STRUCTURES OF STEVIA DITERPENE-GLUCOSIDES : APPLICATION OF ¹³C 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 <u>8-10</u> and <u>20</u> were isolated from enzymatic hydrolysate of a crude glycoside mixture of <u>Stevia paniculata</u> (Compositae).²⁾ For the purpose of structural elucidation of glycosides of this plant and the related species, we have studied ¹³C NMR spectra of kaurene-type diterpenes <u>1-11</u> (7 = methyl ester of <u>20</u>). Pyridine-d₅ (C₅D₅N) was used as the solvent due generally to its high solubility for plant-glycosides. ¹³C Chemical shifts in C₅D₅N differed no more than 1 ppm³ from those in CDCl₃. 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 ¹³C signal assignments shown in Table 1. Comparison of the spectra with those of related compounds⁵ also helped our determination.

¹³C 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. ¹³C 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 $C_5 D_5 N$ assigned according to those reported data in $D_2 O_5^{(7)}$ 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 (G1-1) 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-OH⁷)[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-8-glucoside (& 100.5) was also demonstrated in paeoniflorin⁸⁾, a constituent of paeony root.⁹⁾ The remaining

<pre></pre>			41.0 (40.9) 19.5b(19.2)b 38.3 (38.2) 44.0 (43.9) 56.8 (56.9)	21.3 (21.0) 44.0 (43.9) 49.5 (49.2) 48.2 (48.1) 39.7 (39.6)	19.2 ^b (19.0) ^b 25.1 (24.9) 45.0 (44.8) 39.7 (39.6) 135.6(135.1)	142.2(142.5) 15.4(15.2) 28.7(28.7) 177.6(178.0) 15.4(15.0)	51.1 (51.1) cometer conditions; ita points: A.
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$ \begin{bmatrix} 71.5 & 71.1 & 171.3 & 69.8 & 70.5 \\ 78.5 & 177.9 & 77.9 & 78.4 & 178.3 \\ 62.5 & 62.3 & 62.5 & 62.3 & 162.3 \\ 106.2 & 106.5 & 106.5 & 104.4 & 104.5 \end{bmatrix} $	$\begin{bmatrix} 71.5 & 71.1 & 171.3 & 69.8 & 70.5 \\ 78.5 & 57.9 & [78.4] [78.3] \\ 62.5 & 62.3 & 62.5 & [62.3] [62.3] \\ 106.2 & 106.5 & [104.6] 104.5 \end{bmatrix} $ $\begin{array}{c} 106.2 & 106.2 & 106.4 & 104.6 \\ 104.6 & 104.6 & 104.5 \end{bmatrix} $ (56.7)		[77.9]	[77.6]	77.9	88.0	87.8							E OH		-	
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106.2 106.5 [104.6] [04.6] 104.5] HO ^{662.7} (56.7)	$106.2 \ 106.5 [104.6] 104.5]$ $HO^{62.7} (56.7)$		[78.5] 62.5	[77.8] 62.3	77.9	[78.4] [62.3]	[78.3] [62.3]						71.6			۰ ۲	
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						104.6]	L04.5							2			

5 51 ^aCondition: see Table 1; Value are those assigned tentatively

G1-1 2 3 4 5 6 6 6 62-1 63-1

glucosyl carbon signals of 12 (G1-2 \sim 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 \circ 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 ¹³C NMR spectroscopy (Table 2, Chart 1).10)

Of diterpene-glycosides of S. paniculata, we have now isolated three new crystalline glucosides, named paniculosides I (<u>17</u>), mp 134-136°, $[\alpha]_D^{23}$ -64.9° (MeOH), II (<u>18</u>), mp 246-250°, $[\alpha]_D^{24}$ -52.7° (MeOH), and III (19), mp 153-157°, $[\alpha]_D^{23}$ -125.4° (MeOH). ¹³C NMR signals of aglycone moieties of <u>17</u>, <u>18</u>, and <u>19</u> 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 \circ 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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