Tetrahedron Letters No. 14, pp 1227 - 1230, 1977. Pergamon Press. Printed in Great Britain.

THE STRUCTURES OF SAIKOSAPONIN-E AND ACETYLSAIKOSAPONINS, MINOR COMPONENTS ISOLATED FROM BUPLEURUM FALCATUM L., DETERMINED BY C-13 NMR SPECTROSCOPY

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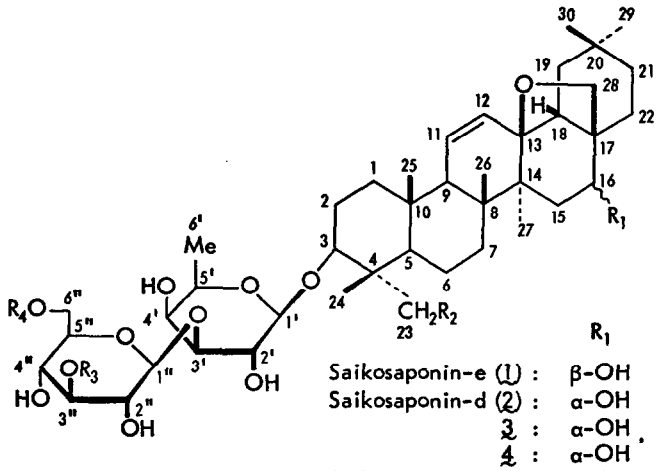
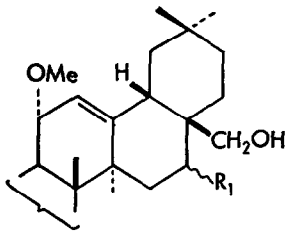
(Received in Japan 5 February 1977; received in UK for publication 23 February 1977)

We report here a successful example of organic structure determination which requires several steps of chemical procedure and has readily been performed using ^{13}C NMR spectroscopy without chemical degradations.

Triterpenoid glycosides, saikosaponin-a (5), -b_1~-b_4, -c, -d (2), and -f were already isolated from the methanolic extract of Bupleurum falcatum L. and their structures had been determined in this laboratory.[1-3] In connection with useful biological activities of this kind of saponins, we searched for minor components of B. falcatum and isolated seven saponins (1, 3, 4, 6, 7, 9, and 10) from the fractions with Rf values higher than those of the known saponins (see TABLE 1). Their 100-MHz ^1H NMR spectra in pyridine-d_5 revealed that five of them, 3, 4, 6, 7, and 9, have one OAc and six angular Me groups, and that 1 has seven angular Me groups and 10 only two angular Me groups (δ_H 0.60 and 0.74).

Recently, Tanaka and coworkers[5] and we[3] demonstrated that ^{13}C NMR spectroscopy is a potential tool for elucidating structures of natural plant glycosides which are unstable against acid hydrolysis, provided





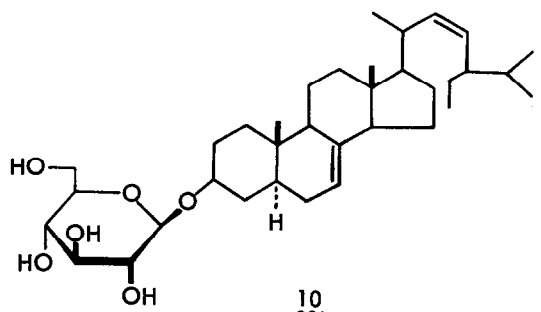
	R_1	R_2	R_3	R_4	
Saikosaponin-e (1) :	β-OH	H	H	H	
Saikosaponin-d (2) :	α-OH	OH	H	H	: Saikosaponin-b_4 (8)
3 :	α-OH	OH	Ac	H	
4 :	α-OH	OH	H	Ac	: 9
Saikosaponin-a (5) :	β-OH	OH	H	H	
6 :	β-OH	OH	H	Ac	
7 :	β-OH	OAc	H	H	

TABLE 1. Physical Properties of Saikosaponin-e (1) and Mono-O-acetylsaikosaponins (3, 4, 6, 7, 9)

	1	3	4	6	7	9
mp	227-230°	219-225°	196-205°	217-224°	223-231°	209-217°
Rf[a]	0.25	0.58	0.40	0.33	0.22	0.31
Anal.	$C_{42}H_{68}O_{12}\cdot 2H_2O$	$C_{44}H_{70}O_{14}\cdot 3H_2O$	$C_{44}H_{70}O_{14}\cdot H_2O$	$C_{44}H_{70}O_{14}\cdot H_2O$	$C_{44}H_{70}O_{14}\cdot H_2O$	$C_{45}H_{74}O_{15}\cdot 2H_2O$
$[\alpha]_D$ (MeOH)	+40.8°	+47.5°	+43.5°	+44.3°	+47.6°	-6.4°
ν_{max}^{KBr} cm^{-1} (OAc)	---	1735	1735	1735	1715	1735
$\delta_H^{C_5D_5N}$[b] OAc	---	1.95	1.96	1.96	2.12	1.95
OMe	---	---	---	---	---	3.28
H-6'	1.44	1.41	1.50	1.50	1.46	1.46

[a] Solvent, EtOAc—EtOH—H_2O (9 : 1 : 0.5), double development. [b] ^1H NMR spectra were taken with a Varian HA-100 spectrometer at 100 MHz; δ_H ±0.01.

that ^{13}C data on their aglycones and sugar moieties are available, and presented some examples of glycosidation shifts, i.e., chemical-shift changes from aglycone and sugar to saponin. The natural-abundance ^1H-noise-decoupled ^{13}C FT NMR spectrum of 1, a crystalline triterpenic glycoside named saikosaponin-e, readily revealed that it is a glucosyl fucoside of saikogenin E, i.e., 13β,28-epoxy-16β-hydroxyolean-11-en-3β-yl O-β-D-glucopyranosyl-(1→3)-β-D-fucopyranoside, upon comparison with the spectra of saikogenin E[3] and saikosaponin-a (5).[3] The ^1H NMR suggested that 10, $C_{35}H_{58}O_6$, mp 272-283°, $[\alpha]_D^{25}$ -30.0° (pyridine), is a glycoside of one of the sterol components already isolated from the plant.[6] Thus, the ^{13}C NMR of 10 was compared with those of authentic α-spinasterol and stigmasterol and revealed that 10 is α-spinasteryl β-D-glucopyranoside.[7]



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On acetylation, the monoacetyl derivatives 3 and 4 afforded saikosaponin-d heptaacetate, 6 and 7 gave saikosaponin-a octaacetate, and 9 gave saikosaponin-b_4 nonaacetate. Since the ^1H NMR of 2-9 showed that the Me singlets of the new saponins, 3, 4, 6, and 9, appear at almost the same positions as those of the corresponding mother compounds, 2, 5, and 8, we inferred that their mono-O-acetyl groups are situated at their sugar moieties. No other useful information was provided by their physical data. Moreover, it seemed laborious to determine the positions of their OAc groups using chemical techniques. However, the acetylation shift rule[8] in ^{13}C NMR spectroscopy can easily solve the problem, if the ^{13}C signals of the mother compounds are fully assigned. We had assigned the signals of 2 and 5 in a

TABLE 2. Carbon-13 Chemical Shifts δ_C (±0.1)[a] of Saikosaponin-e (1) and Mono-O-acetylsaikosaponins (3, 4, 6, 7, 9), and Acetylation Shifts Δδ (in parentheses, ppm)[b]

Carbon No.	1	Δδ[c]	3	Δδ 3-2	4	Δδ 4-2	6	Δδ 6-5	7	Δδ 7-5	9	Δδ 9-8
1	39.0	(+0.2)	38.7[d]	(-0.1)	38.8[d]	(0.0)	39.0	(+0.1)	38.7	(-0.2)	40.2	(-0.1)
2	26.5	(-1.4)	25.9	(+0.1)	25.9	(+0.1)	25.8	(+0.2)	26.0[d]	(+0.4)	26.3[d]	(0.0)
3	88.9	(+10.9)	82.5	(0.0)	82.5	(0.0)	82.4	(-0.1)	82.1	(-0.4)	82.8	(+0.1)
4	39.8	(+0.3)	43.9	(+0.1)	43.9	(+0.1)	43.7	(+0.2)	42.8	(-0.7)	43.8	(0.0)
5	55.9	(+0.6)	48.1	(0.0)	48.1	(0.0)	48.1	(+0.1)	48.9	(+0.9)	48.5	(0.0)
6	18.1	(-0.1)	17.9	(+0.1)	17.9	(+0.1)	17.9	(+0.1)	18.1	(+0.3)	18.6	(0.0)
7	32.2	(+0.3)	31.9	(+0.1)	31.9	(+0.1)	32.0	(+0.1)	32.1	(+0.2)	33.8	(-0.1)
8	42.5	(+0.3)	42.2	(+0.1)	42.2	(+0.1)	42.5	(+0.1)	42.5	(+0.1)	41.0	(0.0)
9	53.2	(+0.2)	53.3	(+0.1)	53.3	(+0.1)	53.3	(+0.1)	53.4	(+0.2)	53.5[e]	(-0.1)
10	36.7	(0.0)	36.7	(+0.1)	36.7	(+0.1)	36.7	(+0.1)	36.7	(+0.1)	38.6	(0.0)
11	132.0	(-0.1)	132.0	(+0.1)	132.0	(+0.1)	132.0	(0.0)	131.8	(-0.2)	76.3	(0.0)
12	131.2	(0.0)	132.0	(+0.1)	132.0	(+0.1)	131.1	(+0.1)	131.4	(+0.4)	122.7	(0.0)
13	84.1	(+0.1)	85.1	(0.0)	85.1	(0.0)	84.1	(+0.1)	84.1	(+0.1)	149.8	(0.0)
14	46.0[d]	(+0.4)	43.8	(+0.1)	43.8	(+0.1)	46.0[d]	(0.0)	46.0[e]	(0.0)	42.3	(0.0)
15	36.2[e]	(-0.1)	35.7	(0.0)	35.7	(0.0)	36.4	(+0.2)	36.2	(0.0)	37.1	(-0.1)
16	64.4	(+0.4)	77.6	(+0.1)	77.6	(+0.1)	64.5	(+0.1)	64.4	(0.0)	74.3	(0.0)
17	47.0[d]	(0.0)	45.6	(+0.1)	45.6	(+0.1)	47.1[d]	(+0.2)	47.1[e]	(+0.2)	42.3	(0.0)
18	52.4	(+0.2)	51.6	(+0.1)	51.6	(+0.1)	52.4	(0.0)	52.4	(0.0)	43.8	(0.0)
19	38.2	(+0.4)	38.9[d]	(+0.1)	38.9[d]	(+0.1)	38.2	(0.0)	38.2	(0.0)	48.5	(0.0)
20	31.6	(0.0)	31.9	(+0.1)	31.9	(+0.1)	31.7	(+0.1)	31.7	(+0.1)	31.2	(0.0)
21	35.0	(+0.3)	37.1	(+0.1)	37.1	(+0.1)	35.0	(0.0)	35.0	(0.0)	35.2	(0.0)
22	25.7[e]	(0.0)	31.3	(+0.1)	31.3	(+0.1)	25.8	(+0.2)	25.8[d]	(+0.2)	30.0	(0.0)
23	28.1	(-0.3)	65.1	(-0.1)	65.2	(0.0)	65.0	(-0.1)	66.4	(+1.3)	65.6	(+0.1)
24	16.3	(+0.4)	12.9	(+0.1)	12.8	(0.0)	12.8	(+0.1)	12.6	(-0.1)	13.4[f]	(-0.1)
25	18.1	(-0.1)	18.8	(+0.1)	18.8	(+0.1)	18.7	(+0.1)	18.4	(-0.2)	17.9[f]	(0.0)
26	19.9	(-0.1)	19.5	(+0.1)	19.5	(+0.1)	19.9	(+0.1)	20.0	(+0.2)	18.8[d]	(0.0)
27	20.9	(0.0)	18.2	(+0.1)	18.2	(+0.1)	20.9	(+0.1)	20.8	(0.0)	26.5[d]	(0.0)
28	73.0	(0.0)	77.9	(+0.1)	77.8	(0.0)	72.9	(0.0)	73.1	(+0.2)	70.2	(0.0)
29	33.7	(0.0)	33.7	(0.0)	33.7	(0.0)	33.7	(0.0)	33.7	(0.0)	33.2	(0.0)
30	23.9	(+0.1)	24.6	(+0.1)	24.6	(+0.1)	23.9	(0.0)	23.9	(0.0)	25.0	(0.0)
1'	106.3	(—)	105.6	(+0.2)	105.6	(+0.2)	105.6	(+0.4)	106.0	(+0.8)	105.6	(+0.1)
2'	71.6	(—)	71.8	(+0.1)	71.6	(-0.1)	71.5	(-0.2)	71.6	(-0.1)	71.6	(-0.2)
3'	85.1	(—)	85.2	(+0.1)	85.4	(+0.3)	85.4	(+0.4)	85.3	(+0.3)	85.4	(+0.1)
4'	71.9	(—)	72.1	(+0.2)	71.7	(-0.2)	71.7	(0.0)	72.1	(+0.4)	71.7	(-0.3)
5'	70.8	(—)	71.0	(+0.1)	71.0	(+0.1)	71.0	(+0.2)	71.0	(+0.2)	71.0	(0.0)
6'	17.0	(—)	17.0	(+0.1)	17.1	(+0.2)	17.1	(+0.2)	17.0	(+0.1)	17.0	(0.0)
1"	105.9	(—)	105.9	(+0.1)	105.6	(-0.2)	105.6	(+0.1)	106.0	(+0.5)	105.6	(-0.3)
2"	75.5	(—)	73.7	(-1.8)	75.4	(-0.1)	75.4	(+0.1)	75.7	(+0.4)	75.3	(-0.3)
3"	78.1	(—)	79.5	(+1.4)	78.1	(0.0)	78.1	(+0.2)	78.3	(+0.4)	78.1	(-0.2)
4"	71.9	(—)	70.0	(-1.9)	72.0	(+0.1)	72.0	(+0.3)	72.1	(+0.4)	72.0	(0.0)
5"	78.1	(—)	78.3	(+0.2)	75.4	(-2.7)	75.4	(-2.5)	78.3	(+0.4)	75.3	(-3.0)
6"	62.9	(—)	62.6	(-0.4)	64.7	(+1.7)	64.6	(+1.8)	63.1	(+0.3)	64.6	(+1.6)
MeCO	—	—	21.0	(—)	20.6	(—)	20.6	(—)	20.8	(—)	20.6	(—)
MeCO	—	—	170.8	(—)	170.6	(—)	170.4	(—)	170.5	(—)	170.7	(—)
MeO	—	—	—	—	—	—	—	—	—	—	51.8[e]	(0.0)

TABLE 2 (continued)

[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 reference (δ_C 0) in 8-mm spinning tubes at 100°; concentrations were about 0.15 mmol/cm^3. FT measurement conditions were as follows: spectral width, 3923 Hz; pulse flipping angle, ca. 36°; acquisition time, 0.6 sec; number of data points, 4820. [b] See text; plus sign denotes a downfield shift. [c] Glycosidation shifts from saikogenin E (see ref 3). [d-f] Assignments may be interchanged in each vertical column.

structural study of other saikosaponins.[3] The signal assignment for 8 was straightforward.

Thus, ^{13}C spectra of the mono-O-acetylsaikosaponins were examined in pyridine-d_5 at 100° and compared with those of their mother compounds. TABLE 2 shows that the signals of α-carbons carrying an acetylated OH group shifted downfield by +1.3~+1.8 ppm and the signals of β-carbons moved upfield by -0.7~-3.0 ppm. Therefore, the positions of the OAc groups were readily pointed out, i.e., compounds 3, 4, 6, 7, and 9 are 3"-O-acetyl- and 6"-O-acetylsaikosaponins-d, 6"-O-acetyl- and 23-O-acetylsaikosaponins-a, and 6"-O-acetylsaikosaponin-b_4 (this appears to be an artifact),[2] respectively.[9]

Acknowledgements. We thank Drs. K. Takeda, H. Minato and Y. Tomita, and Mr. A. Shimaoka of this laboratory for their helpful advice and encouragement.

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