Attempts to synthesise the hypothetical biogenetic intermediate mancunine (**5**, 18,19-double bond) from hirsuteine (**1**, 18,19-double bond) by the same route were unsuccesful.

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Received December 22, 1976

[Chem. Pharm. Bull. 25(4) 844—846 (1977)]

UDC 547.918.02 : 543.422.25.06

Application of ^{13}C NMR Spectroscopy to Chemistry of Natural Glycosides: Rebaudioside-C, a New Sweet Diterpene Glycoside of *Stevia rebaudiana*

From leaves of *Stevia rebaudiana* BERTONI (Compositae), there were isolated three new sweet glycosides, named rebaudioside-C, -D, and -E. Application of ^{13}C NMR spectroscopy as well as chemical evidences led to assign the structure V in Chart 1 to rebaudioside-C.

Keywords——^{13}C NMR of oligoglycosides; PRFT method; Kaurene type diterpenes; rebaudiosides-C, -D, -E; natural sweetener; *Stevia rebaudiana* BERTONI; Compositae

Stevia rebaudiana BERTONI (Compositae), a wild herb of Paraguay is known to contain the sweet glucoside, stevioside(I)[1] and has attracted much attention as a new source of the natural sweetener. Recently, the present authors reported isolation and structural determination of additional sweet glucosides, named rebaudiosides-A(II) and -B(III) from this plant.[2]

The glycoside fraction[2] of the methanolic extract of leaves of this plant was recrystallized from methanol to give I and the mother liquor was subjected to repeated column chromatography on silica gel (solvent $CHCl_3$: MeOH: H_2O(30: 10: 1 homogeneous) and AcOEt: MeOH(10: 1)), affording now three new sweet glycosides, named rebaudiosides-C (yield 0.4%), –D(yield 0.03%) and –E(yield 0.03%) along with I, II, III and steviolbioside-(IV).[2]

Rebaudioside-C(V), colorless needles, mp 215—217°, $[\alpha]_D^{25}$ −29.9° (MeOH) which was crystallized by slow concentration of its methanolic solution, showed its spot[3] between those of I and II on thin-layer chromatogram on silica gel(solvent $CHCl_3$: MeOH: H_2O(15: 6: 1 homogeneous)).

On the basis of our recent study on ^{13}C nuclear magnetic resonance(CMR) of *Stevia* diterpene glycosides,[4] the spectrum[5] of V revealed that V must be a glycoside of steviol(VI), both the 19-COOH and the 13-*tert*-OH of which must be combined with sugar moieties. On hydrolysis with crude hesperidinase,[6] V afforded glucose, rhamnose and VI, while alkaline

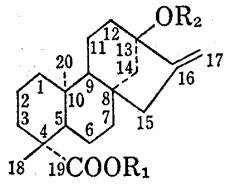
1) E. Mosettig, U. Beglinger, F. Dolder, H. Lichiti, P. Quitt, and J.A. Waters, *J. Am. Chem. Soc.*, **85**, 2305 (1963) and the references cited therein.
2) H. Kohda, R. Kasai, K. Yamasaki, K. Murakami, and O. Tanaka, *Phytochemistry*, **15**, 981 (1976).
3) Visualized as a yellow spot on heating after spraying 10% H_2SO_4.
4) K. Yamasaki, H. Kohda, T. Kobayashi, R. Kasai, and O. Tanaka, *Tetrahedron Letters*, **1976**, 1005.
5) All spectra were taken in C_5D_5N; δ ppm from TMS; at 25°.
6) H. Kohda and O. Tanaka, *Yakugaku Zasshi*, **95**, 246 (1975).

saponification of V yielded 1,6-anhydroglucopyranose[2] and an acidic glycoside(VII), colorless needles(from MeOH), mp 205—207°, $[\alpha]_D^{25}$ −47.9° (MeOH). A set of carbon signals which disappeared on going from V to VII was assigned to a β-glucopyranosyl unit bonded with the 19–COOH of this type of diterpenes[4] (see Table I).

The extensive studies on CMR of mono- and oligo-saccharides have been reported[7] and it has been expected that an α-rhamnopyranoside must be distinguished from its β-anomer by chemical shifts of C–3 and –5 carbons[8] as well as the coupling constant of the 1-^{13}C–1-^{1}H.[9] Recently, the glycosylation shifts of numerous aliphatic alcohols have been also investigated[10]. Partially relaxed Fourier transform method(PRFT) which was reported to facilitate the identification of carbon resonances of individual sugar units in the spectra of oligosaccharides[11] was applied to the spectrum of VII, disclosing that in the region of sugar carbon resonances, a set of resonances with longer spin-lattice relaxation times (T_1) than others can be assigned to an unsubstituted β-glucopyranoside. Further, this PRFT experiment led to assign signals due to other two suger units to an unsubstituted α-rhamnopyranoside and a 2,3-di-O-substituted β-glucopyranoside, in the latter of which the degree of the downfield-displacement of carbon signals of its C-2 and -3 strongly suggested that its C-2 and -3 hydroxyl groups were combined with α-rhamnopyranosyl and β-glucopyranosyl units, respectively.[7,10]

These evidences led to formulation of V and VII as shown in Chart 1. This was further confirmed by the following chemical evidences. Permethylation[12] of VII followed by methanolysis gave methyl 2,3,4-tri-O-methylrhamnopyranoside, methyl 2,3,4,6-tetra-O-methylglucopyranoside and methyl 4,6-di-O-methylglucopyranoside. On mild hydrolysis with 1.5% H_2SO_4 in MeOH: H_2O(1: 1), VII yielded rhamnose and a desrhamno-glycoside-(VIII), which was subjected to methylation[12] followed by methanolysis to give methyl 2,3,4,6-tetra-O-methylglucopyranoside and methyl 2,4,6-tri-O-methylglucopyranoside.

Structural determination of rebaudiosides-D, colorless needles, (from EtOH), mp 283—286°, $[\alpha]_D^{25}$ −22.7° (MeOH) and -E, colorless needles(from MeOH), mp 205—207°, $[\alpha]_D^{25}$



G=β-glucopyranosyl
Rh=α-rhamnopyranosyl

	R_1	R_2		R_1	R_2
I:	-G	-G(2→1)G	VI:	-H	-H
II:	-G	-G(2→1)G, 3→1G	VII:	-H	-G′(2→1)Rh, 3→1G″
III:	-H	-G(2→1)G, 3→1G	VIII:	-H	-G(3→1)G (Δ_{15})
IV:	-H	-G(2→1)G	IX:	-G	-H
V:	-G	-G′(2→1)Rh, 3→1G″	X:	-H	-G

Chart 1

7) P.A.J. Gorin and M. Mazurek, *Can. J. Chem.*, **53**, 1212 (1975); T. Usui, N. Yamaoka, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seto, *J.C.S.* Perkin I, **1973**, 2425; P. Colson and R.R. King, *Carbohydrate Research*, **47**, 1 (1976) and the references cited therein.
8) D.E. Dorman and J.D. Roberts, *J. Am. Chem. Soc.*, **92**, 1355 (1970).
9) K. Bock, I. Lundt, and C. Pedersen, *Tetrahedron Letters*, **1973**, 1037.
10) R. Kasai, M. Suzuo, J. Asakawa, and O. Tanaka, *Tetrahedron Letters*, **1977**, 175; K. Tori, S. Seo, Y. Yoshimura, H. Arita, and Y. Tomita, *Tetrahedron Letters*, **1977**, 179.
11) A. Allerhand and D. Dodfrell, *J. Am. Chem. Soc.*, **93**, 2777 (1971).
12) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).

−34.2°(MeOH), both of which showed lower *Rf* values than II in the thin-layer chromatography (*vide supra*), are under progress.

TABLE I. ^{13}C Chemical Shifts (in C_5D_5N at 25°, 25.15 MHz)

	Aglycone			Sugar moiety			Sugar moiety			
	V	VII		V	VII		Me-G	IX	X[4)]	Me-Rh
C-1	40.8	41.0	G-1	96.1		C-1	105.5	95.7	99.4	102.4
2	19.4	19.7	2	74.2		2	74.9	73.9	75.3	72.6
3	38.5	38.6	3	79.5[a)]		3	78.3	79.1	77.9[c)]	72.0
4	44.0	43.9	4	70.7		4	71.6	71.0	71.5	73.7
5	57.5	57.0	5	78.7[a)]		5	78.3	79.1	78.5[c)]	69.4
6	22.0	22.6	6	62.1		6	62.7	62.0	62.6	18.5
7	41.7	41.7	G′-1	97.7	98.2					
8	42.2	42.5	2	77.4	76.2					
9	54.1	54.1	3	89.9	89.8					
10	39.9	39.8	4	70.3	69.6					
11	20.6	20.7	5	77.4	77.5					
12	38.5	38.0	6	62.4[d)]	62.3					
13	87.3	86.6	G″-1	104.2	104.2					
14	43.2	43.6	2	75.1	75.0					
15	48.6	48.5	3	78.3[b)]	78.4					
16	152.9	154.1	4	71.5	71.4					
17	106.1	105.0	5	78.7[b)]	78.4					
18	28.2	29.3	6	62.6[d)]	62.3					
19	177.2	180.0	Rh-1	102.1	101.6[e)]					
20	15.3	15.9	2	72.5	72.4					
			3	72.0	72.4					
			4	74.2	74.1					
			5	69.8	69.6					
			6	18.8	18.8					

IX: prepared from VI
Me-G: methyl β-D-glucopyranoside
Me-Rh: methyl α-L-rhamnopyranoside
a,b,c,d) Values may be reversed. *e*) $J_{C_1\text{-}H_1}=171\pm1$ Hz

Acknowledgement The authors are grateful to Dr. Y. Miyazaki and Mr. K. Nishi, the Experimental Stations of Medicinal Plants, National Institute of Hygienic Sciences for their kind supply of the cultivated plant material, to Prof. J. Shoji, Showa University for his kind donation of partially methylated glucoses and to Dr. K. Morimoto, Hiroshima Prefectural Institute of Public Health for his valuable advice.

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Received December 23, 1976