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THE OXIDATION OF SOME TERMINAL-SUBSTITUTED POLYHYDRIC ALCOHOLS BY ACETOBACTER SUBOXYDANS[1]

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ABSTRACT

Various terminal-substituted pentitols and hexitols, which possessed either the L-*lyxo*- or the D-*ribo*-configuration at the three contiguous carbon atoms adjacent to the terminal carbon atom, were oxidized to ketoses by *Acetobacter suboxydans*. The terminal hydroxyl group of the polyol was replaced by -H, -*O*Me, -*S*Et, and -*O*Ac groups; several 1,1-dithioacetal derivatives of aldoses were also tested. The preparation of 1-*O*-acetyl-DL-galactitol, 1-deoxy-1-*S*-ethyl-D-arabitol, and 6-deoxy-6-*S*-ethyl-L-sorbose are described. 6-Deoxy-6-*S*-ethyl-L-sorbose was prepared by the microbiological oxidation of 1-deoxy-1-*S*-ethyl-D-glucitol.

INTRODUCTION

The oxidation of unsubstituted sugar alcohols by proliferating cells of *Acetobacter suboxydans* has resulted in the formulation of the Bertrand–Hudson rule for oxidations occurring within the pH range of 5–6.5 (1, 2). Thus a polyol with the D-*erythro* configuration (I) is oxidized mainly at the secondary alcohol group adjacent to the terminal primary alcohol to give a ketose (II). Many examples of this reaction have been reported (3, 4, 5, 6, 7, 8). More recently, cell-free extracts have been used for the oxidation of some polyols in an alkaline environment (optimum pH 7.8), although the reaction is less specific since polyols possessing either the L-*threo*- (III) or D-*erythro*- (I) configurations were utilized (9).

Investigation of the microbiological oxidation of various ω-deoxy-sugar alcohols at pH 5–6.5 has shown that ω(*n*)-deoxy-polyols with the D-*erythro* configuration at the carbon atoms (*n*-2) and (*n*-3) (where *n* equals the number of carbons in the chain) (e.g. VI and VII) gave copper reducing products even when the favorable D-*erythro* configuration was absent at the other end of the chain (10, 11). This configuration is present in L-fucitol (6-deoxy-L-galactitol) (IV), which has been shown to be oxidized at carbon 4 to give 1-deoxy-D-*xylo*-3-hexulose (L-fuco-4-ketose) (V). Hudson, Stewart, and Richtmyer reconciled this evidence with the known specificity of the organism by suggesting that the secondary alcohol at carbon 5 of 6-deoxy-L-galactitol (IV), which can be considered as a *C*-methyl derivative of a pentitol, serves at the enzyme surface in a capacity similar to the primary alcohol of an unsubstituted acyclic polyol. We have investigated the specificity of this microbiological oxidation with a view to the preparation of 3-hexuloses and 3-pentuloses.

A limited number of polyols which possess the favorable D-*ribo*- (VI) or L-*lyxo*- (VII) configuration adjacent to a substituent other than hydroxyl at the terminal carbon atom have been tested as substrates for *A. suboxydans* and some gave reducing products. The substituents included a hydrogen atom (ω-deoxy), ω-*O*-methyl, ω-deoxy-ω-*S*-ethyl, and an ω-*O*-acetyl group. All of the unbranched polyols except 1-deoxy-1-*S*-ethyl-L-arabitol were oxidized to reducing products, which unlike the polyols gave bright yellow or orange spots with *p*-anisidine hydrochloride on paper chromatograms. Similar color reactions were reported for 2-*O*-methyl-3-hexuloses (12) and more commonly for 2-hexuloses (13). Although 1-deoxy-L-arabitol (5-deoxy-L-lyxitol) was oxidized by *A. suboxydans*

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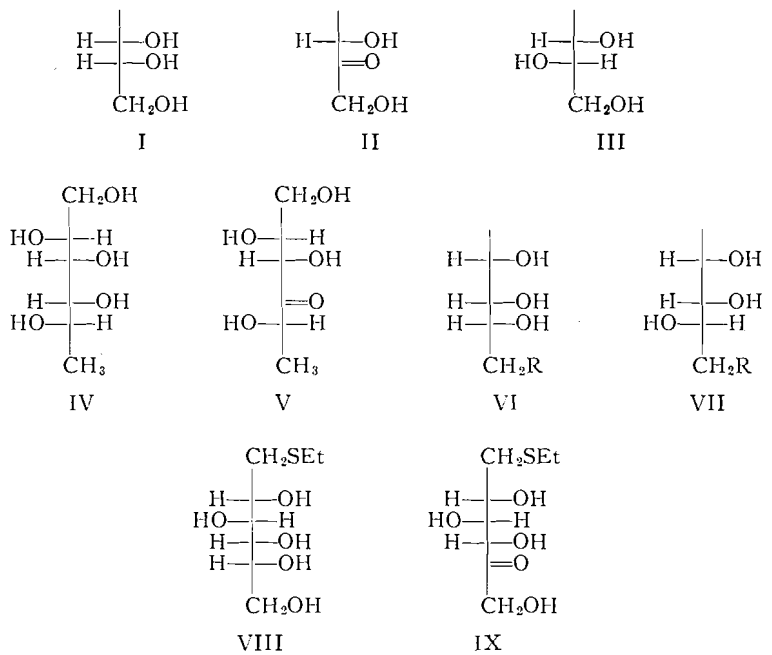
(11) to give a reducing product that resulted in a typical ketose reaction with *p*-anisidine hydrochloride, the substitution of a thioethyl group for a hydrogen atom at carbon-1 in this molecule completely inhibited the oxidation. However, in the cases of 6-deoxy-L-galactitol and 6-deoxy-6-*S*-ethyl-L-galactitol, both substrates yielded reducing compounds in moderate yields. Terminal-branched polyols like diethyldithioacetals (aldose mercaptals) were also tested but they did not appear to be oxidized by *A. suboxydans*, thus supporting the observations of Hudson (1) and Iselin (14).

Estimates of the amounts of reducing sugars formed from the oxidation of various substrates by *A. suboxydans* were made by copper-reduction measurements on aliquots taken from the culture medium at various times during incubation. However, this determination was found to be unsatisfactory when the substrate contained sulphur and unexpectedly high reducing values were obtained; ω-deoxy-ω-thioethyl polyols and dithioacetals reduced the copper reagent when tested in control solutions. An approximate reducing value was obtained by subtracting a 'control reducing value' from the reducing value of the medium which was being tested for biochemical oxidation. The reducing values reported by Bollenback and Underkofler for sugar dithioacetals, when they were used as substrates for *A. suboxydans*, were most likely due to the oxidation of the sulphur atom(s) by the copper reagent (11).

The finding that 6-substituted derivatives of L-galactitol, such as 6-deoxy-L-galactitol (L-fucitol), 1,6-dideoxy-L-galactitol (1,6-dideoxy dulcitol (10)), and 6-deoxy-6-*S*-ethyl-L-galactitol were oxidized by *A. suboxydans* prompted an investigation of the preparation and oxidation of similar derivatives. Two possible synthetic routes to 3-hexuloses might use either L-galactose or DL-galactitol (dulcitol) as starting compounds.

Preferential esterification of the primary alcohol group at carbon 6 of D-galactose and D-glucose has been accomplished by Duff (15, 16) using 50% acetic acid at 100° C. Acetylation of DL-galactitol under slightly modified conditions followed by chromatography on charcoal (25) yielded a crystalline monoacetate as the major product. Periodate



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oxidation experiments revealed that esterification had taken place at one of the primary alcohol groups, and the compound was therefore identified as 1-*O*-acetyl-DL-galactitol (6-*O*-acetyl-DL-galactitol). Since only one-half of the DL-galactitol derivative, the D-isomer (i.e. 6-*O*-acetyl-L-galactitol), can be utilized by *A. suboxydans*, a lower copper reducing value would be anticipated from this substrate than from an L-isomer, such as 6-deoxy-L-galactitol, and this has been verified experimentally.

The oxidation product of 1-deoxy-1-*S*-ethyl-D-glucitol (VIII) has been identified conclusively as 6-deoxy-6-*S*-ethyl-L-sorbose (IX), since after conversion to the 2,3-*O*-isopropylidene derivative, reductive desulphurization gave 6-deoxy-L-sorbose which was characterized as the derived phenylosazone.

At the present time, structural studies are in progress on the products formed by oxidation with *A. suboxydans* of 6-deoxy-6-*S*-ethyl-L-galactitol and 1-*O*-acetyl-DL-galactitol. From the known specificity of the microorganism, these products are probably derivatives of 3-hexuloses.

EXPERIMENTAL

Solutions were concentrated under reduced pressure (*ca.* 15 mm). Optical rotations were determined in water at 23°±3° C unless otherwise stated. Paper chromatography was carried out by the descending method (17) on Whatman No. 1 filter paper using the following solvent systems (v:v): (*a*) ethyl acetate – acetic acid – water (9:2:2); (*b*) ethyl acetate – acetic acid – formic acid – water (18:3:1:4); (*c*) butan-1-ol–ethanol–water (40:11:19); and (*d*) butan-1-ol–pyridine–water (10:3:3). Paper electrophoresis was carried out on Whatman No. 3 filter paper impregnated with 0.05 *M* borate buffer (pH 9.2 at 1800 volts for 1.5 hours). Reducing compounds were detected on paper chromatograms and paper electrophoretograms by the *p*-anisidine hydrochloride reagent (13). Non-reducing compounds were detected on paper chromatograms with an alkaline silver nitrate reagent (18) and on paper electrophoretograms by 3% (w:v) lead tetra-acetate in absolute benzene (19). The rate of movement on paper chromatograms is quoted relative to that of rhamnose (R_{Rh} value). On paper electrophoretograms the mobilities are quoted relative to that of D-glucose (M_G value), and are corrected for endosmosis from the rate of movement of tetra-*O*-methyl-D-glucose relative to the D-glucose standard.

Acetobacter suboxydans (A.T.C.C. No. 621) was kindly supplied by Dr. N. K. Richtmyer. The stock culture was grown on agar slopes on a medium consisting of sorbitol (5%, w:v), yeast extract (0.5% w:v),* and potassium dihydrogen phosphate (0.05% w:v) (10). The substituted polyols under examination (Table II) were made up as a broth containing the polyol (1–3%, accurately weighed), yeast extract (0.5% w:v), potassium dihydrogen phosphate (0.05% w:v), and were fortified with sorbitol (0.1% w:v). Control solutions, made up in duplicate, contained the above constituents except the substituted polyol. The solutions (10 ml) in 75-ml conical flasks were autoclaved (15 p.s.i., 20 minutes), cooled to room temperature, and inoculated with a 72 hours' culture of *A. suboxydans* grown on sorbitol (1 drop). The flasks were stored without agitation at 27° C.

At intervals, an aliquot sample (*ca.* 2 ml) was removed from the medium with a sterile pipette and a little examined directly on paper chromatograms and paper electrophoretograms. The remainder (1 ml) was deproteinized and the reducing power estimated using Somogyi's microcopper reagent (20). Solutions of the sulphur-containing polyols

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were made up to identical concentration for copper-reduction estimation. The reducing values of the two control solutions were then subtracted from the reducing value of the medium containing the sulphur-containing polyols under biochemical test. The reducing values are quoted in moles of reducing sugar (calculated as L-sorbose)/100 moles of polyol.

The results are shown in Table II. The 1,1-diethyldithioacetals of D-arabinose, D-glucose, D-galactose, and the 1,1-dibenzyldithioacetal of D-arabinose were not oxidized by *A. suboxydans* as revealed by the copper-reducing power of the culture medium and the absence of any products on paper chromatograms.

Preparation of Substrates

2-Amino-2-deoxy-D-glucitol and 2-acetamido-2-deoxy-D-glucitol were prepared by borohydride-reduction of the respective aldoses according to the method of Bragg and Hough (21). 5-Deoxy-D-ribitol and 5-*O*-methyl-D-ribitol were prepared from D-ribono-1→4-lactone (22). 1-Deoxy-1-*S*-ethyl-polyols were prepared from the corresponding aldose diethyldithioacetals by partial desulphurization with aged Raney nickel (23).

1-Deoxy-1-S-ethyl-D-arabitol

D-Arabinose diethyldithioacetal (3.0 g) was partially desulphurized (23) to yield 57% of product melting at 124–125° C, and with $|\alpha|_D^{28}$ 13°±1° (*c*, 1.1). Anal. Calc. for $C_7H_{16}O_4S$: C, 42.8; H, 8.2; S, 16.3. Found: C, 43.1; H, 8.2; S, 16.7.

1-Deoxy-1-S-ethyl-D-arabitol Tetraacetate

1-Deoxy-1-*S*-ethyl-D-arabitol was acetylated with acetic anhydride in pyridine. The product crystallized when it was poured into a slurry of chipped ice and water, and it was collected by filtration. Recrystallization from aqueous ethanol yielded flakes that melted at 58° C, and with a $|\alpha|_D$ 38°±4° (*c*, 1.3, chloroform). Anal. Calc. for $C_{15}H_{24}O_8S$: C, 49.4; H, 6.6; S, 8.8. Found: C, 49.8; H, 6.8; S, 8.2.

1-O-Acetyl-DL-galactitol (6-O-Acetyl-DL-galactitol, 1-O-Acetyl-dulcitol)

Dulcitol (100 g) was heated under reflux with 50% acetic acid (100 ml) for 36 hours at 80° C (15, 16). Upon cooling, most of the unreacted dulcitol was recovered by filtration and the soluble acetates were obtained as a white residue upon removal of the solvent. Paper chromatographic examination of the residue indicated at least four constituents which had R_{Rh} values of 0.96, 1.23 (major constituent), 1.90, and 2.22 (solvent *a*). The mixture (55 g) was fractionated on a short charcoal column (10×12 cm) of Ultrasorb S.C.* 120–240 (24, 25). Elution was then carried out stepwise with successive fractions (3 liters) of water, and 1.5, 3, 5, 8, 12.5, and 25% ethanol; the major constituent was found in the 8% ethanol fraction. This fraction was filtered, deionized with Amberlite resins (IR-120 (H) and IR-400 (carbonate)) at 5° C, and concentrated to dryness. The product (18 g—low yield due to an accident) after recrystallization from ethanol melted at 131–133° C and possessed a strong adsorption peak in the infrared at ν_{max} 1745 cm^{-1} (nujol). Sorbitol hexaacetate gave an absorption peak at ν_{max} 1748 cm^{-1} and D-glucose diethyldithioacetal possessed a double-peaked adsorption at ν_{max} 1730 and ν_{max} 1750 cm^{-1}. Anal. Calc. for $C_8H_{16}O_7$: C, 42.9; H, 7.2; OAc, 19.2. Found: C, 43.0; H, 7.1; OAc, 19.7.

Structural Determination by Periodate Oxidation Experiments

Oxidations were carried out in the dark at 18° C.

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The sample (23 mg) was dissolved in water (21 ml) containing 0.3 *M* sodium metaperiodate solution (4 ml). At intervals, aliquots (2 ml) were withdrawn for either the determination of periodate consumption or the estimation of formic acid liberated (26). Formaldehyde was estimated on aliquots that were diluted 10-fold prior to the estimation (27).

TABLE I

The periodate oxidation of 1-*O*-acetyl-DL-galactitol

Hours	0.08	0.25	0.5	2.25	4.0	28
Periodate uptake*	3.7	3.9	3.9	4.0	4.0	
Formic acid*		2.9		2.8		2.8
Formaldehyde*	0.95		0.95		0.95	

*Moles/mole of 1-*O*-acetyl-DL-galactitol.

*6-Deoxy-6-S-ethyl-*L*-sorbose Phenylosazone*

A culture medium containing 1-deoxy-1-*S*-ethyl-D-glucitol was oxidized for 10 days with *A. suboxydans*. A portion of the solution was deproteinized, deionized with Amberlite resins (IR-120 (H) and IR-4B (OH)), and concentrated to a syrup. The components were separated by paper chromatography (Whatman 3MM) in the usual way (solvent *c*). Some of the syrupy ketose (18 mg) was dissolved in a mixture of phenylhydrazine (0.1 ml), acetic acid (0.1 ml), and water (0.5 ml), and heated in a water bath at 70°C for 3 hours. The phenylosazone (18 mg) was washed first with water, followed by benzene, and was finally recrystallized from aqueous ethanol to give a product melting at 185–190° C. Anal. Calc. for $C_{20}H_{26}N_4O_3S$: C, 59.7; H, 6.5; N, 13.9. Found: C, 59.6; H, 6.5; N, 13.7.

*Reductive Desulphurization to 6-Deoxy-*L*-sorbose (via the O-Isopropylidene Derivative)*

A solution of 6-deoxy-6-*S*-ethyl-L-sorbose (0.20 g) in acetone (20 ml) containing concentrated hydrochloric acid (0.2 ml) was shaken at room temperature for 2.5 hours

TABLE II

Substrates			Products						
			Copper-reducing power (days)						
	R_{Rh}[a]	M_G[b]	3	5	6	14	21	R_{Rh}[a,d]	M_G[d]
5-Deoxy-D-ribitol	1.2	0.75	81.5[c]	—	91	72	—	1.7 (Y)[f]	0.89
5-Deoxy-L-lyxitol	1.2	0.83	—	—	—	—	—	1.6 (Y)	0.89
6-Deoxy-L-galactitol (L-fucitol)	1.1	0.95	17.6	—	38	—	50	1.3 (Y)	0.80
5-*O*-Methyl-D-ribitol	1.3	0.72	6.7	—	15.4	22.9	24.4	1.8 (Y)	0.81
6-*O*-Acetyl-DL-galactitol	1.2	—	—	26	—	21.8	—	1.6[g](Y)	—
2-Amino-2-deoxy-D-glucitol	—	—	—	6.9	—	6.9	—	Nil	—
2-Acetamido-2-deoxy-D-glucitol	0.82	—	51	—	—	57	—	0.75 (Y)	—
1-Deoxy-1-*S*-ethyl derivative of:									
-D-Glucitol	1.6	—	19	—	87	116	116	2.1 (Y)	—
-L-Arabitol	1.8	0.85	—	—	—	—	—	Nil	—
-D-Arabitol	1.8	0.83	71	—	75	82	92	2.6 (O)[e]	0.84
-D-Galactitol	1.6	0.84	16	—	24	27	31	2.1 (Y)	0.88

[a]Solvent (*b*) detected with silver nitrate – sodium hydroxide reagent.
[b]Detected with lead tetraacetate reagent.
[c]Moles of reducing substance/100 moles of substrate.
[d]Detected with *p*-anisidine hydrochloride reagent.
[e]Orange color with *p*-anisidine hydrochloride (O).
[f]Yellow color with *p*-anisidine hydrochloride (Y).
[g]Detected with resorcinol reagent (13).

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followed by neutralization with silver carbonate. The filtrate was concentrated to a syrup which was non-reducing and moved on paper chromatograms at a rate considerably faster than the original ketose. The *O*-isopropylidene derivative (0.12 g) was dissolved in ethanol containing freshly prepared Raney nickel W-2 catalyst (6 ml settled volume) (28) and was heated under reflux for 4 hours. After cooling and filtering free of nickel, a syrup (97 mg) was obtained which had $|\alpha|_D$ 18°±2° (*c*, 1.2, ethanol). (Lit. for 2,3-*O*-isopropylidene-L-sorbose $|\alpha|_D$ 17.9° (29).)

A solution of the syrupy *O*-isopropylidene compound (61 mg) was dissolved in a mixture of phenylhydrazine (0.1 ml), acetic acid (0.1 ml), and water (2 ml), and heated in a water bath at 70° C for 3 hours. The phenylosazone which crystallized from the solution during heating was collected by filtration and washed with cold water followed by ether. Its melting point (175–180° C) was not depressed when admixed with an authentic specimen prepared from 6-deoxy-L-sorbose (30). (Lit. melting point 183–185° C (29) and 168–172° C (9).) The infrared adsorption spectra of the derived and authentic specimens were identical over the frequency range of 600–4000 cm^{-1}.

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