[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

Reduction of the Products of Periodate Oxidation of Carbohydrates. IX. Correlation of the Structure of Methyl β-Lactoside and Methyl β-Cellobioside[1]

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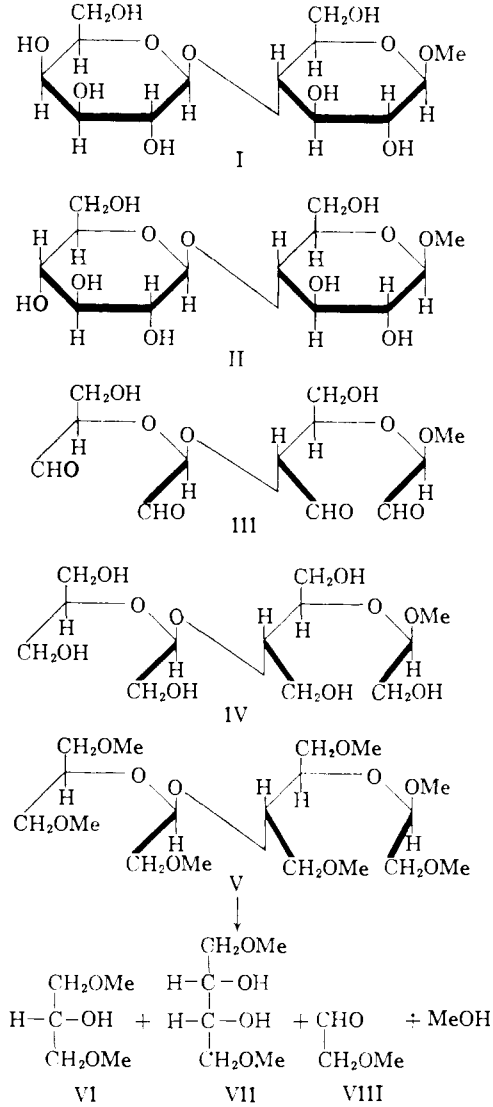
When methyl β-lactoside and methyl β-cellobioside are oxidized with periodate they give rise to the same tetraaldehyde (III). Reduction of III yields the corresponding hexahydric alcohol IV. Hydrolysis of the methylated alcohol V derived from IV has been shown to give rise to 2,3-di-*O*-methyl-glycerol (VI), 1,4-di-*O*-methyl-erythritol (VII) and methoxyacetaldehyde (VIII).

In previous communications[2,3] it was demonstrated that the reduction of the products of periodate oxidation could be used to correlate the structure of the glycosides of pentoses, 6-deoxyhexoses and hexoses. In certain cases this reduction procedure proved to be simpler than the method[4] formerly used which involved the bromine oxidation of the periodate-oxidized glycosides.

This paper deals with the correlation of the structure of the disaccharides, lactose and cellobiose, by periodate oxidation and reduction of their β-glycosides. Both methyl β-lactoside (I) and methyl β-cellobioside (II) were shown by polarimetry and iodimetry to react relatively rapidly with about two molecular proportions of 0.25 *N* periodic acid at room temperature in the dark; thereafter the consumption of the third molar proportion of periodate proceeded more slowly. It is believed that the slowing down of the periodate oxidation of the two glycosides is due to the formation of cyclic, dioxane intermediates,[5-7] although steric hindrance[8] may also be involved. The nature of the product formed after the consumption of two molar proportions of periodate by I and II will form the subject of a later communication.

Reduction of the tetraldehyde III from I and II with sodium borohydride[2,9,10] was shown to give rise to the same hexahydric alcohol IV. After purification by acetylation[10] the alcohol IV was characterized as its hexa-*p*-nitrobenzoate.

Further support for the structure of the hexahydric alcohol IV was provided by an extension of the previous studies[10] on the methylation of the alcohols produced by periodate oxidation and reduction of methyl glycosides. Thus treatment of IV with sodium and methyl iodide in liquid ammonia[11] as formerly described[10] afforded the corresponding methylated derivative V. Hydrolysis of V gave rise to 1,3-di-*O*-methyl-glycerol (VI), 1,4-di-*O*-methyl-erythritol (VII) and methoxyacetaldehyde (VIII). The 1,3-di-*O*-methyl-glycerol was characterized as the *p*-nitrobenzoate,[10] the 1,4-di-O-methyl-erythritol as the di-*p*-toluenesulfonate, and the methoxyacetaldehyde as the *p*-nitrophenylhydrazone.[10]



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(2) F. Smith and J. W. Van Cleve, THIS JOURNAL, **77**, 3091 (1955).

(3) M. Abdel-Akher, J. E. Cadotte, Bertha A. Lewis, R. Montgomery, F. Smith and J. W. Van Cleve, *Nature*, **171**, 474 (1953).

(4) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **59**, 994 (1937).

(5) J. E. Cadotte, G. G. S. Dutton, I. J. Goldstein, Bertha A. Lewis, F. Smith and J. W. Van Cleve, *ibid.*, **79**, 691 (1957).

(6) I. J. Goldstein, B. A. Lewis and F. Smith, *ibid.*, **80**, 939 (1958).

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(8) E. P. Garner, I. J. Goldstein, R. Montgomery and F. Smith, THIS JOURNAL, **80**, 1206 (1958).

(9) M. Abdel-Akher, J. K. Hamilton and F. Smith, *ibid.*, **73**, 4691 (1951).

(10) J. K. Hamilton, G. W. Huffman and F. Smith, *ibid.*, **81**, 2173 (1959).

(11) I. E. Muskat, *ibid.*, **56**, 693 (1934).

This work on the alcohol IV derived from the disaccharides I and II further supports the view,[10] advanced after investigations on the alcohols produced from methyl α-D-glucopyranoside and methyl β-D-xylopyranoside, that methylation studies on the polyalcohols, derived from polysaccharides by periodate oxidation and reduction, may prove to be of some value in determining the structure of carbohydrate polymers. These studies also have provided a further reference compound, 1,4-di-*O*-methyl-erythritol, for subsequent investigations into polymers containing 1→4-linked D-glucose residues.

Experimental

Unless stated otherwise, all evaporations were carried out *in vacuo* at 40–45°.

A. Oxidation of Methyl β-Lactoside (I) with Periodic Acid.—Methyl β-lactoside (16 g., m.p. 208°, $[\alpha]^{25}D$ +2.3° in water (*c* 12),[12] was treated with 0.25 *N* periodic acid in aqueous solution (2 l.) at room temperature in the dark. The specific optical rotation changed from +2.30° (initial value) to a maximum of +25° in about 50 min. after which time the consumption of periodate was 2.3 moles per mole of lactoside. Thereafter the specific rotation gradually fell to a constant minimum, −92.5°, after about 80 hr. At this stage 3.42 moles of periodate had been consumed per mole of lactoside (see Fig. 1). In another experiment carried out at 4° the total periodate consumption after 144 hr. was 3.03 moles per mole of lactoside.

The solution was neutralized with 0.3 *N* barium hydroxide, any excess of the latter being neutralized immediately by the addition of a small piece of Dry Ice (solid CO_2). The solution was filtered and used directly in the next, reduction step.

Reduction of the Tetraaldehyde III with Sodium Borohydride.—To the solution obtained above, sodium borohydride (5 g.) was added quickly with stirring and 3 hr. later a second portion (1 g.) of sodium borohydride was added. After standing overnight the solution was neutralized (Dry Ice) and evaporated to dryness, the process being aided by azeotropic distillation with benzene. The residue so produced contained the hexahydric alcohol IV together with some inorganic salts.

Acetylation of the Hexahydric Alcohol IV.—The dry residue from the previous experiment was acetylated with acetic anhydride (120 ml.) and pyridine (150 ml.) as formerly described.[10] The product was isolated by pouring the reaction mixture into water with subsequent extraction by chloroform. Distillation yielded the hexaacetate of IV as a pale yellow viscous liquid, b.p. (bath temp.) 230–235° (0.003 mm.), $n^{26}D$ 1.4561, $[\alpha]^{25}D$ +12.2° in chloroform (*c* 4).

Isolation of the Hexahydric Alcohol IV.—The acetate of IV was dissolved in dry methanol and treated with a small amount of sodium.[13] After standing 24 hr. at room temperature the reaction mixture was refluxed for 10 min. and then evaporated to dryness to give the hexahydric alcohol IV as a colorless viscous liquid.

In a separate experiment the tetraaldehyde, prepared from methyl β-lactoside as already described, was reduced[2] with hydrogen and a Raney nickel catalyst under pressure at 100°. The hexahydric alcohol IV recovered from the reaction mixture by filtration and evaporation was a clear colorless viscous liquid.

Anal. Calcd. for $C_{12}H_{26}O_{10}$: C, 43.65; H, 7.9. Found: C, 43.5; H, 7.4.

A solution of the hexahydric alcohol IV (0.187 g.) in pyridine (5 ml.) was treated with *p*-nitrobenzoyl chloride (0.943 g.) at 80–90° for 45 min. The reaction mixture was cooled and poured with stirring into a saturated solution (20 ml.) of sodium bicarbonate. The product was extracted with chloroform and the chloroform solution extracted successively with sodium bicarbonate solution and water. Removal of solvent yielded the hexa-*p*-nitrobenzoate of IV as a sirup which solidified on trituration either with methanol or ethanol, m.p. 87–90°, $[\alpha]^{25}D$ +12.0° in chloroform (*c* 5).

(12) F. Smith and J. W. Van Cleve, THIS JOURNAL, **74**, 1912 (1952).
(13) G. Zemplén, *Ber.*, **59**, 1254 (1926).

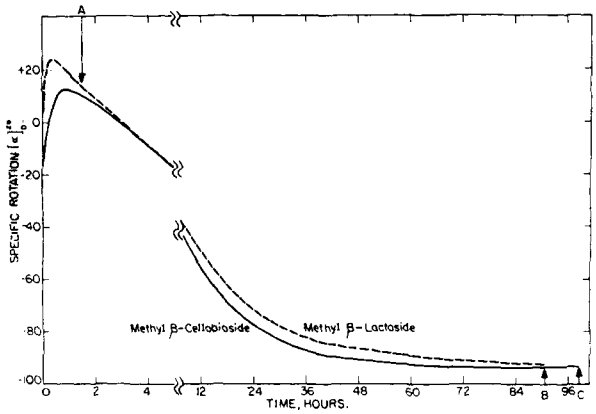

Fig. 1.—Oxidation of methyl β-lactoside and methyl β-cellobioside with 0.25 *N* periodic acid at room temperature, periodate consumption: A, 1.5 hr., 2.3 moles per mole of lactoside and 2.3 moles per mole of cellobioside; B, 90 hr., 3.42 moles per mole of lactoside; C, 98 hr., 3.2 moles per mole of cellobioside.

Anal. Calcd. for $C_{54}H_{44}O_{28}N_6$: C, 52.9; H, 3.6; N, 6.9. Found: C, 52.8; H, 3.7; N, 7.1.

B. Oxidation of Methyl β-Cellobioside (II) with Periodic Acid.—A solution of methyl β-cellobioside (2.00 g., m.p. 193°, $[\alpha]^{17}D$ −19° (water)) in 0.25 *N* periodic acid (250 ml.) was kept at room temperature. The specific rotation changed to a maximum of +13° in about 55 min. at which time the periodate consumption was 2.2 moles per mole of methyl β-cellobioside. The specific rotation fell slowly thereafter to a constant minimum value of −93.9° (after about 8 hr.) and the periodate consumption (tested after 98 hr.) was about 3.2 moles per mole of glycoside (see Fig. 1). After 100 hr. the solution was neutralized with 0.3 *N* barium hydroxide and filtered.

Reduction of the Tetraaldehyde.—The tetraaldehyde III present in the aqueous filtrate produced in the previous experiment was reduced by the addition of sodium borohydride (0.43 g.). After 3 hr. at room temperature, a further portion (0.2 g.) of sodium borohydride was added and the solution allowed to stand overnight. The excess of the sodium borohydride was destroyed with carbon dioxide (Dry Ice) and the solution was evaporated to a sirup. Extraction of the sirup with ethanol served to remove most of the inorganic salts.

Acetylation of the Hexahydric Alcohol IV.—The sirupy residue from the previous experiment was acetylated with acetic anhydride (40 ml.) and pyridine (50 ml.) as already described above for the alcohol from methyl β-lactoside. Distillation of the product gave the hexaacetate of IV as a viscous pale yellow liquid, 1.8 g., b.p. (bath temp.) 230–235° (0.003 mm.), $n^{26}D$ 1.4582, $[\alpha]^{25}D$ +13.4° in chloroform (*c* 6).

Isolation of the Alcohol IV.—Catalytic deacetylation[13] of the acetate of IV with sodium in methanol as already described furnished the hexahydric alcohol IV as a viscous liquid showing $[\alpha]^{27}D$ +9.3° in 65% aqueous ethanol (*c* 6.7).

Treatment of this hexahydric alcohol (IV, 0.142 g.) with *p*-nitrobenzoyl chloride (0.717 g.) in dry pyridine (5 ml.) as described above yielded the corresponding hexa-*p*-nitrobenzoate as a pale yellow solid, m.p. 85–88°, $[\alpha]^{25}D$ +9.0° in chloroform (*c* 5).

Anal. Calcd. for $C_{54}H_{44}O_{28}N_6$: C, 52.9; H, 3.6; N, 6.9. Found: C, 53.1; H, 3.6; N, 6.9.

C. Methylation of the Hexahydric Alcohol IV.—The hexahydric alcohol IV (5.6 g.), obtained above from methyl β-lactoside, was methylated in liquid ammonia (600 ml.) as described previously,[10] five additions of sodium and methyl iodide being employed. Since the hexahydric alcohol appeared to react more slowly with sodium than the two related alcohols from methyl β-D-xylopyranoside and methyl α-D-glucopyranoside, namely, D'-methoxy-diethylene glycol and D'-methoxy-D-hydroxymethyl-diethylene glycol, apparently because the sodio derivative of IV was insoluble in liquid ammonia, a second methylation was applied as

before. After removing the liquid ammonia the product was extracted with chloroform and distilled giving the hepta-*O*-methyl derivative V as a colorless liquid, 1.94 g., b.p. (bath temp.) 185–190° (0.003 mm.), $n^{27}D$ 1.4411, $[\alpha]^{26}D$ +16.9° (pure liquid), +9.7° in methanol (*c* 7). The substance was insoluble in water.

Anal. Calcd. for $C_{18}H_{38}O_{10}$: C, 52.2; H, 9.25; OCH_3, 52.4. Found: C, 52.3; H, 9.25; OCH_3,[14] 50.1.

Hydrolysis of the Heptamethyl Ether V.—A suspension of the methyl ether (V, 1 g.) in *N* sulfuric acid (30 ml.) was refluxed for 3 hr. when the clear solution had become optically inactive. The solution was distilled at atmospheric pressure and the first 5 ml. of distillate was treated with *p*-nitrophenylhydrazine[10] whereby there was produced rapidly methoxyacetaldehyde *p*-nitrophenylhydrazone,[15] m.p. and mixed m.p. 116° (after recrystallization from ethanol–water (2:3)).

The aqueous, acidic distillation residue after removal of the methoxyacetaldehyde–water azeotrope, was saturated with ammonium sulfate and extracted with ether. The dried (Na_2SO_4), combined extracts were evaporated and the sirupy product distilled giving: fraction 1, 1,3-di-*O*-methyl-glycerol (0.102 g.), b.p. (bath temp.) 1 atm., 180–190°, $n^{31}D$ 1.4165. Fraction 2, a mixture of 1,3-di-*O*-methyl-glycerol and 1,4-di-*O*-methyl-erythritol (0.067 g.), b.p. (bath temp.) 145–150° (30 mm.), $n^{31}D$ 1.4223. Fraction 3, 1,4-di-*O*-methyl-erythritol (0.044 g.), b.p. (bath temp.), 180–190° (30 mm.), $n^{27}D$ 1.4385, R_f (butan-1-ol–ethanol–water (4:1:5) 0.65.

Treatment of fraction 1 (52 mg.) in pyridine (3 ml.) with *p*-nitrobenzoyl chloride (150 mg.) as previously described[10] afforded 1,3-di-*O*-methylglycerol *p*-nitrobenzoate, m.p. and mixed m.p. 41.5,[10] after recrystallization from light petroleum ether.

Fraction 3 (40 mg.) was treated in pyridine (3 ml.) with *p*-toluenesulfonyl chloride (150 mg.) for 1 day at room temperature and for 10 min. at 80–85°. The reaction mixture was cooled, poured into ice-water and the crystals of 1,4-di-*O*-methyl-2,3-di-*O*-tosyl-erythritol so formed were filtered, washed with water and recrystallized from ethanol, m.p. and mixed m.p. 140°.

Anal. Calcd. for $C_{20}H_{26}O_8S_2$: C, 52.4; H, 5.7; S, 13.9. Found: C, 52.2; H, 5.8; S, 13.9.

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[CONTRIBUTION FROM DIVISION OF CHEMISTRY, NATIONAL BUREAU OF STANDARDS]

Branched-chain Higher Sugars. I. A 9-Aldo-4-*C*-formyl-nonose Derivative[1,2]

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Two molecules of 5-aldo-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose in alkaline solution combine to form a branched-chain decose derivative (I) by an aldol condensation. An explanation is offered for the occurrence of this condensation, which is not a typical carbohydrate reaction. A proof of the complete structure and configuration of I is presented and the compound is named 9-aldo-4-*C*-formyl-1,2:8,9-di-*O*-isopropylidene-L-*xylo*-L-*ido*-nono-1,4:9,6-difurano-4(1),7-α-pyranose.

Introduction

In a Communication to the Editor,[3] the authors pointed out that branched-chain aldoses containing 8 to 14 carbon atoms can be obtained in reasonably good yield by aldol condensation of suitable sugar derivatives. The present paper describes work establishing the structure of the branched-chain decose derivative I obtained from 5-aldo-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose (II)[4,5] by treatment with alkali.

The aldol condensation occurs with two-carbon and three-carbon hydroxy-aldehydes, hydroxy-ketones and *O*-substituted hydroxy-aldehydes.[6] Although, prior to the present work, a variety of sugars had been synthesized by aldol condensations, the only products reported from tetroses or higher sugars are a heptulose[7] and a dodecitol.[8] (The latter was separated from an electrolyzed alkaline hexose mixture and its synthesis may be considered to have included an aldol condensation.)

The aldol condensation involves two molecules, one in the aldehyde form and the other in the enol form. Presence of both forms is necessary for the reaction to take place. Under the conditions used in aldol condensations, tetroses and higher reducing sugars establish equilibrium states consisting of furanose and pyranose modifications, together with small proportions of the open-chain aldehyde and enol forms. However, the enol form of the sugar is very reactive and undergoes isomerization and degradation reactions in competition with the aldol condensation. On account of this complication and the low concentration of the aldehyde form, reducing sugars in general do not undergo noticeable aldol formation. With substances in which ring closure and isomerization reactions are blocked by substituent groups, the tendency for aldol condensation should be greatly enhanced by the resulting presence of more of the free aldehyde and enediol forms in the reaction mixture. With substance II, used in the present study, the fused-ring structure prevents the aldehyde group at carbon 5 from forming either a pyranose or a furanose ring, and it restricts enolization to the formation of the 4,5-enediol. Hence, it is not surprising that, on treatment with aqueous calcium hydroxide, this compound undergoes aldol condensation to give a product shown to be the branched-chain decose derivative I.

The structure of I was assigned on the basis of the following evidence: (a) The analysis corresponds to the formula $(C_8H_{12}O_5)_n$ and the molecular weight corresponds to the formula $C_{16}H_{24}O_{10}$. This formula is twice that of the monomeric form of the parent substance II. Hence it appears that the

(1) This work was sponsored by the Division of Research, Atomic Energy Commission.

(2) Presented before the Division of Carbohydrate Chemistry at the 132nd Meeting of the American Chemical Society at New York, N. Y., September 11, 1957.

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(4) K. Iwadare, *Bull. Chem. Soc. Japan*, **16**, 40 (1941).

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