**1-Methyl-3-benzylaminopiperidine.**—From 22.6 g. (0.2 mole) of crude 1-methyl-3-piperidone and 23.4 g. (0.2 mole) of benzylamine after hydrogenation in 75 cc. of absolute ethanol in the presence of platinum oxide catalyst was obtained 27.0 g. (66%) of 1-methyl-3-benzylaminopiperidine, b. p. 112–117° (1 mm.); $n^{22.6}$D 1.5299. The dipicrate melted at 191–193°.

*Anal.* Calcd. for $C_{25}H_{26}N_8O_{14}$: C, 45.32; H, 3.96; N, 16.91. Found: C, 45.61; H, 4.22; N, 17.05.

**1-Methyl-3-(β-dimethylaminoethylamino)-piperidine.**—Reductive alkylation of β-dimethylaminoethylamine with 1-methyl-3-piperidone gave 61% of 1-methyl-3-(β-dimethylaminoethylamino)-piperidine, b. p. 121–130° (19–21 mm.). Redistillation gave a product which boiled at 120–123° (17 mm.); $n^{24}$D 1.4675.

*Anal.* Calcd. for $C_{10}H_{23}N_3$: C, 64.81; H, 12.51; N, 22.68. Found: C, 65.02; H, 12.01; N, 22.17.

The tripicrate melted at 216–217° (dec.).

*Anal.* Calcd. for $C_{28}H_{31}N_{12}O_{21}$: C, 38.54; H, 3.70; N, 19.26. Found: C, 38.62; H, 3.53; N, 18.81.

**1-Ethyl-3-(N-benzyl-N-α-pyridylamino)-piperidine.**—A mixture of 20.2 g. (0.0926 mole) of 1-ethyl-3-benzylaminopiperidine, 14.6 g. (0.0926 mole) of α-bromopyridine, 12.8 g. (0.0926 mole) of potassium carbonate and 0.2 g. of copper bronze was heated with stirring for forty-eight hours at 160–170°. After addition of 25 cc. of water and 100 cc. of ether the mixture was filtered, the layers were separated, and the aqueous layer was extracted with ether. Removal of solvent and distillation of the residue gave 11.1 g. of starting material, b. p. 109–120° (0.3–0.6 mm.), and 6.9 g. of an oil which boiled at 170° at 0.3 mm. Redistillation of the latter fraction gave a viscous light yellow tertiary amine, b. p. 155–160° (0.2 mm.). The dipicrate, m. p. 162–163° (dec.), was prepared in ethanol.

*Anal.* Calcd. for $C_{31}H_{31}N_9O_{14}$: C, 49.40; H, 4.15; N, 16.73. Found: C, 49.57; H, 4.7; N, 15.91.

The methyl analog, prepared by the procedure above, was obtained as a slightly impure oil[10] boiling at 163–164°. (0.1 mm.) after redistillation. The picrate and picrolonate were oils, and the hydrochloride deteriorated rapidly on standing.

**N-3-Pyridyl-p-toluenesulfonamide.**—A solution of 12.6 g. (0.134 mole) of 3-aminopyridine in 30 cc. of dry pyridine was treated with 25.6 g. (0.134 mole) of p-toluenesulfonyl chloride and warmed thirty minutes on a steam-bath. After removal of half of the pyridine by distillation under reduced pressure a white solid was precipitated by the addition of 50 cc. of water. This was purified by dissolving in 100 cc. of 5% sodium hydroxide, treating the solution with decolorizing charcoal and reprecipitating with sodium bicarbonate solution. The white N-3-pyridyl-p-toluenesulfonamide, m. p. 190.5–191.5°, weighed 28.8 g. (82%). The melting point was not raised by two crystallizations from xylene.

*Anal.* Calcd. for $C_{12}H_{12}N_2O_2S$: C, 58.04; H, 4.87; N, 11.28. Found: C, 58.31; H, 4.86; N, 11.52.

**N-3-Piperidyl-p-toluenesulfonamide.**—The reduction of 4.15 g. (0.017 mole) of N-3-pyridylamino-p-toluenesulfonamide was accomplished in 20 cc. of methanol containing 30 cc. of 6 *N* hydrochloric acid by the use of Adams platinum catalyst at room temperature. The uptake of hydrogen was very slow. After removal of the catalyst, the solution was evaporated to dryness, taken up in 20 cc. of water, and neutralized. Evaporation of the filtrate after removal of 3.1 g. of starting material which had precipitated, gave 0.6 g. (56% yield based on recovered N-3-pyridyl-p-toluenesulfonamide) of solid. Recrystallization from a large volume of petroleum ether (b. p. 90–110°) gave white N-3-piperidyl-p-toluenesulfonamide, m. p. 125–126°.

*Anal.* Calcd. for $C_{12}H_{18}N_2O_2S$: C, 55.66; H, 7.13; N, 11.02. Found: C, 56.06; H, 6.59; N, 10.93.

Reduction in glacial acetic acid with platinum oxide catalyst or with sodium and ethanol was less successful.

(10) *Anal.* Calcd. for $C_{11}H_{23}N_3$: C, 76.83; H, 8.26; N, 14.93. Found: C, 77.46; H, 8.07; N, 14.86.

## Summary

1. Various syntheses of substituted 3-aminopiperidines have been investigated, of which reductive amination of 1-alkyl-3-piperidones was the most useful.

2. 1-Alkyl-3-amino-, 1-alkyl-3-benzylamino- and 1-alkyl-3-(β-dimethylaminoethylamino)-piperidines were prepared.

3. 1-Ethyl- and 1-methyl-3-(N-benzyl-N-α-pyridylamino)-piperidines were prepared by alkylation of the secondary amines.

KALAMAZOO, MICHIGAN RECEIVED SEPTEMBER 20, 1948

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[CONTRIBUTION FROM THE NORTHERN REGIONAL RESEARCH LABORATORY[1] AND THE CORN INDUSTRIES RESEARCH FOUNDATION]

# Isolation of 6-[α-D-Glucopyranosyl]-D-glucose (Isomaltose) from Enzymic Hydrolyzates of Starch[2]

BY EDNA M. MONTGOMERY,[3] F. B. WEAKLEY[3] AND G. E. HILBERT

## Introduction

Starch is a polymer composed apparently of the repeating unit, maltose, and the cross linking unit, 6-[α-D-glucopyranosyl]-D-glucose (isomaltose). Amylose or the A fraction[4] of starch has been shown to consist almost entirely, if not completely, of maltose units arranged in a linear configuration. Amylopectin or the B fraction[4] of starch, on the other hand, is a branched molecule; it contains the anomalous or branching unit which serves to cross link chains composed of maltose units.[5] On the basis of studies dealing with the structure of the products resulting from the hy-

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) Presented at the 33rd Annual Meeting of Cereal Chemists at Cincinnati, Ohio, on May 25, 1948.

(3) Corn Industries Research Foundation Fellow at the Northern Regional Research Laboratory, Peoria, Illinois.

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

(5) K. H. Meyer, *Naturwissenshaften*, **28**, 397, 564, 722 (1940); K. Freudenberg and H. Boeppel, *ibid.*, **28**, 264 (1940); W. N. Haworth, E. L. Hirst and F. A. Isherwood, *J. Chem. Soc.*, 577 (1937); E. L. Hirst and G. T. Young, *ibid.*, 1471 (1939); C. E. H. Bawn, E. L. Hirst, and G. T. Young, *Trans. Faraday Soc.*, **36**, 880 (1940); K. Myrbäck, B. Ortenblad, and K. Ahlborg, *Biochem. Z.*, **307**, 53 (1940); K. Myrbäck and K. Ahlborg, *ibid.*, **307**, 69 (1940); and K. Ahlborg and K. Myrbäck, *ibid.*, **308**, 187–195 (1941).

drolysis of methylated starch[5] the configuration of the anomalous unit has been deduced to be 6-[α-D-glucopyranosyl]-D-glucose. Until recently all efforts to isolate from hydrolyzates of starch or to synthesize pure, authentic 6-[α-D-glucopyranosyl]-D-glucose or any of its derivatives have failed. A crystalline derivative of 6-[α-D-glucopyranosyl]-D-glucose was for the first time recently described by Georges, Miller and Wolfrom.[6] This was octaacetyl-6-[α-D-glucopyranosyl]-β-D-glucose and was prepared from the acid hydrolyzate of dextran. This acetate as well as other derivatives of 6-[α-D-glucopyranosyl]-D-glucose were prepared independently from the enzymic hydrolyzate of waxy (glutinous) corn starch by Montgomery, Weakley and Hilbert.[7] This paper is an extension of our previous report.

The enzyme used in this work to hydrolyze starch was derived from *Aspergillus oryzae*. The substrate usually was waxy corn starch.

Two procedures for isolating 6-[α-D-glucopyranosyl]-D-glucose from the enzymic hydrolyzate were developed. In the one (I), the enzymic hydrolyzate was subjected to the action of bakers' yeast to remove the fermentable sugars before isolating 6-[α-D-glucopyranosyl]-D-glucose in the form of one of its derivatives from the non-fermentable fraction. The other procedure (II) was devised to eliminate the use of yeast and to avoid the possible criticism that the 6-[α-D-glucopyranosyl]-D-glucose was being introduced by the yeast. In (II) the starch was first exhaustively hydrolyzed by the enzyme until all the fermentable sugar was in the form of glucose. The glucose was then separated from 6-[α-D-glucopyranosyl]-D-glucose by passage through carbon–Celite columns using a modification of the adsorption technique described by Tiselius.[8] The glucose passed through the columns rapidly at *p*H 5–5.5, the small amount adsorbed being easily removed by washing the columns with water. The 6-[α-D-glucopyranosyl]-D-glucose was subsequently removed from the columns by desorption with a dilute solution of phenol in water.[9]

In procedure I, 6-[α-D-glucopyranosyl]-D-glucose was isolated in a crude and amorphous condition. From it, however, three pure crystalline derivatives were readily prepared. By esterification with *p*-nitrobenzoyl chloride, octa-*p*-nitrobenzoyl-6-[α-D-glucopyranosyl]-D-glucose was obtained; with acetic anhydride and pyridine, octaacetyl-6-[α-D-glucopyranosyl]-D-glucose (III); and with acetic anhydride and sodium acetate, octaacetyl-6-[α-D-glucopyranosyl]-β-D-glucose (IV). These three derivatives are interchangeable, thus showing they are derived from the same parent sugar. The configuration of the octa-*p*-nitrobenzoyl and of the octaacetyl (III) derivatives were not determined; the study of the structure of (III) will be carried out later.

Evidence favoring the occurrence of a 1,6-linkage in the octaacetates (III) and (IV) was obtained by a study of their behavior in a solution of hydrogen bromide, acetic acid and acetyl bromide.[10] Toward this reagent (III) and (IV) behaved like the 1,6-disaccharide, gentiobiose, and strikingly different than the 1,4-disaccharide, maltose. The configuration of octaacetate (IV) was established by comparison with the preparation of Georges, Miller and Wolfrom[6] which was made available by Professor Wolfrom. X-Ray diffraction patterns of the two acetates are identical (Fig. 1); the optical rotation, melting points (m. p. of mixture unchanged) and crystalline form are in agreement also. The configuration of the simple, unesterified sugar is, therefore, the predicted one, 6-[α-D-glucopyranosyl]-D-glucose.[11]

Crystalline 6-[α-D-glucopyranosyl]-D-glucose has been obtained directly from solutions fractionated through adsorption following procedure II, or indirectly through the octaacetate (IV). Its most outstanding characteristic is the sluggishness with which it crystallizes. Figure 2 is a photomicrograph of the first crystals obtained of the anomalous unit. A study of the properties of the crystalline sugar, including its rate of hydrolysis in aqueous acid, is under way.

By means of its crystalline derivative (IV), the presence of the anomalous unit has also been established in tapioca starch and in corn starch. For the sake of brevity the experimental details are omitted.

The assumption that the α-1,6-biose unit isolated from the hydrolyzates of starch is actually present in the starch is comparable to the assumption that the maltosidic, α-1,4-linkage occurs in starch. The possibility that the new biose might be formed secondarily by the amylase was investigated with negative results in six experiments: The effect of the amylase was tried on (1) glucose, (2) maltose, (3) 6-[α-D-glucopyranosyl]-D-glucose, (4) amylose, (5) amylose in the presence of the branching unit, *i.e.*, amylopectin or glutinous

(6) L. W. Georges, I. L. Miller, and M. L. Wolfrom, THIS JOURNAL, **69**, 473 (1947). Subsequently, the authors re-established the α-1,6-biose linkage of the disaccharide by periodic acid analyses. (Private communication in reference to paper now in press.)

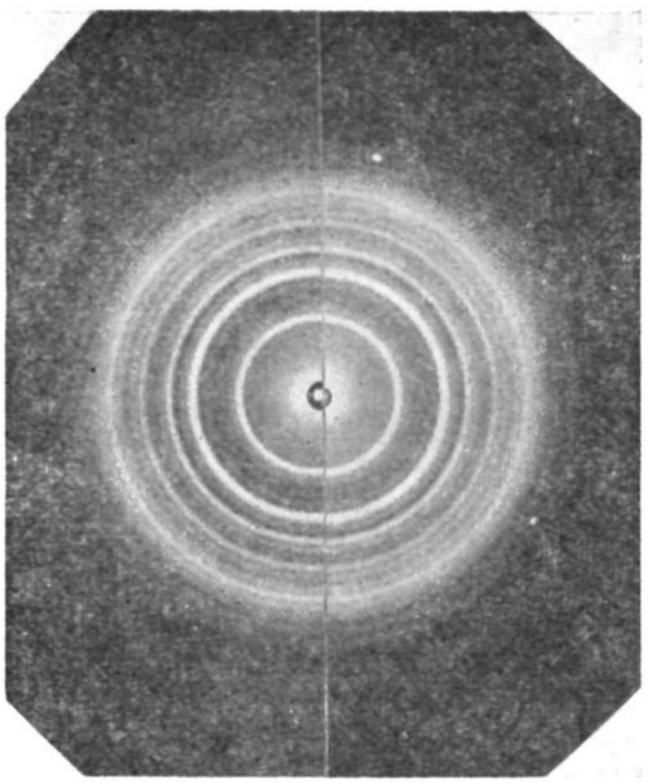
(7) Edna M. Montgomery, F. B. Weakley, and G. E. Hilbert, *ibid.*, **69**, 2249 (1947).

(8) A. Tiselius, *Kolloid Z.*, **105**, 101 (1943); A. Tiselius and L. Hahn, *ibid.*, **105**, 177 (1943).

(9) This procedure for separating monosaccharides from disaccharides can be highly recommended. It was also tested on corn sugar molasses "hydrol," the glucose being readily separated in pure condition. This work is now being prepared for publication.

(10) A. R. Jeanes and G. E. Hilbert, presented before the American Chemical Society, Sept. 11–15 (1944).

(11) The term isomaltose has been applied to this sugar in the past, as well as to sirups, resulting from synthetic endeavors or from the enzymic hydrolysis of starch, which were believed to be 6-[α-D-glucopyranosyl]-D-glucose. Although carbohydrate chemists recognize that the terms isomaltose and 6-[α-D-glucopyranosyl]-D-glucose are synonymous, the name isomaltose is believed by many to be an unfortunate one. A simple and appropriate name for the bulky term 6-[α-D-glucopyranosyl]-D-glucose seems to be "brachiose" (derived from the Greek *brachion* and Latin, *brachium*, meaning branch). One of us (E. M. M.) has referred the name "brachiose" to the Nomenclature Committee of the Sugar Division of the American Chemical Society for formal action.



Reference octaacetate (dextran)
Identified octaacetate (starch)

Fig. 1.—X-Ray diffraction patterns of octaacetyl-6-[α-D-glucopyranosyl]-D-glucose.

corn starch and (6) a fragment of glutinous corn starch. In part (6) the yield of 6-[α-D-glucopyranosyl]-D-glucose was increased to 16.4% as was anticipated.

6-[α-D-Glucopyranosyl]-D-glucose has been found to occur consistently in taka-amylase hydrolyzates of amylopectin in a yield agreeing with that anticipated from the amount of branching calculated from methylation data.

## Experimental

**Materials.—Waxy corn starch,** 25 pounds, was prepared on a pilot-plant scale, the corn being steeped for forty-six hours at 52° in water containing 0.25% sulfur dioxide by weight. The starch was dried at a temperature not exceeding 74°. *Anal.* Methanol extract, 0.19; phosphorus, 0.006; nitrogen, 0.03; alkali lability number, 3.

**The enzyme** was prepared from *Aspergillus oryzae*. It was a taka-amylase[12] furnished by Takamine, Inc.; it contained maltase. As received it was partially purified and only slightly colored. It gave a clear solution in water and even after treatment with hot aqueous mineral acid did not reduce Fehling solution. Possible bacterial contamination was removed by Seitz filtration. The enzyme, reprecipitated from aqueous solution of alcohol, was obtained in colorless condition with slightly increased potency and was so used in this work. It is designated in this paper as the enzyme.

**Amberlite Ion Exchange Columns.**—The resin exchange materials, IR-100 and IR-4, in a moist condition when obtained from the manufacturer, were immersed in a column of water to form beds. These beds were "broken in" by three or four exchange cycles, followed by regeneration and washing to eliminate any color discharge and fines.[13] Each solution was passed through two columns of each type, IR-100 and IR-4 being used alternately.

(12) M. L. Caldwell, R. M. Chester, A. H. Doebbling and G. W. Volz, *J. Biol. Chem.*, **161**, 361-365 (1945).

(13) R. M. McCready and W. Z. Hassid, THIS JOURNAL, **66**, 560 (1944).

Fig. 2.—Crystalline 6-[α-D-glucopyranosyl]-D-glucose.

**Analytical Methods.**—Reducing sugars were determined in the solution containing phenol by the Munson-Walker method[14] and in the earlier fermented preparation by the cuprous titration procedure devised by Shaffer-Hartmann.[15] Reducing powers expressed in terms of glucose as a standard are designated $G_{Cu}$ and in terms of maltose hydrate, $M_{Cu}$.

**Removal of Fermentable Sugars.**—Some fermentations were carried out at 10-12° and *p*H 4 to 5, using washed, bakers' starch-free yeast activated with dextrose; others with the distillers' yeast, *Saccharomyces cerevisiae*, NRRL 567, at 30° and *p*H 6.0.

**Enzymic Hydrolysis of Glutinous Corn Starch for Preparation of Isomaltose (Procedure 1).**—A slurry of 400 g. of the starch (dry weight) and 900 ml. of water was poured rapidly with stirring into 7.5 liters of boiling water; the resulting clear gelatinous paste reached a temperature of 82° at the time of mixing. To this paste, cooled to 38°, was added 2.0 g. of the enzyme dissolved in 20 ml. of water, and 50 ml. of toluene. The mixture was shaken well and maintained at 38° for seventy-two hours. The course of hydrolysis was followed analytically by means of optical rotation and reducing power. The resulting solution, showing only a slight color, was autoclaved, cooled, and treated with bakers' starch-free yeast.[16] The fer-

(14) L. S. Munson and P. H. Walker, *ibid.*, **28**, 663 (1906).

(15) P. A. Shaffer and A. F. Hartmann, *J. Biol. Chem.*, **45**, 349-390 (1921).

(16) An aliquot of the solution was fermented with the yeast NRRL 567. The data obtained show that the action of the two different yeasts was essentially the same; because of the greater convenience in obtaining large quantities of bakers' yeast it was used in most of these studies even though the strain was not known.

mented solution was then freed from yeast, autoclaved, clarified, and passed through the Amberlite ion exchange columns.

To the clear, colorless solution was added 1.0 g. of enzyme and the hydrolysis was continued for four additional days. The autoclaving and exchange column treatment was then repeated and the fermentable sugars removed by treatment with yeast. The solution was treated again in a similar manner until no change in rotation or reducing power could be observed. The final solution was golden in color; after autoclaving and cooling, the proteins were removed with basic lead acetate. This solution was again passed through the exchange columns; no loss in reducing power or change in rotation occurred during the entire treatment. The resulting colorless, clear solution was concentrated *in vacuo* to a volume of 290 ml.; $G_{Cu}$, 111 mg./ml., the observed reading in °S., + 47 (*l*,1).[17]

**Fractionation of the Unfermentable Residue.**—Concentration of the hydrolyzate (290 ml.) described above yielded about 30 ml. of a thick brown sirup which was dissolved in 100 ml. of warm methanol. Extraneous material was precipitated by dropwise addition of 500 ml. of ethanol and 500 ml. of ether at 25°. Precipitation was increased by cooling and maintaining the solution at 0° for forty-eight hours. The precipitated material was essentially non-reducing. On concentration of the supernatant liquid a colorless sirup was obtained (wt. 8.6 g.) and dried to a constant weight over phosphorus pentoxide *in vacuo* at 25°.

By dissolving the sirup in 1 liter of 1 part butanol and 2 parts methanol and concentrating the solution slowly *in vacuo* at 30°, a granular but non-crystalline sugar was precipitated. The precipitate was collected and dried over phosphorus pentoxide *in vacuo;* yield, 6.3 g. The crude isomaltose (I) was a white powder; $[\alpha]^{25}D$ +127.0° (*c*, 2 in water); $M_{Cu}$, 82%.

The crude isomaltose so purified was used in preparing the crystalline derivatives described below.

**Octa-*p*-nitrobenzoyl-6-[α-D-glucopyranosyl]-D-glucose (II).**—Two grams of the crude isomaltose (I) was dissolved in 200 ml. of pyridine; 12.2 g. of *p*-nitrobenzoyl chloride was added and the mixture refluxed under anhydrous conditions for two hours. The resulting golden-colored mixture was poured into ice and water; a cream-colored, coarse, finely divided precipitate separated. This was separated by filtration, and washed with 3% cold sodium bicarbonate, followed by 2% aqueous acetic acid. The residual solid was dried to constant weight at 50° (7.2 g.) and crystallized from 150 ml. of hot acetone. A crop of well-formed, diamond-shaped crystals separated. After four recrystallizations from acetone, octa-*p*-nitrobenzoyl-6-[α-D-glucopyranosyl]-D-glucose reached a constant rotation, $[\alpha]^{25}D$ + 22.0° (*c*, 1.27 in acetonylacetone); m. p. 188°; yield 3.50 g.

*Anal.* Calcd. for $C_{12}H_{14}O_{11}(COC_6H_4NO_2)_8$: C, 53.2; H, 3.02; N, 7.3. Found: C, 53.2; H, 3.27; N, 7.71.

The pure *p*-nitrobenzoate is colorless. It is soluble in hot acetone, almost insoluble in cold acetone in which it forms heavily supersaturated solutions, and progressively less soluble in benzene, carbon tetrachloride, chloroform, ether and alcohol.

**Octaacetyl-6-[α-D-glucopyranosyl]-D-glucose (III).**—Two grams of the crude sugar (I), was acetylated with a mixture of 20 ml. of acetic anhydride and 100 ml. of pyridine at 25° for twenty-four hours. The resulting clear liquid was then heated on a steam-bath for thirty minutes and after cooling was poured into a mixture of ice and water. A heavy, colorless oil was formed and crystallized in ethanol; yield 0.62 g. of well-built needle-like prisms. The product was recrystallized from ethanol; $[\alpha]^{25}D$ +37° (*c*, 1.23 in chloroform), m. p. 175°. Recrystallization did not change these values.

*Anal.* Calcd. for $C_{12}H_{14}O_{11}(CH_3CO)_8$: C, 49.6; H, 5.63; $CH_3CO$, 50.7; molecular weight, 678.6. Found: C, 49.5; H, 5.48; $CH_3CO$, 50.6; molecular weight (Rast), 670.

**Octaacetyl-6-[α-D-glucopyranosyl]-β-D-glucose (IV).**—A second form, the β-form, of the octaacetyl-6-[α-D-glucopyranosyl]-D-glucose was obtained in the following manner: 2 g. of crude sugar similar to I was acetylated at 100 to 110° with 20 ml. of acetic anhydride and 0.5 g. fused sodium acetate. The resulting solution was poured into a mixture of ice and water. The acetylated product was extracted with chloroform; the extract was washed with a dilute solution of sodium bicarbonate in water and then concentrated *in vacuo*. The resulting oil (3.4 g.) was dissolved in 20 ml. of chloroform and by dropwise addition of 200 ml. of isopentane an amorphous solid was precipitated. After four reprecipitations the product was obtained as a colorless amorphous powder; weight 0.6 g. (dried to constant weight at 50° and 2 mm.); $[\alpha]^{25}D$ + 96.2° (*c*, 2.00 in chloroform).

Crystalline octaacetyl-6-[α-D-glucopyranosyl]-β-D-glucose (IV) was obtained by dissolving 2.0 g. of this amorphous acetate in ethanol and by repeatedly allowing the solution to concentrate slowly over calcium chloride in a desiccator over a period of several months. Eventually, long colorless prisms separated; weight, 0.70 g. (dried to constant weight over calcium chloride at 70° *in vacuo*); m. p. 143°; $[\alpha]^{25}D$ +98.2° (*c*, 1.00 in chloroform).

*Anal.* Calcd. for $C_{12}H_{14}O_{11}(CH_3CO)_8$: C, 49.6; H, 5.63; $CH_3CO$, 50.7. Found: C, 49.6; H, 5.73; $CH_3CO$, 50.9.

A mixture of this acetate with a sample of octaacetyl-6-[α-D-glucopyranosyl]-β-D-glucose which was furnished by Dr. Wolfrom and obtained from the acid hydrolyzate of dextran, melted without depression at 143°. Powder X-ray diffraction patterns of these two acetates were identical (Fig. 1).

**X-Ray Diffraction Powder Pattern of Octaacetyl-6-[α-D-glucopyranosyl]-β-D-glucose.**—Patterns were made with CuKα radiation, flat casette, 5-cm. sample to film distance.

| Line | Interplanar spacing, Å. | Estimated intensity[a] |
|---|---|---|
| 1 | 11.00 | V. W. |
| 2 | 9.75 | S. |
| 3 | 8.66 | W. |
| 4 | 7.60 | V. W. |
| 5 | 6.80 | W. |
| 6 | 6.12 | V. S. |
| 7 | 5.63 | W. |
| 8 | 5.25 | S. |
| 9 | 4.94 | M. |
| 10 | 4.73 | V. W. |
| 11 | 4.50 | S. |
| 12 | 4.28 | M. |
| 13 | 4.07 | M. |
| 14 | 3.90 | S. |
| 15 | 3.74 | M. |
| 16 | 3.56 | V. W. |
| 17 | 3.45 | W. |
| 18 | 3.25 | V. W. |
| 19 | 3.11 | W. (broad) |
| 20 | 2.91 | W. |
| 21 | 2.62 | W. |
| 22 | 2.51 | W. |
| 23 | 2.36 | W. |

[a] V = Very; S = Strong; M = Medium; W = Weak.

**Transformation of Octaacetyl-6-[α-D-glucopyranosyl]-D-glucose (III) to Octa-*p*-nitrobenzoyl-6-[α-D-glucopyranoyl]-D-glucose (II).**—One gram of octaacetate (III), dissolved in 100 ml. of methanol, was deacetylated catalytically with barium methylate. The barium was precipitated quantitatively as the sulfate and removed by filtration. The

(17) All rotations were measured at 25° for the D line of sodium using a saccharimeter and the figures in parentheses indicate the length of the tube.

solution was then concentrated *in vacuo* to a dry sirup which was esterified in a mixture of 100 ml. of pyridine and 4.05 g. of *p*-nitrobenzoyl chloride according to the established procedure and the product was purified. There was isolated 1.5 g. of crystalline octa-*p*-nitrobenzoyl-6-[α-D-glucopyranosyl]-D-glucose (II); $[\alpha]^{25}D$ + 22° (*c*, 1 in acetonylacetone); m. p. 188°.

**Transformation of Octaacetyl-6-[α-D-glucopyranosyl]-D-glucose (III) to Octaacetyl-6-[α-D-glucopyranosyl]-β-D-glucose (IV).**—One gram of pure octaacetate (III) was de-esterified catalytically with barium methylate. The resulting sugar was purified and a colorless sirup (0.58 g.) obtained. This sirup was acetylated at 100–110° with acetic anhydride in the presence of fused sodium acetate. After purification of the acetylated material by the established procedure 0.35 g. of crystalline octaacetyl-6-[α-D-glucopyranosyl]-β-D-glucose (IV) was obtained; $[\alpha]^{25}D$ +97.9° (*c*, 1 in chloroform); m. p. 143°.

**Behavior of Octaacetate (III) and (IV) toward Hydrogen Bromide in Acetic Acid and Acetyl Bromide.**—This reagent has been shown by Jeanes and Hilbert[10] to distinguish between 1,6- and 1,4-disaccharides. The test was carried out in a 1:1 solution of saturated hydrogen bromide in acetic acid and acetyl bromide. Exactly enough of each acetate was dissolved in 10 ml. of the ice-cold solution at 25° to produce $7.576 \times 10^{-4}$ mole of acetobromoglucose, on the assumption that the disaccharides would be completely converted to this compound. The solutions of the acetates were maintained at 25° and the course of the reaction followed polarimetrically until the rotation became constant, which it did after twenty to thirty hours. The end rotations in °S. (*l*, 1) were +12.1 and +11.2 for (III) and (IV), respectively. In comparison, the end rotations of acetobromoglucose, β-maltose octaacetate and β-gentiobiose octaacetate were +17.39, +17.2 and +12.2, respectively.

**Enzymic Hydrolysis of Glutinous Corn Starch for Preparation of Isomaltose (Procedure 2).**—As in procedure 1, the starch, 200 g. (dry wt.), in 5% concentration was pasted at 82°, cooled to 38°, and shaken well with 50 ml. of toluene. Ten grams of enzyme was added and the solution was digested at 38° for twenty-four hours. The resulting solution was water-thin. Passed through a Seitz filter under aseptic conditions, the solution was clear and almost colorless. The digestion was then continued for ninety-six hours, or until the reducing power and rotation were constant; the reaction mixture was inactive toward maltase. The solution had a volume of 3.0 l.; °S. (*l*, 2), +23.7°; $G_{Cu}$, 57.9 mg./ml.; total $G_{Cu} \approx 168$ g.; after clarification and removal of inorganic ions by passage through the Amberlite columns IR-100 and IR-4 as in procedure 1 the total $G_{Cu}$ was ≈ 166 g. The solution usually at a *p*H 5–8 was immediately passed onto carbon-Celite columns.

**Separation of Glucose and Isomaltose from Enzymic Hydrolyzate by Means of Carbon-Celite Columns.**—The columns were prepared in Pyrex cylinders 30 cm. × 7¾ cm. with fritted glass bottoms. A ¾ inch layer of glass wool was placed on the fritted glass to prevent fine particles from clogging the disc. The adsorbent was made by mixing mechanically equal parts by volume of Darco G60 and Celite 501. A slurry of the adsorbent in water was poured into the cylinders. The adsorbent was packed with the aid of suction to form columns 21 cm. high. These columns were tamped and then washed with 15 volumes of water. Each unit consisted of 3 cylinders set one above the other, the outlet of the upper cylinder fitting into a stopper in the top of the cylinder immediately below it. Sufficient suction was applied to draw the liquids through at a rate of one drop in two seconds, or about 1.5 liters per hour, a contact factor of 1 per hour.

At *p*H 7 to 8 all the sugars were completely adsorbed and glucose could be removed merely by washing the columns with water. At *p*H 5.5, the glucose was adsorbed only weakly and soon appeared in the effluent. In either case the glucose was completely removed in pure form by aqueous washing of the loaded columns.

After adsorption of the sugars on the columns, water was added at the top of the unit from a controlled feed; the effluent containing the desorbed material was caught at the bottom of the unit in a large suction flask in 1,000-ml. quantities. The eluates obtained were submitted to analysis; the optical rotation and the quantity of reducing material were determined. Expressed in terms of specific rotation, the values served as analytical guides on the separation of glucose from isomaltose; and of isomaltose from the dextrins. The rotations (°S.), $G_{Cu}$ and $M_{Cu}$, and specific rotations of the various eluates are shown in Table I. It is to be noted that the specific rotations of the eluates (A) No. 1 to No. 13 and the tailing fraction which consisted of the concentrated, exhaustive water washings are close to the value for D-glucose, +52°.

TABLE I

FRACTIONATION OF ENZYMIC HYDROLYZATE

| Fractions Water | °S. (*l*, 4)[a] Degrees | $R_{Cu}$[b] M-W Mg./ml. | $[\alpha]^{25}D$ Degrees | Per cent. of total reducing sugar as glucose Glucose |
|---|---|---|---|---|
| (A) | | | | |
| Liter No. 1 | + 3.60 | 5.82 G | +53.5 | |
| 2 | +19.73 | 32.69 G | +52.2 | |
| 3 | +19.40 | 31.89 G | +52.6 | |
| 4 | +10.33 | 17.03 G | +52.5 | |
| 5 | + 6.45 | 10.38 G | +53.8 | |
| 6 | + 5.10 | 8.28 G | +53.3 | |
| 7 | + 4.50 | 7.49 G | +52.0 | |
| 8 | + 3.67 | 5.95 G | +53.4 | |
| 9 | + 3.03 | 4.94 G | +53.1 | |
| 10 | + 3.28 | 5.33 G | +53.3 | |
| 11 | + 2.81 | 4.59 G | +52.9 | |
| 12 | + 2.00 | 3.24 G | +53.4 | |
| 13 | + 1.30 | 2.19 G | +51.4 | |
| Tailings | +18.30 | 30.44 G | +52.0 | |
| | | Total | | 92.5 |
| | | | | Isomaltose[c] |
| Phenol (0.5%) | | | | |
| (B) 6-Liter conc. | +30.40 | 23.18 M | +113.5 | 3.8 |

[a] Solutions were permitted to reach rotational equilibrium. [b] G—calculated as glucose; M—calculated as maltose hydrate. [c] When calculated in terms of maltose hydrate, this value is equivalent to 5.3% of the starch or 12 g. of isomaltose in the 6-liter concentration.

After removal of the glucose by washing the columns with water, the isomaltose was desorbed by washing with 0.5% phenol in water. The data are included in Table I. A thorough analysis of the fraction obtained by desorbing the dextrins (3.5% by weight) was not made at this time.

**α-D-Glucose Monohydrate.**—The combined eluates (A) including the tailings obtained by washing the columns with water were concentrated *in vacuo*. A colorless sirup was obtained; $n^{20}D$ 1.4850. The sirup was kept in motion at 25° for about three hours or until a cake of uniform, well-constructed crystals was formed. The mass was broken up and the crystals washed with 95% ethanol, filtered and air-dried. The product, α-D-glucose monohydrate, was a colorless, finely-divided powder which was readily sieved; m. p., 83°; $[\alpha]^{25}D$ +47.9° (*c*, 1.20 in water).[18] The reducing value of the sugar was that required by theory; the sugar was ash free. The constants of the sugar were not changed by recrystallization. The yield of α-D-glucose monohydrate, isolated in crystalline form, was 81% of the weight of the starch.

**Acetylation of 6-[α-D-Glucopyranosyl]-D-glucose.**—Concentration of one-half of the 0.5% aqueous phenol

(18) National Bureau of Standards Cir. C440, p. 728.

eluate (B), Table I, *in vacuo* to a dry sirup and acetylation of this sirup resulted in the isolation of a colorless sirup rotating +93.4° (*c*, 2 in chloroform). This acetate, dissolved in ethanol, crystallized overnight at 25°. The total yield of pure octaacetyl-6-[α-D-glucopyranosyl]-β-D-glucose, $[\alpha]^{25}D$ +98.0° (*c*, 2 in chloroform), m. p. 143°, was 9.2 g. or 77%.

**Crystallization of 6-[α-D-Glucopyranosyl]-D-glucose.**—Six grams of (IV), $[\alpha]^{26}D$ +98.0° in chloroform, was deactylated catalytically with barium methylate at 0°. The barium was precipitated with 0.5 *N* sulfuric acid and removed by filtration as barium sulfate. A colorless solution, *p*H 7.0, resulted. Concentrated *in vacuo* to a sirup and taken up in ethanol the deacetylated material crystallized during several days as long prisms. Recrystallized under similar conditions the sugar was separated by filtration, washed and dried to constant weight at 98°. The dry crystalline substance melted at 120° and had an equilibrium $[\alpha]^{25}D$ value (*c*, 1.2 in water) of +120° mutarotating downwards. Its $M_{Cu}$ value (*c*, 1) was 97%; yield, 2.3 g.

*Anal.* Calcd. as $C_{12}H_{22}O_{11}$: C, 42.1; H, 6.43. Found: C, 41.9; H, 6.66.

**Acknowledgment.**—The authors are indebted to Dr. N. N. Hellman for the X-ray photographs, Mr. C. H. Van Etten and Mrs. M. B. Wiele for the microanalyses, to Dr. L. J. Wickerham for the distillers' yeast, *Saccharomyces cerevisiae* (NRRL 567), and to Mr. R. W. Haines for the photomicrographs.

## Summary

1. Waxy corn starch was exhaustively hydrolyzed with enzymes and the fermentable sugars removed by fermentation.

2. A disaccharide, apparently the anomalous unit, was isolated from the unfermentable residue in the form of three crystalline derivatives.

3. The three derivatives were shown to be interconvertible.

4. The structure of the disaccharide was proved to be 6-[α-D-glucopyranosyl]-D-glucose through its octaacetyl derivative (IV) which is identical with octaacetyl-6-[α-D-glucopyranosyl]-β-D-glucose isolated from dextran hydrolyzates and identified by periodate analyses of the methyl glycoside of the disaccharide.

5. In a second similar experiment the hydrolyzate was not fermented. The principal products, D-glucose and isomaltose, were separated by sorption and desorption on carbon–Celite columns.

6. The preparative procedure for 6-[α-D-glucopyranosyl]-D-glucose from starch has been simplified. The new disaccharide has been isolated for the first time as a crystalline substance.

PEORIA 5, ILLINOIS RECEIVED SEPTEMBER 16, 1948

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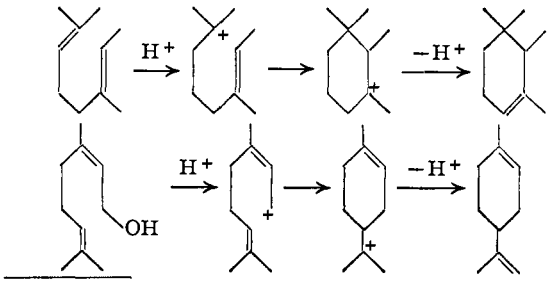
[CONTRIBUTION FROM THE STERLING CHEMICAL LABORATORY OF YALE UNIVERSITY]

# A New Synthesis of the Cyclopentane Ring with Notes on the Biological Origin of Terpenes and Sterols[1,2]

BY PHILIP G. STEVENS[3] AND SAMUEL C. SPALDING, JR.[4]

The great rarity in nature of monocyclic cyclopentanes stands in sharp contrast to the abundance of cyclohexanes. This is particularly noticeable in the terpene field in which, thus far, no authentic case of the occurrence of a monocyclic cyclopentane terpenoid, sesqui, or diterpenoid is known to exist.[5] Indeed the only cyclopentane rings in this field are those in which the five-membered ring is fused[6] to another larger ring such as in the azulenoids, in perhaps a few complex pentacyclic triterpenes, and in the partially isoprenic steroids.

In the case of the terpenes, sesqui-terpenes, and their derivatives, the mode of formation in nature is considered to involve a cyclization of an acyclic dienic[7] precursor, in the manner typified either by the formation in the presence of acids of cyclomethylgeraniolene from methylgeraniolene, or by the formation of dipentene from geraniol.[8] In each of these ring formations, a carbonium ion appears to be formed first, which then adds to a double bond—a mechanism similar to that proposed by Whitmore for the polymerization of isobutene[9]




(1) Presented before the Organic Division of the American Chemical Society at Atlantic City, N. J., April 15, 1947.

(2) From a dissertation submitted in partial fulfillment for the degree of Doctor of Philosophy by Samuel C. Spalding, Jr., in 1947 to Yale University.

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(5) The structures advanced for lanceol (Bradfield, Francis, Penfold and Simonsen, *J. Chem. Soc.*, 1619 (1936) and for nepatalic acid (McElvain, Bright and Johnson, THIS JOURNAL, **63**, 1558 (1941); McElvain, Walters and Bright, *ibid.*, **64**, 1828 (1942)) are too uncertain for these substances to be considered as exceptions to this statement; and the cyclopentyl ketone of Naves (*Helv. Chim. Acta*, **27**, 51 (1944)) is not a terpenoid. In any case it is probably a degradation product of fenchene. The cyclopentyl terpenoid isolated by Schmidt (*Ber.*, **80**, 528 (1947)) from Spanish eucalyptus oil (yield only 0.005%) may be an artifact.

(6) Bridged ring terpenes like camphene, etc., are considered to be cyclohexanes, not cyclopentanes.

(7) Or trienic, etc.

(8) Compare Butz, Butz and Geddes, *J. Org. Chem.*, **5**, 179 (1940).

(9) Compare Bloomfield, *J. Chem. Soc.*, 289 (1943).