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Isolation, Characterization, and Nuclear Magnetic Resonance Spectra of New Saponins from the Roots of *Bupleurum falcatum* L.¹⁾

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A systematic separation of a saponin fraction of the root of *Bupleurum falcatum* L. furnished seven new saponins, *i.e.*, five monoacetylsaikosaponins (15—18, 20) and saikosaponins-e (19) and -f (21), besides the known saikosaponins-a, -b₂, -b₄, -c, and -d. α -Spinasteryl β -D-glucoside (22) was also isolated. The structures of these new saponins were determined by means of carbon-13 nuclear magnetic resonance spectroscopy.

Keywords—*Bupleurum falcatum* L.; separation of saikosaponins; saikogenins A-G; saikosaponins-a, -b₁, -b₂, -b₃, -b₄, -c, -d, -e, -f; monoacetylsaikosaponins; α -spinasteryl β -D-glucoside; ¹³C NMR spectroscopy; acetylation shift

The roots of *Bupleurum falcatum* L. (Mishima-saiko in Japanese) are used as an important Chinese drug.³⁾ In 1953, Takeda, Hamamoto, and Kubota⁴⁾ started chemical studies on constituents of the plant in this laboratory. Later, Shibata and co-workers,^{5,6)} and Kubota and co-workers⁷⁻⁹⁾ independently clarified the structures of its triterpenoid sapogenins, named saikogenins A—G (1—7). Kubota and Hinoh¹⁰⁾ also reported the structure elucidation of the major saponin constituents, *i.e.*, saikosaponins-a through -d (8—11). Several years later, Shimaoka *et al.*¹¹⁾ reinvestigated the components of the saikosaponins-a and -b, and pointed out that Kubota's "saponin-a" contained saikosaponins-b₁ (12, trace) and -b₃ (13, 10%), and "saponin-b" consisted of saikosaponins-b₂ (9, 87%) and -b₄ (14, 13%).

The accumulation of knowledge on the structure of these saponins obtained in this laboratory⁷⁻¹¹⁾ as well as recent investigations of the ¹³C nuclear magnetic resonance (NMR) spectra of oleanene-type triterpenes, including saikogenins and saikosaponins,^{1a,12,13)} prompted us to examine other minor saponins in this plant. Thus, we carried out a systematic separation of saponins and isolated seven new saikosaponin analogs (15—21) and α -spinasteryl β -D-glucoside (22).¹⁴⁾ Their structures were assigned mainly by ¹³C NMR spectroscopy.

The plant material was extracted with methanol and the methanolic extract was partitioned between butan-1-ol and water. The organic layer was concentrated and the residue

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Another pair of new saponins **17**, mp 217–224°, $[\alpha]_D +44.3^\circ$, and **20**, mp 223–231°, $[\alpha]_D +47.6^\circ$, showed an IR band at 1735 and 1715 cm^{-1} as well as an ^1H NMR singlet signal at δ_H 1.96 and 2.12, respectively; these results indicate the presence of an acetyl group in their molecules. On acetylation they yielded saikosaponin-a octaacetate.¹¹⁾ Comparison of their ^{13}C NMR signals with those of their mother saponin **8** clarified the positions of the acetyl group (see underlined figures in Table I),¹⁷⁾ and indicated that **17** and **20** are 6''-O-acetyl- and 23-O-acetylsaikosaponins-a, respectively.

Chart 2. Structures of Saikogenins and Saikosaponins

The fifth new saponin **18**, mp 209—217°, $[\alpha]_D -6.4^\circ$, also showed an acetyl absorption at 1735 cm^{-1} in its IR spectrum. In the ^1H NMR spectrum, it gave, besides an acetyl singlet at δ_H 1.95, a singlet signal due to a methoxyl group at 3.28. Acetylation of **18** yielded saikosaponin-b₄ nonaacetate.¹¹⁾ By comparison of its ^{13}C NMR spectrum with that of the mother saponin **14** (Table I),¹⁹⁾ **18** was found to be 6''-O-acetylsaikosaponin-b₄. This compound appears to be an artifact¹¹⁾ derived from **16**.

The sixth new saponin **19**, mp 227—230°, $[\alpha]_D +40.8^\circ$, did not show any acetyl absorptions in its IR and ^1H NMR spectra. The ^{13}C NMR signals revealed that it consisted of the aglycone of saikosaponin-c, *i.e.*, saikogenin E (**5**), and the sugar moiety of saikosaponin-a,

TABLE I. ^{13}C and ^1H NMR Spectral Data in Pyridine- d_5 ^{a)}

	6 ^{b)}	8 ^{c)}	17 ^{d)}	20 ^{d)}	7 ^{b)}	11 ^{c)}	15 ^{c)}	16 ^{d)}	5 ^{b)}	19 ^{d)}	10 ^{c)}	23 ^{b)}	21 ^{d)}	3	1	12	4	9	13	14 ^{c)}	18 ^{c)}	24	22	
C-1	38.8	38.9	39.0	38.7	38.9 ^{f)}	38.8 ^{f)}	38.9 ^{f)}	38.9 ^{f)}	38.8	39.0	38.8	39.4	39.1	38.8	38.4	38.7	38.7	38.7	38.7	40.2	40.3	40.2	37.3	37.7
C-2	27.5	25.6	25.8	26.0 ^{f)}	27.6	25.8	25.9	25.9	27.9	26.5	26.4	28.1	26.4	28.1	27.6	25.8	27.6	25.9	26.3	26.3	26.3	31.6	30.2	
C-3	74.2	82.5	82.4	82.1	74.2	82.5	82.5	82.5	78.0	88.9	89.2	78.3	89.3	78.5	73.1	82.4	74.4	82.6	82.6	82.7	82.8	71.0	77.9	
C-4	43.0	43.5	43.7	42.8	43.0	43.8	43.9	43.9	39.5	39.8	39.7	39.4	39.5	39.6	43.1	43.7	43.0	43.7	43.9	43.8	43.8	38.2	35.1	
C-5	49.3	48.0	48.1	48.9	49.2	48.1	48.1	48.1	55.3	55.9	55.7	56.0	56.1	55.7	48.2	48.1	49.4	48.2	48.3	48.5	48.5	40.5	40.4	
C-6	18.2	17.8	17.9	18.1	18.2	17.8	17.9	17.9	18.2	18.1	18.1	18.9	18.7	19.0	18.6	18.8	18.9	18.8	18.6	18.6	18.6	29.8	30.2	
C-7	32.0	31.9	32.0	32.1	31.9	31.8	31.9	31.9	31.9	32.2	32.2	33.3	33.4	33.1	32.4 ^{f)}	32.2	32.5	32.5	33.6	33.9	33.8	117.6	117.9	
C-8	42.5	42.4	42.5	42.5	42.1	42.1	42.2	42.2	42.2	42.5	42.4	40.4 ^{f)}	40.4 ^{f)}	40.7	40.5	40.8	41.3	41.4	41.1	41.0	41.0	139.6	139.8	
C-9	53.3	53.2	53.3	53.4	53.2	53.2	53.3	53.3	53.0	53.2	53.1	47.4	47.4	54.6	54.5	54.7	54.2	54.2	52.1	51.8	51.8	49.7	50.1	
C-10	36.9	36.6	36.7	36.7	36.8	36.6	36.7	36.7	36.7	36.7	36.6	37.4	37.1	37.3	36.8	36.9	37.1	36.9	38.4	38.6	38.6	34.4	34.8	
C-11	132.0	132.0	132.0	131.8	131.9	131.9	132.0	132.0	132.1	132.0	132.0	24.0	24.0	127.1	127.1	127.1	126.3	126.3	76.2	76.3	76.3	21.7	22.0	
C-12	131.2	131.0	131.1	131.4	131.9	131.9	132.0	132.0	131.2	131.2	131.1	122.7	122.8	125.8	125.7	125.7	126.3	126.3	122.9	122.7	122.7	39.7	40.0	
C-13	84.1	84.0	84.1	84.1	85.0	85.1	85.1	85.1	84.0	84.1	84.0	144.1	144.0	136.7	136.4	136.8	136.5	137.1	148.1	149.8	149.8	43.4	43.8	
C-14	46.0	46.0	46.0	46.0	43.9	43.7	43.8	43.8	45.6	46.0	45.9	44.1	44.1	44.6 ^{f)}	44.4	44.6 ^{f)}	42.2	42.2	44.6	42.3	42.3	55.3	55.5	
C-15	36.3	36.2	36.4	36.2	35.7	35.7	35.7	35.7	36.3	36.2	36.2	36.9	36.8	35.1 ^{g)}	34.8 ^{g)}	35.1 ^{g)}	32.8	32.8	37.0	37.2	37.1	23.1	23.4	
C-16	64.4	64.4	64.5	64.4	77.5	77.5	77.6	77.6	64.0	64.4	64.4	67.0	67.2	76.5	76.5	76.5	68.8	68.8	66.9	74.3	74.3	28.5	28.6	
C-17	46.8	46.9	47.1	47.1	45.6	45.5	45.6	45.6	47.0	47.0	47.0	41.1 ^{f)}	41.1 ^{f)}	44.8 ^{f)}	44.4	44.8 ^{f)}	45.4	45.4	43.9 ^{f)}	43.8 ^{g)}	43.8 ^{g)}	56.2	56.7	
C-18	52.4	52.4	52.4	52.4	51.5	51.5	51.6	51.6	52.2	52.4	52.3	44.9	44.9	133.7	133.3	133.6	133.0	133.0	44.3 ^{f)}	42.3 ^{g)}	42.3 ^{g)}	12.2	12.3	
C-19	38.2	38.2	38.2	38.2	38.7 ^{f)}	38.7 ^{f)}	38.7 ^{f)}	38.8 ^{f)}	37.8	38.2	38.1	47.4	47.4	38.7	38.4	38.7	39.2	39.2	47.3	48.5	48.5	13.0	13.0	
C-20	31.6	31.6	31.7	31.7	31.9	31.8	31.9	31.9	31.6	31.6	31.6	31.1	31.0	32.8	32.7	32.8	32.8	32.8	31.1	31.2	31.2	40.8	40.7	
C-21	35.0	35.0	35.0	35.0	37.0	37.0	37.1	37.1	34.7	35.0	34.9	34.4	34.4	35.4 ^{g)}	35.1 ^{g)}	35.4 ^{g)}	35.8	35.8	34.4	35.2	35.2	21.1	21.1	
C-22	25.7	25.6	25.8	25.8 ^{f)}	31.3	31.2	31.3	31.3	25.7	25.7	25.7	26.4	26.4	30.2	29.9	30.2	24.9	24.9	26.3	30.0	30.0	138.1	138.4	
C-23	69.0	65.1	65.0	66.4	69.0	65.2	65.1	65.2	28.4	28.1	28.1	28.8	28.4	28.5	67.4	65.2	65.6	65.3	65.4	65.6	129.7	130.2		
C-24	12.1	12.7	12.8	12.6	12.6	12.8	12.9	12.8	15.9	16.3	16.3	16.4	17.0	15.8	12.6	12.9	12.2	12.9	13.4	13.5	13.4	51.4	51.6	
C-25	18.5	18.6	18.7	18.4	18.6	18.7	18.8	18.8	18.2	18.1	18.1	15.8	15.7	18.3	18.6	18.5	18.6	18.6	17.9	17.9	17.9	31.9	32.2	
C-26	19.9	19.8	19.9	20.0	19.5	19.4	19.5	19.5	20.0	19.9	19.8	17.1	17.1	17.2	17.1	17.3	17.5	17.5	18.6	18.8	18.8	21.4	21.6	
C-27	20.9	20.8	20.9	20.8	18.0	18.1	18.2	18.2	20.9	20.9	20.8	27.1	27.1	22.1	22.0	22.1	22.2	22.3	26.3	26.5	26.5	19.1	19.3	
C-28	73.0	72.9	72.9	73.1	77.9	77.8	77.9	77.8	73.0	73.0	72.9	69.4	69.3	64.1	64.0	64.1	64.7	65.6	69.2	70.2	70.2	25.4	25.6	
C-29	33.7	33.7	33.7	33.7	33.7	33.7	33.7	33.7	33.7	33.7	33.7	33.3	33.3	25.0	24.8	25.0	25.3	25.3	33.2	33.2	33.2	12.2	12.3	
C-30	23.9	23.9	23.9	23.9	24.6	24.5	24.6	24.6	23.8	23.9	23.9	24.2	24.3	32.3	32.3 ^{f)}	32.8	32.5	32.5	24.3	25.0	25.0			
C-1'	105.3	105.6	106.0		105.4	105.5	105.6		106.3	106.1		106.1				105.5	105.5	105.5	105.5	105.6		102.6		
C-2'	71.7	71.5	71.6		71.7	71.8	71.6		71.6	75.2		75.2				71.8	71.8	71.8	71.8	71.6		75.3		
C-3'	85.0	85.4	85.3		85.1	85.2	85.4		85.1	76.8		76.9				85.2	85.2	85.3	85.3	85.4		78.6		
C-4'	71.8	71.7	72.1		71.9	72.1	71.7		71.9	80.2		80.2				72.1	72.0 ^{g)}	72.0	71.7			72.3		
C-5'	70.8	71.0	71.0		70.9	71.0	71.0		70.8	75.5		75.5				71.0	71.0	71.0	71.0	71.0		77.9		
C-6'	16.9	17.1	17.0		16.9	17.0	17.1		17.0	69.2		69.3				17.0	17.0	17.0	17.0	17.0		63.3		
C-1''	105.7	105.6	106.0		105.8	105.9	105.6		105.9	102.6		102.6				106.0	105.9	106.0	105.9	105.6				
C-2''	75.4	75.4	75.7		75.5	73.7	75.4		75.5	72.2 ^{g)}		72.3 ^{g)}				75.7	75.6	75.6	75.6	75.3				
C-3''	78.0	78.1	78.3		78.1	79.5	78.1		78.1	72.6 ^{g)}		72.6 ^{g)}				78.3	78.2	78.3	78.3	78.1				
C-4''	71.8	72.0	72.1		71.9	70.0	72.0		71.9	73.8		73.8				72.1	72.1	72.1 ^{g)}	72.0	72.0				
C-5''	78.0	75.4	78.3		78.1	78.3	75.4		78.1	70.5		70.5				78.3	78.1	78.3	78.3	75.3				
C-6''	62.9	64.6	63.1		63.0	62.6	64.7		62.9	18.1		18.2				63.1	63.1	63.0	63.0	64.6				
C-1'''											104.8		104.8											
C-2'''											74.7		74.7											
C-3'''											78.2		78.3											
C-4'''											71.8		71.9											
C-5'''											77.9		78.0											
C-6'''											62.9		62.9											
OMe																			53.8	53.6	53.5			
MeCO			170.4	170.5			170.8	170.6													170.7		H-18	
MeCO			20.6	20.8			21.0	20.6												20.6	0.63	0.60		
																						H-19		
H-23									1.23	1.27	1.25	1.25	1.30	1.25								0.85	0.74	
H-24	1.07 ^{f)}	0.89	0.90	0.87	1.08	0.89	0.88	0.89	0.98	0.92 ^{f)}	0.92	0.96	0.85	0.99	1.06 ^{h)}	0.87	1.08	0.90	0.88	0.99	0.89		H-21	
H-25	1.06 ^{f)}	1.00	1.00	0.96	1.08	1.02	1.02	1.02	1.04	0.94 ^{f)}	0.96	1.08	1.00	1.04	1.04 ^{h)}	0.98	1.08	1.02	1.08	1.11	1.11	1.09	1.10	
H-26	1.43	1.37	1.38	1.34	1.39	1.34	1.34	1.32	1.41	1.33	1.33	1.36	1.36	0.97 ^{h)}	0.98 ^{h)}	0.98 ^{h)}								

i.e., a glucosyl-fucosyl group (see Table I). Since this is a new combination of the aglycone and sugar moiety, **19** was named saikosaponin-e.

Fractions 2 and 3 in Chart 1 were separated into pairs of known saponins,¹¹ **8** and **11**, and **9** and **14**, respectively. The existence of saikosaponins-b₁ (**12**) and -b₃ (**13**) was suggested as their peracetates on thin-layer chromatography (TLC) of an acetylation product of crude saponin-a.¹¹ However, these two saponins could not be isolated because of their low contents and the co-existence of rather large amounts of other compounds.

Fraction 4 proved to correspond to a "saponin-c" fraction from its chromatographic behavior.¹¹ Although Kubota and Hinoh reported the isolation of longispinogenin (**23**) on degradation of "saponin-c",⁹ no saponin possessing **23** in the molecule has so far been isolated from this plant. Thus, we inspected the ¹³C NMR spectrum of this fraction to find that it was composed of signals of two saponins having the same sugar moiety; one component was saikosaponin-c (**10**) and the other was the seventh new saponin, named saikosaponin-f (**21**), which was shown to contain longispinogenin (**23**) in the molecule (Table I). The fraction was separated by chromatography after acetylation to give **10**-decaacetate and **21**-undecaacetate, both of which were hydrolyzed to regenerate pure saikosaponins-c, mp 205–209°, [α]_D +5.7°, and -f, mp 203–206°, [α]_D –16.9°, respectively. The latter is considered to be the saponin which had furnished **23** in Kubota's experiment.⁹

In conclusion, we systematically separated twelve saikosaponins (**8**–**11**, **14**–**21**) together with α -spinasteryl β -D-glucoside (**22**)¹⁵ from the roots of *B. falcatum*; the structures of seven new saponins (**15**–**21**) were determined by ¹³C NMR spectroscopy without any chemical degradation. The present results show ¹³C NMR spectroscopy to be a powerful tool for elucidating the chemical structures of natural plant glycosides, and also to be practically useful in identifying saponins of known structure.

Experimental

Melting points were determined on a Yanagimoto micro melting point determination apparatus and are uncorrected. Unless otherwise stated, rotations were taken in MeOH. Silica gel used for column chromatography was Kieselgel 60 (Merck). Thin-layer chromatography was carried out on pre-coated TLC plates of Kieselgel 60 F₂₅₄ (Merck) with double development of each solvent system.

¹H NMR spectra were taken with a Varian XL-100, HA-100, or A-60A spectrometer using pyridine-*d*₅ solutions containing tetramethylsilane (TMS) as an internal standard at ordinary probe temperatures. ¹³C NMR spectra were recorded on a Varian NV-14 FT NMR spectrometer at 15.087 MHz using pyridine-*d*₅ solutions containing TMS as an internal reference in 8 mm spinning tubes at 100° (in order to avoid line broadening). Usual FT NMR measurement conditions were as follows: spectral width, 3923 Hz; acquisition time, 0.6 sec; pulse width 10–20 μ sec (pulse flipping angle, 15–30°); number of data points, 4820; numbers of transients, 10000–100000. The ¹³C NMR signals were assigned by using known chemical-shift rules, ¹H single frequency off-resonance decoupling techniques, and by chemical-shift comparisons with many other compounds already reported.^{1a,12,13} Chemical shifts are expressed in terms of δ values (ppm downfield from TMS). Accuracies of δ _H and δ _C are within 0.02 and 0.1 ppm, respectively.

The ¹³C chemical shift data are listed in Table I, which also includes the methyl ¹H chemical shift data with tentative assignments.

Extraction and Fractionation of Crude Saponin—Dried and cut roots of *Bupleurum falcatum* L.⁴ (1 kg) were extracted with MeOH containing 0.1% pyridine⁹ (4 $\times$ 3 l) at room temperature for 3 days. Material from the MeOH extract (168 g) was partitioned three times with a butan-1-ol–H₂O system and the organic layer was concentrated *in vacuo*. The resulting residue (53 g) was mixed with Celite (30 g) and the mixture was triturated with petroleum ether (3 $\times$ 100 ml) to remove oily components (26 g). The Celite which had adsorbed crude saponin was put on top of a column of silica gel (500 g) and the column was eluted successively with CHCl₃ (1 l), CHCl₃–EtOH (9:1) (1 l), CHCl₃–EtOH (8:2) (1 l), CHCl₃–EtOH (7:3) (1 l, Fr. 1), CHCl₃–MeOH–H₂O (7:3:0.3) (1 l, Fr. 2), CHCl₃–MeOH–H₂O (6:4:0.4) (500 ml, Fr. 3+500 ml, Fr. 4), and MeOH (1 l).

α -Spinasteryl β -D-Glucoside (22**)**—Fr. 1 (5.8 g) was chromatographed on silica gel (200 g) and eluted successively with CHCl₃–EtOH (9:1) (1 l), CHCl₃–EtOH (85:15) (200 ml, Fr. 5+250 ml, Fr. 6+500 ml, Fr. 7), CHCl₃–EtOH (8:2) (400 ml, Fr. 8), CHCl₃–EtOH (7:3) and MeOH (200 ml). Fr. 5 (816 mg) was repeatedly chromatographed on silica gel using CHCl₃–MeOH–H₂O (8:2:0.2) to give a **22**-rich fraction (320 mg, Fr. 15), which was further purified by chromatography of its acetylation product.¹⁵ The purified glyco-

side (285 mg) was crystallized from CHCl_3 -MeOH to yield **22** as colorless plates, mp 272–283°, $[\alpha]_D^{25} -30.0^\circ$ ($c=0.95$, pyridine). *Anal.* Calcd for $\text{C}_{35}\text{H}_{58}\text{O}_6$: C, 73.13; H, 10.17. Found: C, 72.92; H, 10.19. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400 (br), 1075, 1030.

3''-O-Acetylsaikosaponin-d (15)—Fr. 6 (905 mg) was repeatedly chromatographed on silica gel with EtOAc-EtOH- H_2O (9:1:0.5) to give a **15**-rich fraction (305 mg, Fr. 16), which was again chromatographed on silica gel using CHCl_3 -MeOH- H_2O (8:2:0.2) to afford Fr. 21 (131 mg). The fraction was precipitated from MeOH-ether, yielding **15** as a white powder, mp 219–225°, $[\alpha]_D^{21.5} +47.5^\circ$ ($c=1.03$). *Anal.* Calcd for $\text{C}_{44}\text{H}_{70}\text{O}_{14} \cdot 3\text{H}_2\text{O}$: C, 60.32; H, 8.74. Found: C, 60.56; H, 8.84. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400 (br), 1730, 1250, 1075 (br).

Saponin **15** (30 mg) was acetylated with Ac_2O in pyridine at room temperature overnight. The crude product (41 mg) was purified by chromatography on silica gel with benzene-EtOAc (3:2) to give saikosaponin-d heptaacetate (23 mg) as a white powder, mp 178–183° (from ether-pentane), $[\alpha]_D^{23} +52.0^\circ$ ($c=0.54$). *Anal.* Calcd for $\text{C}_{56}\text{H}_{82}\text{O}_{20}$: C, 62.55; H, 7.69. Found: C, 62.45; H, 7.97. This was identical with a similar acetylation product of saikosaponin-d on the basis of mixed mp determination, TLC [*Rf* 0.28, CHCl_3 -acetone (9:1); *Rf* 0.39, benzene-EtOAc (3:2)], and comparison of ^{13}C NMR spectra.

6''-O-Acetylsaikosaponin-d (16)—Fr. 7 (971 mg) was repeatedly chromatographed on silica gel with EtOAc-EtOH- H_2O (9:1:0.5), giving a **16**-rich fraction (241 mg, Fr. 17) and a fraction containing **17** (406 mg, Fr. 18). Fr. 17 was rechromatographed on silica gel (50 g) using CHCl_3 -MeOH- H_2O (8:2:0.2) to give Fr. 22 (149 mg), which was treated with MeOH-ether to yield **16** as a white powder, mp 196–205°, $[\alpha]_D^{24} +43.5^\circ$ ($c=1.01$). *Anal.* Calcd for $\text{C}_{44}\text{H}_{70}\text{O}_{14} \cdot \text{H}_2\text{O}$: C, 62.91; H, 8.61. Found: C, 62.76; H, 8.69. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400 (br), 1735, 1245, 1075 (br).

Compound **16** was acetylated as described above to give an acetate, mp 178–183°, $[\alpha]_D^{25.5} +50.4^\circ$ ($c=1.04$), which was identical with saikosaponin-d heptaacetate (mixed mp, TLC and ^{13}C NMR spectrum).

6''-O-Acetylsaikosaponin-a (17)—Fr. 18 (406 mg) was repeatedly chromatographed on silica gel with CHCl_3 -MeOH- H_2O (8:2:0.2) to yield Fr. 23 (57 mg), which was precipitated from MeOH-ether to afford **17** as a white powder, mp 217–224°, $[\alpha]_D^{24} +44.3^\circ$ ($c=1.04$). *Anal.* Calcd for $\text{C}_{44}\text{H}_{70}\text{O}_{14} \cdot \text{H}_2\text{O}$: C, 62.91; H, 8.61. Found: C, 62.92; H, 8.38. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3420 (br), 1735, 1245, 1070 (br).

Saponin **17** was acetylated with Ac_2O in pyridine at 80° for 2 hr. The product was purified by chromatography [silica gel, benzene-EtOAc (3:2)] to give saikosaponin-a octaacetate,¹¹ mp 165–169°, $[\alpha]_D^{23} +68.1^\circ$ ($c=0.54$). *Anal.* Calcd for $\text{C}_{58}\text{H}_{80}\text{O}_{21}$: C, 62.35; H, 7.58. Found: C, 62.48; H, 7.77. Its identity was confirmed by mixed mp determination, TLC [*Rf* 0.46, CHCl_3 -acetone (9:1); *Rf* 0.48, benzene-EtOAc (3:2)], and ^{13}C NMR spectroscopy.

6''-O-Acetylsaikosaponin-b₄ (18)—Fr. 8 (762 mg) was repeatedly chromatographed on silica gel with EtOAc-EtOH- H_2O (9:1:0.5) to provide Fr. 19 (320 mg) and Fr. 20 (154 mg). Further chromatography of Fr. 19 using CHCl_3 -MeOH- H_2O (8:2:0.2) gave Fr. 24 (24 mg) and Fr. 25 (192 mg). Fr. 24 was precipitated from MeOH-Et₂O to give **18** as a white powder, mp 209–217°, $[\alpha]_D^{24.5} -6.4^\circ$ ($c=1.01$). *Anal.* Calcd for $\text{C}_{45}\text{H}_{74}\text{O}_{15} \cdot 2\text{H}_2\text{O}$: C, 60.65; H, 8.82. Found: C, 60.34; H, 8.46.

Compound **18** was acetylated as described for **17**, yielding saikosaponin-b₄ nonaacetate,¹¹ mp 153–157°, $[\alpha]_D^{23} -12.7^\circ$ ($c=0.53$), which was identical with an authentic sample on the basis of mixed mp, TLC [*Rf* 0.37 CHCl_3 -acetone (9:1); *Rf* 0.41, benzene-EtOAc (3:2)], and ^{13}C NMR spectroscopy. *Anal.* Calcd for $\text{C}_{61}\text{H}_{90}\text{O}_{23}$: C, 61.50; H, 7.62. Found: C, 61.92; H, 7.75. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3420 (br), 1735, 1245, 1075 (br).

Saikosaponin-e (19)—Fr. 25 (192 mg) was chromatographed on silica gel (60 g) with EtOAc-EtOH- H_2O (9:1:0.5) to give Fr. 27 (137 mg), which was precipitated from MeOH-ether, yielding **19** as a white powder, mp 227–230°, $[\alpha]_D^{24} +40.8^\circ$ ($c=1.05$). *Anal.* Calcd for $\text{C}_{42}\text{H}_{68}\text{O}_{12} \cdot 2\text{H}_2\text{O}$: C, 62.97; H, 9.06; Found: C, 62.75; H, 9.22. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400 (br), 1080 (br).

Acetylation of **19** gave its heptaacetate, mp 176–178°, $[\alpha]_D^{24} +64.1^\circ$ ($c=1.01$). *Rf* 0.55, CHCl_3 -acetone (9:1); *Rf* 0.62, benzene-EtOAc (3:2). *Anal.* Calcd for $\text{C}_{56}\text{H}_{82}\text{O}_{19}$: C, 63.50; H, 7.80. Found: C, 63.72; H, 7.85.

23-O-Acetylsaikosaponin-a (20)—Fr. 20 (154 mg) was purified by chromatography on silica gel (50 g) with CHCl_3 -MeOH- H_2O (8:2:0.2) to give Fr. 26 (117 mg), which was precipitated from MeOH-ether, affording **20** as a white powder, mp 223–231°, $[\alpha]_D^{24} +47.6^\circ$ ($c=1.01$). *Anal.* Calcd for $\text{C}_{44}\text{H}_{70}\text{O}_{14} \cdot \text{H}_2\text{O}$: C, 62.91; H, 8.61. Found: C, 62.94; H, 8.56. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3420 (br), 1715, 1250, 1075 (br).

Saponin **20** was acetylated as described for **17** to give an acetate, mp 166–170°, $[\alpha]_D^{25.5} +70.0^\circ$ ($c=1.05$), which was identified as 8-octaacetate¹¹ by mixed mp determination, TLC, and ^{13}C NMR.

Isolation of Saikosaponins-a (8) and -d (11)—Fr. 2 (4.1 g) was chromatographed on silica gel (300 g) using EtOAc-EtOH- H_2O (8:2:1) to give an 11-rich fraction (1.58 g, Fr. 9) and an 8-rich fraction (2.49 g, Fr. 10). Fr. 9 was acetylated in a usual manner and the product (2.57 g) was chromatographed on silica gel (150 g) with benzene-EtOAc (3:2) to furnish 11-heptaacetate (897 mg). Fr. 10 was treated similarly to yield 8-octaacetate (2.27 g) and 11-heptaacetate (348 mg). TLC of the middle fraction (806 mg) eluted between the above acetates showed spots of *Rf* 0.45 and 0.38 [CHCl_3 -acetone (9:1)], corresponding to those of saikosaponins-b₁ and -b₃, which partially overlapped the spots of other compounds.

The acetates of **8** (2.27 g) and **11** (1.24 g) were separately hydrolyzed with 2% KOH-MeOH under reflux for 3 hr to give **8** (1.45 g), mp 237–240°, $[\alpha]_D^{25} +57.0^\circ$ ($c=1.06$), and **11** (862 mg), mp 256–259°, $[\alpha]_D^{25}$

+51.8° ($c=1.01$, $\text{CHCl}_3\text{-MeOH}$ (1:1)).

Isolation of Saikosaponins-b₂ (9) and -b₄ (14)—Fr. 3 (1.4 g) was repeatedly chromatographed on silica gel with $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (7:3:0.3) to give Fr. 11 (291 mg) and Fr. 12 (453 mg), which were precipitated from MeOH-ether to yield **14**, mp 232—236°, $[\alpha]_{\text{D}}^{25.5} -27.2^\circ$ ($c=1.04$), and **9**, mp 231—238°, $[\alpha]_{\text{D}}^{25.5} -11.5^\circ$ ($c=1.0$), respectively.

Saikosaponins-c (10) and -f (21)—Fr. 4 (910 mg) was acetylated with Ac_2O (2 ml) in pyridine (3 ml) at 90° for 4 hr. Water was added to the reaction mixture, which was extracted with CHCl_3 . The CHCl_3 layer was washed with H_2O , dried over Na_2SO_4 and concentrated to give a product (1.3 g). The product was chromatographed on silica gel (100 g) with $\text{CHCl}_3\text{-acetone}$ (9:1) to furnish Fr. 13 (380 mg) and Fr. 14 (755 mg).

Fr. 13 was hydrolyzed with 2% KOH-MeOH under reflux for 3 hr to give **21** as a white powder (241 mg), mp 203—206° (from MeOH-ether), $[\alpha]_{\text{D}}^{24} -16.9^\circ$ ($c=1.0$). *Anal.* Calcd for $\text{C}_{48}\text{H}_{80}\text{O}_{17}\cdot 2\text{H}_2\text{O}$: C, 59.73; H, 8.77. Found: C, 59.43; H, 8.67. **21**-Undecaacetate, mp 144—147° (from ether-pentane), $[\alpha]_{\text{D}}^{25} -13.8^\circ$ ($c=1.01$). *Anal.* Calcd for $\text{C}_{70}\text{H}_{102}\text{O}_{28}$: C, 60.42; H, 7.39. Found: C, 60.16; H, 7.22.

Fr. 14 was hydrolyzed similarly to yield **10** as a white powder (480 mg), mp 205—209° (from MeOH-ether), $[\alpha]_{\text{D}}^{24} +5.7^\circ$ ($c=1.01$). *Anal.* Calcd for $\text{C}_{48}\text{H}_{78}\text{O}_{17}\cdot 3\text{H}_2\text{O}$: C, 58.76; H, 8.63. Found: C, 59.04; H, 8.56. **10**-Decaacetate, mp 154—156° (from $\text{Et}_2\text{O-pentane}$), $[\alpha]_{\text{D}}^{25} 0^\circ$; $[\alpha]_{\text{D}}^{25} +7.1^\circ$ ($c=1.01$) *Anal.* Calcd for $\text{C}_{68}\text{H}_{98}\text{O}_{27}$: C, 60.61; H, 7.33. Found: C, 60.73; H, 7.30.