o-phenylenediamine[60] and silver nitrate,[61] but none were detected.

Oxidation of Methyl 4-*O*-Methyl-α-D-glucopyranoside.—The α-anomer was oxidized under the same conditions as the β-glucoside. Identification of glyoxylic acid, 2-*O*-methyl-D-erythrono-γ-lactone[33] and glyoxal was made in the same manner. Results are shown in Table III.

(60) M. C. Lanning and S. S. Cohan, *J. Biol. Chem.*, **189**, 109 (1949).

(61) E. L. Hirst, L. Hough and J. K. N. Jones, *J. Chem. Soc.*, 928 (1949).

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LAFAYETTE, INDIANA

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

Stevioside. IV. Evidence that Stevioside is a Sophoroside

BY ERIK VIS[1] AND HEWITT G. FLETCHER, JR.

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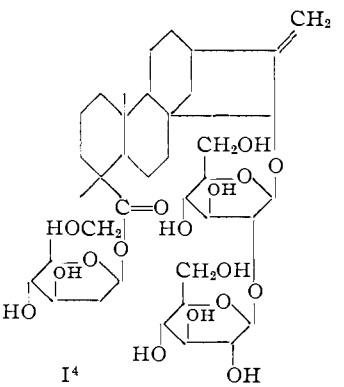
Treatment of steviolbioside heptaacetate with hydrogen bromide in glacial acetic acid has been found to give α-acetobromosophorose (3,4,6-tri-*O*-acetyl-2-*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucosyl bromide). The disaccharide residue in stevioside is, therefore, a sophorosyl group.

Structural studies of stevioside,[2,3] the intensely sweet principle of *Stevia Rebaudiana* Bertoni, have shown that the substance contains one glucopyranose unit attached to a carboxyl group and two glucopyranose units linked to $C_1 \rightarrow C_2$ and thence to an alcoholic hydroxyl group. These facts may be represented by I, it being understood that precise details of the structure of the aglucon remain to be elucidated. Since stevioside is not attacked by the enzymes usually employed in settling questions of anomerism and attempts to cleave the disaccharide fragment as a whole failed, the configurations of the three anomeric carbons were left open in the original work. Subsequently a study of a simple



I[4]

analog of a portion of the stevioside molecule[5] indicated with some certainty that the ester-linked sugar residue is a β-D-glucopyranosyl group. We now wish to report evidence bearing on the configuration of the linkage joining the glucose residues of the disaccharide portion.

As described earlier,[2] treatment of stevioside with alkali results in loss of the ester-linked sugar residue and formation of a glucoside which was given the trivial designation of "steviolbioside." Acetylation of this substance has now provided an amorphous heptaacetate which, in glacial acetic acid, is readily cleaved by hydrogen bromide to give a relatively insoluble acetobromobiose. The physical constants of this substance agreed closely with those of α-acetobromosophorose; treatment with silver acetate converted the product to β-sophorose octaacetate, identification being confirmed through comparison with authentic material. Since sophorose has been unequivocally demonstrated to be 2-*O*-(β-D-glucopyranosyl)-D-glucose,[6] the configuration of the linkage joining the two glucose units in stevioside may now be considered established. While the configuration of the sophorose–steviol linkage remains unproved, it is very probably β also.

To the authors' knowledge stevioside is but the second sophoroside to be found, the first being kaempferol sophoroside which occurs in the fruit of the widespread ornamental tree *Sophora japonica* L.[6a,7]

While the present work was not directly concerned with the fate of the aglucon in the cleavage, ketoisostevic acid (isosteviol) was isolated after one rather prolonged run. That an unrearranged product such as hydroxydehydroisostevic acid (steviol)[3] or its acetate might be isolable under milder conditions is not excluded.

Experimental[8]

Steviolbioside Heptaacetate.—Anhydrous steviolbioside (2.5 g.) was dissolved in 10 ml. of dry pyridine and the solution cooled to −70°. Acetic anhydride (3.5 ml.) was then added and the solution slowly warmed to 20°. After 20 hr. at 20° and 4 hr. at 60°, the reaction mixture was poured into 200 ml. of 1% aqueous acetic acid at 0°. The white powder thus precipitated was removed after 1 hr., dissolved in ether and the solution washed successively with dilute

(1) Chemical Foundation Fellow, 1956.

(2) H. B. Wood, Jr., R. Allerton, H. W. Diehl and H. G. Fletcher, Jr., *J. Org. Chem.*, **20**, 875 (1955).

(3) E. Mosettig and W. R. Nes, *J. Org. Chem.*, **20**, 884 (1955).

(4) The hydrogen atoms attached to the rings of the glucose moieties have been omitted for clarity.

(5) H. B. Wood, Jr., and H. G. Fletcher, Jr., THIS JOURNAL, **78**, 207 (1956).

(6) (a) K. Freudenberg, H. Knauber and F. Cramer, *Chem. Ber.*, **84**, 144 (1951); (b) J. Rabaté, *Bull. soc. chim.*, **7**, 565 (1940); (c) K. Freudenberg and K. Soff, *Ber.*, **69**, 1245 (1936); (d) K. Freudenberg, H. Toepffer and C. C. Andersen, *ibid.*, **61**, 1750 (1928).

(7) J. Rabaté and J. Dussy, *Bull. soc. chim. biol.*, **20**, 459, 467 (1938).

(8) Melting points are corrected.

sulfuric acid, aqueous sodium bicarbonate and water. Moisture was removed with sodium sulfate and the solution concentrated; dropwise addition of pentane yielded a fine powder (3.60 g., 99%) which was reprecipitated from diisopropyl ether by the addition of pentane and dried *in vacuo* (0.1 mm.) at 80° for 5 hr. Attempts to obtain this amorphous product in crystalline form failed. After chromatography on alumina it showed $[\alpha]^{20}D$ −24.3° in glacial acetic acid (*c* 4.85) and $[\alpha]^{20}D$ −28.3° in ethanol (*c* 4.26).

Anal. Calcd. for $C_{31}H_{42}O_4(O_2C_2H_3)_7COOH$: neut. equiv., 117.1. Found: neut. equiv., 116.7.

Cleavage of Steviolbioside Heptaacetate with Hydrogen Bromide in Glacial Acetic Acid.—A solution of 65 mg. of the heptaacetate in 1.5 ml. of glacial acetic acid was mixed with 0.13 ml. of a solution containing *ca.* 30% by weight of hydrogen bromide in glacial acetic acid. Polarimetric observations showed a minimum rotation at 5 minutes followed by a slow dextromutarotation to a positive value. After 36 hr. crystallization began and, after 50 hr., the crystals were removed. Recrystallized from dichloromethane–ether and dried *in vacuo* at 90°, the material (15 mg., 31%) melted with decomposition at 195–197° and rotated $[\alpha]^{20}D$ +94.2° in chloroform (*c* 0.43). Freudenberg and his coworkers[6] have reported m.p. 196° and $[\alpha]^{20}D$ +95.6° ($CHCl_3$) for α-acetobromosophorose.

In another experiment wherein a solution containing 1.100 g. of steviolbioside heptaacetate, 6.5 ml. of glacial acetic acid, 2.2 ml. of AcOH–HBr and 0.8 ml. of acetic anhydride was held at 20° for 3 days and then chilled, α-acetobromosophorose was obtained in 8% yield. The filtrate was diluted with ether, washed with aqueous sodium bicarbonate and extracted with aqueous sodium hydroxide. After acidification to *p*H 4 the alkaline extract was immediately extracted with ether which, in turn, was washed with sodium bicarbonate, water and then dried over sodium sulfate. Concentration and the addition of pentane gave 24 mg. of prisms which, recrystallized from ether–pentane, melted at 231–232° and did not depress the melting point of keto-isostevic acid (isosteviol).

β-Sophorose Octaacetate.—A mixture of 60 mg. of α-acetobromosophorose, 20 mg. of silver acetate and 0.5 ml. of glacial acetic acid was heated at 100° for 1 hr. After cooling and diluting with ethyl acetate, the silver bromide was filtered off and the filtrate concentrated *in vacuo* to a thick sirup. Upon addition of methanol crystallization took place. Recrystallized from dichloromethane–pentane, the product melted at 192° either alone or in admixture with a sample of authentic β-sophorose octaacetate.

Acknowledgment.—We wish to express our indebtedness to Professor Karl Freudenberg for his gift of authentic sophorose derivatives.

BETHESDA 14, MARYLAND

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

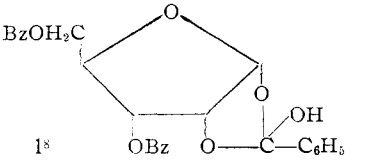
Evidence that the Supposed 3,5-Di-*O*-benzoyl-1,2-*O*-(1-hydroxybenzylidene)-α-D-ribose is Actually 1,3,5-Tri-*O*-benzoyl-α-D-ribose

BY ROBERT K. NESS AND HEWITT G. FLETCHER, JR.

RECEIVED APRIL 5, 1956

The more dextrorotatory of the two D-ribofuranose tribenzoates obtained in the acidic or neutral hydrolysis of tri-*O*-benzoyl-D-ribofuranosyl bromide has been shown to be 1,3,5-tri-*O*-benzoyl-α-D-ribose. Some implications arising from this conclusion particularly the probable non-existence of stable cyclic orthoacid structures in the carbohydrate field, are discussed.

In earlier papers[1,2] we have described the hydrolysis of amorphous 2,3,5-tri-*O*-benzoyl-D-ribosyl bromide as giving rise to two crystalline ribofuranose tribenzoates, one melting at 142–143°[3] and rotating +85.3°[3] in chloroform and the other melting at *ca.* 111–112° and rotating +68.8→66.2° ($CHCl_3$, 2 days). The structure of the latter ester was unequivocally proved by a synthesis involving the hydrogenolysis of benzyl β-D-ribofuranoside tribenzoate. The former, more dextrorotatory substance[4] proved to be stable in acidic solution but was rapidly converted by mild alkali into 2,3,5-tri-*O*-benzoyl-β-D-ribose. These properties plainly indicated that the substance was not simply an anomeric form of 2,3,5-tri-*O*-benzoyl-D-ribose but strongly suggested the possibility of a cyclic orthoacid structure, a 2-hydroxy-1,3-dioxolane. Such structures, while rarely mentioned in the literature, have nevertheless been postulated in certain cases.[5,6] The most firmly established of these at the time of our earlier work appeared to be the D-talose monobenzoate which Pigman and Isbell[7] had obtained in 1937 through the perbenzoic acid oxidation of D-galactal. Like our ribose tribenzoate, this D-talose monobenzoate was relatively stable in dilute acid solution but mutarotated rapidly in the presence of very dilute alkali. In order to throw light upon the structure of our D-ribofuranose tribenzoate we attempted the deliberate synthesis of 3,5-di-*O*-benzoyl-1,2-*O*-(1-hydroxybenzylidene)-α-D-ribofuranose (I). To this



end, tri-*O*-benzoyl-D-ribofuranosyl bromide was condensed with benzyl alcohol in the presence of

(1) R. K. Ness, H. W. Diehl and H. G. Fletcher, Jr., THIS JOURNAL, **76**, 763 (1954).

(2) R. K. Ness and H. G. Fletcher, Jr., *ibid.*, **76**, 1663 (1954).

(3) Melting points are corrected. Unless otherwise specified rotations are specific rotations for the D line of sodium at 20°, concentration being expressed in g. per 100 ml. of solution.

(4) F. Weygand and F. Wirth [*Chem. Ber.*, **85**, 1000 (1952)] had previously and independently obtained this substance through the acidic hydrolysis of a partially benzoylated adenosine and designated it as 2,3,5-tri-*O*-benzoyl-D-ribose.

(5) N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **58**, 2534 (1936).

(6) *Cf.* E. Pacsu, *Advances in Carbohydrate Chem.*, **1**, 77 (1945), for a review.

(7) W. W. Pigman and H. S. Isbell, *J. Research Natl. Bur. Standards*, **19**, 189 (1937).

(8) The ring-attached hydrogen atoms in this and succeeding formulas have been omitted for greater clarity.