the water soluble–sulfuric acid soluble γ_4 subfraction and on hemicellulose B. These results are shown in Table VII.

The samples were removed from the 10° bath and were allowed to remain at room temperature (24°) in the dark for 2 months. At the end of this time the periodate consumption was well in excess of 1 mole consumed per mole anhydro sugar unit. The excess periodate was destroyed with ethylene glycol and the solution of the polyaldehyde dialyzed until free of iodate. This solution was concentrated under diminished pressure to a small volume and the polyaldehyde reduced to the corresponding polyalcohol by sodium borohydride (200% excess). The polyalcohol solution was adjusted so that the concentration of sulfuric acid was 1 *N* and the solution was hydrolyzed for 6 hours on a boiling water-bath. The hydrolysate was neutralized with barium carbonate and the excess barium ions removed by slurrying with a portion of Amberlite IR-120 cation-exchange resin. The deionized solution was concentrated *in vacuo* to a sirup and dry methanol was added and removed by vacuum distillation (this procedure was repeated several times) in order to remove the last trace of borates as the highly volatile methyl borate. Similar studies were carried out on the original water soluble γ_4, the sulfuric acid soluble γ_4 subfraction and on hemicellulose B. Paper partition chromatographic analyses in all cases showed the presence of a small amount of unoxidized xylose indicating the presence of a branch in the polysaccharides. The irrigating solvents employed were ethyl acetate–acetic acid–water (3:1:3) and (9:2:2). In the experiment with the latter solvent a check was made for the presence of glycerol and this was shown to be present in large amounts as expected.

Acetylation of the Glucomannan.—The β-fraction (5 g.) containing 8.3% moisture was dispersed in water (15 ml.) in a 250-ml. centrifuge cup and allowed to stand for 16 hours at room temperature. The mixture was flooded with ethanol (10–12 volumes), spun down and the precipitated polysaccharide washed with ethanol (170 ml.). The alcohol wet hemicellulose was transferred with pyridine (150 ml.); acetic anhydride (100 ml.) was added to the mixture and it was stirred and heated on a boiling water-bath for 20 hours under a nitrogen atmosphere. During this time the reaction mixture had turned quite dark and only a small quantity of the glucomannan remained undissolved. The glucomannan acetate was isolated by filtering the reaction mixture through sintered glass and pouring the filtrate into water. The product was isolated by filtration, and dried (yield 7 g. or 86%). The acetyl analysis was 47.5% and the product showed $[\alpha]^{25}D$ −24.2° (*c* 5.0, tetrachloroethane–ethanol 9:1). The theoretical acetyl content of a fully acetylated hexosan of infinite chain length is 44.8%. The intrinsic viscosity of the glucomannan acetate in tetrachloroethane–ethanol 9:1 was found to be 0.21.

Acetylation of a Xylan Polyuronide.—The γ_4-water soluble–sulfuric acid insoluble fraction (5 g.) was acetylated by a similar method to that employed for the glucomannan. The notable difference in the two acetylations was that the polyuronide acetate was insoluble in the acetylation medium and hence the latter was not filtered but was isolated by pouring directly into water. The yield of the acetate was 7.35 g. (90.5% of theoretical), the acetyl content was 40.8% and it showed $[\alpha]^{25}D$ −72.5° (*c* 5.0, tetrachloroethane–ethanol 9:1). The theoretical acetyl content for a fully acetylated pentosan of infinite chain length is 39.8%. The intrinsic viscosity of the acetate in tetrachloroethane–ethanol 9:1 was found to be 0.31.

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SHELTON, WASHINGTON

[CONTRIBUTION FROM THE NORTHERN UTILIZATION RESEARCH BRANCH[1]]

The Preparation, Properties and Structure of the Disaccharide Leucrose

BY FRANK H. STODOLA, E. S. SHARPE AND H. J. KOEPSELL

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Leucrose has been prepared from sucrose in 7.9% yield by the action of dextransucrase derived from the bacterium *Leuconostoc mesenteroides* (NRRL B-512F). The disaccharide gave fructose and glucose on hydrolysis and showed a low order of reactivity with hypoiodite. Complete methylation of leucrose phenylosotriazole produced a hepta-*O*-methyl derivative which on hydrolysis with acid gave 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4,6-tri-*O*-methyl-D-*arabino*-hexose phenylosotriazole. The disaccharide was shown to belong to the α-glucosyl series. On the basis of these data the structure of leucrose has been established as 5-*O*-α-D-glucopyranosyl-D-fructose (Fig. 1 in text).

In a preliminary note[2] we reported the isolation of a new disaccharide, leucrose, which was produced as a by-product in the synthesis of dextran from sucrose by the bacterium *Leuconostoc mesenteroides* (NRRL B-512F). In the present paper a method is described for the preparation of this sugar in quantity; in addition, the chemical and physical properties of the disaccharide are summarized and a complete structure proof is presented which shows that leucrose is 5-*O*-α-D-glucopyranosyl-D-fructose.

A study of the factors influencing leucrose formation showed that the best yields could be obtained at high sucrose concentration. The sucrose solution was treated with partially purified dextransucrase for six days, the dextran was removed by alcohol precipitation and the fructose by a yeast fermentation. Crystalline leucrose was obtained directly from the remaining sugars.

Leucrose, which crystallizes as a monohydrate melting at 156–158°, shows slight mutarotation, going from a value of $[\alpha]^{25}D$ −8.2° at 7 minutes to a constant value of −7.6° in less than one hour. It is not readily hydrolyzed by acids which is in conformance with the finding of Wise, *et al.*,[3] that leucrose shows only 19% fructose by their anthrone method under conditions which gave 100% yields of fructose with sucrose, melezitose and raffinose.

Leucrose forms a phenylosazone (m.p. 186–188°) which crystallizes as yellow needles from ethyl ace-

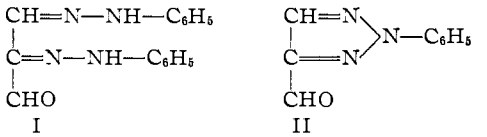
(1) One of the Branches of the Agricultural Research Service, U. S. Department of Agriculture.

(2) F. H. Stodola, H. J. Koepsell and E. S. Sharpe, THIS JOURNAL, **74**, 3202 (1952).

(3) C. S. Wise, R. J. Dimler, H. A. Davis and C. E. Rist, *Anal. Chem.*, **27**, 33 (1955).

tate saturated with water. The osazone could be oxidized with copper sulfate to the phenylosotriazole (m.p. 108–109°), the heptaacetate of which melts at 150–151°.

Early structural work had established that leucrose is a D-glucosyl-D-fructose since it showed a low order of reactivity with hypoiodite and since its phenylosotriazole could be hydrolyzed to D-glucose and D-glucose phenylosotriazole. That the point of attachment of the glucosyl group to the fructose moiety was not at position 3 or 4 followed from the fact that leucrose phenylosazone was readily cleaved by sodium metaperiodate to the aldehyde I. Under similar conditions this product was *not* formed with the phenylosazones of the 1,3-di-

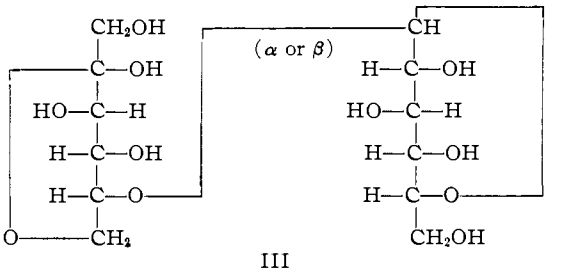


I II

saccharides turanose and laminaribiose or the 1,4-disaccharides maltose and cellobiose, whereas the phenylosazones of the 1,6-disaccharides isomaltose and gentiobiose behaved in the same manner as the leucrose derivative in giving aldehyde I. This work has now been confirmed with the phenylosotriazoles. Leucrose phenylosotriazole and gentiobiose phenylosotriazole both yielded aldehyde II, whereas cellobiose phenylosotriazole did not. These observations established with reasonable certainty that the glucosyl linkage could not be at either position 3 or 4. Only the elimination of either position 5 or 6 was needed to permit the assignment of a tentative structure to leucrose.

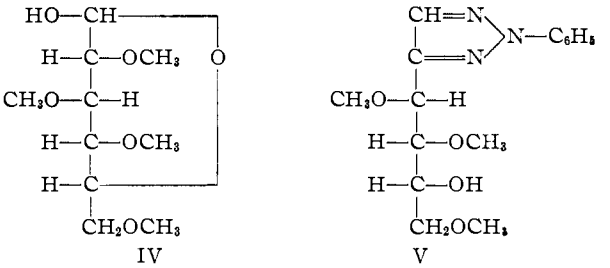
In preliminary studies a comparison of the phenylosazones had been used as a means of rejecting position 6 as a possibility. However, since these derivatives leave much to be desired for comparative purposes this work has now been repeated with the more suitable phenylosotriazoles. Leucrose phenylosotriazole (m.p. 108–109°) was easily distinguished from the phenylosotriazole of isomaltose[4] which melted at 175–176°. On the other hand comparison with gentiobiose phenylosotriazole could not be made directly since this compound is known only in the form of its ethyl alcoholate[5] so it was necessary to compare the heptaacetates. It was found that, although leucrose phenylosotriazole heptaacetate has about the same melting point as the corresponding gentiobiose derivative, the melting point of the mixture was depressed and the X-ray patterns of the two compounds were different. These new data on the triazoles, therefore, supported our earlier elimination of position 6 and thereby established tentatively a 5-*O*-D-glucosyl-D-fructose structure for leucrose.

With these structural deductions as a basis it was possible to proceed to methylation studies for final proof. Direct methylation of leucrose itself could not be used since the same trimethylfructose would result whether the glucosidic linkage is at position 5 or 6 so methylation of the phenylosotriazole was undertaken. If it is assumed that leucrose has the structure III, then methylation of its phenyloso-



III

triazole, followed by hydrolysis, should give 2,3,4,6-tetra-*O*-methyl-D-glucose (IV) and 3,4,6-tri-*O*-methyl-D-*arabino*-hexose phenylosotriazole (V).



IV V

The most direct route to V, a compound which has not been previously described in the literature, appeared to be from inulin. Haworth and Learner[6] have shown that permethylated inulin gives 3,4,6-tri-*O*-methylfructose on hydrolysis. With this method of approach it was possible to arrive at a suitable crystalline derivative through the following sequence of reactions: inulin → triacetyl inulin → trimethyl inulin → 3,4,6-tri-*O*-methylfructose → 3,4,6-tri-*O*-methyl-D-*arabino*-hexose phenylosazone (m.p. 85°) → 3,4,6-tri-*O*-methyl-D-*arabino*-hexose phenylosotriazole (gum) → 3,4,6-tri-*O*-methyl-D-*arabino*-hexose phenylosotriazole 3,5-dinitrobenzoate (m.p. 127°).

Having, thereby, made available a suitable reference compound, we undertook methylation of leucrose phenylosotriazole. Almost complete methylation was obtained by two treatments with dimethyl sulfate (30% NaOH) and four treatments with Purdie's reagent. Hydrolysis of the permethylated product with 90% formic acid gave a mixture which was separated on the basis of solubility in water. From the soluble fraction the known 2,3,4,6-tetra-*O*-methyl-D-glucose could be isolated in 42% yield. The water-insoluble portion gave a 22% yield of a 3,5-dinitrobenzoate which was shown by mixed melting point test and X-ray diffraction patterns to be identical with the corresponding compound from inulin. These methylation studies therefore show with reasonable certainty that leucrose is a 5-*O*-D-glucopyranosyl-D-fructose.

The remaining question of whether leucrose is an α- or a β-glucoside was resolved by taking advantage of the work of Freudenberg and v. Oertzen[7] on the synthesis of β-5-glucosidoglucose. Starting with a

(4) A. Thompson and M. L. Wolfrom, THIS JOURNAL, **76**, 5173 (1954).

(5) W. T. Haskins, R. M. Hann and C. S. Hudson, *ibid.*, **70**, 2288 (1948).

(6) W. N. Haworth and A. Learner, *J. Chem. Soc.*, 619 (1928).

(7) K. Freudenberg and K. v. Oertzen, *Ann.*, **574**, 37 (1951).

sample of 5-(β-tetraacetylglucosido)-1,2-diacetone-3,6-diacetylglucose kindly supplied by Professor Freudenberg, we have been able to prepare the crystalline heptaacetate of 5-*O*-β-D-glucopyranosyl-D-*arabino*-hexose phenylosotriazole (m.p. 168–169°) and show that it is different from the corresponding leucrose derivative which melts at 150–151°.

All of these data are in accord with the conclusion that the structure of leucrose is 5-O-α-D-glucopyranosyl-D-fructose, a fructopyranose form of which may be formulated as shown in Fig. 1.

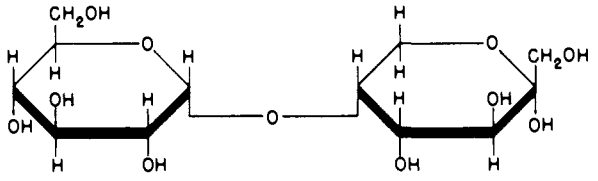


Fig. 1.—5-*O*-α-D-Glucopyranosyl-D-fructopyranose.

So far as we know, no naturally occurring 1,5-disaccharide has previously been isolated. It is worthy of mention, however, that the polysaccharide galactocarolose isolated by Raistrick[8] is made up of galactose units linked in the 1,5-positions.

Leucrose, being a 5-substituted fructose, cannot form a furanose ring and hence a consideration of its rotation might be expected to throw further light on the complex mutarotation of fructose which Isbell and Pigman[9] ascribe largely to the pyranose–furanose interconversion rather than to the normal α–β pyranose change. The negligible mutarotations of tetra-*O*-acetyl-D-fructopyranose and 1,3,4,5-tetra-*O*-methyl-D-fructose were cited by these workers in support of their hypothesis; leucrose with its slight mutarotation provides still another example in its favor. The recent studies of Bell[10] on the thermal mutarotation of some methylated fructoses also substantiates the similar work of Isbell and Pigman[9] with fructose.

Experimental[11]

Preparation of Leucrose.—To sucrose (600 g.), made up to a volume of 842 ml. with distilled water containing *p*H 5 phosphate buffer, was added 15 ml. of a partially purified enzyme solution[12] from *Leuconostoc mesenteroides* (NRRL B-512F) containing 30,000 dextransucrase units. Small amounts of toluene and thymol were added as preservatives and the reaction mixture was incubated at 27°. At the end of two days 5 ml. of enzyme solution containing 10,000 dextransucrase units was added. After a total of six days a reducing power determination showed that the reaction had gone to completion.

Absolute ethanol (6.5 l.) was added slowly with rapid stirring to the reaction mixture to bring the alcohol concentration to about 88%. The supernatant liquor was decanted, the precipitated dextran gum (A) washed with 250-ml. portions of 90% alcohol and the washings added to the supernatant solution. This solution was concentrated *in vacuo* at about 35° to one liter. Three liters of water was added and the concentration repeated. The one liter of concentrate was diluted to 1.5 l. with water, the *p*H adjusted to 5 and 75 g. of fresh baker's yeast added. After standing overnight at 27° the solution was centrifuged to remove yeast cells. Paper chromatographs showed sucrose, fructose and glucose to be absent; the largest spot observed was that of leucrose.

After deionization the solution was concentrated *in vacuo* to a thick sirup weighing 71.9 g., which was dissolved in 15 ml. of water on the steam-bath, cooled to room temperature, and seeded. After several hours the crystalline mass was worked with methanol and the crystals were filtered off, yielding 15.7 g. of crude leucrose. A second crop brought the total to 21.9 g. The sugars in the mother liquor were separated on a Whistler–Durso carbon column[13] into a disaccharide fraction (5% ethanol eluate) and a higher oligosaccharide fraction (15% ethanol eluate). From the disaccharide fraction was obtained 15.5 g. of crude crystalline leucrose and a mother liquor (B) which was saved for the isolation of isomaltulose to be described elsewhere.

To obtain the sugars thrown down with the dextran, the gum A was treated as follows after being dissolved by autoclaving with one l. of water. Precipitation at 88% alcohol concentration gave 213.3 g. of dextran powder and a supernatant liquor (plus washings) which was reduced to a sirup (54.8 g.) free of sucrose, glucose and fructose. This yielded 12.4 g. of crude crystalline leucrose and a mother liquor, which was placed on a carbon column. The disaccharide mixture from this column gave 10.6 g. of crystalline leucrose along with a mother liquor, which was added to the solution B mentioned above.

For further purification the samples of crystalline leucrose were combined (60.4 g.) and recrystallized as follows. A portion (10.8 g.) was dissolved in 325 ml. of boiling methanol and filtered through a fluted paper. Addition of an equal volume of slightly moist ethyl acetate yielded a somewhat cloudy solution which gave a heavy deposit of well-formed bars on the sides of the flask. After 26 hours at room temperature more ethyl acetate (250 ml.) was added and the solution was refrigerated overnight. The clear liquid was decanted and the crystals were rinsed twice with ethyl acetate–methanol (2:1), twice with ethyl acetate, and twice with low boiling petroleum ether.

The rest of the 60.4-g. sample was purified in the same way to give a total of 42.2 g. (calculated as anhydrous material) of pure leucrose as the first crop. The rotation of this crop was $[\alpha]^{25}D$ −7.5° at equilibrium (1 hr.) (*c* 4, H_2O). A second crop (5.3 g.) had $[\alpha]^{25}D$ −7.6° under the same conditions. This recovery of 47.5 g. of leucrose represents a yield of 7.9% based on the 600 g. of sucrose used. The rotations (*c* 4, H_2O) of the third (7.1 g.) and fourth crops (2.9 g.) were −4.5° and +5.4°, respectively, at 24 hours.

As early work had indicated that the addition of maltose could increase the yield of leucrose, a run was made as described above except that 60 g. of maltose was added to the 600 g. of sucrose. The first crop of pure, recrystallized leucrose weighed 35.8 g. and had an equilibrium rotation (1 hr.) of $[\alpha]^{25}D$ −7.6° (*c* 4, H_2O) and the second crop (5.7 g.) −7.6°. The yield for the first two crops (41.5 g.) was 6.9%, showing that maltose had no appreciable effect on the yield of leucrose.

Properties of Leucrose.—Leucrose, which is not very sweet, dissolves quite readily in water at room temperature and about one-fifth of its weight of water at 100°. It is moderately soluble in boiling methanol (about 30 g. per l.) but almost insoluble in hot absolute alcohol or hot ethyl acetate. It crystallizes in bars from slightly moist methanol–ethyl acetate as the monohydrate, which melts at 156–158°. Under anhydrous conditions it was also obtained several times as the crystalline anhydrous form, which melted at 161–163°. The anhydrous product rapidly takes up a mole of water on standing in air. The monohydrate readily lost its water in 1 hr. at 78° (1 mm.) and then analyzed as follows.

Anal. Calcd. for $C_{12}H_{22}O_{11}$: C, 42.12; H, 6.45. Found: C, 41.9; H, 6.34.

Leucrose shows some mutarotation, the $[\alpha]^{25}D$ at 7 minutes being −8.2° (*c* 4, H_2O) and −7.6° at equilibrium, which is reached in less than 1 hour.

The reducing power of leucrose was 46% that of fructose as shown by the method of Somogyi.[14] That the reducing

(8) W. N. Haworth, H. Raistrick and M. Stacey, *Biochem. J.*, **31**, 640 (1937).

(9) H. S. Isbell and W. W. Pigman, *J. Research Natl. Bur. Standards*, **20**, 773 (1938).

(10) D. J. Bell, *J. Chem. Soc.*, 1231 (1953).

(11) All melting points are capillary and corrected.

(12) H. M. Tsuchiya, N. N. Hellman, H. J. Koepsell, J. Corman, C. S. Stringer, S. P. Rogovin, M. O. Bogard, G. Bryant, V. H. Feger, C. A. Hoffman, F. R. Senti and R. W. Jackson, THIS JOURNAL, **77**, 2412 (1955).

(13) R. L. Whistler and D. F. Durso, *ibid.*, **72**, 677 (1950).

(14) M. Somogyi, *J. Biol. Chem.*, **160**, 61 (1945).

part of the molecule contains the fructose moiety was determined by hypoiodite oxidation. Using the Willstätter–Schudel method,[15] leucrose was oxidized to the extent of 9%; under the same conditions turanose (3-*O*-α-D-glucopyranosyl-D-fructose) gave a value of 11%.

The suitability of leucrose as a substrate for yeast growth was determined as follows by Dr. L. J. Wickerham of this Laboratory.[16] Solutions (0.5%, 4.5 ml.) of leucrose were pipetted into 16 mm. test-tubes which were plugged and sterilized at 15 pounds pressure for 15 minutes. Nitrogen was provided by the addition of 0.5 ml. of 10 × nitrogen base medium per tube. The leucrose medium was very lightly inoculated with a yeast inoculum grown in a 0.1% glucose inoculum medium. The tubes were examined after 15 days with the following results. No growth was observed in 15 days with *Saccharomyces globosus* (Y-409), *Torulaspora rosei* (Y-1567), *Candida monosa* (Y-1079), *Candida krusei* (Y-301), *Hansenula saturnus* (Y-1304), *Mycoderma cerevisiae* (Y-946) and *Zygosaccharomyces ashbyi* (Y-1598). Good growth resulted in 15 days with *Candida tropicalis* (Y-619), *Candida krusoides* (Y-305), *Candida guilliermondii* (Y-488) and *Torulopsis laurentii* (Y-199). A strain of *Saccharomyces cerevisiae* (Y-567) was intermediate in its action.

Leucrose can be distinguished from other sugars by means of paper chromatography. With butanol–pyridine–water, leucrose moves to a position between maltose and isomaltose, maltose being the fastest-moving of the three. Leucrose gives a red-brown color with the urea-phosphoric acid spray[3] in contrast to the blue gray color shown by the more readily hydrolyzed fructose-containing disaccharides such as sucrose, turanose and maltulose.

Leucrose was unaffected by emulsin, yeast invertase or honey invertase.

Hydrolysis of Leucrose.—As leucrose hydrolyzes rather slowly in acid solution, some difficulty was experienced in finding conditions which would give extensive hydrolysis without excessive destruction of the fructose formed. After hydrolysis at 95° (1 hr.) in 0.5 *N* HCl, paper chromatograms showed considerable unchanged leucrose in addition to glucose and fructose. A study of conditions indicated that hydrolysis at 95° for 3 hr. in 0.25 *N* HCl was the most satisfactory. A sample of leucrose was hydrolyzed in this manner, the solution freed of ions with a monobed resin, and the hydrolysis products were separated on heavy filter paper using *n*-butyl alcohol–pyridine–water (6:4:3) as the developing solvent. The fructose fraction obtained in this way (82 mg. from 367 mg. of hydrolysate) was converted in part to crude crystalline fructose methylphenylosazone (44 mg. from 70 mg. of the fructose fraction).[17] Crystallization from ethyl acetate gave 30 mg. of bright orange crystals (m.p. 151–153°), the identity of which was established by a comparison of X-ray diffraction patterns and by mixed melting point tests with a known sample prepared from fructose.

Leucrose Phenylosazone.—Leucrose (500 mg.), phenylhydrazine hydrochloride (1 g.), and sodium acetate (1.5 g.) were dissolved in 7.5 ml. of water and heated 1 hr. at 100°. An oil which separated changed to a solid on standing in the refrigerator overnight. Crystallization from water-saturated ethyl acetate (100 mg. in 5 ml.) gave 84 mg. of orange-yellow needles melting at 186–188°.

Anal. Calcd. for $C_{24}H_{32}N_4O_9$: C, 55.38; H, 6.20; N, 10.77. Found: C, 55.2; H, 6.04; N, 11.1.

Leucrose Phenylosotriazole.—The phenylosazone (1.51 g.) was refluxed for 30 min. with 1.1 equivalents (803 mg.) of copper sulfate pentahydrate in 15 ml. of water. The solution was filtered, deionized, and lyophilized to 645 mg. of white powder which gave needles on slow evaporation of a methanol solution. The compound could not be recrystallized readily; it could, however, be obtained as long needles by slow evaporation of a seeded solution in methanol–ethyl acetate; m.p. 108–109°.

Anal. Calcd. for $C_{18}H_{25}O_9N_3$: C, 50.6; H, 5.90; N, 9.84. Found: C, 50.2; H, 5.92; N, 9.92.

Leucrose Phenylosotriazole Heptaacetate.—Leucrose phenylosotriazole (100 mg.) was heated for 2 hr. on the steam-bath with 0.8 ml. of acetic anhydride and 25 mg. of sodium acetate. Addition of water gave 147 mg. of crude crystalline acetate. Recrystallization from alcohol gave blades (124 mg.) melting at 150–151°.

Anal. Calcd. for $C_{32}H_{39}N_3O_{16}$: C, 53.26; H, 5.45; N, 5.82. Found: C, 53.1; H, 5.44; N, 6.04.

The X-ray pattern of this compound was different from that of gentiobiose phenylosotriazole heptaacetate (m.p. 148–149°) kindly furnished by W. T. Haskins. The mixture melted at 129–138°.

Hydrolysis of Leucrose Phenylosotriazole.—Leucrose phenylosotriazole (432 mg.) in 15 ml. of 0.5 *N* HCl was heated at 100° for 1.5 hr. The crude glucose phenylosotriazole (174 mg., 64.8%) was filtered off and the filtrate saved for the isolation of glucose.

Purification of the crude osotriazole gave 138 mg. of pure product (m.p. 197–198°, $[\alpha]^{25}D$ −80.6°; 51.4%). These constants are in agreement with published values.[18] A mixture with an authentic sample (m.p. 198–199°) melted at 198–199°.

For isolation of glucose, the solution was decolorized and concentrated to a gum which yielded 82 mg. (45%) of crude glucose on crystallization from glacial acetic acid. Recrystallization gave pure D-glucose ($[\alpha]^{25}D$ +52°), the X-ray pattern of which was identical with that of a known sample.

Periodate Cleavage of Osazones.—Leucrose phenylosazone (52 mg., 0.0001 mole) was dissolved in 10 ml. of hot 66% alcohol and the solution was cooled to room temperature. A solution of 85.6 mg. (0.004 mole) of sodium metaperiodate in 0.5 ml. of water was added. Orange needles, which appeared in about 5 min., were filtered off after 30 min. The product (18.7 mg.) on crystallization from alcohol gave 17.1 mg. (66%) of the pure osazone of mesoxalaldehyde,[19] m.p. 199–200° dec. Identity was established by mixed melting point test and X-ray diffraction patterns.

Under similar conditions the phenylosazones of isomaltose and gentiobiose rapidly gave the aldehyde in yields of 54 and 66%, respectively. On the other hand only white inorganic salts were obtained after several hours with the phenylosazones of turanose, laminaribiose, maltose and cellobiose.

Periodate Cleavage of Phenylosotriazoles.—Leucrose phenylosotriazole (42.7 mg., 0.0001 mole) was dissolved in 0.2 ml. of water. To this solution was added 85.6 mg. (0.0004 mole) of sodium metaperiodate in 9.5 ml. of water. There was immediate precipitation of an oil which crystallized in less than one minute. After 5 min. the crystals of 2-phenyl-4-formyl osotriazole were filtered off; 11.5 mg. (67%), m.p. 67–68.5°. There was no depression in melting point on admixture with an authentic sample melting at 68–69°. Under similar conditions, 43 mg. of gentiobiose phenylosotriazole ethyl alcoholate, kindly supplied by W. T. Haskins, gave 11 mg. of the aldehyde. With cellobiose phenylosotriazole there was no cloudiness of the reaction mixture in 25 minutes.

3,4,6-Tri-*O*-methyl-D-*arabino*-hexose Phenylosotriazole-3,5-dinitrobenzoate.—Trimethyl inulin was prepared in 92% yield from triacetyl inulin by the method of Haworth and Learner[6] and hydrolyzed in oxalic acid solution to 3,4,6-tri-*O*-methylfructose, which was purified on a cellulose column (developing solvent: water-saturated methyl ethyl ketone containing ammonia). The phenylosazone of m.p. 83–85° (lit.[20] 81–82°), obtained in 54% yield, was converted to the phenylosotriazole, which was a gum. Benzoylation in pyridine with 3,5-dinitrobenzoyl chloride gave a 74% yield of 3,4,6-tri-*O*-methyl-D-*arabino*-hexose phenylosotriazole-3,5-dinitrobenzoate in the form of pale yellow bars; m.p. 127–128°.

Anal. Calcd. for $C_{22}H_{23}O_9N_5$: C, 52.52; H, 4.61; N, 14.31. Found: C, 52.5; H, 4.56; N, 14.3.

Permethylated Leucrose Phenylosotriazole and its Hydrolysis.—Leucrose phenylosotriazole (2.0 g.) was methylated at 55° with 60 ml. of dimethyl sulfate and 160 ml. of 30% sodium hydroxide. Found OCH_3, 27.7%; calcd. 41.3%. Repetition of the process raised the methoxyl content to 29.8%. Four methylations with silver oxide–methyl iodide gave 2.15 g. of a gum having 40.2% methoxyl (97.3% of theory).

(15) R. Willstätter and G. Schudel, *Ber.*, **51**, 780 (1918).

(16) L. J. Wickerham, U. S. Department of Agriculture Technical Bulletin No. 1029 (1951).

(17) C. Neuberg and I. Mandl, *Arch. Biochem.*, **11**, 451 (1946).

(18) R. M. Hann and C. S. Hudson, THIS JOURNAL, **66**, 735 (1944).

(19) E. Chargaff and B. Magasanik, *ibid.*, **69**, 1459 (1947).

(20) T. N. Montgomery, *ibid.*, **56**, 419 (1934).

Some difficulty was experienced in finding hydrolysis conditions vigorous enough to break the glucosidic linkage without too extensive demethylation of the hexose phenylosotriazole moiety. The first satisfactory results were obtained as follows. Permethylated leucrose phenylosotriazole (422 mg.) was heated for seven hours at 95° with 5 ml. of 90% formic acid. The hydrolysate was concentrated *in vacuo* to a gum which was dissolved in 0.2 ml. of methanol. Dropwise addition of 1 ml. of water threw out an oil. The aqueous methanol solution was pipetted off and the oil was washed five times with water. The combined solutions were saved for the isolation of tetramethylglucose.

A portion of the oil (155 mg. of a total 205 mg.) was converted to the 3,5-dinitrobenzoate (28 mg., 9.4% based on permethylated leucrose phenylosotriazole). Crystallization from methanol yielded 19.4 mg. of product melting at 125.5–126.5°. The melting point upon admixture with the 3,5-dinitrobenzoate from inulin (m.p. 127–128°) was 125.5–126.5°. The X-ray diffraction patterns of the derivatives were the same.

The tetramethylglucose was identified as follows. The aqueous solution was evaporated to gummy crystals which were purified by sublimation and crystallization from petroleum ether; yield 77 mg. (42%); m.p. 88–92°. The X-ray diffraction pattern was the same as that of an authentic sample of 2,3,4,6-tetra-*O*-methyl-D-glucose (m.p. 89–93°).

Hydrolysis of permethylated leucrose phenylosotriazole under milder conditions (1 hr. at 95° in 90% formic acid) led to a 22% yield of the 3,5-dinitrobenzoate.

5-*O*-β-D-Glucopyranosyl-D-*arabino*-hexose Phenylosotriazole Heptaacetate.—5-β-(Tetraacetylglucopyranosyl)-1,2-acetone-3,6-diacetyl-D-glucose (317 mg.), prepared by Freudenberg and v. Oertzen,[7] was saponified with sodium methoxide. Deionization and lyophilization gave 165 mg. of 5-β-D-glucopyranosyl-1,2-diacetone-D-glucose in the form of a white powder (86%). The acetone group was removed by heating with 1.9 ml. of 0.1 *N* HCl for 2 hr. at 70°. Deionization and lyophilization yielded 121 mg. (82%) of 5-glucosylglucose. Heating at 100° for 1 hr. with phenylhydrazine and sodium acetate gave 107 mg. (58%) of an amorphous osazone (m.p. 135–140°). This was converted to the phenylosotriazole (70.3 mg., 80%) which on acetylation with acetic anhydride and pyridine gave 107 mg. (90%) of the crude heptaacetate. Crystallization from alcohol gave bars melting at 168–169°.

Anal. Calcd. for $C_{32}H_{39}N_3O_6$: C, 53.26; H, 5.45; N, 5.82. Found: C, 53.2; H, 5.52; N, 6.06.

The infrared spectrum and the X-ray diffraction patterns of this β-form of the heptaacetate were distinctly different from those of leucrose phenylosotriazole heptaacetate (m.p. 150–151°).

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PEORIA, ILLINOIS

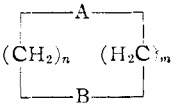
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF CALIFORNIA AT LOS ANGELES]

Macro Rings. XIII. Synthesis and Properties of 1,7-Cyclododecadiyne and Related Compounds[1]

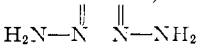
BY DONALD J. CRAM AND NORMAN L. ALLINGER

RECEIVED OCTOBER 14, 1955

Compounds I have been prepared with $n = m = 4$ and A = B = —C≡C—, A = —C≡C— and B = —CHOHCO—, A = —C≡C— and B = —COCO—, and A = B = —CH=CH— (*cis*); and with $n = m = 3$, and A = B = —CH=CH— (*cis*), A = —CH=CH— (*cis*), B = —C≡C—, A —CH=CH— (*cis*) and B = —CHOHCO—, A = —CH=CH— (*cis*), and B = —COCO—, and A = —CH=CH— (*cis*) and B = —CHOHCHOH— (*meso*). An anomalous transannular reaction was encountered when compound I ($n = m = 4$, A = —C≡C— and B = C—C—) was treated with mercuric oxide, a bicyclic diene being the product.

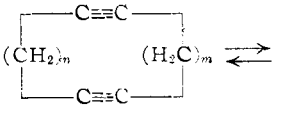


I

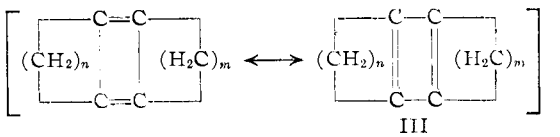


Cyclobutadiene has to date evaded numerous attempts at its synthesis, although the dibenzo substance (biphenylene) and related compounds have been prepared repeatedly.[2] Recent calculations indicate that cyclobutadiene will not be stabilized by any appreciable resonance energy.[3] Synthetic experiments directed at preparation of the compound by conventional methods have produced other products, usually those derived by ring rupture and including acetylene.[4] This last observation plus the fact that the conversion of two molecules of acetylene into one of cyclobutadiene would involve very little molecular reorganization suggests that *in principle* such a dimerization is possible, although such a reaction is of course unknown.

In the present work a molecule has been so designed (II) as to encourage any tendency for acetylene dimerization that might exist.[5] Since the acetylenic linkages in II with $n = m = 3$ or 4 are properly situated to provide valency tautomer-



II



III

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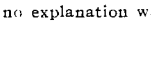
(2) (a) M. P. Cava and J. F. Stucker, *Chemistry & Industry*, 446 (1955); (b) W. Baker, M. P. V. Boarland and J. F. W. McOmie, *J. Chem. Soc.*, 1476 (1954); (c) W. Baker, *ibid.*, 258 (1945); (d) W. C. Lothrop, THIS JOURNAL, **63**, 1187 (1941).

(3) D. P. Craig, *J. Chem. Soc.*, 3175 (1951).

(4) (a) R. Willstätter and W. von Schmaedel, *Ber.*, **38**, 1992 (1905); (b) E. R. Buchman, A.C.S. Meeting Abstracts, New York, N. Y., Sept., 1954, p. 9-O.

(5) The structurally similar compound  has been reported [Lespieau, *Compt. rend.*, **188**, 502 (1929)]; it was obtained in small yield, was poorly characterized, and no explanation was offered for its unexpected properties.