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Note

The acid-catalyzed decarboxylation of D-xyluronic, D-galacturonic, and D-*glycero*-D-*gulo*-hepturonic acid*†

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In acidic solution at elevated temperatures, hexuronic acids undergo decarboxylation with the resultant formation of 2-furaldehyde, reductic acid (2,3-dihydroxycyclopenten-1-one), and traces of 5-formyl-2-furoic acid[1–4]. Because the decarboxylation is very nearly quantitative, the reaction has been used as an analytical method[5] for hexuronic acids. Although a number of mechanistic proposals are in print that attempt to explain this reaction[1–3,6] and, as a result, the kinetics of these reactions have been extensively studied, such studies have dealt almost exclusively with hexuronic acids. In two reports (which are exceptions), Machida[7] reported that D-xyluronic and D-arabinuronic acids both undergo decarboxylation, but that yields of carbon dioxide are not theoretical (kinetic data were not reported in this case), and Ikawa and Link[8] showed that D-threuronic acid readily decomposes to pyruvaldehyde, presumably by elimination of carbon dioxide, but again, no kinetic data were collected.

For purposes of mechanistic studies, it would be interesting to determine whether quantitative decarboxylation is a general feature in dehydration of uronic acids, or if it is unique to hexuronic acids only. For these reasons, methyl β-D-*glycero*-D-*gulo*-heptopyranosiduronic acid (**1**, a new compound) was prepared and characterized, and its rate of decarboxylation (and yields of carbon dioxide) were measured relative to D-xyluronic acid (**2**) and D-galacturonic acid (**3**).

Compound **1** was prepared by catalytic oxidation[9] of the known and well characterized methyl β-D-*glycero*-D-*gulo*-heptopyranoside[10]. Although isolated as a syrupy glycoside, the following data indicate the constitution of the product. During the oxidation, one mol of hydrogencarbonate was consumed, indicating the production of a monocarboxylic acid. High-pressure liquid chromatography (h.p.l.c., column A) indicated complete reaction of the starting material; oxidation of the

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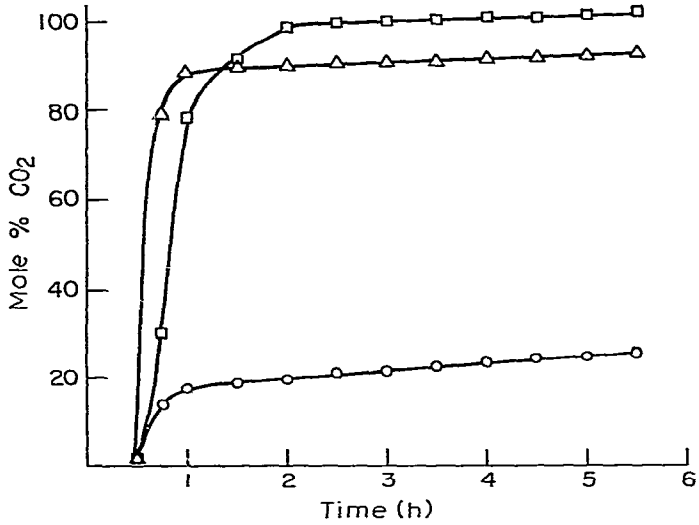

Fig. 1. Rates of evolution of carbon dioxide from β-D-*glycero*-D-*gulo*-heptopyranosiduronic acid (**1**, ○), D-xyluronic acid (**2**, △), and D-galacturonic acid (**3**, □).

product with nitric acid converted it into a material having the same m.p. as that reported by Kiliani[11] for the aldarolactone. Finally, when the presumed heptopyranosiduronic acid was converted into the trimethylsilyl derivative and subjected to mass spectrometry, the following peaks were observed: *m/e* (%): 73 (100), 129 (27), 59 (26), 204 (24), 217 (11), and 421 (2). These data are supportive[12] for retention of the pyranoside ring during the reactions.

After isolation of the glycoside and conversion into the free acid, h.p.l.c. (column A) indicated 2 interconvertible peaks (T_R = 11 and 26 min) representing, respectively, the free acid and a lactone. This conclusion was supported by the fact that paper chromatography (irrigant *A*) showed 2 spots, one of which gave a positive lactone test with the hydroxamic acid spray-reagent. Inspection of models indicates that the compound could readily exist as a (7→4)-lactone when in the pyranoside form.

The D-xyluronic acid (**2**) used in this work was prepared as methyl 1,2-*O*-isopropylidene-α-D-xylofuranuronate as described by Morgenlie[13].

Fig. 1 shows plots of the rate of evolution of carbon dioxide for **1**, **2**, and **3** versus time in boiling 3.29M hydrochloric acid under reflux. For **1** and **2**, the derivatized compounds were used directly as, under conditions of decarboxylation, hydrolysis of the protecting groups would be much faster than dehydration. It was also shown, in parallel experiments, that neither methanol nor acetone, at the levels produced by hydrolysis, interfered with the measurements of carbon dioxide yield.

For initial rate-measurements, data points were collected for each 5 min of the reaction for the first 0.5 h. The data fitted pseudomolecular, first-order plots, and rate constants were calculated to be ($sec^{-1} \times 10^{-4}$) [1.4 for **1**], 9.1 for **2**; and 2.2

for **3**. Although comparative rate-data are not available for **1** and **2**, the value for **3** (2.2×10^{-4} sec^{-1}) is in the range expected for a hexuronic acid. Anderson and Garbutt[1] reported a value of 1.6×10^{-4} sec^{-1} for D-glucuronic acid under comparable conditions, and values of 3.6 and 2.6 $sec^{-1} \times 10^{-4}$ for D-glucuronic acid and D-galacturonic acids[14], respectively, in slightly stronger (19%) hydrochloric acid at comparable temperatures.

Overall yields of carbon dioxide arising from each compound were determined by extrapolation to zero time of the linear portion of the curve. As expected, D-galacturonic acid gave nearly theoretical yields (98% of theory) of carbon dioxide, and D-xyluronic acid gave 88% of theory. The hepturonic acid, however, gave only 16%. In all instances, paper-chromatographic studies indicated that, when these yields were attained, the starting materials had completely decomposed.

The data clearly show that all three uronic acids readily decompose in acid solution, but that only the hexuronic and penturonic acids do so in theoretical or near-theoretical yields. Thin-layer chromatography of chloroform extracts of acid-treated solution of either the penturonic acid or the hepturonic acid indicated that a number of u.v.-absorbing compounds were produced. Although these have not yet been isolated, they do not correspond to any of the dehydration products produced from hexoses or hexuronic acids. During dehydration, u.v. absorption appeared at 253 (for **2**) and at 275 nm (for **1**).

EXPERIMENTAL

Materials and methods. — Ultraviolet spectra were obtained on a Coleman Model 124 double-beam spectrometer and n.m.r. spectra were obtained with a Varian T-60 spectrometer. For paper chromatography, the following irrigants were used: (*A*) 18:3:1:4 (v/v) ethyl acetate–acetic acid–formic acid–water and (*B*) 40:11:19 (v/v) butanol–ethanol–water. The benzidine spray-reagent[16] was used to visualize chromatograms. T.l.c. was performed on silica gel HG-254 (Brinkmann) with the following irrigants: (*A*) 9:1 (v/v) chloroform–methanol; (*B*) 49:1 (v/v) benzene–methanol; (*C*) 4:1 (v/v) benzene–ethanol, and (*D*) 4:5:5 (v/v) chloroform–methanol–acetic acid. Chromatograms were made visible by u.v. irradiation or by charring for 20 min at 100° after being sprayed with 10% ethanolic sulfuric acid. High-performance liquid chromatography (h.p.l.c.) was performed on a Waters model 501 instrument equipped with a refractive-index detector. The columns were packed with (A) BioRad Aminex cation-exchange resin charged in the calcium form, with water at 75° as the irrigant, or (B) a Waters Bondapak carbohydrate column with an acetonitrile–water azeotrope as the irrigant. Gas–liquid chromatography (g.l.c.) was performed in conjunction with a CEC model 91–110 mass spectrometer using (A) 2.5% GC-SE-52 as the liquid phase and 80–100 mesh Chromosorb or (B) 2% DEGA on 180–200 mesh Chromosorb W as the solid phase. Prior to separation, carbohydrates were converted by standard methods into their trimethylsilyl ethers.

Preparation of methyl 1,2-O-isopropylidene-α-D-xylofuranuronate. — This compound was prepared from 1,2-*O*-isopropylidene-α-D-glucofuranose by treatment with silver carbonate on Celite as described by Morgenlie[13]. The material was purified by sublimation at 80° and 0.4 mm. The compound isolated was chromatographically pure by t.l.c. (irrigant *D*), by h.p.l.c. (irrigant *A*), had m.p. 104–105° (lit.[13] m.p. 104–106°), and had an n.m.r. spectrum in chloroform-*d* identical to that reported for the original compound.

Preparation of methyl β-D-glycero-D-gulo-heptopyranoside. — The starting material for this synthesis, D-*glycero*-D-*gulo*-heptose [m.p. 191° (lit. m.p. 191–192°), $[\alpha]_D^{25}$ −19.2° (*c*, 3.5, water); reported $[\alpha]_D^{25}$ −20° (*c*, 3.5 water)] was prepared from the lactone by reduction with sodium borohydride as described by Wolfrom and Thompson[15]. The heptose was converted into the pyranoside by using the procedure described by Angyal[10] and coworkers. The crystalline material had m.p. 170° (lit. m.p. −170°), $[\alpha]_D^{25}$ −74.7° (*c*, 1.0 water). The compound was chromatographically pure, showing, for the acetate (pyridine–acetic anhydride), a single peak in g.l.c. using column A, and also a single peak for the trimethylsilyl derivative (column B). The compound had T_R 19.0 min by h.p.l.c. (column A).

Preparation of methyl β-D-glycero-D-gulo-heptopyranosiduronic acid (**1**). — This compound was prepared from the foregoing methyl heptopyranoside by oxidation with platinum on charcoal as described by Marsh and Levvy[9]. The starting material (2.95 g) was dissolved in water (100 mL), 1.4 g of catalyst added, and the suspension stirred at 55° with oxygen gas being continuously bubbled into the solution. The pH of the solution was maintained at 7.7 by the addition of 0.5M sodium hydrogencarbonate solution, and when an equivalent amount of solution (based on amount of sugar used) had been added, the reaction was stopped by filtration through Celite. The resulting filtrate was passed through a column containing 30 mL of Dowex-50 (H^+) and the product was then adsorbed on a column (1 × 65 cm) of Amberlite IRN-78 resin charged in the formate form. After washing with water, the uronic acid was eluted with 0.05M formic acid (2.8 L). Fractions containing 100 mL were collected and examined by h.p.l.c. (column A), and those containing the major component (T_R 11.0 min) were pooled, extracted with ether, and the aqueous phase was neutralized with sodium hydroxide and evaporated to a syrup. The sodium salt had $[\alpha]_D^{25}$ −51.8° (*c*, 2.67, water) and showed the following ^{1}H-n.m.r. data in deuterium oxide relative to 4,4-dimethyl-4-silapentanesulfonic acid: δ 4.04 (3-proton singlet) δ 5.10, 1-proton doublet, J 3.18 Hz, δ 4.10–4.90 multiplet (5 protons). When a solution of this compound was converted into the free acid, by use of Dowex-50 (H^+), and was heated during 12 h at 80°, a second compound was produced having T_R 21 min in h.p.l.c. (column A). This compound was collected by preparative h.p.l.c. The resulting solution gave a positive lactone-test (hydroxamic acid), and on evaporation to a syrup and conversion into the trimethylsilyl derivative, it had T_R 6.0 min by g.l.c. (column B). On treatment with sodium hydroxide, followed by deionization and concentration, it was converted into the original compound, as evidenced by h.p.l.c. (column A).

Measurement of decarboxylation rate. — These data were collected by measurement of the amount of carbon dioxide evolved, using an apparatus and conditions essentially as described by Whistler and co-workers[5]. From plots of total carbon dioxide evolved, both the total yield and the rate of carbon dioxide production were measured.

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