Anal. Calcd. for $C_{14}H_{22}O_8$: C, 52.82; H, 6.97; CH_3CO, 54.1. Found: C, 53.06; H, 6.82; CH_3CO, 54.3.

The Two 1,6-Didesoxy-2,3,4,5-dibenzylidene-L-mannitols.—A mixture of 3 g. of 1,6-didesoxy-L-mannitol, 12 ml. of concentrated hydrochloric acid and 10 ml. of benzaldehyde was kept at 0° for 2 days. The solution was neutralized with aqueous ammonia and extracted with chloroform. The residue obtained upon concentration of the chloroform extract was dissolved in absolute ethanol and overnight in the refrigerator the solution deposited 0.7 g. of thick needles. After 4 recrystallizations from a mixture of chloroform and pentane the product, of m.p. 159–160° and $[\alpha]^{20}D$ −47.5° in chloroform (*c* 1), was identified as the 1,6-didesoxy-2,3,4,5-dibenzylidene-L-mannitol that is enantiomorphous with the D form, of m.p. 159–160° and $[\alpha]^{20}D$ +49.5°, reported earlier from this Laboratory.[13b]

The ethanolic mother liquor from the 0.7 g. of thick needles was concentrated to a sirup that was dissolved in ether, diluted with pentane, and chilled to −10°. Additional crystalline material was obtained and by fractional crystallization from chloroform and pentane it could be separated to yield more of the levorotatory dibenzylidene compound described above and a second, lower-melting, dextrorotatory benzylidene compound. The latter crystallized as white prisms, melted at 131–134°, showed $[\alpha]^{20}D$ +12.3° in chloroform (*c* 0.2), and also had the composition of a 1,6-didesoxy-2,3,4,5-dibenzylidene-L-mannitol. Upon hydrolysis by heating with 80% acetic acid for 2 hours on the steam-bath the original 1,6-didesoxy-L-mannitol was recovered.

Anal. Calcd. for $C_{20}H_{22}O_4$: C, 73.59; H, 6.80. Found (159–160° isomer): C, 73.52; H, 6.87; (131–134° isomer) C, 73.54; H, 6.87.

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BETHESDA, MARYLAND

[CONTRIBUTION FROM THE RADIOCHEMISTRY LABORATORY, DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

6-C^{14}-D-Glucose and 6-C^{14}-D-Glucuronolactone[1]

BY JOHN C. SOWDEN

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6-C^{14}-D-Glucose has been prepared from D-glucose through the following successive intermediates: 1,2-isopropylidene-D-glucofuranose, 5-aldo-1,2-isopropylidene-D-xylofuranose, 6-C^{14}-1,2-isopropylidene-D-glucofuranuronic acid and 6-C^{14}-1,2-isopropylidene-D-glucofuranurono-γ-lactone. The isotopic carbon was introduced through a cyanohydrin condensation between NaC^{14}N and 5-aldo-1,2-isopropylidene-D-xylofuranose. The radiochemical yield of 6-C^{14}-D-glucose from NaC^{14}N was aproximately 10% while, as an alternative product, 6-C^{14}-D-glucuronolactone could be obtained in a radiochemical yield of approximately 18%.

Since their introduction a few years ago, C^{14}-labeled sugars have found extensive application for studies of chemical and, especially, biochemical reaction mechanisms. In the aldose sugar series, biosynthetic labeling has made available uniformly-labeled D-glucose[2] and D-galactose[3] as well as C_3,C_4-labeled D-glucose.[4] General synthetic methods applicable to C_1-labeling include the nitromethane[5] and cyanohydrin[6] syntheses. In addition, C_1-labeled D-xylose also may be obtained from C_1-labeled D-glucose by degradation.[7] No general synthetic method is available for the preparation of sugars with specific labeling in other than C_1.

The preparation of 6-C^{14}-D-glucose was of interest for the study of certain chemical and biochemical reactions involving scission of the D-glucose carbon chain. In addition 6-C^{14}-D-glucuronic acid should prove of value in elucidating the biochemistry of this physiologically important substance. The present synthesis, leading to both the labeled sugar and uronic acid, is unique for the glucose structure.

The reaction sequence employed is shown in the accompanying reaction scheme. The terminal carbon of D-glucose first is removed as formaldehyde by the cleavage of 1,2-isopropylidene-D-glucofuranose with sodium metaperiodate.[7,8] Introduction of the radioactive label then is accomplished by condensation of the resulting 5-aldo-1,2-isopropylidene-D-xylofuranose (I) with NaC^{14}N to give, after hydrolysis, a mixture of acetonated 6-C^{14}-L-iduronic acid and 6-C^{14}-D-glucuronic acid (II). The latter can be isolated by crystallization in about 20% yield based on the cyanide.

The use of excess 5-aldo-1,2-isopropylidene-D-xylofuranose in the cyanohydrin condensation to increase the radiochemical yield based on NaC^{14}N is limited by the following practical considerations: The sirupy dialdehyde, I, retains tenaciously a portion of the formaldehyde arising in the periodate cleavage and thus, with a large excess of dialdehyde, the yield of labeled uronic acids may be lowered seriously by the accompanying formation of labeled glycolic acid. Moreover, in the alkaline hydrolysis that follows the cyanohydrin condensation, appreciable amounts of unreacted dialdehyde give rise to troublesome quantities of sugar destruction products.

(1) Presented before the Division of Sugar Chemistry, 121st Meeting of the American Chemical Society, Milwaukee, Wisconsin, March 30–April 3, 1952.

(2) L. G. Livingston and G. Medes, *J. Gen. Physiol.*, **31**, 75 (1947); S. Aronoff, A. Benson, W. Z. Hassid and M. Calvin, *Science*, **105**, 644 (1947); E. W. Putman, W. Z. Hassid, G. Krotkov and H. A. Barker, *J. Biol. Chem.*, **173**, 785 (1948).

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(4) A. K. Solomon, B. Vennesland, F. W. Klemperer, J. M. Buchanan and A. B. Hastings, *J. Biol. Chem.*, **140**, 171 (1941); H. G. Wood, N. Lifson and V. Lorber, *ibid.*, **159**, 475 (1945); D. B. Zilversmit, I. L. Chaikoff, D. D. Feller and E. J. Masoro, *ibid.*, **176**, 389 (1948).

(5) J. C. Sowden, *Science*, **109**, 229 (1949); *J. Biol. Chem.*, **180**, 55 (1949).

(6) D. E. Koshland, Jr., and F. H. Westheimer, THIS JOURNAL, **71**, 1139 (1949); **72**, 3383 (1950).

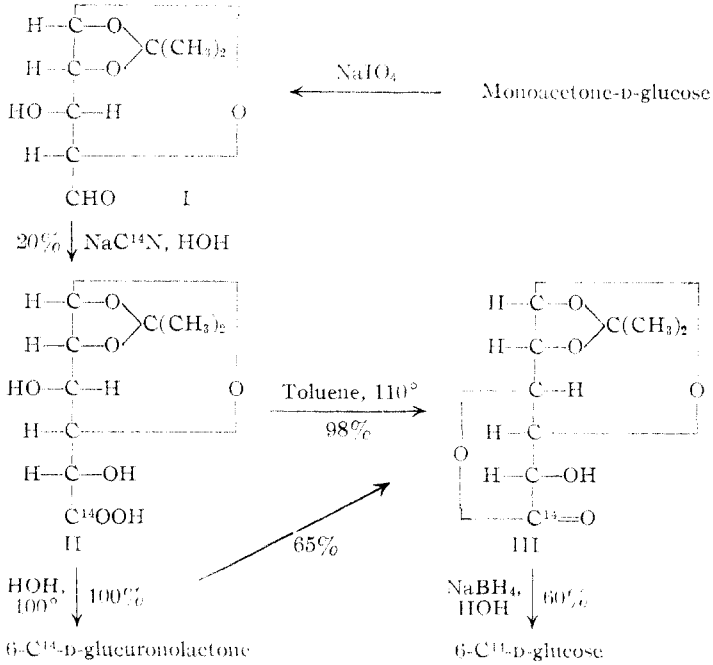
(7) J. C. Sowden, *ibid.*, **73**, 5496 (1951).

(8) K. Iwadare, *Bull. Chem. Soc. Japan*, **16**, 40 (1941).

The lactonization of 1,2-isopropylidene-D-glucuronic acid had been accomplished previously by heating II *in vacuo* and isolating the acetonated lactone, III, by sublimation.[9] Somewhat more convenient, especially for radiochemical work, is the simple refluxing of a suspension of II in toluene employed in the present synthesis.

The mild experimental conditions under which sodium borohydride reduces sugars[10] and sugar acid lactones[11] are admirably suited for the reduction of the acid- and alkali-sensitive[9] monoacetone-D-glucuronolactone to monoacetoneglucose. The latter, without isolation, then is converted to D-glucose by acid hydrolysis of the isopropylidene group.

Following the isolation of crystalline 6-C^{14}-1,2-isopropylidene-D-glucuronic acid, an appreciable amount of 6-C^{14}-D-glucuronolactone may be isolated from the residual reaction sirup by isotopic dilution. If the labeled lactone is the product of choice, the acetonated glucuronic acid may be converted readily to the unsubstituted labeled lactone by evaporation of its hot aqueous solution. If, on the other hand, it is desired to convert labeled glucuronolactone obtained from the synthesis to labeled glucose, this may be accomplished in moderate yield by acetonation[9] followed by reduction and hydrolysis as indicated in the reaction scheme.



Experimental

6-C^{14}-1,2-Isopropylidene-D-glucuronic Acid and 6-C^{14}-D-Glucuronolactone.—A solution containing 8.8 g. of monoacetoneglucose in 100 ml. of water was stirred with 8.8 g. of sodium metaperiodate for 30 minutes at 15–20°. The solution then was concentrated to dryness at reduced pressure at a bath temperature not exceeding 50°. Following the addition of anhydrous sodium sulfate, the residue was extracted by shaking with three 50-ml. portions of chloroform. The chloroform extract was clarified with anhydrous sodium sulfate, filtered, and concentrated at reduced pressure to a sirup. To remove formaldehyde, the sirup was concentrated further at a bath temperature of 40° for two hours using a mechanical pump protected by a trap containing activated carbon at −70°.

The sirupy 5-aldo-monoacetone-D-xylose, weighing approximately 6 g., was dissolved in 25 ml. of water at room temperature, the solution cooled to 0°, and an ice-cold solution of 1.27 g. of sodium cyanide[12] containing two millicuries of $NaC^{14}N$ (Tracerlab) in 75 ml. of water was added. (The synthesis was carried to this stage without interruption.) After standing at 0° for 70 hours and at room temperature for 20 hours in a stoppered flask, the solution was transferred to a beaker and heated on the steam-bath for 3.5 hours. *This operation and all subsequent ones were performed in an efficient hood lined with blotting paper.* The dark solution was cooled with ice-water and passed through a column containing 50 ml. of Amberlite IR-100-H[13]. Ice-water (100 ml.) was employed to wash the column. The combined effluent and washings, kept cold with ice-water, were decolorized by two successive rapid treatments with decolorizing carbon combined with suction filtration. After titration to the phenolphthalein end-point with aqueous sodium hydroxide (16 meq.), the solution was concentrated to dryness at reduced pressure. The residual sodium salts were boiled briefly with 50 ml. of absolute ethanol, cooled, filtered, and washed with ethanol and ether. The white, amorphous salts (3.1 g.*)[14] were dissolved in 25 ml. of water and the solution suction-filtered from a small amount (110 mg.)* of insoluble material. The filtrate was passed through a column containing 40 ml. of Amberlite IR-100-H and the column washed with 50 ml. of water. The effluent was extracted immediately with ten 40-ml. portions of ethyl acetate. After drying with sodium sulfate, filtration and concentration, the ethyl acetate extract yielded a partly crystalline residue. The sirupy fraction was removed by trituration with a mixture of ethyl acetate and ether (1:3) and then with ether. Recrystallization of the residue from acetone by the addition of petroleum ether (Skellysolve F) yielded 535 mg. of monoacetone-D-glucuronic acid,[15] m.p. 143–144°, and a second crop of less pure material weighing 195 mg., m.p. 136–138°; yield 12.5% based on sodium cyanide.

The residual sirup obtained by combination and concentration of the trituration and recrystallization liquors was dissolved in water, the solution heated on the steam-bath for three hours, and then concentrated in a stream of dry air on the steam-bath. A few drops of ethanol were added to the residual sirup and seeding then produced 75 mg. of impure D-glucuronolactone, m.p. 155–158°. The filtrate again was dissolved in water after the addition of 1.0 g. of nonradioactive D-glucuronolactone.[16] Concentration on the steam-bath in a current of dry air then produced 1.0 g. of the lactone, m.p. 168–172°, showing 18% of the specific radioactivity of the undiluted radioactive lactone. The combined radiochemical yield of monoacetone-D-glucuronic acid and D-glucuronolactone is thus approximately 19%.

In several non-radioactive trial experiments, the above ethyl acetate extraction of monoacetone-D-glucuronic acid was replaced by rapid concentration of the acidic aqueous solution at reduced pressure. This variation in procedure usually gave lower yields of the sensitive acetonated acid. Moreover, the latter was frequently contaminated with a second crystalline product (m.p. 128–130°, $[\alpha]D$ 87.5°, in water) believed

(9) L. N. Owen, S. Peat and W. J. G. Jones, *J. Chem. Soc.*, 339 (1941).

(10) M. Abdel-Akher, J. K. Hamilton and F. Smith, THIS JOURNAL, **73**, 4691 (1951).

(11) M. L. Wolfrom and H. B. Wood, *ibid.*, **73**, 2933 (1951).

(12) C. S. Hudson, *ibid.*, **73**, 4498 (1951).

(13) Product of Rohm and Haas Co., Philadelphia, Pa.

(14) Starred yields and properties were determined in parallel nonradioactive experiments.

(15) L. Zervas and P. Sessler, *Ber.*, **66**, 1326 (1933).

(16) The author is indebted to Dr. T. J. Schoch, Corn Products Refining Co., Argo, Ill., for this material.

to be 1,2-isopropylidene-L-iduronolactone. Further investigation of this by-product is planned.

D-Glucuronolactone from Monoacetone-D-glucuronic Acid*.[16]—A solution containing 100 mg. of monoacetone-D-glucuronic acid, m.p. 144–145°, in a few ml. of water was heated on the steam-bath for two hours, and then evaporated to dryness on the steam-bath. Seeding of the residual sirup and the addition of a few drops of ethanol then produced 75 mg. (quantitative) of D-glucuronolactone, m.p. 168–172°, $[\alpha]_D$ 20°, in water.

6-C^{14}-D-Glucose.—A suspension of 535 mg. of 6-C^{14}-monoacetone-D-glucuronic acid, m.p. 143–144°, in 25 ml. of toluene was heated under reflux for three hours. The resulting clear solution was cooled and diluted with 15 ml. of petroleum ether (Skellysolve F). The ensuing crystallization was completed by gradual dilution with an additional 35 ml. of petroleum ether. The acetonated lactone separated as well-formed needles adhering to the surface of the flask. The product was isolated by decantation of the solvents and washed in place, by decantation, with petroleum ether; yield, 485 mg. (98%), m.p.* 119–120°.

The acetonated lactone was dissolved in 40 ml. of water containing 150 mg. of sodium borohydride. One-half hour later, an additional 100 mg. of sodium borohydride was rinsed in with 5 ml. of water. After standing four hours at room temperature, the solution was acidified by the dropwise addition of 2 ml. of 6 *N* sulfuric acid and then heated for one hour on the steam-bath. The cooled solution then was passed through a column containing in successive bands 15 ml. of Duolite A-4,[17] 15 ml. of Amberlite IR-100-H, and 15 ml. of Duolite A-4. The combined effluent and washings were concentrated at reduced pressure to a colorless sirup. Crystallization from 3–4 ml. of 95% ethanol yielded 185 mg. of 6-C^{14}-D-glucose, m.p. 145–146°, $[\alpha]_D$ 52.5°, equil. in water*.

The crystallization filtrate and washings (ethanol) were diluted with water and then used to dissolve 500 mg. of non-radioactive D-glucose. Concentration and crystallization then yielded 500 mg. of 6-C^{14}-D-glucose showing 10% of the specific radioactivity shown by the undiluted radioactive sugar. The yield of 6-C^{14}-D-glucose from 6-C^{14}-monoacetone-D-glucuronolactone is thus approximately 60%.

Acetonation of D-Glucuronolactone*.[9]—D-Glucuronolactone (240 mg.) was stirred in a closed flask (magnetic stirrer) for 2.5 hours with 15 ml. of acetone containing 0.1 ml. of sulfuric acid. The resulting clear solution then was passed through a column containing 8 ml. of Duolite A-4 moistened with acetone. Concentration of the effluent left a residue of crystalline monoacetone-D-glucuronolactone. Recrystallization from ether–petroleum ether gave 190 mg. (64%) of the product, m.p. 119–120°.

Radioactivity of Products.—The undiluted 6-C^{14}-D-glucuronolactone and 6-C^{14}-D-glucose described above were calculated from the original dilution of $NaC^{14}N$ to give approximately 10^6 dis./min./mg. When counted as thin layers (*ca.* 1 μg./sq. cm.) in the Nucleometer,[18] each product showed approximately 5×10^5 ct./min./mg.

(17) Product of Chemical Process Co., Redwood City, Cal. After regeneration and washing, this resin, prior to use, was treated with several volumes of 5% sodium chloride solution and then washed free of chloride ion.

(18) Manufactured by Radiation Counter Laboratories, Chicago, Ill.

ST. LOUIS, MISSOURI

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE STATE UNIVERSITY OF IOWA]

The Stability of the Tetramethylammonium Polyiodides in Ethylene Chloride[1]

BY ROBERT E. BUCKLES, JAMES P. YUK AND ALEXANDER I. POPOV

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The three known tetramethylammonium polyiodides, $(CH_3)_4NI_3$, $(CH_3)_4NI_5$ and $(CH_3)_4NI_9$, have been studied spectrophotometrically in ethylene chloride. The pentaiodide and the enneaiodide were shown to have absorption spectra consistent with the view that in dilute solutions they are virtually completely dissociated into the triiodide and iodine. The method of continuous variation applied to mixtures of iodine and tetramethylammonium monoiodide gave evidence that the only stable polyiodide in very dilute solutions in ethylene chloride is the triiodide. The dissociation of the triiodide into the monoiodide and iodine was calculated to have a pK value of 7.0 ± 0.2 at 25°.

A fairly large number of quaternary ammonium polyiodides including triiodides, pentaiodides, heptaiodides and enneaiodides have been reported.[2]

The stability and identity of each of these solid polyiodides has been studied to some extent as a part of its preparation and isolation. In some cases there have been more detailed studies such as that of the system: tetramethylammonium iodide–iodine, which has been reported[3] to show the tri-, penta- and enneaiodides with congruent m.p.'s whereas the m.p. of the heptaiodide was incongruent. Attempts to isolate the tetramethylammonium heptaiodide have not been successful.[2a] X-Ray diffraction studies of the crystals of tetraethylammonium triiodide[4] and tetramethylammonium pentaiodide[5] have led to the determination of the crystal structure in each case.

From an early study[6] of the solubilities of mixtures of iodine and tetramethylammonium iodide in nitrobenzene it was concluded that the enneaiodide was the highest polyiodide which existed in solution. These results, however, merely point up the fact that the enneaiodide was insoluble enough to precipitate under certain conditions and do not give any information as to the relative concentrations of the various polyiodides in solution.

The ultraviolet absorption spectrum of *p*-bromophenyltrimethylammonium triiodide has been measured.[7] Also the spectra of mixtures of quaternary ammonium iodides with iodine in aqueous alcohol have been reported.[8] In neither case was any study of the identity of the absorbing species carried out. It has been suggested on the basis of some electrical conductivity measurements

(1) Part of this work was carried out under Contract No. At(11-1)-72, Project No. 7, with the Atomic Energy Commission. Presented before the Organic Division of the American Chemical Society, Milwaukee, Wisconsin, April 1, 1952.

(2) (a) F. D. Chattaway and G. Hoyle, *J. Chem. Soc.*, **123**, 654 (1923); (b) H. McCombie and T. H. Reade, *ibid.*, **123**, 141 (1923); (c) D. Strömholm, *J. prakt. Chem.*, **67**, 345 (1903); (d) A. Geuther, *Ann.*, **240**, 66 (1887); (e) R. Muller, *ibid.*, **108**, 1 (1858); (f) Weltzien, *ibid.*, **91**, 33 (1854).

(3) F. Olivari, *Atti. accad. Lincei*, **17**, II, 717 (1908).

(4) R. C. L. Mooney, *Phys. Rev.*, **53**, 851 (1938).

(5) R. J. Hach and R. E. Rundle, THIS JOURNAL, **73**, 4321 (1951).

(6) H. M. Dawson and E. C. Goodson, *J. Chem. Soc.*, **85**, 796 (1904).

(7) F. L. Gilbert, R. R. Goldstein and T. M. Lowry, *ibid.*, 1092 (1931).

(8) C. K. Tinkler, *ibid.*, **93**, 1611 (1908).