ration. After final extraction and concentration the compound was precipitated with acetone, filtered and dried in a vacuum oven at 60°. The yield was 0.4 g. and the specific rotation of the compound was found to be +87° (*c* 1, water).

Reducing Power.—Samples of 1.6 mg. of the compound, of maltose and of turanose were used for the determination of reducing values with Reagent 60 of Shaffer and Somogyi.[8] The values expressed in ml. of 0.005 *N* sodium thiosulfate were as follows: 4.46 for the new compound, 5.45 for maltose and 4.35 for turanose. The oligosaccharide and turanose possess approximately 80% of the reducing value of maltose.

Paper Chromatography.—Droplets of 0.01 ml. of the solutions of the new compound, glucose, maltose, turanose, lactose, isomaltose, cellobiose and the original reaction mixtures were placed near the bottom of a square (20 cm.) of filter paper (Eaton and Dikeman No. 613). The filter paper was rolled in a cylinder and placed in a solvent of *n*-butyl alcohol–pyridine–water (6:4:3 by volume). Four ascents of the solvent were used for developing the chromatogram. The finished strips were sprayed with copper sulfate reagent[11] and heated in an oven at 100° for 10 minutes in which time the areas at which carbohydrates were present turned yellow. The apparent R_f values obtained by dividing the distance to which the compounds had moved by the total height of the paper are recorded in Table I.

Fermentation Tests.—Samples of 0.2 ml. of 2% solutions of the new oligosaccharide, glucose, maltose and isomaltose were mixed with 0.2 ml. of 20% suspension of bakers' yeast. Aliquots of the digest were analyzed for reducing sugars at 0, 6, 24 and 48 hour reaction periods by paper chromatography. The analyses showed that under these conditions the glucose and maltose had completely disappeared from the reaction mixture in 24 and 48 hours, respectively, while the new oligosaccharide and isomaltose remained unchanged.

Acid Hydrolysis of the Oligosaccharide.—One ml. of a solution containing 4 mg. of the oligosaccharide was mixed with 1 ml. of 0.1 *N* hydrochloric acid. The test-tube containing the mixture was stoppered tightly and heated in an oven at 100° for 2 hours. Samples removed during heating at 0, 0.5, 1 and 2 hour intervals were placed on paper chromatograms and analyzed for reducing sugars. Examination of the sprayed chromatograms showed that only glucose and the unhydrolyzed compound were present during the course of the hydrolysis. Further, in the 2-hour period the compound appeared to be completely hydrolyzed to glucose. Samples of 0.4 ml. of the 2-hour hydrolysate (equivalent to 0.8 mg. of the compound) and 0.8 mg. of glucose were used for determination of reducing values. The reducing values expressed in ml. of 0.005 *N* sodium thiosulfate were 5.46 for the acid hydrolysate of the compound and 5.42 for 0.8 mg. of glucose. The acid treatment had, therefore, converted the new compound quantitatively to glucose.

Preparation of Phenylosazone Derivative.—A solution of 0.1 g. of the oligosaccharide, 0.2 g. of phenylhydrazine hydrochloride and 0.3 g. of sodium acetate in 2 ml. of water was heated in a boiling water-bath for 30 minutes. The compound that precipitated from solution was collected on a filter, air-dried, and recrystallized from 3 ml. of ethyl alcohol; m.p. 203–205° dec., mixed m.p. with turanose phenylosazone 203–206° dec., literature value 204–206°.[7] The X-ray diffraction pattern[12] for this compound gave the following data: 5.42[13] — 50[14]; 5.13 — 40; 4.68 — 20 (double); 4.27 — 80; 4.05 — 80; 3.51 — 10; 3.32 — 100; 3.17 — 5; 2.69 — 50; 2.21 — 20; 2.10 — 10.

Preparation of Turanose Phenylosazone.—A solution of 0.2 g. of turanose, 0.4 g. of phenylhydrazine hydrochloride and 0.6 g. of sodium acetate in 5 ml. of water was heated in a boiling water-bath for 30 minutes. The phenylosazone which formed on cooling was collected on a filter and air-dried. It was recrystallized from 10 ml. of ethyl alcohol; m.p. 205–206° dec., literature values 200–205°[15] dec., and 204–206°.[7] The X-ray diffraction data for the turanose phenylosazone was: 5.42[13] — 40[14]; 5.13 — 40; 4.68 — 20 (double); 4.25 — 70; 4.05 — 70; 3.50 — 10; 3.32 — 100; 3.16 — 10; 2.69 — 50; 2.21 — 10; 2.10 — 5. Comparison of these values with those in the preceding section shows that the phenylosazone of turanose and of the oligosaccharide are identical and, therefore, the glucose units of the oligosaccharide must be joined by the same type of linkage as is present in turanose, namely, the α-1,3-linkage.

(12) We wish to thank Mr. W. C. Robison, Electrical Engineering Department and the University Instrument Laboratory, University of Nebraska, for the X-ray patterns.

(13) Interplanar spacings, Å., CuKα radiation.

(14) Relative intensities on basis of 100 for the strongest line.

(15) C. S. Hudson, *J. Org. Chem.*, **9**, 470 (1944).

LINCOLN, NEBRASKA

[CONTRIBUTION FROM THE NATIONAL BUREAU OF STANDARDS]

Synthesis of Maltose-1-C^{14}, Maltobiono-δ-lactone-1-C^{14}, and Lithium Maltobionate-1-C^{14} from 3-(α-D-Glucopyranosyl)-D-arabinose[1,2]

BY HORACE S. ISBELL AND ROBERT SCHAFFER

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A method is presented for the production of maltose-1-C^{14} from sodium cyanide-C^{14} and 3-(α-D-glucopyranosyl)-D-arabinose in 42% yield. The process includes the intermediate production of lithium maltobionate-1-C^{14} trihydrate and maltobiono-δ-lactone-1-C^{14}. Lithium maltobionate trihydrate is the first metallic salt of maltobionic acid to be crystallized. It was prepared not only by the cyanohydrin synthesis but also by the electrolytic oxidation of maltose in the presence of lithium bromide and lithium bicarbonate. Crystalline maltobiono-δ-lactone is likewise a new substance. Concentration of Methyl Cellosolve (ethylene glycol monomethyl ether) solutions of maltobionic acid in the presence of seed crystals leads to a slow conversion of the acid to the crystalline lactone.

Numerous applications of C^{14}-labeled maltose can be envisioned in the fields of enzymology, biochemistry and nutrition. Hence the development of a method for the synthesis of maltose-1-C^{14}, similar to the methods previously described for the production of other sugars[3–6] was undertaken. The C^{14}-labeled products were prepared by the following steps:

(1) Part of a project on the development of methods for synthesis of radioactive carbohydrates, sponsored by the Atomic Energy Commission.

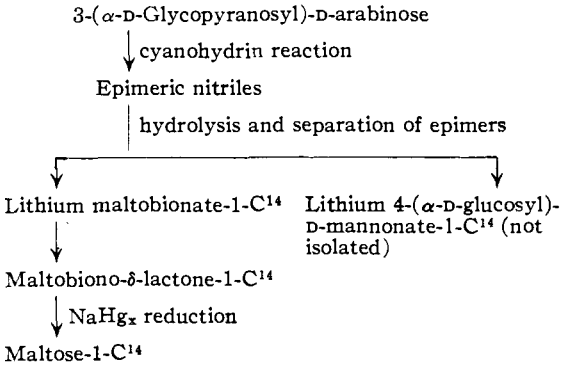
(2) Presented before the Division of Carbohydrate Chemistry at the 126th Meeting of the American Chemical Society, New York, N. Y., September, 1954.

(3) H. S. Isbell, J. V. Karabinos, H. L. Frush, N. B. Holt, A. Schwebel and T. T. Galkowski, *J. Research Natl. Bur. Standards*, **48**, 163 (1952).

(4) H. L. Frush and H. S. Isbell, *ibid.*, **50**, 133 (1953).

(5) H. S. Isbell and J. V. Karabinos, *ibid.*, **48**, 438 (1952); H. L. Frush and H. S. Isbell, *ibid.*, **51**, 167, 307 (1953); **54**, 267 (1955).

(6) H. S. Isbell, H. L. Frush and N. B. Holt, *ibid.*, **53**, 217, 325 (1954); H. S. Isbell, H. L. Frush and R. Schaffer, *ibid.*, **54**, 201 (1955).



Both lithium maltobionate trihydrate and maltobiono-δ-lactone are new crystalline compounds. Apart from the brucine salt,[7] these substances are the only known crystalline derivatives suitable for the purification of maltobionic acid. A supply of the lithium salt was prepared by oxidation of maltose in the presence of lithium bromide and lithium bicarbonate, by use of the electrolytic process of Isbell and Frush.[8] The sugar, 3-(α-D-glucopyranosyl)-D-arabinose, was first reported by Zemplen[9] and later by Gakhokidze.[10] The crystalline sugar employed here was prepared by Isbell and Moyer.[11] It has been found, in general accord with the results previously obtained in similar studies with D-arabinose[3] and with 3-(β-D-galactosyl)-D-arabinose,[4] that the maximum yield of the gluconic epimer (maltobionic nitrile) is obtained with reaction mixtures containing sodium carbonate.

For the preparation of C^{14}-labeled maltose the cyanohydrin reaction was conducted in the presence of sodium carbonate, and the maltobionate epimer was separated as crystalline lithium maltobionate-1-C^{14} trihydrate in a 21% chemical yield. By the use of non-radioactive lithium maltobionate as carrier, additional amounts of the radioactive salt were isolated, giving a radiochemical yield of 54%.

The lithium salt was treated with cation-exchange resin to liberate maltobionic-1-C^{14} acid. The acid was converted to maltobiono-δ-lactone-1-C^{14} by lyophilization of the solution and digestion of the dry residue with Methyl Cellosolve (ethylene glycol monomethyl ether) in the presence of seed crystals. When the lactonization appeared to be complete, the material was reduced with sodium amalgam and a sodium acid oxalate buffer. Several exploratory reductions of non-radioactive maltobiono-δ-lactone gave yields up to 94% by analysis. Reduction of the crude lactone obtained from lithium maltobionate-1-C^{14} gave a 77% radiochemical yield of maltose-1-C^{14}. When the resin used in the purification of the maltose-1-C^{14} was eluted with aqueous acetic acid, 17% of maltobionic-1-C^{14} acid was recovered. The high proportion of unreduced acid indicates that the lactonization step in the radiochemical preparation was not entirely complete. Nevertheless the over-all radiochemical yield of maltose-1-C^{14} based on the sodium cyanide-1-C^{14} used in the cyanohydrin reaction was 42%.

(7) J. W. E. Glattfeld and M. T. Hanke, THIS JOURNAL, **40**, 973 (1918).

(8) H. S. Isbell and H. L. Frush, *J. Research Natl. Bur. Standards*, **6**, 1145 (1931).

(9) G. Zemplen, *Ber.*, **59**, 2408 (1926).

(10) A. M. Gakhokidze, *J. Gen. Chem. (USSR)*, **18**, 60 (1948); *C. A.*, **42**, 4949 (1948).

(11) H. S. Isbell and J. D. Moyer, Abstracts of Am. Chem. Soc., New York, N. Y., Sept. 12, 1954, p. 24D.

Experimental

Lithium Maltobionate Trihydrate from β-Maltose.—Lithium maltobionate for use as carrier was prepared from maltose by electrolytic oxidation.[8] A carbon dioxide-saturated solution containing 9.26 g. of lithium carbonate, 8.0 g. of lithium bromide monohydrate and 90 g. of β-maltose hydrate in one liter of water was placed in a 2-liter, 3-necked flask equipped with graphite electrodes 22 mm. in diameter and a mechanical stirrer. A direct current of about 0.5 ampere was passed through the solution with continuous stirring. After 32 hr. the reaction was stopped, the electrolyzed solution was passed through a filter coated with decolorizing carbon and the filtrate was concentrated under reduced pressure to 125 ml. The concentrate was gradually mixed with 2-propanol and seed crystals of lithium maltobionate trihydrate. The product, which crystallized rapidly, was separated by filtration, washed with aqueous 2-propanol and dried. Additional crystalline lithium maltobionate was obtained by concentrating the mother liquor, seeding and adding 2-propanol. The yield, 89 g., corresponded to 85% of the maltose used. Lithium maltobionate trihydrate was recrystallized in high yield by dissolving the salt in an equal weight of hot water, seeding and, as the crystallization proceeded, gradually adding 2 volumes of 2-propanol.

The new compound, which crystallizes from aqueous 2-propanol as prisms, corresponds by analysis to a trihydrate.

Anal. Calcd. for $C_{12}H_{27}O_{15}Li$: C, 34.5; H, 6.5; Li, 1.7. Found: C, 34.4; H, 6.4; Li, 1.7.

A sample of the hydrate lost no weight when placed in a desiccator over calcium chloride at a pressure of 20 mm. for 20 hours, but a weight loss equivalent to three molecules of water was found after 2 hours at 105° under vacuum. The hydrate melts at 106.5–108° and resolidifies with the formation of needle-like crystals. The salt turns slightly yellow at 190° with progressive decomposition as the temperature is increased.

The $[\alpha]^{20}D$ of the trihydrate is +97.3° (*c* 8.7, H_2O). Addition of an equivalent of sulfuric acid to the salt resulted in a specific rotation of +104.1 ± 1° for maltobionic acid which changed in 12 hr. to +106 ± 1°.

Preparation of Maltobiono-δ-lactone from Lithium Maltobionate Trihydrate.—An ice-cold aqueous solution of 5 g. of lithium maltobionate trihydrate was passed through a column containing 24 ml. of ice-cold Amberlite IR-120 resin.[12] The column was washed with 250 ml. of ice-cold water. The effluent was freeze-dried, and the residue was dissolved in 20 ml. of Methyl Cellosolve. The solution was heated to 90° for 5 minutes, seeded with crystalline lactone and concentrated at 50°, under a gentle current of air. The material was stored at 50° for two weeks with the occasional addition of a few milliliters of Methyl Cellosolve followed by reconcentration under the stream of air. The crude, crystalline product was finally triturated with Methyl Cellosolve, filtered and washed with Methyl Cellosolve. The dried product weighed 4.0 g. By concentrating the filtrate and seeding, additional lactone was obtained. The product was recrystallized from 25 parts of boiling Methyl Cellosolve by filtering through decolorizing carbon, concentrating to a thin sirup and seeding. The thin pointed crystalline leaflets thus obtained melt at 192–195°.

Anal. Calcd. for $C_{12}H_{20}O_{11}$: C, 42.4; H, 5.9. Found: C, 42.2; H, 6.1.

The $[\alpha]^{20}D$ +123 ± 1° initially, changed in 12 hours to +111° ± 1° (*c* 2, H_2O).

Reduction of Maltobiono-δ-lactone to Maltose.[13]—The reductions were conducted at 0° in a glass tube 200 mm. × 25 mm., having a side arm 50 mm. × 15 mm. for the introduction of sodium amalgam and fitted with an efficient stirrer. The lactone was placed in the tube, and the sodium acid oxalate, water and sodium amalgam were added in rapid order and with vigorous stirring. After 2 hours the

(12) Resinous Products Division of Rohm and Haas Co., Philadelphia, Pa.

(13) H. S. Isbell, U. S. Patent 2,606,918 (1952).

stirring was stopped and the aqueous solution was separated from the mercury, neutralized with dilute alkali and diluted to 100 ml. Aliquots of the solution were analyzed for maltose by the Scales method.[14] In each of four reductions of non-radioactive maltobiono-δ-lactone with various proportions of lactone, sodium amalgam and sodium acid oxalate as buffer the yield of maltose by analysis ranged from 91 to 94%. Best results were obtained with 0.5 millimole of the lactone, 2.6 g. of sodium acid oxalate, 20 ml. of water and 3 g. of 5% sodium amalgam in pellet form.

Yields of Lithium Maltobionate from Cyanohydrin Reactions Determined by Isotopic Dilution Analysis.—Three reaction mixtures were prepared by freezing separately in each flask 0.058 g. of 3-(α-D-glucosyl)-D-arabinose dissolved in 1 ml. of water and 1 ml. of an aqueous solution containing 0.16 millimole of sodium cyanide-C^{14} (112 μc.) and 0.45 millimole of sodium hydroxide. To reaction mixture I, 0.03 g. of solid carbon dioxide was added and the vessel was securely stoppered. A 1-ml. solution of 0.45 millimole of sodium bicarbonate was added to reaction mixture II. No addition was made to reaction mixture III. The three mixtures were kept at 8° for 3 days followed by one day at room temperature. Eighty milligrams of sodium carbonate was then added to I, and 40 mg. of sodium carbonate to II and to III. Then 160-mg. portions of lithium maltobionate trihydrate were added to each flask and the solutions were heated to 80° for 5 hours, at which time the evolution of ammonia had ceased. After being cooled at 0°, each solution was passed into a column containing 10 ml. of ice-cold Amberlite IR-120H and then washed through the resin with ice-water. To remove dissolved carbon dioxide each eluent was swirled in a flask for a few minutes while under reduced pressure. The solutions were neutralized with lithium hydroxide and concentrated to thin sirups under reduced pressure. Lithium maltobionate-1-C^{14} trihydrate crystallized from the solutions after they were seeded and treated with 2-propanol. The products were recrystallized from aqueous 2-propanol to constant specific radioactivity. The radioactivity was determined by direct count in formamide solution.[15] The products from mixtures I, II and III had activities of 0.0535, 0.269 and 0.114 microcurie per milligram, respectively. These values correspond to the production of lithium maltobionate-1-C^{14} in yields of 7.9, 45.7 and 17.4%.

Lithium Maltobionate-1-C^{14} Trihydrate.—An ice-cold solution of 2.81 g. of 3-(α-D-glucosyl)-D-arabinose (9.0 millimoles) in 65 ml. of water was mixed with an ice-cold solution containing 8.85 millimoles of sodium cyanide-C^{14} (11.2 mc.) and 9.6 millimoles of sodium carbonate in 25 ml. of water. The reaction mixture was kept at 8° for 3 days and then at room temperature for 1 day. After the addition of 0.53 g. of sodium carbonate, the colorless solution was heated at 80° until the evolution of ammonia ceased. The dark solution that resulted was passed into a column containing 220 ml. of ice-cold Amberlite IR-100H and then washed from the resin with about one liter of ice-water. The effluent in a flask was swirled under reduced pressure for a few minutes to remove carbon dioxide and then neutralized with aqueous lithium hydroxide. The neutral solution was concentrated under vacuum to about 25 ml., treated with 75 ml. of methanol and passed through a filter to remove the amorphous precipitate that formed. The filtrate was concentrated at reduced pressure to about 5 ml., treated with a few drops of 2-propanol and seeded with crystals of lithium maltobionate trihydrate. As the crystallization proceeded, additional 2-propanol was put in. A total of 0.976 g. of crystalline material was obtained upon removing the mother liquor, washing the product with aqueous 2-propanol and drying. Recrystallization from aqueous 2-propanol yielded 0.779 g. of lithium maltobionate-1-C^{14} trihydrate having an activity of 2353 μc. By the use of a total of 3.0 g. of non-radioactive lithium maltobionate trihydrate as carrier, an additional 3670 μc. of radioactive salt was isolated. The radiochemical yield (6023 μc.) of lithium maltobionate-1-C^{14} trihydrate was 54% of the radioactivity of the sodium cyanide-C^{14}.

β-Maltose-1-C^{14} Hydrate.—An ice-cold solution of 212 mg. of lithium maltobionate-1-C^{14} trihydrate (641 μc.) in 10 ml. of water was passed through a column containing 7 ml. of ice-cold Amberlite IR-120H. The resin was washed with ice-water until about 100 ml. of effluent was collected. The effluent was lyophilized and the residue was dissolved in Methyl Cellosolve, transferred into a reduction tube and seeded with crystalline lactone. After being heated at 90° for a few minutes, the tube was kept at 50° under a gentle air stream. Whenever the crystallizing solution became solid, about 0.5 ml. of Methyl Cellosolve was added, and the mixture was reheated to 90° and then reconcentrated at the lower temperature. After this process had been continued for 10 days, the partly crystallized material was dried over calcium chloride under vacuum.

For the reduction, the tube containing the lactone was partly immersed in an ice–water slurry, stirring was begun and through the side-arm of the reduction-tube, 2.6 g. of crystalline sodium acid oxalate, 20 ml. of ice-cold water and 4.0 g. of 3.7% sodium amalgam pellets were added in rapid order. Vigorous stirring was continued for 2.5 hr. The aqueous solution was separated from the mercury and undissolved salts and then neutralized with dilute sodium hydroxide. Addition of 3 volumes of methanol precipitated a large crop of inorganic salt, which was removed by filtration. On concentration of the filtrate to about 10 ml. and addition of 100 ml. of methanol, a second crop of salt precipitated and was separated. The filtrate was concentrated to a sirup, dissolved in 25 ml. of ice-cold water and passed in series through a column containing 25 ml. of ice-cold Amberlite IR-120H and a column containing 25 ml. of Duolite A-4 resin.[16] The sugar solution was washed through the resins with 250 ml. of ice-cold water and lyophilized. The residue was dissolved in methanol and the solution was filtered and reconcentrated. The sirup was dissolved in water filtered through decolorizing carbon and finally concentrated to a volume of about 1 ml. Seed crystals of maltose hydrate and a few drops of 2-propanol were added. The β-maltose-1-C^{14} hydrate that crystallized was separated from the mother liquor and washed with aqueous 2-propanol. Recrystallization from aqueous 2-propanol yielded 98 mg. of β-maltose-1-C^{14} hydrate with a total radioactivity of 344 μc. By use of the carrier maltose an additional 147 μc. of the radioactive sugar was obtained to give a radiochemical yield of 77%.

Passage of 300 ml. of 10% aqueous acetic acid through the column containing Duolite A-4 liberated 107 μc. of D-maltobionic-1-C^{14} acid, which was recovered as the lithium salt after lyophilizing the acetic acid effluent and neutralizing an aqueous solution of the residue with lithium hydroxide. Thus 17% of the initial radioactive salt was recovered after the lactonization and reduction steps.

Acknowledgment.—We thank R. Paulson of this Bureau for the determinations of carbon, hydrogen and lithium.

WASHINGTON 25, D. C.

(14) F. J. Bates and associates, *Polarimetry, Saccharimetry and the Sugars*, NBS Circular 440, p. 189 (1942).

(15) A. Schwebel, H. S. Isbell and J. D. Moyer, *J. Research Natl. Bur. Standards*, **53**, 221 (1954).

(16) Product of the Chemical Process Co., Redwood City, Calif.