in vacuo*. Addition of water and evaporation were repeated for removal of the HCl. The residue showed the spot of the same R_f value and colour as that of D-fucose on TLCs developed with AcOEt–EtOH–H_2O (5:2:1 v/v) or toluene–acetone–H_2O (1:7:1 v/v).

(b) *By GLC.* Prosaikogenin F (3 mg) in 5% dry HCl–MeOH (1 ml) was heated in a sealed tube at 105° overnight. The mixture was diluted with $CHCl_3$ and extracted with water. The water layer was treated with Amberlite IR–4B(OH) and the filtrate was evaporated *in vacuo* to dryness. The residue was dissolved in pyridine and treated with hexamethyldisilazane and trimethylchlorosilane at 100° for 1 min. The mixture was diluted with $CHCl_3$, washed with water, dried over Na_2SO_4 and evaporated to dryness. The sample was identical with methyl fucoside-TMS prepared by the same treatment of D-fucose, in the retention times (9·95, 11·4 and 13·8 min) and the pattern of peaks,[7] on the GLC operated with the following conditions: A Shimazu Gas Chromatograph GC 1B equipped with a hydrogen flame ionization detector; a 3 m column packed with 5% Ucon LB 500X on Shimarite W (80–100 mesh); column temp 173°; and carrier N_2 flow rate 20 ml/min.

Degradation of the saponin b *with periodate-alkali*

The procedure was the same as described for the degradation of the saponin *a*. The saponin *b* (300 mg) was treated with $NaIO_4$ (300 mg) in aq EtOH to give an intermediate (273 mg). Treatment with boiling KOH–EtOH for 1 hr and extraction of the mixture with AcOEt afforded a prosapogenin (86 mg), which was divided into 2 portions.

One half was purified by prep TLC and the corresponding fraction (25 mg) was crystallized from acetone giving *prosaikogenin* D, m.p. 200–205°. The sugar moiety was proved to be fucose as in prosaikogenin F.

The other half was treated again with $NaIO_4$ and with KOH–EtOH to give a crude aglycone (16 mg), which on prep TLC afforded saikogenin D (2 mg).

Degradation of the saponin c *with periodate-alkali*

The saponin *c* (140 mg) in 95% EtOH (3 ml) was treated with $NaIO_4$ (140 mg) in water (1·5 ml) at room temp overnight. The mixture was diluted with AcOEt, washed with water and evaporated to give a residue (120 mg). The intermediate was refluxed with 3% KOH-aq EtOH for 1 hr under N_2 atm. The mixture was diluted with water, acidified carefully and extracted with AcOEt. The extract was washed with 5% $NaHCO_3$ aq and water and evaporated to give a residue (27 mg). The product was chromatographed on Al_2O_3 (2 g). The fractions (9 mg) eluted with benzene–$CHCl_3$ (4:1) was crystallized from AcOEt, giving *saikogenin* E (5 mg). Elution with benzene–$CHCl_3$ (1:1) afforded a fraction (8 mg), which on crystallization from AcOEt yielded longispinogenin (1 mg).

Degradation of the saponin mixture (a, b *and* c) *with periodate-alkali*

Crude amorphous saponins (38 g) which was already washed with AcOEt was chromatographed on Florisil (800 g) and elutions with AcOEt–MeOH (9:1) containing pyridine (0–2%) afforded a mixture (17·8 g) of the saponins *a*, *b* and *c*. To the saponins in 95% EtOH (210 ml), a soln of $NaIO_4$ (17·8 g) in water (180 ml) was added dropwise with stirring at 20°. The mixture was allowed to stand at room temp overnight. The product was separated by addition of water as a ppt (15·6 g) and extraction of the filtrate with AcOEt afforded an additional crop (0·70 g). The combined material was refluxed with a soln of KOH (6·3 g) in 95% EtOH (190 ml) and water (20 ml) under N_2 atm for 1 hr. The mixture was concentrated to a half volume, diluted with water, acidified with dil HCl under cooling and extracted with AcOEt. The extract was washed with 5% $NaHCO_3$ aq and sat NaCl soln, dried over Na_2SO_4 and evaporated to give a residue (3·40 g). The intermediate was again treated with $NaIO_4$ and with KOH-aq EtOH in the same manner as described above to give a mixture of aglycones (1·56 g), which was chromatographed on Al_2O_3 (50 g). Elution with benzene–$CHCl_3$ (4:1) gave a fraction (200 mg), which on recrystallization from AcOEt afforded saikogenin E (154 mg), m.p. 250–270°. Elutions with benzene–$CHCl_3$ (1:1) and $CHCl_3$ yielded a fraction (60 mg) which on recrystallization from AcOEt gave longispinogenin (35 mg), m.p. 241–245°. Elution with $CHCl_3$–MeOH (99:1) gave a fraction (206 mg), which on recrystallization from AcOEt afforded crude saikogenin F (159 mg), m.p. 248–263°. Elutions with $CHCl_3$–MeOH (49:1–19:1) afforded a fraction (256 mg) which was recrystallized from $CHCl_3$ giving saikogenin D (114 mg), m.p. 254–262°.

Saikogenin E (Ia) *and its diacetate* (Ib)

Saikogenin E, after recrystallization from AcOEt, was obtained as plates, which began to sinter at 265° and melted at 283°. $[\alpha]_D$ +108° (*c* 1·07). $\varepsilon_{206\,m\mu}$ 5450. ν_{max} 3445, 3330, 1189, 1180, 1139, 1091, 1061, 1051, 1043, 1025, 995, 972, 955, 903, 883 cm^{-1}. (Found: C, 78·86; H, 10·82. $C_{30}H_{48}O_3$ requires: C, 78·89; H, 10·59%.) GLC[3] relative retention time to cholestane 1·00 (7·4 min) was 6·50 for saikogenin E TMS derivative.

Saikogenin E was treated with Ac_2O–pyridine (1:2) at room temp overnight. The product was isolated through extraction with ether and recrystallized from n-hexane giving the diacetate Ib as prisms, m.p. 207–211°. $[\alpha]_D$ +111° (*c* 1·07). ν_{max} 1736, 1245, 1020, 1010, 984, 975, 915 cm^{-1}. (Found: C, 75·76; H, 9·89; $C_{34}H_{52}O_5$ requires: C, 75·51; H, 9·69%).

Acid treatment of saikogenin E (Ia)

A soln of saikogenin E (9 mg) in 95% EtOH (3 ml) and 10% H_2SO_4 (0·5 ml) was refluxed for 1 hr. The mixture was diluted with water, and the EtOH was evaporated. The residue was extracted with ether and the ether soln was washed with 5% $NaHCO_3$ aq and water, dried over Na_2SO_4 and evaporated to give the residue (8 mg), which on GLC examination[3] was shown to be a mixture of saikogenins B and C in an approx ratio of 1:5. Recrystallization from AcOEt gave pure saikogenin C, m.p. 291–294°, identical in all respects with an authentic specimen.[2]

Hydrogenolysis of saikogenin E (Ia)

A soln of saikogenin E (50 mg) in glacial AcOH (10 ml) was magnetically stirred with Adams' catalyst (25 mg) in a H_2 atm for 2 hr. The catalyst was filtered off and the filtrate was evaporated *in vacuo*. The residue was taken with AcOEt, washed with $NaHCO_3$aq and water and evaporated. Recrystallization of the residue from AcOEt gave needles (41 mg) of longispinogenin, m.p. 245–248°, which was identified with an authentic sample.[3]

Saikogenin F (IIa) *and its triacetate* (IIb)

Saikogenin F on recrystallization from $CHCl_3$ was obtained as plates combined with $CHCl_3$, which was not lost by heating *in vacuo* at 100° for 5 hr, m.p. 265–273°. $[\alpha]_D$ +96° (+108° on calculation as $CHCl_3$-free based on the analytical result) (*c* 0·60). $\varepsilon_{205\,m\mu}$ 5600. ν_{max} 3360, 1042, 977, 904, 884, 763, 754 cm^{-1}. (Found: C, 68·82; H, 9·14. $C_{30}H_{48}O_4 \cdot \frac{1}{2}CHCl_3$: C, 68·81; H, 9·18%.) Recrystallization from acetone afforded another crystalline form, m.p. 265–273°, combined with acetone, which showed the IR band at 1710 cm^{-1}.

Saikogenin F was acetylated by allowing to stand with Ac_2O–pyridine (1:2) at room temp overnight. The product isolated by extraction with ether was recrystallized from n-hexane giving the triacetate IIb, m.p. 205–208·5°. $[\alpha]_D$ +110° (*c* 0·57). ν_{max} 1740, 1250, 1036, 1021, 1008, 986, 971, 962, 912 cm^{-1}. (Found: C, 72·25; H, 9·22. $C_{36}H_{54}O_7$ requires: C, 72·21; H, 9·09%.)

Acid treatment of saikogenin F (IIa)

A mixture of saikogenin F (11 mg) in 2N H_2SO_4aq (0·5 ml) and 95% EtOH (1 ml) was refluxed for 1 hr. To the mixture, ether and 5% $NaHCO_3$aq were added and the ether soln was washed with water, dried and evaporated to dryness (9 mg). Recrystallization from MeOH afforded saikogenin A (5 mg), m.p. 286–290°, which was identified with an authentic sample.[2]

Isolation of the intact saponins exhibiting no UV absorption

The dried, cut root (2·4 kg) of *Bupleurum falcatum* L was extracted 3 times with EtOH (6 l.) containing pyridine (30 g) under reflux for 6 hr. The EtOH soln was evaporated *in vacuo* and the combined residue (482 g) was dispersed into a mixture (1·2 l.) of n-BuOH–water (2:1). The upper layer was washed with water and concentrated *in vacuo* to a small volume to which ether was added gradually. The resulting ppt was collected by filtration and washed with ether to yield crude saponins (74 g) which showed no UV absorption characteristic of conjugated dienes. TLC developed with $CHCl_3$–MeOH–H_2O (30:10:1 v/v) exhibited 2 spots corresponding to the saponins *a* and *c* but no spot of the saponin *b*. However, TLC developed with AcOEt–EtOH–H_2O (40:10:5 v/v) showed 3 spots of the saponins *d* (R_f 0·38), *a* (R_f 0·34) and *c* (R_f 0·21). When the saponins were treated with dioxan (1 ml) containing 10% H_2SO_4 (1 drop) at room temp overnight, the saponin *d* was transformed into *b* with no change of the saponins *a* and *c*.

The above crude saponins (30 g) were dissolved in $CHCl_3$–MeOH (9:1) and adsorbed on a column of Florisil (900 g). The fractions (7·46 g) eluted with $CHCl_3$–MeOH (9:1) containing pyridine (1–2%) were shown on TLC to consist of the saponins *d* and *a*. Trituration of the fraction with AcOEt gave amorphous saponins (6·76 g). Further elutions with $CHCl_3$–MeOH–pyridine (80:20:2) and with AcOEt–MeOH–pyridine (80:20:2) afforded fractions (5·23 g) which were shown on TLC to be a mixture of the saponins *d*, *a* and *c*.

Degradation of the saponin mixture (d *and* a) *with periodate-alkali*

The purified saponins *d* and *a* mixture (5·50 g) in 95% EtOH (220 ml) and pyridine (1·1 g) was treated with $NaIO_4$ (11·0 g) in water (110 ml) at room temp overnight. The mixture was concentrated and, after addition of water, extracted with AcOEt. Evaporation of the extract gave the residue (5·03 g), which was treated with a soln of KOH (5·0 g) in 95% EtOH (90 ml) and water (10 ml) for 1 hr on a steam bath under N_2 atm. The mixture was concentrated, diluted with water, acidified under cooling and extracted with AcOEt. The extract was washed with 5% $NaHCO_3$aq and water, dried and evaporated to give a neutral product (1·26 g). Acidification of the $NaHCO_3$ layer with HCl and extraction with AcOEt afforded an acidic fraction (2·86 g). The neutral product was again treated with $NaIO_4$ and with KOH-aqEtOH in the same manner as described above to give crude aglycone (425 mg). Chromatography on Al_2O_3 was eluted with $CHCl_3$ and $CHCl_3$–MeOH (99:1) to give a crystalline fraction (73 mg). The fraction was separated into 2 fractions by prep TLC developed with toluene–AcOEt (1:2) on Silica Gel G. The more mobile one (20 mg) on crystallization from MeOH afforded saikogenin G (12 mg), m.p. 230–242°. The less mobile fraction (36 mg) on crystallization from $CHCl_3$ gave saikogenin F (31 mg).

Saikogenin G (IIIa) *and its diacetate* (IIIb)

Saikogenin G, after recrystallization from MeOH, was obtained as prisms, m.p. 238–245°. $[\alpha]_D$ +83° (*c* 0·49 in $CHCl_3$–MeOH (4:1)). $\varepsilon_{205\,m\mu}$ 5500. ν_{max} 3485, 3320, 1052, 1040, 1018, 984, 976, 954, 944, 907, 888 cm^{-1}. (Found: C, 76·51; H, 10·35. $C_{30}H_{48}O_4$ requires: C, 76·22; H, 10·24%.)

Saikogenin G in Ac_2O–pyridine (1:3) was allowed to stand in a refrigerator overnight. Extraction with ether in the usual manner gave an amorphous product, which was characterized as the diacetate IIIb by the NMR (Table 1) and IR spectra. $\nu_{max}^{CHCl_3}$ 3615, 3450, 1729, 1037, 985, 910, 890 cm^{-1}.

Acid treatment of saikogenin G (IIIa)

A soln of saikogenin G (10 mg) in 95% EtOH (3 ml) and 10% H_2SO_4 aq (0·5 ml) was refluxed for 1 hr. The mixture was diluted with ether, washed with dil $NaHCO_3$ aq and water, dried and evaporated to dryness (9 mg). Recrystallization from $CHCl_3$ yielded saikogenin D, m.p. 255–260°, which was identical with an authentic sample.[2]

Transformation of olean-12-ene-3β,11α,16β,28-tetrol and -3β,11β,16β,28-tetrol into saikogenin E

To a soln of olean-12-ene-3β,11α,16β,28-tetrol[3] (10 mg) in dioxan (1 ml), 10% H_2SO_4 (1 drop) was added and the mixture was allowed to stand at room temp for 1 hr. The mixture was diluted with ether, washed with dil $NaHCO_3$ aq and water and evaporated. Recrystallization of the residue from AcOEt–MeOH afforded saikogenin E (7 mg) as plates, m.p. 255–270°.

The epimer,3β,11β,16β,28-tetrol,[3] on the same treatment as above also yielded saikogenin E. These products were identical with an authentic sample.

Modified degradations of the saponin mixture (d *and* a)

The purified saponins *d* and *a* mixture (3·0 g) was treated with $NaIO_4$ in the same manner as described above. Aldehyde intermediates (2·64 g) isolated by extraction with AcOEt were divided into halves and subjected to the procedures (a) and (b).

(a) *By the Goldstein's method.*[11] To a soln of the intermediates (1·32 g) in 95% EtOH (50 ml) and water (10 ml), $NaBH_4$ (650 mg) was added. The mixture was allowed to stand at room temp overnight, acidified with dil HCl under cooling and extracted with AcOEt. The extract was washed with 5% $NaHCO_3$ aq and water, dried and evaporated to dryness (1·03 g). The residue was dissolved in dioxan (60 ml) containing *p*-toluenesulphonic acid (60 mg) and warmed at 60° for 30 min. The soln was neutralized with 5% $NaHCO_3$ aq and evaporated *in vacuo*. The residue was taken with AcOEt, washed with water and evaporated to dryness (855 mg). The prosapogenins were again treated successively with $NaIO_4$, $NaBH_4$ and *p*-toluene–sulphonic acid, in the same manner as described above. The product (600 mg) was chromatographed on Al_2O_3 (20 g) and elutions with benzene–$CHCl_3$ (1:1) and $CHCl_3$ gave crystalline fractions (183 mg), which were shown on TLC to consist of at least 3 unidentified compounds. Elutions with $CHCl_3$–MeOH (99:1) afforded a fraction (132 mg), which was separated into 2 fractions by prep TLC. The more mobile one (48 mg) was expected to contain a small amount of saikogenin G, which, however, could not be isolated because the majority of the fraction was an unidentified compound. The less mobile fraction (41 mg) on crystallization from AcOEt afforded saikogenin F (30 mg). Further elutions of the chromatography with $CHCl_3$–MeOH (49:1) afforded fractions (54 mg) which on crystallization from $CHCl_3$ yielded saikogenin D (16 mg).

(b) *By the Barry degradation.*[12] To a soln of the above-mentioned aldehydes (1·32 g) in 95% EtOH (78 ml) was added a soln of phenylhydrazine–HCl (1·04 g) and $AcONa \cdot 3H_2O$ (1·56 g) in water (26 ml). The mixture was refluxed for 1 hr and extracted with AcOEt. The extract was washed with water, dried and evaporated. The residue (1·81 g) was purified by chromatography on Al_2O_3 and eluates with $CHCl_3$–MeOH (19:1 and 9:1) were collected. The prosapogenins (645 mg) were again treated with $NaIO_4$ and phenylhydrazine in the same manner as described above. The crude product (730 mg) was chromatographed on Al_2O_3 and eluates with $CHCl_3$ and $CHCl_3$–MeOH (99:1) were collected. The fraction (177 mg) was separated into 2 fractions by prep TLC. The more mobile one (42 mg) on crystallization from MeOH gave saikogenin G (15 mg) and the less mobile fraction (63 mg) was crystallized from $CHCl_3$ to give saikogenin F (45 mg).

A modified degradation of the intact crude saponins

The intact crude saponins (40 g) were treated, in the first step degradation, with $NaIO_4$ and KOH-aq EtOH as described above. The obtained prosapogenins (6·90 g) were treated successively with $NaIO_4$ and phenylhydrazine. The crude product (9·6 g) was chromatographed on Al_2O_3 (300 g). Fractions (2·91 g)

eluted with benzene–$CHCl_3$ (2:1–1:1) and $CHCl_3$ were rechromatographed on alumina and saikogenin E (230 mg) and longispinogenin (35 mg) were isolated after crystallization of the respective fractions from AcOEt. Fractions (1·20 g) eluted with $CHCl_3$–MeOH (99:1) were crystallized from $CHCl_3$ giving crystals (560 mg), which were separated into saikogenins G (136 mg) and F (400 mg) by prep TLC developed with toluene–AcOEt (1:2) on Silica Gel G.

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