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

Degradation of Amylopectin to Panose

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By the use of a combination of carbon and silicate chromatography a trisaccharide, 4-α-isomaltopyranosyl-D-glucose or panose, has been isolated as its crystalline glycitol dodecaacetate from a reduced acid hydrolyzate of amylopectin. The hydrolysis was carried to 70% completion since calculations showed that at this point a maximum yield of trisaccharide would be produced. The isolation of panose, which contains one α-1,6-link, from this hydrolyzate constitutes further evidence for the conception that branching occurs at the six position of D-glucose in the amylopectin molecule.

Considerable indirect evidence has accumulated that amylopectin is a branched polymer of D-glucose with the point of branching at C-6 of the D-glucose molecule. This concept is further supported by the isolation of isomaltose (6-α-D-glucopyranosyl-D-glucose), characterized as its crystalline β-octaacetate,[2] from the enzymic[3] or acid[4] hydrolyzate of amylopectin, evidence being offered that isomaltose was preformed in the molecule and was not an artifact. We wish to report herein the isolation of panose[5] (4-α-isomaltopyranosyl-D-glucose[6,7]), characterized as its crystalline alditol dodecaacetate, from the acid hydrolysis (with subsequent reduction) of amylopectin.

In a previous publication[8] a statistical analysis of the hydrolytic degradation of branched chain molecules of the amylopectin or glycogen type was made whereby the yields of those oligosaccharides which contain one α-1,6-glycosidic linkage could be calculated for any desired degree of hydrolysis. A detailed treatment was recorded for the case of isomaltose. An exactly similar calculation can be made for the three possible trisaccharides from amylopectin containing one α-1,6-link employing a branching frequency of 1:25. This shows that a maximum yield of 0.91% trisaccharide will occur at 68.9% of complete hydrolysis. Since there are three possible trisaccharides containing one α-1,6- and one α-1,4-link, the maximum yield of each will be 0.91/3 or 0.3%.

The amylopectin (waxy maize starch) hydrolysis (2% carbohydrate in 0.05 *N* sulfuric acid at 98°) in the work herein reported was accordingly carried out to *ca.* 70% completion (24 hr.), the course of the reaction being followed by copper reduction. The hydrolyzate material was subjected to a preliminary fractionation by the method of selective elution from carbon as developed by Whistler and Durso.[9] The fractions were then acetylated (sodium acetate and acetic anhydride) and further separated by silicate chromatography. The zones thus obtained which were considered most likely to contain trisaccharides were deacetylated and reduced to the corresponding glycitols with Raney nickel catalyst and hydrogen under pressure. Upon reacetylation with subsequent silicate chromatography, one of these zones yielded crystalline material (nuclei being at hand from previous work[7]) adequately identified as panitol dodecaacetate by melting point, rotation and X-ray powder diffraction pattern. The yield of pure material was 0.1% in comparison with a calculated maximum of 0.3%.

Maltotriose, as its crystalline β-hendecaacetate, has been isolated from enzymic[2,10] and acid[4,8] hydrolyzates of amylose and amylopectin but panose is the first trisaccharide containing the α-1,6-link which has been identified, on a crystalline basis, in an acid hydrolyzate of amylopectin. This constitutes further degradative evidence for branching at C-6 of D-glucose in the amylopectin molecule. Confirmatory to previous work,[4] small amounts of maltose and isomaltose were isolated as their β-octaacetates from the reaction mixture. The fraction normally expected to contain D-glucose was not investigated. Boissonnas[11] has pointed out the chromatographic simplification effectable by reducing an aldose to the alditol (glycitol). It is hoped that further application of the chromatographic resolution of such reduced hydrolyzates may yield other crystalline derivatives of these difficultly crystallizable starch hydrolytic fragments.

Experimental

Hydrolysis of Amylopectin.—Amylopectin in the form of waxy maize starch (50 g.) was stirred vigorously in 2500 ml. of 0.05 *N* H_2SO_4 while heating in a boiling water-bath. The course of the hydrolysis was followed by the copper-reduction method of Somogyi.[12] The reducing sugar was calculated as glucose and the hydrolysis was continued until the reaction had reached 70% completion (24 hr.). The sulfuric acid was removed by passing the solution through a 500 × 30 mm. (i.d.) column of Duolite A-4.[13] The neutral solution was then evaporated under reduced pressure to 1250 ml.

Fractionation of Amylopectin Hydrolyzate.—The above hydrolyzate was placed on a 275 × 105 mm. (i.d.) column of activated carbon (Darco G-60[14]–Celite,[15] 1:1 by wt.). The D-glucose was removed from the column by washing with water (9 liters) until the effluent became negative to Benedict solution. This first portion of effluent was discarded. A second portion of sugar was obtained by washing the column with 5% ethanol (15 liters) until the effluent again became negative to Benedict solution. A third portion of material was obtained by washing the column with 15% ethanol

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

(2) M. L. Wolfrom, L. W. Georges and I. L. Miller, THIS JOURNAL, 69, 473 (1947); *ibid.*, 71, 125 (1949).

(3) Edna M. Montgomery, F. B. Weakley and G. E. Hilbert, *ibid.*, 69, 2249 (1947); *ibid.*, 71, 1682 (1949).

(4) M. L. Wolfrom, J. T. Tyree, T. T. Galkowski and A. N. O'Neill, *ibid.*, 72, 1427 (1950); *ibid.*, 73, 4927 (1951).

(5) S. C. Pan, A. A. Andreasen and P. Kolachov, *Science*, 112, 115 (1950); S. C. Pan, L. W. Nicholson and P. Kolachov, THIS JOURNAL, 73, 2547 (1951).

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

(7) M. L. Wolfrom, A. Thompson and T. T. Galkowski, THIS JOURNAL, 73, 4093 (1951).

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

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

(10) M. L. Wolfrom, L. W. Georges, A. Thompson and I. L. Miller, *ibid.*, 71, 2873 (1949).

(11) R. A. Boissonnas, *Helv. Chim. Acta*, 30, 1689 (1947).

(12) M. Somogyi, *J. Biol. Chem.*, 160, 61 (1945).

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

(14) A product of Darco Dept., Atlas Powder Company, New York, N. Y.

(15) No. 535, a product of Johns-Manville Co., New York, N. Y.

(8 liters). The 5% and 15% ethanol effluents were evaporated to sirups under reduced pressure and further dried by distillation with methanol under reduced pressure; yields 5 g. and 6 g., respectively.

β-Isomaltose Octaacetate.—The 5 g. of amorphous material from the 5% ethanol effluent was acetylated with 2 g. of sodium acetate and 40 ml. of acetic anhydride at a temperature just below the boiling point of the mixture. The excess acetic anhydride was hydrolyzed by stirring with 200 g. of ice and water; yield 9 g. of acetylated sirup. This material was dissolved in 90 ml. of benzene and chromatographed on two 275 × 80 mm. (i.d.) columns of Magnesol[14]–Celite[15] (5:1 by wt.) and each was developed with 3000 ml. of benzene–*t*-butyl alcohol (50:1 by vol.). Four zones were located by streaking the extruded columns with permanganate indicator (1% $KMnO_4$ in 10% NaOH). The sectioned zones were eluted with acetone and the acetone was removed by evaporation under reduced pressure. The material from the three top zones failed to crystallize; that in the zone near the bottom crystallized from ethanol; yield 360 mg., m.p. 144–145° unchanged on admixture with authentic β-isomaltose octaacetate, $[\alpha]^{22}D$ +96° (*c* 2.9, chloroform). These values agree with those (143–144°, +97°) accepted[2] for β-isomaltose octaacetate.

Panitol Dodecaacetate.—The material (6 g.) from the 15% ethanol effluent from the carbon column was acetylated in the manner just described. The resulting acetylated material was chromatographed on Magnesol–Celite as described above except that 3000 ml. of benzene–*t*-butyl alcohol (35:1 by vol.) was used as the developing agent. Four zones appeared on the column. The zone material was removed from the sectioned column by elution with acetone. After removal of the acetone the zone failed to crystallize. The benzene–*t*-butyl alcohol effluent was evaporated to dryness to give β-maltose octaacetate; yield 0.5 g., m.p. 155–156°, $[\alpha]^{23}D$ +63° (accepted values: 159–160°, +63°). The material (0.9 g., from the second zone from the top of the column was dissolved in 10 ml. of 0.05 *N* $NaOCH_3$ in methanol and allowed to remain at 5° overnight. It was then diluted with 50 ml. of water and passed successively through ion exchange columns (150 × 20 mm. i.d.) of Amberlite 120[17] and Duolite A-4[13]. The solution and washings were evaporated to 50 ml. under reduced pressure. The sugar was then hydrogenated at 1800 p.s.i. and 80° for 3 hr. in the presence of 2 g. of Raney nickel catalyst. After filtration and removal of the solvent by evaporation under reduced pressure, the resultant amorphous material was again acetylated with sodium acetate (0.3 g.) and acetic anhydride (7 ml.) as described above. The resulting sirup crystallized from ethanol; yield 35 mg., m.p. 138–144°. The mother liquor was evaporated to a sirup and redissolved in 30 ml. of benzene. This solution was placed on a column (275 × 80 mm. i.d.) of Silene[18]–Celite[15] (5:1 by wt.) and developed with 4 liters of benzene–*t*-butyl alcohol (75:1 by vol.). Three zones appeared on the column which were sectioned and eluted with acetone. Crystalline material was obtained from the zone near the column top; yield 90 mg., m.p. 140–145°. The combined portions of crystalline material (125 mg.) were further purified by three recrystallizations from ethanol; yield 70 mg. (0.1%), m.p. 147–148° unchanged on admixture with authentic panitol dodecaacetate (m.p. 148.5–150°, $[\alpha]^{25}D$ +120° in chloroform[7]), $[\alpha]^{25}D$ +118° (*c* 2.6, chloroform). X-Ray powder diffraction data: 8.51[19] −70,[20] 6.96–20, 5.73–5, 4.66–100, 4.08–20, 3.36–40, 3.17–5, 2.97–10, 2.67–10, 2.35–5. The X-ray diagram was identical with that of an authentic sample of panitol dodecaacetate.

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

(17) A product of Rohm and Haas Co., Philadelphia, Pennsylvania.

(18) A product of the Columbia Chemical Co., Barberton, Ohio.

(19) Interplanar spacing, Å.; $CuK\alpha$ radiation.

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

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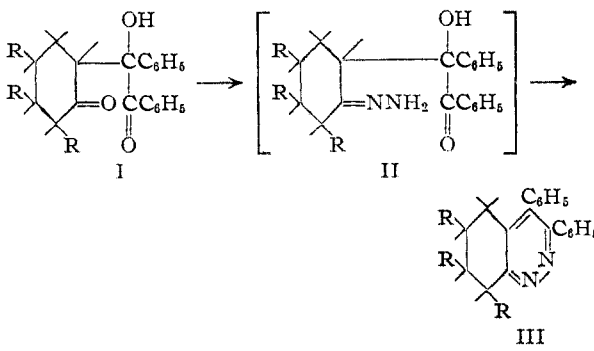
Some 3,4-Diphenylcinnolines and Related Compounds

BY C. F. H. ALLEN AND J. A. VANALLAN

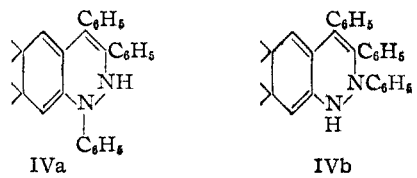
2-(Hydroxydesyl)-cyclohexanones have been converted into 5,6,7,8-tetrahydrocinnolines by treatment with hydrazine. These are the first reduced cinnolines, other than 1,2-dihydro derivatives, to be described. A variety of substances result on dehydrogenation in the presence of a palladium catalyst. The principal product is an indole, but a true cinnoline is also formed; the reaction is not a useful source of cinnolines. The cinnolines described in this paper form salts with one equivalent of an alkyl halide. Improved procedures are given for the preparation of benzil monophenylhydrazone and 3,4-diphenylcinnoline.

When 2-(hydroxydesyl)-4(or 5, or 6)-methylcyclohexanones (I)[1] are treated with hydrazine, water is eliminated as with other 1,4-diketones and reduced cinnolines (1,2-diazanaphthalenes) (III) are formed.



I II III

The intermediate hydrazone (II) cannot be isolated unless phenylhydrazine or 2,4-dinitrophenylhydrazine is used. These two derivatives have been previously described.[2] While the monophenylhydrazone is readily cyclized, all attempts to cyclize the dinitro derivative failed. No attempt has been made to determine which of the two most likely structures is correct for the tetrahydrocinnoline (IVa, b) derived from phenylhydrazine.



IVa IVb

The tetrahydrocinnolines (III) do not evolve methane when treated with methylmagnesium iodide; hence, it is unlikely that any hydrogen is attached to nitrogen. Since these substances do

(1) C. F. H. Allen and J. A. VanAllan, *J. Org. Chem.*, 16, 716 (1951).

(2) C. F. H. Allen, *Can. J. Research*, 4, 264 (1931).