1-Vinylnaphthalene acted both as diene and dienophile in undergoing a Diels–Alder type of dimerization to 1-(1′-naphthyl)-1,2,3,4-tetrahydrophenanthrene.

The results of the reactions have added support to current electronic interpretations of the Diels–Alder reaction.

ANN ARBOR, MICHIGAN RECEIVED MARCH 11, 1949

[CONTRIBUTION FROM THE SUGAR RESEARCH FOUNDATION LABORATORY, DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

The Preparation and Proof of Structure of 1,2:5,6-Dicyclohexylidene-D-glucofuranose[1]

BY ROBERT C. HOCKETT,[2] ROBERT ELLSWORTH MILLER[3] AND ALLEN SCATTERGOOD

Acetone is the only ketone whose reaction products with D-glucose have been fully characterized and determined structurally.[4] The products of the reaction, the water-soluble and non-reducing "monoacetoneglucose" and "diacetoneglucose" have been unequivocally shown to be 1,2-isopropylidene-D-glucofuranose and 1,2:5,6-diisopropylidene-D-glucofuranose, respectively.[5]

We have now found that the acid-catalyzed condensation of D-glucose with cyclohexanone forms a crystalline non-reducing dicyclohexylidene-glucose (I) which, in contrast to "diacetoneglucose," is water-insoluble. A 14-experiment yield study in which the variables were mole ratio of cyclohexanone to glucose, sulfuric acid concentration, and time, indicated that the best crude yield that could be secured under anhydrous conditions at room temperature was about 40%.

Structure

One hydroxyl group was shown to be present in I by the preparation in crystalline form of a monobenzoate (IIa), a benzenesulfonate (IIb) and a *p*-toluenesulfonate (tosylate, IIc). We were unable to secure the acetylation product or the methylation product of I in the crystalline condition. The preparation of these monoesters of non-reducing I showed that four of the five hydroxyl groups of a hemiacetal form of the D-glucose were blocked, probably by the formation of two heterocyclic rings. When compound IIb was heated under reflux with sodium iodide in acetic anhydride, practically no insoluble sodium benzenesulfonate was formed. This indicated that the benzenesulfonate group of IIb would undergo a displacement reaction only with considerable difficulty. In the absence of a neighboring hydroxyl group, an arylsulfonate group reluctant to undergo a displacement reaction is usually considered to be attached to a secondary carbon atom[6] or to have the back side approach of the replacing group blocked.[7] Thus it seemed probable that the hydroxyl group of I was on a ring which was blocked by other attached groups so that the approach of a replacing group was made difficult.

The location of the hydroxyl group of I was ascertained by an acid-catalyzed methanolysis of tosylate IIc followed by an acetylation of the sirupy product (III). Crystalline methyl 3-tosyl-2,4,6-triacetyl-β-D-glucopyranoside (IV) resulted, and demonstrated that carbon atom number three of I was the site of the hydroxyl group. Compound IV had previously been obtained by Peat and Wiggins[8] through a similar series of reactions starting with 1,2:5,6-diisopropylidene-D-glucofuranose (diacetoneglucose).

The location and size of the acetal rings of I was determined by the discovery that one of the two cyclohexylidene groups of I could be removed selectively. Controlled acid-catalyzed methanolysis of I gave a non-reducing crystalline monocyclohexylidene-D-glucose (V). Product V, obtained in good yield, could readily be recrystallized from water in contrast to 1,2-isopropylidene-D-glucofuranose ("monoacetone-glucose") which is very soluble in water.

The benzoylation of V gave a crystalline tribenzoate (VI) demonstrating the presence of three hydroxyl groups in V. The acid-catalyzed hydrolysis of VI gave 3,5,6-tribenzoyl-D-glucofuranose (VII) previously obtained by Fischer and Rund[9] from 1,2-isopropylidene-D-glucofuranose ("monoacetoneglucose"). Compound VII was isolated as its carbon tetrachloride addition product first observed by Fischer.

(1) A portion of the material in this paper was presented before the Division of Sugar Chemistry and Technology at the 115th meeting of the American Chemical Society, San Francisco, California, March 27, 1949.

(2) Present address: Sugar Research Foundation, Inc., 52 Wall Street, New York 5, N. Y.

(3) The material presented in this paper is taken from a thesis submitted to the Department of Chemistry of the Massachusetts Institute of Technology in partial fulfillment of the requirements for the degree of Doctor of Philosophy by Robert Ellsworth Miller whose present address is: The American Sugar Refining Company, Research and Development Division, Philadelphia 48, Pennsylvania.

(4) Kranslein, Dickhauser and Voss, U. S. Patent 1,902,866 (March 28, 1933) reported a "dicyclohexanoneglucose of m. p. 134°" which they formulated as a 1,2:5,6-dicyclohexylidene-D-glucofuranose in the patent. No analytical results, specific rotation, yield, or proof of structure were given.

(5) (a) K. Freundenberg and A. Doser, *Ber.*, **56**, 1243 (1923); (b) Anderson, Charlton and Haworth, *J. Chem. Soc.*, 1329 (1929).

(6) (a) Bell, Friedmann and Williamson, *ibid.*, 252 (1937); (b) Oldham and Rutherford, THIS JOURNAL, **54**, 366 (1932).

(7) Bartlett and Knox, *ibid.*, **61**, 3184 (1939).

(8) Peat and Wiggins, *J. Chem. Soc.*, 1092 (1938).

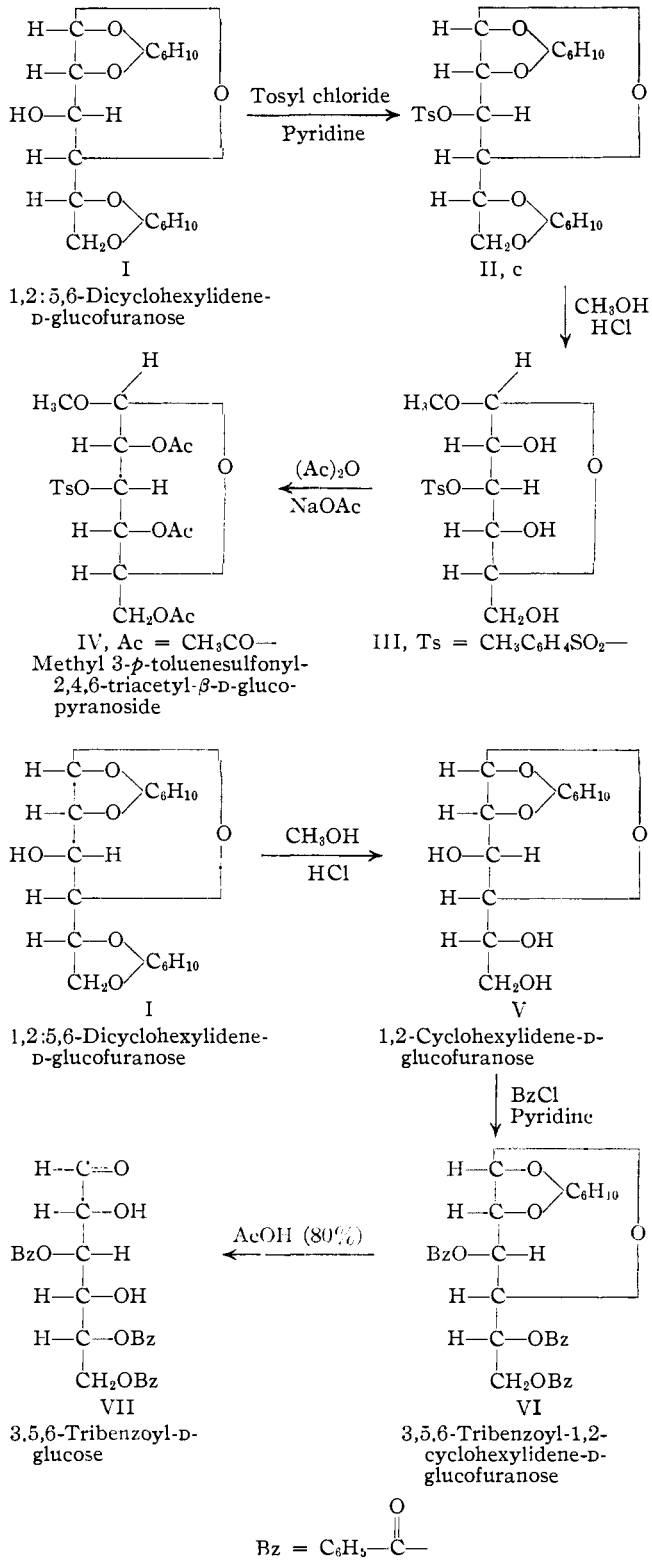
(9) Fischer and Rund, *Ber.*, **49**, 88 (1916).

The isolation of VII from VI indicated that the three benzoyl groups in VI and thus the three hydroxyl groups in V are in the 3, 5 and 6 positions. Since V was obtained by the removal of one cyclohexylidene residue from I (which had previously been shown to contain one hydroxyl group in position 3) the two hydroxyl groups thus liberated (positions 5 and 6) must be the site of one of the cyclohexylidene residues.

Since V is non-reducing the aldehyde group originally present at carbon atom number one in the glucose molecule must be blocked in some manner. Positions 2 and 4 must also be blocked since it has been demonstrated above that V has only three hydroxyl groups which are located on carbon atoms 3, 5 and 6. The most probable explanation for the blocking of positions 1, 2 and 4 in V is the presence of two heterocyclic rings involving these three positions. Of the structures that can be written for such a monocyclohexylideneglucose the one most probable is 1,2-cyclohexylidene-D-glucofuranose, V. Thus I becomes 1,2:5,6-dicyclohexylidene-D-glucofuranose.

Two other possibilities remain to be considered. They are (1) the inversion of the configuration of one or more of the asymmetric carbon atoms of the glucose molecule and (2) the wandering of a tosyl group or acetal ring during one or more of the reactions carried out. Results of parallel experiments under similar conditions conducted by many other workers on reactions of 1,2:5,6-diisopropylidene-D-glucofuranose make both of these possibilities unlikely.

Determination of the position of the hydroxyl group in 1,2:5,6-dicyclohexylidene-D-glucofuranose is shown in I, IIc, III, IV; preparation and structure determination of 1,2-cyclohexylidene-D-glucofuranose in I, V, VI, VII.



I
1,2:5,6-Dicyclohexylidene-D-glucofuranose

II, c

IV, Ac = CH_3CO—
Methyl 3-*p*-toluenesulfonyl-2,4,6-triacetyl-β-D-glucopyranoside

III, Ts = $CH_3C_6H_4SO_2$—

V
1,2-Cyclohexylidene-D-glucofuranose

VII
3,5,6-Tribenzoyl-D-glucose

VI
3,5,6-Tribenzoyl-1,2-cyclohexylidene-D-glucofuranose

Experimental

1,2:5,6-Dicyclohexylidene-D-glucofuranose, I.—Anhydrous D-glucose (45 g., 0.25 mole, Merck reagent grade) was added to a mixture of cyclohexanone (100 ml., 1 mole, du Pont commercial grade) and concentrated sulfuric acid (6.5 ml., d. 1.84) and the reaction mixture was shaken at room temperature for twelve hours. Commercial *n*-heptane (250 ml.) was then added and the mixture was heated until the solids had dissolved and two liquid layers had formed. The *n*-heptane layer was decanted from the dark oily layer, allowed to cool and then refrigerated. The product (36.8 g., 43.2%) crystallized overnight. It softened at 105° (uncor.) and melted at 109–113° (uncor.). The compound was recrystallized from methylcyclohexane (6 ml./g.) and then from *n*-heptane (20 ml./g.) to give a product which melted at 131.4–132.4° (cor.) and showed a rotation in alcohol $[\alpha]^{31}D$ −2.20° (*c*, 2.27) (0.5683 g. in 25 ml. solution gave α −0.10° in a 2-dcm. tube). The rotation in chloroform was $[\alpha]^{31}D$ + 1.65° (*c*, 2.10) (1.0509 g. in 50 ml. of solution gave α + 0.14° in a 4-dm. tube). The compound is soluble in acetone, alcohol, chloroform, benzene and methyl "cellosolve." It is soluble in warm isobutanol, hot *n*-heptane and hot methylcyclohexane but is insoluble in water. It does not reduce hot Fehling solution.

Anal. Calcd. for $C_{18}H_{28}O_6$: C, 63.51; H, 8.29. Found: C, 63.36; H, 8.13.

3-Benzoyl-1,2:5,6-dicyclohexylidene-D-glucofuranose, IIa.—1,2:5,6-Dicyclohexylidene-D-glucofuranose (6.8 g., 0.02 mole) was added to a mixture of benzoyl chloride (3.7 g., 0.026 mole, Hooker Chemical Company) in anhydrous pyridine (10 ml., 0.124 mole). The reaction mixture was chilled until the initial heat evolution had dissipated. After standing at room temperature for three days the mixture was chilled, cold water (10 ml.) was added with chilling and shaking and the turbid solution was refrigerated. An oil separated which did not crystallize. The oil was extracted with chloroform (100 ml.) and water was bubbled through the chloroform solution overnight. The chloroform layer was removed, dried over granular calcium chloride and then concentrated under reduced pressure at 45–50° to a thick, yellow sirup. This sirup was dissolved in hot methanol (50 ml.) and the solution was allowed to cool to room temperature. An oil formed which upon standing and scratching with a glass rod crystallized. This product (5.20 g., 58.5%) melted at 109–110.3° (uncor.). The compound was recrystallized from *n*-heptane (5 ml./g.) and then from methanol (8 ml./g.) to a product which melted at 111.2–112.2° (cor.) and showed a rotation in chloroform $[\alpha]^{26}D$ −37.0° (*c*, 1.2) (0.6210 g. in 50 ml. of solution gave α −0.92° in a 2-dm. tube). The substance is soluble in acetone and chloroform, slightly soluble in methanol, methyl "cellosolve" and alcohol. It is soluble in warm *n*-heptane and warm isobutanol but is insoluble in water.

Anal. Calcd. for $C_{25}H_{32}O_7$: C, 67.54; H, 7.26. Found: C, 67.14; H, 7.59.

3-Benzenesulfonyl-1,2:5,6-dicyclohexylidene-D-glucofuranose, IIb.—Benzenesulfonyl chloride (14 ml., 0.109 mole, Eastman Kodak Company, "Eastman" grade) was added in one portion to a solution of 1,2:5,6-dicyclohexylidene-D-glucofuranose (34.0 g., 0.1 mole) in anhydrous pyridine (50 ml., 0.62 mole). A heat evolution was not observed. After being shaken at room temperature overnight the reaction mixture was chilled; cold water (50 ml.) was added slowly with shaking and the turbid solution was refrigerated. A sirup formed which did not crystallize. The liquid layer was decanted and discarded. The sirup was triturated with six portions (25 ml. each) of cold water and these water washings were discarded. The residue was dissolved in warm absolute alcohol (100 ml.), decolorized with charcoal and filtered. The clear filtrate upon refrigeration gave a product (24 g., 50%) melting at 90–92° (uncor.). Concentration of the mother liquor gave a second crop of 7.14 g. (total yield 65%). The product after recrystallization from *n*-heptane (2.5 ml./g.) and then from absolute alcohol (5 ml./g.) melted at 94.6–95.4° (cor.) and showed a rotation in chloroform $[\alpha]^{28}D$ −61.4° (*c*, 1.71) (0.8585 g. in 50 ml. of solution gave α −2.11° in a 2-dm. tube). The compound is soluble in acetone and chloroform. It is soluble in warm alcohol, warm isobutanol, warm methyl "cellosolve" and hot *n*-heptane but is insoluble in water.

Anal. Calcd. for $C_{24}H_{32}O_8S$: C, 59.98; H, 6.71; S, 6.67. Found: C, 59.80; H, 6.79; S, 6.63.

Attempted Replacement of the Benzenesulfonate Group in 3-Benzenesulfonyl-1,2:5,6-dicyclohexylidene-D-glucofuranose, IIb.—A solution of 3-benzenesulfonyl-1,2:5,6-dicyclohexylidene-D-glucofuranose (1.20 g., 0.0025 mole) and sodium iodide (0.5 g., 0.0034 mole, Merck reagent grade) in acetone (25 ml.) was boiled under reflux for three days. No precipitate was formed.

A solution of 3-benzenesulfonyl-1,2:5,6-dicyclohexylidene-D-glucofuranose (1.20 g.) and sodium iodide (0.5 g.) in acetic anhydride (25 ml.) was heated under reflux for twenty-four hours. The reaction mixture became very black but only a negligible amount of a precipitate formed.

3-*p*-Toluenesulfonyl-1,2:5,6-dicyclohexylidene-D-glucofuranose, IIC.—1,2:5,6-Dicyclohexylidene-D-glucofuranose (6.80 g., 0.02 mole) was added to a solution of *p*-toluenesulfonyl chloride (4.8 g., 0.025 mole, Eastman Kodak Company, "Eastman" grade) in anhydrous pyridine (10 ml., 0.124 mole). The solid immediately dissolved without any heat evolution. After being shaken at room temperature for ten days the reaction mixture was chilled; cold water (10 ml.) was added slowly with chilling and shaking and the resultant turbid solution was refrigerated. A white sirup formed which did not crystallize. The liquid layer was decanted and discarded. The sirup was triturated with three portions (20 ml. each) of cold water and these washings were discarded. The residue was dissolved in chloroform (150 ml.) and water was bubbled through the chloroform solution overnight. The chloroform layer was dried over granular calcium chloride and then concentrated under reduced pressure to a sirup. This residue was dissolved in warm methanol (50 ml.) and the methanol solution was allowed to stand in an open beaker overnight. A crystalline product (7.5 g., 75.8%) of m. p. 88.5–90° (uncor.) was thus obtained. The compound was alternately recrystallized from isobutanol (4 ml./g.) and from *n*-heptane (7 ml./g.) to a constant m. p. of 89.7–91.1° (cor.) and then showed a rotation in chloroform $[\alpha]^{26}D$ −67.6° (*c*, 0.18) (0.0924 g. in 50 ml. of solution gave α −0.50° in a 4-dm. tube). The compound is soluble in acetone and chloroform; slightly soluble in ethyl acetate and methyl "cellosolve." It is soluble in hot alcohol, hot *n*-heptane and hot 80% alcohol but is insoluble in water.

Anal. Calcd. for $C_{25}H_{34}O_8S$: C, 60.71; H, 6.93; S, 6.48. Found: C, 60.41; H, 7.20; S, 6.48.

Conversion of 3-*p*-Toluenesulfonyl-1,2:5,6-dicyclohexylidene-D-glucofuranose, IIc, to Methyl 3-*p*-Toluenesulfonyl-2,4,6-triacetyl-D-glucopyranoside, IV.—3-*p*-Toluenesulfonyl-1,2:5,6-dicyclohexylidene-D-glucofuranose (19 g., 0.038 mole) was dissolved in warm, anhydrous methanol containing hydrogen chloride (500 ml., 1.97 g. hydrogen chloride/100 ml. solution). The reaction mixture was refluxed under a water-cooled condenser, protected from the atmosphere by a calcium chloride drying tube, until a constant angular rotation was observed (approximately twenty-seven hours). The light yellow solution was then neutralized with excess silver carbonate and filtered. The residue was washed with four portions of methanol (50 ml. each) and discarded. The filtrate and washings were combined and concentrated under reduced pressure to a sirup. The sirup was dissolved in chloroform and some residual silver *p*-toluenesulfonate was removed by filtration. The chloroform filtrate was then concentrated under reduced pressure at 45° to a thick sirup. The sirup was dissolved in a hot mixture of acetic anhydride (200 ml.) and anhydrous sodium acetate (15 g.) The reaction mixture was heated at 85–90° on a steam-bath for three hours and was then poured into a vigorously stirred mixture of ice and water (1 liter). An oil separated which did not crystallize. The mixture was extracted with four portions of chloroform (50 ml. each) and the combined chloroform extracts were concentrated to a thin sirup under reduced pressure at 50°. The sirup was dissolved in anhydrous benzene (50 ml.) and the resulting solution was concentrated under reduced pressure to a thick sirup. Ether was added (50 ml.); the sirup slowly dissolved and a crystalline material soon began to appear. Refrigeration gave 4.7 g. (26.2%) of product melting at 131–132° (uncor.). After several recrystallizations from methanol the product melted at 135.5–137° (cor.) and showed a rotation in chloroform $[\alpha]^{25}D$ −18.6° (*c*, 0.6), (0.1513 g. in 25 ml. of solution gave α −0.45° in a 4-dm. tube).

Anal. Calcd. for $C_{20}H_{26}O_{11}S$: C, 50.62; H, 5.52; S, 6.76. Found: C, 50.49; H, 5.57; S, 6.77.

An authentic sample of methyl 3-*p*-toluenesulfonyl-2,4,6-triacetyl-D-glycopyranoside, IV, was obtained from Dr. L. F. Wiggins. His sample melted at 135–135.5° (cor.). A mixture of his sample and our product melted at 135–136° (cor.).

Acid-catalyzed Methanolysis of 1,2:5,6-Dicyclohexylidene-D-glucofuranose, I.—1,2:5,6-Dicyclohexylidene-D-glucofuranose (3.4034 g., 0.01 mole) was dissolved in commercial methanol (45 ml.) in a 50-ml. volumetric flask.

Methanolic hydrogen chloride (1.12 *N*, 0.45 ml.) was added and the reaction mixture was immediately diluted to the mark with commercial methanol. (The reaction mixture was 0.20 molar in 1,2:5,6-dicyclohexylidene-D-glucofuranose and 0.01 *N* in hydrogen chloride.) The reaction mixture was thoroughly mixed; transferred to a 2-dm. polarimeter tube and the reaction was followed polarimetrically. The curve (angular rotation *versus* time) is plotted on Fig. 1.

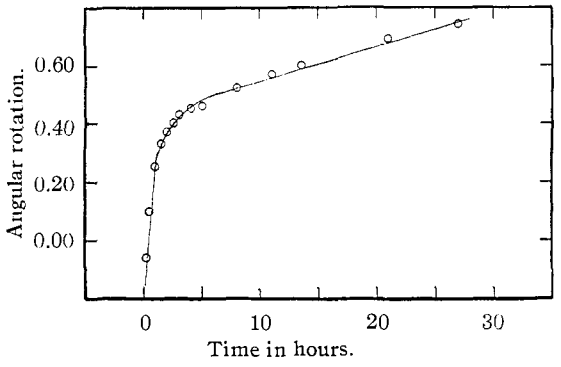


Fig. 1.—Acid-catalyzed methanolysis of 1,2:5,6-dicyclohexylidene-D-glucofuranose: 0.2 molar in dicyclohexylidene-D-glucose, 0.01 molar in hydrogen chloride.

1,2-Cyclohexylidene-D-glucofuranose, V.—1,2:5,6-Dicyclohexylidene-D-glucofuranose (68 g., 0.2 mole) was dissolved in commercial methanol (980 ml.) in a one-liter volumetric flask and methanolic hydrogen chloride (1.12 *N*, 9.0 ml.) was then added. The reaction mixture was diluted to the mark with methanol and allowed to stand at room temperature for four hours. (The reaction mixture was 0.2 molar in 1,2:5,6-dicyclohexylidene-D-glucofuranose and 0.01 *N* in hydrogen chloride. These are the same concentrations as those used in following the reaction polarimetrically.) Excess solid sodium bicarbonate was added and then 10% aqueous sodium hydroxide until the mixture became alkaline to litmus. The solution was filtered and the filtrate was concentrated under reduced pressure at 45–50° to a thick, sirupy-solid mass. Water (100 ml.) and *n*-heptane (200 ml.) were added and the mixture was heated until two clear liquid layers were obtained. The two layers were separated, allowed to cool to room temperature and then refrigerated. From the *n*-heptane layer, starting material (16.2 g.) was recovered, while the aqueous solution yielded crystalline 1,2-cyclohexylidene-D-glucofuranose (29.5 g., 74.5% based on 1,2:5,6-dicyclohexylidene-D-glucofuranose consumed) of m. p. 145–148° (uncor.). Concentration of the aqueous filtrate gave a second crop of product, 4.85 g. (total yield 87%). The compound after recrystallization from isobutanol (7 ml./g.) and from water (3 ml./g.) melted at 151.7–153.1° (cor.) and showed a rotation in acetone $[\alpha]^{29}D$ +4.00° (*c*, 1.4), (0.1376 g. in 10 ml. of solution gave α +0.11° in a 2-dm. tube). The compound is soluble in acetone and only slightly soluble in water. It is soluble in hot alcohol, hot isobutanol and hot methyl "cellosolve." The compound does not reduce hot Fehling solution.

Anal. Calcd. for $C_{12}H_{20}O_6$: C, 55.37; H, 7.75. Found: C, 55.01; H, 7.71.

3,5,6-Tribenzoyl-1,2-cyclohexylidene-D-glucofuranose, VI.—Benzoyl chloride (4 ml., 0.034 mole, Hooker Chemical Company) was added in one portion with vigorous stirring to a chilled mixture of 1,2-cyclohexylidene-D-glucofuranose (2.60 g., 0.01 mole) in anhydrous pyridine (10 ml., 0.12 mole). The reaction mixture was kept overnight in a 50° bath and the mixture was then poured into a vigorously stirred mixture of ice and water (400 ml.). An oil formed which slowly solidified. The solid was removed by filtration, washed several times with water and dried. The product (5.34 g., 93%) melted at 123.5–125° (uncor.). The compound after recrystallization from isobutanol (10 ml./g.) and then from *n*-heptane (20 ml./g.) melted at 124.6–125.6° (cor.) and showed a rotation in chloroform $[\alpha]^{26}D$ −90.4° (*c*, 0.62), (0.3139 g. in 50 ml. of solution gave α −2.27° in a 4-dm. tube). The compound is soluble in acetone, benzene, chloroform and ethyl acetate. It is soluble in hot isobutanol, hot *n*-heptane and hot methyl "cellosolve" but is insoluble in water.

Anal. Calcd. for $C_{33}H_{22}O_9$: C, 69.21; H, 5.63. Found: C, 68.80; H, 5.64.

3,5,6-Tribenzoyl-D-glucofuranose-Carbon Tetrachloride, VII.—3,5,6-Tribenzoyl-1,2-cyclohexylidene-D-glucofuranose (26.3 g., 0.046 mole) was dissolved in hot 80% acetic acid (600 ml.) and the reaction mixture was refluxed for five and one-third hours. The solution was allowed to cool to room temperature and was then seeded with starting material but nothing crystallized from the reaction mixture while standing overnight. (Previous experimentation had shown that the starting material while soluble in hot 80% acetic acid crystallized readily from the cool solution.) The reaction mixture was concentrated under reduced pressure at 45–50° to a light yellow, thick sirup. The sirup was taken up in room temperature carbon tetrachloride (200 ml.); the solution was seeded with 3,5,6-tribenzoyl-D-glucofuranose-carbon tetrachloride[10] and refrigerated. The product (20.8 g., 70%) crystallized overnight. The compound after several recrystallizations from carbon tetrachloride (15 ml./g.) softened at 65° (cor.) and melted at 69–78° (cor.).

Anal. Calcd. for $C_{28}H_{24}O_9Cl_4$: C, 52.03; H, 3.74; Cl, 21.94. Found: C, 51.88; H, 3.85; Cl, 20.81.

Mutarotation of 3,5,6-Tribenzoyl-D-glucofuranose-Carbon Tetrachloride.—A solution of 0.5030 g. of 3,5,6-tribenzoyl-D-glucofuranose-carbon tetrachloride which had been prepared from 1,2-cyclohexylidene-D-glucofuranose and recrystallized five times from carbon tetrachloride was made in absolute alcohol in a 25-ml. volumetric flask. The alcohol was added at zero time and the solution was made up to 25 ml. and transferred to a jacketed 2-dm. polarimeter tube. In Fig. 2 the specific rotation observed during the mutarotation is plotted against the time for two different temperatures.

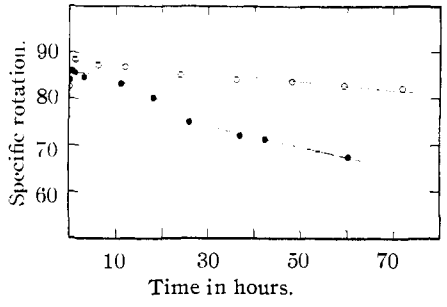


Fig. 2.—Mutarotation of 3,5,6-tribenzoyl-D-glucose in alcohol: ● at 30°; ○ at 20°.

Not all the mutarotation values which have been reported for this compound by Fischer and Rund[9] could be checked. The temperature at which these workers made their observations was not stated, but if it was 20° or lower the final part of the curve may have been so slow that the change was not detected.

Summary

1. Condensation of D-glucose with cyclohexanone has been found to form crystalline 1,2:5,6-dicyclohexylidene-D-glucofuranose (I). Three crystalline esters of this substance have also been prepared.

(10) Prepared by Dr. Franklin E. Morris, M. I. T. Chemistry Department Thesis, 1945, from 1,2-isopropylidene-D-glucofuranose.

2. Preferential hydrolysis of I has been found to yield crystalline 1,2-cyclohexylidene-D-glucofuranose. A crystalline tribenzoate of this substance has also been secured.

3. Proof of structure of all these substances is offered.

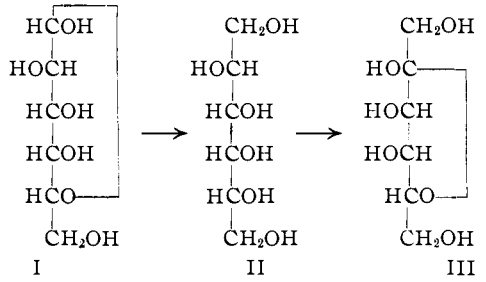
CAMBRIDGE 39, MASSACHUSETTS
RECEIVED APRIL 23, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF WISCONSIN]

The Synthesis of D-Tagatose by Biochemical Oxidation and by an Improved Chemical Method[1]

BY EZRA L. TOTTON AND HENRY A. LARDY

In connection with work being carried out in this Laboratory on the intermediary metabolism of galactose, it became necessary to prepare D-tagatose (III). Since a wide variety of polyalcohols containing *cis*-hydroxyl groups at one end of the carbon chain had been oxidized to ketoses by *Acetobacter* species,[2] it was decided to test the action of *Acetobacter suboxydans* on D-talitol (II) as a means of preparing D-tagatose (III).



D-Talitol (II) was prepared for this purpose by the catalytic reduction of D-altrose (I). The latter was synthesized by methods developed by Robertson and Griffith[3] and Richtmyer and Hudson.[4]

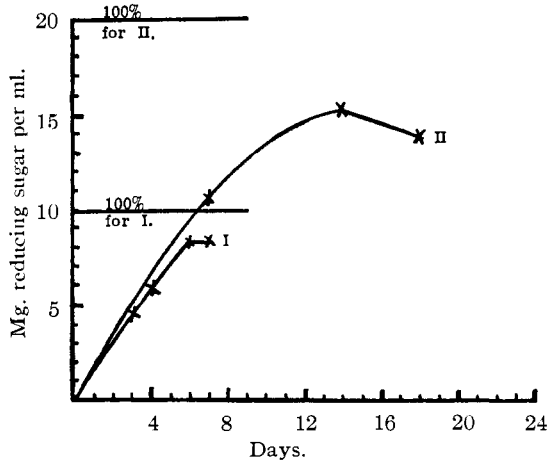


Fig. 1.—Oxidation of 1 and 2% solutions of D-talitol by *A. suboxydans*.

The D-talitol was rapidly oxidized by growing cultures of *A. suboxydans*. At concentrations of talitol below 5%, D-tagatose (III) was produced in yields of 75 to 84%. The rate of oxidation at 30° is shown in Fig. 1. When more concentrated solutions (5 to 16%) of talitol were employed, about 50% of the talitol was converted to tagatose. The yields quoted were based on a copper reduction method[5] standardized against pure D-tagatose. The bacterial oxidation product from D-talitol was demonstrated to be D-tagatose, since it gave a strongly positive test with Selivanov's reagent, was only very slightly oxidized by alkaline iodine, and gave a *p*-bromophenylosazone and a phenylosazone which were identical with those prepared from galactose.

The oxidation of D-talitol (II) to D-tagatose (III) by *A. suboxydans* offers a synthesis of D-tagatose which might be of preparative value should D-talitol become more readily available.

Since the synthesis of sufficient D-talitol to meet our needs for preparing tagatose would have been too laborious, we used the chemical procedure of Reichstein and Bosshard[6] for the preparation of larger quantities of tagatose. However, when using their procedure considerable difficulty was experienced in isolating, consistently, the tagatose from the mixture of isomerized galactose. The method was therefore modified by the use of lead acetate rather than absolute ethanol[6] to precipitate proteins and gums following the fermentation of the remaining galactose. The excess lead ions were then removed with an IR-100 ion exchange resin.[7] This modification eliminated many of the difficulties involved in the isolation of the product, and insured reproducible results.

It was also found that the rate of removal of galactose from the isomerized mixture may be increased by the use of a strain of yeast which rapidly adapts to galactose fermentation. The baker's yeast used by Reichstein and Bosshard required four days to ferment the excess galactose.

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(2) Bertrand, *Compt. rend.*, **126**, 762 (1898); Hann, Tilden and Hudson, THIS JOURNAL, **60**, 1201 (1938); Anderson and Lardy, *ibid.*, **70**, 594 (1948).

(3) Robertson and Griffith, *J. Chem. Soc.*, 1193 (1935).

(4) Richtmyer and Hudson, THIS JOURNAL, **65**, 740 (1943).

(5) Schaffer and Somogyi, *J. Biol. Chem.*, **100**, 695 (1933).

(6) Reichstein and Bosshard, *Helv. Chim. Acta*, **17**, 753 (1934).

(7) From Resinous Products Co., Philadelphia, Pennsylvania.