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

# Maltotriose and its Crystalline β-D-Hendecaacetate

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In previous communications[2] there was described the isolation of a crystalline trisaccharide hendecaacetate (m. p. 134–136°, $[\alpha]^{25}D$ + 86° in chloroform) from the acetylated enzymic (malt amylases) hydrolyzate of waxy maize starch (amylopectin). The acetate chromatographic technics[3] developed in this Laboratory made the separation of the pure compound from the complex mixture possible, although it was present only in small quantities.

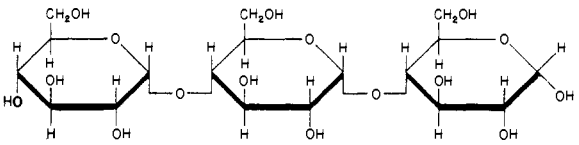
Herein evidence is presented to demonstrate that this trisaccharide hendecaacetate is β-maltotriose hendecaacetate (a trisaccharide with two α-D 1,4 linkages). The acetate was first converted into the corresponding amorphous hendecamethyl ether of unknown and probably mixed anomeric form, by employing three different technics in succession. The general procedure (dimethyl sulfate and alkali) of Haworth, Hirst and Webb[4] was employed initially and this was followed by that of Purdie and Irvine[5] using silver oxide and methyl iodide. Essentially the theoretical methoxyl content was then attained by a final treatment using sodium in liquid ammonia followed by reaction with methyl iodide as described by Muskat[6] and modified by Hendricks and Rundle.[7] The sirupy methyl ether exhibited $[\alpha]^{25}D$ + 122° and $[\alpha]^{25}$ 578 + 128° in chloroform, the latter value being in agreement with that cited by Freudenberg and co-workers[8] for a similar product (of unknown anomeric constitution) prepared by the methylation of a starch acetolyzate.

Acid hydrolysis of the methyl ether gave a sirup which was chromatographically separated into two crystalline compounds, 2,3,6-trimethyl-D-glucose and 2,3,4,6-tetramethyl-D-glucose, characterized by melting point, mixed melting point, rotation and the crystalline derivatives 2,3,6-trimethyl-β-D-glucose 1,4-diacetate and 2,3,4,6-tetramethyl-D-glucose anilide. The chromatographic technic employed[3] allowed this separation to be effected utilizing only 1 g. of the crystalline acetate. The molar ratio of the 2,3,6-trimethyl-D-glucose to 2,3,4,6-tetramethyl-D-glucose was 2 to 1 with the recovery of the chromatographed, crystalline substances of good purity being approximately 90%. This demonstrates that there are two 1,4-D-glucosidic linkages per molecule, assuming the normal pyranoid rings in each D-glucose unit.

The deacetylation of the crystalline hendecaacetate yielded the free sugar, obtained as an amorphous solid, which to date has resisted crystallization; $[\alpha]^{23}D$ + 160° (water). This sugar was not fermented by a commercial bakers' yeast that did not ferment maltose. Myrbäck and co-workers[9,10] have stated that sirupy products assumed by them to be or to contain maltotriose were fermented by a maltose-fermenting yeast.

One mole of the free sugar was partially hydrolyzed by a commercial maltase preparation at 26–27° in forty-four hours into 1.7 moles of D-glucose and 0.2 mole of maltose with recovery of 0.1 mole of the original trisaccharide, as determined by weights of the products isolated. The products of hydrolysis as well as the unchanged sugar were isolated as acetates using a chromatographic technic.[3] The free sugar was not hydrolyzed by emulsin. The fact that the sugar was hydrolyzed by maltase but not by emulsin establishes the two 1,4-D-glucosidic linkages as being α-D. The conversion to maltose gives final support to the assignment of the maltotriose structure to the trisaccharide. The experiments further illustrate the value of chromatographic technics in the analysis and separation of sugar mixtures.

Since the acetate of maltotriose was prepared[2] by the hot sodium acetate acetylation procedure and since it exhibits a specific rotation of $[\alpha]^{25}D$ +86° in chloroform, we have considered the compound to be the β-D-anomer of 4-[4-(α-D-glucopyranosyl)-α-D-glucopyranosyl]-β-D-glucopyranose hendecaacetate.



## Experimental[11]

**Methyl Decamethylmaltotrioside.**[12]—Following the general procedure of Haworth, Hirst and Webb,[4] 1.000 g. of maltotriose hendecaacetate (m. p. 134–136°, $[\alpha]^{25}D$ +86° in chloroform)[2] was dissolved in 3 ml. of acetone in a conical, four-necked flask fitted with a mechanical stirrer, a condenser, two dropping funnels and a capillary to introduce nitrogen. While maintaining an atmosphere of ni-

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

(2) M. L. Wolfrom, L. W. Georges, Alva Thompson and I. L. Miller, THIS JOURNAL, **71**, 2873 (1949); L. W. Georges, I. L. Miller and M. L. Wolfrom, *ibid.*, **69**, 473 (1947).

(3) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, *ibid.*, **67**, 527 (1945).

(4) W. N. Haworth, E. L. Hirst and J. I. Webb, *J. Chem. Soc.*, 2681 (1928).

(5) T. Purdie and J. C. Irvine, *ibid.*, **83**, 1021 (1903); **85**, 1049 (1904).

(6) I. E. Muskat, THIS JOURNAL, **56**, 693, 2449 (1934).

(7) B. C. Hendricks and R. E. Rundle, *ibid.*, **60**, 2563 (1938).

(8) K. Freudenberg, K. Friedrich, Ilse Bumann and K. Soff, *Ann.*, **494**, 41 (1932).

(9) K. Myrbäck and Elsa Leissner, *Arkiv. Kemi, Mineral. Geol.*, **17A**, No. 18 (1944).

(10) K. Myrbäck and W. Thorsell, *Svensk Kem. Tid.*, **55**, 178 (1943).

(11) All melting points are uncorrected.

(12) Anomeric composition unknown.

trogen and an external bath at 30-35° and vigorously stirring, 3 ml. of 30% sodium hydroxide and 2 ml. of dimethyl sulfate were added through the dropping funnels in small quantities over a period of two hours. The bath temperature was raised to 35-40° and vigorous stirring was continued until a small aliquot of the reaction mixture did not reduce Fehling solution (about four hours after adding the methylating reagents). The temperature of the bath was then increased to 55° and 5 ml. of dimethyl sulfate and 9 ml. of 30% sodium hydroxide were added in portions over a period of one and one-half hours with continued stirring. The bath temperature was then raised to 100° for one-half hour. The reaction flask was cooled and the solution was neutralized with 6 *N* sulfuric acid, sufficient water being added to dissolve any precipitated sulfate. This aqueous solution was extracted four times with 5-ml. portions of chloroform. Solvent removal from the combined, dried chloroform extracts left a sirup which was dried to constant weight in a vacuum desiccator containing Dehydrite (anhydrous magnesium perchlorate); yield 414 mg.

The aqueous solution remaining after chloroform extraction was evaporated to a thick slurry. Absolute ethanol (25 ml.) was added and the precipitated sodium sulfate was removed by filtration. The filtrate was again evaporated to a slurry, and a second portion of 25 ml. of absolute ethanol was added. Filtration and concentration of the filtrate to 3 ml. was followed by remethylation with 3 ml. of dimethyl sulfate and 5 ml. of 30% sodium hydroxide at 55°. This treatment was followed by reaction at 100° for one-half hour. The aqueous solution obtained was treated as previously described; yield of dried sirup 81 mg.

The sirups obtained were combined (495 mg.) and subjected to the general methylation procedure described by Purdie and Irvine.[5] The combined material was dissolved in 10 ml. of methyl iodide and placed in a flask fitted with a mechanical stirrer and a reflux condenser. An amount of 2 g. of silver oxide was added under gentle refluxing maintained subsequently for four hours. Excess methyl iodide was allowed to evaporate and the residue was repeatedly extracted with anhydrous ether (total volume 50 ml.). Evaporation of the ether left a sirup which was dried to constant weight over Dehydrite under reduced pressure; yield 490 mg.

This sirup (490 mg.) was dissolved in 5 ml. of liquid ammonia in an unsilvered Dewar flask and subjected to the Muskat[6] methylation procedure as modified by Hendricks and Rundle.[7] A solution of 60 mg. of sodium in 20 ml. of liquid ammonia was added in portions such that the blue color of the liquid ammonia solution persisted for one hour after the last addition (total volume 10 ml.). Excess methyl iodide (5 ml.) was added in portions and the ammonia was allowed to evaporate. The reaction mixture was transferred to a 25-ml. round-bottomed flask fitted with a reflux condenser. A further amount of 5 ml. of methyl iodide was added under gentle refluxing maintained for four hours. The mixture was then held overnight at room temperature. Excess methyl iodide was allowed to evaporate and the residue was extracted repeatedly with anhydrous ether. The combined ether solution (50 ml.) was treated with activated charcoal. Solvent removal left a sirup which was dried to constant weight at 78°, under reduced pressure, over phosphorus pentoxide; yield 488 mg. (72%), $[\alpha]^{25}D$ +122° (*c* 1.91, chloroform), $[\alpha]^{25}578$ +128° (*c* 1.91, chloroform). Freudenberg and co-workers[8] report: $[\alpha]^{18}_{578}$ +129.9° (*c* 1.57, chloroform) for a preparation of methyl decamethylmaltotrioside of undetermined anomeric admixture.

*Anal.* Calcd. for $C_{18}H_{21}O_5(OCH_3)_{11}$: C, 52.87; H, 8.26; $OCH_3$, 51.8. Found: C, 53.05; H, 8.30; $OCH_3$, 51.0.

**Hydrolysis of Methyl Decamethylmaltotrioside.**—The general procedure described by West and Holden[13] was applied. An amount of 98.5 mg. of methyl decamethylmaltotrioside was dissolved in 1 ml. of chloroform and 2.5 ml. of 2 *N* hydrochloric acid was added. Steam, generated at atmospheric pressure, was bubbled into the mixture. The chloroform was rapidly distilled leaving the methylated trisaccharide in a state of fine suspension. The reaction vessel was submerged in a bath, maintained at 104-106° and the current of steam was passed through for two and one-half hours at such a rate as to maintain the volume constant at 3-5 ml. The cooled solution was neutralized with silver carbonate and residual silver ion was removed with hydrogen sulfide. Distillation of the solution under reduced pressure left a sirup which was dried to constant weight in a vacuum desiccator over Dehydrite; yield 99 mg. This sirup (99 mg.) was dissolved in 25 ml. of chloroform containing 0.5% ethanol and the solution was placed on a chromatographic column packed with acid-washed[14] Magnesol[15]-Celite[16] (5:1 by wt.) (200 × 35 mm. diam.[17]) and developed with 350 ml. (5 column lengths) of benzene–ethanol (100:1 by vol.). Extrusion of the column and streaking with a permanganate indicator (1 part of potassium permanganate, 10 parts of sodium hydroxide and 100 parts of water) located two zones, the first about one-fifth of a column length from the top and the second near the middle of the column. These zones were sectioned and each section was eluted with 50 ml. of acetone. From the eluate of the top zone, solvent removal left 61 mg. (92%) of crystalline material; m. p. 112-114°, $[\alpha]^{24}D$ +65° (*c* 1.78, methanol, equilibrium). This substance was recrystallized from ether; yield 45.3 mg., melting point and mixed melting point with authentic 2,3,6-trimethyl-D-glucose 117.5-118°, $[\alpha]^{27}D$ +67.5° (*c* 1.69, methanol, equilibrium).[18]

An amount of 17.0 mg. of this material was acetylated by heating at 98° with acetic anhydride (5 ml.) and anhydrous sodium acetate (25 mg.). The product obtained on pouring the cooled reaction mixture into an excess (150 g.) of ice and water was extracted with chloroform and the washed (with aqueous sodium bicarbonate) and dried extract was concentrated to a sirup; yield 50.2 mg. This sirup was dissolved in 25 ml. of benzene and placed on a chromatographic column packed with acid-washed[14] Magnesol[15]-Celite[16] (5:1 by wt.) (200 × 35 mm. diam.[17]) and developed with two column lengths (130 ml.) of benzene–ethanol (100:1 by vol.). A zone near the middle of the extruded column was located by means of the permanganate streak indicator. This was sectioned and eluted with 50 ml. of acetone. Evaporation of the solvent left 36.9 mg. of a sirup, which when dissolved in a minimum of petroleum naphtha (b. p. 90-100°)-ether (4:1 by vol.) and allowed to stand overnight in the cold gave crystals; yield 15 mg., m. p. 57-61°. Pure material was obtained on further crystallization from the same solvent; m. p. 66-66.5°. Micheel and Hess[19] reported for 2,3,6-trimethyl-β-D-glucose 1,4-diacetate, m. p. 67-68°. A mixed melting point with an authentic sample showed no depression.

Solvent removal from the acetone eluate of the second zone of the above-described chromatogram left 30.6 mg. (87%) of crystals; m. p. 81-86°, $[\alpha]^{22}D$ +89.5° (*c* 2.24, ethanol, equilibrium). Recrystallization from petroleum naphtha (b. p. 90-100°)–ether (3:1 by vol.) left 22.3 mg.

(13) E. S. West and R. F. Holden, THIS JOURNAL, **56**, 930 (1934).

(14) A mixture of 5 parts (by wt.) of Magnesol and 1 part of Celite was suspended with efficient stirring in a solution composed of 1 part (by vol.) of concentrated hydrochloric acid and 3 parts of water. The amount of Magnesol-Celite added to the diluted acid was regulated so that a very thin paste resulted. After stirring for sixty minutes the slurry was filtered and washed free of chloride ion and the water was displaced with acetone. The material was dried at room temperature overnight and then for two hours at 110°. The adsorbent was cooled, and only that portion which passed a 200-mesh (per linear inch) sieve was used in the chromatographic procedures.

(15) A product of Westvaco Chlorine Products Co., South Charleston, West Virginia.

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

(17) Dimensions of the adsorbent.

(18) M. L. Wolfrom and L. W. Georges, THIS JOURNAL, **59**, 602 (1937).

(19) F. Micheel and K. Hess, *Ber.*, **60**, 1898 (1927).

of material, melting point and mixed melting point with authentic 2,3,4,6-tetramethyl-D-glucose 91–92°, $[\alpha]^{26}D$ +84.3° (*c* 1.62, ethanol, equilibrium).[6] An amount of 17 mg. of this material was converted to the anilide according to the procedure of Irvine and Moodie[20]; m. p. 134–135°, unchanged on admixture with an authentic specimen of like melting point.

Evaporation of the effluent from the above-described chromatogram left 4.0 mg. of an unidentified sirup.

**Maltotriose.**—Two grams of β-maltotriose hendecaacetate was dissolved in 40 ml. of absolute methanol and cooled to 0°. A solution of 2.4 ml. of 0.4 *N* barium methoxide was added, and the whole was kept at 0° for twenty-four hours. Then to the solution 200 ml. of cold water was added and the ionic material was removed by passage through Amberlite[21] resins IR-100 and IR-4. The effluent was concentrated to a sirup under reduced pressure. The residual water was removed by repeatedly stirring with absolute ethanol and evaporating to dryness at room temperature in a vacuum desiccator containing anhydrous calcium chloride. All attempts to crystallize the amorphous solid have failed; $[\alpha]^{23}D$ +160° (*c* 2.36, water).

*Anal.* Calcd. for $C_{18}H_{32}O_{16}$: C, 42.86; H, 6.39. Found: C, 42.32; H, 6.39.

The free sugar was not hydrolyzed by emulsin and was not fermented by bakers' yeast,[22] which also failed to ferment maltose but fermented D-glucose rapidly.

**Hydrolysis of Maltotriose by Maltase.**—An amount of 98.0 mg. (1 mole) of maltotriose was dissolved in 0.5 ml. of 0.1 *N* acetate buffer (*p*H 4.7) and 0.5 ml. of an aqueous solution containing 1 mg. of a commercial purified maltase (Maltase 20[23]) preparation. The resultant solution was diluted to a volume of 2.52 ml. The enzymic reaction was followed polarimetrically. After twenty-six hours at 26–27°, hydrolysis to D-glucose was 71% complete; after forty-four hours, 82% complete (as determined polarimetrically). At the end of the latter period, the solution was evaporated to a sirup under reduced pressure. Residual water was removed by azeotropic distillation with absolute ethanol at reduced pressure. To the solids remaining were added 5 ml. of acetic anhydride and 50 mg. of anhydrous sodium acetate. Acetylation was conducted at 110–120° for one hour. The solution was then cooled and poured into 100 g. of ice and water. After hydrolysis of the excess acetic anhydride was complete, the aqueous solution containing suspended material was extracted with three 5-ml. portions of chloroform. The combined chloroform solution was repeatedly washed with a saturated aqueous solution of sodium bicarbonate, dried over anhydrous sodium sulfate, and evaporated to a solid; yield 196 mg. This crude product was dissolved in 25 ml. of benzene and placed on a chromatographic column packed with Magnesol[15]-Celite[16] (5:1 by wt.) (200 × 35 mm. diam.[17]) and developed with 200 ml. of benzene-ethanol (100:1 by vol.). A zone about one-third of a column length from the bottom of the extruded column was located by means of the permanganate streak indicator. This was sectioned and eluted with 50 ml. of acetone. Evaporation of the solvent left 140 mg. of crystals which were recrystallized from methanol; yield 128 mg. (1.7 moles), melting point and mixed melting point with β-D-glucopyranose pentaacetate 129–130°, $[\alpha]^{25}D$ +5° (*c* 3.58, chloroform).

A second zone about one-third of a column length from the top was sectioned and eluted with 50 ml. of acetone. Solvent removal left 30 mg. (0.2 mole) of material which crystallized from 95% ethanol; melting point and mixed melting point with β-maltose octaacetate 152–153.5°, mixed melting point with β-D-glycopyranose pentaacetate 114–119°, mixed melting point with β-maltotriose hendecaacetate 121–129°. A third zone at the top of the column was treated in the same fashion to yield 21 mg. (0.1 mole) of material which crystallized from 95% ethanol; melting point with β-maltotriose hendecaacetate 131–133°, mixed melting point with β-D-glucopyranose pentaacetate 105–109°, mixed melting point with β-maltose octaacetate 123–130°.

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## Summary

A crystalline trisaccharide hendecaacetate isolated from the acetylated enzymic hydrolyzate of amylopectin in the form of waxy maize starch[2] was shown to be β-maltotriose hendecaacetate. The methylation and acid hydrolysis of the acetate yielded crystalline 2,3,6-trimethyl-D-glucose and crystalline 2,3,4,6-tetramethyl-D-glucose, separated by chromatographic technics, and further characterized as the crystalline 2,3,6-trimethyl-β-D-glucose 1,4-diacetate and 2,3,4,6-tetramethyl-D-glucose anilide. The molar ratio of 2,3,6-trimethyl-D-glucose to 2,3,4,6-tetramethyl-D-glucose was 2:1.

Amorphous maltotriose, prepared through its crystalline β-hendecaacetate, was not fermented by bakers' yeast, was not hydrolyzed by emulsin, and was partially hydrolyzed by a maltase preparation into D-glucose and maltose (identified as the crystalline β-D-acetates separated chromatographically).

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(20) J. C. Irvine and Agnes M. Moodie, *J. Chem. Soc.*, **93**, 95 (1908).

(21) A product of the Resinous Products Division of the Rohm and Haas Co., Philadelphia, Pennsylvania.

(22) Manufactured by Standard Brands, Inc., New York, N. Y.

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