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Note

Loss of C-5 hydrogen during conversion of D-glucuronic acid into methyl α-D-glucopyranoside*

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When heated in anhydrous, methanolic hydrogen chloride, D-glucuronolactone is converted into methyl (methyl D-glucopyranosid)uronate[1] (**1**) The latter, on reduction with lithium aluminum hydride in aprotic solvents or with sodium borohydride in aqueous medium, has been reported to give methyl α-D-glucopyranoside (**3**) in 35% yield[2] In our studies of the mechanism of 5-epimerization during the biosynthesis of heparin from precursor uridine 5′-(α-D-glucopyranosyluronic acid pyrophosphate)[3], we sought a convenient method for the conversion in high yield of D-glucuronic acid into **3** on a micromolar scale Examination of the reduction of **1** with sodium borohydride in methanol revealed that the reaction occurs with essentially complete loss of H-5, but not of hydrogen at other carbon atoms Details are presented in this Note

EXPERIMENTAL

Materials — ^{3}H-Labeled D-glucose was purchased from the Radiochemical Center, Amersham–Searle U S A, and 2-^{3}H-labeled glycerol was purchased from ICN Pharmaceuticals, Irvine, CA Uridine 5′-(α-D-glucopyranosyluronic acid pyrophosphate), specifically ^{3}H-labeled in various positions, was prepared from D-[2-^{3}H]glucose (5 mCi/μmol), D-[3-^{3}H]glucose (2100 μCi/μmol), D-[4-^{3}H]glucose (6100 mCi/μmol), or D-[5-^{3}H]glucose (2400 μCi/μmol) by the method previously described[3] Phosphate diesterase I (from *Crotalus adamanteus* venom) and alkaline phosphatase (from *Escherichia coli*) were obtained from Worthington Biochemical Corporation, Freehold, New Jersey, and glycerol dehydrogenase and L-glutamic dehydrogenase from Sigma, St Louis, Missouri Methanol was dried by distillation from magnesium methoxide and stored over molecular sieves Paper chromatography

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was performed on Whatman No 1 paper in the following solvents *A*, 7 3 (v/v) ethanol–M ammonium acetate, pH 7 5, *B*, 4 1 5 (v/v/v) 1-butanol–ethanol–water (upper phase), *C* 14 7 10 (v/v/v) 1-butanol–propanoic acid–water[4], and *D*, 7 2 1 (v/v/v) 1-propanol–ethyl acetate–water Carbohydrates were detected with alkaline silver nitrate[3], glycerol and 1,3-dihydroxy-2-propanone with potassium permanganate spray (3 ml M aqueous potassium permanganate diluted to 100 ml with acetone), and glutamic acid with ninhydrin[6]

Preparation of D-*glucuronic acid from uridine 5'-(*α-D-*glucopyranosyluronic acid pyrophosphate)* — Incubation mixtures contained the following in a final volume of 60 μl Tris HCl pH 7 5 2 5 μmol, magnesium chloride, 0 4 μmol ^{14}C-labeled or specifically ^{3}H-labeled uridine 5' (α-D-glucopyranosyluronic acid pyrophosphate) 0 5 mCi, and phosphate diesterase I (1 mg) After 1 h at 25°, the mixture was kept for 2 min at 100° Alkaline phosphatase (5 μl) was added to the cooled mixture and, after a further 45 min at 25°, the mixture was subjected to electrophoresis on Whatman 3 MM paper in 0 05M ammonium formate, pH 3 6 The single radioactive compound present which had the mobility of D-glucuronic acid, was eluted with water purified by paper chromatography in solvent *A*, and finally eluted with water

Synthesis of methyl γ-D-*glucopyranoside* (**3**) *from* D-*glucuronic acid* — Unlabeled D-glucuronic acid (1 mg) was mixed with specifically ^{3}H-labeled, or ^{14}C-uniformly labeled (0 2 mCi) D-glucuronic acid, obtained as already described in a Teflon-lined screw-cap tube and the mixture was dried at 37° in a vacuum Dry methanol (1 ml) and acetyl chloride (70 μl) were added, and the tube was tightly capped After 4 h at 85° the mixture was cooled to 25° solid sodium borohydride (40 mg) was added all at once (the temperature of the mixture at this point rose to 45°), and the tube was immediately capped and maintained for 12 h at 25° The mixture was then diluted with 5 ml of water and the pH was adjusted to 2 0 with Dowex-50 (H^+) to decompose the excess of borohydride The resin was removed by filtration and boric acid present in the mixture was removed by repeated evaporation of methanol from the product under diminished pressure The salt-free residue was subjected to paper chromatography in solvent *B* Radioactivity was located by strip scanning, and the labeled compound was eluted with water and rechromatographed in solvent *C* The product was identified as **3** by cochromatography with authentic, unlabeled **3** in solvents *B* and *C* Only the γ anomer was detectable in the product upon chromatography in solvent *C* under conditions that separate the anomers Upon heating in M HCl for 30 min at 100° only one radioactive compound, having the chromatographic mobility of D-glucose in solvent *D*, was released from the presumed methyl D-glucopyranoside

Compound **3** was degraded by a modification of the method of Smith *et al* [7] After treatment with periodate as described by these authors, formic acid was distilled from the mixture at room temperature The residue was treated with sodium borohydride with subsequent hydrolysis by hydrochloric acid[7] The glycerol and glycoaldehyde formed were separated by paper electrophoresis on Whatman No 1 filter paper in 0 1M sodium hydrogensulfite, 0 01M sodium acetate buffer, pH 4 7, and the radioactive compounds were located with a Nuclear-Chicago 4π strip counter

Glycerol eluted from electrophoretograms was further purified by paper chromatography in solvent *D* and eluted with water The glycerol isolated was incubated in 50 μl of 0 5M ammonium carbonate, pH 8 5, containing 100 μg of glycerol dehydrogenase, 1 μmol of NAD^+, 0 5 μmol of 2-oxoglutarate, and 100 μg of L-glutamic dehydrogenase After 24 h at 25°, the mixture was subjected to chromatography on Whatman No 1 paper in solvent *D* Under these conditions, the label of [2-^{3}H]-glycerol could be recovered quantitatively in D-glutamic acid

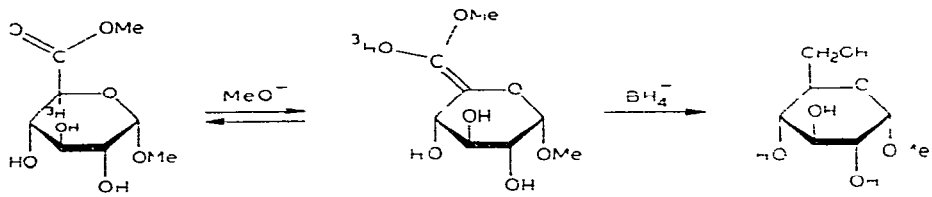
RESULTS AND DISCUSSION

Uniformly ^{14}C-labeled D-glucuronic acid in 1% methanolic hydrogen chloride was heated in a sealed tube for 4 h at 82° the acid was neutralized with silver carbonate, and the silver salts were filtered off Methanol was removed under a stream of nitrogen and the product was reduced with aqueous sodium borohydride by addition of sodium borohydride (40 mg) in 1 ml of water After subsequent processing, as described, the mixture was resolved into five components by paper chromatography in solvent *C* The best yield of methyl α-D-glucopyranoside (**3**) thus isolated, based on radioactivity, was about 15% When solid sodium borohydride (40 mg) was added directly to the anhydrous mixture without prior removal of hydrochloric acid or methanol ^{14}C-labeled **3** could be isolated from the mixture in 95% yield Also, D-[2-^{3}H]- D-[3-^{3}H]- and D-[4-^{3}H]-glucuronic acid gave **3** in >95% yield (based on radioactivity) Methyl α-D-glucopyranoside (**3**) from D-[2-^{3}H]glucuronic acid yielded glycolaldehyde as the sole radioactive product of periodate degradation in the case of D-[3-^{3}H]glucuronic acid, only [^{3}H]formate resulted D-[4-^{3}H]Glucuronic acid yielded [1-^{3}H]glycerol (shown by the inability of glycerol dehydrogenase and glutamic dehydrogenase to transfer the ^{3}H to 2-oxoglutarate) These results show that the only ^{3}H label present in each of the labeled methyl α-D-glucopyranosides was present at the position of original labeling of the parent D-glucose used for preparation of uridine 5′-(α-D-glucopyranosyluronic acid pyrophosphate) However under identical conditions, D-[5-^{3}H)glucuronic acid lost >85% of the tritium, methyl α-D-[^{3}H]-glucopyranoside was isolated in less than 15% yield (based on radioactivity) indicating loss of at least 85% of the initial label at C-5 No other labeled material could be detected on chromatograms Conversion of authentic D-[5-^{3}H]glucose into methyl α-D-[^{3}H]glucopyranoside by the method of Fischer[8] proceeded with no loss of label, and periodate degradation of this compound indicated that the label was located at C-5 (as shown by transfer of all of the label from [^{3}H]glycerol to 2-oxoglutarate by the combined action of glycerol dehydrogenase and glutamic dehydrogenase)

Loss of H-5 during conversion of methyl (methyl D-glucopyranosid)uronate (**1**) into **3** probably does not involve 4 5-dehydration such as has been demonstrated with D-glucopyranosiduronate 4-sulfonates[9] or with α-D-glucopyranosyl-(1→4)-γ-D-glucopyranosiduronates[10] In the latter two examples, the presence of a good leaving-group at C-4 promotes β-elimination with the resulting formation of a 4 5-un-

saturated product However, as compound **1** is unsubstituted at C-4, there is no promoting effect of a leaving group possible Furthermore, involvement in the reaction of dehydration leading to a double bond between C-4 and C-5, followed by rehydration, might be expected to occur with some randomization of configuration at C-4 and C-5, yielding a mixture of methyl glycosides However, such randomization did not occur, as only D-glucose was detectable in a hydrolyzate of the methyl glycoside prepared by way of D-glucuronic acid

When the series of reactions, described for conversion of D-glucuronic acid into **3** was performed with sodium methoxide instead of sodium borohydride, loss of 5-^{3}H (by conversion into a volatile product, presumably methanol) was in the order of 10%, suggesting that basic conditions contribute to labilization of H-5 In the light of this observation, the formation of **3** from **1** may reasonably be explained as follows



1 2 3

The intermediate **2** resulting from base-catalyzed enolization of **1** very rapidly exchanges ^{3}H with the medium Removal of **2** from the reaction by reduction with BH_4^- shifts the equilibrium between **1** and **2** to the right thereby allowing extensive ^{3}H exchange to occur As no other hexopyranosides are formed the overall reaction must proceed with retention of the original configuration at C-5

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