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

Hiroshi Ishii, Miharu Nakamura, Shujiro Seo, Kazuo Tori, Takehiko Tozyo, and Yohko Yoshimura

Shionogi Research Laboratory, Shionogi and Co., Ltd.[2)]

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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, -b_2, -b_4, -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, -b_1, -b_2, -b_3, -b_4, -c, -d, -e, -f; monoacetylsaikosaponins; α-spinasteryl β-D-glucoside; ^{13}C 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 Kubota[4)] started chemical studies on constituents of the plant in this laboratory. Later, Shibata and co-workers,[5,6)] and Kubota and co-workers[7–9)] independently clarified the structures of its triterpenoid sapogenins, named saikogenins A—G (**1**—**7**). Kubota and Hinoh[10)] 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-b_1 (**12**, trace) and -b_3 (**13**, 10%), and "saponin-b" consisted of saikosaponins-b_2 (**9**, 87%) and -b_4 (**14**, 13%).

The accumulation of knowledge on the structure of these saponins obtained in this laboratory[7–11)] as well as recent investigations of the ^{13}C 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 ^{13}C 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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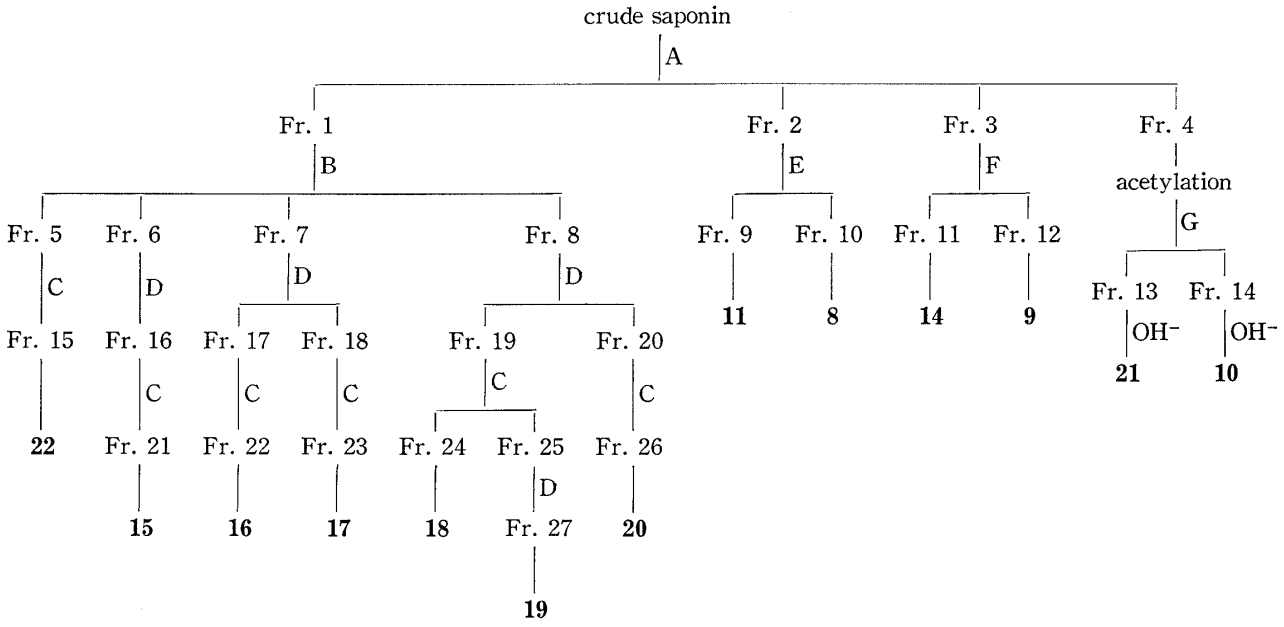

solvent system A: $CHCl_3$–MeOH–H_2O in various ratios
B: $CHCl_3$–EtOH in various ratios
C: $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2)
D: EtOAc–EtOH–H_2O (9 : 1 : 0.5)
E: EtOAc–EtOH–H_2O (8 : 2 : 1)
F: $CHCl_3$–MeOH–H_2O (7 : 3 : 0.3)
G: $CHCl_3$–acetone (9 : 1)

Chart 1. Separation of Saikosaponins by Silica Gel Chromatography

obtained was washed with petroleum ether to remove oily components, giving a crude saponin mixture. The saponin mixture was separated by column chromatography as shown in Chart 1.

Fraction 1 was subjected to repeated chromatography to give, in order of increasing polarity, compound **22**, four monoacetylated saponins (**15**—**18**), saikosaponin-e (**19**), and another monoacetylated saponins (**20**). Compound **22**, mp 272—283°, $[\alpha]_D$ −30.0°, showed only two singlet methyl signals in its 100-MHz ^1H NMR spectrum; this suggests that it is a glycoside of one of the sterol components already isolated from this plant.[4,15] The ^{13}C NMR spectrum revealed that **22** is α-spinasteryl β-D-glucoside of known structure,[14] by comparison with the spectra of α-spinasterol (**24**) and methyl β-D-glucoside (see the underlined figures for **22** in Table I, which show the glycosidation shifts).[16–18]

A pair of new saponins **15**, mp 219—225°, $[\alpha]_D$ +47.5°, and **16**, mp 196—205°, $[\alpha]_D$ +43.5°, exhibited an infrared (IR) absorption band at 1730 and 1735 cm^{-1}, and an ^1H NMR singlet signal at δ_H 1.95 and 1.96, respectively, due to an acetyl group. Further, the NMR spectra showed that the two compounds have six angular methyl groups, as do the known saikosaponins. When acetylated with acetic anhydride in pyridine they afforded the same saikosaponins-d heptaacetate; these results indicate that their mother saponin is saikosaponin-d (**11**). Thus, the ^{13}C NMR spectra of **15** and **16** were compared with that of **11** to examine the acetylation shifts[19] and the results (see underlined figures in Table I) demonstrated that **15** and **16** are 3″-O-acetyl- and 6″-O-acetylsaikosaponins-d, respectively. Saponin **15** was also isolated by Yamasaki *et al.*,[20] who determined its structure in a similar way.

15) K. Takeda and T. Kubota, *Chem. Pharm. Bull.*, **6**, 536 (1958).
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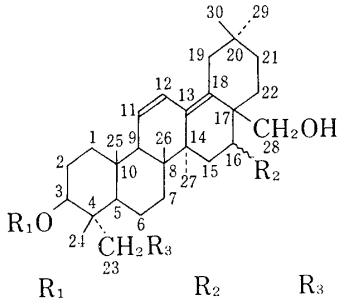
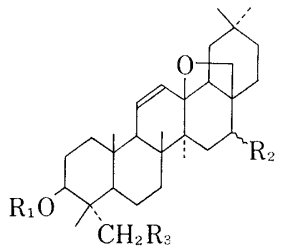
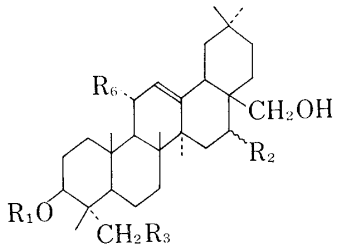
Another pair of new saponins **17**, mp 217—224°, $[\alpha]_D$ +44.3°, and **20**, mp 223—231°, $[\alpha]_D$ +47.6°, showed an IR band at 1735 and 1715 cm^{-1} as well as an ^1H NMR singlet signal at δ_H 1.96 and 2.12, respectively; these results indicate the presence of an acetyl group in their molecules. 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.



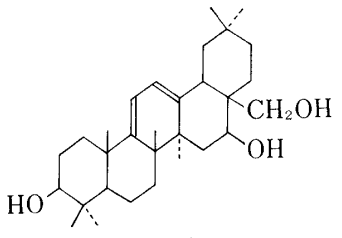
	R_1	R_2	R_3	R_4	R_5
1	H	β-OH	OH	—	—
3	H	β-OH	H	—	—
4	H	α-OH	OH	—	—
9	Glc-Fuc-	α-OH	OH	H	H
12	Glc-Fuc-	β-OH	OH	H	H



	R_1	R_2	R_3	R_4	R_5
5	H	β-OH	H	—	—
6	H	β-OH	OH	—	—
7	H	α-OH	OH	—	—
8	Glc-Fuc-	β-OH	OH	H	H
10	Glc-Glc- (Rham)	β-OH	H	—	—
11	Glc-Fuc-	α-OH	OH	H	H
15	Glc-Fuc-	α-OH	OH	Ac	H
16	Glc-Fuc-	α-OH	OH	H	Ac
17	Glc-Fuc-	β-OH	OH	H	Ac
19	Glc-Fuc-	β-OH	H	H	H
20	Glc-Fuc-	β-OH	OAc	H	H



	R_1	R_2	R_3	R_4	R_5	R_6
13	Glc-Fuc-	β-OH	OH	H	H	OCH_3
14	Glc-Fuc-	α-OH	OH	H	H	OCH_3
18	Glc-Fuc-	α-OH	OH	H	Ac	OCH_3
21	Glc-Glc- (Rham)	β-OH	H	—	—	H
23	H	β-OH	H	—	—	H



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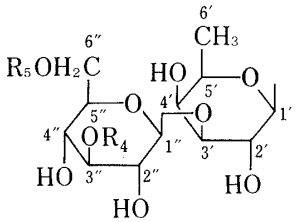
Glc-Fuc- :



Glc-Glc- (Rham) :

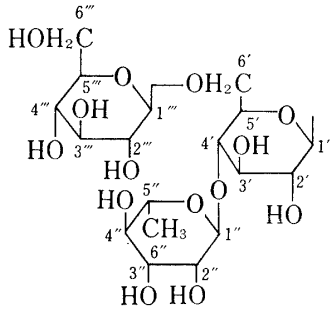


Chart 2. Structures of Saikogenins and Saikosaponins

The fifth new saponin **18**, mp 209—217°, $[\alpha]_D$ −6.4°, 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-b_4 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-b_4. This compound appears to be an artifact[11] derived from **16**.

The sixth new saponin **19**, mp 227—230°, $[\alpha]_D$ +40.8°, 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[d]	16[f]	5[b]	19[d]	10[d]	23[b]	21[d]	3	1	12	4	9	13	14[d]	18[f]	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	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[e]	43.8[e]	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[e]	42.3[e]	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	65.5	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	105.6	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		72.1	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[e]		72.3[e]			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[e]		72.6[e]			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[i]	0.98[h]	1.05[f]	1.05[f]	1.39	1.14	1.15	H-26(27)	
H-27	1.10	1.10	1.10	1.12	1.62	1.60	1.60	1.59	1.15	1.14	1.12	1.06	1.02	1.16	1.09	1.09	1.68	1.68	1.12	1.84	1.85	0.91	0.90
H-29	0.94[g]	0.94[g]	0.94[g]	0.94[g]	1.04	1.02	1.02	1.02	0.97[f]	0.96[f]	0.96[f]	1.02[g]	1.02[g]	0.89[h]	0.91[i]	0.92[h]	1.01[f]	1.01[f]	1.00	1.00	1.00	H-29	
H-30	0.91[g]	0.90[g]	0.90[g]	0.91[g]	0.97	0.96	0.95	0.96	0.93[f]	0.92[f]	0.92[f]	0.96[g]	0.96[g]	0.89[h]	0.88[i]	0.87[h]	0.94[f]	0.93[f]	0.94	0.93	0.94	0.93	0.93
H-6′		1.41	1.49	1.46		1.41	1.41	1.47		1.42						1.46		1.47	1.41	1.39	1.45		
H-6″											1.57		1.65										
OAc			1.96	2.12			1.95	1.96													1.95		
OMe																			3.23	3.25	3.27		

a) ^{13}C spectra were observed at 100° to avoid line broadening at 15 MHz.
b) For the signal assignments, see Ref. (13), where data obtained in $CDCl_3$–CD_3OD and data on the peracetates observed in $CDCl_3$ are also reported.
c) Taken from Ref. (1a),where data on the dihydrosaikosaponins are also given.
d) Taken from Ref. (1b).
e) Assignments in Refs. (1a) and (1b) should be interchanged in each vertical column.
f—i) Assignments may be interchanged in each vertical column.

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-b_1 (**12**) and -b_3 (**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 ^{13}C 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**-undeca-acetate, both of which were hydrolyzed to regenerate pure saikosaponins-c, mp 205—209°, $[\alpha]_D$ +5.7°, and -f, mp 203—206°, $[\alpha]_D$ −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 ^{13}C NMR spectroscopy without any chemical degradation. The present results show ^{13}C 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 F_{254} (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. ^{13}C 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 μsec (pulse flipping angle, 15—30°); number of data points, 4820; numbers of transients, 10000—100000. The ^{13}C 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 ^{13}C 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% pyridine[9] (4×3 l) at room temperature for 3 days. Material from the MeOH extract (168 g) was partitioned three times with a butan-1-ol–H_2O 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 (3×100 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 $CHCl_3$ (1 l), $CHCl_3$–EtOH (9: 1) (1 l), $CHCl_3$–EtOH (8: 2) (1 l), $CHCl_3$–EtOH (7: 3) (1 l, Fr. 1), $CHCl_3$–MeOH–H_2O (7: 3: 0.3) (1 l, Fr. 2), $CHCl_3$–MeOH–H_2O (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 $CHCl_3$–EtOH (9: 1) (1 l), $CHCl_3$–EtOH (85: 15) (200 ml, Fr. 5+250 ml, Fr. 6+500 ml, Fr. 7), $CHCl_3$–EtOH (8: 2) (400 ml, Fr. 8), $CHCl_3$–EtOH (7: 3) and MeOH (200 ml). Fr. 5 (816 mg) was repeatedly chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (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° (c=0.95, pyridine). *Anal.* Calcd for $C_{35}H_{58}O_6$: C, 73.13; H, 10.17. Found: C, 72.92; H, 10.19. IR ν_{max}^{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° (c=1.03). *Anal.* Calcd for $C_{44}H_{70}O_{14}\cdot 3H_2O$: C, 60.32; H, 8.74. Found: C, 60.56; H, 8.84. IR ν_{max}^{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° (c=0.54). *Anal.* Calcd for $C_{56}H_{82}O_{20}$: 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° (c=1.01). *Anal.* Calcd for $C_{44}H_{70}O_{14}\cdot H_2O$: C, 62.91; H, 8.61. Found: C, 62.76; H, 8.69. IR ν_{max}^{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° (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° (c=1.04). *Anal.* Calcd for $C_{44}H_{70}O_{14}\cdot H_2O$: C, 62.91; H, 8.61. Found: C, 62.92; H, 8.38. IR ν_{max}^{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° (c=0.54). *Anal.* Calcd for $C_{58}H_{80}O_{21}$: 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-b_4 (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–Et_2O to give **18** as a white powder, mp 209—217°, $[\alpha]_D^{24.5}$ −6.4° (c=1.01). *Anal.* Calcd for $C_{45}H_{74}O_{15}\cdot 2H_2O$: C, 60.65; H, 8.82. Found: C, 60.34; H, 8.46.

Compound **18** was acetylated as described for **17**, yielding saikosaponin-b_4 nonaacetate,[11] mp 153—157°, $[\alpha]_D^{23}$ −12.7° (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 $C_{61}H_{90}O_{23}$: C, 61.50; H, 7.62. Found: C, 61.92; H, 7.75. IR ν_{max}^{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° (c=1.05). *Anal.* Calcd for $C_{42}H_{68}O_{12}\cdot 2H_2O$: C, 62.97; H, 9.06; Found: C, 62.75; H, 9.22. IR ν_{max}^{KBr} cm^{-1}: 3400 (br), 1080 (br).

Acetylation of **19** gave its heptaacetate, mp 176—178°, $[\alpha]_D^{24}$ +64.1° (c=1.01). *Rf* 0.55, $CHCl_3$–acetone (9: 1); *Rf* 0.62, benzene–EtOAc (3: 2). *Anal.* Calcd for $C_{56}H_{82}O_{19}$: 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° (c=1.01). *Anal.* Calcd for $C_{44}H_{70}O_{14}\cdot H_2O$: C, 62.91; H, 8.61. Found: C, 62.94; H, 8.56. IR ν_{max}^{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° (c=1.05), which was identified as **8**-octaacetate[11] 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-b_1 and -b_3, 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° (c=1.06), and **11** (862 mg), mp 256—259°, $[\alpha]_D^{25}$

+51.8° (c=1.01, $CHCl_3$–MeOH (1: 1)).

Isolation of Saikosaponins-b_2 (9) and -b_4 (14)——Fr. 3 (1.4 g) was repeatedly chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (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°, $[\alpha]_D^{25.5}$ −27.2° (c=1.04), and **9**, mp 231—238°, $[\alpha]_D^{25.5}$ −11.5° (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 $CHCl_3$–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), $[\alpha]_D^{24}$ −16.9° (c=1.0). *Anal.* Calcd for $C_{48}H_{80}O_{17}\cdot 2H_2O$: C, 59.73; H, 8.77. Found: C, 59.43; H, 8.67. **21**-Undecaacetate, mp 144—147° (from ether–pentane), $[\alpha]_D^{25}$ −13.8° (c=1.01). *Anal.* Calcd for $C_{70}H_{102}O_{28}$: 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), $[\alpha]_D^{24}$ +5.7° (c=1.01). *Anal.* Calcd for $C_{48}H_{78}O_{17}\cdot 3H_2O$: C, 58.76; H, 8.63. Found: C, 59.04; H, 8.56. **10**-Decaacetate, mp 154—156° (from Et_2O–pentane), $[\alpha]_D^{25}$ 0°; $[\alpha]_{365}^{25}$ +7.1° (c=1.01) *Anal.* Calcd for $C_{68}H_{98}O_{27}$: C, 60.61; H, 7.33. Found: C, 60.73; H, 7.30.