ganisms. Thanks are also due to W. N. Eigel and M. D. Pierson for the use of their laboratory facilities.

Registry No. α-CD, 10016-20-3; β-CD, 7585-39-9; CDase, 37288-41-8.

LITERATURE CITED

Anderson, G. H.; Robbins, F. M.; Dominigues, F. I.; Long, C. L. *Toxicol. Appl. Pharmacol.* **1963**, *5*, 257.
Burkitt, D.; Painter, N. In "Refined Carbohydrate Foods and Disease"; Burkitt, D.; Trowell, H. C., Eds.; Academic Press: London, 1975; pp 71–83.
Conrad, E. C.; Palmer, J. K. *Food Technol.* (Chicago) **1976**, *30*, 84.
Dekker, J.; Palmer, J. K. *J. Agric. Food Chem.* **1981**, *29*, 480.
French, D.; McIntire, R. L. *J. Am. Chem. Soc.* **1950**, *72*, 5148.
Gerloczy, A.; Fonagy, A.; Szejtli, J. In "Proceedings of the First International Symposium on Cyclodextrins"; Szejtli, J., Ed.; Reidel Publishing Co.: Dordrecht, Holland, 1981; pp 101–108.
Holdeman, L. V.; Cato, E. P.; Moore, W. E. C. "Anaerobe Laboratory Manual", 4th ed.; Virginia Polytechnic Institute and State University: Blacksburg, VA, 1977.
Johnson, J. L. *Appl. Environ. Microbiol.* **1980**, *39*, 407.
Kondo, H.; Nakatani, H.; Hiromi, K. *Agric. Biol. Chem.* **1981**, *45*, 2369.
Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
McFeeters, R. F. *Anal. Biochem.* **1979**, *103*, 302.
Palmer, J. K. *Anal. Lett.* **1975**, *8*, 215.
Palmer, J. K. In "GLC and HPLC Determination of Therapeutic Agents, Part 3"; Tsuji, K., Ed.; Marcel Dekker: New York, 1979; pp 1317–1339.
Pitha, J. *Life Sci.* **1981**, *29*, 307.
Radley, J. A. "Starch and its Derivatives", 4th ed.; Chapman and Hall: London, 1968; pp 290–301.
Robyt, J.; French, D. *Arch. Biochem. Biophys.* **1964**, *104*, 338.
Saenger, W. In "Proceedings of the First International Symposium on Cyclodextrins"; Szejtli, J., Ed.; Reidel Publishing Co.: Dordrecht, Holland, 1981; pp 141–150.
Salyers, A. A.; West, S. E. H.; Vercellotti, J. R.; Wilkins, T. D. *Appl. Environ. Microbiol.* **1977**, *33*, 319.
Szabo, P.; Ferenczy, T.; Serfozo, J.; Szejtli, J. In "Proceedings of the First International Symposium on Cyclodextrins"; Szejtli, J., Ed.; Reidel Publishing Co.: Dordrecht, Holland, 1981; pp 115–122.
Szejtli, J. In "Proceedings of the First International Symposium on Cyclodextrins"; Szejtli, J., Ed.; Reidel Publishing Co.: Dordrecht, Holland, 1981; pp 469–480.
Szejtli, J.; Gerloczy, A.; Fonagy, A. *Drug Res.* **1980**, *30*, 808.
Varel, V. H.; Bryant, M. P. *Appl. Microbiol.* **1974**, *28*, 251.
Vercellotti, J. R.; Salyers, A. A.; Wilkins, T. D. *Can. J. Biochem.* **1977**, *55*, 1190.
Wilkens, T. D.; Walker, C. B. *Appl. Microbiol.* **1975**, *30*, 831.
Yamada, T.; Komiya, T.; Akaki, M. *Agric. Biol. Chem.* **1980**, *44*, 1411.

Received for review May 7, 1984. Accepted August 22, 1984. Presented as part of the 186th National Meeting of the American Chemical Society, Washington, DC, Sept 1983, AGFD 212.

Diterpenoid Sweeteners. Synthesis and Sensory Evaluation of Biologically Stable Analogues of Stevioside

Grant E. DuBois,*[1] Leonard A. Bunes, Paul S. Dietrich, and Rebecca A. Stephenson

Two analogues, 2′,3′,19-tri-*O*-(sodiosulfopropyl)steviolmonoside (**6**) and 2″,3″,3′,19-tetra-*O*-(sodiosulfopropyl)steviolbioside (**7**), of the biologically labile diterpenoid triglycoside Stevioside (**1**) were prepared. Under conditions simulating those of the human GI tract, **6** and **7** were found to be completely stable while **1** was converted completely to the aglycon, steviol. The purpose of this work was to obtain sweet analogues of **1** possessing a safety advantage over **1** due to minimal GI-tract absorption resulting from increased molecular weight and charge. Neither **6** nor **7** exhibited significant sweet taste quality. The sensory properties of **1**, nine other analogues of **1**, and six other nonnutritive sweeteners, including saccharin, Acesulfam-K, sodium cyclamate, Aspartame, neohesperidin dihydrochalcone, and monoammonium glycyrrhizzinate, are also presented.

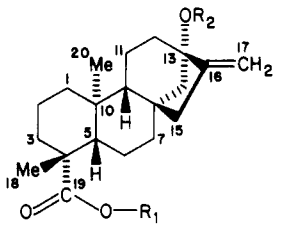
The public interest in development of safe nonnutritive substitutes for carbohydrate sweeteners is very high. This has been demonstrated most conclusively by the strong consumer acceptance of Aspartame, a dipeptide sweetener composed of the two natural amino acids, L-Phe and L-Asp. In this case, safety for human consumption was anticipated from the natural nutrient composition. Our approach toward the development of a safe nonnutritive sweetener has been entirely different. Our strategy assumes that a sweet compound that is nonabsorbable through the gastrointestinal (GI) tract will be devoid of toxicity. Earlier, we reported results of a study on the effect of charge and molecular weight on GI-tract absorption of some dihydrochalcone sweeteners (Wingard et al., 1978). In essence, a cutoff for significant (≤1%) absorption was found to occur at approximately 1000 daltons. In addition, increased negative charge was observed to reduce absorption. This result would be anticipated if one assumes a phospholipid membrane model for GI-tract cells. Here anionic species would experience charge repulsion during approach to the membrane.

Recently, we reported that the 19-*O*-β-D-glucosyl moiety of Stevioside (**1**) (Scheme I) could be replaced by a sodiosulfopropyl moiety to give **2**, a compound where the sweet taste character was not only retained but also improved considerably (DuBois et al., 1981b). As a bonus, it found that, whereas **1** is rapidly metabolized to the potentially toxic (Vignais et al., 1966) aglycon, steviol (**3**), **2** gives only the dessophoro derivative **4**. Although it was found that the 13-*O*-β-D-sophorosyl moiety of **2** could not be substituted by other polar groups (e.g., sodiosulfopropyl) without loss of sweet taste character, it has been deter-

Chemical Synthesis Laboratory, Dynapol, Palo Alto, California.
[1] Present address: NutraSweet Group, G. D. Searle & Co., Skokie, IL 60076.

0021-8561/84/1432-1321$01.50/0

Scheme I



1, (Stevioside), R_1=β-D-glucosyl; R_2=β-D-sophorosyl
2, R_1=$(CH_2)_3SO_3Na$; R_2=β-D-sophorosyl
3, (steviol), R_1=R_2=H
4, R_1=$(CH_2)_3SO_3Na$, R_2=H
5, (Alpha G-P), R_1=β-D-glucosyl [α-D-(4-1)-glucosyl]$_x$;
R_2=β-D-sophorosyl [α-D-(4-1)-glucosyl]$_y$, $x + y = 9$
6, R_1=$(CH_2)_3SO_3Na$; R_2=2,3-di-*O*-(sodiosulfopropyl)-β-D-glucosyl
7, R=$(CH_2)_3SO_3Na$;
R_2=2',3,3'-tri-*O*-(sodiosulfopropyl)-β-D-sophorosyl
8, (Rubusoside), R_1=R_2=β-D-glucosyl
9, (Rebaudioside A), R_1=β-D-glucosyl;
R_2=3-*O*-β-D-glucosyl-β-D-sophorosyl
10, (steviolbioside), R_1=H; R_2=β-D-sophorosyl
11, (steviolmonoside), R_1=H; R_2=β-D-glucosyl
12, R_1=$(CH_2)_3SO_3Na$; R_2=β-D-glucosyl

mined that the sophorose moiety may be modified to give material of improved sensory properties. Thus, Miyake has described Alpha G-P, in which glucose units have been appended to the terminal glucose moieties of **1** in an α_{1-4} manner (Miyake, 1980). Although this compound also lacks metabolic stability, it exhibits improved sweet taste character over **1**. Therefore, for the reason of obtaining a completely metabolically stable analogue of **2**, we elected to prepare **6** and **7**. It was hoped that, since the sulfopropyl moiety has found general utility in replacing carbohydrate functionality without loss of sweet taste character (DuBois et al., 1977, 1981b) in dihydrochalcone and stevioside sweeteners, these compounds would be sweet. Further, it was expected that these compounds would be metabolically stable and be poorly absorbed across the GI tract.

EXPERIMENTAL SECTION

Sensory Evaluation. The compounds evaluated by the human sensory panel were either synthesized as described under synthetic Procedures or were obtained from the following preparations or commercial sources: Stevioside (**1**), **2**, steviol potassium salt (**3**), **4**, and steviolbioside sodium salt (**10**) were obtained as described earlier (DuBois et al., 1981b). Alpha G-P was obtained from the Toyo Sugar Refining Co., Japan. The analysis of this sample has been described (DuBois and Lee, 1983). Rebaudioside A was isolated by preparative radial chromatography (silica gel PF-254; $CHCl_3$–CH_3OH–H_2O) of a crude *Stevia rebaudiana* extract (Steviron F) obtained from Morita Kagaku Kogyo Co., Ltd., Japan. The remaining compounds that were evaluated were either obtained from Sigma Chemical Co. or prepared as described in the synthetic procedures given below except for Acesulfam potassium salt (Hoechst Pharmaceutical Co.), neohesperidin dihydrochalcone (Nutrilite Products, Inc., Buena Park, CA), and monoammonium glycyrrhizzinate (Maruzen Pharmaceutical Co., Ltd., Japan).

Biological Studies. The biological stabilities of **6** and **7** were assayed by methods developed earlier (Wingard et al., 1980) and employing Stevioside (**1**) as a control. The changes in concentration per unit time were quantitated by HPLC. No change in the concentrations of either **6** or **7** was observed under conditions that quantitatively degrade **1** to steviol.

The absorption study on ^{14}C-labeled **2** was carried out by the method described for steviol-*17*-^{14}C (Wingard et al., 1980). The labeled **2** was prepared (Hale et al., 1981) by alkylation of steviolbioside (**10**) with 1,3-propanesultone-*5*-^{14}C by the method described earlier for preparation of **2** (DuBois, et al., 1981b).

Synthetic Procedures. All organic starting materials and reagents were obtained from Aldrich Chemical Co. except for Stevioside (Morita Chemical Co., Japan). Stevioside was obtained in pure form by recrystallization (EtOH) of the commercial product, Steviron F. Solvents used were reagent grade and obtained from either J. T. Baker or Fisher Scientific Co. Dimethylformamide (DMF) and dimethyl sulfoxide (Me_2SO) were distilled in vacuo from CaH_2 and stored over activated (400 °C, 3 h) molecular sieves (J. T. Baker Chemical Co.) prior to use.

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer Model 137 infrared spectrometer. Proton magnetic resonance spectra were recorded on either a Varian T-60A spectrometer or a Brüker 100-MHz instrument and are reported as parts per million (ppm) relative to tetramethylsilane. Combustion analyses were preformed by the Microanalysis Laboratory, Stanford University, Stanford, CA. Neutralization equivalents were obtained by potentiometric titration vs. 0.100 N NaOH. Sulfonic acid salts were first converted to the protic form by ion exchange on Bio-Rad AG MP-50 cation-exchange resin (acid form). Values given were calculated for the sodium salts and are corrected for water content, which was determined by Karl Fischer titration.

Analytical thin-layer chromatography (TLC) was carried out on prelayered silica gel F-254 plates (E. Merck, Darmstadt, West Germany) visualizing with either UV light or sulfuric acid charring. Preparative radial chromatography was carried out on a Harrison Research (Palo Alto, CA) Chromatotron using a 4-mm silica gel PF-254 rotor. High-pressure liquid chromatography was carried out on a Waters Associates Instrument equipped with a Model 660 solvent programmer and two Model 6000A pumps. Analytical work was carried out on a μBondapak C-18 reverse-phase column (30 cm × 3.9 mm i.d.) eluting with a linear program (15 min, 2 mL/min) of 10–40% MeCN in 0.005 M KH_2PO_4 (pH 3.4) unless otherwise indicated. The detector employed was a Schoeffels Model SF 770 spectroflow monitor equipped with a Model GM 770 monochromator. Detection was carried out at 200 nm.

Unless otherwise indicated, all reactions were carried out under an inert atmosphere of argon with vigorous magnetic stirring. The final products were assayed for purity by a combination of (1) proton titration, after ion exchange of sulfonic acid sodium salts to the acid form on Bio-Rad AG MP 50 cation exchange resin, (2) HPLC, and (3) Karl Fischer analyses. Titrations were carried out with a Brinkman Metrohm Herisau Potentiograph E576.

Caution: 1,3-propanesultone has been shown to be a potent carcinogen in animals (Druckey et al., 1968; Doak et al., 1976; Ulland et al., 1971). Reactions employing it should be handled with extreme caution.

Rubusoside (**8**). This compound was prepared by the method very briefly described by Kaneda et al. (1977). Thus, 4.60 g of Stevioside was incubated at 37 °C with 4.60 g of Sanzyme R in 460 mL of McIlvain's buffer until TLC analysis ($CHCl_3$–CH_3OH–H_2O, 15:10:2) indicated the reaction to be complete (5 days). The reaction mixture was then diluted with 800 mL of water, cooled to 5 °C, and extracted with 1-butanol (3 × 300 mL). The combined extracts were concentrated in vacuo to yield 4.11 g of a tan solid. This crude product was recrystallized twice from absolute methanol to give 0.874 g of colorless clusters: mp

178.5–181 °C [lit. (Kaneda et al., 1977) mp 178–182 °C]; TLC ($CHCl_3$–MeOH–H_2O, 15:10:2) R_f = 0.42; HPLC, t_R = 10.8 min; Neutralization equivalents (saponification product): calcd, 642.7; found, 649.2. This sample was found to be identical, by chromatographic and mixed melting point analyses, to an authentic sample (Tanaka et al., 1981).

Steviolmonoside (**11**). This compound was prepared by the method very briefly described by Kaneda et al. (1977). Thus, 4.06 g of 13,19-*O*-di-β-D-glucosylsteviol was added to a mixture of 95 mL of CH_3OH and 95 mL of 10% NaOH. The resultant reaction mixture was refluxed for 1.5 h, cooled to 5 °C, and then acidified, by dropwise addition of ice-cold 40% H_2SO_4, to pH 3. The precipitate thus obtained was filtered on a Büchner funnel and dried to give 2.7 g of a tan solid. Recrystallization from 2-butanone–CH_3OH yielded 1.16 g of light tan crystals: mp 217–221 °C; TLC ($CHCl_3$–CH_3OH–H_2O, 15:10:2) R_f = 0.68; HPLC, t_R = 18.7 min. Neutralization equivalents: calcd. 480.6; found, 483.5.

13-O-β-D-Glucosyl-19-O-(sodiosulfopropyl)steviol (**12**). To a solution of 471 mg (0.98 mmol) of steviolmonoside in 7 mL of dry DMF was added 149 mg (1.08 mmol) of anhydrous K_2CO_3 and 144 mg (1.18 mmol) of 1,3-propanesultone. The resultant reaction mixture was stirred at ambient temperature for 21 h. At this point, TLC analysis ($CHCl_3$–CH_3OH–H_2O,15:10:2) showed all starting materials (R_f = 0.58) to be consumed to give one product (R_f = 0.32). Ten percent KOH (0.42 mL) was added to the reaction mixture, which was then stirred overnight at 40 °C. Twenty milliliters of water was then added and the pH adjusted to 5 with 3% HCl. Concentration in vacuo yielded 900 mg of a white solid. The crude product was converted to the sodium form by passage through a column of 400 g of Bio-Rad AG-MP 50 ion-exchange resin (Na form). The desired product was then obtained in pure form by preparative radial chromatography ($CHCl_3$–CH_3OH–H_2O) and lyophilization to yield 394 mg of **12** as a flocculent white solid: HPLC, t_R = 14.7 min. Neutralization equivalents: calcd. 624.7; found, 637.6.

Steviolmonoside 4',6'-Benzylidene Acetal (**13**). Ten millilters of 0.1% $HClO_4$ in DMF was added to a solution of 481 mg (1.00 mmol) of steviolmonoside and 761 mg (5.0 mmol) of benzaldehyde dimethyl acetal in 25 mL of dry DMF. The resultant reaction mixture was stirred at 45–50 °C for 4 h. TLC analysis ($CHCl_3$–CH_3OH–H_2O,15:5:0.5) at this time indicated that all starting materials (R_f = 0.40) had been consumed to yield one product (R_f = 0.69). The reaction mixture was then poured into water and extracted with ether (4 × 30 mL), the combined portions of which were washed with water (5 × 30 mL), dried ($MgSO_4$), and concentrated in vacuo to give 670 mg of a glassy solid. Recrytallization from absolute CH_3OH yielded 290 mg (51%) of colorless needles: mp 163–168 °C [lit. (Kaneda et al., 1977) mp 167–170 °C]; HPLC, t_R = 11.6 min.

2',3',19-Tri-O-(sodiosulfopropyl)steviolmonoside (**6**). A stock solution of 0.50 M potassium dimsylate in Me_2SO was prepared by addition of 0.60 g (15 mmol) of KH (2.42 g of 24.9% mineral oil suspension washed 3 times with hexane) to 30 mL of Me_2SO. A stock solution of 2.0 M 1,3-propanesultone in Me_2SO was then prepared by dissolution of 1.83 g (15 mmol) in 7.5 mL of Me_2SO. To a solution of 569 mg (1.00 mmol) of steviolmonoside, 4',6'-benzylidene acetal and 5 mg of triphenylmethane in 5 mL of Me_2SO was added 0.50 M potassium dimsylate stock solution until a persistent red color was observed (6.6 mL, 3.3 mmol). One and a half milliliters of the propanesultone stock solution (3.0 mmol) was then added dropwise. The red color was instantly discharged. This procedure was repeated until TLC analysts ($CHCl_3$–CH_3OH–H_2O,15:5:0.5) showed the reaction mixture to contain a single product (R_f = 0.03). Five additions were required. Excess propanesultone was then decomposed by addition of 8 mL of 10% KOH and heating at 40–50 °C for 16 h. The acetal was then hydrolyzed by pouring the reaction mixture into 160 mL of 0.25 N H_2SO_4 and stirring the resultant homogeneous solution overnight at ambient temperature. The solution was then neutralized by the addition of 10% KOH and concentrated in vacuo to give 21.3 g of a yellow solid. HPLC analysis indicated the presence of a single product having t_R = 11.6 min. The crude product was converted to the sodium salt and fractionated by ion-exchange chromatography over a column of 400 g of Bio-Rad AG MP-50 (Na) ion-exchange resin. This product was then desalted by chromatography over a column of Bio-Rad AG 11A8 ion retardation resin to yield 1.93 g of a yellow solid. The desired product (440 mg, 44%) was then obtained following preparative radial chromatography as a very hydroscopic white solid: TLC ($CHCl_3$–CH_3OH–H_2O,15:10:2) R_f = 0.17; HPLC, t_R = 11.6 min; IR (KBr) 2.90 (O—H), 5.84 (C=O), 6.04 (C=C), 8.5 (S=O), 9.6 (S=O) μm; NMR (CD_3SOCD_3) δ 0.80 (s, 3 H, 20-CH_3), 1.14 (s, 3 H, 18-CH_3), 4.78 (br s, 2H, 17-CH_2). Neutralization equivalents: calcd 304.3; found, 301.0.

Steviolbioside 4',6',4'',6''-Bis(benzylidene acetal) (**14**). To a solution of 1.29 g (2.00 mmol) of steviolbioside and 1.52 g (10.0 mmol) of benzaldehyde dimethyl acetal in 20 mL of DMF was added 1 drop of 70% $HClO_4$. The resultant homogeneous solution was stirred at 45 °C for 8 h at which point 1.52 g of additional benzaldehyde dimethyl acetal was added and stirring continued at 45 °C for 8 h. The reaction mixture was then poured into 200 mL of water and extracted with ether (3 × 50 mL). The combined extracts were washed with water (6 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo to yield 1.53 g of a white solid. Preparative radial chromatography ($CHCl_3$–CH_3OH–H_2O) yielded 1.26 g (77%) of **14** as a colorless glass. Recrystallization (CH_3OH) yielded **14** as hygroscopic colorless granules: mp 195–205 °C; TLC ($CHCl_3$–CH_3OH, 95:5) R_f = 0.16; HPLC (10–100% MeCN in 0.005 M KH_2PO_4) t_R = 15.3 min; IR (KBr) 2.92 (OH), 5.83 (C=O) μm; NMR (CD_3SOCD_3) δ 0.91 (s, 3 H, 20-CH_3), 1.12 (s, 3 H, 18-CH_3), 4.63 (d, J = 8 Hz, 1 H, 17-H), 4.79 (d, J = 8 Hz, 1 H, 17-H), 7.39 (s, 10 H, Ph-H). Anal. ($C_{46}H_{58}O_{13}·H_2O$): C, H.

2'',3',3'',19-Tetra-O-(sodiosulfopropyl)steviolbioside (**7**). This compound was prepared by a procedure exactly analogous to the preparation of **6**. Thus, 1.06 g (1.24 mmol) of **14** was treated repetitively with potassium dimsylate and 1,3-propanesultone in Me_2SO until HPLC analysis showed the presence of only one component (t_R = 12.4 min). Acetal hydrolysis followed by ion exchange to the sodium salt, ion-retardation chromatography, and recrystallization (CH_3OH–ether) yielded 733 mg (48%) of **7** as a fine, white solid: mp 109–112 °C dec: IR (KBr) 2.92 (OH), 5.87 (C=O), 6.10 (C=C), 8.4 (S=O), 9.6 (S=O) μm; NMR (CD_3SOCD_3) δ 0.80 (s, 3 H, 20-CH_3), 1.13 (s, 3 H, 18-CH_3). Neutralization equivalents: calcd. 304.8; found, 299.0. Anal. ($C_{44}H_{70}O_{25}S_4Na_4·H_2O$): C, H, S, Na.

Sensory Evaluation. The experimental compounds described below were evaluated by a human sensory panel according to the procedures described by Swartz and Furia (1977). An application of this methodology to the dihydrochalcone class of sweeteners is extensively discussed in our earlier work (DuBois et al., 1981a). The same criteria, regarding purity and absence of toxicity, which

Table I. Sensory Evaluation of Stevioside, Stevioside Analogues, and Other Sweeteners[a]

compd	judgments	concn, ppm	I_p[b]	P_w[c]	P_m[c]	taste character: sweet	bitter	other	sweet/bitter, other
1 (Stevioside)	12	500	0.9 (0.1)	190 (10)	440 (30)	62 (10)	30 (10)	8 (5)	62/38
2	12	534	0.8 (0.1)	160 (10)	360 (20)	92 (6)	4 (6)	4 (4)	92/8
3 (steviol K salt)	1	2000	0.5	25	23	0	100	0	0/100
4	1	2000	1	50	70	0	100	0	0/100
5 (Alpha G-P)	12	1690	1.0 (0.1)	60 (5)	300 (30)	88 (4)	8 (4)	4 (4)	88/12
6	6	1250	0.2 (0.2)	16 (16)	40 (40)	8 (9)	67 (41)	25 (45)	8/92
7	6	1000	0.5 (0.2)	50 (20)	160 (70)	23 (19)	27 (27)	52 (36)	23/77
8 (Rubusoside)	12	650	1.0 (0.1)	150 (10)	290 (30)	69 (7)	26 (8)	5 (5)	69/31
9 (Rebaudioside A)	12	638	1.1 (0.1)	170 (10)	470 (20)	85 (5)	12 (6)	3 (2)	85/15
10 (steviolbioside Na salt)	12	1000	1.0 (0.1)	100 (10)	190 (20)	65 (9)	35 (10)	0 (1)	65/35
11 (steviolmonoside Na salt)	12	834	1.3 (0.2)	160 (30)	230 (40)	33 (12)	61 (14)	6 (6)	33/67
12	12	1250	1.2 (0.2)	100 (10)	170 (30)	40 (10)	50 (10)	10 (4)	40/60
sucrose	12	10000	1.0 (0)	1.0 (0)	1.0 (0)	100 (0)	0 (0)	0 (0)	100/0
saccharin	12	330	1.0 (0.1)	300 (30)	180 (20)	85 (6)	12 (8)	3 (2)	85/15
Acesulfam K salt	12	1000	1.0 (0.05)	100 (5)	55 (3)	83 (8)	16 (8)	1 (1)	83/17
sodium cyclamate	12	5000	1.1 (0.1)	22 (2)	13 (1)	94 (3)	2 (2)	4 (3)	94/6
aspartame	12	750	1.0 (0)	130 (0)	85 (0)	100 (1)	0 (1)	0 (0)	100/0
neohesperidin dihydrochalcone	12	250	1.2 (0.1)	480 (40)	860 (70)	81 (8)	3 (2)	16 (8)	81/19
glycyrrhizzic acid monoammonium salt	12	3000	1.0 (0.1)	33 (3)	90 (8)	84 (8)	3 (4)	13 (8)	84/16

[a] Sensory data are reported as follows: mean value ($2S_m$). [b] Sample intensity relative to 10% sucrose; compound concentrations were chosen, when possible, by the panel supervisor to yield solutions having taste intensity comparable to the 10% sucrose reference. [c] P_w = compound potency calculated on a weight basis; P_m = compound potency calculated on a molar basis.

were applied to this earlier work, were applied to the materials described herein. Since none of them showed any toxicity, they were subjected to sensory analysis by a trained panel of judges. Panelists were required to carry our magnitude estimation (vs. 10% sucrose) and taste quality (percent sweet, sour, salty, bitter, and other) evaluations in one sensory session. From this analysis, comparative taste potency data, calculated on both weight (P_W) and molar (P_m) bases, and taste quality data were obtained.

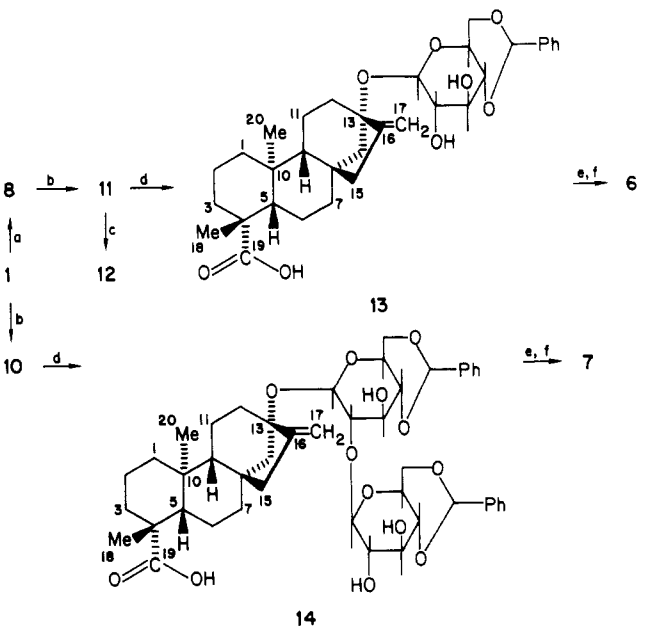
RESULTS AND DISCUSSION

The compounds that provide the subject of this report were synthesized as illustrated in Scheme II. The details of the synthetic methods are described under Experimental Section. The conversion of Stevioside (1) to the desglucosyl derivative 8 was achieved with the aid of Sanzyme R (Sankyo Co., Japan), a crude amylase preparation from *Aspergillus oryzae* (Kaneda et al., 1977). This desglucosyl compound has recently been found to be the natural sweet principle of *Rubus chingii* Hu, the leaves of which have been used as a sweet tea in the southern part of China (Tanaka et al., 1981), and has been named Rubusoside. The results of sensory evaluation of all the compounds synthesized are given in Table I. In addition, for the purpose of comparison, the results of sensory evaluation of some Stevioside analogues and other common sweeteners are also presented in Table I.

Exceedingly few nonnutritive sweeteners exhibit the high taste quality of sucrose. Of all the examples given in Table I, Aspartame taste most closely approximates the taste of sucrose. Sodium cyclamate and synthetic Stevioside analogue 2 are both high-quality sweeteners, but each of them fall slightly short of sucrose and Aspartame. Of the naturally occurring steviol glycosides, Rebaudioside A (9) exhibits the cleanest taste character. Even 9, however, exhibits a bitter taste component comparable to that of saccharin and therefore would be expected to be marginally acceptable for food use.

Although, on first principles, it may be thought that the synthetic Stevioside analogue 2, due to its anionic charge and rather high molecular weight (M_r 804), may be poorly absorbed through the GI tract, this was not observed to be the case. In a study on the absorption of 2 (Hale et al., 1981), ^{14}C-labeled 2 was administered to rats under conditions identical with those employed in an earlier study (Wingard et al., 1980) on the absorption of steviol. The absorption of 2 was determined to be 15%. Although this is an improvement over the quantitative absorption to be expected from 1 or 9, it remains too high for our purposes. Subsequently, it was found (Dietrich, 1981) that 2, on incubation with rat cecal contents, under the earlier described conditions (Wingard et al., 1980), was quantitatively converted within 2 days to the dessophoro compound 4. Thus, the higher than anticipated absorption of 2 is likely due to metabolism to 4, which is then more efficiently absorbed due to its lower molecular weight (M_r 480).

Scheme II[a]



[a] (a) Sanzyme R. (b) NaOH. (c) K_2CO_3, 1,3-propanesultone, DMF. (d) $PhCH(OCH_3)_2$, $HClO_4$, DMF. (e) KCH_2SOCH_3, 1,3-propanesultone, Me_2SO. (f) H_3O^+.

For the present work, we reasoned that, since the rather poor taste quality of Stevioside was dramatically improved by substitution of sodiosulfopropyl for the 19-*O*-β-D-glucosyl moiety (i.e., 1 → 2), substitution of the 2′-*O*-, 3′-*O*- and 19-*O*-β-D-glucosyl moieties of Rebaudioside A (9) with the same polar group (i.e., 9 → 6) may resut in a compound with improved taste quality. We further reasoned that substitution of the 2′- and 3′-*O*-β-D-glucosyl moieties of 9 with sodiosulfopropyl may result in a biologically stable compound that, due to its increased charge and molecular weight (M_r 846), may be nearly nonabsorbable through the GI tract. In fact, when 6 was incubated with rat cecal contents under conditions that quantitatively degrade 1, no change of 6 could be detected. Thus, low absorption of 6 would be anticipated. However, the taste character of 6 is very poor. The compound is essentially completely bitter with only a hint of sweet taste.

In related work, we prepared the tetra-*O*-sodiosulfopropyl derivative 7 (M_r 1130) of steviolbioside (10) for reasons analogous to those described above. As was described for 6, 7 was completely stable to the enzymes of the rat cecum. Unfortunately, as was observed for 6, 7 was mainly bitter in taste character with only weak sweetness.

On inspection of the data on Table I, several conclusions are possible. First, since 2 is a better quality sweetener that Stevioside (1), the 19-O carbohydrate moiety of 1 is not involved in receptor binding interaction. It is probable that the 19-*O*-β-D-glucosyl functionality of 1 may be substituted by a variety of polar groups without a major change in taste properties. Second, although Rubusoside is exceptional, it appears that for the presence of a high-quality sweet taste, the 13-O substituent must minimally be a disaccharide. The compounds lacking 13-O carbohydrate functionality (3, 4) or containing only a monosaccharide (11, 12) are totally and predominantly bitter in taste character, respectively. Compounds containing 13-O disaccharide substituents (1, 2, 10) are mainly sweet while those containing 13-O tri- or polysaccharide substituents (9, 5) exhibit taste quality equaling or exceeding that of saccharin. The fact that compounds that contain 13-O substituents as different as those present in 5 and 9 are both high-quality sweeteners shows that the structural constraints on the 13-O substituent are not severe. Thus, it is likely that the sweet taste receptor does not bind in any specific fashion to the 13-O substituent. The polar moiety at this position probably functions to potentiate molecular hydrophilicity, so as to allow optimum interaction with the sweet taste receptor while minimizing the more general hydrophobic interactions involved in bitter taste elicitation. Negative charge on the 13-O substituent, however, seems to be highly detrimental. In 6 and 7, which are analogues of this type, the charge may be disfavoring interaction of nearby essential binding functionality of a region of the receptor protein, which is also negatively charged.

In summary, our results show that is possible to prepare biologically stable Stevioside analogues. Molecular weights can be achieved where absorption through the GI tract is expected to be minimal. Although the sensory properties of the sodiosulfopropyl-substituted analogues 6, and 7 were poor, the rather high taste quality of the glucose-substituted analogue 5 clearly shows that high molecular weight Stevioside analogues may have attractive sensory properties. One might consider replacement of the metabolically *labile* glucose moieties of 5 with other biologically *stable* groups so as to obtain nonabsorbable analogues with attractive sweet taste properties. This concept will provide the subject of future work.

ACKNOWLEDGMENT

We thank G. McGarraugh for analytical studies, F. Enderlin for biological safety studies, and J. Lee for the sensory studies described in this work. In addition, we are grateful to O. Tanaka for a generous sample of Rubusoside and for advice on its enzyme-promoted preparation from Stevioside (Tanaka, 1980).

Registry No. 1, 57817-89-7; 2, 79184-06-8; 3 (K salt), 92013-86-0; 4, 92013-87-1; 5, 86752-59-2; 6, 92013-88-2; 7, 87972-91-6; 8, 64849-39-4; 9, 58543-16-1; 10 (Na salt), 79200-40-1; 11 (Na salt), 87078-05-5; 12, 87956-99-8; 13, 71769-08-9; 14, 87972-89-2; 1,3-propanesultone, 1120-71-4; sucrose, 57-50-1; saccharin, 81-07-2; acesulfam K salt, 55589-62-3; sodium cyclamate, 139-05-9; aspartame, 22839-47-0; neohesperidin, 13241-33-3; dihydrochalcone, 1083-30-3; glycyrrhizic acid monoammonium salt, 53956-04-0.

LITERATURE CITED

Dietrich, P. S., Dynapol, Palo Alto, CA, unpublished data, 1981.
Doak, S. M. A.; Simpson, B. J. E.; Hunt, P. F.; Stevenson, D. E. *Toxicology* **1976**, *6*, 139.
Druckey, H.; Kruse, H.; Preussmann, R. *Naturwissenschaften* **1968**, *55*, 449.
DuBois, G. E.; Crosby, G. A.; Stephenson, R. A. *J. Med. Chem.* **1981a**, *24*, 408–428.
DuBois, G. E.; Crosby, G. A.; Stephenson, R. A.; Wingard, R. E. *J. Agric. Food Chem.* **1977**, *25*, 763–772.
DuBois, G. E.; Dietrich, P. S.; Lee, J. F.; McGarraugh, G. V.; Stephenson, R. A. *J. Med. Chem.* **1981b**, *24*, 1269–1271.
DuBois, G. E.; Lee, J. F. *Chem. Senses* **1983**, *7*, 237–247.
Hale, R. L.; Enderlin, F. E.; Ng. S., Dynapol, Palo Alto, CA, unpublished results, 1981.
Kaneda, N.; Kasai, R.; Yamasaki, K.; Tanaka, O. *Chem. Pharm. Bull.* **1977**, *25*, 2466–2467.
Miyake, T. British Patent 2 027 423A, 1980.
Swartz, M. L.; Furia, T. E. *Food Technol. (Chicago)* **1977**, *11*, 51–67.
Tanaka, O., Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Hiroshima, Japan, personal communication, 1980.
Tanaka, T.; Kohda, H.; Tanaka, O.; Chen, F.-H.; Chou, W.-H.; Leu, J.-L. *Agric. Biol. Chem.* **1981**, *45*, 2165–2166.
Ulland, B.; Finkelstein, M.; Weisburger, E. K.; Rice, J. M.; Weisburger, J. H. *Nature (London)* **1971**, *230*, 460.
Vignais, P. V.; Duee, E. D.; Vignais, P. M.; Huet, J. *Biochim. Biophys. Acta* **1966**, *118*, 465–483.
Wingard, R. E.; Brown, J. P.; Enderlin, F. E.; Dale, J. A.; Hale, R. L.; Seitz, C. T. *Experientia* **1980**, *36*, 519–520.
Wingard, R. E.; Crosby, G. A.; DuBois, G. E. *CHEMTECH* **1978**, *8*, 616–621.

Received for review April 16, 1984. Accepted July 23, 1984.