Note

A crystalline, nonreducing anhydro-octulose, presumably 2,7-anhydro-L-*glycero*-β-D-*manno*-octulopyranose

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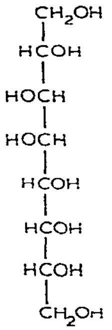
(Received January 29th, 1972)

In my penultimate research paper[1], I described the preparation and properties of the second known crystalline octulose and the first crystalline methyl octuloside; the structure and configuration of the latter were proved by degradation to the enantiomorph of a known methyl heptuloside. Hoping to obtain similar results in a different series, I started with D-*erythro*-D-*galacto*-octitol (**1**), which had been isolated from the avocado[2], and oxidized it by means of *Acetobacter suboxydans*; because of the configuration of the octitol, the resulting syrupy octulose could only have been L-*glycero*-D-*manno*-octulose (**2**). When this octulose was boiled with methanol in the presence of the cation-exchange resin Amberlite IR-120 (H^+), which had been thoroughly prewashed with methanol, the resulting product contained two new compounds, one of which was presumed to be the expected methyl octuloside **3**. Unfortunately, neither compound crystallized, and so the mixture was subjected to chromatography on a column of powdered cellulose; elution was effected with butyl alcohol half-saturated with water.

When the appropriate eluate fractions were combined and evaporated, one of the two new compounds crystallized spontaneously. After being recrystallized, it melted at 80–85°, and was partially solvated; it showed a specific rotation of $[\alpha]_D^{20}$ $-83.3°$ (in water; corrected for content of solvent); and it did not reduce hot Fehling solution. The results obtained on analysis for carbon and hydrogen corresponded to the values calculated for either a methyl octuloside (such as **3**) or an anhydro-octulose (such as **5**). To distinguish between these two possibilities, recourse was had to nuclear magnetic resonance spectroscopy, mass spectrometry, and periodate oxidation. The n.m.r. spectrum showed no peak corresponding to a methyl group, whereas the previously described methyl D-*glycero*-α-L-*gluco*-octulopyranoside[1] showed a strong peak at δ 3.27 p.p.m. Mass spectroscopy of the per-*O*-(trimethylsilyl) derivative of the compound indicated that it had a molecular weight corresponding to that of a pentakis-*O*-(trimethylsilyl) derivative of an anhydro-octulose, rather than to that of a hexakis(trimethylsilyl) ether of a methyl octuloside. Finally, on periodate oxidation, the compound consumed about two moles of reagent per mole and yielded no formaldehyde. This result was to be expected of the anhydro-octulose **5**, whereas the methyl octuloside 3 should consume there moles of reagent per mole, and yield one mole of formaldehyde.

The structure of the new compound thus seems to have been satisfactorily proved to be that of an anhydro-octulose. Had it been the methyl L-*glycero*-α-D-*manno*-octulose (3) expected, its complete configuration could have been established by oxidation with one equivalent of periodate followed by reduction with borohydride to the known methyl α-D-*manno*-heptulopyranoside[3] (4), as had been done similarly in the preceding publication[1]. However, since this is the first example of a crystalline anhydro-octulose, its complete configuration could not be definitively proved; there were no previous examples with which to compare it, and lack of both time and material prevented further experimentation. Nevertheless, its configuration as 2,7-anhydro-L-*glycero*-β-D-*manno*-octulopyranose (5) can reasonably be assumed to be correct, because (*a*) its specific rotation (−83.3°) is very close to that of the lower homolog 2,7-anhydro-β-D-*manno*-heptulopyranose (−80.4°)[4], and (*b*) when D-*manno*-heptulose was heated with methanol and Amberlite IR-120 (H^+) resin, the resulting product was shown, by paper chromatography, to contain 2,7-anhydro-β-D-*manno*-heptulopyranose.



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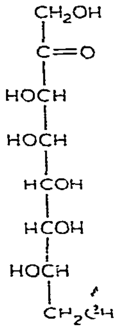

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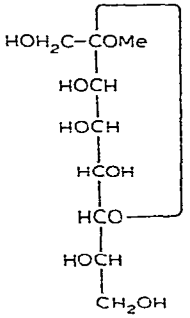

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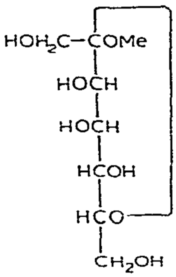

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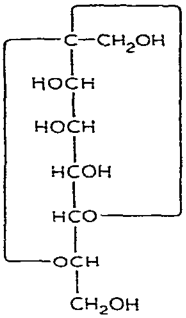

5

EXPERIMENTAL

L-glycero-D-manno-*Octulose* (2) *from* D-erythro-D-galacto-*octitol* (1) *by the action of* Acetobacter suboxydans.—A total of 700 mg of the octitol (1), isolated from the avocado[2], was incubated with a culture of *Acetobacter suboxydans* (ATCC No. 621) for 15 days at 31° by the procedure that had been used earlier in this laboratory[5]. Deproteinization, de-ionization, and evaporation yielded 690 mg of syrupy octulose (2) that, according to paper-chromatographic examination, contained only a trace of unchanged octitol; through an oversight, the octulose was not further characterized.

Treatment of the octulose (2) *with methanol in the presence of Amberlite IR-120* (H^+) *resin. Isolation of 2,7-anhydro*-L-glycero-β-D-manno-*octulopyranose* (5).—A solution of the dried, syrupy octulose (690 mg) in methanol (100 ml) was boiled under a reflux condenser for 14 h with 8.8 g (dry weight) of Amberlite IR-120 (H^+) ion-exchange resin that had been thoroughly prewashed with methanol. The mixture was cooled and filtered, and the filtrate was stirred with some Duolite A-4 (OH^-) ion-exchange resin to remove any acidic material, and with some Darco X decolorizing carbon to remove the yellow color. The suspension was filtered, and the filtrate was evaporated to a colorless syrup (450 mg) that did not crystallize. A paper chromatogram, with an orcinol–hydrochloric acid spray, revealed three spots that indicated a trace of the starting octulose and two faster-moving components.

These three components were separated on a column (effective size 33 × 4 cm) of powdered cellulose by eluting with butyl alcohol half-saturated with water. After a forerun of 250 ml, fractions (14 ml) were collected mechanically. Fractions 46–65 yielded 160 mg of crystalline material (the anhydro-octulose **5**); the second new compound (presumably the methyl octuloside **3**) was mostly eluted in fractions 91–115, but did not crystallize.

The crystalline product was recrystallized twice from methanol by the slow addition of ethyl acetate; the radiating clusters of small prisms thus obtained (75 mg) melted at 80–85° and contained some solvent of crystallization. The specific rotation of $[\alpha]_D^{20}$ $-83.3°$ (*c* 1.8, water) has been corrected for a loss of 1.7% found when a separate sample was dried to constant weight under a high vacuum.

Anal. Calc. for $C_8H_{14}O_7$: C, 42.34; H, 6.35. Found (dried sample): C, 42.07; H, 6.83.

N.m.r. studies.—When crystalline methyl D-*glycero*-α-L-*gluco*-octulopyranoside[1] was dissolved in deuterium oxide and its n.m.r. spectrum measured, the methyl group appeared strongly at δ 3.27 p.p.m.; this singlet was absent from the similarly measured spectrum of compound **5**.

Mass-spectrographic studies.—Compound **5** was converted into its per(trimethylsilyl) derivative by means of Trisil. The reaction was complete within 5 min (as determined by g.l.c.). The mass spectrum, determined at 70 eV on an LKB-9000 mass spectrometer, showed *m/e* 582; a pentakis-*O*-(trimethylsilyl) derivative of an anhydro-octulose ($C_{23}H_{54}O_7Si_5$) requires *m/e* 582, whereas a hexakis-*O*-(trimethylsilyl) derivative of a methyl octuloside ($C_{27}H_{66}O_8Si_6$) would require *m/e* 686.

Periodate oxidation studies.—When compound **5** (7.40 mg) was dissolved in an excess of 15 mM aqueous sodium metaperiodate and the consumption of oxidant was monitored spectrophotometrically, essentially by the method of Aspinall and Ferrier[6], the reaction was complete within 4 h. The oxidation consumed 1.80 moles of periodate per mole of compound; had a correction been made for the content of solvent in crystalline **5**, this value would have been closer to 1.9 moles. A test for formaldehyde[7] in the final solution was negative.

For comparison, sedoheptulosan (2,7-anhydro-β-D-*altro*-heptulopyranose) monohydrate consumed 2.03 moles of periodate per mole and yielded no formaldehyde, as expected. Galactitol, on the other hand, gave, after oxidation, a deep-purple color in the test for formaldehyde with the chromotropic acid reagent[7].

ACKNOWLEDGMENTS

The author thanks Dr. Cornelis P. J. Glaudemans, of this Section on Carbohydrates, for assistance with the inoculations of *Acetobacter suboxydans*; Miss Paula M. Parisius and Mrs. Alice L. Wong, both of this Laboratory of Chemistry, for performing the microanalyses; Dr. G. Gilbert Ashwell, of this Institute, for the periodate oxidation studies; and Dr. Robert J. Highet and Mr. Edward A. Sokoloski, both of the National Heart and Lung Institute, for the n.m.r. spectroscopy and mass spectrometry studies.

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