Chem. Pharm. Bull. 25(12)3437—3439(1977)

UDC 547.918.02.08 : 543.422.25.06 : 581.192

Application of ^{13}C NMR Spectroscopy to Chemistry of Plant Glycosides: Rebaudiosides-D and -E, New Sweet Diterpene-Glucosides of *Stevia rebaudiana* Bertoni

From the leaves of *Stevia rebaudiana* Bertoni, additional two sweet glucosides, named rebaudiosides-D (**4**) and -E (**5**) were isolated. On the basis of ^{13}C NMR evidences as well as the results of chemical and enzymatic hydrolysis, the structures of **4** and **5** were assigned to be β-sophorosyl ester of steviol-13-O-[β-D-glucopyranosyl (1—2)][β-D-glucopyranosyl-(1—3)]-β-D-glucopyranoside and β-sophorosyl ester of steviol-13-O-β-sophoroside, respectively.

In connection with the structure determination, β-sophorosyl ester of *ent*-kaur-16-en-19-oic acid was prepared and the ^{13}C chemical shift and the coupling constant of its ester anomeric carbon were discussed. The formulation of **4** and **5** was finally substantiated by their preparation from the known compounds.

Keywords——^{13}C NMR; oligoglycosides; kaurene type diterpenes; rebaudioside-D, -E; natural sweetener; *Stevia rebaudiana* Bertoni; Compositae; preparation of β-sophorosyl ester

Stevia rebaudiana Bertoni(Compositae) is known to contain the sweet diterpene-glucoside, stevioside (**1**) which has been expected to be a natural sweetener.[1] We previously reported isolation and structure determination of another major sweet glucoside, named rebaudioside-A (**2**).[2] Further investigation of this plant led to isolation of new minor sweet glycosides designated as rebaudiosides-C (**3**), -D (**4**), and -E (**5**).[3] The structure of **3** was already reported preliminarily.[3] Very recently, Kobayashi, *et al.* reported isolation and structure determination of two glycosides of the same plant, named dulcosides-A and -B,[4] the latter of which is identical with our rebaudioside-C (**3**). The present communication concerns with the structure study of **4** and **5**.

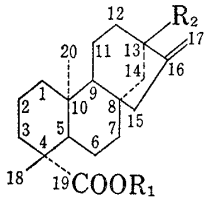
On alkaline saponification, rebaudioside-D (**4**), colorless needles, mp 283—286°, $[\alpha]_D^{25}$ −22.7° (MeOH) yielded rebaudioside-B (**6**),[2] the 13-O-triglucoside of steviol (**7**), which was already obtained from **2** by the same treatment and was formulated as illustrated in Table I. Enzymatic hydrolysis of **4** with crude hesperidinase[5] afforded glucose and **6**. Field desorption (FD) mass spectrum of **4** exhibited a peak at *m/e* 1151 which is attributable to $M^+ + 23 =$ steviol—$(glucose)_5$+Na. These evidences as well as comparison of ^{13}C NMR spectrum of **4** with those of **2** and **6** indicated that **4** must be a diglucosyl ester of **6**.

The anomeric carbon signal of β-monoglucosyl ester such as **1**, **2**, and **3** has been found to appear near δ 95.5.[2,3,6] However, a signal assignable to an ester glucosyl anomeric carbon of **4** was observed at somewhat higher field (δ 93.7, $^1J_{C-H}$=164 Hz). This strongly suggested that the ester diglucosyl moiety of **4** would be β-sophorosyl (=2-O-(β-glucopyranosyl)-β-glucopyranosyl) group, because on going from β-glucose to β-sophorose, the anomeric carbon signal of the reducing unit is found to be shielded by the substitution at its vicinal hydroxyl

1) E. Mosettig, U. Beglinger, F. Dolder, H. Lichiti, P. Quitt, and J.A. Waters, *J. Am. Chem. Soc.*, **85**, 2305 (1963).
2) H. Kohda, R. Kasai, K. Yamasaki, K. Murakami, and O. Tanaka, *Phytochemistry*, **15**, 981 (1976) (Conversion of **1** to **2**: N. Kaneda, R. Kasai, K. Yamasaki, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **25**, 2466 (1977).): Rebaudioside-B (**6**) in this report seems now to be an artifact formed from **2** by accidental partial hydrolysis during the process of the isolation.
3) I. Sakamoto, K. Yamasaki, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **25**, 844 (1977).
4) M. Kobayashi, S. Horikawa, I.H. Degrandi, J. Ueno, and H. Mitsuhashi, *Phytochemistry*, **16**, 1405 (1977).
5) H. Kohda and O. Tanaka, *Yakugaku Zasshi*, **95**, 246 (1975).
6) K. Yamasaki, H. Kohda, T. Kobayashi, R. Kasai, and O. Tanaka, *Tetrahedron Lett.*, **1976**, 1005.

TABLE I. ^{13}C Chemical Shifts of **4**, **5** and **8** (Sugar moiety only)[a] (in C_5D_5N at 25°, 25.15 MHz)

	19-O-Glucoses		13-O-Glucoses		
Rebaudioside D (**4**)					
	G	G′	G	G′	G″
1	93.7	105.5	97.6	104.5	104.5
2	81.0	76.3[b]	80.8	76.0[b]	75.3[b]
3	77.3	78.4	88.0	78.4	78.4
4	70.9	72.3[c]	70.0	71.6[c]	72.1[c]
5	78.4	78.4	78.4	78.4	78.4
6	63.1	62.3	63.1	62.3	62.3
Rebaudioside E (**5**)					
	G	G′	G	G′	
1	93.4	105.4	97.7	106.4	
2	81.1	77.0	84.2	76.2	
3	77.6	78.3[d]	78.0[d]	78.3[d]	
4	70.8	71.8[e]	71.6[e]	71.1[e]	
5	78.9[d]	78.0[d]	78.0[d]	78.3[d]	
6	62.8	62.2	62.8	62.2	
19-Sophorosyl kaurenoate (**8**)					
	G	G′			
1	93.5	104.8			
2	79.9	75.9			
3	78.0	78.3			
4	70.9	71.9			
5	78.8	78.3			
6	63.0	62.2			



G=β-glucopyranosyl
Rh=α-rhamnopyranosyl

	R_1	R_2
1	-G	-OG(2→1)G
2	-G	-OG(2→1)G, (3→1)G
3	-G	-OG(2→1)Rh, (3→1)G
4	-G(2→1)G′	-OG(2→1)G′, (3→1)G″
5	-G(2→1)G′	-OG(2→1)G′
6	-H	-OG(2→1)G, (3→1)G
7	-H	-OH
8	-G(2→1)G′	-H
9	-H	-H
10	-H	-OG(2→1)G

a) Aglycone carbon chemical shifts are within 1.0 ppm differences to corresponding 19-O-monoglucosyl esters, except C-19 and C-20 of **4** (175.8 and 16.8, respectively) and C-19 and C-20 of **5** (175.7 and 16.7, respectively).[2,9]
b, c, d, e) Values may be interchanged.

group.[7] In order to confirm this, a model compound, β-sophorosyl ester (**8**) of *ent*-kaur-16-en-19-oic acid (**9**) was prepared by condensation of **9** with acetobromosophorose in the presence of Ag_2CO_3-Celite[8] followed by deacetylation with $BaOCH_3$. The β-anomeric configuration of the ester glucosyl linkage of **8** was confirmed by the coupling constant of its anomeric proton signal (δ 6.06, doublet J=7.0 Hz). The signal due to the ester glucosyl anomeric carbon of **8** appeared at the almost same position (δ 93.5) with the same coupling constant ($^1J_{C-H}$=164 Hz) as that of **4**, supporting the formulation of **4** mentioned above.

The structure of **4** was finally established by its preparation from **6**. Peracetate of **6** was subjected to β-sophorosylation in the same way as the preparation of **8** to give an ester sophoroside, which was proved to be identical with **4** by comparison of thin-layer chromatograms (TLC), ^{13}C NMR spectra and other physical constants.

Acid hydrolysis of rebaudioside-E (**5**), colorless needles, mp 205—207°, $[\alpha]_D^{25}$ −34.2° (MeOH) afforded glucose and the migrated aglycone, isosteviol,[1] while alkaline saponification of **5** yielded steviolbioside (**10**), 13-O-sophoroside of **7**, [2] which was already obtained from **1**

7) T. Usui, N. Yamaoka, K. Matsuda, K. Tsuzimura, H. Sugiyama, and S. Seto, *J.C.S. Perkin I*, **1973**, 2425.
8) J. Hartenstein and G. Satzinger, *Liebigs Ann.*, **1974**, 1763.
9) K. Yamasaki, H. Kohda, T. Kobayashi, N. Kaneda, R. Kasai, O. Tanaka, K. Nishi, *Chem. Pharm. Bull.* (Tokyo), **25**, 2895 (1977).

by the same treatment. The FD mass spectrum of **5** showed a peak at *m/e* 989 which is assignable to $M^+ + 23 =$ steviol$-$(glucose)$_4$+Na. Further, the anomeric carbon signal of the ester glucosyl linkage of **5** was observed at almost the same position (δ 93.4) with the same coupling constant ($^1J_{C-H} = 164$ Hz) as those of **4** and **8**. These evidences strongly suggested that **5** must be formulated as β-sophorosyl ester of **10**. Comparison of the other carbon signals of **5** with those of **1**, **4**, **8** and **10** also supported this formulation. The structure of **5** was finally substantiated by its preparation from **10**. β-Sophorosylation of peracetate of **10** in the same procedure as that of **4** and **8** gave an ester sophoroside which was proved to be identical with **5** by comparison of TLC, ^{13}C NMR spectra, and other physical constants.

Acknowledgement The authors are grateful to Dr. Y. Miyazaki and Mr. K. Nishi, National Institute of Hygienic Sciences for their kind supply of the plant material, and to Dr. K. Morimoto, Hiroshima Prefectural Institute of Public Health for his valuable advice. This work was supported by the Grant- in-Aid from the Ministry of Education, Science and Culture, which is gratefully acknowledged.

Hiroshima Prefectural Institute of Public Health
1-5-70 Ujina Kanda, Hiroshima-shi, 734 Japan
Ikunori Sakamoto

Institute of Pharmaceutical Sciences,
Hiroshima University School of Medicine
1-2-3 Kasumi, Hiroshima-shi, 734 Japan
Kazuo Yamasaki
Osamu Tanaka[10)]

Received October 11, 1977

10) Correspondence should be addressed to O. Tanaka.