charides and D-xylose with the eventual disappearance of one after another of the oligosaccharides unti the presence of only D-xylose was indicated. The final optical rotation of the solution also was that calculated for D-xylose alone.

Another aliquot of each oligosaccharide solution was hydrolyzed completely, and the solution was then neutralized with 0.05 N sodium hydroxide and titrated iodometrically as described above. The equivalent weights of the hydrolysates are reported in Table II.

Periodate Oxidation.—A sample sufficient to produce about 10 mg. of formic acid was weighed into a 500-ml. stoppered bottle and dissolved in distilled water. Following addition by pipet of 100 ml. of 5% potassium chloride solution and 20 ml. of 0.3 M sodium periodate, the reaction was allowed to proceed in the dark at 15° with shaking. A pair of 10-ml. aliquots were withdrawn at intervals and filtered through coarse sintered glass. The first 10-ml. portion was used to determine the formic acid produced and to determine the total amount of iodine in the form of iodate.[13] The second portion was used for the determination of the excess periodate.[14] The amount of periodate consumed by the sugar was found by subtracting the periodate remaining from the iodate produced.

The periodate oxidation data are shown in Table III. The calculated values are based on pyranose rings linked 1,4'.

Osazones. **Phenylxylobiosazone.**—Five-tenths gram of the crystalline xylobiose, 1 g. of phenylhydrazine hydrochloride, 1.5 g. of sodium acetate pentahydrate and 12 ml. of distilled water were mixed in a 50-ml. erlenmeyer flask. The flask was loosely stoppered and placed in boiling water. After 20 minutes with occasional shaking, to avoid supersaturation, 0.25 g. of yellow needle-like crystals formed. They were twice recrystallized from either 60% ethanol or a mixture of 1,4-dioxane and petroleum ether (b.p. 65–75°). m.p. 195–196° dec., $[\alpha]^{22}D$ −22.5 → −77.0 (33 hr., c 0.65 in a 7:3 mixture of pyridine and absolute ethanol).

Anal. Calcd. for $C_{22}H_{28}O_7N_4 \cdot H_2O$: N, 11.71. Found: N, 11.5.

Phenylxylotriosazone.—Xylotriose treated in the same way for osazone formation gave yellow crystalline needles after 25 minutes of heating and on slight cooling of the solution, yield 0.23 g.

The material was twice recrystallized from 95% ethanol, m.p., 214–215° dec., $[\alpha]^{26}D$ → −53.5 (12 hr., c 0.41 in a 7:3 mixture of pyridine and absolute ethanol).

Anal. Calcd. for $C_{27}H_{36}O_{11}N_4 \cdot H_2O$: N, 9.17. Found: N, 9.2.

LAFAYETTE, INDIANA

(13) F. Brown, S. Dunstan, T. G. Halsall, E. L. Hirst and J. K. N. Jones, *Nature*, **156**, 285 (1945); T. G. Halsall, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1427 (1947).

(14) Fleury and Lange, *J. pharm. chim.*, **17**, 107, 196 (1933).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

The Structure of Maltotriose[1]

BY A. THOMPSON[2] AND M. L. WOLFROM

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An improved method for the isolation of maltotriose as its hendecaacetate is presented. The method for proving the structure of a trisaccharide, first established with panose, has now been employed to confirm the structure of maltotriose as assigned by Sugihara and Wolfrom by other methods. The acetylated partial hydrolyzate of the alditol was resolved by silicate column chromatography and the components β-D-glucose pentaacetate, D-glucitol (sorbitol) hexaacetate, β-maltose octaacetate and maltitol nonaacetate were adequately identified in the crystalline state. This definitely confirms the structure 4-α-maltopyranosyl-D-glucose (I) for maltotriose.

Maltotriose[3] merits the interest of chemists in the carbohydrate and allied fields because it is one of the hydrolytic products of starch. It is therefore highly desirable to find better methods for preparing this sugar in a pure condition. We wish to describe herein a method for separating maltotriose from complex carbohydrate mixtures in which it may occur. The method used is in part that of Whistler and Durso[4] for separating sugars by means of a carbon column. The maltotriose in a commercial hydrolyzate of starch can be concentrated considerably by this means. When the aqueous solution of the complex carbohydrate mixture is placed on a carbon column, the monosaccharides are not adsorbed. Oligosaccharides are then removed by developing with successively higher concentrations of ethanol in water. Pure crystalline β-maltotriose hendecaacetate can then be prepared by means of silicate column chromatography of the acetylated concentrate.

Previously, Wolfrom, Thompson and Galkowski[5] used a method for the determination of the structure of trisaccharides which involved marking the monosaccharide unit containing the reducing group by hydrogenation to the corresponding alcohol (alditol), followed by partial hydrolysis with separation and identification of the acetylated fragments in the hydrolyzate by means of silicate column chromatography. This method in principle was also employed by French[6] in a structural study of panose. We wish to report herein the extension of this technique in confirming the structure of maltotriose which was first established, in a not entirely unequivocal manner, by Sugihara and Wolfrom,[7] through the employment of methylation procedures and enzymic partial hydrolysis.

It is advantageous to determine the conditions of hydrolysis which will produce the maximum quantities of disaccharide material. In the hydrolysis of panitol, in which two kinds of linkages exist, the reaction time t to bring about hydrolysis to maximum quantities of disaccharide material was calculated[5] to be

$$t = \frac{\log[(k_1 + k_2)/k_2]}{k_1} \quad (1)$$

when the reaction was carried out with a sugar concentration of 2% in a 0.05 N sulfuric acid solution

(1) Reported in *Abstracts Papers Am. Chem. Soc.*, **121**, 5P (1952).

(2) Corn Industries Research Foundation Associate of The Ohio State University Research Foundation (Project 203).

(3) M. L. Wolfrom, L. W. Georges, A. Thompson and I. L. Miller, THIS JOURNAL, **71**, 2873 (1949); L. W. Georges, I. L. Miller and M. L. Wolfrom, *ibid.*, **69**, 473 (1947).

(4) R. L. Whistler and D. F. Durso, *ibid.*, **72**, 677 (1950).

(5) M. L. Wolfrom, A. Thompson and T. T. Galkowski, *ibid.*, **73**, 4093 (1951).

(6) D. French, *Science*, **113**, 352 (1951).

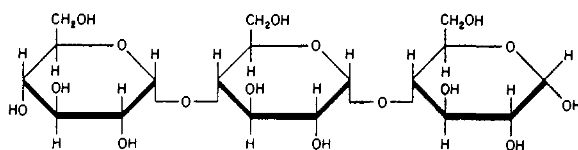
(7) J. M. Sugihara and M. L. Wolfrom, *ibid.*, **71**, 3357 (1949).

at the boiling point. Since in maltotriitol only one kind of linkage exists, k_1 becomes equal to k_2 and the equation reduces to

$$t = \frac{\log 2}{k} \quad (2)$$

In these calculations it is assumed that each type of link hydrolyzes at the same rate as in maltose or isomaltose regardless of where it occurs. The first-order specific reaction constant for the 4-α-D-glucosyl link in maltose has been determined[8] and found to be k_2 = 0.0846 (hr.$^{-1}$, $\log_e$, 99.5°). The value for t in equation (2) then becomes 8.2 hours.

The hydrolysis was interrupted after eight hours. The products isolated from the acetylated hydrolyzate were β-D-glucose pentaacetate, D-glucitol (sorbitol) hexaacetate, β-maltose octaacetate, maltitol nonaacetate and a sirup presumably the acetate of maltotriitol which has not as yet been crystallized and characterized. The only trisaccharide which can produce this group of hydrolytic products is 4-α-maltopyranosyl-D-glucose or O-α-D-glucopyranosyl-(1 → 4)-O-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose (I), a formulation confirmatory to that established by Sugihara and Wolfrom.[7]



Maltotriose

Experimental

Preparation of β-Maltotriose Hendecaacetate.—One hundred grams of commercial corn sirup[9] was dissolved in 1000 ml. of water and placed on a column (350 × 110 mm., diam.) of Darco G-60[4,10] mixed with Celite[4,10] (1:1 by wt.) and washed successively with water and 3% ethanol. Washing was continued in each case until the effluents reacted negatively toward Benedict reagent. These solutions contained D-glucose and maltose and were discarded. The column was then washed with 15% ethanol until again the washings were negative to Benedict solution. The effluent (8000 ml.) was evaporated under reduced pressure to 1000 ml. This solution was again placed on a Celite-(Darco G-60) column (350 × 80 mm., diam.) and washed with water and 3% ethanol, these washings being discarded. The column was then washed with 8% ethanol until the effluent was negative to Benedict solution, about 8000 ml. of solution being collected in this fraction. This solution was then evaporated under reduced pressure to a sirup which was further dried to an amorphous powder by repeatedly dissolving in methanol and evaporating under reduced pressure; yield 10 g. This material was carefully acetylated by heating, just below the boiling point, with 5 g. of sodium acetate and 100 ml. of acetic anhydride; yield 17 g. of acetate (see below for preparative details). The acetylated mixture was chromatographed on Magnesol[11]-Celite (5:1 by wt.) columns (275 × 80 mm., diam.) in 5-g. portions using 2500 ml. of a benzene-(t-butyl alcohol) solution (75:1 by vol.); yield 5.5 g. of β-maltotriose hendecaacetate[3]; m.p. 134–136°, $[\alpha]^{26}D$ +89.5° (c 2.9, chloroform).

Reduction and Hydrolysis of Maltotriose.—Six grams of crystalline β-maltotriose hendecaacetate was dissolved in 78 ml. of 0.05 N $NaOCH_3$ in dry methanol and allowed to stand overnight at 5°. It was then diluted with 50 ml. of water and deionized by passing successively through columns (100 × 25 mm., diam.) of Amberlite 120[12] and Duolite A-4.[13] The solution was combined with the washings, evaporated under reduced pressure to 100 ml. and then placed in a bomb with 2.0 g. of Raney nickel under 2000 p.s.i. of hydrogen and shaken at 80° for 3 hr. The filtered reaction mixture was evaporated to a sirup under reduced pressure and dried to an amorphous powder by repeatedly distilling with methanol under reduced pressure; yield 2.8 g. of material negative to Benedict solution.

The above amorphous material was dissolved in 140 ml. of 0.05 N H_2SO_4 and refluxed for 8 hr. The sulfuric acid was removed from the cooled solution by passage through a column (100 × 25 mm., diam.) of Duolite A-4.[13] The effluent was evaporated to a sirup and further dried by repeatedly distilling with methanol under reduced pressure. The dry material was acetylated with 1.5 g. of sodium acetate and 30 ml. of acetic anhydride by heating just short of the boiling point. The reaction mixture was poured into 200 ml. of water and allowed to stand for 2 hr. It was then extracted portionwise with a total of 100 ml. of chloroform. The chloroform extract was washed with water, aqueous sodium bicarbonate and again with water, dried with Na_2SO_4, filtered and evaporated to a sirup under reduced pressure.

Chromatography of the Acetylated Hydrolyzate.—The above sirup was dissolved in 20 ml. of benzene and placed on a column (275 × 80 mm., diam.) of Magnesol-Celite (5:1 by wt.) and developed with 6000 ml. of benzene-(t-butyl alcohol) (300:1 by vol.). Four zones were located by streaking the extruded column with permanganate indicator (1% $KMnO_4$ in 10% NaOH). These zones were numbered from the bottom to the top and sectioned, zone 4 being divided into two equal parts. The sugar acetate in each was removed by extraction with acetone and the acetone solutions evaporated to sirups.

The material from zone 1 crystallized from ethanol; yield 0.4 g., m.p. 120–125°. This crystalline substance was rechromatographed on a column (175 × 35 mm., diam.) of Magnesol-Celite (5:1 by wt.) using 1000 ml. of benzene-(t-butyl alcohol) (300:1 by vol.). Part of the substance was washed from the column and was removed by evaporating the developing solvent and crystallizing from ethanol; m.p. 126–128° unchanged on admixture with an authentic specimen, $[\alpha]^{27}D$ +4.6° (c 3.8, chloroform). This identifies the substance as β-D-glucopyranose pentaacetate.

The sirup from zone 2 crystallized from ethanol; yield 0.65 g., m.p. 93–97°. A portion of this material (0.3 g.) was rechromatographed on a column (175 × 35 mm., diam.) of Magnesol-Celite (5:1 by wt.) using 750 ml. of benzene-(t-butyl alcohol) (200:1 by vol.). The material in the principal zone was isolated by acetone elution and was crystallized from ethanol; m.p. 97–99° unchanged on admixture with an authentic specimen, $[\alpha]^{27}D$ +9.8° (c 4.6, chloroform). These constants are those accepted for D-glucitol (sorbitol) hexaacetate.

The material from zone 3 crystallized from ethanol; yield 0.2 g., m.p. 153–155° unchanged on admixture with an authentic specimen. After further recrystallization from ethanol it was identified as β-maltose octaacetate; m.p. 154–156°, $[\alpha]^{27}D$ +64° (c 3.1, chloroform).

The material from the bottom half of zone 4 was crystallized from ethanol; yield 0.25 g., m.p. 90° with sintering at 80°. A further quantity of 0.20 g. of crystalline material was obtained by rechromatographing the top half of zone 4 on a column (210 × 45 mm., diam.) of Magnesol-Celite (5:1 by wt.) and developing with 1500 ml. of benzene-(t-butyl alcohol) (75:1 by vol.). A sirup (0.75 g.) obtained from the top part of this column by acetone elution was assumed to be maltotriitol dodecaacetate and was not further investigated since this substance has not been crystallized and therefore not characterized. The combined crystalline material from zone 4 was recrystallized from ethanol; m.p. 95–97° with sintering at 87°, $[\alpha]^{24}D$ +78° (c 4.1, chloro-

(8) M. L. Wolfrom, E. N. Lassettre and A. N. O'Neill, *Science*, **73**, 595 (1951).

(9) Kindly furnished by the A. E. Staley Manufacturing Co., Decatur, Illinois; a partially hydrolyzed (*ca.* 65%) corn starch produced by the successive action of mineral acid and mold amylase, *ca.* 80% solids.

(10) M. L. Wolfrom, A. Thompson, A. N. O'Neill and T. T. Galkowski, THIS JOURNAL, **74**, 1062 (1952).

(11) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, *ibid.*, **67**, 527 (1945).

(12) A product of the Resinous Products and Chemical Co., Philadelphia, Pa.

(13) A product of the Chemical Process Co., Redwood City, California.

form), X-ray powder diffraction data[14]: 7.97[15] –20[16], 7.10–5, 6.55–5, 5.63–10, 5.50–10, 4.65–10, 4.29–100, 3.98–40, 3.75–20, 3.66–10, 3.41–10, 3.20–10, 3.01–10, 2.84–5, 2.70–10, 2.37–5, 2.16–10, 2.01–5. The substance is therefore identified as maltitol nonaacetate.[5]

(14) Reported in ref. 5 but herein further developed with more lines.

(15) Interplanar spacing, Å., CuK_α radiation.

(16) Relative intensity as percentage strongest line; estimated visually.

COLUMBUS 10, OHIO

[CONTRIBUTION FROM THE BOTANICAL INSTITUTE, FACULTY OF SCIENCE, UNIVERSITY OF TOKYO]

Studies on Flavanone Glycosides. IV. The Glycosides of Ripe Fruit Peel and Flower Petals of *Citrus Aurantium* L.

BY SHIZUO HATTORI, MASAMI SHIMOKORIYAMA AND MOTOTAKE KANAO

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From the ripe fruit peel of Japanese bitter orange (*Citrus aurantium* f. *Kabusu* and *C. aurantium* var. *cyathifera*), two flavonoid glycosides have been isolated, namely naringin and rhoifolin. Details of the isolation and identification of these two glycosides are reported. The finding of these two flavonoid glycosides in the ripe fruit peel of the Japanese bitter orange is contradictory to previous reports of other workers that hesperidin is the only flavanone glycoside present in the ripe fruit of the *Citrus aurantium* cultivated in Europe. In the flower petals, however, of *C. aurantium* f. *Kabusu*, hesperidin has been found to be the only flavonoid glycoside present.

Introduction

Kolle and Gloppe[1] have reported that the ripe fruit peel of *Citrus aurantium* L., cultivated in Europe, contains one flavanone glycoside, hesperidin. The unripe fruit peel, however, contains not only the hesperidin, but also its isomer neohesperidin. Zemplén and Tettamanti[2] tentatively gave neohesperidose, the disaccharide of neohesperidin, a structure of 1-L-rhamnoside-4-D-glucose.

In order to see if the peel of Japanese bitter oranges contain either hesperidin or neohesperidin, or both, the ripe fruits of *C. aurantium* f. *Kabusu* and var. *cyathifera* have been investigated. Contrary to expectation, neither a trace of hesperidin nor of neohesperidin could be found. Rather, two different flavonoid glycosides, naringin and rhoifolin have been isolated and identified. Rhoifolin is the 7-rhamnoglucoside of apigenin and has been previously isolated by Hattori and Matsuda[3] from the leaves of *Rhus succedenea.*

Naringin has also been previously isolated from the fruit peels of an edible citrus, *Citrus grandis* Osbeck.[4] Asahina and Inubuse[5] also found naringin in the flower petals of this citrus. From the flower petals of *C. Aurantium* f. *Kabusu*, however, not naringin, but rather hesperidin has been the only flavanone glycoside obtained.

Experimental

Isolation of the Glycosides from the Ripe Fruit Peel.—Two kg. of finely divided fresh peels of *C. aurantium* f. *Kabusu*, obtained from 25 ripe fruit in February, 1951, was extracted five times with hot ethanol (a total of 11 l.) for one hour. After concentration *in vacuo* of the combined extracts to about 400 ml., 2 l. of ethanol was added and the precipitate then filtered off. The ethanolic filtrate was again evaporated, this time to 250 ml., water was added, and the mixture was further evaporated to about 200 ml. The resulting aqueous solution was treated with ether, and then saturated with chloroform by shaking. After 2–3 days, the colorless needles which had precipitated were filtered, and washed with a small quantity of water and then with ether; yield 10 g. Approximately the same yield was obtained from 2 kg. of the peels of *C. aurantium* var. *cyathifera*.

When 8 g. of the crude crystals was extracted with 20 parts of boiling ethyl acetate, approximately 4 g. dissolved. The residue was extracted again with 50 ml. of the solvent, and the mixture filtered. The filtrate was evaporated, and this new residue was redissolved in ethyl acetate and filtered. On evaporation to 40 ml., crystallization occurred. After repeated recrystallizations from water, colorless crystals of this glycoside I (naringin) were obtained; yield about 3 g.

The residue (of the original 8 g. of crude crystals) after the ethyl acetate extractions, was recrystallized from 20 ml. of 50% ethanol (or methanol). Almost colorless, minute, crystalline needles of the glycoside II (rhoifolin) were obtained; yield about 2.5 g.

Identification of the Glycosides of the Ripe Fruit Peel.—The crystals of glycoside I melted first at 80–83°, then solidified, and melted finally at 193–194°. A mixture of this glycoside with an authentic sample of naringin showed no depression of melting point.

Anal. Calcd. for $C_{27}H_{32}O_{24}\cdot 6H_2O$: C, 47.08; H, 6.44; water of crystallization, 15.7. Found: C, 47.08; H, 6.12; water of crystallization, 15.9.

Hydrolysis of the glycoside I yielded naringenin and the sugars glucose and rhamnose. The latter were identified by their osazones, and part of the aglycon was converted to its diacetate, m.p. 140–143°.[6] Mixed melting point determination of authentic naringenin with the aglycon, m.p. 245–247°; and of authentic naringenin diacetate with the diacetate of the aglycon showed no change. Glycoside I is, therefore, naringin.

Rhoifolin.—The crystals of glycoside II melted at 250–265° after sintering at 200–205°. When mixed with rhoifolin isolated from the leaves of *Rhus succedanea*, this substance showed no depression of melting point.

Anal. Calcd. for $C_{27}H_{30}O_{14}\cdot 6H_2O$: C, 47.08; H, 6.44; water of crystallization, 16.3. Found: C, 47.16; H, 6.12; water of crystallization, 15.7.

Hydrolysis of glycoside II produced rhamnose and glucose, which were identified as the osazones, and an aglycon with a melting point above 340°. The aglycon gave a diacetate, m.p. 192–193°,[7] and a triacetate, m.p. 180–182°. The melting points of the diacetate and the triacetate were not altered when mixed with authentic apigenin di- and triacetate (m.p. 192–193° and 180–182°). Glycoside II, therefore, was apigenin-7-rhamnoglucoside, which is rhoifolin.

For the determination of the position of the sugar residue in rhoifolin (glycoside II), 0.4 g. of the glycoside was put into a flask with 4 ml. of dimethyl sulfate. To this mixture was then added, drop by drop, a solution of 4 g. of sodium

(1) F. Kolle and K. E. Gloppe, *Pharm. Zentralhalle*, **77**, 421 (1936).

(2) G. Zemplén and A. K. Tettamanti, *Ber.*, **71**, 2511 (1939).

(3) S. Hattori and H. Matsuda, *Arch. Biochem. Biophys.*, in press.

(4) S. Hattori, M. Hasegawa and M. Kanao, *Acta Phytochimica*, **15**, 199 (1949).

(5) Y. Asahina and M. Inubuse, *J. Pharm. Soc. Japan*, **48**, 868 (1938).

(6) M. Shimokoriyama, *J. Chem. Soc. Japan*, **70**, 234 (1949).

(7) M. Shimokoriyama, *Bull. Chem. Soc. Japan*, **16**, 284 (1941).