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The Chemical Studies on Oriental Plant Drugs. XVIII.[1] The Constituents of *Bupleurum* spp. (3).[2] Saikogenins E[3] and G

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A genuine sapogenin, saikogenin E, was isolated by applying the Smith-type controled degradation procedure for saikoside II fraction, one of the main saponins of *Bupleurum falcatum* L. It was also obtained by alkaline degradation of the periodate oxidized saponin. The structure of saikogenin E (Ia) was elucidated and saikogenins B and C were proved to be the artifacts derived from it. Saikogenin G, the genuine sapogenin corresponding to saikogenin D, was also obtained by modified de Mayo-type degradation method and its structure was established as (Va).

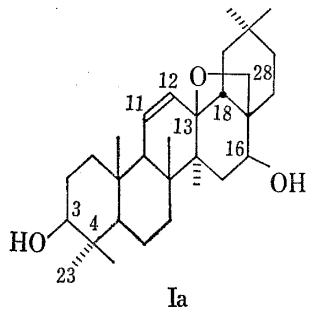
Previously we reported the isolation of saikogenin A as a major sapogenin of saikosides Ia and Ib fraction, and discussed its structure as being represented by oleana-11,13(18)-diene-3β,16β or 22β, 23,28-tetraol.[5] Afterwards, Kubota, *et al.*[6] confirmed the position of the secondary hydroxyl in 16β, and at the same time they forwarded the structures of saikogenins B (IV), C (III) and D (VI), which were obtained by the acid hydrolysis of crude saikoside, as being represented by oleana-9(11),12-diene-3β,16β,28-triol, oleana-11,13(18)-diene-3β,16β, 28-triol, and oleana-11,13(18)-diene-3β,16α,23,28-tetraol, respectively.

In the earlier stage of study, the characteristic UV-absorption of conjugated double bonds was noted as being represented by the saponins and sapogenins of *Bupleurum* roots.[5] However it has been shown that saikoside II purified by preparative thin-layer chromatography exhibits no significant UV-absorption maximum above 210 mμ. Therefore the conjugated double bonds in saikogenins C (III) and B (IV) which are derived from saikoside II fraction on acid hydrolysis would be artifacts.

Under a mild condition, by Smith-type controled degradation procedure[7] using stepwise sodium metaperiodate, sodium borohydride and 0.1 N methanolic sulfuric acid or *p*-toluenesulfonic acid in methanol at room temperature, saikoside II fraction afforded a genuine sapogenin, named saikogenin E (Ia). Saikogenin E was also obtained by the degradation of the sugar part[8] of saikoside II, using sodium metaperiodate and alkali, subsequently. Saikogenin E, $C_{30}H_{48}O_3$, mp 289°, $[\alpha]_D^{20}$ +112°, shows no UV-absorption of conjugated double bonds. On treatment with diluted sulfuric acid-methanol for 30 minutes on a boiling water bath saikogenin E yielded saikogenin C, which was identified with authentic specimen.[6] The UV-spectrum of mother liquor of saikogenin C showed that saikogenin B, having a homoannular conjugated diene system, was also produced in this reaction. Thus it has been concluded that saikogenins C and B are the artifacts derived from the genuine sapogenin, saikogenin E.

1) Part XVII: S. Shibata, T. Ando, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **14**, 1157 (1966).
2) (2) S. Shibata, I. Kitagawa, and H. Fujimoto, *Chem. Pharm. Bull.* (Tokyo), **14**, 1023 (1966).
3) Preliminary report: See N. Aimi and S. Shibata, *Tetrahedron Letters*, **No. 39**, 4721 (1966).
4) Location: *Hongo, Tokyo.*
5) S. Shibata, I. Kitagawa, and H. Fujimoto, *Tetrahedron Letters*, **No. 42**, 3783 (1965); *Chem. Pharm. Bull.* (Tokyo), **14**, 1023 (1966).
6) T. Kubota, F. Tanami, and H. Hinoh, *Tetrahedron Letters*, **No. 7**, 701 (1966).
7) F. Smith and A.M. Unrau, *Chem. Ind.* (London), **1959**, 881; I.J. Goldstein, G.W. Hay, B.A. Lewis, and F. Smith, "Methods in Carbohydrate Chemistry," ed. R.L. Whistler, Vol. V, Academic Press, N.Y. & London, 1965, p. 361.
8) J.J. Dugan, P. de Mayo, *Can. J. Chem.*, **43**, 2033 (1965).

Saikogenin E afforded diacetate, $C_{34}H_{52}O_5$, mp 213°, $[\alpha]_D^{21}$ +118°, whose IR-spectrum showed the absence of hydroxyl and UV-spectrum gave no evidence for the existence of conjugated double bonds. The NMR-spectrum (100 Mc, in $CDCl_3$) of saikogenin E indicated the presence of 7 tertiary methyl groups (τ 8.91—9.23), a methylene adjacent to an ether linkage (τ 6.90 (1H), 6.11 (1H), AB type doublets J=7.7 cps) and two olefinic protons (τ 4.59 (1H), q. J=11.5, 3 cps; τ 4.17 (1H), d. J=11.5 cps, AB part of ABX system). The NMR-spectrum of saikogenin E diacetate (60 Mc, in $CDCl_3$) showed two acetyl groupings (τ 8.01 (6H), s.) in addition to the presence of the functional groupings as given by saikogenin E, such as the methylene adjacent to the ether linkage (τ 6.84 (1H), τ 6.07 (1H) AB type doublets J=7.5 cps) and the olefinic protons (τ 4.65 (1H) br. d., τ 4.18 (1H) d. AB part of ABX system J=10.5 cps). The signals of protons in saikogenin E at τ 6.78 (1H) and τ 5.78 (1H) were shifted to τ 5.60 (1H) and τ 4.60 (1H), respectively, in the NMR-spectrum of the diacetate. This would indicate that the hydroxyls of saikogenin E are secondary locating at 3β and 16β, corresponding to those of saikogenin C.

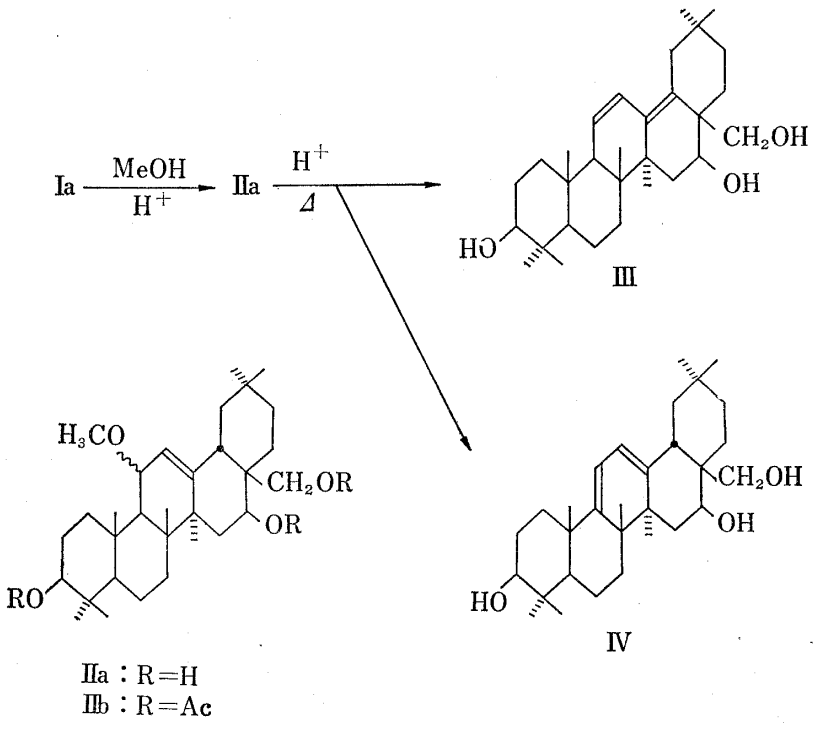


Ia

Chart 1

The above results provided a conclusive evidence for the structure of saikogenin E as being represented by the formula (Ia).

In the case of Smith-type degradation of saikoside II fraction, obtaining saikogenin E, another sapogenin was isolated accompanied by a small amount of saikogenins B and C mixture. This sapogenin (IIa), $C_{31}H_{52}O_4$, mp 222—225°, $[\alpha]_D^{22}$ −23.7°, was also formed from saikogenin E by the action of *p*-toluenesulfonic acid in methanol at room temperature. The NMR-spectrum of its triacetate (IIb), $C_{37}H_{58}O_7$, mp 169—172° $[\alpha]_D^{16}$ +45.5°, indicated the presence of 7 tertiary methyls (τ 9.14—8.65), 3 acetyls (τ 7.97 (6H), 7.91 (3H)), 1 methoxyl (τ 6.78 (3H), s.), $C_{(11)}$-H (τ 6.10 (1H)), $C_{(28)}$-H_2 (τ 5.95 (2H), br. s.), $C_{(3)}$-H (τ 5.50 (1H) q.), $C_{(12)}$-H (τ 4.60 (1H), d. J=3 cps), and $C_{(16)}$-H (τ 4.50, (1H)).



IIa : R=H
IIb : R=Ac

Chart 2

From these evidences, the formula IIa was given to this intermediate sapogenin, which gave saikogenins C (III) and B (IV) on heating with diluted methanolic sulfuric acid as formulated in Chart 2.

The configuration of methoxyl groups of IIa and IIb is likely to be β, by the possible mechanism of formation from Ia.

Kubota, *et al.*[9] reported that saikogenin D was obtained by twice de Mayo-type degradation[4] of saikoside Ib fraction which exhibited UV-absorption of heteroannular diene, while they isolated saikogenin F, a genuine sapogenin corresponding to saikogenin A, from saikoside Ia, which gave no UV-absorption of conjugated double bonds.

Recently we have found that saikoside Ib fraction can be separated into 2 portions, Ib-1 and Ib-2, the former contains no conjugated double bonds and the latter involves a heteroannular diene system. The thin-layer chromatogram of crude saikoside is shown in Fig. 1.

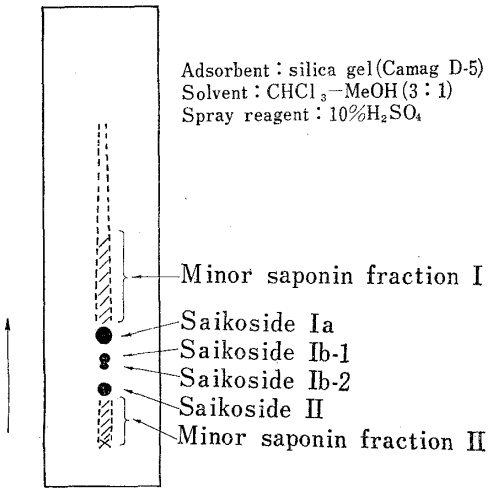


Fig. 1.

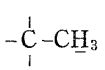
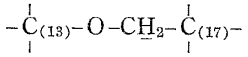
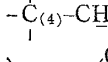
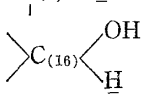
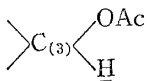
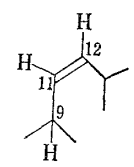
Seeking for the genuine sapogenin corresponding to saikogenin D (VI), we performed de Mayo-type alkaline degradation[8] of metaperiodate-oxidized saikoside fraction which exhibited no significant UV-absorption of conjugated diene, while it gave saikogenins D and A on acid hydrolysis.

The genuine sapogenin, saikogenin G (Va), $C_{30}H_{48}O_4$, mp 242—247°, $[\alpha]_D^{26}$ +45°, which was obtained along with saikogenin F[9] by applying de Mayo's procedure twice to the above saponin gave no UV-absorption maximum above 210 mμ.

Diacetate of saikogenin G (Vb) showed in its IR-spectrum the presence of a non-acetylated hydroxyl along with O-acetyls. On treatment with dil. sulfuric acid in methanol, saikogenin G was easily converted into saikogenin D.

The functional groupings and the partial structure of saikogenin G are proved by the NMR-spectrum of the diacetate as shown in the following table:

Table I. NMR-Spectrum of Saikogenin G Diacetate (Vb) (measured at 100 Mc, in $CDCl_3$)

	τ-values
	9.17 (3H), 9.07 (3H), 9.03 (6H), 8.94 (3H), 8.74 (3H)
CH_3COO-	7.97 (3H, singlet), 7.93 (3H, singlet)
	6.80 (1H), 6.52 (1H) (AB type doublets, J=7 cps)
	6.28 (1H), 6.11 (1H) (AB type doublets, J=11 cps)
	5.98 (1H, br. doublet, J=5 cps)
	5.19 (1H, br. quartet, J_1=10, J_2=6 cps)
	4.54 (1H), 4.11 (1H) (AB type doublets, J=10 cps) The bands at τ 4.54 are split (J=3 cps) by the coupling with $C_{(9)}$-H

9) T. Kubota and H. Hinoh, *Tetrahedron Letters*, **No. 41**, 5045 (1966).

Thus the structure of saikogenin G is formulated as (Va) correlating with saikogenin D (VI).

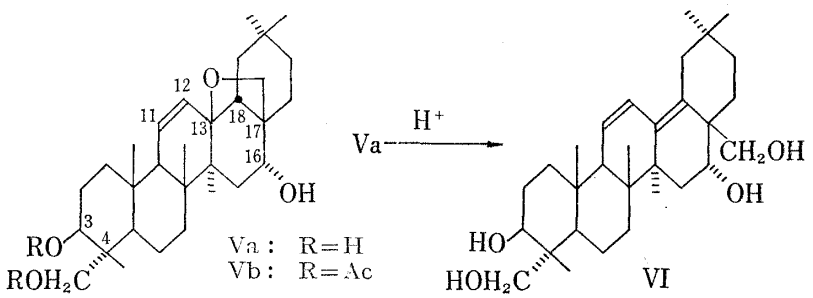


Chart 3

The configuration of $C_{(18)}$–H in saikogenin G seems to be β, since longispinogenin is occurring in the same plant, whose C_{18}–H was established to possess β–configuration.[10]

As reported by Kubota, *et al.*[9] the genuine sapogenin corresponding to saikogenin A is saikogenin F (Va, 16β–OH instead of 16α–OH), which was proved to be identical with protosaikogenin A which had been isolated by us independently. Therefore, it has now been concluded that all the sapogenins of *Bupleurum* root which possess heteroannular or homoannular diene system are artifacts formed during the course of acid hydrolysis of saponins.

Experimental

All melting points were uncorrected. Optical rotations were measured with Yanagimoto Photo–Magnetic Direct Reading Polarimeter Model OR–20. The ultraviolet absorption spectra were measured in ethanol solution with Cary self–recording spectrophotometer Model 11, infrared spectra with Japan Spectroscopic Co. Model DS–402 G Spectrophotometer. The nuclear magnetic resonance spectra were measured by a Varian Model HR–100 (100 Mc) or a Japan Electron Optics Lab. JNM–3H–60 spectrometer, with Me_4Si as internal standard, respectively. Gas-liquid chromatographic analyses were carried out with a Shimadzu Model GC–1B Gas Chromatograph attached with a hydrogen flame detector. The operating conditions were as follows; Glass column (4 mm *i.d.*×3.2 m) packed with 1.5% SE–30 on Chromosorb W 60—80 mesh; column temperature: 245°; carrier gas: N_2, 70 ml/min. Trimethylsilyl derivatives were prepared as usual, and their ralative retention times (r.r.t.) to cholestane (9.2 min) are shown. The high resolution mass spectrum was measured by a JMS–OIS spectrometer (Japan Electron Optics Lab.), electron accelerating voltage 60 eV. Silica gel, Camag D–5, was used for all the thin–layer chromatographic analyses.

Saikogenin E (Ia)——i) The crude saponin of the root of *Bupleurum falcatum* L. was chromatographed on silica gel column using $CHCl_3$–MeOH (9:1) as the solvent to separate saikoside II fraction. Saikoside II fraction (6 g) dissolved in water (1.1 liter) was added with $NaIO_4$ (10 g), and the mixture was stirred at room temperature for 12 hr in the dark. The mixture was extracted with BuOH, and the BuOH layer was washed subsequently with $NaHCO_3$ and water.

Evaporation of the solvent left a yellowish white powder (4.7 g), which was dissolved in EtOH (165 ml) and added with $NaBH_4$ (2.5 g). The mixture was stirred for 12 hr at room temperature, and an excess of $NaBH_4$ was decomposed carefully by the addition of AcOH. After adding H_2O the reaction mixture was extracted with BuOH, and the BuOH layer was washed with $NaHCO_3$ and H_2O. On evaporation of the solvent, an alcoholic product was obtained. Yield: 4.5 g.

The alcoholic substance (3.5 g) was dissolved in MeOH (70 ml) and added with 0.3 N H_2SO_4 (35 ml). The mixture was stirred for 48 hr at room temperature, and then extracted with benzene. After washing with $NaHCO_3$ and H_2O the solvent was removed to give a faint yellow syrup which was crystallized from MeOH to form saikogenin E, mp 280—289°, $[\alpha]_D^{20}$ +112° (c=0.39 in $CHCl_3$). Yield: 140 mg. *Anal.* Calcd. for $C_{30}H_{48}O_3 \cdot CH_3OH$: C, 76.18; H, 10.72. Found: C, 75.80; H, 10.38. Dried *in vacuo* at 110°. *Anal.* Calcd. for $C_{30}H_{48}O_3$; C, 78.95; H, 10.53. Found: C, 79.04; H, 10.32. UV: End absorption λ^{EtOH} 205 mμ (log ε 3.70). IR: ν_{max}^{KBr} 3460, 3350 cm^{-1} (OH), no C=O.

ii) The product (550 mg) by the oxidation of saikoside II fraction with $NaIO_4$ was dissolved in 5% KOH (50 ml), and the solution was heated for 1.5 hr under stirring and N_2–streaming. The reaction mixture was neutralized with dil. HCl and extracted with ether.

10) C. Djerassi, L.E. Geller, and A.J. Lemin, *J. Am. Chem. Soc.*, **76**, 4089 (1954).

On triturating the ethereal extract with benzene and removal of the solvent saikogenin E was obtained. Yield: 10 mg.

Saikogenin E Diacetate (Ib)——On acetylation of saikogenin E (35 mg) with Ac_2O and pyridine by standing for 48 hr at room temperature diacetate, mp 205—213° (from MeOH), $[\alpha]_D^{21}$ +118° (c=1.1, $CHCl_3$) was obtained. Yield: 26 mg. *Anal.* Calcd. for $C_{34}H_{52}O_5$: C, 75.51; H, 9 .69. Found: C, 75.70; H, 9.33. UV: End absorption λ^{EtOH} 205 mμ (log ε 3.66). IR: ν_{max}^{Nujol} 1750, 1740, 1240 cm^{-1}; no OH.

Acid Treatment of Saikogenin E——Refluxing the mixture of saikogenin E (13 mg), 10% H_2SO_4 (3 ml) and MeOH (6 ml) for 30 min gave saikogenin C, mp 295—301° (yield: 7 mg), which was recrystallized from acetone and identified with the authentic sample kindly supplied by Dr. Kubota, Shionogi Co. The mother liquor of reaction mixture separated from saikogenin C showed UV-absorption maximum of homoannular diene at 282 mμ indicating the formation of saikogenin B.

11-Methoxyoleana-12-ene-3β,16β,28-triol (IIa)——i) In the case of Smith-type degradation of saikoside II fraction, the mother liquor of reaction mixture separated from saikogenin E crystallizing out was chromatographed over Al_2O_3 (Wölm grade II) with benzene: $CHCl_3$ (1:1), where the compound (IIa) (70 mg) was obtained along with saikogenin E (30 mg) and a small amount of saikogenins B and C mixture.

The compound (IIa) was recrystallized from acetone to give mp 222—225°, $[\alpha]_D^{22}$ −23.6° (c=0.89 in pyridine), *Anal.* Calcd. for $C_{31}H_{52}O_4$: C, 76.18; H, 10.72. Found: C, 75.72; H, 10.55. UV: End absorption λ^{EtOH} 210 mμ (log ε 3.80). IR: ν_{max}^{KBr} 3360 cm^{-1} (OH); no C=O.
Mass spectrum:

470	$C_{31}H_{50}O_3$	M–H_2O
456	$C_{30}H_{48}O_3$	M–CH_3OH
452	$C_{31}H_{48}O_2$	M–$2H_2O$
438	$C_{30}H_{46}O_2$	M–(CH_3OH+H_2O)
425	$C_{29}H_{45}O_2$	M–(H_2O+CH_2O+CH_3)
420	$C_{30}H_{44}O$	M–($2H_2O$+CH_3OH)
408	$C_{29}H_{44}O$	M–(H_2O+CH_3OH+CH_2O)

ii) A solution of saikogenin E (20 mg) in MeOH (5 ml) was mixed with p-TsOH•H_2O (5 mg) and stirred for 24 hr at room temperature. After neutralization with dil. $NaHCO_3$ the reaction mixture was extracted with ether to obtain a solid.

The formation of the compound (IIa) was proved by thin-layer ($CHCl_3$: MeOH 11:1) and gas-liquid chromatography (trimethylsilyl deriv., r.r.t. 5.20).

Compound (IIa) Acetate (IIb)——Prepared by the acetylation of compound (IIa) with Ac_2O and pyridine at room temperature and recrystallized from MeOH to give mp 189—195°, after drying *in vacuo* at 110°, mp 169—172°. $[\alpha]_D^{16}$ +45.5° (c=0.4 in $CHCl_3$). *Anal.* Calcd. for $C_{37}H_{58}O_7$: C, 72.27; H, 9.51. Found: C, 72.83; H, 9.37. IR: $\nu_{max}^{CCl_4}$ 1745 cm^{-1}; no OH.

Acid Treatment of Compound (IIa)——Refluxing a mixture of compound (IIa) (10 mg), MeOH (5 ml) and 20% H_2SO_4 (1 ml) for 30 min afforded saikogenin C, mp 299—303°, which was recrystallized from Me_2CO and identified with the authentic sample by a mixed fusion, and the comparison of IR-spectra, thin-layer chromatograms ($CHCl_3$: MeOH 11:1) and gas-liquid chromatograms (trimethylsilyl deriv., r.r.t. 5.42).

The presence of saikogenin B in the reaction mixture was proved by the UV-spectrum (absorption maximum at 281 mμ) and by the gas-liquid chromatography of the trimethylsilylate (r.r.t. 4.16).

Saikogenin G (Va)——The crude *Bupleurum* saponin (saikoside) (35.4 g) was chromatographed over silica gel (600 g) using subsequently the following solvent systems: $CHCl_3$ (1 liter), $CHCl_3$–MeOH (15:1) (2.6 liter), $CHCl_3$–MeOH (9:1) (25.9 liter), $CHCl_3$–MeOH (6:1) (5.2 liter), $CHCl_3$–MeOH (3:1) (6.4 liter). From the fraction eluted with $CHCl_3$–MeOH (9:1) a saikoside fraction (2.17 g) was isolated, which forms saikogenins D and A on acid hydrolysis, nevertheless shows no remarkable UV-absorption of conjugated double bond system. This fraction containing saikosides Ia, Ib-1, Ib-2 and a small portion of minor saponin fraction I (3.3 g) was suspended in an aqueous solution of $NaIO_4$ (6.6 g in 700 ml) and stirred overnight in the dark cold room. The reaction mixture was extracted with BuOH, and the BuOH-extract was evaporated after washing with water. The residue was suspended in a solution of KOH (10 g) in H_2O (150 ml)–EtOH (50 ml) and refluxed for 1 hr under N_2-stream. The reaction mixture was extracted with BuOH. The extract was evaporated to give intermediate product mixture (1.85 g) which yielded saikogenins D and A on acid hydrolysis, nevertheless it still possesses sugar moieties giving lower *Rf*-values than those of saikogenins D and A. Then the intermediate product mixture was subjected again to the oxidation with $NaIO_4$ (3.7 g) in MeOH (100 ml)–H_2O (300 ml). The reaction product was treated with KOH (5 g) in H_2O (75 ml)–EtOH (25 ml) as described above.

The reaction mixture was extracted with ether to obtain sapogenins which were fractionated by silica gel (10 g) column chromatography using subsequently the following solvent systems: $CHCl_3$ (750 ml), $CHCl_3$–ether (3:1) (550 ml), $CHCl_3$–ether (1:1) (450 ml), ether (350 ml), ether–MeOH (30:1) (500 ml).

Saikogenin G (16 mg) was isolated from the fraction eluted with $CHCl_3$–ether (3:1), and recrystallized from MeOH to give mp 242—247°, $[\alpha]_D^{26}$ +45° (c=0.71 in pyridine) UV (EtOH): End absorption only; IR ν_{max}^{KBr}~3500 cm^{-1} (OH), no C=O. *Anal.* Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.07; H, 10.18.

Saikogenin F (10 mg) was obtained from the later part of elution with $CHCl_3$–ether (3:1).

Saikogenin G Diacetate (Vb)——Saikogenin G (35 mg) was acetylated with Ac_2O (0.7 ml) and pyridine (2.1 ml) under ice-cooling for 2 days. The crude product was purified by chromatography over silica gel in benzene to give amorphous diacetate (22 mg) which exhibited single spot on the thin-layer chromatogram developed with $CHCl_3$–MeOH (9:1).

UV (EtOH): End absorption only; IR: $\nu_{max}^{CCl_4}$ cm^{-1} 3635 (OH), 1745 (OAc).

Saikogenin D (VI) derived from Saikogenin G (Va)——A solution of saikogenin G (10 mg) in 0.1 N H_2SO_4 (3 ml)–MeOH (9 ml) was warmed at 45° under stirring for 30 min. The reaction mixture which was neutralized with Na_2CO_3 and diluted with water was extracted with ether. The residue obtained on evaporation was chromatographed over silica gel plate using $CHCl_3$–MeOH (5:1) as the solvent. The saikogenin D fraction was triturated with ether and then recrystallized from AcOEt to obtain pure saikogenin D (2 mg) which was identified with the authentic sample kindly supplied by Dr. Kubota, Shionogi Co., by mixed fusion (mp 263.5—267°), and the comparison of IR-spectra (KBr) and thin-layer chromatograms developed with $CHCl_3$–MeOH (5:1).

Acknowledgement We are grateful to Dr. T. Kubota, Shionogi Research Laboratory for identification of saikogenins C, D and F. Thanks are also due to Dr. T. Hino, National Institute of Radiological Sciences, and Miss M. Ohnishi, National Cancer Center Research Institute for the measurement of NMR spectra. We are also indebted to Japan Electron Optics Laboratory Co. for the measurement of high resolution mass spectrum, and to Yakuri Kenkyukai for grant.