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CARBON-13 NMR SPECTRA OF SAIKOSAPONINS A, C, D and F ISOLATED FROM BUPLEURUM FALCATUM L.

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Carbon-13 NMR spectroscopy promises to be a powerful tool in elucidating structures of natural glycosides without chemical degradation. First, however, we feel that more ^{13}C NMR data should be accumulated on both aglycones and saccharides. Several papers have been published dealing with this problem.[1-4] In the course of studies of saikosaponins isolated from Bupleurum falcatum L. in this laboratory,[5,6] the search for minor components and their structure determination has become important, since the useful biological activities of their analogues were revealed.[7] The ^{13}C NMR method is probably the most suitable, because the aglycones of these glycosides are very unstable against acid hydrolysis. We have already determined ^{13}C NMR spectra of several saikogenins and their derivatives with full signal assignments.[8] In this paper, we report chemical-shift differences between the ^{13}C signals of known saikosaponins-d (2), -a (4), and -c (6),[5,9] and dihydro derivatives (8, 10),[10] and those of their aglycones (1, 3, 5, 7, and 9, respectively)[8] and saccharides, and further apply our results to determine the structure of saikosaponin-f (12), a new genuine glycoside isolated from the methanolic extract of B. falcatum.

^{13}C NMR spectra of methyl β-D-glucopyranoside (13),[4,11-13] methyl β-D-fucopyranoside (14),[13] and methyl α-L-rhamnopyranoside (15)[13] in pyridine-d_5 were examined as model saccharides; the ^{13}C signals in 13-15 were assigned by comparison with literature data in D_2O.[11-13] ^{13}C spectra of 2, 4, 8, and 10 in pyridine-d_5 were determined at 100° to avoid significant signal broadening.[14] The signal assignments were fairly straightforward by comparison with those in their aglycone moieties, 1, 3, 7, and 9, and sugar moieties, 13-15 (see the TABLE). Thus, ^{13}C chemical-shift changes from aglycone[8] and methyl glycoside to saponin, i.e., glycosidation shifts, are also shown in the TABLE. Characteristic signal shifts[2-4] are caused at the α-, β-, and γ-positions of the OH group in which the glycosidation took place; in particular, α-CH signals are shifted downfield by about +8.3 ppm, while β-CH_2 and β-C< signals move by about -1.8 and +0.7 ppm, respectively.

During this study, we found that the ^{13}C signals of a saikosaponin-c fraction were composed of those of two saponins having the same sugar moiety; one component was saikosaponin-c (6)[5] and the other a new saponin named saikosaponin-f (12). The ^{13}C spectrum clearly showed that the latter has longispinogenin (11) as the aglycone, which has been obtained by degradation of a similar fraction.[15] Thus, the saikosaponin-c fraction was separated by column chromatography after acetylation: saikosaponin-c,

TABLE Carbon-13 Chemical Shifts δ_C (±0.1)[a] of Saikosaponins and Dihydrosaikosaponins, and Glycosidation Shifts Δδ (in parentheses in ppm)[b]

Carbon No.	2	Δδ2-1	4	Δδ4-3	8	Δδ8-7	10	Δδ10-9	6	Δδ6-5	12	Δδ12-11
1	38.8[c]	(-0.1)	38.9	(+0.1)	39.2	(0.0)	39.1	(-0.1)	38.8	(-0.3)	39.1	(-0.3)
2	25.8	(-1.8)	25.7	(-1.8)	25.9	(-1.7)	25.9	(-1.7)	26.4	(-1.5)	26.4	(-1.7)
3	82.5	(+8.3)	82.5	(+8.3)	82.8	(+8.4)	82.7	(+8.3)	89.2	(+11.2)	89.3	(+11.0)
4	43.8	(+0.8)	43.5	(+0.5)	43.5	(+0.7)	43.5	(+0.7)	39.7	(+0.2)	39.5	(+0.1)
5	48.1	(-1.1)	48.0	(-1.3)	48.3	(-1.4)	48.2	(-1.2)	55.7	(+0.1)	56.1	(+0.1)
6	17.8	(-0.4)	17.8	(-0.4)	18.5	(-0.4)	18.4	(-0.4)	18.1	(-0.1)	18.7	(-0.2)
7	31.8	(-0.1)	31.9	(-0.1)	33.2	(-0.1)	33.0	(0.0)	32.2	(+0.1)	33.4	(+0.1)
8	42.1	(0.0)	42.4	(-0.1)	40.5[c]	(0.0)	40.5[c]	(0.0)	42.4	(+0.2)	40.4[c]	(0.0)
9	53.2	(0.0)	53.2	(-0.1)	47.5	(0.0)	47.5	(+0.1)	53.1	(+0.1)	47.4	(0.0)
10	36.6	(-0.2)	36.6	(-0.3)	37.1	(-0.3)	37.0	(-0.3)	36.6	(-0.2)	37.1	(-0.3)
11	131.9	(0.0)	132.0	(0.0)	24.0	(0.0)	24.0	(0.0)	132.0	(-0.1)	24.0	(0.0)
12	131.9	(0.0)	131.0	(-0.2)	122.4	(0.0)	122.7	(0.0)	131.1	(-0.1)	122.8	(+0.1)
13	85.1	(+0.1)	84.0	(-0.1)	145.2	(0.0)	144.1	(0.0)	84.0	(0.0)	144.0	(-0.1)
14	43.7	(-0.2)	46.0	(0.0)	42.3	(0.0)	44.2	(0.0)	45.9	(+0.1)	44.1	(0.0)
15	35.7	(0.0)	36.2	(-0.1)	35.1	(0.0)	37.0	(0.0)	36.2	(+0.1)	36.8	(-0.1)
16	77.5	(0.0)	64.4	(0.0)	74.4	(0.0)	67.2	(+0.1)	64.4	(0.0)	67.2	(+0.2)
17	45.5	(-0.1)	46.9	(+0.1)	41.1[c]	(-0.1)	41.1[c]	(0.0)	47.0	(0.0)	41.1[c]	(0.0)
18	51.5	(0.0)	52.4	(0.0)	42.9	(+0.1)	44.9	(-0.1)	52.3	(+0.1)	44.9	(0.0)
19	38.7[c]	(0.0)	38.2	(0.0)	48.3	(+0.2)	47.5	(+0.1)	38.1	(0.0)	47.4	(0.0)
20	31.8	(-0.1)	31.6	(0.0)	31.1	(+0.1)	31.0	(0.0)	31.6	(0.0)	31.0	(-0.1)
21	37.0	(0.0)	35.0	(0.0)	37.0	(+0.4)	34.4	(0.0)	34.9	(0.0)	34.4	(0.0)
22	31.2	(-0.1)	25.6	(-0.1)	29.5	(+0.1)	26.4	(-0.1)	25.7	(0.0)	26.4	(0.0)
23	65.2	(-3.8)	65.1	(-3.9)	65.5	(-4.2)	65.6	(-3.7)	28.1	(-0.4)	28.4	(-0.4)
24	12.8	(+0.6)	12.7	(+0.6)	13.4	(+0.8)	13.4	(+0.7)	16.3	(+0.5)	17.0	(+0.6)
25	18.7	(+0.1)	18.6	(+0.1)	16.4	(+0.1)	16.3	(+0.1)	18.1	(-0.1)	15.7	(-0.1)
26	19.4	(-0.1)	19.8	(-0.1)	17.4	(0.0)	17.2	(0.0)	19.8	(-0.2)	17.1	(0.0)
27	18.1	(+0.1)	20.8	(-0.1)	27.5	(+0.1)	27.1	(0.0)	20.8	(-0.1)	27.1	(0.0)
28	77.8	(-0.1)	72.9	(-0.1)	70.2	(0.0)	69.3	(-0.2)	72.9	(-0.1)	69.3	(-0.1)
29	33.7	(0.0)	33.7	(0.0)	33.2	(-0.1)	33.2	(-0.1)	33.7	(0.0)	33.3	(0.0)
30	24.5	(-0.1)	23.9	(0.0)	25.3	(0.0)	24.3	(0.0)	23.9	(+0.1)	24.3	(+0.1)

		Δδ2-14	Δδ4-14	Δδ8-14	Δδ10-14		Δδ6-13	Δδ12-13
1'	Fuc	105.4 (-0.2)	105.3 (-0.3)	105.5 (-0.2)	105.4 (-0.2)	Glc	106.1 (+0.7)	106.1 (+0.7)
2'		71.7 (-0.4)	71.7 (-0.4)	71.7 (-0.4)	71.7 (-0.4)		75.2 (+0.2)	75.2 (+0.2)
3'		85.1 (+9.9)	85.0 (+9.8)	85.2 (+10.0)	85.1 (+10.1)		76.8 ((-1.6)	76.9 (-1.5)
4'		71.9 (-0.7)	71.8 (-0.8)	71.9 (-0.7)	71.9 (-0.7)		80.2 (+8.2)	80.2 (+8.2)
5'		70.9 (-0.5)	70.8 (-0.6)	70.9 (-0.5)	70.9 (-0.5)		75.5 (-2.5)	75.5 (-2.5)
6'		16.9 (+0.1)	16.9 (+0.1)	17.0 (+0.2)	16.9 (+0.1)		69.2 (+6.2)	69.3 (+6.3)

		Δδ2-13	Δδ4-13	Δδ8-13	Δδ10-13		Δδ6-15	Δδ12-15
1"	Glc	105.8 (+0.4)	105.7 (+0.3)	105.9 (+0.5)	105.8 (+0.4)	Rham	102.6 (+0.1)	102.6 (+0.1)
2"		75.5 (+0.5)	75.4 (+0.4)	75.6 (+0.6)	75.5 (+0.5)		72.6 (+0.6)	72.6 (+0.6)
3"		78.1 (-0.3)	78.0 (-0.4)	78.2 (-0.2)	78.1 (-0.3)		72.2 (-0.5)	72.3 (-0.4)
4"		71.9 (-0.1)	71.8 (-0.2)	71.9 (-0.1)	71.9 (-0.1)		73.8 (+0.1)	73.8 (+0.1)
5"		78.1 (+0.1)	78.0 (0.0)	78.2 (+0.2)	78.1 (+0.1)		70.5 (+1.1)	70.5 (+1.1)
6"		63.0 (0.0)	62.9 (-0.1)	62.9 (-0.1)	62.9 (+0.1)		18.1 (-0.2)	18.2 (-0.1)

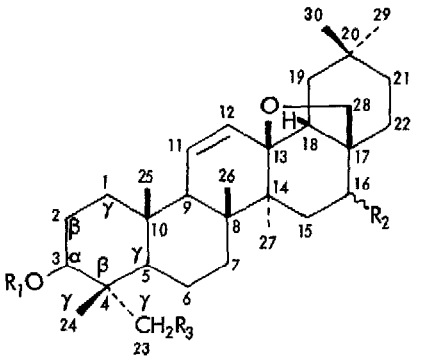
TABLE (Continued)

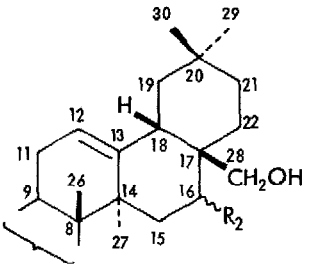
Carbon No.		δ	$\Delta\delta_{6-13}$	12	$\Delta\delta_{12-13}$
1‴	Glc	104.8	(-0.6)	104.8	(-0.6)
2‴		74.7	(-0.3)	74.7	(-0.3)
3‴		78.2	(-0.2)	78.3	(-0.1)
4‴		71.8	(-0.2)	71.9	(-0.1)
5‴		77.9	(-0.1)	78.0	(0.0)
6‴		62.9	(-0.1)	62.9	(-0.1)

[a] ^{13}C NMR spectra were recorded on a Varian NV-14 FT NMR spectrometer at 15.087 MHz in pyridine-d_5 with TMS as an internal standard (δ_C 0) in 8-mm spinning tubes at 100°; concentrations were about 0.1-0.3 mmol/cm^3. FT measurement conditions were: spectral width, 3923 Hz; pulse flipping angle, ca. 36°; acquisition time, 0.6 sec; number of data points, 4820.

[b] See text; the plus sign denotes a downfield shift.

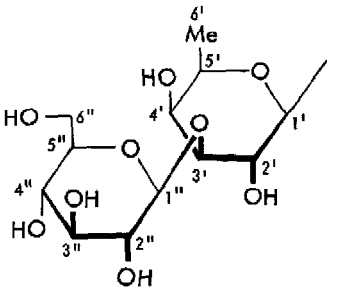
[c] Assignments may be reversed in each vertical column.



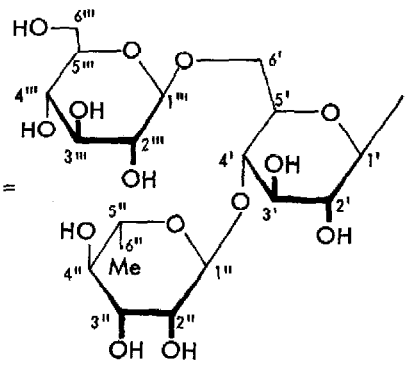


	R_1	R_2	R_3	
Saikogenin G (1):	H	α-OH	OH :	23-Hydroxyprimulagenin A (7)
Saikosaponin-d (2):	Glc-Fuc	α-OH	OH :	Dihydrosaikosaponin-d (8)
Saikogenin F (3):	H	β-OH	OH :	23-Hydroxylongispinogenin (9)
Saikosaponin-a (4):	Glc-Fuc	β-OH	OH :	Dihydrosaikosaponin-a (10)
Saikogenin E (5):	H	β-OH	H :	Longispinogenin (11)
Saikosaponin-c (6):	Rham-(Glc)-Glc	β-OH	H :	Saikosaponin-f (12)

Glc-Fuc =



Rham-(Glc)-Glc =



$C_{48}H_{78}O_{17}\cdot 3H_2O$, mp 205-209°, $[\alpha]_D^{24}$ +5.7°; peracetate, mp 154-156°, $[\alpha]_D^{25}$ 0°: saikosaponin-f, i.e., 16β,28-dihydroxyolean-12-en-3β-yl α-L-rhamnopyranosyl-(1→4)-,β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside, $C_{48}H_{80}O_{17}\cdot 2H_2O$, mp 203-206°, $[\alpha]_D^{24}$ -16.9°; peracetate, mp 144-147°, $[\alpha]_D^{25}$ -13.8°. The ^{13}C NMR results of 6 and 12 isolated here are also included in the TABLE; the signals of the sugar moiety were assigned by comparison with those in gentiobiose[12] and 15. Similar glycosidation shifts are indicated. However, it should be noted that the glycosidation shifts were considerably affected by the neighboring substituent(s); e.g., the 23-CH_2OH (γ-position) signals are considerably shifted (ca. -4 ppm) in 2, 4, 8, and 10, but the 23-Me signals are a little in 6 and 12. Furthermore, it should be emphasized that the C-5 (γ-position) signal in 15 was significantly shifted downfield by the glycosidation.

The present method and data should be useful in determining structures of natural glycosides, and further applications will be reported soon.

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