Synthesis and Taste of Some Analogs of Stevioside

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Some analogs of stevioside (I), a sweet diterpene glycoside, were synthesized. Coupling of hepta-*O*-acetylsteviolbioside with hexa-*O*-acetyl-2-*O*- α -L-rhamnopyranosyl- α -D-glucopyranosyl bromide, hexa-*O*-acetyl-2-*O*- α -L-rhamnopyranosyl- α -D-galactopyranosyl bromide and hexa-*O*-acetyl-2-*O*- α -L-quinovopyranosyl- α -D-galactopyranosyl bromide, in the presence of silver carbonate-celite in dichloroethane followed by deacetylation yielded steviol-13-*O*- β -sophoroside-19-*O*-(2-*O*- α -L-rhamnopyranosyl- β -D-glucopyranosylester) (VIII), steviol-13-*O*- β -sophoroside-19-*O*-(2-*O*- α -L-rhamnopyranosyl- β -D-galactopyranosylester) (IX) and steviol-13-*O*- β -sophoroside-19-*O*-(2-*O*- α -L-quinovopyranosyl- β -D-glucopyranosylester) (X), respectively. Methylation of steviolbioside heptaacetate followed by deacetylation yielded steviolbioside-19-*O*-methylester (XII). Reduction of stevioside and XII with hydrogen gave dihydrostevioside (XIV) and dihydrosteviolbioside-19-*O*-methylester. The relative sweetness of I, VIII, IX, X, steviolbioside, XII, dihydrosteviolbioside, XIV and XV as expected by the percentage of that of sucrose were 210, 300, 230, 90, 6, 30 and 3, respectively.

Stevioside (I)¹⁾ is a sweet diterpene glycoside obtainable from a Paraguayan wild herb, *Stevia rebaudiana* Bertonii which belongs to the Composite family, and is now used as a food additive in Japan. Its chemical structure including absolute configuration was finally determined by Mossetig *et al.*²⁾ as steviol-13-*O*- β -sophoroside-19-*O*- β -D-glucopyranosylester. Recently Kohda *et al.*³⁾ and Sakamoto *et al.*⁴⁾ found several new analogous sweet glycosides in this plant and named them rebaudioside-A, -C, -D and -E, respectively. Independantly, Kobayashi *et al.*⁵⁾ reported the presence of two new diterpene glycosides in the same plant and named them dulcoside -A, and -B, respectively. The latter compound was later demonstrated to be identical with rebaudioside-C by Sakamoto *et al.*⁴⁾ Kasai *et al.*^{6,7)} reported the relationship between the chemical structure and sweetness of stevioside based on the taste of the above mentioned glycosides and many other enzymatically and chemically prepared stevioside analogs. A number of dihydrochalcone glycosides were synthesized and their structure-taste relationship were examined.^{8~12)} Hesperetin dihydrochalcone-4'- β -D-glucopyranoside (II) and -4'- β -D-galactopyranoside (III)

have been reported to be as sweet as saccharin by Horowitz and Gentili.¹²⁾ When α -L-rhamnose or α -L-quinovose is linked to the C-2 hydroxyl group of D-glucose or D-galactose in II or III the sweetness of the resulting glycosides are enhanced ten⁹⁾ and three times¹⁰⁾ on the molar basis, respectively. If the mechanism of the sweet response of the taste receptor site to the dihydrochalcone glycosides and stevioside are common, replacement of esterified D-glucose of I by 2-*O*- α -L-rhamnopyranosyl- β -D-glucopyranose, 2-*O*- α -L-rhamnopyranosyl- β -D-galactopyranose or 2-*O*- α -L-quinovopyranosyl- β -D-glucopyranose would enhance the sweetness considerably. In this paper the synthesis of some analogs of stevioside and the results of the examination of their taste are reported. Coupling of hepta-*O*-acetylsteviolbioside (steviol-13-*O*- β -sophoroside)¹³⁾ (IV) with hexa-*O*-acetyl-2-*O*- α -L-rhamnopyranosyl- α -D-glucopyranosyl bromide (V),¹⁴⁾ hexa-*O*-acetyl-2-*O*- α -L-rhamnopyranosyl- α -D-galactopyranosyl bromide¹⁵⁾ (VI), and 2-*O*- α -L-quinovopyranosyl- α -D-glucopyranosyl bromide¹⁶⁾ (VII) in the presence of silver carbonate-celite¹⁷⁾ in 1,2-dichloroethane by the method of Sakamoto *et al.*⁴⁾

- I. X = β -D-glucose
 VIII. X = 2-O- α -L-rhamnopyranosyl- β -D-glucopyranosyl
 IX. X = 2-O- α -L-rhamnopyranosyl- β -D-galactopyranosyl
 X. X = 2-O- α -L-quinovopyranosyl- β -D-glucopyranosyl
 XI. X = H
 XII. X = CH₃
 XIII. X = H, and -CH₃ instead of =CH₂
 XIV. X = β -D-glucopyranosyl and -CH₃ instead of =CH₂
 XV. X = CH₃, and -CH₃ instead of =CH₂

followed by deacetylation yielded steviol-13-O- β -sophoroside-19-O-(2-O- α -L-rhamnopyranosyl- β -D-glucopyranosylester) (VIII), steviol-13-O- β -sophoroside-19-O-(2-O- α -L-rhamnopyranosyl- β -D-galactopyranosylester) (IX) and steviol-13-O- β -sophoroside-19-O-(2-O- α -L-quinovopyranosyl- β -D-glucopyranosylester) (X), respectively.

Removal of D-glucose at the carboxyl group of I afforded steviolbioside (XI)¹⁸⁾ which has hitherto been reported to be tasteless. Unexpectedly, however, it was found to be considerably sweet in the present study. In order to clarify the role of the carboxyl group of XI for the appearance of sweetness, methylation of the carboxyl group of IV with diazomethane followed by deacetylation was carried out to yield steviol-13-O-sophoroside-19-O-methylester (XII).

Since Mossetig *et al.*¹⁹⁾ reported that XI readily absorbed hydrogen at the double bond to produce dihydrosteviolbioside (XIII) whose taste was not described, the role of the double bond of steviol in the elicitation of sweetness of I was also examined. In the presence of palladium-carbon (10%) I and XII were hydrogenated to yield dihydrostevioside (XIV) and dihydrosteviol-13-O- β -sophoroside-19-O-methylester (XV), respectively. The tastes of

TABLE I. TASTE AND RELATIVE SWEETNESS OF STEVIOL GLYCOSIDES AND SUCROSE

The sweetness of test samples was compared with that of sucrose and saccharin. An aqueous solution of 0.6% (w/v) sucrose was judged as its threshold level. The level of each test sample was calculated from its concentration which is required to attain the sweetness equivalent to that of 0.6% sucrose solution.

Compound	Taste	Relative Sweetness	
		Molar	Weight
I	Sweet	515	210
VIII	"	880	300
IX	"	880	300
X	"	684	225
XI	"	173	90
XII	Bitter sweet	186	90
XIII	"	11	6
XIV	Sweet	58	30
XV	Bitter sweet	6	3
Saccharin	Sweet	211	300
Sucrose	"	1	1

VIII, IX, X, XI, XII, XIII, XIV and XV are summarized in Table I. Unexpectedly, VIII and IX were about only 1.5 times as sweet as I, and X was almost the same as I on a weight basis. Thus, it is suggested that the attachment of α -L-rhamnose or α -L-quinovose at the C-2 hydroxyl group of the esterified D-glucose of I does not significantly affect the sweetness in contrast to the case of dihydrochalcones. On the other hand, dulcoside-A, and -B whose structure have been determined as β -D-glucopyranosylesters of steviol-13-O-(2-O- α -L-rhamnopyranosyl- β -D-glucopyranoside) and steviol-13-O-[β -D-glucopyranosyl-(1 $\rightarrow$ 2)] [α -L-rhamnopyranosyl-(1 $\rightarrow$ 3)]- β -D-glucopyranoside], respectively, were 0.1 times⁵⁾ or 0.25 times²⁰⁾ as sweet as I as had been reported. Ishima *et al.*²⁰⁾ concluded that the attachment of α -L-rhamnose instead of D-glucose to the glucose moiety of the C-13 position of I inhibits the elicitation of sweetness considerably. From the above result it is suggested that the mechanism of interaction between the taste cells and two sugar moieties at the opposite ends of steviol in the elicitation of sweetness is different. Recently, Hodge and Inglett²¹⁾ investigated the relationship between the chemi-

cal structure and sweetness of five natural intensely sweet glycosides, *i.e.* stevioside, glycyrrhizin, osladin, neohesperidin dihydrochalcone and naringin dihydrochalcone. They concluded that those compounds have common structural features, namely, (1) U-shaped hydrophobic aglycone, (2) a disaccharide with an internal (1→2)-D-glucosidic linkage, and (3) a second hydrophilic group such as sugars or carboxylates or phenolate anions. Methylation of the carboxyl group of **XI** gave **XII** which is as sweet as **XI** although the latter has a somewhat bitter taste. The result does not coincide with the above hypothesis of Hodge *et al.* The fact that the degrees of sweetness of **XIII**, **XIV** and **XV** greatly decrease as compared with **I** indicates that the double bond of steviol plays an important role for the emergence of intense sweetness. Kasai *et al.*⁷⁾ reported that steviol is entirely tasteless while steviol-13-*O*-β-D-glucopyranoside and steviol-19-*O*-β-D-glucopyranosylester are sweet although less than **I**. Accordingly, essential factors affecting the emergence of sweetness of **I** are thought to be the double bond of steviol and sugars with Shallenberger's AH-B system²²⁾ attached at the C-19 positions of aglycone. The double bond of **I** does not correspond to Kier's site²³⁾ because its position is far from the AH-B system of sugars.

EXPERIMENTAL

Mps, IR, and NMR spectra were measured with the equipments reported previously.²⁴⁾ Thin-layer chromatographic analyses were carried out on silica gel plates with chloroform-methanol-water (60:40:8).

*I) General procedure for the preparation of steviol-13-*O*-β-sophoroside-19-*O*-β-glycosylester.* In a well dried Erlenmeyer flask steviolbioside heptaacetate (1 mmol), preheated at 100°C for 2 hr, and α-acetobromosugars (2 mmol) were dissolved in anhydrous 1,2-dichloroethane and silver carbonate-celite (4mmol) was added to the solution. Being protected from the light the solution was heated till boiling under vigorous stirring and a small quantity of water was distilled off. After that, the reaction mixture was heated at 100~120°C under continuous stirring for 2 hr. After the reaction was over, the filtrate was distilled off under reduced pressure and the residual syrup was deacetyl-

ated with 0.1 N sodium methoxide in methanol at 0°C for 2 hr and then neutralized with Amberlite IR-120 (H⁺). The filtrate was distilled under reduced pressure to give a syrupy residue which was dissolved in methanol. Crystallization began instantly at 0°C. After a while, the crystals were collected and recrystallized from methanol to yield pure compound.

i) Steviol-13-*O*-β-sophoroside-19-*O*-(2-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosylester) (**VIII**).

A mixture of **IV** (2.86 g), **V** (5.1 g) and silver carbonate-celite (8 g) in dichloroethane (120 ml) was treated as described above and **VIII** was obtained as colorless needles in a yield of 1.06 g (36.6%), mp 207~210°C. $[\alpha]_D^{15} - 44.4^\circ$ ($c=1.67$, C₆H₅N). TLC *R_f* 0.58. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3350 (OH), 2920 (CH₂), 1720 (CO-O). *Anal.* Found: C, 52.47; H, 7.50; Calcd. for C₄₄H₇₀O₂₂·3H₂O: C, 52.59; H, 7.56%. **VIII** was acetylated with a mixture of acetic anhydride and pyridine to give its decaacetate, mp 125~129°C. $[\alpha]_D^{15} - 14.0^\circ$ ($c=0.5$, C₆H₅N). NMR $\delta_{\text{MeSi}}^{\text{CDCl}_3}$: 0.70~2.34 (methyl, methylene and rhamnosyl methyl protons of steviol); 1.87~2.17 (sugar's acetoxyl protons); 3.34~5.27 (sugar protons). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 2920 (CH₂), 1750 (C=O). *Anal.* Found: C, 56.17; H, 6.47; Calcd. for C₇₀H₈₈O₃₅: C, 56.14; H, 6.41%.

ii) Steviol-13-*O*-β-sophoroside-19-*O*-(2-*O*-α-L-rhamnopyranosyl-β-D-galactopyranosylester) (**IX**).

A mixture of **IV** (1.4 g), silver carbonate-celite (3.92g), and **VI** (2.5 g) in dichloroethane (42 ml) was treated as described above and **IX** was obtained as colorless needles in a yield of 0.71 g (50.0%), mp 226~229°C. TLC *R_f* 0.58. $[\alpha]_D^{15} - 44.2^\circ$ ($c=1.65$, C₆H₅N). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3400 (OH), 2920 (CH₂), 1740 (CO-O). *Anal.* Found: C, 51.87; H, 7.34; Calcd. for C₄₄H₇₀O₂₂·3H₂O: C, 52.59; H, 7.56%. Tridecaacetate, mp 137~140°C. $[\alpha]_D^{15} - 28.0^\circ$ ($c=0.5$, CHCl₃). NMR $\delta_{\text{MeSi}}^{\text{CDCl}_3}$: 0.63~2.60 (methyl and methylene protons of steviol); 1.13 (doublet, *J*=6 Hz, rhamnosyl methyl protons); 1.80~2.20 (sugar acetoxyl protons); 3.27~5.60 (sugar protons). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 2900 (CH₂), 1750 (CO-O), 1380 (CH₃). *Anal.* Found: C, 56.01; H, 6.46; Calcd. for C₇₀H₈₈O₃₅: C, 56.14; H, 6.46%.

iii) Steviol-13-*O*-β-sophoroside-19-*O*-(2-*O*-α-L-quinovopyranosyl-β-D-glucopyranosylester) (**X**).

A mixture of **IV** (2.9 g), silver carbonate-celite (8.1 g), and **VII** (5.2 g) in dichloroethane (63 ml) was treated as described above and **X** was obtained as plates in a yield of 1.6 g (48.4%), mp 203~205°C. $[\alpha]_D^{12} - 52.5^\circ$ ($c=4.0$, C₆H₅N). TLC *R_f* 0.58. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3410 (OH), 2960, 2900, 2880 (CH₂), 1750 (CO-O). *Anal.* Found: C, 50.79; H, 7.76; Calcd. for C₄₄H₇₀O₂₂·5H₂O: C, 50.76; H, 7.82%. Tridecaacetate, mp 134~137°C. $[\alpha]_D^{15} - 39.0^\circ$ ($c=2.0$, CHCl₃). NMR $\delta_{\text{MeSi}}^{\text{CDCl}_3}$: 0.53~2.60 (methyl, methylene and quinovosyl methyl protons); 1.85~2.16 (sugar acetoxyl protons); 3.36~

5.70 (sugar protons). *Anal.* Found: C, 55.79; H, 6.39; Calcd. for $C_{70}H_{76}O_{86}$: C, 56.14; H, 6.41%.

2) *Steviol-13-O-β-sophoroside-19-O-methylester* (XII). **IV** (5.0 g) was dissolved in dichloromethane (25 ml) and an excess amount of diazomethane in dichloromethane (25 ml) was added and the reaction mixture was allowed to stand overnight at room temperature. After acetic acid was added to decompose excess diazomethane the solution was distilled off and the residue (5.3 g) was deacetylated with 0.1 N sodium methoxide in methanol at 0°C for 2 hr. and then the solution was acidified with 10% sulfuric acid. The crystalline precipitate was collected and recrystallized from water to yield pure needles in a yield of 3.38 g (96.6%), mp 171~174°C. $[\alpha]_D^{11} - 33.0^\circ$ ($c=1.0$, 50% EtOH). IR $\nu_{\max}^{KBr}$ cm^{-1} : 3320 (OH), 2920, 2880, 2840 (CH_2), 1720 (CO-O). *Anal.* Found: C, 55.92; H, 7.79; Calcd. for $C_{38}H_{62}O_{13} \cdot 3H_2O$: C, 55.77; H, 8.16%. **XII** was acetylated with a mixture of acetic anhydride and pyridine to give its acetate (**XII'**), mp 109~113°C. $[\alpha]_D^{15} - 8.0^\circ$ ($c=0.5$, $CHCl_3$). IR $\nu_{\max}^{KBr}$ cm^{-1} : 2940, 2880, 2800 (CH_2), 1750 (CO-O), 1370 (CH_3). *Anal.* Found: C, 59.11; H, 6.90; Calcd. for $C_{47}H_{66}O_{20}$: C, 59.36; H, 6.94%. NMR $\delta_{MeSi}^{CDCl_3}$: 0.76~2.40 (methyl and methylene protons of steviol and methyl protons of rhamnose); 1.90~2.10 (sugar acetoxyl protons); 3.36~5.10 (sugar protons).

3) *Dihydrostevioside* (dihydrosteviol-13-O-β-sophoroside-19-O-β-D-glucopyranosylester (XIV). **I** (500 mg) was dissolved in ethanol (30 ml) and palladium-carbon (10%) (300 mg) was added. The mixture was shaken under a hydrogen atmosphere for 2 hr. The filtrate was distilled off to leave a white amorphous powder (270 mg, 54.0%) which could not be crystallized after all. Easily soluble in methanol and water. Mp 201~203°C. $[\alpha]_D^{15} - 45.5^\circ$ ($c=1.67$, H_2O). IR $\nu_{\max}^{KBr}$ cm^{-1} : 3300 (OH), 2920, 2880, 2850 (CH_2), 1720 (CO-O), 1380 (CH_3). *Anal.* Found: C, 54.03; H, 7.76; Calcd. for $C_{39}H_{62}O_{18} \cdot 2H_2O$: C, 54.15; H, 7.60%. Acetate, 123~125°C. $[\alpha]_D^{15} - 22.2^\circ$ ($c=1.13$, $CHCl_3$). IR $\nu_{\max}^{KBr}$ cm^{-1} : 3300 (OH), 2920, 2850, 2880 (CH_2), 1720 (CO-O), 1380 (CH_3). *Anal.* Found: C, 56.33; H, 6.54; Calcd. for $C_{60}H_{84}O_{28}$: C, 56.78; H, 6.62%. NMR $\delta_{MeSi}^{CDCl_3}$: 0.73~2.50 (methyl and methylene protons of dihydrosteviol); 0.75 (singlet, methyl protons); 1.03 (doublet, $J=6$ Hz, methyl protons); 1.26 (singlet, methyl protons); 2.0~2.26 (sugar acetoxyl protons); 3.46~5.33 (sugar protons); 5.80 (doublet, $J=8$ Hz, anomeric protons of esterified glucose).

4) *Dihydrosteviol-13-O-β-sophoroside-19-O-methylester* (XV). **XII** (0.5 g) in 50% ethanol (30 ml) was reduced with hydrogen as described above give **XV** in a yield of 0.31 g (62.0%), mp 165~169°C. TLC

Rf 0.79. $[\alpha]_D^{14} - 13.2^\circ$ ($c=1.67$, 50% EtOH). IR $\nu_{\max}^{KBr}$ cm^{-1} : 3360 (OH), 2920, 2880, 2840 (CH_2), 1700 (CO-O). *Anal.* Found: C, 57.21; H, 8.35; Calcd. for $C_{38}H_{64}O_{13} \cdot 2H_2O$: C, 57.06; H, 8.36%. Acetate, mp 100~105°C. $[\alpha]_D^{15} - 14.0^\circ$ ($c=0.5$, $CHCl_3$). IR $\nu_{\max}^{KBr}$ cm^{-1} : 2940, 2880 (CH_2), 1750 (CO-O), 1370 (CH_3). *Anal.* Found: C, 58.97; H, 7.11; Calcd. for $C_{47}H_{71}O_{20}$: C, 59.05; H, 7.43%. NMR $\delta_{MeSi}^{CDCl_3}$: 0.67~2.34 (methyl and methylene protons of dihydrosteviol), 0.80 (singlet, methyl protons); 1.00 (doublet, $J=6$ Hz, methyl protons); 1.14 (singlet, methyl protons); 1.90~2.10 (sugar acetoxyl protons); 3.27~5.07 (sugar protons); 3.54 (singlet, methoxyl protons).

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