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Note

^{14}C-Tracer studies on the conversion of methyl β-D-*ribo*-hexosid-3-ulose into reductic acid (2,3-dihydroxycyclopenten-1-one)*

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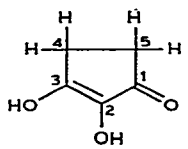
By mechanisms not completely understood, reductic acid (2,3-dihydroxycyclopenten-1-one, **1**, shown with the appropriate carbon atoms numbered) is produced from pentoses[1,2], 2-furaldehyde[2,3], and hexuronic acids[1,4] (and from polymers containing such constituents) by treatment with acid at elevated temperatures.



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Labeling experiments with ^{14}C-labeled starting-materials[2,4] have shown that, for the foregoing compounds, no single order of the original carbon chain can be assigned in the derived reductic acid. Some time ago, Theander reported[5] that **1** is also produced, in much higher yields than from these compounds, from methyl β-D-*arabino*-hexopyranosid-2-ulose (**2**) and its related D-*ribo*-3-keto isomer (**3**). From compound **3**, no *ribo*-hexos-3-ulose could be detected, but a small amount of D-*erythro*-pentulose was isolated. Although mechanisms have been proposed[6] for the conversion, no confirmatory experiments have ever been undertaken to evaluate them. This report presents the results of radiochemical tracer experiments that show the relationship between the carbon atoms of **1** and **3** and also reports on the decomposition of the 4-keto isomer (**4**).

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RESULTS AND DISCUSSION

Pure samples of methyl β-D-glucopyranoside, labeled with ^{14}C at either C-1, C-2, or C-6 were prepared. These compounds were each converted into a mixture of **2**, **3**, and **4** by oxidation with bromine[7]. The products were separated by chromatography as the corresponding, more-stable O-methyloxime derivatives, from which the parent keto compounds were regenerated by mild treatment with acid. Glycoside **3** from the three, differently labeled methyl β-D-glucosides, respectively, was treated with dilute, aqueous sulfuric acid and the resulting **1** isolated. The overall yields are low in this conversion, and a considerable time-period elapsed between the isolation of **3**, and its subsequent chemical degradation. (Compound **1** was isolated in Sweden and then sent to the U.S. for determination of the position of the label.) As a result, a considerable amount of **1** appeared to have decomposed during transit. The exact amount of **1** remaining was measured spectrophotometrically at 270 nm (assuming $\varepsilon = 9{,}000$), and then the sample was diluted with a measured amount of inert **1**, purified to constant radiochemical activity, and the distribution of label determined in the diluted sample.

To determine the distribution of the ^{14}C-isotope ion **1**, the compound was first converted into succinic acid by oxidation with permanganate. This permits the activity at C-2 of reductic acid to be determined by measurement of the differences in the activities of succinic acid and the starting **1**. The activity on the carboxyl carbon atoms of succinic acid, which represent the chemically equivalent C-1 and C-3 of **1** and the methylene carbon atoms of succinic acid, representing the equivalent carbon atoms (C-4 and C-5) of **1**, were determined by Curtius degradation (the eventual conversion of succinic acid to ethylenediamine dihydrochloride, representing the methylene carbon atoms of succinic acid) as described in previous reports[4,8].

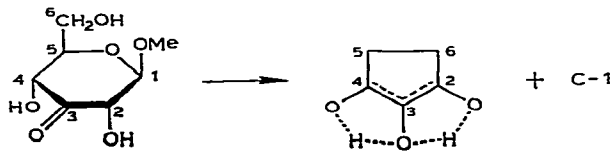
For the case of [1-^{14}C]-**3**, having a specific activity of 1.58 μCi per mmol, 5.0 g gave rise to 2.02 mg of **1**. The sample, in 25 mL of ethanol, was identified as **1** from its characteristic u.v. spectrum and by comparison of its t.l.c. mobility with that of an authentic sample. Compound **1** was diluted with exactly 2.000 g of inert material and, after evaporation to dryness, it was purified by alternate recrystallization from ethanol followed by sublimation at 0.2 mmHg and 150° to constant activity. All subsequent samples of **1** were processed in the same manner. Had C-1 of **3** been retained in **1**, then the expected activity would be 1.6×10^{-3} μCi per mmol. The value determined was 7.3×10^{-5} μCi per mmol. Thus, a negligible proportion (4.5%) of the activity of [1-^{14}C]-**3** is found in **1**, indicating that C-1 of **3** is lost during the conversion.

Compound [2-^{14}C]-**3** (5.0 g, specific activity 0.146 μCi per mmol) was treated to give 7.125 mg of **1**, 6.500 mg of which was diluted with 1.000 g of inert **1**. The calculated activity of **1**, assuming that it contains all the activity of [2-^{14}C]-**3**, is 0.95×10^{-3} μCi per mmol; the value found was 1.09×10^{-3} μCi per mmol, indicating that, within experimental error, all of the activity is retained. Likewise, the succinic acid obtained from **1** had an activity of 0.91×10^{-3} μCi per mmol and

the ethylenediamine dihydrochloride obtained from the succinic acid had an activity of 1.28×10^{-4} μCi per mmol. Thus, nearly all (91.5%) of the total activity contained by the [2-^{14}C]-**3** and **1** is found in the equivalent carbon atoms 1 and 3 of **1**.

The [6-^{14}C]-**3** had specific activity 1.995 μCi per mmol. Reaction of 5.0 g gave 3.33 mg of **1**, which was diluted with 2.000 g of inert **1**. The calculated activity (for complete radiochemical conversion) is 3.32×10^{-3} μCi per mmol; the value determined was 3.30×10^{-3} μCi per mmol. The succinic acid obtained from **1** had a specific activity of 3.4×10^{-3} μCi per mmol and the ethylenediamine dihydrochloride obtained from the succinic acid had a specific activity of 3.30×10^{-3} μCi per mmol. Thus C-6 of **3** corresponds to C-4 and C-5 of **1**.

The data collected show that C-1 of **3** is lost during its conversion into **1**, that C-2 of **3** corresponds to C-1 and C-2 of **1**, and that C-6 of **3** corresponds to C-4 and C-5 of **1**. The relationship between the carbon atoms of compound **3** and compounds **1** are shown in the accompanying scheme. The numbering shown for **1** corresponds to the respective carbon atoms of compound **3**. These labeling data are consistent with the mechanism proposed for this conversion, as given in a prior article[6].



3 **1**

On treatment of **4** (the isomeric glycosid-4-ulose) with acid, no reductic acid was detected. The major product proved to be 3-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one[9], as evidenced by a comparison of its chromatographic (t.l.c.), mass-spectral, and n.m.r. properties with those of an authentic sample.

EXPERIMENTAL

Materials and methods. — T.l.c. was performed on silica gel HF plates with 9:1 (v/v) chloroform–acetic acid as the irrigant. Reductive acid was detected by u.v. irradiation. Spectra were recorded with a Perkin–Elmer Hitachi, Model 124, double-beam, grating instrument. Radiochemical determinations were made with a Packard Tri-Carb scintillation counter with Aquasol (Beckman) as the counting medium. Efficiencies were determined by using [^{14}C]toluene as the internal standard. Labeled sugars were obtained from New England Nuclear Corp.

Synthesis of methyl [^{14}C]glucosides. — In a typical experiment, 20 μCi of sugar (<1 mg) was diluted with 10 g of inert, anhydrous D-glucose and converted into methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (by the silver carbonate procedure) via tetra-*O*-acetyl-α-D-glucopyranosyl bromide[10]. The resulting crystals

were repeatedly crystallized from ethanol to constant activity and were then conventionally *O*-deacetylated with sodium methoxide in methanol.

Preparation of **2**, **3**, *and* **4**. — In a typical experiment, methyl β-D-[6-^{14}C]glucopyranoside (5.0 g) was treated with bromine in aqueous solution at pH 7 and the resulting mixture of keto derivatives was converted into the corresponding, more-stable *O*-methyloximes as previously described[7]. The evaporated product was extracted with boiling chloroform (10 × 40 mL, reflux). The extract was dried (sodium sulfate), evaporated, and the product fractionated on a column (100 × 2 cm) of silica gel with 40:10:1 ethyl acetate–chloroform–pyridine as irrigant. The *O*-methyloximes of methyl β-D-*arabino*-hexopyranosid-2-ulose (**2**, 0.67 g), methyl β-D-*ribo*-hexopyranosid-3-ulose (**3**, 0.48 g), and methyl β-D-*xylo*-hexopyranosid-4-ulose (**4**, 0.77 g) were isolated and, in each case, found identical with authentic samples[7].

Conversion of **3** *into* **1**. — Methyl β-D-*ribo*-hexopyranosid-3-ulose (0.40 g), obtained after treatment of the corresponding *O*-methyloxime with cation-exchange resin[7], was treated with 0.25M sulfuric acid (3 h, 100°)[5]. The solution was then continuously extracted by ethyl acetate (2 × 24 h) and the product (200 mg) obtained after evaporation of the dried (sodium sulfate) extract was fractionated on a column (14 × 1 cm) of silica gel with 40:10:1 chloroform–methanol–water as solvent. Reductic acid (**1**, 30 mg) was thus isolated chromatographically pure, but was further purified by alternate sublimation and crystallization, after dilution and before the subsequent degradation-reactions.

*Isolation of 3-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one after treatment of methyl* β-D-xylo-*hexopyranosid-4-ulose* (**4**) *with acid.* — When **4** (110 mg) was treated with 0.25M sulfuric acid as described for **3**, no reductic acid could be detected. The main, low-molecular product (15 mg) was isolated chromatographically from the ethyl acetate extract after fractionation on a column (30 × 1 cm) of silica gel with 40:10:1 chloroform–methanol–water as solvent. It was shown to be identical (t.l.c., m.s., and n.m.r.) with an authentic sample of 3-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one[9].

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