Anal. Calcd. for $C_7H_6N_2 \cdot HCl$: C, 54.37; H, 4.57; N, 18.12; Cl, 22.94. Found: C, 54.33; H, 4.50; N, 18.28; Cl, 22.89.

A picrate of the 3-cyanomethylpyridine melted at 161.3°.

Anal. Calcd. for $C_7H_6N_2 \cdot C_6H_3N_3O_7$: C, 44.94; H, 2.61; N, 20.18. Found: C, 44.99; H, 2.68; N, 19.98.

Bromination of 3-Cyanomethylpyridine.—The reaction of bromine with 3-cyanomethylpyridine in glacial acetic acid solvent appeared to proceed normally with the evolution of HBr and resulted in the formation of a quantitative yield of light amber oil when the acetic acid was removed under vacuum. The product could not be obtained crystalline and failed to give any identifiable product when treated with aluminum chloride and benzene.

α-Phenyl-α-(3-pyridyl)-acetonitrile.—Phenylacetonitrile (117 g., 1 mole) was heated at 80° with 300 ml. of toluene and one mole of sodium amide for two hours. The resulting mixture was cooled and 3-bromopyridine (159.1 g., 1 mole) was added over a ten-minute period. The mixture was refluxed for one hour, cooled, water added, and the toluene layer extracted with dilute hydrochloric acid. The acid extract was made basic with sodium hydroxide and extracted with benzene. The benzene layer was dried and distilled to give 79.9 g. of recovered 3-bromopyridine and 35.1 (36%) of α-phenyl-α-(3-pyridyl)-acetonitrile, b.p. 152–157° (2 mm.), m.p. 58–62°. Recrystallization gave a product melting at 63–65°.

Anal. Calcd. for $C_{13}H_{10}N_2$: C, 80,37; H, 5.19; N, 14.44. Found: C, 80.46; H, 5.18; N, 14.52.

This formed a picrate melting at 148–150°. An extended reflux period decreased the yield and although excess sodium amide increased the yield slightly (42%) the product could not be purified as readily.

2-Phenyl-2-(3-pyridyl)-4-dimethylaminobutyronitrile.—A mixture of α-phenyl-α-(3-pyridyl)-acetonitrile (78.7 g.), benzene (200 ml.) and sodamide (16.5 g.) was held with stirring at 40–50° for 1.5 hours. Ammonia was evolved and an oil separated. The mixture was cooled to 20° and β-dimethylaminoethyl chloride (42.5 g.) added over a ten-minute period. The temperature rose spontaneously to 30° and after stirring one hour the reaction mixture was refluxed for 1.5 hours. Water was added and the benzene layer extracted with 500 ml. of 2 *N* hydrochloric acid. Neutralization of the acid extract with ammonium hydroxide caused separation of an oil layer which was removed by benzene extraction. Distillation of the dried benzene extracts gave 64.2 g. (59.7% yield) of a clear viscous oil, b.p. 152° (0.15 mm.). A picrate melted at 193.5–196.5°.

Anal. Calcd. for $C_{17}H_{19}N_3 \cdot 2C_6H_3N_3O_7$: C, 48.13; H, 3.46. Found: C, 48.58; H, 3.68.

4-Phenyl-4-(3-pyridyl)-6-dimethylamino-3-hexanone.—To the Grignard solution from 0.2 mole of ethyl bromide in 60 ml. of ether was added 2-phenyl-2-(3-pyridyl)-4-dimethylaminobutyronitrile (14 g., 0.053 mole) dissolved in 20 ml. of toluene; a heavy gum formed. The mixture was refluxed for six hours and then hydrolyzed by cold 6 *N* hydrochloric acid. The acid solution was neutralized with ammonium hydroxide until magnesium hydroxide just precipitated. The oil which separated was extracted with benzene and after drying over potassium carbonate was distilled to give 6.1 g. of material, b.p. 115–145° (0.05 mm.) and 3.5 g. b.p. 145–146° (0.05 mm.). The latter material formed a picrate, m.p. 192–194°.

Anal. Calcd. for $C_{19}H_{24}N_2O \cdot 2C_6H_3N_3O_7$: C, 49.34; H, 3.96. Found: C, 49.01; H, 4.07.

A hydrochloride was formed in absolute isopropyl alcohol–benzene mixture, m.p. 133–136° dec.

Anal. Calcd. for $C_{19}H_{24}N_2O \cdot 2HCl \cdot 2H_2O$: C, 56.24; H, 7.46; N, 6.91; Cl, 17.49. Found: C, 56.83; H, 7.24; N, 6.96; Cl, 17.60.

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

Acid Degradation of Amylopectin to Isomaltose and Maltotriose[1]

BY M. L. WOLFROM, J. T. TYREE,[2] T. T. GALKOWSKI[2] AND A. N. O'NEILL

Mild acetolysis of amylopectin (waxy maize starch) with subsequent conversion of the acetolysate to the β-D-acetate mixture and silicate column chromatography of this led to the isolation in crystalline form of β-D-glucopyranose pentaacetate, β-maltose octaacetate (and heptaacetate) and β-maltotriose hendecaacetate. Since calculation showed that the maximum amount of isomaltose would be expected at 90% hydrolysis, amylopectin was hydrolyzed to this point and the hydrolysate was converted to the β-D-acetate mixture which was chromatographed on silicate columns. There was isolated in crystalline form β-D-glucopyranose pentaacetate, β-maltose octaacetate and β-isomaltose octaacetate (1% yield). Evidence is presented that this amount of the latter is not a reversion (resynthesis) product and therefore offers further definitive evidence, on a crystalline basis, for the 6-α point of branching in amylopectin.

Indirect evidence exists that amylopectin is a two-dimensional polymer branched at C_6. It is desirable to place this evidence upon a definitive basis through the isolation, from an amylopectin hydrolysate, of the disaccharide isomaltose, 6-(α-D-glucopyranosyl)-D-glucose, containing the point of branching together with evidence that the disaccharide is not a product of reversion (resynthesis). It is known that both acids and some enzymes are capable of causing re-synthesis in hydrolysates. Thus an enzyme preparation from *Aspergillus niger* NRRL 337 acts upon maltose to form a trisaccharide[3] containing both maltose and isomaltose disaccharide linkages.[4] It is established that high sugar concentrations and high acidity favor resynthesis by acids.

Action upon amylopectin of the α- and β-amylases of malt does not yield isomaltose,[5] although levoglucosan (1,6-anhydro-D-glucopyranose) is isolable from such an enzymic hydrolysate after subsequent treatment with an amylase preparation from *Aspergillus oryzae*.[6] This same enzyme preparation (containing maltase) acts directly upon amylopectin to give isomaltose,[7] characterizable as its crystalline β-octaacetate. This disaccharide was not isolable from reaction mixtures containing the enzyme and the various substances, other than

(1) A preliminary report, by the same authors, of the acid degradation of amylopectin to isomaltose has appeared in THIS JOURNAL, **72**, 1427 (1950).

(2) Research Associate (J. T. T.) and Research Fellow (T. T. G.) of the Corn Industries Research Foundation (Project 203 of The Ohio State University Research Foundation).

(3) 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).

(4) D. French, *Science*, **113**, 352 (1951); M. L. Wolfrom, A. Thompson and T. T. Galkowski, THIS JOURNAL, **73**, 4093 (1951).

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

(6) Edna M. Montgomery and G. E. Hilbert, *ibid.*, **68**, 916 (1946).

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

amylopectin, present in the hydrolysate. This constitutes evidence, based upon enzymic hydrolysis, that isomaltose is preformed in amylopectin.

We are concerned herewith in establishing isomaltose as a preformed acid hydrolysis product of amylopectin. Following the procedure employed successfully with cellulose,[8] conditions of mild acetolysis were applied to amylopectin (waxy maize starch) but the only crystalline products isolated by chromatographic techniques were β-D-glucopyranose pentaacetate, β-maltose octaacetate (and heptaacetate) and maltotriose hendecaacetate; to obtain these the acetolysis mixture of α-D-acetates was converted to the more readily crystallizable β-D-anomers by deacetylation and reacetylation with sodium acetate and acetic anhydride. Calculations, first applied to glycogen[9] and based upon the relative rates of hydrolysis (4:1) of the maltose and isomaltose linkages under the conditions employed, showed that appreciable amounts of isomaltose would be formed only in the latter stages of hydrolysis. The calculated results for amylopectin (Fig. 1), employing a frequency ratio of 24:1 for the maltose and isomaltose linkages and substituting this in the previously reported formulas, show that the maximum concentration of isomaltose would occur at 91% hydrolysis where 3.4% isomaltose (42% of the original 8%) could be expected. The chromatographic resolution of such a nearly completely hydrolyzed polysaccharide was first effected with glycogen[9] where the frequency ratio (12:1) of the two linkages is more favorable; the crystalline β-D-acetates of D-glucopyranose, maltose, isomaltose and maltotriose were isolated. A control with amylose showed that if any isomaltose were formed as a reversion product under these conditions, the amounts present were below the limits of detectability for the methods employed.

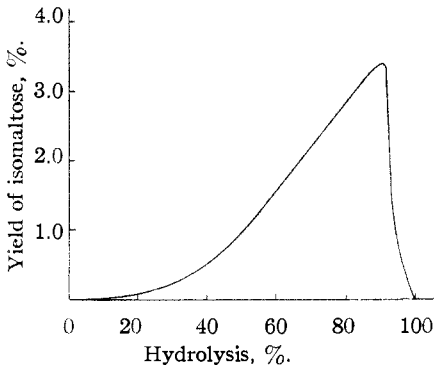

Fig. 1.—Yield of isomaltose as a function of the degree of hydrolysis of amylopectin; calculated according to the formulas of ref. 9 and employing a frequency ratio of 24:1 and a relative hydrolytic rate of 1:4 for the maltose:isomaltose disaccharide linkages.

Hydrolytic conditions (0.4% substrate in 0.08 *N* hydrochloric acid at 100°) closely comparable to those (2% substrate in 0.05 *N* hydrochloric acid) employed with glycogen, were then applied to amylopectin (waxy maize starch) and the course of the reaction was followed by copper reduction methods. The results are shown in Fig. 2 together with those from one experiment at a lower acidity. The reaction is acid-catalyzed and is kinetically first order, in agreement with the results of Swanson and Cori.[10] Our data are not sufficiently accurate to detect a predictable[9] decrease in the specific reaction constant toward the end of the reaction. The experiment at the higher acidity was interrupted at 90% completion, and the acetylated (with sodium acetate and acetic anhydride) hydrolysate was subjected to chromatographic resolution on columns of Magnesol[11]–Celite[11] employing benzene–*t*-butyl alcohol as developer. There was thus isolated in crystalline form: β-D-glucopyranose pentaacetate, β-maltose octaacetate and β-isomaltose octaacetate. Identification was effected by melting point, mixed melting point, rotation and, in the case of the isomaltose derivative, X-ray powder diffraction data. The amount of pure β-isomaltose octaacetate actually isolated was 1% (original amylopectin basis) and so compares with the 3.4% calculated. A blank experiment was performed wherein amylose was substituted for amylopectin and no crystalline β-isomaltose octaacetate was isolable by the methods employed. Therefore, while it is not excluded that a small amount of isomaltose may be formed as a reversion product under these hydrolytic conditions, our method of analysis does not detect it. The crystalline β-isomaltose octaacetate, on the basis of the evidence presented, is believed to exist preformed in the amylopectin and is thus not an artifact.

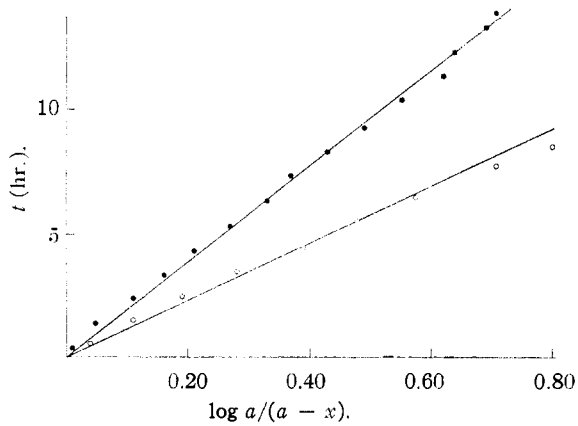

Fig. 2.—Rate of hydrolysis of amylopectin (waxy maize starch) (*c* 0.40) at 100° in hydrochloric acid: ●, 0.040 *N* ($k = 0.11 \pm 0.02$ hr.$^{-1}$); ○, 0.082 *N* ($k = 0.19 \pm 0.03$ hr.$^{-1}$); $k = 2.303 \log a/(a - x)$ wherein x was determined by copper reduction (ref. 15) and k by a statistical average of the slopes between the points on the curves.

Experimental

Acetolysis of Amylopectin.—Using the general procedure of Hess and Dziengel[12] for the mild acetolysis of cellulose, an amount of 50 g. of amylopectin (waxy maize starch) was added with agitation to a cooled mixture of 240 ml. of acetic anhydride, 160 ml. of glacial acetic acid and 30 ml. of concentrated sulfuric acid. After standing at room tempera-

(8) E. E. Dickey and M. L. Wolfrom, THIS JOURNAL, **71**, 825 (1949).

(9) M. L. Wolfrom, E. N. Lassettre and A. N. O'Neill, *ibid.*, **73**, 595 (1951); M. L. Wolfrom and A. N. O'Neill, *ibid.*, **71**, 3857 (1949).

(10) Marjorie A. Swanson and C. F. Cori, *J. Biol. Chem.*, **172**, 797 (1948).

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

(12) K. Hess and K. Dziengel, *Ber.*, **68**, 1594 (1935).

ture for 60 hr., the mixture was heated slowly to 80° and maintained at this temperature until the solid material had completely dissolved (*ca.* 30 min.). The clear dark red solution was filtered through a sintered glass funnel and the filtrate poured slowly and with continuous stirring into 6 l. of ice and water, neutralized with sodium bicarbonate and allowed to stand overnight. The precipitated acetolysate was removed by filtration, washed on the filter with water until the filtrate was neutral to litmus, and dried to constant weight in a vacuum desiccator; yield 85 g.

Deacetylation of the Amylopectin Acetolysate.—An amount of 56 g. of the previously described amylopectin acetolysate was dissolved in 2000 ml. of absolute methanol and cooled to 0°. A solution of 100 ml. of 0.42 *N* barium methoxide was added and the whole was kept at 0° for 48 hr. Then to the solution sufficient cold water was added to dissolve the precipitate and the ionic material was removed by passage through Amberlite exchange resins IR-100 and IR-4.[13] The effluent was concentrated to a sirup under reduced pressure and the residual water was removed by repeated trituration with absolute ethanol. The resulting sirup was frothed and dried in a vacuum desiccator to an amorphous white powder; yield 20 g.

Preparation of the Mixture of β-D-Acetates of the Amylopectin Acetolysate.—The above mixture of free sugars (20 g.) was acetylated with 15 g. of fused sodium acetate and 150 ml. of acetic anhydride by initiating the reaction at a bath temperature of 120° and allowing it to proceed for 1 hr. at 100°. The mixture was then cooled to room temperature, poured with stirring into 1 l. of ice and water, and allowed to stand with occasional stirring for 6 hr. The sirupy acetate mixture was extracted with five 200-ml. portions of chloroform and the extract was washed with water until neutral, dried over anhydrous calcium sulfate and finally concentrated under reduced pressure to a thick sirup which was frothed and dried to constant weight in a vacuum desiccator; yield 38 g.

Chromatographic Resolution.—An amount of 8.0 g. of the above mixture of β-D-acetates was dissolved in 180 ml. of benzene and added at the top of a 265 × 74 mm. (diam.)[14] column of Magnesol[11]–Celite[11] (5:1 by wt.). The chromatogram was developed with 6 l. of benzene–*t*-butyl alcohol (75:1 by vol.). The material in the effluent was recovered by solvent removal under reduced pressure and was recrystallized from an ether–petroleum ether mixture. It was identified as β-D-glucopyranose pentaacetate; yield 2.2 g., m.p. 133–134° unchanged on admixture with an authentic specimen, $[\alpha]^{25}D$ +4° (*c* 5.0, chloroform).

An alkaline permanganate streak[11] on the extruded column indicated three zones: one located on the bottom half of the column (Zone A); another about one-third from the column top (Zone B); and the more highly adsorbed material at the top (Zone C). The column was sectioned and the individual zones were eluted with acetone. The acetone was then removed by distillation under reduced pressure.

The material obtained on concentration of the eluate from Zone A was dissolved in 85 ml. of benzene and rechromatographed on a 240 × 54 mm. (diam.) column of Magnesol–Celite (5:1 by wt.) by development with 2600 ml. of benzene–*t*-butyl alcohol (75:1 by vol.). Two zones were located on the extruded column. The material from the bottom zone was crystallized from absolute ethanol and recrystallized from the same solvent. It was identified as β-maltose octaacetate; yield 0.92 g., m.p. 159–160° unchanged on admixture with an authentic sample, $[\alpha]^{25}D$ +63° (*c* 1.2, chloroform).

The sirup obtained on solvent removal from the eluate of the zone at the top of the column crystallized from absolute ethanol and was identified as β-maltose heptaacetate; yield 15 mg., m.p. 179–181° unchanged on admixture with an authentic specimen.

The sirupy material from Zone B was dissolved in 50 ml. of benzene and added at the top of a 215 × 44 mm. (diam.) column of Magnesol–Celite (5:1 by wt.). The chromatogram was developed with 1800 ml. of benzene–*t*-butyl alcohol (75:1 by vol.). The column was extruded and an alkaline permanganate streak located two zones: the first at the column top and the second about one-third of a column length from the top. These zones were sectioned and each section was eluted with acetone. From the eluate of the top zone, solvent removal left about 10 mg. of a sirup which crystallized from absolute ethanol and was identified as β-maltose heptaacetate; m.p. 179–180°, unchanged on admixture with an authentic specimen. Solvent removal from the acetone eluate of the second zone of the above-described chromatogram left 95 mg. of a sirup which on crystallization and recrystallization from absolute ethanol yielded 80 mg. of crystalline material which was identified as β-maltotriose hendecaacetate; m.p. and mixed m.p. with authentic β-maltotriose hendecaacetate 133–135°, $[\alpha]^{24}D$ +86° (*c* 1.0, chloroform). Wolfrom and associates[5] report for this substance: m.p. 134–136°, $[\alpha]^{25}D$ +86° (*c* 1.5, chloroform).

Attempts to further resolve the sirupy material obtained from Zone C of the original chromatogram into additional crystalline compounds were unsuccessful.

Acetylated Acid Hydrolysate of Amylopectin.—An amount of 20 g. of amylopectin (waxy maize starch) was added at the boiling point to 5 l. of 0.082 *N* HCl. The course of the hydrolysis was followed by reducing value (Fig. 2), employing the procedure of Somogyi,[15] and the reaction was stopped at 10 hr. (*ca.* 90% completion). The rapidly cooled solution was passed over Duolite A-4[16] anion exchange resin. The sirup obtained on solvent removal below 40° under reduced pressure was frothed and acetylated under stirring with acetic anhydride (200 ml.) and fused sodium acetate (10 g.) for 4 hr. at 95–100° (bath temperature) and 0.5 hr. at 120–125°. Crushed ice (1500 g.) was added to the cooled solution under stirring (45 min.) and neutralization to *p*H 6.0 was effected with solid sodium bicarbonate. Solvent removal from the dried chloroform extract of this mixture left a sirup; yield 39 g. (dried over P_2O_5 under reduced pressure).

Chromatographic Resolution.—The above described material (39.0 g.) was made up to 500 ml. with benzene and an aliquot of 75 ml. (5.85 g. of sirup) was placed on a 250 × 75 mm. (diam.) column of Magnesol–Celite (5:1 by wt.) and developed with 2700 ml. of benzene–*t*-butyl alcohol (75:1 by vol.). A zone extending from the middle to the bottom of the extruded column was located by the permanganate streak indicator. Solvent removal from the acetone eluate of this zone yielded material which was crystallized twice from ethanol and identified as β-D-glucopyranose pentaacetate; m.p. 128.5–129.5°, mixed unchanged, $[\alpha]^{24}D$ +4.3° (*c* 2.9, chloroform). The acetone eluate material from a zone about one-third from the top of the column was rechromatographed on a 250 × 55 mm. (diam.) column of Magnesol–Celite (5:1 by wt.) with 1200 ml. of benzene–*t*-butyl alcohol (75:1 by vol.) as developer. The permanganate streak indicator located two zones on the extruded column. The material from the acetone eluate of the lower zone was crystallized from ethanol and identified as β-maltose octaacetate; m.p. 155–156°, mixed m.p. 156–157°, $[\alpha]^{25}D$ +62° (*c* 3.1, chloroform). The material from the acetone eluate of the upper zone was rechromatographed as before on a 220 × 45 mm. (diam.) column with 1500 ml. of developer. The acetone eluate material from a zone located one-third of a column length from the top of the extruded column was again chromatographed as above on a 220 × 45 mm. (diam.) column with 3000 ml. of developer. The acetone eluate of the material from the main zone near the column center was crystallized from ethanol and identified as β-isomaltose octaacetate; yield 50 mg. (1%, basis original starch), m.p. 144–145°, mixed unchanged, $[\alpha]^{25}D$ +96° (*c* 2.0, chloroform), X-ray powder diffraction diagram[7] identical with that of an authentic specimen. Wolfrom, Georges and Miller[17] report for this substance: m.p. 143–144°, $[\alpha]^{25}D$ +97° (*c* 2.7, chloroform).

Acid Hydrolysis of Amylose.—Corn amylose, prepared from defatted corn starch according to the procedure of Schoch,[18] was substituted for amylopectin in the above-described procedure and no β-isomaltose octaacetate nor β-gentiobiose octaacetate (generally considered to be a D-glucose "reversion" product) was found in the chromatographed, acetylated hydrolysate.

COLUMBUS 10, OHIO RECEIVED MARCH 15, 1951

(13) Products of the Resinous Products Division of the Rohm and Haas Co., Philadelphia, Pennsylvania.

(14) Adsorbent dimensions.

(15) M. Somogyi, *J. Biol. Chem.*, **160**, 61 (1945); P. A. Shaffer and M. Somogyi, *ibid.*, **100**, 695 (1933).

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

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

(18) T. J. Schoch, *Cereal Chem.*, **18**, 121 (1941); THIS JOURNAL, **64**, 2957 (1942).