Synthesis and Characterization of D-*threo*-2,3-Dihydroxy-4-pentenal[1]

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The chemical degradation of coenzyme B_{12} by acid hydrolysis or cyanide treatment yields a carbohydrate fragment which has been characterized as D-*erythro*-2,3-dihydroxy-4-pentenal (I).[2-4] This paper describes the synthesis and characterization of the D-*threo* isomer II of this substance and outlines a general route for the synthesis of such compounds.

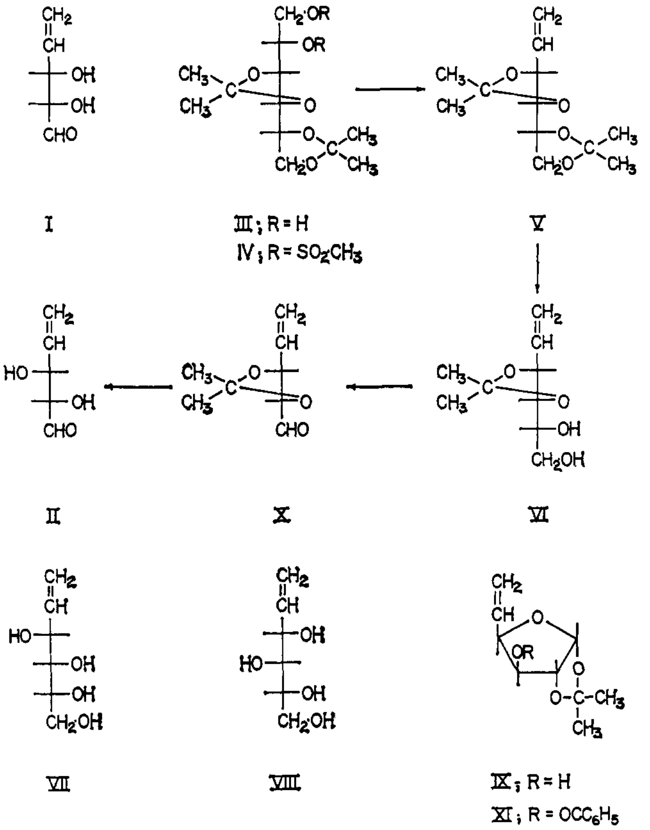
3,4:5,6-Di-*O*-isopropylidene-D-sorbitol (III) was prepared from D-sorbitol by the method of Bourne, *et al.*[5] Reaction with methanesulfonyl chloride gave the disulfonyl derivative IV in good yield. This compound, on heating with sodium iodide in acetone, underwent the expected reaction,[6] liberating 2 moles of sodium methanesulfonate and resulting in formation of the unsaturated sugar V. The rotation of V was similar to that reported for the isomeric substance "1,2:3,4-di-*O*-isopropylidene-5,6-mannitoleen."[7]

Selective acid hydrolysis of V removed the 5,6-isopropylidene residue to give VI. Stronger acid hydrolysis gave 5,6-didehydro-5,6-dideoxy-D-*arabo*-hexitol (VII), which had an R_f value similar to, but different from, that of authentic 5,6-didehydro-5,6-dideoxy-L-*xylo*-hexitol (VIII). The latter was prepared, for comparison, from 5,6-didehydro-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuranose (IX) by acid hydrolysis followed by borohydride reduction.

On oxidation with sodium periodate, VI consumed approximately 1 mole of oxidant and liberated 1 mole of formaldehyde within 30 min., which confirmed that the primary hydroxyl group was free. Quantitative oxidation of VI with periodate and isolation of the product gave 2,3-*O*-isopropylidene-D-*threo*-2,3-dihydroxy-4-pentenal (X), which on mild acid treatment gave the desired sugar, D-*threo*-2,3-dihydroxy-4-pentenal (II).

The structure of II was confirmed as follows. On oxidation with periodate, it consumed approximately 2 moles of oxidant and produced 2 moles of formate. The aldehyde formed by periodate oxidation gave a 2,4-dinitrophenylhydrazone identical with acrolein 2,4-dinitrophenylhydrazone. Ozonolysis, followed by reduction with sodium borohydride, gave a reaction product which migrated with threitol on paper chromatography. Threose was the reaction product, identified by paper chromatography, when II was reduced with borohydride and then subjected to ozonolysis. Hydroxylation of II by treatment with osmium tetroxide gave a reaction product which contained approximately equal amounts of xylose and arabinose.

CHART I



These same reactions were employed by Hogenkamp and Barker[3] in the identification of the sugar derived from coenzyme B_{12}, and the analogous results obtained here confirm the correctness of their structural deductions.

The synthetic method outlined above serves as a general method for the synthesis of such compounds. An alternate approach starting with IX was not successful. Benzoylation of IX yielded the 3-*O*-benzoate XI which was hydrolyzed in acid to remove the acetone group. It was expected that removal of C-1 by periodate oxidation would afford a derivative of II. However, investigation, by thin layer chromatography, of the product of acid hydrolysis of XI revealed two components instead of one as expected. Oxidation of the hydrolysate with sodium metaperiodate gave anomalous results, less than 1 molar equiv. of oxidant being consumed, and very little formate being produced. Since these results persisted even though the experimental conditions were altered, this approach was abandoned.

Experimental

Methods.—Paper chromatography was carried out using Whatman No. 1 paper with 1-butanol–95% ethanol–water (4:1:1, solvent A). Tetroses and pentoses were run in 1-butanol–pyridine–water (10:3:3, solvent B), and tetritols in ethyl acetate–pyridine–water saturated with boric acid (10:4:3, solvent C). Table I gives some R_f values. Column chromatography was done on neutral alumina (100–200 mesh) supplied by Bio-Rad Laboratories, Richmond, Calif.

Free sugars were determined with aniline phthalate or alkaline silver nitrate, while α-glycols were detected with a periodate–benzidene reagent or alkaline silver nitrate.

3,4:5,6-Di-*O*-isopropylidene-D-sorbitol (III).—This was prepared from 1,2:3,4:5,6-tri-*O*-isopropylidene-D-sorbitol (m.p. 47.5°) by the method of Bourne, *et al.*[5] It had m.p. 55–56° as reported.[5]

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3,4:5,6-Di-*O*-isopropylidene-1,2-di-*O*-methanesulfonyl-D-sorbitol (IV).—The above isopropylidene compound III, 3.17 g., in 30 ml. of pyridine was treated with 5.5 g. (4 molar equiv.) of methanesulfonyl chloride with cooling, and the reaction was left for 20 hr. at 2°. Isolation by chloroform extraction gave a sirupy product weighing 4.65 g. (93%), $[\alpha]^{23}\mathrm{D}$ +4.3° (*c* 3.8, chloroform).

Anal. Calcd. for $C_{14}H_{26}O_{10}S_2$: C, 40.22; H, 6.22; S, 15.31. Found: C, 40.68; H, 6.17; S, 14.94.

5,6-Didehydro-5,6-dideoxy-1,2:3,4-di-*O*-isopropylidene-D-*arabo*-hexitol (V).—The dimesyl derivative IV, 4.49 g., was dissolved in 87 ml. of acetone containing 12 g. of sodium iodide and the solution was heated at 105° for 5 hr. The mixture was cooled and filtered. The precipitate of sodium methanesulfonate weighed 3.15 g., 95% of the expected amount.[8] The filtrate and acetone washings were evaporated to dryness and the residue was shaken with 100 ml. of water containing 20 g. of sodium thiosulfate. This solution was extracted with chloroform, five 25-ml. portions, and the chloroform extract was dried and evaporated to dryness. The product was a mobile sirup (2.1 g., 86%) with $[\alpha]^{23}\mathrm{D}$ −3.6° (*c* 4.23, chloroform).

Anal. Calcd. for $C_{12}H_{20}O_4$: C, 63.16; H, 8.77. Found: C, 63.18; H, 8.76.

5,6-Didehydro-5,6-dideoxy-3,4-*O*-isopropylidene-D-*arabo*-hexitol (VI).—Compound V, 1.87 g., dissolved in 10 ml. of 65% aqueous acetic acid, was left at room temperature for 16 hr. The mixture was diluted with 8 ml. of water and the solution was neutralized with barium carbonate, filtered, and evaporated to dryness. The residue was extracted with acetone, five 15-ml. portions, and the extracts were re-evaporated to dryness. The final residue was dissolved in a little chloroform and chromatographed on a column of neutral alumina. Chloroform–ethanol (1:1) eluted the product as a sirup (0.7 g., 50%) with $[\alpha]^{23}\mathrm{D}$ +16.1° (*c* 3.25, ethanol).

Anal. Calcd. for $C_9H_{16}O_4$: C, 57.45; H, 8.51. Found: C, 57.27; H, 8.37.

Oxidation of VI with Sodium Periodate.—A solution of sodium metaperiodate, 0.1 g. in 5 ml. of water, was added to 0.032 g. of the sugar in 5 ml. of ethanol and the mixture was left at room temperature. A blank was run. Periodate consumption was determined by titration with arsenite solution and formaldehyde was estimated colorimetrically.[9] The number of moles of periodate consumed per mole of sugar was 0.97 (15 min.) and 0.98 (30 min.). The number of moles of formaldehyde released per mole of sugar after 30 min. was 1.08.

5,6-Didehydro-5,6-dideoxy-D-*arabo*-hexitol (VII) and 5,6-Didehydro-5,6-dideoxy-L-*xylo*-hexitol (VIII).—5,6-Didehydro-5,6-dideoxy-1,2:3,4-di-*O*-isopropylidene-D-*arabo*-hexitol, 10 mg., in 3 ml. of 65% aqueous acetic acid, was heated under reflux for 2 hr. and gave a chromatographically pure product (VII) on evaporation of the solvent (Table I).

TABLE I

CHROMATOGRAPHIC PROPERTIES OF VARIOUS INTERMEDIATES

Compd.	R_f value in solvent A
II	0.76[a]
VI	0.85
VII	0.43
VIII	0.49
X	0.90

[a] Slight streaking.

5,6-Didehydro-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuranose, 0.1 g. [m.p. 62–64°, $[\alpha]^{25}\mathrm{D}$ −56.2° (chloroform); lit.[10] m.p. 62–65°, $[\alpha]\mathrm{D}$ −51.5°], was dissolved in 4 ml. of 0.1 *N* hydrochloric acid and the solution was heated at 100° for 75 min. The cooled solution was passed through Amberlite CG45-resin (carbonate form) and evaporated to a sirupy residue. This was dissolved in 2 ml. of water and treated with sodium borohydride (about 25 mg.) for 18 hr. at room temperature. The mixture was treated with a few drops of glacial acetic acid, passed through Dowex-50 (H^+), and evaporated to dryness. The residue was dissolved in methanol and re-evaporated to dryness three times. The residue (VIII) was chromatographically pure.

2,3-*O*-Isopropylidene-D-*threo*-2,3-dihydroxy-4-pentenal (X).—About 0.7 g. of VI was dissolved in 10 ml. of water and treated with 1.0 g. (1.25 molar equiv.) of sodium metaperiodate at room temperature. After 30 min., investigation of an aliquot showed 0.98 molar equiv. of oxidant consumed. After 45 min., the solution was saturated with sodium chloride and extracted exhaustively with chloroform. The chloroform extract was evaporated to dryness giving the product as a sirup (0.54 g., 93%) with $[\alpha]^{23}\mathrm{D}$ +12.6° (*c* 2.11, ethanol).

Anal. Calcd. for $C_8H_{12}O_3$: C, 61.4; H, 7.7. Found: C, 60.4; H, 8.0.

D-*threo*-2,3-Dihydroxy-4-pentenal (II).—The isopropylidene derivative X, 0.17 g., dissolved in 6.4 ml. of dioxane and 4.8 ml. of 0.2 *N* sulfuric acid, was heated at 70° for 1 hr. The solution was diluted with ethanol, neutralized with barium carbonate, filtered, and evaporated to give a sirupy product (0.1 g., 80%) with $[\alpha]^{23}\mathrm{D}$ +12.7° (*c* 2.82, ethanol). Its paper chromatographic properties are indicated in Table I.

Anal. Calcd. for $C_5H_8O_3$: C, 51.72; H, 6.90. Found: C, 51.6; H, 7.13.

Oxidation of D-*threo*-2,3-Dihydroxy-4-pentenal.—The material (25.6 mg.) was dissolved in water containing sodium metaperiodate (0.15 g.) to give 5 ml. of solution. Periodate consumption was determined by titration with arsenite solution and formate release by titration with sodium hydroxide. The number of moles of periodate consumed per mole of sugar was: 1.68 (14 min.), 1.86 (30 min.), and 1.95 (120 min.). The number of moles of formic acid released after 120 min. was 1.72.

After 120 min. the solution was saturated with sodium chloride and extracted exhaustively with ether. The ether extract was treated with 1.8 ml. of a solution of 2,4-dinitrophenylhydrazine, prepared by dissolving 0.4 g. of 2,4-dinitrophenylhydrazine in 2 ml. of concentrated sulfuric acid, then adding 3 ml. of water and 10 ml. of ethanol. The solution was gently warmed to remove the ether solvent and the precipitate was collected by filtration. It had m.p. 160° (lit.[11] m.p. 165°) and an infrared spectrum identical with that of authentic acrolein 2,4-dinitrophenylhydrazone prepared under the same conditions.

Ozonolysis and Reduction of II.—The pentenal (20 μmoles) in 25 ml. of methanol was treated with ozone (150 μmoles) at −70° for 15 min. Excess ozone was removed by bubbling nitrogen gas through the solution, and a little triethyl phosphite was added. The solution was evaporated to dryness and the residue was dissolved in 5 ml. of water. This solution was treated with 5 mg. of sodium borohydride at room temperature for 20 hr. A few drops of acetic acid was added and the solution was passed through a column of Dowex-50 (H^+). The eluate was evaporated to dryness; the residue was dissolved in methanol and re-evaporated to dryness three times. On paper chromatography (solvent C), the residue gave one spot corresponding in R_f to threitol.

Reduction and Ozonolysis II.—The pentenal was treated as in the above experiment except the order of oxidation and reduction was reversed. The final product on paper chromatography (solvent B) gave a spot corresponding in R_f to that of threose.

Osmium Tetroxide Treatment of II.—The pentenal (10 μmoles) in 1 ml. of water was treated with 2 ml. of 2.5% aqueous osmium tetroxide at room temperature for 2 hr. During this time the solution became black. The solution was extracted three times with excess carbon tetrachloride and the water layer was evaporated to dryness. The residue was triturated with ethanol and subjected to paper chromatography. The reaction product was a mixture of xylose and arabinose, which appeared visually to be of equal concentration. A control experiment using pure xylose failed to turn black, and the only detectable product was xylose.

3-*O*-Benzoyl-5,6-didehydro-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuranose (XI).—IX, 1.3 g., dissolved in 8 ml. of pyridine, was treated with 2.2 ml. (2.7 molar equiv.) of benzoyl chloride at room temperature for 16 hr. The product was isolated by chloroform extraction to give a sirup. The sirup was extracted with petroleum ether (b.p. 60–70°) and the soluble extract was evaporated to dryness. The product crystallized from aqueous ethanol (1.68 g., 83%), m.p. 69–71°, $[\alpha]^{23}\mathrm{D}$ −50.5° (*c* 1.11, chloroform).

Anal. Calcd. for $C_{16}H_{18}O_5$: C, 66.2; H, 6.2. Found: C, 66.1; H, 6.4.

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