the hydrolysis mixtures were dried and distilled to dryness. The residues were fractionally distilled under reduced pressure to remove some vanillonitrile and long chain alcohol and to recover the desired esters as colorless oils which solidified to low melting solids. Data are given in Table II.

Acknowledgment.—The authors wish to thank Mr. Donald McDonnell for the analyses and spectra reported in this paper.

APPLETON, WISCONSIN RECEIVED JANUARY 27, 1951

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

4-α-Isomaltopyranosyl-D-glucose

BY M. L. WOLFROM, A. THOMPSON[1] AND T. T. GALKOWSKI[1]

The crystalline trisaccharide of D-glucose, herein designated panose, obtained by Pan, Kolachov and associates through the action on maltose of cultures of the mold *Aspergillus niger* NRRL 337, has been reduced to the amorphous glycitol (panitol), characterized as a crystalline dodecaacetate. The acetylated partial hydrolyzate of this glycitol has been chromatographically separated into the following components, all of which have been adequately identified as crystalline substances: panitol dodecaacetate, sorbitol hexaacetate, β-D-glucopyranose pentaacetate, β-isomaltose octaacetate and maltitol nonaacetate. This definitively establishes the structure I (4-α-isomaltopyranosyl-D-glucose) for panose. Calculations have been made for the rate of production of the hydrolytic products of such a trisaccharide alcohol.

The discovery of 4-α-isomaltopyranosyl-D-glucose, a possible hydrolytic product of amylopectin, is an event of major interest in the study of the chemical structure of starch. The synthesis of this new trisaccharide by the action on maltose of an apparent enzyme system contained in the culture filtrate of *Aspergillus niger* NRRL 337 has been reported by Pan, Kolachov and co-workers,[2,3] who demonstrated that the crystalline trisaccharide was composed of D-glucose units. French[4] has proposed for the sugar the structure 4-[6-(α-D-glucopyranosyl)-α-D-glucopyranosyl]-D-glucose or 4-α-isomaltopyranosyl-D-glucose. The sugar and its aldonic acid were partially hydrolyzed by this worker and paper chromatographic evidence was presented for the presence of isomaltose, maltose and D-glucose in the hydrolyzate of the former and of isomaltose (no maltose) and D-glucose in the latter. Further evidence for the presence of one 6-α and one 4-α link was based on the relative positions of the spots from maltotriose, the unknown trisaccharide and a possible trisaccharide from the α-1,6-dextran series. While an excellent exploratory method, paper chromatography does not identify a substance beyond doubt.

We wish to report herein a definitive proof for the structure of this new trisaccharide based upon the isolation and identification of crystalline substances. The sugar was reduced to the corresponding alcohol, thus marking the D-glucose unit possessing the reducing group. The amorphous trisaccharide alcohol was purified through its crystalline dodecaacetate (m.p. 148–150°, $[\alpha]^{27}D$ +120° in chloroform). The trisaccharide alcohol was then partially hydrolyzed to produce mono- and disaccharide sugars and alcohols. The acetylated hydrolyzate was separated into its five components by silicate chromatography[5] and each was adequately identified as a crystalline substance.

There are three possible trisaccharides of D-glucose joined by one 6-α and one 4-α glycosidic linkage. Reduction of these substances to the glycitols with subsequent partial hydrolysis would produce D-glucose, sorbitol (D-glucitol) and unchanged material from all. In addition, one structure would yield maltitol and isomaltose; a second, maltose and isomaltitol; and a third, maltitol and isomaltitol.

Assuming the structure cited first above, it is desirable to calculate the degree of hydrolysis at which the optimum yield of disaccharide materials would be produced. Herein the assumption is made that the two types of linkages, 6-α and 4-α, would each hydrolyze at their constant rate regardless of location in the disaccharide or trisaccharide cleavage fragment and regardless of the state of reduction of the fragment. The first order specific reaction constants for the 6-α and 4-α unions have been determined[6] in isomaltose and maltose and found to be $k_1 = 0.0208$ and $k_2 = 0.0846$ (hr.$^{-1}$, $\log_e$), respectively, when the hydrolysis is carried out in 2% concentration in 0.05 N sulfuric acid at 100°. Such a hydrolytic reaction can be expressed by the equation $C = C_0e^{-kt}$ in which C_0 and C are the concentrations of the material at initial time and after time t, respectively. If the initial quantity of material is taken as one mole, the equation reduces to $C = e^{-kt}$. The total amount of material (either disaccharide or trisaccharide units) in moles containing 6-α links at time t could then be represented by

$$C' = e^{-k_1t} \quad (1)$$

and the amount containing 4-α links would be

$$C'' = e^{-k_2t} \quad (2)$$

Then $(1 - e^{-k_1t})$ equals the number of moles of material, whether di- or trisaccharide, in which 6-α links have been destroyed and likewise $(1 - e^{-k_2t})$ equals the number of moles in which 4-α links have been destroyed. It follows then that the number of moles of starting material in which both links are broken is

$$(1 - e^{-k_1t})(1 - e^{-k_2t})$$

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

(2) S. C. Pan, A. A. Andreasen and P. Kolachov, *Science*, **112**, 115 (1950).

(3) S. C. Pan, L. W. Nicholson and P. Kolachov, *Abstracts Papers Am. Chem. Soc.*, **118**, 113A (1950); THIS JOURNAL, **73**, 2547 (1951).

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

(5) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, **67**, 527 (1945),

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

Maltitol (M) arises when the 6-α link alone is broken and this can be expressed as

$$M = (1 - e^{-k_1 t})\, e^{-k_2 t} \quad (3)$$

Likewise, the amount of isomaltose (I) present is

$$I = (1 - e^{-k_2 t}) e^{-k_1 t} \quad (4)$$

By setting the differential of equation (3) equal to zero and solving for t, one obtains the equation

$$t = \frac{\log (k_1 + k_2)/k_2}{k_1} \quad (5)$$

in which t is the time required to obtain a maximum yield of maltitol. Substituting the known values cited above for k_1 and k_2, the time required to obtain a maximum yield of maltitol is 10.7 hours. Similarly, the time required to obtain a maximum yield of isomaltose is found to be 19.0 hours.

One molecule of D-glucose arises from the terminal non-reducing unit through scission of the 6-α link. A second molecule of D-glucose requires for its formation the scission of both 4-α and 6-α links. Therefore the quantity of D-glucose (G) present is

$$G = (1 - e^{-k_1 t}) + (1 - e^{-k_1 t})(1 - e^{-k_2 t}) \quad (6)$$

$$= (1 - e^{-k_1 t})(2 - e^{-k_2 t}) \quad (7)$$

One molecule of sorbitol (D-glucitol) results when a 4-α link is broken. Therefore, the amount of sorbitol (S) present is

$$S = (1 - e^{-k_2 t}) \quad (8)$$

The amount of unchanged material (P) in which neither 6-α nor 4-α links are broken can be expressed

$$P = e^{-k_1 t}\, e^{-k_2 t} \quad (9)$$

$$= e^{-(k_1 + k_2)t} \quad (10)$$

The above equations allow the calculation of the quantities of the various materials present in a panitol hydrolyzate (Fig. 1). The validity of the equations was tested in a trial run and the observed rotations were found to be in excellent agreement with those calculated from the curves of Fig. 1.

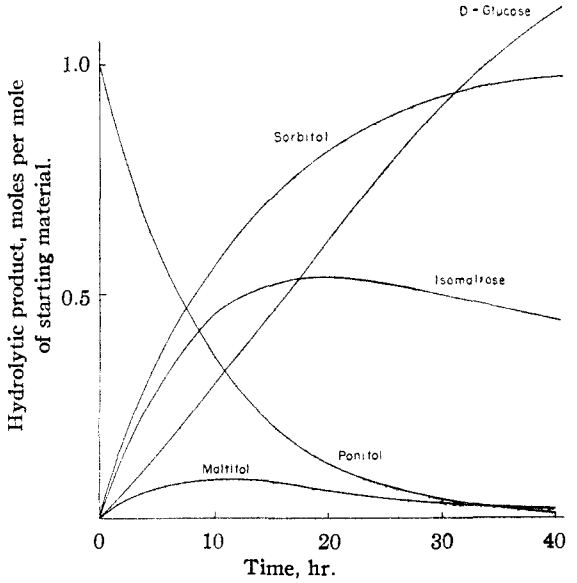
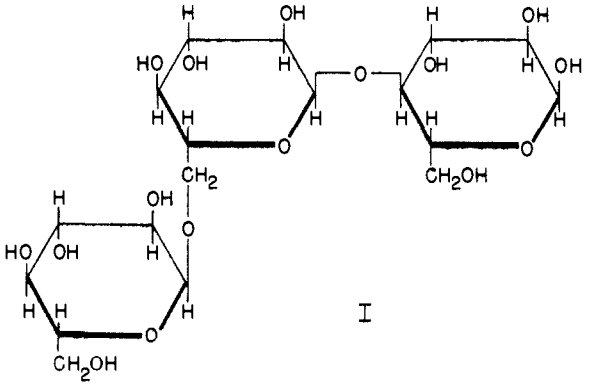


Fig. 1.—Calculated formation of hydrolytic products from panitol (2%) in 0.5 N H_2SO_4 at 100°. Calcd. (panitol basis) $[\alpha]^{25}D$ at 5, 7 and 10.7 hr.: +106°, +97°, +87°, resp.; found: +106°, +101°, +87°.

Maltitol is the disaccharide unit present in the smallest quantities and for isolation purposes the hydrolysis was interrupted at a time close to that calculated (10.7 hr.) to yield the maximum quantity of this substance. The products chromatographically isolated from the acetylated (sodium acetate–acetic anhydride) hydrolyzate and definitely identified as crystalline acetates were: the dodecaacetate of unchanged trisaccharide alcohol, β-D-glucopyranose pentaacetate, D-glucitol (sorbitol) hexaacetate, maltitol nonaacetate and β-isomaltose octaacetate. The only trisaccharide (reduced to the alcohol) which can produce this combination of products is 4-α-D-isomaltopyranosyl-α-D-glucopyranose (I). The α-D assignment for the free reducing unit rests upon the fact that Pan and co-workers[8] determined that the sugar showed downward mutarotation. The pyranose ring is highly probable in this terminal D-glucose moiety but is not rigidly established. The only other adequately characterized trisaccharide containing D-glucose residues joined in α-D union is maltotriose.[7] A short non-systematic name for the new trisaccharide is desirable and the name panose (panitol) is suggested. It is of interest that panose is a rather readily crystallizable sugar and differs in this respect from isomaltose (6-α-D-glucopyranosyl-D-glucose).[8]



Experimental

4-α-Isomaltopyranosyl-D-glucitol Dodecaacetate.—A solution of 5.0 g. of crystalline 4-α-isomaltopyranosyl-α-D-glucose[9] in 50 ml. of water, in which 2 g. of Raney nickel catalyst was suspended, was agitated for 12 hr. with hydrogen (1800 p.s.i.) at 80°. The solution was then filtered and evaporated under reduced pressure below 50° to a sirup. This residue was further dried by distillation with absolute ethanol under reduced pressure. The amorphous material was treated with 2 g. of fused sodium acetate and 25 ml. of acetic anhydride at a temperature just below the boiling point until it was all in solution (about 15 min.). After hydrolysis of the acetic anhydride in 300 ml. of ice and water the acetate crystallized; yield 8.3 g., m.p. 147–149°. Pure material was obtained on further crystallization from abs. ethanol; m.p. 148.5–150°, $[\alpha]^{27}D$ +120° (c 4.0, chloroform[10]).

Anal. Calcd. for $C_{18}H_{22}O_{16}(CH_3CO)_{12}$: C, 49.90; H, 5.78; ($CH_3CO$), 11.88 ml. 0.1 N NaOH per 100 mg.; mol. wt., 1010.9. Found: C, 49.87; H, 5.75; (CH_3CO), 11.77 ml.; mol. wt. (Rast), 1054.

(7) L. W. Georges, I. L. Miller and M. L. Wolfrom, THIS JOURNAL, **69**, 473 (1947); M. L. Wolfrom, L. W. Georges, A. Thompson and I. L. Miller, *ibid.*, **71**, 2873 (1949); J. M. Sugihara and M. L. Wolfrom, *ibid.*, **71**, 3357 (1949).

(8) Isomaltose has never been obtained crystalline in this Laboratory.

(9) Kindly furnished by Dr. S. C. Pan of the Research Laboratories, Joseph E. Seagram and Sons, Inc., Louisville, Kentucky.

(10) All chloroform employed contained *ca.* 0.5% ethanol.

4-α-Isomaltopyranosyl-D-glucitol.—Seven grams of 4-α-isomaltopyranosyl-D-glucitol dodecaacetate suspended in 90 ml. of 0.05 *N* $NaOCH_3$ was maintained at room temperature for 2 hr. and then overnight at ice-box temperature. The resultant suspension was dissolved by the addition of 50 ml. of water and passed successively through Amberlite IR-120[11] and Duolite A-4[12] ion exchange columns (150 × 20 mm., diam.). The solution was then evaporated to a sirup under reduced pressure below 50° and dried by distillation under reduced pressure with absolute ethanol; yield 3.5 g. of amorphous material, $[\alpha]^{23}D$ +133° (*c* 1.7, water).

Anal. Calcd. for $C_{18}H_{34}O_{16}$: C, 42.68; H, 6.76. Found: C, 42.61; H, 7.06.

Hydrolysis of 4-α-Isomaltopyranosyl-D-glucitol.—4-α-Isomaltopyranosyl-D-glucitol (3.3 g.) was dissolved in 150 ml. of 0.05 *N* H_2SO_4 and refluxed for 7 hr. During this time the specific rotation of the solution changed from +133° to +101° (calcd. +97°, panitol basis). The reaction was stopped and the solution was passed through a column (150 × 20 mm., diam.) of Duolite A-4[12] to remove the sulfuric acid. The water was removed by evaporation under reduced pressure below 50° and finally dried by distillation with absolute ethanol under reduced pressure.

Acetylation of Hydrolysis Products of 4-α-Isomaltopyranosyl-D-glucitol.—The residue of partially hydrolyzed trisaccharide alcohol was heated just short of boiling with 20 ml. of acetic anhydride in 2 g. of fused sodium acetate until solution was effected (*ca.* 15 min.). The excess acetic anhydride was hydrolyzed by pouring the cooled mixture into 200 ml. of ice and water and the resultant mixture was extracted with chloroform. The extract was washed with an aqueous solution of sodium bicarbonate and then with water, dried with anhydrous sodium sulfate and the chloroform removed under reduced pressure.

Separation and Identification of the Components of the Acetylated Mixture.—The mixed acetates from the above procedure were dissolved in 50 ml. of benzene, placed on a Magnesol[13]-Celite[14] (5:1 by wt.) column (275 × 80 mm., diam.) and developed with 2500 ml. of benzene–*t*-butyl alcohol (75:1 by vol.). Three zones were located by streaking the extruded column with the permanganate indicator (1% $KMnO_4$ in 10% NaOH). The sectioned zones were eluted with acetone and the acetone removed under reduced pressure.

The material from the zone near the bottom of the column was crystallized from ethanol; yield 0.57 g., m.p. 127–130°. It was recrystallized from abs. ethanol; m.p. 128–130° unchanged on admixture with an authentic specimen of β-D-glucopyranose pentaacetate (m.p. 130–131°), $[\alpha]^{23}D$ +4.5° (*c* 4.6, chloroform). This fraction was thus identified as β-D-glucopyranose pentaacetate.

The material from the zone near the middle of the column was crystallized from abs. ethanol; yield 0.98 g., m.p. 97–100°. It was recrystallized from abs. ethanol; m.p. 98–100°, $[\alpha]^{23}D$ +9.4° (*c* 4.2, chloroform). These values identify this fraction as D-glucitol (sorbitol) hexaacetate.

The material from the third zone near the top of the column was dissolved in 50 ml. of benzene and placed on a fresh column of Magnesol–Celite of the same size as above and developed with 4000 ml. of benzene–*t*-butyl alcohol (75:1 by vol.). Upon extrusion and application of the indicator, two zones appeared near the top of the column. The sectioned zones were eluted with acetone and the solvent removed under reduced pressure. The material from the lower zone was dissolved in abs. ethanol and crystallized upon standing overnight; yield 0.72 g., m.p. 143–145°. Recrystallization was effected from abs. ethanol; m.p. 143–145°, $[\alpha]^{27}D$ +98° (*c* 4.5, chloroform). This fraction was thus identified as β-isomaltose octaacetate for which reported[15] constants are: m.p. 143–144°, $[\alpha]^{25}D$ +97° (*c* 3, chloroform).

The material from the zone near the column top was crystallized from abs. ethanol; yield 1.34 g., m.p. 140–144°. Since purification of this material was not effected by further recrystallization, a portion (0.6 g.) was rechromatographed on a Magnesol–Celite (5:1 by wt.) column (230 × 50 mm., diam.) using 5000 ml. of benzene–*t*-butyl alcohol (50:1 by vol.) as developer. The principal zone near the top of the column was removed, eluted with acetone and its content obtained as a sirup by solvent removal under reduced pressure. The material crystallized from abs. ethanol; m.p. 147–149°, $[\alpha]^{25}D$ +120° (*c* 4.1, chloroform). This fraction was thus identified as unchanged 4-α-isomaltopyranosyl-D-glucitol dodecaacetate.

The mother liquors from the crystallization of the β-isomaltose octaacetate and the 4-α-isomaltopyranosyl-D-glucitol dodecaacetate were combined and concentrated to a sirup. This sirup was dissolved in 30 ml. of benzene, placed on a column of Magnesol–Celite (5:1 by wt.) (230 × 50 mm., diam.) and developed with 2500 ml. of benzene–*t*-butyl alcohol (50:1 by vol.). Three zones appeared in the upper half of the column. The sectioned zones were eluted with acetone and the acetone solutions were evaporated under reduced pressure to sirups which were crystallized from ethanol. The crystalline substances from the top and bottom zones were identified by melting point as the acetate of unchanged starting material and β-isomaltose octaacetate, respectively. The middle zone material (yield 0.10 g., m.p. 91–95°) was recrystallized from ethanol; m.p. 76–80° on grinding in a mortar, $[\alpha]^{25}D$ +81° (*c* 1.8, chloroform). X-Ray powder diffraction data: 7.99[16]–20[17]; 5.59–5; 4.27–100; 4.00–40; 3.75–20; 3.40–5; 2.71–5; identical with that of a known sample of maltitol nonaacetate.[18] These constants identify this fraction as maltitol nonaacetate. The peculiar melting behavior is characteristic of our current preparations of this substance and may be indicative of some polymorphism.

Acknowledgment.—We are pleased to acknowledge the counsel of Prof. E. N. Lassettre of this department.

COLUMBUS 10, OHIO RECEIVED MARCH 5, 1951

(11) A product of Rohm and Haas Company, Philadelphia 5, Pennsylvania.

(12) A product of the Chemical Process Company, Redwood City, California.

(13) A product of Westvaco Chlorine Products Corp., South Charleston, West Virginia.

(14) A product of Johns-Manville Co., New York, N. Y.

(15) M. L. Wolfrom, L. W. Georges and I. L. Miller, THIS JOURNAL, **71**, 125 (1949).

(16) Interplanar spacing, Å., Cu Kα radiation.

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

(18) M. L. Wolfrom and T. S. Gardner, THIS JOURNAL, **62**, 2553 (1940).