Synthesis of L-glucurone. Conversion of D-glucose into L-glucose

WALTER SOWA
Ontario Research Foundation, Sheridan Park, Ontario
Received May 5, 1969

L-Glucurone (**2**) was readily prepared on a small scale by treatment of D-*glycero*-D-*gulo*-heptonolactone (**1**) with a molar equivalent of periodic acid; thin-layer chromatography was used for its isolation. On a larger scale pure crystalline L-glucurone was obtained in over 80% yield from 3,5;6,7-di-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptonolactone (**4**) in two steps consisting of concomitant hydrolysis and oxidation of **4** with periodic acid followed by treatment of the intermediate oxidation product with trifluoroacetic acid. L-Glucose was prepared from L-glucurone by borohydride reduction and hydrolysis of the 1,2-*O*-isopropylidene derivative. Since **1** was derived from D-glucose, the result of this series of reactions was the conversion of D-glucose into its enantiomer L-glucose.

Canadian Journal of Chemistry, 47, 3931 (1969)

The first chemical synthesis of a uronic acid was carried out in 1890 by Fischer who obtained D-glucuronic acid and its lactone by reduction of D-glucaro-1,4-lactone with sodium amalgam in acid solution (1). Niemann and Link in 1934 used the same procedure for the enantiomer L-glucuronic acid (2). The starting material, L-glucaro-1,4-lactone, was prepared via calcium L-glucarate from nitric acid oxidation of D-gulono-1,4-lactone. The yield of L-glucurone (**2**), the form in which the L-glucuronic acid was isolated, was about 1% based on calcium L-glucarate. L-Glucuronic acid is of interest because it has been reported to occur naturally, along with D-glucuronic acid, as a metabolic product of the action of rat kidney enzymes on inositol (3). Other workers, however, were unable to detect L-glucuronic acid and have found that inositol is converted into D-glucuronic and L-gulonic acids (4). This discrepancy has not been explained.

The biochemical and pharmacological importance of D-glucuronic acid (5) has led to a thorough investigation of methods for its synthesis, especially those based on oxidation of suitably protected D-glucose derivatives (6). Undoubtedly these procedures can be applied to the synthesis of L-glucuronic acid from L-glucose, but this is not attractive because L-glucose is not readily available. The present publication describes a facile synthesis of L-glucurone (**2**) from D-*glycero*-D-*gulo*-heptonolactone (**1**), a starting material easily prepared from ordinary D-glucose by the Kiliani synthesis (7). This work is an example of the application of a simple and general method for the preparation of uronic acids directly as their lactones, namely selective periodate cleavage of the exocyclic glycol system of an aldonolactone. The procedure has been used on two previous occasions. Woods and Neish treated 2-*C*-hydroxymethyl-D-glucono-1,4-lactone with an equimolar amount of periodate and obtained 4-*C*-hydroxymethyl-L-xyluronolactone, which crystallized as the acid (8). Similarly, Hulyalkar and Perry oxidized D-galactono-1,4-lactone to obtain L-lyxuronic acid, isolated as methyl L-lyxuronate (9). Essentially, oxidative removal of the primary alcohol of an aldonolactone results in inversion of the molecule with the penultimate carbon atom of the aldonolactone becoming C-1 of the uronic acid lactone.

Trial experiments followed by paper chromatography indicated that D-*glycero*-D-*gulo*-hepto nolactone (**1**) is oxidized by an equimolar quantity of periodic acid to yield approximately equal amounts of arabinose (**3**), glucurone (**2**), and unreacted starting material. The arabinose and glucurone arise from glycol cleavage of the heptonolactone at C_2—C_3 and C_6—C_7, respectively. Apparently, 2-*O*-glyoxylyl-D-arabinose, the initial product expected from oxidation at C_2—C_3, is unstable under the experimental conditions which were used. The products were identified as D-arabinose and L-glucurone by isolation as the crystalline derivatives D-arabinose diethyl dithioacetal and 2,4,5-tri-*O*-acetyl-L-glucurone diethyl dithioacetal.

Two procedures were developed for the preparation of L-glucurone. Small quantities (ca. 25 mg) were very simply obtained by treatment of D-*glycero*-D-*gulo*-heptonolactone with an equimolar amount of periodic acid followed by thin-layer chromatography (t.l.c.) of the oxidation mixture on cellulose. The L-glucurone was either crystallized directly or converted to the triacetate.

For larger scale work it was desirable to improve the yield of L-glucurone and avoid chromatographic isolation. It was therefore necessary to prevent cyclic glycol cleavage and ensure that oxidation of the exocyclic triol (C_5—C_6—C_7) was restricted to C_6—C_7. This was accomplished by using a suitably substituted starting material, the 3,5;6,7-di-*O*-isopropylidene derivative (**4**) of D-*glycero*-D-*gulo*-heptonolactone.

Isopropylidene acetals involving primary alcohol groups are often acid-labile as, for example, in the case of exocyclic acetal groups of di-*O*-isopropylidene hexofuranoses (11). A similar sensitivity to acid has been found with the C_6—C_7 isopropylidene group of **4** (12).[1] It was advantageous to effect both hydrolysis and oxidation of **4** in one step with periodic acid. The product, presumably 2,4-*O*-isopropylidene-*aldehydo*-L-glucurone (**5**), gave positive reactions with Fehling's solution and Schiff reagent. According to t.l.c. the oxidation product was a mixture of two compounds both of which gave a distinctive purple color with the *p*-anisidine hydrochloride spray reagent. The two compounds were probably **5** in the form of lactone and acid. Treatment of the syrupy mixture with 90% trifluoroacetic acid for hydrolysis of the isopropylidene group (13) afforded crude crystalline L-glucurone in almost theoretical yield based on **4**. The yield of pure recrystallized L-glucurone was over 80%.

L-Glucose was prepared from L-glucurone by procedures previously developed for the enantiomeric compound. This involved temporary blocking of the reducing group as the 1,2-*O*-isopropylidene acetal (14), followed by reduction of the lactone with sodium borohydride in the presence of boric acid (15). The crude 1,2-*O*-isopropylidene-L-glucose which was obtained was hydrolyzed with trifluoroacetic acid to L-glucose. Since D-glucose was the precursor of D-*glycero*-D-*gulo*-heptonolactone from which the L-glucurone was derived, this overall sequence of reactions constitutes the conversion of D-glucose into L-glucose.

[1]In two publications Brimacombe and co-workers discuss the acetonation of **1** to yield **4** and refer to an analogous case (25) stating "acid-catalyzed acetonation of D-glucurono-δ-lactone diethyl dithioacetal yields a 2,4-*O*-isopropylidene derivative, presumably with contraction in size of the lactone ring" (10), and "D-glucurono-6,2-lactone diethyl dithioacetal, which yields a 2,4-*O*-isopropylidene derivative, presumably with contraction in size of the lactone ring" (12). The assumption of ring contraction is erroneous and has apparently arisen from a typographical error in Gorin's paper in which compounds referred to as δ-lactones were actually γ-lactones. This is indicated by infrared (i.r.) data for the isopropylidene derivative of D-glucurone diethyl dithioacetal (absorption at 1780 cm^{-1}). Moreover the starting material "D-glucurono-δ-lactone diethyl dithioacetal prepared by the method of Zinner and Dassler" was in fact the γ-lactone (26).

Experimental

Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. Paper chromatography was carried out by the descending method on Whatman No. 4 paper using ethyl acetate–pyridine–water (10:4:3 v/v) as developing solvent. Thin-layer chromatography was performed on silica gel G with development in ethyl acetate. Spray reagents for detection of compounds were *p*-anisidine hydrochloride (16) and alkaline silver nitrate (17); for t.l.c. 50% sulfuric acid was also used. Chromatograms sprayed with *p*-anisidine hydrochloride or sulfuric acid were heated at 120° for visualization. Solutions were concentrated under reduced pressure using a rotary evaporator and a water bath at a temperature of 50° or less.

L-*Glucurone (2) from Unsubstituted* D-*Glycero*-D-*gulo*-*heptonolactone (1)*

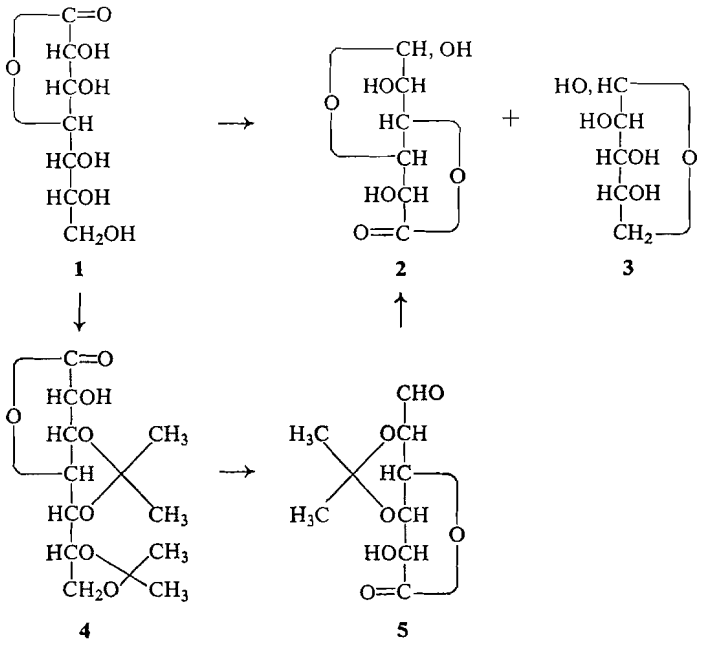
(a) Characterization of Products

In small scale (mmole) experiments followed by paper chromatography, solutions of **1** (7) were treated with an equimolar amount of periodic acid at 5° and at room temperature, and for periods ranging from a few min to 1 h. Under all conditions there appeared to be roughly equal amounts of arabinose (**3**), glucurone (**2**), and unreacted **1**. The products were characterized from an oxidation carried out on a larger scale.

Solutions of periodic acid (4.56 g) and **1** (4.16 g), each in 100 ml of water, were mixed and left for 1 h at room temperature. Barium acetate (2.8 g) in water (25 ml) was added to the mixture, the precipitate was removed by filtration and the filtrate was evaporated. The residue was treated with hydrochloric acid (5 ml) and ethanethiol (5 ml) for 1 h at 10°. Ice and water (15 ml) were added to the reaction and the mixture was extracted with ethyl acetate (5 × 50 ml). The aqueous layer was evaporated to dryness and the residue was crystallized from methanol–water to recover unoxidized heptonolactone (1.5 g, 36%), m.p. 152–154°. The combined ethyl acetate extracts were washed with saturated sodium bicarbonate solution (30 ml) and water (2 × 30 ml) and dried over anhydrous sodium sulfate. The solution was concentrated to about 30 ml and cooled to yield D-arabinose diethyl dithioacetal (0.9 g), m.p. 126–127° and $[\alpha]_D^{23}$ −11.2° (*c*, 3.67 in methanol). The reported values are m.p. 126° and $[\alpha]_D^{24}$ −11.0° (*c*, 3.69 in methanol) (18).

Anal. Calcd. for $C_9H_{20}O_4S_2$: C, 42.16; H, 7.89; S, 25.01. Found: C, 42.54; H, 7.60; S, 25.11.

The mother liquor from the D-arabinose diethyl dithioacetal was evaporated to a syrup which was dried over phosphorus pentoxide in a vacuum desiccator. The residue (2.3 g) was acetylated with acetic anhydride (10 ml) and pyridine (8 ml) for 5 h at 4°. The mixture was poured into ice and water containing sodium bicarbonate and extracted with ether. The combined ether



extracts were washed successively with cold dilute sulfuric acid, water, saturated sodium bicarbonate solution and water and then dried over anhydrous sodium sulfate. The ether solution was evaporated to a residue which was redissolved in ether–petroleum ether to crystallize 2,4,5-tri-*O*-acetyl-L-glucurone diethyl dithioacetal (1.0 g from 4 crops); m.p. 113–114° and $[\alpha]_D^{23}$ −53.3° (*c*, 4.30 in chloroform). Literature values for 2,4,5-tri-*O*-acetyl-D-glucurone diethyl dithioacetal are m.p. 113–114° and $[\alpha]_D^{25}$ +58° (*c*, 4.1 in chloroform) (19); and m.p. 110–112° and $[\alpha]_D^{16}$ +53.9° (*c*, 1.61 in methanol) (20).

Anal. Calcd. for $C_{16}H_{24}O_8S_2$: C, 47.04; H, 5.93; S, 15.69. Found: C, 46.83; H, 5.81; S, 15.59.

(b) L-Glucurone

L-Glucurone was conveniently prepared in small amounts directly from **1** by using t.l.c. for its isolation. A solution of periodic acid (H_5IO_6, 228 mg) in water (5 ml) was added to a solution of D-*glycero*-D-*gulo*-heptonolactone (208 mg) in water (5 ml). The reaction mixture was left for 15 min at room temperature and barium acetate (137 mg) was added. The precipitate was removed by filtration and the filtrate was evaporated to a syrup (*ca.* 210–220 mg). Preparative t.l.c. of the mixture on microcrystalline cellulose (21) (0.5 mm layers on 2 plates 20 × 20 cm) using double development in acetone–water (19:1 v/v) gave syrupy L-glucurone. Yields ranged from 40 to 52 mg. For crystallization the syrupy product (42 mg) was first dissolved in hot acetic acid. The solution was then evaporated and the residue was crystallized from methanol–ether to obtain L-glucurone (28 mg, 16% yield from **1**) with m.p. 174–176° and $[\alpha]_D^{23}$ −19° (*c*, 2.07 in water). The reported values for L-glucurone are m.p. 169–172° and $[\alpha]_D^{25}$ −18.5° (*c*, 0.6 in water) (2); for D-glucurone m.p. 180° and $[\alpha]_D^{23}$ +18.55° (*c*, 1.914 in water) (22).

Anal. Calcd. for $C_6H_8O_6$: C, 40.91; H, 4.59. Found: C, 41.22; H, 4.59.

Syrupy L-glucurone (52 mg) isolated by t.l.c. was acetylated by treatment for 1 h with acetic anhydride (0.5 ml) containing boron trifluoride etherate (0.05 ml). The mixture was poured into ice and water and the resulting precipitate was recrystallized from methanol to obtain 1,2,5-tri-*O*-acetyl-β-L-glucurone (53 mg) having m.p. 195–196° and $[\alpha]_D^{21}$ −85.4° (*c*, 2.58 in chloroform). Literature values for 1,2,5-tri-*O*-acetyl-β-D-glucurone are m.p. 193–194° and $[\alpha]_D^{20}$ +89.6° (*c*, 2.21 in chloroform) (23).

Anal. Calcd. for $C_{12}H_{14}O_9$: C, 47.68; H, 4.68. Found: C, 47.69; H, 4.66.

L-Glucurone (2) from 3,5;6,7-di-O-isopropylidene-D-glycero-D-gulo-heptonolactone (4)

A solution of periodic acid (H_5IO_6, 2.28 g) in water (25 ml) was added to **4** (10) (2.88 g) in an equal volume of water and the mixture was shaken for 1 h at room temperature. Barium carbonate (1.5 g) was added and shaking was continued for 15 min. The neutralized mixture was filtered and the filtrate was evaporated to dryness. The residue was extracted with acetone and the extract was concentrated to obtain a hygroscopic syrup (2.10 g) having $[\alpha]_D^{22}$ −83° (*c*, 1.9 in acetone). This product, which gave positive reactions with Fehling's solution and Schiff reagent, was a mixture of two compounds according to t.l.c. Both produced a distinctive purple color with the *p*-anisidine hydrochloride spray reagent. The major compound was mobile with a tendency to streak, while the minor compound remained on the starting line. The mixture (1.00 g) was hydrolyzed with 90% trifluoroacetic acid (13) (10 ml) for 10 min at room temperature. Evaporation of the hydrolyzate gave crude crystalline L-glucurone, m.p. 165–167°, (0.81 g,

yield 97% from **4**). On recrystallization from acetic acid (10 ml) the product (0.69 g, yield 83% from **4**) had m.p. 180–181° and $[\alpha]_D^{22}$ −18° (*c*, 2.0 in water).

Anal. Calcd. for $C_6H_8O_6$: C, 40.91; H, 4.59. Found: C, 41.06; H, 4.52.

L-Glucose

1,2-*O*-Isopropylidene-L-glucurone, m.p. 122–123° and $[\alpha]_D^{22}$ −68° (*c*, 1.2 in water), was prepared from L-glucurone and acetone using sulfuric acid catalyst according to procedures described for the D-glucurone derivative (14) except that the calculated quantity of ammonium hydroxide instead of barium carbonate or sodium carbonate was used for neutralization of the sulfuric acid. The corresponding constants recorded for 1,2-*O*-isopropylidene-D-glucurone are m.p. 120° and $[\alpha]_D^{18}$ +70° (*c*, 1.0 in water) (14).

Anal. Calcd. for $C_9H_{12}O_6$: C, 49.99; H, 5.60. Found: C, 50.03; H, 5.56.

1,2-*O*-Isopropylidene-L-glucurone was reduced in boric acid solution with sodium borohydride (15). A stirred solution of the sugar (0.54 g) in 0.4 *M* boric acid (15 ml) was cooled in an ice-bath and treated with 0.3 *M* sodium borohydride (25 ml) added over 30 min. The reaction was stored at 4° for 22 h with a further addition of 0.3 *M* sodium borohydride (15 ml) after 4 h. The solution was neutralized with dilute acetic acid, passed through Rexyn 101 (H^+) (Fisher Scientific Co., Ltd.) and evaporated to dryness. Some of the borate was removed from the residue by adding methanol and evaporating to dryness several times. The mixture was then hydrolyzed with 90% trifluoroacetic acid (6 ml) for 15 min at room temperature. The hydrolyzate, after concentration to dryness, was repeatedly treated with methanol until the remaining borate was removed. The syrupy product was crystallized from ethanol–water to obtain 2 crops of L-glucose (390 mg) having m.p. and mixed m.p. 146–147° and $[\alpha]_D^{24}$ −53° (*c*, 3 0 in water). Reported values for α-L-glucose are m.p. 146–147° and $[\alpha]_D^{22}$ −53° (at equilibrium; *c*, 2.6 in water) (24).

Acknowledgments

The author is grateful to Mrs. Brigitte Licht for technical assistance. This work was made possible by funds received from the Province of Ontario through the Department of Trade and Development.

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