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STRUCTURES OF STEVIA DITERPENE-GLUCOSIDES : APPLICATION OF ^{13}C NMR

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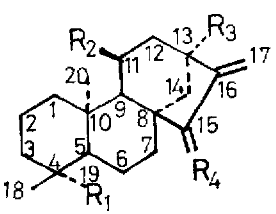
The application of ^{13}C NMR spectroscopy to the structural elucidation of plant-glycosides has not been extensive although this technique offers potential advantages over other spectroscopic and chemical methods, especially for terpenoid-glucosides with aglycones unstable to acid hydrolysis.$^{1)}$

Four aglycones 8-10 and 20 were isolated from enzymatic hydrolysate of a crude glycoside mixture of *Stevia paniculata* (Compositae).$^{2)}$ For the purpose of structural elucidation of glycosides of this plant and the related species, we have studied ^{13}C NMR spectra of kaurene-type diterpenes 1-11 (7 = methyl ester of 20). Pyridine-d_5 (C_5D_5N) was used as the solvent due generally to its high solubility for plant-glycosides. ^{13}C Chemical shifts in C_5D_5N differed no more than 1 ppm$^{3)}$ from those in $CDCl_3$. Application of chemical shift theory (especially α, β, γ, and δ effects of OH and C=O)$^{4)}$ and the results from the ^1H single-frequency off-resonance or selective decoupling technique led us to the ^{13}C signal assignments shown in Table 1. Comparison of the spectra with those of related compounds$^{5)}$ also helped our determination.

^{13}C NMR spectra of several steviol-glucosides 12-14 were then investigated (Table 2). Stevioside (14) is known as a sweet glucoside in *S. rebaudiana*.$^{6)}$ *Alkaline saponification of 14 yielded 13, which afforded 12 on enzymatic partial* hydrolysis. ^{13}C Signals in 12-14 were assigned in comparison with those in the aglycone 4 as well as those in several methyl glucosides and glucobiosides in C_5D_5N assigned according to those reported data in D_2O.$^{7)}$ As expected, signals due to aglycone carbons adjacent to the glucosyl bond (underlined in Table 2) appear at positions significantly different (>1 ppm) from those of 4, while other aglycone carbons resonate at essentially the same positions as 4. With respect to sugar signals, the anomeric carbon (Gl-1) bound directly to the *tert*-OH at C-13 resonates at an abnormally high field (δ 97.6-99.4) compared to β- glucosides of sterically less hindered *prim*- or *sec*-OH$^{7)}$ [*e. g.* δ 105.5 for methyl β-glucoside (21)]. This finding will be useful for elucidation of location and configuration of glucoside linkage. Such a high-field shift for the anomeric carbon signal in a bridge-head-*O*-β-glucoside (δ 100.5) was also demonstrated in paeoniflorin$^{8)}$, a constituent of paeony root.$^{9)}$ The remaining

Table 1 ^{13}C Chemical Shifts[a]



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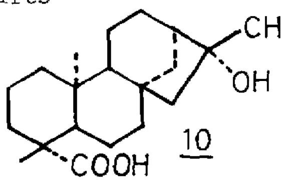

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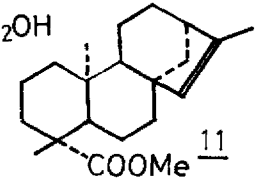

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	1	2	3	4	5	6	7	8	9	10	11
R_1	CH_3	COOH	COOMe	COOH	COOMe	COOMe	COOMe	COOH	COOH		
R_2	H	H	H	H	H	H	H	OH	OH		
R_3	H	H	H	OH	H	H	H	H	H		
R_4	H_2	H_2	H_2	H_2	=O	—H ···OH	—OH ···H	=O	—OH ···H		
C-1	40.5 (40.5)	41.1	40.8 (40.9)	41.1	40.0 (39.9)	40.9 (40.9)	40.9 (40.7)	40.2	40.9	41.1	41.0 (40.9)
2	18.9^{b}(18.7)^{b}	19.8	19.5 (19.2)	19.8	19.3 (18.8)	19.6 (19.2)	19.6 (19.2)	19.6	19.9	19.8	19.5^{b}(19.2)^{b}
3	42.2 (42.1)	38.6	38.2 (38.1)	38.6	38.3 (38.0)	38.3 (38.1)	38.3 (38.1)	38.5	38.5	38.7	38.3 (38.2)
4	33.3 (33.3)	43.8	43.9 (43.9)	43.9	43.9 (43.7)	44.0 (43.9)	44.0 (43.9)	43.9	44.0	43.9	44.0 (43.9)
5	56.2^{c}(56.3)^{c}	57.1	56.9 (57.2)	57.1	56.2 (56.1)	57.1 (57.1)	56.7 (56.4)	56.4	56.7	57.0	56.8 (56.9)
6	20.4 (20.3)	22.5	22.2 (21.9)	22.6	20.6 (20.0)	21.7 (21.1)	22.2 (21.6)	20.9	22.3	22.9	21.3 (21.0)
7	41.3 (41.3)	41.5	41.4 (41.4)	41.8	36.6^{b}(36.4)^{b}	36.6^{b}(36.3)^{b}	36.8 (36.3)	37.3	36.5	42.7	44.0 (43.9)
8	44.3 (44.3)	44.4	44.4 (44.3)	41.8	52.5 (52.3)	48.2 (47.8)	46.3 (45.7)	51.0	45.4	44.9	49.5 (49.2)
9	56.1^{c}(56.1)^{c}	55.2	55.1 (55.2)	54.3	51.8 (51.5)	54.0 (53.5)	46.0 (45.5)	63.3	55.3	56.3	48.2 (48.1)
10	39.5 (39.4)	39.9	39.6 (39.5)	39.8	40.3 (40.6)	39.9 (39.7)	39.4 (39.1)	39.3	38.3	40.0	39.7 (39.6)
11	18.3^{b}(18.2)^{b}	18.6	18.6 (18.4)	20.8	18.7 (18.2)	18.7 (18.3)	18.3 (18.3)	65.3	65.7	18.9	19.2^{b}(19.0)^{b}
12	33.5 (33.3)	33.3	33.3 (33.2)	40.7	32.4 (32.1)	33.1 (32.6)	33.9 (33.1)	41.6	42.6	26.8	25.1 (24.9)
13	44.3 (44.0)	44.2	44.2 (43.9)	79.8	38.3 (38.0)	42.8 (42.3)	40.7 (40.1)	37.8	39.9	45.8	45.0 (44.8)
14	40.0 (39.9)	39.9	39.8 (39.7)	47.4	34.1^{b}(33.6)^{b}	36.2^{b}(35.3)^{b}	39.4 (38.9)	34.9	39.4	37.8	39.7 (39.6)
15	49.4 (49.2)	49.2	49.1 (49.0)	48.1	209.6 (210.3)	82.6 (82.7)	82.2 (82.5)	208.8	82.9	53.9	135.6 (135.1)
16	155.8 (156.0)	155.7	155.7 (155.8)	157.6	150.2 (149.4)	161.1 (160.3)	159.6 (158.4)	151.9	159.4	81.6	142.2 (142.5)
17	103.4 (102.8)	103.5	103.6 (103.1)	102.9	113.9 (114.2)	107.8 (108.2)	104.4 (104.7)	110.8	105.6	66.4	15.4 (15.2)
18	33.7 (33.7)	29.3	28.6 (28.8)	29.3	28.6 (28.5)	28.7 (28.8)	28.7 (28.7)	29.3	29.3	29.3	28.7 (28.7)
19	21.7 (21.7)	179.9	177.5 (178.0)	180.0	177.5 (177.7)	177.7 (178.0)	177.7 (178.0)	179.9	180.0	180.1	177.6 (178.0)
20	17.7 (17.6)	16.0	15.5 (15.4)	15.9	15.5 (15.3)	15.9 (15.7)	15.8 (15.5)	16.0	15.9	16.0	15.4 (15.2)
COOMe			51.1 (51.1)		51.2 (51.0)	51.1 (51.2)	51.1 (51.1)				51.1 (51.1)

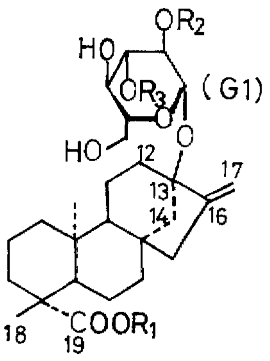
[a] δ ppm from internal TMS in C_5D_5N or $CDCl_3$ (in parentheses); JEOL JNM-PFT-100 NMR spectrometer at 25.15 MHz; concentration: 0.08-0.4 mM/ml; temperature: 25°; using 10 mm tubes; FT NMR conditions; spectral width: 5 KHz; pulse flipping angle: 45°; acquisition time: 0.4 sec; number of data points: 4096; transient time: 2-3 sec; number of transient: 600-10000.

[b,c] Values in any vertical column may be reversed although those given here are preferred.

Table 2 ^{13}C Chemical Shifts for Stevioside (14) and Related Glucosides in C_5D_5N, δ[a]

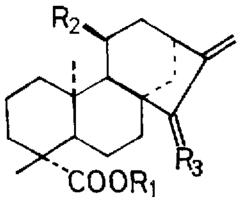
	12	13	14	15	16	17	18	19
C-1	41.0	40.9	40.7	41.0	40.9	40.8	40.5	39.9
2	19.7	19.7	19.2	19.8	19.4	19.6	19.5	19.2
3	38.4	38.5	38.1	38.7	38.3	38.4	38.3	38.1
4	43.8	43.8	43.9	43.8	44.0	44.2	44.1	44.0
5	56.9	56.9	57.3	57.0	57.4	57.2	57.1	56.8
6	22.5	22.4	22.0	22.5	22.2	22.0	21.8	20.5
7	41.6	41.5	41.5	41.8	41.8	36.7	36.3	37.0
8	42.1	42.4	42.5	42.2	42.4	46.3	45.4	50.8
9	54.1	54.1	53.8	54.1	54.0	46.1	55.2	63.1
10	39.7	39.6	39.7	39.8	39.8	39.6	38.3	39.2
11	20.6	20.5	20.6	20.6	20.7	18.4	65.7	65.3
12	38.4	37.2	36.6	37.8	37.3	33.8	42.5	41.4
13	86.4	86.1	85.9	86.8	86.6	40.7	39.9	37.7
14	44.6	44.7	44.3	44.5	44.5	39.6	39.9	34.8
15	48.2	47.9	47.5	47.9	47.9	82.3	82.8	208.8
16	153.7	154.0	154.3	153.7	153.9	159.7	159.4	151.8
17	104.9	104.9	104.5	104.6	104.5	104.3	105.5	110.7
18	29.2	29.2	28.2	29.3	28.3	28.6	28.6	28.5
19	180.0	180.1	177.0	180.2	177.0	176.9	176.8	176.6
20	15.7	15.9	15.4	16.1	15.5	16.1	15.7	15.8
G*-1			95.6		95.6	95.7	95.7	95.7
2			73.8		73.8	74.0	73.9	73.9
3			79.0		78.8	79.0	79.2	79.2
4			70.8		70.8	71.1	71.1	71.0
5			79.0		78.8	79.0	79.2	79.0
6			61.9		62.3	62.1	62.1	62.1
G1-1	99.4	97.6	97.7	97.8	97.9			
2	75.3	84.1	84.3	80.5	80.7			
3	[77.9]	[77.6]	77.9	88.0	87.8			
4	71.5	71.1	[71.3]	69.8	70.5			
5	[78.5]	[77.8]	77.9	[78.4]	[78.3]			
6	62.5	62.3	62.5	[62.3]	[62.3]			
G2-1		106.2	106.5	[104.4]	[104.5]			
G3-1				[104.6]	104.5			

[a] Condition: see Table 1; Values in square brackets are those assigned tentatively



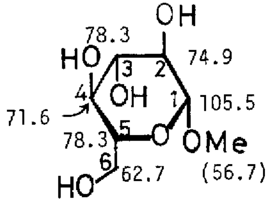
	R_1	R_2	R_3
12	H	H	H
13	H	Glc (G2)	H
14	Glc (G*)	Glc (G2)	H
15	H	Glc (G2)	Glc (G3)
16	Glc (G*)	Glc (G2)	Glc (G3)

Glc: β-glucopyranosyl



	R_1	R_2	R_3
17	Glc (G*)	H	$<^{OH}_{H}$
18	Glc (G*)	OH	$<^{OH}_{H}$
19	Glc (G*)	OH	=0
20	H	H	$<^{OH}_{H}$

CHART 1



21

glucosyl carbon signals of 12 (Gl-2∿6) appear at practically the same position as 21. The anomeric carbon of the ester-glucoside of 14 (G*-1) was found at a higher field (δ 95.6) and other ester-glucosyl signals (G*-2∿6) were observed at somewhat shifted positions from those of 21 owing to the esterification effect.

Besides 13 and 14, new sweet steviol-glucosides, named rebaudioside A (16), mp 242-244°, $[\alpha]_D^{24}$ -20.8° (MeOH), and B (15), mp 193-195°, $[\alpha]_D^{24}$ -45.4° (MeOH) were recently isolated from *S. rebaudiana*. In determining the structures, the identification of genuine aglycones and the elucidation of location and configuration of glucosyl linkage were achieved mainly by ^{13}C NMR spectroscopy (Table 2, Chart 1).[10]

Of diterpene-glycosides of *S. paniculata*, we have now isolated three new crystalline glucosides, named paniculosides I (17), mp 134-136°, $[\alpha]_D^{23}$ -64.9° (MeOH), II (18), mp 246-250°, $[\alpha]_D^{24}$ -52.7° (MeOH), and III (19), mp 153-157°, $[\alpha]_D^{23}$ -125.4° (MeOH). ^{13}C NMR signals of aglycone moieties of 17, 18, and 19 appear at almost the same positions, except for C-19 (and also C-18 in case of 18 and 19), as those of 7 (methyl ester of 20), 9, and 8, respectively. The carbon signals due to C-18, C-19, and sugar moieties are practically the same as C-18, C-19, and G*-1∿6 of 14 or 16, thus establishing formulations of 17, 18, and 19, respectively, as 19-β-glucopyranosyl ester of 20, 9, and 8 without further spectroscopic procedures or chemical degradations (Table 2, Chart 1).

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