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APPLICATION OF C-13 NMR TO THE STRUCTURAL ELUCIDATION OF ACYLATED PLANT GLYCOSIDES

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Although partially acylated glycosides are known to be distributed widely in plant kingdom, it is not always easy to determine their acylated position, especially when an acyl group is located in sugar moieties.

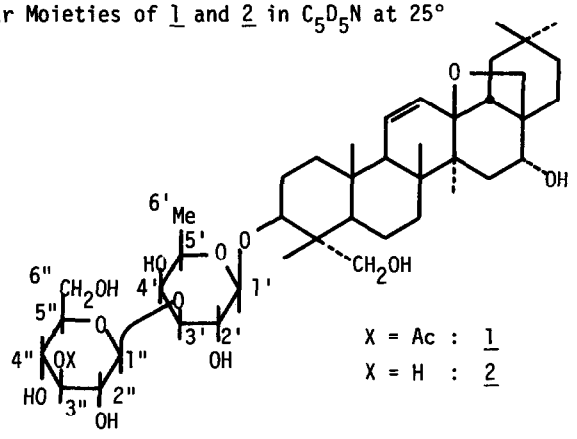
C-13 NMR spectroscopy is expected to have essential advantages for structural studies on compounds of such a type, since recently regularities of esterification shifts in carbon resonances have been investigated extensively[1-3]. On acylation, a carbinyl carbon (α-C) is somewhat deshielded, while a β-carbon resonance is displaced obviously upfield. For instance, on going from β-glucopyranose to its 3-O-acetyl derivative, the signals of α-C (3-C) and β-C (2-C and 4-C) were reported to be displaced by +1.4 and -1.8 ppm, respectively, while signals due to other carbons remained almost unaffected[4]. The present communication deals with determination of allocation of acyl groups in several types of naturally occurring acyl-glycosides by C-13 NMR spectroscopy. In addition to ordinary measurements, partially relaxed Fourier transform (PRFT) procedures were also used to differentiate carbon signals of monosaccharide units from each other in the spectra of oligoglycosides[5].

A number of saponins named saikosaponins have been isolated from roots of *Bupleurum falcatum* (Japanese name; Mishimasaiko)[6,7]. Further investigation of the roots of this plant led to isolation of a new minor saponin (1), white powder $[\alpha]_D^{27}$ +41.8° (MeOH). Its IR bands (Nujol), 1730 and 1240 cm^{-1} and ^1H NMR signal δ 1.95 (3H s) in C_5D_5N indicated the presence of an acetoxyl group. Alkaline saponification of 1 gave a desacetyl-saponin which was proved to be identical with saikosaponin-d (2)[6]. The presence of fragment peaks at m/e 711 $[(TMSi)_5(CH_3CO)(glucosyl\text{-}fucosyl)^+]$ and 421 $[(TMSi)_3(CH_3CO)(glucosyl)^+]$ and the absence of a peak at m/e 451 $[(TMSi)_4(glucosyl)^+]$ in the mass spectrum of trimethylsilylated-1 (TMSi-1) showed that

the acetoxyl group of 1 must be located on the terminal glucopyranosyl moiety of 2. The position of this acetoxyl group was elucidated by comparison of C-13 NMR spectrum of 1 with that of 2. Referring to the recent publication of C-13 NMR assignments of saikosaponins[8], application of PRFT technique to the spectrum of 1 led to distinguish carbon signals of its terminal glucopyranosyl moiety (C-1" - C-6") from those of its fucosyl and aglycone moieties (Table I). On going from 2 to 1, C-3" signal is displaced downfield by 0.8 ppm and C-2" and C-4" are shielded by 2.2 and 2.4 ppm, respectively, while other signals remain almost unaffected. On consideration of the acetylation shift mentioned above[1-4], it follows that 1 can be formulated as C-3"-O-acetyl-saikosaponin-d. The same saponin was recently isolated from the same plant also by Ishii et al[9].

Table I ^{13}C Chemical Shifts (δ_C) of Sugar Moieties of 1 and 2 in C_5D_5N at 25°

		1	2	
fucosyl	C-1'	105.9	106.5	
	2'	70.8	70.9	
	3'	84.8	84.9	
	4'	72.0	72.0	
	5'	71.5	71.7	
	6'	17.2	17.2	Δδ
glucosyl	1"	105.9	105.8	+0.1
	2"	73.4	75.6	-2.2
	3"	79.1	78.3	+0.8
	4"	69.1	71.5	-2.4
	5"	78.3	78.6	-0.3
	6"	62.0	62.7	-0.7
	Ac	21.1		
		170.7		



Several flavonoid-glycosides were isolated from fruits of *Rosa multiflora*. Of these, multiflorin A (3) was proved to be a monoacetate of multiflorin B (4, glucorhamnoside of kaempferol)[10], but the allocation of its acetyl group has been left unidentified. Referring to the published data of C-13 NMR assignment of flavonoids[11], carbon signals of 3 and 4 were assigned with the aid of PRFT experiments (Table III). The comparison of the spectrum of 3 with that of 4, under the consideration of the acetylation shifts led to establish the position of the O-acetyl group of 3 to be at C-6 (G-6 in Table III) of the terminal glucopyranosyl moiety.

As mentioned below, two oxalyl glucosides have been isolated from a rhubarb[12]. For the purpose of elucidation of disposition of their oxalyl groups, several alkyl oxalates were prepared and the oxalylation shifts of C-13 NMR spectra were examined (Table II).

Table II Oxalylation Shifts in DMSO-d_6; $R_1OOC\text{-}COOR_2$

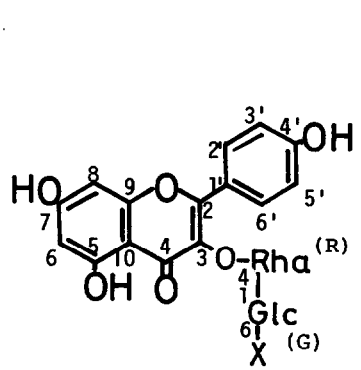
R_1	R_2	δ_{CO}	$(\Delta\delta)^a$	$\delta_{\alpha\text{-}C}$	$(\Delta\delta)^b$	$\delta_{\beta\text{-}C}$	$(\Delta\delta)^b$
H	H	162.1					
H	Et	160.5	(-1.6)	61.6	(+4.6)	13.8	(-4.8)
		160.7	(-1.4)				
H	*t*-Bu	160.7	(-1.4)	83.4	(+16.5)	27.3	(-3.9)
		161.3	(-0.8)				
Me	Me	158.4	(-3.7)	53.5	(+4.4)		
Et	Et	158.4	(-3.7)	63.1	(+6.1)	14.1	(-4.5)
iso-Pr	*iso*-Pr	157.3	(-4.8)	70.6	(+8.6)	21.0	(-4.2)
⬡	⬡	157.2	(-4.9)	75.0	(+6.8)	30.7	(-4.6)

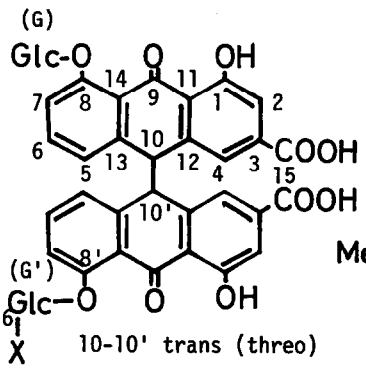
a: Chemical shift difference from oxalic acid
b: Chemical shift defference from original alcohol

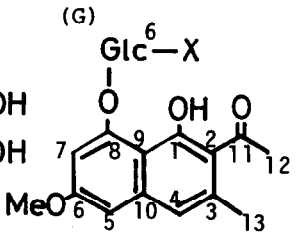
As in the case of other acylation

Table III ^{13}C Chemical Shifts (δ_C) in DMSO-d_6 at 25°[a]

		3	4			5	6			7	8	
aglycone moieties	C-2	157.1	157.3		C-1[c]	158.9	158.7		C-1	151.1	150.9	
	3	134.3	134.4		2	118.3	118.2		2	123.2	123.0	
	4	177.5	177.6		3	135.4	135.2		3	136.9	136.8	
	5	156.4	156.5		4	124.0	123.9		4	118.6[b]	118.8[b]	
	6	98.6	98.9		5	120.4	120.4		5	103.3[b]	103.0[b]	
	7	164.1	164.3		6	135.4	135.2		6	158.2[b]	158.2[b]	
	8	93.8	93.9		7	116.4	116.5		7	101.2[b]	101.1[b]	
	9	160.9	161.3		8	157.6*	157.7		8	155.1	155.3	
	10	104.1	104.2		9	186.3	186.4		9	108.7	108.5	
	1'	120.5	120.5		10	53.9	54.0		10	133.6	133.6	
	2'	130.6	130.7		11	122.4[b]	122.4[b]		11	204.3	204.3	
	3'	115.4	115.5		12	138.4[b]	138.2[b]		12	32.2	32.0	
	4'	159.8	160.0		13	142.3[b]	142.3[b]		13	19.4	19.4	
	5'	115.4	115.5		14	121.5	121.5		OMe	55.3	55.2	
	6'	130.6	130.7		15	165.5	165.5					
												Δδ
sugar moieties	R-1	101.8	101.9		G-1	103.6	103.7		G-1	102.4[b]	102.7[b]	-0.3
	2	70.1	70.4		2	73.4	73.5		2	73.2	73.3	-0.1
	3	69.6	69.9		3	75.2	75.2		3	75.8	76.1	-0.3
	4	82.1	81.9		4	69.7	69.7		4	69.9	69.8	+0.1
	5	68.8	69.0		5	77.4	77.5		5	74.4	77.7	-3.3
	6	17.0	17.5	Δδ	6	60.7	60.7	Δδ	6	63.3	60.5	+2.8
	G-1	104.6	104.7	-0.1	G'-1	103.4	103.7	-0.3				
	2	74.1	74.5	-0.4	2	73.4	73.5	-0.1				
	3	76.2	76.7	-0.5	3	75.0	75.2	-0.2				
	4	70.1	69.9	+0.2	4	69.7	69.7	0				
	5	73.7	76.9	-3.2	5	73.9	77.5	-3.6				
	6	63.6	61.1	+2.5	6	64.2	60.7	+3.5				
	Ac	20.5			Oxalyl	166.7			Oxalyl	168.9		
		170.1				167.7				170.5		







X = Ac : 3 X = Oxalyl : 5 X = Oxalyl : 7

X = H : 4 X = H : 6 X = H : 8

Rha = α-L-Rhamnopyranosyl, Glc = β-D-Glucopyranosyl

[a] Taken with a JEOL PFT-100 spectrometer at 25.15 MHz; $\delta_C \pm 0.1$, as 0.1-0.17 M solutions. [b] Values may be interchanged. [c] C-1, 2, and 3... also represented C-1', 2', and 3'..., respectively, except C-8 (* $\delta_{C\text{-}8'} = 157.3$).

shifts, the α-carbon was found to be deshielded and the β-carbon being shielded on oxalylation though the magnitudes of the shift are somewhat larger than those of the acetylation shift. The γ-carbon signal was observed at essentially the same position as that of the original alcohol. As in the case of acetylation and benzoylation etc., the α-carbon of *tert*-BuOH was demonstrated to be more deshielded than those of primary and secondary alcohols also on oxalylation[1,2,13].

Sennoside E (5), one of the bianthrone glucosides of the rhizome of the rhubarb has been characterized to be a monooxalyl ester of sennoside A (6)[14], the oxalylated position of which has been left unidentified. Comparison of the spectra of both compounds 5 and 6 indicated that on going from 6 to 5, the G'-6 signal is shifted by +3.5 ppm and G'-5 signal by -3.6 ppm, while other carbon resonances of both compounds appear at essentially the same positions except for the presence of oxalyl carbon peaks in 6 (Table III). This concluded that the position of the O-oxalyl group of 5 must be limited to G'-6.

Another oxalyl glucoside, 7, yellow powder, $[\alpha]_D^{18}$= -102°(H_2O c=0.25) was recently isolated also from the rhizome of the rhubarb along with torachrysone-8-O-β-D-glucopyranoside (8). The structure of 7 was now established to be 8-O-(6'-oxalyl)-β-D-glucopyranosyl torachrysone, similarly by comparison of the C-13 NMR spectra of 7 and 8 (Table III).

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REFERENCES and NOTES

1. D. E. Dorman, D. Bauer and J. D. Roberts, *J. Org. Chem.*, 40, 3729 (1975).
2. S. E. Pelletier, Z. Djurmati and C. Pape, *Tetrahedron*, 32, 995 (1976).
3. Y. Terui, K. Tori and N. Tsuji, *Tetrahedron Lett.*, 621 (1976).
4. M. R. Vignon and J. A. Vottero, *Tetrahedron Lett.*, 2445 (1976).
5. D. Doddrell and A. Allerhand, *J. Am. Chem. Soc.*, 93, 2777 (1971).
6. T. Kubota and H. Hinoh, *Tetrahedron Lett.*, 303 (1968).
7. A. Shimaoka, S. Seo and M. Minato, *J. C. S. Perkin I*, 2043 (1975).
8. K. Tori, Y. Yoshimura, S. Seo, K. Sakurawi, Y. Tomita and H. Ishii, *Tetrahedron Lett.*, 4163 (1976), K. Tori, S. Seo, Y. Yoshimura, M. Nakamura, Y. Tomita and H. Ishii, *Tetrahedron Lett.*, 4167 (1976).
9. H. Ishii, S. Seo, K. Tori, T. Tozyo and Y. Yoshimura, *Tetrahedron Lett.*, the preceding paper.
10. S. Takagi, M. Yamaki, K. Masuda and M. Kubota, *Yakugaku Zasshi*, 96, 1217 (1976).
11. H. Wagner, V. M. Chari and J. Sonnenbichler, *Tetrahedron Lett.*, 1799 (1976); K. R. Markham and B. Ternai, *Tetrahedron*, 32, 2607 (1976).
12. Japanese rhubarb "Shinshū-Daiō", a hybrid between *Rheum coreanum* Nakai and *R. Palmatum* L. cf. T. Matsuoka and R. Hatta, *Takeda Kenkyusho Nempo*, 29, 776 (1970).
13. J. B. Stothers, ^{13}C NMR Spectroscopy, p.169, Academic Press, New York (1972).
14. H. Oshio, S. Imai, S. Fujioka, T. Sugawara, M. Miyamoto and M. Tsukui, *Chem. Pharm. Bull. (Tokyo)*, 22, 823 (1974).