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

Isomaltitol

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Successive carbon and silicate chomatography leads to the preparative isolation of isomaltose (6-α-D-glucopyranosyl-D-glucose), as its crystalline β-D-octaacetate, from commercial hydrol; β-gentiobiose octaacetate can be obtained simultaneously. Isomaltitol and its nonaacetate are described in crystalline form. The structure of isomaltitol is defined by periodate oxidation; that of maltitol is not. Silicate column chromatographic resolution of the mixture resulting from an acetylated, reduced, partial acid hydrolyzate of glycogen yielded sorbitol hexaacetate and the nonaacetates of maltitol and isomaltitol.

The discovery that 6-α-D-glucopyranosyl-D-glucose (isomaltose), the first crystalline derivative of which was prepared by Wolfrom, Georges and Miller,[2] is an integral part of the amylopectin molecule[3,4] has aroused considerable interest in this sugar. It is therefore desirable to find a source of supply and suitable methods for its isolation in working quantities. One of the better sources of isomaltose[5,6] is "hydrol," the residue or molasses from the commercial production of D-glucose from corn starch. The carbohydrates occurring in hydrol can be fractionated directly by the use of a carbon column,[7] thus concentrating the isomaltose fraction, which, after acetylation and subjection to silicate chromatography, yields as much as 5.0 g. of pure β-isomaltose octaacetate from 100 g. of raw hydrol. A second fraction from the carbon column yields on acetylation β-gentiobiose octaacetate[8] which can be isolated by direct crystallization; the yield is about one-half that of the β-isomaltose octaacetate.

In the study of starch hydrolyzates it is sometimes convenient to reduce the mixture of sugars to aldiotols. This eliminates the anomers and the less complex mixture tends toward better crystallization[9,10] and the easier isolation of components. We wish to report herein the preparation of crystalline isomaltitol (6-α-D-glucopyranosyl-D-glucitol, m.p. 165.5–167°, $[\alpha]^{28}D$ +89° in water) and of crystalline isomaltitol nonaacetate (m.p. 114–115°, $[\alpha]^{29}D$ +70° in chloroform). These are essential reference compounds in the study of reduced amylopectin hydrolyzates. Thus, when the related polysaccharide glycogen was submitted to partial acid hydrolysis with subsequent reduction, acetylation and chromatography on Magnesol[11] and Silene,[12] there was obtained, in agreement with previous work[13] on the corresponding aldose acetates, the following crystalline products: sorbitol (D-glucitol) hexaacetate, maltitol nonaacetate and isomaltitol nonaacetate.

Periodate oxidation experiments (Table I) show that the isomaltitol molecule consumes six moles of periodate and produces essentially four moles of formic acid and one mole of formaldehyde. These values provide further definitive evidence of the presence of a 6-D-glucopyranosyl link in isomaltose and isomaltitol (Fig. 1). We find, on the other hand, that the maltitol[14,15] structure is not susceptible to definition by periodate oxidation. Maltitol behaves anomalously toward periodate in that it is extensively oxidized with occurrence of no critical plateaus in the oxidation curve. This is due to the intermediate formation of a substituted malonaldehyde (second stage in Fig. 2) which undergoes immediate further oxdation[16–18] leading to the final production of formic and carbonic acids and formaldehyde. It is the experience of this Laboratory[19] that periodate oxidation results on many polyhydroxy compounds show a trend toward over-consumption of oxidant and underproduction of formic acid. This may be due to the hydrolytic stability of some types of formate

TABLE I

OXIDATION OF MALTITOL AND ISOMALTITOL IN 0.0025 *M* SOLUTION WITH 0.04 *M* SODIUM METAPERIODATE AT 25°

Substance	Time, hr.	Moles per mole substance: Oxidant consumed[a]	Formaldehyde formed[b]	Formic acid formed[c]
Isomaltitol	0.50	5.4		
	3.25	6.0		
	13.00	6.0	0.9	3.8
Maltitol[d]	0.1	4.7		1.4
	2.4	7.5		3.1
	24	9.1		
	54	9.4		5.0
	288	10.7		6.2
	530	11.6	2.7	

[a] In the dark. [b] By dimedon method. [c] As acidity to methyl red. [d] Prepared through its crystalline nonaacetate.

(1) Corn Industries Research Foundation Associate (A. T.) and Fellow (T. T. G.) of The Ohio State University Research Foundation (Project 203).

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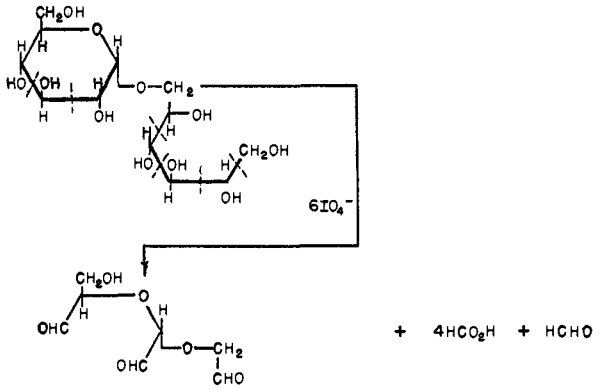

Fig. 1.—Periodate oxidation of isomaltitol.

esters and to the oxidation of a hydrogen atom in the alpha position to a carbonyl group which oxidation results in the formation of carbonic rather than of formic acid. Such a reaction is shown in the final stages of the postulated series of changes of Fig. 2 which predict the data given for maltitol in Table I.

Experimental

β-Isomaltose Octaacetate and β-Gentiobiose Octaacetate from Hydrol.—Hydrol[20] (100 g., *ca.* 75% solids) was diluted to 500 ml. and filtered through a column (370 × 115 mm., diam.) of Darco G-60[21]–Celite[11] (1:1 by wt.). The column was washed with water (8 liters) until the effluent reacted negative to Benedict reagent. This solution, which normally contains monosaccharides, was discarded. The column was then washed successively with 5% and 15% ethanol–water solutions until the effluent reacted negative to Benedict reagent in each case. The 5% effluent was evaporated to a sirup under reduced pressure below 50° and the sirup was further dried by distillation under reduced pressure with methanol; yield 7.5 g. This material was acetylated with 3.0 g. of anhydrous sodium acetate and 30 ml. of acetic anhydride at a temperature just below the boiling point. It was then poured into 200 ml. of ice and water. After the excess acetic anhydride was hydrolyzed, the sirupy insoluble material was dissolved in benzene, dried with anhydrous sodium sulfate, and concentrated under reduced pressure to a sirup; yield 11.0 g. This material was then chromatographed in 5-g. portions on Magnesol[11]–Celite (5:1 by wt.) on columns (250 × 80 mm., diam.) by development with 3 liters of benzene:*t*-butyl alcohol (75:1 by vol.). After extrusion and streaking with indicator (1% $KMnO_4$ in 10% NaOH), the β-isomaltose octaacetate was found in a zone near the middle of the column. The zones from these columns were removed, eluted with acetone, filtered and evaporated to a sirup. The combined sirupy material crystallized from ethanol upon standing at room temperature overnight; yield 5.2 g., m.p. 141–143°. The material was further purified by recrystallization from ethanol; m.p. 144–145°, $[\alpha]^{26}D$ +97.2° (*c* 4.3, chloroform). These constants are in close agreement with the accepted values[2] (m.p. 143–144°, $[\alpha]^{25}D$ +97°) for β-isomaltose octaacetate.

The 15% effluent was evaporated to a sirup under reduced pressure and dried by repeated distillation with methanol under reduced pressure below 50°; yield 6.7 g. This material was acetylated by heating with 3 g. of anhydrous sodium acetate and 35 ml. of acetic anhydride just below the boiling point until all the sugar was in solution. The reaction mixture was poured into 200 ml. of ice and water. After the acetic anhydride was hydrolyzed, the precipitated sirup was removed by decantation, dissolved in ethanol and allowed to crystallize; yield 2.5 g., m.p. 186–189°, $[\alpha]^{27}D$ −5.6° (*c* 4.4, chloroform). This is in substantial agreement with the accepted values of m.p. 193° and $[\alpha]^{20}D$ −5.3° for β-gentiobiose octaacetate.

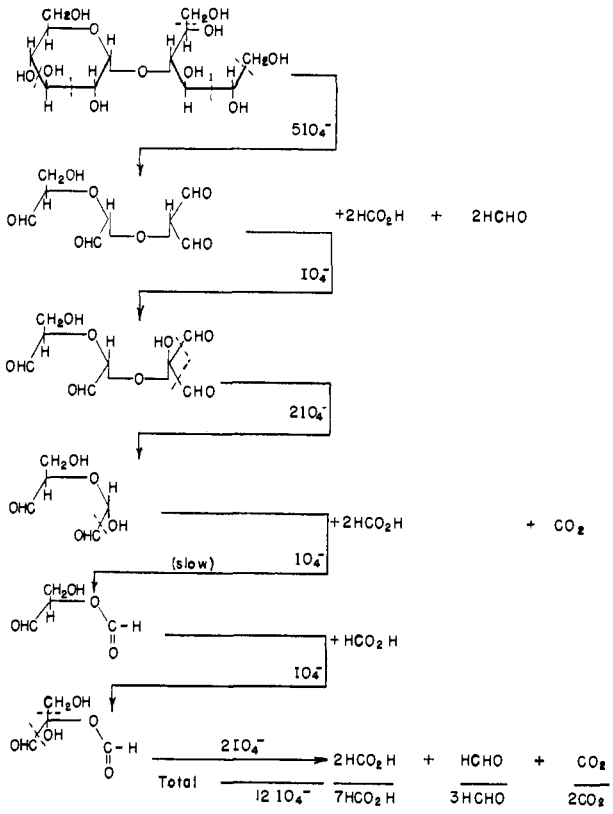

Fig. 2.—Postulated course of periodate oxidation of maltitol.

Isomaltitol.—The sirup obtained from the deacetylation[2] of 5 g. of pure β-isomaltose octaacetate was dissolved in 50 ml. of water containing 0.5 g. of Raney nickel catalyst and shaken in a bomb at 80° under 1700 p.s.i. of hydrogen for 4 hr. The solution was filtered and evaporated to a sirup under reduced pressure. The sirup was dissolved in methanol and again evaporated under reduced pressure. The amorphous material was then crystallized from methanol; yield 2.0 g., m.p. 163–165°. Purification was effected by recrystallization from methanol; m.p. 165.5–167°, $[\alpha]^{23}D$ +89° (*c* 4, water); X-ray powder diffraction data: 6.11[22] −10,[23] 5.67–30, 5.08–90, 4.53–100, 4.24–10, 4.01–65, 3.67–10, 3.49–50, 3.21–70, 3.05–5, 2.92–20, 2.71–25, 2.52–50, 2.27–40, 2.16–35, 2.00–20, 1.88–10, 1.78–5, 1.69–10, 1.61–10. Periodate oxidation data on this substance are recorded in Table I.

Anal. Calcd. for $C_{12}H_{24}O_{11}$: C, 41.85; H, 7.02. Found: C, 41.93; H, 6.78.

Isomaltitol Nonaacetate.—Isomaltitol (1.5 g.) was treated with 0.5 g. of anhydrous sodium acetate and 10 ml. of acetic anhydride at a temperature just below the boiling point of the solution until all the material had dissolved. It was then poured into 75 ml. of ice and water and stirred occasionally until the excess acetic anhydride hydrolyzed. The sirupy material crystallized from ethanol; yield 2.0 g., m.p. 111–114°. The material was further purified by recrystallization from ethanol; m.p. 114–115°, $[\alpha]^{29}D$ +70° (*c* 1.0, chloroform).

Anal. Calcd. for $(C_{12}H_{15}O_{11})(CH_3CO)_9$: C, 49.85; H, 5.85; ($CH_3CO$), 12.31 ml. of 0.1 N NaOH per 100 mg. Found: C, 49.80; H, 5.78; (CH_3CO), 12.48 ml.

Chromatographic Resolution of a Reduced and Acetylated Partial Hydrolyzate from Glycogen.—An amount of 12 g. of a glycogen hydrolyzate carried to 60% completeness[13] was dissolved in 130 ml. of 10% aqueous ethanol and reduced with 10 g. of Raney nickel catalyst at 60° and 1100 p.s.i. of hydrogen for 12 hr. followed by an additional 12 hr. at 80° and 1900

(20) Mother liquor or molasses from the commercial crystallization of D-glucose; furnished by the Corn Products Refining Co., Argo, Illinois.

(21) Decolorizing charcoal; a product of Darco Department, Atlas Powder Co., New York, N. Y.

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

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

p.s.i. The catalyst was removed by filtration and the sirup obtained on solvent removal was dried by repeated distillation of added ethanol under reduced pressure and finally in a desiccator under reduced pressure. The product was dissolved in 180 ml. of freshly distilled and dried pyridine and 80 ml. of this was removed under reduced pressure in order to further dry the reaction mixture. The solution was cooled to 0°, 100 ml. of acetic anhydride was added in small amounts under vigorous shaking, and the whole was maintained at 5° for 82 hr. The viscous acetylation mixture was then poured into 800 ml. of ice and water and the separated sirup was removed by decantation and washed by decantation with several portions of cold water. The resultant solid was dissolved in chloroform and washed successively with water, dilute aqueous cadmium chloride (to remove pyridine as the insoluble cadmium chloride complex) and again with water, dried over Na_2SO_4 and concentrated under reduced pressure. The resultant sirup was frothed and dried in a vacuum desiccator to an amorphous, white solid; yield 22.1 g.

An amount of 3.0 g. of the above acetate mixture was dissolved in 50 ml. of benzene and chromatographed on a 240 × 54 mm. (diam.) column of Magnesol–Celite (5:1 by wt.) by development with 1500 ml. of benzene–*t*-butyl alcohol (75:1 by vol.). By means of the alkaline permanganate[11] streak three zones were located on the extruded column: (A), on the bottom half; (B), one-third of a column length from the top; and (C), at the column top.

The acetone eluate material from each zone was isolated. That from zone A was recrystallized from absolute ethanol and was identified as sorbitol hexaacetate; yield 1.52 g., m.p. 98–99° unchanged on admixture with an authentic specimen, $[\alpha]^{26}D$ +9.3° (*c* 3.0, chloroform).

The sirup from zone B was dissolved in 85 ml. of benzene and rechromatographed as before, employing 2300 ml. of developer. Three zones were obtained. The lower one yielded a further small amount (50 mg.) of sorbitol hexaacetate (m.p. 98–99°) isolated in the same manner. Crystallization from absolute ethanol of the material from the top zone produced maltitol nonaacetate[10,15]; yield 420 mg., m.p. 83–85° unchanged on admixture with an authentic specimen, $[\alpha]^{24}D$ +84.2° (*c* 1.0, chloroform).

The sirup (0.66 g.) from zone C was dissolved in 50 ml. of benzene and added at the top of a 210 mm. × 44 mm. (diam.) column of Silene-EF[12]–Celite (5:1 by wt.). The chromatogram was developed with 900 ml. of benzene–*t*-butyl alcohol (100:1 by vol.). The alkaline permanganate streak located three zones on the extruded column. The acetone eluate from the lowest zone yielded a further amount of maltitol nonaacetate; yield 32 mg., m.p. 82–83°. The acetone eluate from the next higher zone was rechromatographed in the same manner and again yielded two zones. The acetone eluate of the lower zone was dissolved in abs. ethanol and from this isomaltitol nonaacetate crystallized; yield 17 mg., m.p. and mixed m.p. with an authentic specimen (m.p. 114–115°) 113–114°.

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The Reaction of α,β-Unsaturated Aldehydes with Nitro Compounds[1,2]

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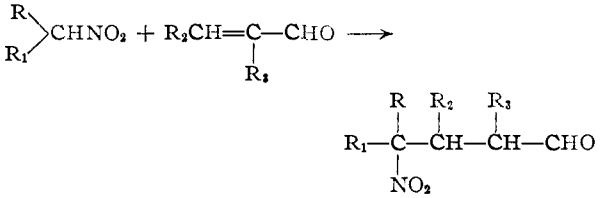
The reactions of primary and secondary nitroparaffins with α,β-unsaturated aldehydes in alcohol solutions containing basic catalysts have been found to yield γ-nitrobutyraldehydes. These aldehyde products have been characterized as their 2,4-dinitrophenylhydrazones. One of the compounds, γ-nitrocaproaldehyde, was converted to the corresponding 4-amino-1-hexanol and this reduction product showed no evidence of vicinal hydroxyl and amino groups in the test with periodic acid. γ-Nitrocaproaldehyde was also hydrogenated at elevated temperatures, and one of the reduction products was α-ethylpyrrolidine. The γ-nitroaldehyde structure has therefore been clearly substantiated.

Several references to the reactions of α,β-unsaturated aldehydes with nitroparaffins have appeared recently.[3,4,5] In all of the experiments recorded, the conditions which were employed apparently resulted in the formation of unsaturated nitro alcohols by the addition of the nitroparaffin to the carbonyl group of the α,β-unsaturated aldehyde.

On the basis of previous studies involving malonate systems and α,β-unsaturated aldehydes,[6] it seemed probable that under the proper conditions, the nitroparaffins might also react with α,β-unsaturated aldehydes to yield nitroaldehyde compounds resulting from the 1,4-addition of one mole of nitroparaffin to one mole of α,β-unsaturated aldehyde. Accordingly, this possibility was first studied with secondary nitro compounds, in which the presence of the single hydrogen atom on the α-carbon atom might be expected to promote the 1,4-addition with fewer side reactions. 2-Nitropropane was selected for these tests. By carrying out the addition in alcoholic solution and in the presence of a small molar ratio of a strong alkaline catalyst, the desired γ-nitroaldehydes were obtained in modest yields.

From these encouraging results, the reaction was extended to primary nitroparaffins such as 1-nitropropane. In each of the instances studied, 1-nitropropane also reacted with the α,β-unsaturated aldehydes in the 1,4-manner to yield γ-nitrobutyraldehydes. The compounds studied and their structures may be summarized in accordance with the general equation



I, R = R_1 = CH_3; R_2 = R_3 = H
II, R = R_1 = R_3 = CH_3; R_2 = H
III, R = R_1 = R_2 = CH_3; R_3 = H
IV, R = C_2H_5; R_1 = R_2 = R_3 = H
V, R = C_2H_5; R_1 = R_2 = H; R_3 = CH_3
VI, R = C_2H_5; R_1 = R_3 = H; R_2 = CH_3

The isolation of the aldehyde compounds I–VI was accomplished by distillation of the crude reaction mixtures after neutralization and washing.

(1) Paper No. 117, Journal Series, General Mills, Inc., Research Dept.

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