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THE ACID-CATALYZED DEHYDRATION OF (METHYL β-D-*glycero*-D-*gulo*-HEPTOPYRANOSID)URONIC ACID*

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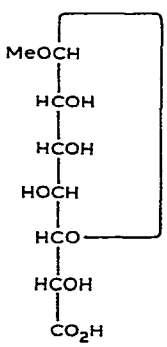
ABSTRACT

The major product soluble in organic solvents, isolated by hydrolysis and subsequent dehydration of (methyl β-D-*glycero*-D-*gulo*-heptopyranosid)uronic acid (**1**) in M sulfuric acid solution at 100°, is 5-hydroxymethyl-2-furaldehyde (**2**), presumably produced *via* loss of C-7 by decarboxylation. The mechanism of the conversion was studied by synthesis of the [1-^{14}C]**1** and conversion to 5-hydroxymethyl-2-fur[^{14}C]aldehyde (**2**-^{14}C). Conversion of **1** into **2**-^{14}C in acidified D_2O, followed by measurement of C-bound D by ^{1}H-n.m.r.–m.s. showed that **2**-^{14}C was labeled only at D-3 (40 atom %) of the furan ring. The major portion of the water-soluble materials was shown (by m.s.) to be D-*glycero*-D-*gulo*-hepturonic acid, and lactone derived therefrom, resulting from the hydrolysis of **1**.



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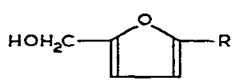

2 R = CHO
3 R = CO_2H

INTRODUCTION

The acid-catalyzed dehydration of uronic acids, particularly of hexuronic acids, is well known, but the mechanism of the reaction is still undetermined, although a

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number of suggestions have been made[1-4]. The hexuronic acids are known to be decarboxylated quantitatively to give 2-furaldehyde, reductic acid (2,3-dihydroxycyclopenten-1-one), and 5-formyl-2-furoic acid[5], but quantitative data are lacking for other types of uronic acids. Although some penturonic acids[6] and D-threuronic acid[7] are known to decompose in acidic media, no quantitative data were reported. Previously we prepared D-xyluronic acid and (methyl β-D-*glycero*-D-*gulo*-heptopyranosid)uronic acid (**1**), and compared the decarboxylation rates and yields of carbon dioxide with those given by D-galacturonic acid, which is known to be decarboxylated quantitatively[8]. D-Galacturonic and D-xyluronic acids are decarboxylated at similar rates, giving a yield of carbon dioxide corresponding to 88% of the theoretical value, but when **1** had completely disappeared, a yield of only 16% of carbon dioxide was measured. The present paper reports the dehydration of **1** in acidic media at elevated temperature, and some isotope-tracer experiments relative to the production of 5-hydroxymethyl-2-furaldehyde (**2**), presumably a decarboxylation product, which is produced in the reaction.

RESULTS AND DISCUSSION

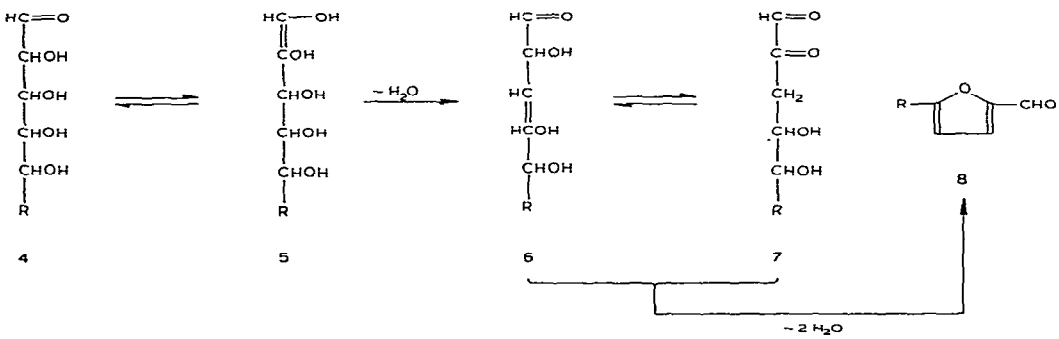
The observation that complete disappearance of **1** occurs in acidic media with only a 16% yield of carbon dioxide[8] suggests a complex dehydration mechanism involving multiple pathways. T.l.c. examination of the reaction mixture verified this conclusion, as several degradation products were obtained. The ethyl acetate–extractable portion of the dehydration reaction mixture contained, as a major component, a compound having t.l.c. properties identical to those of **2**. This compound was isolated by preparative t.l.c. and was shown to be **2** (a) by m.s., which showed two major fragments, the first at *m/z* 126 (85%) being M^+, and the second at *m/z* 97 (100%) corresponding to $M^+ - CHO$, and (b) by its ^{1}H-n.m.r. and u.v. spectra, which were both identical to those of an authentic sample.

To study further the mechanism of this conversion, D-*glycero*-D-*gulo*-[1-^{14}C]-heptose was synthesized and then converted into [1-^{14}C] **1**. When this compound was treated with acid and the resulting **2** isolated and purified, it retained the majority of the radiochemical activity initially present in the starting compound, indicating that **2**, produced in the reaction, is a decarboxylation product. Compound **2** was then oxidized to the furoic acid **3**, and the carboxyl group removed by treatment with mercuric chloride, to give a 2-chloromercuri-5-hydroxymethyl derivative[9] that had only 7% of the activity of **2**-^{14}C. Thus, C-1 of the original **1** corresponds to the aldehyde carbon atom of **2**. The same agreement has been demonstrated for 2-furaldehyde produced from pentoses[10] and hexuronic acids[5]. The procedure for preparing chloromercuri derivatives from **2**, reported herein, is a general one for removing carboxyl groups from furan rings[9], but has not previously been reported for decarboxylating **3**. The structure of the 2-chloromercuri derivative was ascertained by its ^{1}H-n.m.r. spectrum, elemental analysis, and mass spectrum.

In order to interpret the mechanism of the reaction, the dehydration of **1** to **2**

was performed in acidified deuterium oxide, and the carbon-bound deuterium atoms in **2** were estimated by ^{1}H-n.m.r. signal-diminution measurements. The data show a 40% deuterium exchange at C-3 of the furan ring, but no detectable exchange at other positions. The incorporation of deuterium at C-3 of **2** is significant, because it clearly demonstrates that **2** produced in the reaction did not arise *via* a hexose impurity, and that hexoses are not produced as intermediates in the dehydration sequence, as hexoses are dehydrated to **2** in acidic media with essentially no solvent-proton exchange at any position on the furan ring[11]. These observations are in general agreement with isotope-exchange data for the dehydration–decarboxylation[4] of D-glucuronic acid to **2**, which contained no isotope at the aldehyde carbon and was labeled preponderantly at C-3 (35%) of the furan ring, although smaller amounts of isotope were found at C-4 and -5 of the furan ring as well.

It is generally accepted that aldose sugars and uronic acids (**4**) are dehydrated in acidic media *via* a 1,2-enediol (**5**) to an enolic form (**6**) of a 3-deoxyglycosulose (**7**), and then to **8** (see Scheme 1).



Incorporation of deuterium or tritium at C-3 of the furan ring may, therefore, be accounted for by equilibration of **6** and **7** in the isotopic water. The findings herein for **1** are also consistent with the mechanism of dehydration *via* intermediates analogous to **4**–**8**.

It is noteworthy that no carbon-bound deuterium was found at the hydroxymethyl group of **2**. Presumably, this group corresponds to C-6 of **1**, and, as such, must gain one hydrogen atom during the decarboxylation–dehydration sequence. Whatever the mechanism for the decarboxylation, it is obvious that one of the hydrogen atoms of the hydroxymethyl group of **2** cannot be solvent-derived. Otherwise, it would contain at least 50% of deuterium. The evidence for this labeling sequence as described is quite conclusive. In addition to the ^{1}H-n.m.r. data, which show incorporation only at C-3, the mass spectrum for the deuterated **2** shows a peak at *m/z* 127 corresponding to $M^+ + 1$, indicating a monodeuterated material, but no peak at *m/z* 128 (for a dideuterio derivative) was observed.

EXPERIMENTAL

General methods. — Melting points are uncorrected. T.l.c. was performed on Silica gel HG-254 (Brinkmann) plates using 1 : 1 (v/v) hexane–ethyl acetate as the irrigant. The plates were examined under u.v. irradiation, and then sprayed with 10% sulfuric acid in ethanol and heated for 10 min at 100° to give charred spots on a white background. High-performance, liquid chromatography (l.c.) was conducted on columns packed with Aminex cation-exchange resin (Ca^{2+}, BioRad), using water at 75° as the irrigant, and a refractive-index detector. N.m.r.-spectra were recorded at 60 MHz. Electron-impact mass spectra (e.i.m.s.) were recorded following direct insertion or gas chromatography using 3% of SP 2250 on Supelcoport. Radiochemical determinations were made with a Beckman LS 7000 scintillation counter.

Methyl β-D-glycero-D-gulo-*heptopyranoside.* — The starting material for this preparation, D-*glycero*-D-*gulo*-heptose, was prepared from the corresponding lactone by reduction with sodium borohydride as described by Wolfrom and Thompson[12]. The glycoside was prepared by the procedure described by Angyal *et al.*[13] with the following modifications: at the end of the reaction (7 h), when cold, the mixture was made neutral with sodium methoxide and evaporated. The residue was then dissolved in water in order to give a 2–3% solution, which was treated with Dowex 50 (H^+) and then with Dowex SBr 1-X8 (OH^-) ion-exchange resins. Evaporation gave the expected (and previously observed[8]) mixture of α-D- (R_F 43 min, l.c.) and β-D-pyranoside (R_F 19 min, l.c.). The pure β-D form was isolated by crystallization, m.p. 170° (lit.[13] m.p. 170°), $[\alpha]_D^{25}$ −74.7° (*c* 1.0, water). This compound showed one peak, on g.l.c. of the *O*-acetyl (acetic anhydride–pyridine) and the *O*-trimethylsilyl derivatives, respectively.

Preparation of sodium (*methyl* β-D-glycero-D-gulo-*heptopyranosid*)*uronate* (*sodium salt of* **1**). — This compound, already described by Madson and Feather[8], was obtained from the foregoing methyl heptopyranoside by oxidation with platinum on charcoal as described by Marsh and Levvy[14]. To the aqueous solution (10 g in 150 mL) of the starting material was added the catalyst (5 g), and oxygen was bubbled into the stirred suspension at 55°. The pH was maintained between 7.5 and 8 by addition of M sodium hydrogencarbonate solution. After an equivalent amount of this solution had been added (3–4 h), the mixture was filtered on a sintered-glass funnel. The filtrate was evaporated, and the residue was converted into the acid form by passage of its solution, in water, through a column of Dowex 50 (H^+) ion-exchange resin. Pure material (R_F 11.0 min, l.c.) was isolated by elution from a column of Amberlite IRN-78 (CO_2^-, by treatment with 50mM formic acid) anion-exchange resin, as described by Madson and Feather[8]. The free acid (1.0 g) in water (25 mL) was neutralized with M sodium hydroxide, and the solution evaporated. The residue was dissolved in hot methanol (200 mL) and filtered. The filtrate was used in the following experiments.

Dehydration reaction. — A stirred solution of the sodium salt of **1** (2 g, 8 mmol), concentrated sulfuric acid (0.25 mL), and M sulfuric acid (20 mL) was kept

for 2 h at 100°. After cooling to 20°, a stoichiometric amount of sodium hydroxide dissolved in a small amount of water was added. In studies of the composition of the organic extract, barium carbonate was substituted for sodium hydroxide, to give an essentially ion-free, easily extractible solution. After filtration, the solution was extracted 3 times with 30-mL portions of ethyl acetate. The combined organic phases were dried (sodium sulfate), evaporated, weighed, and purified by t.l.c. The major component (R_F 0.3) was found to be **2** (yield <1%) by m.s. [*m/z* 126 (M^+, 85%), 97 (M^+ — CHO, 100%)], and by comparison of t.l.c., u.v., and n.m.r. data with those of an authentic sample.

Water-soluble products. — The aqueous phase was evaporated, and the residue extracted with methanol (40 mL). The mixture obtained after evaporation (0.6 g) was separated by l.c. Two major components, which were interconvertible, were obtained, the first (25 mg) having R_T 15 min, and the second (170 mg) R_T 28 min. They were converted[15] to the per-*O*-trimethylsilyl derivatives for g.l.c.–m.s. analysis by adding anhydrous pyridine (3 drops) and then *N*-trimethylsilylimidazole (0.2 mL), and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.6 mL) (Pierce Chemical Co., Rockford, IL 61105) to 1 mg of product.

The second component was converted into the *O*-methyloxime-*O*-(trimethylsilyl) derivative by use of the following procedure[15]. To the product (1 mg) in a vial were added methanol (0.3 mL), the reagent (0.2 mL) [obtained by dissolving *O*-methylhydroxylamine hydrochloride (300 mg) in dry methanol (1.0 mL) and pyridine (1.78 mL)], and 1-dimethylamino-2-propanol (0.22 mL). The mixture was heated for 15–20 min at 70° and, when cold, was evaporated under a stream of dry air (10 min). The residue was then treated with *N*-trimethylsilylimidazole and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide, as described earlier.

The second component was converted into the *O*-benzyloxime-*O*-(trimethylsilyl) derivative as described earlier for the *O*-methyl derivative, except that *O*-benzylhydroxylamine hydrochloride (570 mg) was used instead of *O*-methylhydroxylamine hydrochloride (300 mg). The following g.l.c.–m.s. data (*m/z* ±2) were collected for various derivatives of both fractions indicating that the first fraction is **1** and the second fraction is a mixture of **1** and its lactone.

O-*Benzyloxime*-O-(*trimethylsilyl*) *derivative of the 2nd component*:

(*a*) Acid (*E* or *Z*), R_T (240°) 8 min; m.s.: *m/z* 670 (M^+ — C_7H_7) and 542 (M^+ — CO_2SiMe_3 — $CHOSiMe_3$).

(*b*) Acid (*E* or *Z*), R_T (240°) 9.3 min; m.s.: *m/z* 732, 670 (M^+ — C_7H_7), and 542 (M^+ — CO_2SiMe_3 — $CHOSiMe_3$).

(*c*) Lactone, R_T (240°) 11.4 min; m.s.: *m/z* 599 (M^+) and 584 (M^+ — Me).

O-Methyloxime-O-(trimethylsilyl) derivative of 2nd component:

(*a*) Acid (*E* or *Z*), R_T (190°) 7.3 min; m.s.: *m/z* 653 (M^+ — MeOH), 568 (M^+ — CO_2SiMe_3), and 466 (M^+ — CO_2SiMe_3 — $CHOSiMe_3$).

(*b*) Acid (*E* or *Z*), R_T (190°) 9.2 min; m.s.: *m/z* 670 (M^+ — Me) and 466 (M^+ — CO_2SiMe_3 — $CHOSiMe_3$).

(*c*) Lactone, R_T (190°) 10.7 min; m.s.: *m/z* 523 (M^+), 508 (M^+ — Me), and 479 (M^+ — CO_2).

O-(*Trimethylsilyl*) *derivative of 2nd component*:

(*a*) Lactone, R_T (190°) 8.3 min; m.s.: *m/z* 494 (M^+) and 479 (M^+ — Me).

(*b*) Acid and lactone, R_T (190°) 9.4 and 8.3 min; m.s.: *m/z* 641 (M^+ acid — Me), 494 (M^+ of lactone), and 479 (M^+ of lactone — Me).

O-(*Trimethylsilyl*) *derivative of 1st component*:

(*a*) Acid (α- or β-D anomer), R_T (190°) 6.7 min; m.s.: *m/z* 641 (M^+ — Me).

(*b*) Acid (α- or β-D anomer), R_T (190°) 9.4 min; m.s.: *m/z* 641 (M^+ — Me).

Conversion of **1** *into* **2** *in acidified deuterium oxide solution.* — To the sodium salt of **1** (10 g, 38 mmol) was added, twice, deuterium oxide (5 mL), followed by evaporation. Concentrated sulfuric acid (3.5 mL) was similarly treated. The two materials were added to deuterium oxide (37 mL), and the dehydration reaction was conducted as described earlier. The mass spectrum of purified **2** showed the incorporation of one deuterium atom; m.s.: *m/z* 126, 127 (M^+), 98 (M^+ — CHO), and 97. ^{1}H-n.m.r. data indicated that the incorporation of D occurred of C-3 of the furan ring (~40% of incorporation).

D-glycero-D-gulo-[^{14}C]*Heptono-1,4-lactone.* — The method described by Fischer[16] and later by Hudson *et al.*[17] was used for the preparation of the labeled compound with some slight modifications: sodium (^{14}C) cyanide (250 μCi, ~1.6 mg), inert sodium cyanide (5.4 g, 110 mmol), and calcium chloride hexahydrate (13.2 g, 60 mmol) were dissolved in water (170 mL). After filtration, D-glucose (18 g, 100 mmol) was added, and the solution was stirred for 24 h at room temperature. Calcium hydroxide (7.4 g, 100 mmol) was then added. The precipitate was removed by filtration and washed with cold, aqueous calcium hydroxide solution until a negative chloride test for the filtrate was obtained. Water (200 mL) was added to the precipitate, and the mixture was treated with a slight excess of Dowex 50 (H^+) cation-exchange resin. The syrup obtained by evaporation crystallized after several days in the cold. The crystals were filtered off and washed with ethanol. The first crop afforded 4.2 g (20%) of a product having a specific activity of 2 μCi/mmol. This product was diluted with inert D-*glycero*-D-*gulo*-heptono-1,4-lactone (15.8 g) and was then reduced[12] to D-*glycero*-D-*gulo*-[1-^{14}C]heptose.

Sodium 5-hydroxymethyl-2-[*2-*^{14}*C*]*furoate* (*sodium salt of* **3**-^{14}*C*). — Crude **2** (20 mg) resulting from the dehydration of **1**-^{14}C (1.780 g, 6.85 mmol, 0.44 mCi/mol) was diluted with inert **2** (380 mg) before purification by t.l.c. To convert **2**-^{14}C into **3**-^{14}C, silver oxide (1 g) was added in small portions to purified and diluted **2**-^{14}C (152 mg, 1.21 mmol, 4.3 μCi/mol) in water (15 mL), and the pH was kept at 10 by dropwise addition of M sodium hydroxide solution to the stirred solution. At the end of the reaction (~15 min), the suspension was filtered on a Celite pad, and Dowex 50 (H^+) cation-exchange resin was added to the solution. After removal of the resin, the solution was made neutral with a stoichiometric amount of 0.1M sodium hydroxide and evaporated to give 162 mg (82%, 4.3 μCi/mol); ^{1}H-n.m.r. (D_2O, external Me_4Si): δ 7.0 (d, 1 H, H-4), 6.45 (d, 1 H, H-3), and 4.6 (s, 2 H, CH_2OD).

2-Chloromercuri-5-(hydroxymethyl)furan. — This compound was prepared by use of mercuric chloride as described by Gilman and Wright[9]. To the sodium salt of 3-^{14}C (104 mg, 0.6 mmol, 4.3 μCi/mol) in water (10 mL) was added mercuric chloride (198 mg, 0.7 mmol) in water (15 mL). The solution was stirred for 1 h at room temperature, and then filtered. The filtrate was boiled until no more carbon dioxide was evolved. When cold, the solution was filtered and concentrated. The precipitate that formed was filtered off, stirred with methanol (15 mL), filtered off, and the filtrate evaporated. To the residue, ethyl acetate (20 mL) was added and, after filtration of the insoluble portion, the solution was evaporated to give 197 mg (93%) of a product having a residual radioactivity <0.3 μCi/mol (<7% of the radioactivity of starting-compound 3); m.p. 120°; t.l.c.: R_F 0.5; ^{1}H-n.m.r. (CD_3CN, external Me_4Si): δ 6.2 (s, 2 H, H-3, -4), 4.3 (s, 2 H, CH_2), and 3.0 (br. s, 1 H, OH); m.s.: *m/z* 334 (M^+, 15%) and 202 (Hg^+, 11%).

Anal. Calc. for $C_5H_5ClHgO_2$: C, 18.02; H, 1.50. Found: C, 17.95; H, 1.59.

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