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STEVIOSIDE. I. THE STRUCTURE OF THE GLUCOSE MOIETIES

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In certain areas of Paraguay there is found a small wild shrub of the composite family known locally as Kaà hê-ê which, because of the remarkable sweetness of its leaves, has been the object of botanical, physiological, and chemical investigation for many years. Initially named *Eupatorium Rebaudianum* by Bertoni in 1899 (1), this relatively rare plant was later recognized by him as belonging to the genus Stevia and was given the name *Stevia Rebaudiana* Bertoni (2) which it still bears.[3] Cultivation of the plant has been carried out in Paraguay both as a horticultural curiosity and on a larger scale but the latter is expensive since the seed is usually sterile and reproduction must be made by subdivision. Chemical investigation of the plant may be said to have begun in 1908 with the work of Rasenack (4) who extracted the leaves with hot alcohol and obtained a crystalline glycoside which, on acid hydrolysis, afforded a second crystalline product. However, the physical constants and analytical values reported for these substances leave their nature still in doubt. In the following year Dieterich (5) extracted the leaves and twigs with water and obtained two fractions: (a) a crystalline, alcohol-soluble part named *eupatorine* and (b) an amorphous, alcohol-insoluble part, designated *rebaudine*. Neither was adequately characterized and it was not until 1931 that real progress in this field was made. In that year Bridel and Lavieille (6) reinvestigated *S. Rebaudiana* and, through aqueous alcoholic extraction of the leaves, obtained a pure, crystalline glycoside which proved to be some 300 times as sweet as sucrose and thus was (and still is, to the authors' knowledge) the sweetest natural product and the only non-nitrogenous or carbohydrate-containing substance of high sweetening power yet discovered (7). The glycoside, named by these authors *stevioside*, was found to form a hydrate of indefinite composition, its water content varying with atmospheric moisture. While the substance was unattacked by emulsin, rhamnodiastase, air-dried brewers' yeast, and powder of *Aspergillus niger*, hydrolysis with 5% sulfuric acid at 100° afforded a crystalline non-sugar product, presumed to be the aglucon, and crystalline D-glucose as the only sugar component. In a succeeding paper (8) the same authors reported the successful enzymatic hydrolysis of stevioside using either the digestive juice or hepato-pancreatic juice of the vineyard snail (escargot; *Helix pomatia*). However, while the sugar thus liberated

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[3] A botanical description of the various Stevias, including Rebaudiana, may be found in (3).

was identical with that obtained on acid hydrolysis, the non-sugar product was different from, but isomeric with, the one obtained earlier. The substance liberated enzymatically was presumed to be the true aglucon and given the name *steviol*, while the product of acid hydrolysis was called *isosteviol*. Acid was found to convert steviol to isosteviol. Analytical values indicated the formula $C_{20}H_{30}O_3$ for the aglucon and, from this and from the yield of D-glucose afforded by stevioside on hydrolysis, Bridel and Lavieille arrived at the formula $C_{38}H_{60}O_{18}$ for stevioside, *i.e.*, steviol condensed with three D-glucose residues. Analyses of rigorously dried stevioside confirmed this formula. Bridel and Lavieille found that stevioside is a neutral substance but, except for establishing the lack of toxicity of the glucoside toward the guinea pig, rabbit, and cock (9), nothing further seems to have been done with regard to the structure of stevioside during the intervening years although attention has repeatedly been drawn to this apparently unique substance (7, 10–13).

An investigation of a complex glycoside such as stevioside may logically be divided into two parts: first, the structure and mode of attachment of the sugar residues and, second, the structure of the aglycon. In the present case these aspects were dealt with by independent but closely collaborating groups. This paper describes what has been found concerning the sugar moieties in stevioside while the following one (14) is devoted to the structure of the aglucon and its transformation products.

The procedures which Bridel and Lavieille (6) employed for the extraction of stevioside may be considerably simplified by the use of ion-exchange resins, materials which were, of course, not available at the time of the earlier work. Aqueous extraction of the leaves and twigs of the air-dried sample of *S. Rebaudiana* available to us, followed by deionization, led to the isolation of nearly pure stevioside in 7% yield. Analysis confirmed the formula, $C_{38}H_{60}O_{18}$, arrived at by Bridel and Lavieille (8). The glucoside was found to consume five moles of periodate while two moles of formic acid were liberated. Methylation, followed by hydrolysis and paper chromatography of the methylated sugars liberated, showed that only two of these latter were formed, one being present in roughly twice the quantity of the other. These preliminary data indicate that the three glucose units are most probably not linked separately to the three oxygen functions of the aglucon. A choice among the numerous remaining possibilities was made in an unexpected fashion. Strong alkali was found to attack stevioside readily; upon subsequent acidification with acetic acid a new, relatively insoluble substance without detectable sweetness was obtained. Analysis showed that one of the three sugar residues had been removed and the new glucoside was, therefore, given the trivial name of steviolbioside. The fate of the sugar residue removed by alkali proved to be interesting, for it was isolated as levoglucosan (III, 1,6-anhydro-β-D-glucopyranose). Furthermore, steviolbioside was found to be a titratable acid and its infrared absorption spectrum showed it to have a carboxyl group. Chemical evidence (14) indicated that the carboxyl group in steviolbioside and in isosteviol is a highly hindered one. Since this is apparently the first occasion when the hydrolysis of a sugar carboxylic ester has been observed to give a glycosan, the reaction will be discussed later in this paper.

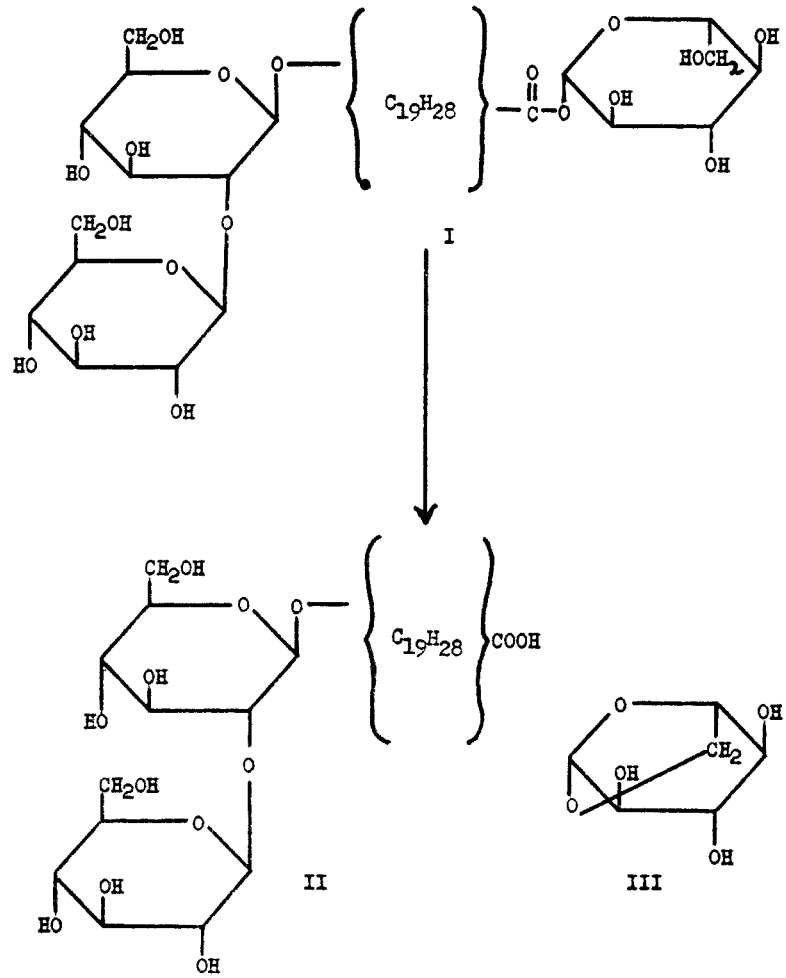

FIGURE 1

Since stevioside is practically without reducing power,[4] it follows from the above evidence that one glucopyranose moiety is attached at C_1 to a carboxyl group. Since only one oxygen function remains in the aglucon the two remaining glucose moieties must be joined together in stevioside and steviolbioside. The latter compound was found to consume 3 moles of lead tetraacetate and thus it is evident that the linkage between the sugar residues is at C_2 or C_4. Methylation of steviolbioside followed by acid hydrolysis afforded the methyl ester of isosteviol (14) and a mixture of partially methylated sugars which were tentatively identified through paper chromatography as 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4,6-tri-*O*-methyl-D-glucose. The former of these was then isolated in crystalline form as its crystalline 1-azoate (15). The cellulose column technique

[4] Even the most highly purified samples of stevioside reduce Fehling solution to a slight extent and it seems probable that a portion of the carboxyl-attached glucose residue is removed in reducing form (rather than as levoglucosan) by the action of alkali.

of Hough, Jones, and Wadman (16) was used to resolve the mixture of methylated sugars and 3,4,6-tri-*O*-methyl-D-glucose was obtained in crystalline form. The x-ray diffraction pattern of the latter was identical with authentic material. Borate ionophoresis of the unknown gave an M_G value (17) identical with that of authentic material and effectively differentiated it from 2,3,6-tri-*O*-methyl-D-glucose. It is apparent, then, that the glucose moieties in steviolbioside are attached as in II and that stevioside may be represented by I. The evidence adduced here throws no light, of course, on the configuration of the anomeric carbon atoms. The stability of the substance toward the usual α- and β-glucosidases blocks the normal path by which such questions are resolved. Furthermore, while infrared spectra have been shown to be useful in distinguishing between anomers (18–20), the infrared spectra of stevioside and steviolbioside appear to offer no clear answer in this case.

It appears, then, that the structure of the sugar moieties of stevioside is as unusual as its sweetness. On the one hand, C_2-linked glucose units are relatively rare, having thus far been found only in the hemicellulose from Iceland moss (21), in the polysaccharide from the crown-gall (22), and in sophorose (2-*O*-β-D-glucopyranosyl-D-glucose) which occurs as a glycoside of kaempferol in the fruit of *Sophora japonica* L. (23–26) and is formed in low yield by the action of emulsin on glucose (27).[5] On the other hand, carbohydrate esters of sterically hindered acids have not, to the authors' knowledge, been found in nature before.

Levoglucosan has been formed by a number of reactions (28) and the action of alkali on aryl β-D-glucopyranosides may be cited as the most widely studied of these. The work of McCloskey and Coleman (29) and of Lemieux and Brice (30) has led to the elaboration of a clear picture for the mechanism of this reaction, a mechanism which is dependent upon the initial loss of a negative aryloxy ion from C_1. Now nothing of this sort has hitherto been observed in the hydrolysis of sugar *esters* because these normally hydrolyze through nucleophilic attack by OH^- on the carbonyl carbon atom. With the esters of sterically hindered acids, however, the carbonyl carbon is relatively inaccessible for such attack and scission takes place primarily between the oxygen and C_1 of the sugar with formation of the same intermediate as obtained by the action of alkali on aryl β-D-glucopyranosides. The seemingly diverse reactions are, therefore, practically identical. The formation of levoglucosan through the alkaline hydrolysis of sterically hindered esters of D-glucose is under investigation and will be the subject of a future paper.

In the course of the above work a quantity of authentic 3,4,6-tri-*O*-methyl-D-glucose was required. While syntheses for this substance have recently been devised by Sundberg, McCloskey, Rees, and Coleman (31) and by Greville and Northcote (32) the starting materials used by these authors are not as readily

[5] In contrast to stevioside, both kaempferol sophoroside (26) and sophorose itself (25) are cleaved by emulsin. While sophorose is readily split from kaempferol sophoroside by hot dilute acid, attempts to obtain a disaccharide from stevioside and steviolbioside by similar means have thus far failed. Whether stevioside is a sophoroside or not remains, therefore, in doubt.

accessible as methyl 4,6-*O*-benzylidene-2-*O*-*p*-tolylsulfonyl-α-D-glucopyranoside. A relatively simple and easy synthesis of 3,4,6-tri-*O*-methyl-D-glucose from this latter substance is described here.

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EXPERIMENTAL[6]

The isolation of stevioside from Stevia Rebaudiana *Bertoni.* Two hundred grams of air-dried and coarsely ground leaves and stems of *Stevia Rebaudiana* Bertoni[7] was mixed with 10 g. of powdered calcium carbonate and extracted with 1200 ml. of water in a pharmaceutical percolator at room temperature for about 20 hrs. After removal of the solvent by draining, the residue was washed with 300 ml. of water and the excess solvent was removed by use of a food press. The cake was stirred with 600 ml. of water and repressed. This latter process was carried out twice more and the combined filtrate and pressings stirred with Amberlite IR-120(H). The precipitate which formed, together with the resin, was removed by filtration on a bed of Hyflo Super-Cel.[8] The filtrate was then deionized by successive passage through columns of Amberlite IR-120(H) (45 x 800 mm.) and Duolite A-4 (45 x 800 mm.) and the effluent was concentrated *in vacuo* at 45–50° (bath) to a honey-like sirup.[9] Dissolved in 175 ml. of methanol and left at +5° overnight, this material deposited crude crystalline stevioside which, washed with 50 ml. of methanol and dried at 40°, weighed 20.2 g. The greenish solid was extracted with 4 parts of boiling dioxane and the residue was removed by filtration on a bed of Filter-Cel. Addition of one part of methanol to the filtrate initiated crystallization. After the mixture had stood at room temperature overnight, 14 g. (7%) of colorless, nearly pure stevioside melting at 198–202° and rotating −38.8° in water (*c*, 0.64) was obtained. Further recrystallization from dioxane-methanol gave, with little loss, pure stevioside; dried at 100° *in vacuo* it melted at 196–198° (Kofler) and showed a rotation of −39.3° in water (*c*, 5.7). In absolute alcohol a rotation of −29.6° (*c*, 3.56) was found. Bridel and Lavieille (6) reported an "instantaneous m.p." of 238–239° (Maquenne block) and a rotation in 95% alcohol of −31.81° (anhydrous basis).

Anal. Calc'd for $C_{38}H_{60}O_{18}$: C, 56.70; H, 7.51.

Found: C, 56.67; H, 7.40.

On standing in the air, the anhydrous material rapidly absorbs moisture. The relatively insoluble crystalline hydrate obtained from aqueous solution (6) loses water of crystallization on standing in the air. The final moisture content of stevioside was found to vary with the atmospheric humidity. The material shows absorption characteristic of an ester at 5.73 μ; in addition, a second peak at 5.78 μ is, by analogy with the behavior of dihydroisosteviol (14), indicative of hydration.

Oxidation of stevioside with sodium metaperiodate. Anhydrous stevioside (102 mg.), dissolved in a small amount of water, was treated with 5.0 ml. of 0.4905 *M* sodium metaperi-

[6] Melting points are corrected. Rotations are specific rotations for the D line of sodium at 20° unless otherwise specified. Concentration is expressed in g. of substance per 100 ml. of solution.

[7] The material employed in this research was imported from Paraguay some years ago.

[8] Omission of this pretreatment with IR-120(H) makes subsequent passage through ion-exchange columns rather difficult.

[9] While the material is highly colored at this stage the use of decolorizing carbon is to be avoided since Darco G-60 was found to adsorb the glycoside tenaciously.

odate and the solution was made up to 25 ml. After 20 hours at room temperature analysis showed the consumption of 5.20 moles of periodate and the production of 2.04 moles of formic acid.

Methylation of stevioside and hydrolysis of methylstevioside. Anhydrous stevioside (10.4 g.) was dissolved in a mixture of 35 ml. of methyl iodide and 30 ml. of dioxane. One gram of Drierite was added and the solution was heated to boiling. Seven-gram portions of silver oxide were added at 30-minute intervals until a total of 35 g. had been used, the boiling solution being stirred vigorously throughout the addition and for two hours afterward. Filtration and concentration gave a sirup which was subjected to the same procedure. After seven methylations the solution of the product was treated with carbon and, after concentration, gave a sirup which weighed 12 g.

Anal. Calc'd for $C_{38}H_{49}O_7(OCH_3)_{11}$: OCH_3, 35.6. Found: OCH_3, 34.56.

Further methylation failed to raise the methoxyl content of the material. A sample (170 mg.) was dissolved in 8 ml. of methanol and 4 ml. of water, the solution was treated with 3 ml. of concentrated hydrochloric acid, and heated at 100° until a cooled sample showed no further change in optical rotation (*ca.* 2.5 hrs.). Acid was removed with silver carbonate and, after removal of silver ions, the filtrate was concentrated *in vacuo* to a heavy sirup (77 mg.) which, from 0.5 ml. of ether at 0°, gave crystalline isosteviol (m.p. 225°, mixture m.p. 228–229°). The strongly reducing filtrate (20 mg.) was chromatographed on paper using a butanol-ethanol-ammonia-water (40:10:1:49) system. Two components were observed. One, R_F 0.88, gave a spot with aniline hydrogen phthalate of approximately twice the intensity of the second, R_F 0.78.

Action of alkali on stevioside: steviolbioside (II) *and levoglucosan* (III). Stevioside (4.4 g.) was heated in 110 ml. of 10% aqueous potassium hydroxide at 100° for one hour. After cooling, the solution was acidified with glacial acetic acid, *ca.* 18 ml. being required. The fine, white crystalline precipitate (3.44 g., 95%) thus obtained was recrystallized from methanol as fine needles which, dried at 100° and 0.03 mm. for two hours, melted at 188–192° and rotated in pure dioxane −37.4° (*c*, 1.4).

Anal. Calc'd for $C_{32}H_{50}O_{13}\cdot H_2O$: C, 58.17; H, 7.93; H_2O, 2.73.
Found: C, 58.20; H, 8.16; H_2O, 2.71.

The infrared spectrum showed a strong peak at 5.92 μ (carboxyl), a weaker one at 6.03 μ, and a broad band at 3.65–4.25 (carboxyl-hydroxyl). Again, by analogy with dihydroisosteviol (14), solvation is indicated.

Hydrolysis of a sample of steviolbioside with warm 10% sulfuric acid afforded isosteviol identical with that obtained *via* the acid hydrolysis of stevioside (14). This identity was confirmed through the preparation of the methyl ester of isosteviol (14).

When the above-mentioned hydrate was held at 161° and 0.02 mm. for 2 hours, anhydrous steviolbioside was obtained.

Anal. Calc'd for $C_{32}H_{50}O_{13}$: C, 59.80; H, 7.84; N.E. 643.
Found: C, 59.74; H, 8.06; N.E. 636.

The mother liquor from the alkaline hydrolysis of stevioside was concentrated and the semi-solid residue thus obtained was dried by azeotroping benzene therefrom. Then it was acetylated with 100 ml. of pyridine and 100 ml. of acetic anhydride. After 45 minutes on the steam-bath the reaction mixture was concentrated *in vacuo* and diluted with ice-water. The product was extracted with methylene chloride and the extract was dried over sodium sulfate, filtered through decolorizing carbon, and concentrated *in vacuo*. From 15 ml. of ethanol the residue afforded 0.65 g. (41%) of product, m.p. 110–111° either alone or in admixture with authentic levoglucosan triacetate. In absolute alcohol the material rotated −50.5° (*c*, 1.24); Lemieux and Brice (30) report a rotation of −51° for levoglucosan triacetate in absolute alcohol (*c*, 1.1).

Lead tetraacetate oxidation of steviolbioside. Steviolbioside (0.308 g.) was oxidized with lead tetraacetate in glacial acetic acid using the technique of Hockett and McClenahan (33). After 140 hours, 3.15 moles of oxidant had been consumed per mole of steviolbioside used.

Methylation of steviolbioside. Steviolbioside (13 g.) was methylated three times with a mixture of acetone, methyl sulfate, and sodium hydroxide, using the usual technique, to give a light yellow, viscous sirup which rotated −30° in chloroform (*c*, 2.6).

Anal. Calc'd for $C_{32}H_{42}O_8(OCH_3)_8$: OCH_3, 32.88. Found: OCH_3, 32.25.

Hydrolysis of methylated steviolbioside. Methylated steviolbioside (4 g.) was mixed with 150 ml. of 5% sulfuric acid and sufficient ethanol to insure solution. The mixture was refluxed 12 hours and the alcohol then was removed *in vacuo*. The product which precipitated (1.15 g., m.p. 197–199°) was recrystallized from absolute ethanol: m.p. 201–202°. Mixed with the methyl ester of isosteviol prepared through the action of diazomethane on isosteviol (14) it melted at 202–203°. The filtrate was freed of acid through the addition of a deficiency of barium hydroxide followed by passage through Duolite A-4. The colorless solution then was concentrated *in vacuo* to a stiff sirup (1.84 g.) which gave a strong Fehling test and was used in the following experiments. Paper chromatography, using a butanol-ethanol-water system (34, 35) and aniline hydrogen phthalate as indicator, showed the product to consist of two sugar components. Relative migration rates found were as follows, authentic samples being run with the unknown.

	R_F	R_{TG}
Spot #1	0.78	1.00
2,3,4,6-Tetra-*O*-methyl-D-glucose	.78	1.00
Spot #2	.65	0.82
3,4,6-Tri-*O*-methyl-D-glucose	.65	0.82

Isolation of 1-O-p-phenylazobenzoyl-2,3,4,6-tetra-O-methyl-D-glucose. The general procedure used was patterned after that described by Coleman, Rees, Sundberg, and McCloskey (15). A sample (0.5 g.) of the mixture of methylated sugars obtained through the hydrolysis of methylated steviolbioside was dissolved in 40 ml. of dry pyridine and the solution was treated with 1.5 g. of *p*-phenylazobenzoyl chloride.[10] The orange solid obtained (700 mg.) was chromatographed on silica (15) to yield a band which on elution afforded 116 mg. of crystalline material. Recrystallization from acetone-pentane gave material of m.p. 123–125° and $[\alpha]_{6438}^{20}$ −28.0° (*c*, 1, $CHCl_3$). Further recrystallization failed to change these values. An authentic sample of 2,3,4,6-tetra-*O*-methyl-D-glucose, similarly azoylated and purified, melted at 124–125° and rotated $[\alpha]_{6438}^{20}$ −28.2° (*c*, 0.95, $CHCl_3$). A mixture of the authentic and stevioside-derived samples showed no depression in m.p.[11]

Anal. Calc'd for $C_{23}H_{28}N_2O_7$: C, 62.15; H, 6.35; N, 6.30.

Found: C, 62.33; H, 6.42; N, 6.54.

Isolation of crystalline 3,4,6-tri-O-methyl-D-glucose. Following the procedure of Hough, Jones, and Wadman (16), 400 mg. of the mixture of partially methylated sugars obtained through the hydrolysis of methylated steviolbioside was chromatographed on a column of powdered cellulose. A chromatographically homogeneous fraction (128 mg.) thus obtained was crystallized from ether-pentane: m.p. 95–98° either alone or in admixture with an authentic sample of 3,4,6-tri-*O*-methyl-D-glucose provided by Prof. Coleman. The x-ray diffraction patterns of the two substances were identical.

[10] Commercial samples of *p*-phenylazobenzoyl chloride are often found to be low in chlorine content owing to hydrolysis but such material may readily be reworked according to the procedure of Coleman, Nichols, McCloskey, and Anspon (36).

[11] A 1-*O*-*p*-phenylazobenzoyl-2,3,4,6-tetra-*O*-methyl-D-glucose, m.p. 125–126°, has been described by Coleman and coworkers (15) as rotating −36° ($CHCl_3$, Hg-Cd lamp with Wratten F filter). The light source used for the present measurements was a tungsten lamp with a carefully calibrated Schmidt & Haensch monochromator. The precise wavelength setting used was checked through measurement of the rotation of the plane of polarized light caused by a standard quartz plate.

A second, chromatographically homogeneous fraction (180 mg.) was also obtained and both fractions were submitted to borate ionophoresis (17). The zones were developed with aniline hydrogen phthalate after preliminary spraying with 5% trichloroacetic acid and the following values were obtained:

Substance	M_G
Glucose	1.00
3,4,6-Tri-*O*-methyl-D-glucose	0.37
Fraction I from methylated steviolbioside	.37
2,3,6-Tri-*O*-methyl-D-glucose	.00
2,3,4,6-Tetra-*O*-methyl-D-glucose	.00
Fraction II from methylated steviolbioside	.00

It is to be noted that 3,4,6- and 2,3,6-tri-*O*-methyl-D-glucose, one of which is to be expected because of the behavior of steviolbioside with lead tetraacetate, are sharply differentiated by means of borate ionophoresis.

Methyl 2-O-p-tolylsulfonyl-α-D-glucopyranoside. Methyl 4,6-*O*-benzylidene-2-*O*-*p*-tolylsulfonyl-α-D-glucopyranoside (10 g.), prepared by the method of Robertson and Griffith (37), was dissolved in 60 ml. of 2% (w/w) methanolic hydrogen chloride and the solution was boiled under reflux until a cooled sample showed no further significant change in rotation (55 mins.). Acid then was removed with silver carbonate (10 g.) and, after filtration through decolorizing carbon, the solution was concentrated *in vacuo* to a sirup. Water (50 ml.) was added and the resulting solution was reconcentrated *in vacuo*. The residue gave from 27 ml. of absolute alcohol 6.68 g. (84%) of crystalline material which, recrystallized from ethyl acetate and then from *n*-propyl alcohol, was obtained as needles melting at 139–140° and rotating +82.2° in chloroform (*c*, 2.0). Brown, Fasman, Magrath, and Todd (38) reported m.p. 138–139° but gave no specific rotation for this substance.

3,4,6-Tri-O-methyl-D-glucose. Methyl 2-O-*p*-tolylsulfonyl-α-D-glucopyranoside (3.0 g.) was methylated six times with methyl iodide and silver oxide to give 2.39 g. of a light yellow sirup.

Anal. Calc'd for $C_{13}H_{14}O_4S(OCH_3)_4$: OCH_3, 31.8. Found: OCH_3, 31.5.

Reductive detosylation was readily carried out through the use of sodium amalgam in the usual fashion (39) and the product, methyl 3,4,6-tri-*O*-methyl-α-D-glucopyranoside, was purified by distillation at 180–190° (bath) and 0.3 mm.: 1.20 g. Hydrolysis of the glucoside was accomplished according to the method of Sundberg, McCloskey, Rees, and Coleman (31), acid finally being removed through the use of Duolite A-4. Removal of water *in vacuo* gave a sirup which, from ether-pentane, afforded 0.4 g. of crystalline material melting at 77–80°. After repeated recrystallization from ether-pentane the product melted at 78–79° and showed a rotation of +77.6° in water (*c*, 1.36) at equilibrium. The latter figure is in excellent agreement with recorded values (31, 32); the melting point suggests that the crystalline substance was the α-anomer since Coleman and his coworkers (31) reported the α-anomer as melting at 78–80° while the β-anomer melted at 97–98°.

SUMMARY

Stevioside, the remarkably sweet glucoside which occurs to the extent of some 7% in the air-dried leaves of the Paraguayan plant *Stevia Rebaudiana* Bertoni, has been investigated with respect to the structure of the glucose moieties present. Of the three glucopyranose units, one is esterified at C_1 by a highly hindered carboxyl group in the aglucon. The remaining two glucose units are joined together with the unusual C_2 linkage and then to the aglucon. As far as the glucose

residues are concerned, stevioside may be represented as I although the anomeric configurations of the sugar residues remain in doubt.

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