which precipitation is mediated by multiple units of D-galactose[14] instead of D-glucuronic acid. The specific polysaccharide of Type XIV pneumococcus is composed of D-galactose, D-glucose and N-acetylglucosamine.[15] It is therefore probable that the D-galactose-containing portion of GA is much the same in both the rhamnose-poor fraction precipitated by Type II antiserum and in the residual gum. Since the proportion of D-glucuronic acid to L-arabinose and D-galactose is not greatly changed by precipitation with either antiserum, it is probable that the rhamnose-poor fraction precipitated by Type II serum contains the D-glucuronic acid in a steric arrangement more closely resembling the as yet only partially elucidated positions of the acid units in SII[5] than is characteristic of the D-glucuronic acid in the unprecipitated portion. This interpretation would indicate isomeric differences as well as differences in composition among the components of GA, but further chemical studies are clearly necessary to establish its validity. At any rate, the immunochemical data serve to indicate what directions such studies might take. Immunochemical tests of any fractions obtained would also be helpful in judging the validity of assigned structures.

(14) M. Heidelberger, THIS JOURNAL, **77**, 4308 (1955), esp. Table II.

(15) W. F. Goebel, P. B. Beeson and C. L. Hoagland, *J. Biol. Chem.*, **129**, 455 (1939); M. Heidelberger, S. A. Barker and M. Stacey, *Science*, **120**, 781 (1954).

Since glacial acetic acid is sometimes useful in the fractionation of polysaccharides,[16] 2 g. of gum was dissolved in 10 ml. of water, with neutralization, and precipitated with 16 ml. of glacial acetic acid in the cold. The precipitate, fraction D, was centrifuged off in the cold and the gum in the supernatant (fraction E) precipitated with chilled alcohol. Both fractions were redissolved in cold water, neutralized, and precipitated with alcohol; yields: D, 0.7 g.; E, 1.1 g. Analyses are given in Table I. As the differences in composition were minor, most of another portion of gum was precipitated by a larger proportion of acetic acid. Eight per cent. of the amount taken was recovered from the supernatant by precipitation with alcohol. This fraction, F, showed appreciable deviations from the composition of the larger fractions, as also noted in Table I. Both L-rhamnose and D-glucuronic acid were low in this fraction, while L-arabinose tended to be high in all of the more soluble fractions

Attempts to split off labile L-arabinose and L-rhamnose in 45% acetic acid at room temperature, 20–28°, for three weeks failed completely, nor was there any change in composition after another three weeks at 37°.

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(16) M. Heidelberger and F. E. Kendall, *J. Exp. Med.*, **53**, 625 (1931).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, QUEEN'S UNIVERSITY, KINGSTON, ONTARIO]

The Synthesis of 3-Hexuloses. Part 1. 2-O-Methyl-L-xylo-3-hexulose[1]

BY J. K. N. JONES

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The preparation of 2-O-methyl-L-xylo-3-hexulose from L-ascorbic acid is described.

1-Desoxy-D-xylo-3-hexulose was prepared by bacterial oxidation of L-fucitol some years ago.[2] This was the only known member of the 3-hexuloses. The present paper describes a synthesis of 2-O-methyl-L-xylo-3-hexulose (I) and a general procedure for the preparation of isomeric 3-hexulose derivatives. These isomeric ketoses are important as possible intermediates in the breakdown of hexoses by alkalies. The 3-pentuloses are possible intermediates in the biological fixation of carbon dioxide.[3]

The starting material in this synthesis of I was L-ascorbic acid (II) which was converted first to its 2,3-di-O-methyl ether (III) and thence by rearrangement to the well characterized crystalline amide of the methyl glycoside of a 3-hexulonic acid (IV).

The amide was then converted to the acid and thence to the lactone[4] V which was reduced with lithium aluminum hydride to the glycoside VI. This material was very labile to acids and gave on hydrolysis compound I, characterized as the 2,5-dichlorophenylhydrazone. The structure of the ketose was confirmed when it was oxidized by periodate to formic acid and an ester VII. This ester on methylation with silver oxide and methyl iodide gave a product which yielded, with alcoholic ammonia, glycolamide (VIII) and the amide[5] of 2,3-di-O-methyl D-glyceronic acid (glyceric acid) (IX). The glycolamide arises probably from the oxidation by silver oxide of the substituted glycolaldehyde derivative VII. 2-O-Methyl D-glyceronamide (X) resulted when VII was boiled with methanolic hydrogen chloride and the resultant ester was treated with alcoholic ammonia. The isolation of this amide, which was identical with a synthetic specimen,[6] and of the amide IX proved the configuration of C_2 in I and thus showed for the first time that the amide IV is a derivative of L-xylose. The configuration of the glycosidic methoxyl group is as yet unknown, but it is probably α-glycosidic.

When D-*arabo*-ascorbic acid was methylated

(1) Paper presented before the Division of Carbohydrate Chemistry at the 128th Meeting of the American Chemical Society in Minneapolis, Minnesota, September, 1955.

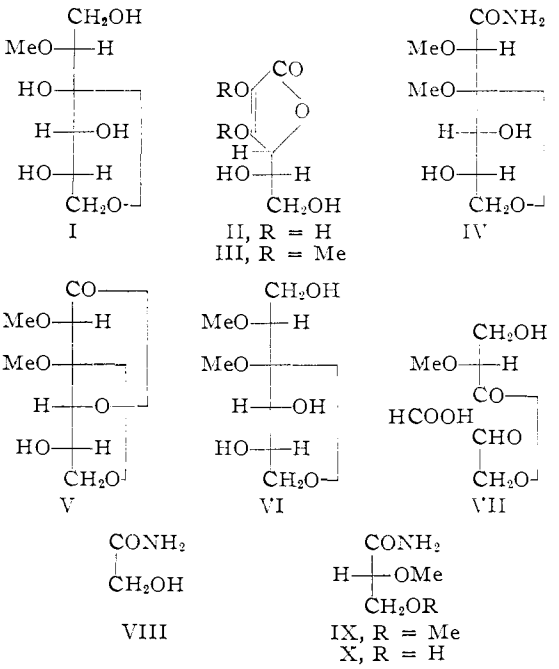
(2) L. E. Stewart, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **74**, 2206 (1952).

(3) A. T. Wilson and M. Calvin, *ibid.*, **77**, 5948 (1955).

(4) W. N. Haworth, E. L. Hirst, F. Smith and W. J. Wilson, *J. Chem. Soc.*, 829 (1937).

(5) P. F. Frankland and N. L. Gebhard, *ibid.*, **87**, 866 (1905).

(6) J. K. N. Jones, *Can. J. Chem.*, **34**, 310 (1956).



I II, R = H III, R = Me IV V VI VII VIII IX, R = Me X, R = H

with diazomethane and the resultant 2,3-di-*O*-methyl ether converted as described above to the isomeric methyl 2-*O*-methyl-3-hexulosides at least two products resulted. These were detected chromatographically. On acidic hydrolysis the glycosidic methoxyl group was eliminated and two sirupy compounds, most probably 2-*O*-methyl-D-*ribo*-3-hexulose and 2-*O*-methyl-D-*arabo*-3-hexulose, resulted. From this mixture a crystalline 2,5-dichlorophenylhydrazone of unknown configuration was prepared.

In a similar manner D-*gluco*-ascorbic acid was converted to a mixture of 2-*O*-methyl-3-heptuloses, from which a 2,5-dichlorophenylhydrazone of unknown configuration was isolated. Work on the structure of these and on the synthesis of the parent sugars is in progress.

Experimental

Chromatography was carried out by the descending method on Whatman No. 1 filter paper[7] using (a) ethyl acetate:acetic acid:formic acid:water (18:3:1:4) or (b) 1-butanol:pyridine:water (10:3:3) parts (v./v.) as the mobile phase. Sugars and derivatives were located on the chromatograms with either ammoniacal silver nitrate or a *ca.* 3% solution of *p*-anisidine hydrochloride[8] in 1-butanol:water.

The rate of movement of the various compounds on the chromatogram is quoted relative to that of rhamnose (*i.e.*, R_{Rh} value). Solutions were concentrated under reduced pressure and at a bath temperature of 40° or less. Optical rotations were determined in water and at 20 ± 2° unless otherwise stated.

Preparation of Methyl 2-*O*-Methyl-L-*xylo*-3-hexuloside.—The amide (6.0 g.) of methyl 2-*O*-methyl-L-*xylo*-3-hexulonoside (isodimethyl-L-ascorbic acid) was converted to the barium salt by the method of Haworth, *et al.*[4] The filtered solution was passed down a column of Amberlite resin IR-120 and the acidic effluent concentrated to a sirup (5.4 g.). The product, which was a lactone, was dissolved in tetrahydrofuran (20 ml.) (or methylal) and reduced by adding its solution to lithium aluminum hydride (2 g.) in tetrahydrofuran (10 ml.) at 30°. After 2 hr. ethyl acetate, followed by water, was added to the reaction mixture which was then filtered. The filtrate was passed down columns of Amberlite resin IR-120 and IR-4B. Chromatographic examination of the solutions before and after passage through the ion exchange columns indicated that some hydrolysis of the glycosidic methoxyl group had occurred. Before deionization the solution, which did not reduce Fehling solution, contained one component (solvent b) with R_{Rh} 1.51. After passage through the ion exchange columns, the solution was reducing and contained three components (4.1 g.) with R_{Rh} 1.51, 1.17 and 1.10, the second greatly predominating. When a portion of this solution was heated with 0.2 *N* hydrochloric acid for 2 hr. at 60°, the component with R_{Rh} 1.51 disappeared and was replaced by components with R_{Rh} 1.17 and 1.10.

Fractionation of the Mixture of Sugars.—Fractionation of the sirup (4.0 g.) was carried out on a cellulose column,[9] using a mixture of 1-butanol:light petroleum (b.p. 100–120°) (4:1 v./v.) and gave three fractions: Fraction I (0.2 g.), $[\alpha]_D$ +27° (*c* 2.7, in methanol) containing two components of R_{Rh} 1.51 and 1.17 in approximately equal amounts. Fraction II (3.1 g.), $[\alpha]_D$ +24° (*c* 1.4, in methanol) contained one component of R_{Rh} 1.17. *Anal.* Found: OMe, 15.0. Fraction III (0.8 g.) contained two components of R_{Rh} 1.17 and 1.0.

Preparation of 2,5-Dichlorophenylhydrazone.—A portion (0.1 g.) of fraction II was dissolved in methanol (5 ml.) containing 2,5-dichlorophenylhydrazine (75 mg.), and the solution was boiled until paper chromatographic examination of a portion indicated that all the sugar had reacted (1 hour). The solvent was allowed to evaporate and the 2,5-dichlorophenylhydrazone then crystallized. It was recrystallized from methanol and acetone, m.p. 161°, $[\alpha]_D$ +93 ± 4° (*c* 0.3, in acetone).

Anal. Calcd. for $C_{13}H_{18}N_2O_5Cl_2$: C, 44.2; H, 5.1; N, 7.9; Cl, 20.0; OMe, 8.8. Found: C, 44.5; H, 5.15; N, 7.4; Cl, 20.2; OMe, 8.6.

Periodate Oxidation of Fraction II (a).—A portion (100 mg.) of fraction II was oxidized with sodium metaperiodate solution. At intervals samples were withdrawn, ethylene glycol was added to the solutions and the formic acid was titrated. After 2 hr. the titer became constant, 1.1 moles of free acid was present in the solution and a substance which behaved as an ester was detected. Addition of excess of 0.01 *N* alkali followed by back titration gave an uptake of alkali corresponding to the liberation of 2.1 moles of acid per mole of sugar. The consumption of periodate was 2.3 moles per mole of sugar at the end of 2 hr. Structure I requires the consumption of 2 moles of metaperiodate with the liberation of one mole of formic acid and the formation of an ester.

(b) Fraction II (1.44 g.) was dissolved in water (10 ml.) and oxidized with sodium metaperiodate (3.7 g.) in water (20 ml.). After 24 hr. acetone was added and the solution was filtered to remove inorganic salts. The filtrate was concentrated to dryness and extracted with acetone. Concentration of the extract gave a sirup (1.09 g.), $[\alpha]_D$ +38°, which was methylated with Purdie reagents. The product (0.89 g.), $[\alpha]_D$ +35° (*c* 8.9, in acetone), was distilled giving fractions; (a) b.p. 75–80° (15 mm.), $n^{25}D$ 1.4290, 0.3 g.; (b) b.p. 80–100° (15 mm.), $n^{25}D$ 1.4380 (0.2 g.).

Each of these fractions was dissolved in methanol and the solutions were saturated with ammonia. On standing the amide of glycolic acid crystallized. The crystals were collected and recrystallized from methanol, m.p. 119°, not depressed on admixture with an authentic specimen.

Anal. Calcd. for $C_2H_5NO_2$: C, 32.0; H, 6.67; N, 18.67. Found: C, 32.1; H, 6.6; N, 18.45.

On standing, the residue recovered from the mother liquors of the glycolamide deposited long needles of 2,3-di-*O*-methyl-D-glyceronamide, m.p. 78°, $[\alpha]_D$ +50 ± 5° (*c* 1.1 in methanol), after recrystallization from ether or light petroleum ether. Frankland and Gebhard[5] report m.p. 77° and $[\alpha]_D$ −54° (in methanol) for the amide of 2,3-di-*O*-methyl-L-glyceronamide.

(c) A portion (1.75 g.) of fraction II was dissolved in water (25 ml.) and oxidized with a solution of sodium metaperiodate (12 g.) in water (50 ml.) for 24 hr. at 30°. Acetone was then added to the solution which was filtered. The filtrate was concentrated and the residual sirup (1.07 g.) examined chromatographically. One substance was de-

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(8) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 1702 (1950).

(9) L. Hough, J. K. N. Jones and W. H. Wadman, *ibid.*, 2511 (1949).

tected on the chromatogram (solvent b). It moved at the rate of 2,3,4,6-tetra-*O*-methyl-D-glucose, and gave a yellow-orange color with the *p*-anisidine hydrochloride spray. The sirup, $[\alpha]_D$ +30° (*c* 9.6, in methanol), was boiled with methanolic hydrogen chloride in order to convert VIII to the ester of 2-*O*-methyl-D-glyceronic acid plus the dimethyl acetal of glycolaldehyde. It was anticipated that the latter compound would be unaffected by alcoholic ammonia whereas the glyceronic acid derivative would yield an amide. Accordingly the solution from the methanolysis was neutralized (Ag_2CO_3), saturated with ammonia and after 24 hr. at 0°, it was evaporated to dryness.[2,3] Crystalline 2-*O*-methyl-D-glyceronamide separated, m.p. and mixed m.p. with an authentic specimen,[6] m.p. 88°, $[\alpha]_D$ +78 ± 6° (*c* 0.3 in methanol) remained after recrystallization from acetone–ether mixture.

Anal. Calcd. for $C_4H_9NO_3$: C, 40.3; H, 7.6; N, 11.8. Found: C, 40.4; H, 7.2; N, 11.9.

Conversion of D-*arabo*-Ascorbic Acid to a 3-Hexulose Derivative.—The iso-di-*O*-methyl derivative[10] (5 g.) was reduced by adding its solution in methylal (25 ml.) to a solution of lithium aluminum hydride (5 g.) in methylal (20 ml.). After 3 hr. excess of lithium aluminum hydride was destroyed by addition of ethyl acetate followed by water and the solution was filtered. The filtrate was deionized (Amberlite resins IR-120 and IR4B) and concentrated to a sirup (3.6 g.). Chromatographic examination of this sirup (solvent b) indicated the presence of four substances with R_{Rh} 1.08, 1.6, 1.65 and 1.85.

The sirup was dissolved in *N* formic acid and the solution was heated at 80°. After 3 hr. chromatographic examination of the solution indicated the presence of two major components with R_{Rh} 1.08 and 1.43 (solvent b). The sirup (3.4 g.) remaining after evaporation of the solvents was fractionated on a column of cellulose using 1-butanol:light petroleum (b.p. 100–120°) as solvent, and the following fractions were obtained: fraction I (0.2 g.), $[\alpha]_D$ −16° (*c* 0.2 in methanol); fraction II (2.5 g.), $[\alpha]_D$ −12° (*c* 2.5 in methanol); fraction III (0.6 g.), $[\alpha]_D$ −5° (*c* 0.6 in methanol). Fractions I and III were mixtures, fraction II consisted mainly of the compound with R_{Rh} 1.08 (solvent b).

A sample of this fraction was heated with an equivalent of 2,5-dichlorophenylhydrazine in methanol. Concentration of the solution yielded a crystalline hydrazone derivative, m.p. 134°, $[\alpha]_D$ +12 ± 2° (*c* 0.2 in acetone).

Anal. Calcd. for $C_{13}H_{18}N_2O_5Cl_2$: N, 7.9; OMe, 8.8; Cl, 20.0. Found: N, 8.0; OMe, 8.8; Cl, 19.4.

Conversion of D-Glucoascorbic Acid to a 3-Heptulose Derivative.—2,3-Di-*O*-methyl-D-glucoascorbic acid[11] (2.73 g.) was converted to the isomeric lactone of methyl 2-*O*-methyl-3-heptulonoside and thence by reduction with lithium aluminum hydride to methyl 2-*O*-methyl-3-heptuloside (2.24 g.). Chromatographic examination of the sirup, after its solution had been deionized on Amberlite resins IR-120 and IR-4B, indicated the presence of five components, the major ones having R_{Rh} 1.42 and 0.75 (solvent b). The sirup was dissolved in 0.1 *N* sulfuric acid (50 ml.) and the solution heated on the steam-bath. The observed rotation changed from +1.06 to −2.8° (constant value) in 4 hours. Chromatographic examination of the solution now showed the presence of two materials with R_{Rh} 0.86 and 0.72, the former predominating (solvent b). In solvent a the rates of movement were R_{Rh} 0.95 and 1.04, the slower moving component predominating. The solution was neutralized ($BaCO_3$), filtered and concentrated to a sirup (2.0 g.). A portion of the material when heated with an alcoholic solution of 2,5-dichlorophenylhydrazine until the sugar could no longer be detected chromatographically gave a derivative, m.p. 154°, after recrystallization from ethyl acetate.

Anal. Calcd. for $C_{14}H_{20}N_2O_6Cl_2$: C, 44.0; H, 5.26; Cl, 18.4; OMe, 8.1. Found: C, 44.1; H, 5.7; Cl, 18.2; OMe, 8.5.

A sample of the sirupy mixture of sugars when oxidized with sodium metaperiodate consumed 3.5 moles of periodate and produced 2.2 moles of formic acid per mole of heptulose derivative.

Acknowledgment.—The author thanks the National Research Council of Canada, and Queen's University for grants which made this work possible.

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[CONTRIBUTION FROM THE BANTING AND BEST DEPARTMENT OF MEDICAL RESEARCH, UNIVERSITY OF TORONTO]

Formation of Symmetric Azo-compounds from Primary Aromatic Amines by Lead Tetraacetate

BY ERICH BAER AND ANTHONY L. TOSONI

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It is shown that oxidation of primary aromatic amines with lead tetraacetate provides a simple method for the preparation of symmetrically substituted azo compounds.

Several years ago while carrying out an oxidation with lead tetraacetate (LTA) in the presence of an aromatic amine, the writers observed the unexpected development of an intense color. This was soon traced to the oxidative effect of the reagent on the amine. A cursory investigation of this reaction with 2,4-dichloroaniline, 2,4,6-tribromoaniline and 4-bromoaniline as substrates disclosed that the oxidation of substituted primary aromatic amines with LTA gives complex mixtures of colored oxidation products that contain fair amounts of symmetric azo compounds. In the case of the three anilines under investigation, the azobenzenes were isolated in yields ranging from 27–36% of the theory. LTA thus becomes another member of a group of oxidizing agents (potassium permanganate,[1,2] potassium ferricyanide,[3] sodium hypobromide,[4] chromic acid anhydride,[5,6] manganese dioxide[7] and lead peroxide[7]) which are capable of converting primary aromatic amines to azo compounds with varying degrees of efficiency. The oxidation of the aromatic amines by LTA to symmetric azo compounds most likely involves the formation of free radicals. This assumption finds strong support in Goldschmidt and Wurzschmitt's

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(6) F. Meyer and K. Dahlem, *Ann.*, **326**, 338 (1903).
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