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Publisher's version / Version de l'éditeur:

https://doi.org/10.1139/v54-044

Canadian Journal of Chemistry, 32, 4, pp. 340-344, 1954-04-01

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A CHEMICAL SYNTHESIS OF D-TREHALOSE[1]

BY R. U. LEMIEUX AND H. F. BAUER

ABSTRACT

Chromatographic separation of the products formed on heating a mixture of the anomers of 2,3,4,6-tetra-*O*-acetyl-D-glucose and tri-*O*-acetyl-D-glucosan ⟨1,5⟩ α ⟨1,2⟩ at 100° C. afforded D-trehalose and the *neo*-D-trehalose of Haworth and Hickinbottom. Evidence was obtained to show that the latter compound is in fact α-D-glucopyranosyl β-D-glucopyranoside.

Three diastereoisomeric D-glucopyranosyl D-glucopyranosides are theoretically possible. The α,α-, β,β-, and α,β-forms have been termed trehalose (7), *iso*-trehalose (3, 7), and *neo*-trehalose (6), respectively. D-Trehalose occurs naturally in many lower plants and trehala (5). The substance has not previously been synthesized by chemical means. Leloir and Cahib (8) have recently reported an enzymic synthesis. *iso*-D-Trehalose (12) was first obtained by Fischer and Delbrück (3) as a by-product in the preparation of 2,3,4,6-tetra-*O*-acetyl-D-glucose. The identity of *neo*-D-trehalose is obscure. Vogel and Debowska-Kurnicka (19) obtained a substance, m.p. 68–70°, $[\alpha]_D$ +68.1 (chloroform), by condensing 2,3,4,6-tetra-*O*-acetyl-D-glucose in the presence of zinc chloride and phosphorus pentoxide. The product was assumed to be *neo*-D-trehalose octaacetate since the rotation was that expected on the basis of Hudson's rules of isorotation (7). Haworth and Hickinbottom (6) reacted 2,3,4,6-tetra-*O*-acetyl-β-D-glucose in benzene solution with Brigl's anhydride (2) to form the heptaacetate of a disaccharide termed *neo*-trehalose. The octaacetate melted at 140–141° C. with specific rotation +82° (chloroform). Sharp and Stacey (18) have recently questioned the identity of this substance and have reported the preparation of *neo*-D-trehalose octaacetate, m.p. 120°, specific rotation, +67° (chloroform), both by reaction of β-acetofluoroglucose with silver carbonate, "anhydrone", and iodine in chloroform and by treating 2,3,4,6-tetra-*O*-acetyl-α-D-glucose with phosphorus pentoxide in chloroform. More recently, Micheel and Hagel (14) reacted acetobromoglucose in acetone with about 0.4 moles of water in the presence of mercuric cyanide to form *iso*-trehalose octaacetate and a *neo*-trehalose octaacetate, m.p. 140°, $[\alpha]_D$ +80° (chloroform). These constants are in close agreement with those reported by Haworth and Hickinbottom (6). Since the present work was completed, a paper has appeared wherein Bredereck, Höschele, and Ruck (1) report melting 2,3,4,6-tetra-*O*-acetyl-β-D-glucose with zinc chloride to form *iso*-trehalose octaacetate and a *neo*-trehalose octaacetate, m.p. 140° C., $[\alpha]_D$ +64.4° (chloroform). We have reinvestigated the reaction of Brigl's anhydride with the anomeric 2,3,4,6-tetra-*O*-acetyl-D-glucoses.

A mixture of the anomeric 2,3,4,6-tetra-*O*-acetyl-D-glucoses (about 65% α-anomer) was heated in a small amount of benzene with an equimolar amount

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[1] *Manuscript received December 18, 1953.*
Contribution from the National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan. Issued as Paper No. 166 on the Uses of Plant Products and as N.R.C. No. 3207.

of tri-*O*-acetyl-D-glucosan ⟨1,5⟩ α ⟨1,2⟩ (Brigl's anhydride) (2) for 36 hr. at 100° C. The product was deacetylated and the disaccharides were isolated by preparative paper chromatography (15). This fraction was acetylated and the resulting mixture of sugar acetates was subjected to chromatographic separation on a column of Magnesol-Celite (5: 1) according to the general procedure of McNeely, Binkley, and Wolfrom (13). Two crystalline substances were isolated. One of the materials was identified as D-trehalose octaacetate. The other substance possessed the melting point and rotation reported by Haworth and Hickinbottom (6) for *neo*-D-trehalose octaacetate. Deacetylation of these substances afforded D-trehalose and a substance with the physical constants reported by Haworth and Hickinbottom for *neo*-trehalose (6).

Lemieux (9) has reviewed the previous experience in the preparation of α-D-glucopyranosides by reaction of alcohols with Brigl's anhydride and prepared β-D-maltose octaacetate by heating 1,2,3,6-tetra-*O*-acetyl-β-D-glucose with the anhydride and acetylating the product. Lemieux and Huber (11) have synthesized sucrose by reaction of the anhydride with 1,3,4,6-tetra-*O*-acetyl-D-fructose and deacetylation of the product. These syntheses as well as the synthesis of D-trehalose reported herein show that Brigl's anhydride has a strong tendency to form α-D-glucopyranosides when reacted with the secondary alcoholic or hemiacetal hydroxyl groups of partially acetylated sugars. Therefore, it is to be expected that Haworth and Hickinbottom (6) did in fact prepare an α-D-glucopyranoside on reacting Brigl's anhydride with 2,3,4,6-tetra-*O*-acetyl-β-D-glucose. There can be little doubt that the substance we have isolated is identical with the *neo*-D-trehalose reported by Haworth and Hickinbottom (6). A sample was hydrolyzed and paper chromatography showed the hydrolyzate to contain only glucose. Therefore, the nonreducing substance must be a D-glucosyl-D-glucoside. Periodate oxidation of the trehalose-type molecule should liberate two moles of formic acid and no formaldehyde. The new technique developed by Perlin (16) to measure the amount of formic acid liberated in periodate oxidations was used to study the periodate oxidation both of trehalose and of the *neo*-trehalose of Haworth and Hickinbottom. In each case, two moles of formic acid were liberated per mole of disaccharide and no appreciable amount of formaldehyde was formed. Therefore, the *neo*-trehalose of Haworth and Hickinbottom must be a D-glucopyranosyl D-glucopyranoside. The rotation in chloroform of the octaacetate, +82°, when compared (7) to those of D-trehalose octaacetate, +163°, and *iso*-D-trehalose octaacetate, −18.4° (12), leaves no doubt that the substance is in fact α-D-glucopyranosyl β-D-glucopyranoside.

EXPERIMENTAL

Octaacetates of D-*Trehalose and neo*-D-*Trehalose*

A solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucose (4), 4 gm., in 50 ml. of 96% ethanol was kept at 50° C. for 40 hr. The solution was concentrated *in vacuo* to a dry sirup which was dissolved in 50 ml. of dry ether. Skellysolve F was added to a slight turbidity and after seeding with starting material the solution was kept overnight at 0° C. The crystals which separated were removed by filtration and the filtrate was evaporated *in vacuo* to a sirup $[\alpha]_D^{22}$ +93° (chloroform). The substance (about 65% α-anomer), 2.15 gm., was dissolved in 25 ml. dry benzene. The solution was concentrated by distillation at atmospheric pressure to about 5 ml. volume and transferred to a tube which contained 1.90 gm. of Brigl's anhydride (2). The tube was sealed and heated at 100° C. for 36 hr. in a temperature-controlled oil bath. The product was deacetylated in methanolic ammonia and a 20% aqueous solution of the free sugars was applied as uniform streaks to 50 × 50 cm. sheets of Schleicher-Schuell No. 470A filter paper for chromatographic separation. About one milliliter of the solution was applied to each sheet. The sheets were attached to strips of Whatman No. 1 filter paper for downward irrigation (15) with butanol–pyridine–water (6:4:3). The band in the resulting chromatogram which was expected to contain disaccharides was readily detected on strips cut from the sheets by the periodate–permanganate spray reagent (10). After isolation by extraction with water and solvent removal, the disaccharides were acetylated with acetic anhydride and sodium acetate in the usual manner. The sirupy sugar acetates were dissolved in about 5 ml. of benzene and added to the top of a 160 × 35 mm. (in diam.) column of Magnesol-Celite (5:1) (12) wetted with benzene. The chromatogram was developed with 1.5 liter of 60:1 benzene – tertiary butanol mixture, the column was extruded and a streak was applied to the length of the column by spraying a freshly prepared aqueous solution of 1% potassium permanganate in 2.5 *N* aqueous sodium hydroxide through a 2 mm.-wide slit cut from a sheet of plexiglass. A diffuse zone extended from 8 to 60 mm. from the top of the column and a sharp zone from 65 to 90 mm. from the top of the column. Elution of the top zone with acetone gave 10 mgm. of a substance, m.p. 141–142° C., $[\alpha]_D^{25}$ + 81.8° (*c*, 0.4 in chloroform), after two crystallizations from ethanol. Haworth and Hickinbottom (6) have reported *neo*-D-trehalose octaacetate to melt at 140–141° C. with $[\alpha]_D$ +82° (*c*, 5 in chloroform). The bottom zone was eluted with acetone to yield, after two crystallizations from ethanol, 15 mgm. of a substance, m.p. 100–101° C., $[\alpha]_D^{25}$ +163° (*c*, 0.1 in chloroform). The melting point was undepressed by authentic D-trehalose octaacetate, m.p. 100–101° C. The infrared spectrum of the synthetic substance pressed with potassium bromide (17) into a window and measured with a Perkin–Elmer Model 21 spectrometer was identical to the spectrum of the authentic sample of D-trehalose octaacetate obtained under the same conditions.

D-*Trehalose*

The synthetic D-trehalose octaacetate was deacetylated in methanolic ammonia and the product was recrystallized from aqueous ethanol. The melting point of the substance, m.p. 97–98° C., was unaffected by mixture with an authentic sample of D-trehalose dihydrate of the same melting point.

neo-D-*Trehalose*

The synthetic *neo*-D-trehalose octaacetate was deacetylated with methanolic ammonia. The product obtained on crystallization from 90% ethanol sintered at 141–145° C. and melted in the range 195–210° C. All attempts to obtain a sharply melting product were unsuccessful. Haworth and Hickinbottom (6) had the same difficulty and reported *neo*-trehalose to melt at 210–220° C. with sintering at 145–150° C. A sample was hydrolyzed in *N* hydrochloric acid. Paper partition chromatography using butanol–ethanol–water (5: 1: 4) and butanol–pyridine–water (6: 4: 3) detected only glucose in the hydrolyzate.

Periodate Oxidations

The disaccharides, 0.004 mM., were oxidized at 16° C. in 3.2 ml. of solution containing 0.05 mM. sodium periodate and 0.042 mM. sodium bicarbonate and saturated with carbon dioxide according to the procedure developed by Perlin (16) using a Warburg apparatus to follow the liberation of carbon dioxide. The results of typical runs are plotted in Fig. 1. Only negligible amounts of formaldehyde could be detected in the oxidation mixture after 24 hr. reaction time using the chromotropic acid reagent.

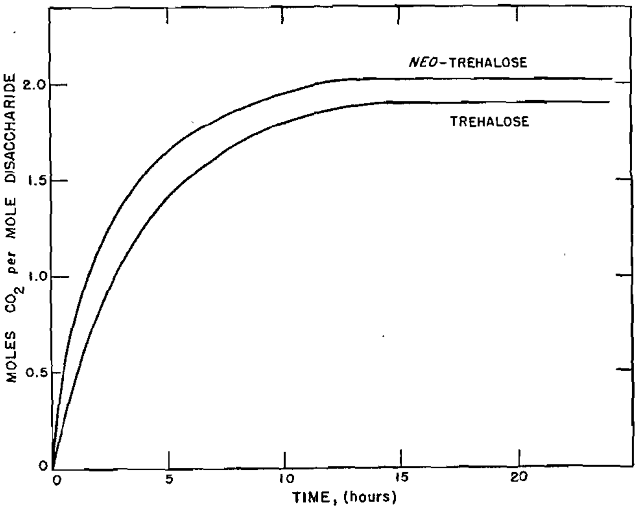

FIG. 1. The rates of liberation of formic acid on periodate oxidation of trehalose and *neo*-trehalose measured by the method of Perlin (16).

ACKNOWLEDGMENT

The authors are indebted to Dr. A. S. Perlin for kindly providing them with detailed instructions for carrying out the periodate oxidations.

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