diheterosorbosan I hexaacetate and the crystalline product, isolated in the same manner, was recrystallized from ethanol; yield 940 mg., m.p. 177–179°, $[\alpha]^{25}D$ −19° (*c* 2.1, chloroform), needles; main X-ray powder diffraction lines[13]: 7.85 (3), 5.17 (2), 4.37 (1), 3.94 (4), 3.16 (5). The substance was soluble in benzene, chloroform and in warm ethanol and ether.

Anal. Calcd. for $C_{24}H_{32}O_{16}$: C, 49.99; H, 5.60; mol. wt., 576.5. Found: C, 50.27; H, 5.73; mol. wt. (Rast), 582.

The combined aqueous decantate and washings from the acetylation was neutralized to *p*H 6–7 with sodium bicarbonate and extracted with six 10-ml. portions of chloroform. The combined chloroform extracts were washed with water, *N* HCl, saturated aqueous $NaHCO_3$ and again with water. The sirup obtained on solvent removal from the dried chloroform solution was added to the sirupy mother liquor material from the crystallization of the above acetate; total yield 800 mg. This material was dissolved in 100 ml. of benzene and added to the top of a 30 × 7.7 cm. (diam.) column of Magnesol[15]–Celite[12] (5:1 by wt.) and the chromatogram was developed with 2800 ml. of benzene–ethanol (100:1 by vol.). The permanganate indicator (1% $KMnO_4$ in 2.5 *N* NaOH) streak located a main zone on the extruded column 100–150 mm. from the column top which was desorbed with 1500 ml. of acetone; yield 400 mg. (total 1.34 g. or 76%) of the above acetate, m.p. 171–173.5° raised to 179° by one recrystallization from benzene–ether (1:10), mixed m.p. 177–179°. The X-ray diffraction patterns were identical.

Diheterosorbosan II (V or VI).—An amount of 500 mg. of diheterosorbosan II hexaacetate was deactylated as described above for diheterosorbosan I hexaacetate and the product, isolated in the same manner, was crystallized from ethanol; yield 180 mg., m.p. 188–189°, $[\alpha]^{26}$ 0.00° (*c* 1 in water and in methanol; throughout the visible portion of the spectrum), prisms, Benedict (−) but (+) after hydrolysis with concd. HCl at 100°; main X-ray powder diffraction lines[13]: 6.39 (4), 5.13 (3), 4.78 (2), 4.49 (1), 3.22 (5). The substance was soluble in water and methanol and was insoluble in ethanol.

Anal. Calcd. for $C_{12}H_{20}O_{10}$: C, 44.44; H, 6.22. Found: C, 44.43; H, 6.25.

Periodate assay (moles per mole of reductant; 0.05 *M* $NaIO_4$ in 0.004 *M* reductant, 26 ± 3°, 7 days required for complete reaction): oxidant consumed, 3.1; formic acid (acidity toward brom cresol purple), 1.0; formaldehyde, 0.0.

COLUMBUS 10, OHIO

(15) A hydrated magnesium acid silicate produced by the Westvaco Chlorine Products Corp., South Charleston, West Virginia.

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

Threitan and Erythritan and their Reaction with Periodate[1]

BY H. KLOSTERMAN AND F. SMITH

RECEIVED MAY 24, 1952

Dehydration of L-threitol and erythritol with 50% sulfuric acid has provided the corresponding 1,4-anhydrides, L-threitan (III) and erythritan (IV), respectively. Both III and IV react with one molecular proportion of sodium periodate and periodic acid; IV, with a *cis*-glycol structure, undergoes cleavage at a greater rate than III which has a *trans*-glycol structure.

Periodic acid and its sodium and potassium salts have been extensively used in determination of the structure of carbohydrates.[2] The results of a large number of experiments have shown that this oxidizing agent cleaves the bond between two carbon atoms carrying hydroxyl groups. It matters little whether the glycol grouping is of the *cis* or the *trans* type although the former is attacked at a greater rate. From these observations the failure of a polyhydroxy compound to react with periodate has been taken to indicate the absence of a 1,2-diol grouping. More recently, however, the danger inherent in using such negative evidence in structural studies has been demonstrated by the fact that both β-1,6-anhydro-D-glucofuranose (I) and α-1,6-anhydro-D-galactofuranose (II), which possess a glycol group, have been found to be completely resistant to periodate oxidation.[3–5] This resistance was attributed to the stereochemical effects caused by the presence of two interlocking rings. Inspection of molecular models of I and II shows that this type of structure with two interlocking rings, which is known to induce unexpected properties in carbohydrate compounds,[6–8] results in the molecules of I and II being essentially rigid and the hydroxyl groups assuming a fixed *trans* configuration. The fixed *trans* positions of the OH groups at C_2 and C_3 thus prevent the formation of an intermediate chelate complex with the periodate ion which appears to be necessary before cleavage can take place.[9]

The importance of this observation, in connection with the use of periodate oxidation in structural studies of the polysaccharides, is that certain sugar residues might not undergo cleavage by periodate, if the stereochemical arrangement of the polysaccharide molecule forces them to assume a rigid conformation in which the adjacent hydroxyl groups take up a fixed *trans* position with respect to each other. Such a contingency might possibly affect the behavior of the interior but not the terminal units, and hence is unlikely to interfere with the very convenient periodate method of end-group analysis.[10–12]

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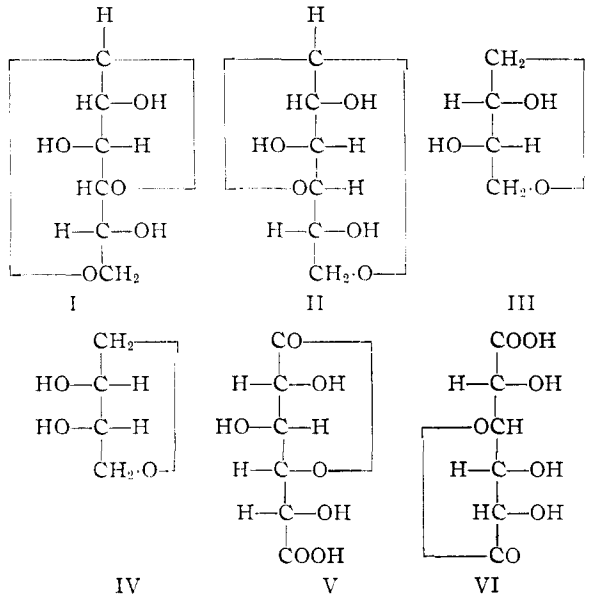

In an attempt to obtain more evidence concerning resistance of compounds I and II to periodate oxidation it seemed desirable to study the effect of periodate upon L-threitan (III) and erythritan (IV) the former of which contains the same 1:4 anhydro ring system with a *trans*-glycol grouping as that present in the two compounds I and II. If resistance to periodate oxidation of the *trans*-glycol grouping in the case of I and II is not influenced by the presence of the second (1:6) anhydro ring, it would be expected that L-threitan (III) would be stable to periodate while erythritan (IV) would undergo cleavage in the usual manner. On the other hand, if molecular rigidity resulting from the two interlocking rings is the factor which determines the behavior of I and II, it would be expected that both L-threitan and erythritan, having a certain degree of stereochemical freedom, would be cleaved by periodate with the latter perhaps undergoing cleavage at a greater rate than the former. This paper is concerned with the synthesis of L-threitan and erythritan and a study of their reaction with sodium periodate and periodic acid. Certain related compounds also have been examined.

The L-threitan required for these model experiments was prepared as follows: reduction of either amyl or butyl L-tartrate with lithium aluminum hydride in tetrahydrofuran[13] afforded crystalline L-threitol. Although the latter gives a crystalline ditosyl compound the yield is low and it is best to characterize the L-threitol as the dibenzylidene compound. In order to obtain good yields of L-threitol the intermediate metal complex, formed during the reduction, should be decomposed for example, by acetylation.[13,14] Dehydration of L-threitol with 50% sulfuric acid, by prolonged heating at 120°, afforded the required crystalline L-threitan (III) which in turn yielded a crystalline di-*p*-nitrobenzoate. Dehydration of erythritol proceeded quite readily by boiling with 50% sulfuric acid to give the required erythritan (IV),[15] a very hygroscopic liquid, which may be characterized as its di-*p*-nitrobenzoate.

Upon treatment with periodate both III and IV undergo cleavage, one molecular proportion of oxidant being consumed in each case. The reaction was, however, noticeably slower in the case of the *trans* compound (III) especially when periodic acid was used instead of sodium periodate (see Tables I and II).

TABLE I

OXIDATION WITH 0.1 *N* $NaIO_4$ AT 5° IN AQUEOUS SOLUTION

	Compound	Time, hr.	Moles of $NaIO_4$ consumed per mole of compound: Found	Calcd.
(1)	L-Threitol	2	3.00	3
(2)	Erythritol	2	3.04	3
(3)	L-Threitan	2	0.45	1
		4	.66	
		22	1.01	
(4)	Erythritan	2	0.92	1
		4	.94	
		22	.99	
(5)	D-Glucosaccharo-1,4-lactone	0.5	1.30	3
		22	2.80	
(6)	D-Glucosaccharo-3,6-lactone	0.5	3.18	3
		22	3.83	

TABLE II

OXIDATION WITH 0.1 *N* HIO_4 AT 5° IN AQUEOUS SOLUTION

	Compound	Time, hr.	Moles of HIO_4 consumed per mole of compound: Found	Calcd.
(1)	L-Threitan	0.5	0.12	1
		1.0	.25	
		2	.43	
		4	.58	
		6	.62	
		48	.95	
(2)	Erythritan	0.5	.73	1
		1	.90	
		2	.97	
		4	.97	
(3)	D-Glucosaccharo-1,4-lactone	0.5	1.18	3
		1	1.20	
		5	1.70	
		8	1.80	
		48	2.20	
(4)	D-Glucosaccharo-3,6-lactone	0.5	2.93	3
		1	2.97	
		5	3.00	
		8	3.18	
		48	3.40	

Similar results were obtained in a study of the periodate oxidation of the two glucosaccharolactones (V) and (VI). The former (V), having a *trans*-glycol structure, reacted at a much slower rate than VI which possesses the *cis*-glycol structure.[16] The rate of reaction was again found to be dependent on the *p*H as in the case of L-threitan (III) and erythritan (IV), since reaction with sodium periodate proceeded more rapidly than with periodic acid (see Tables I and II).

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The experimental evidence recorded herein lends strong support to the view expressed by Alexander, *et al.*,[4] that the resistance of I and II to periodate oxidation is due to the adjacent hydroxyl groups at C_2 and C_3 being held in a fixed *trans* position with respect to each other, as a result of the stereochemical rigidity imposed by the two interlocking anhydro-rings. As these authors point out, it would be particularly interesting to examine the effect of periodate on β-1,6-anhydro-α-D-mannofuranose.

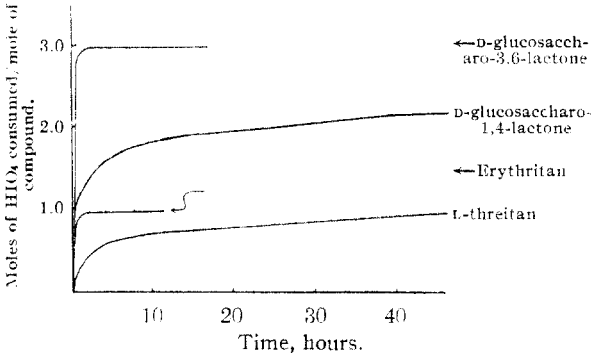

Fig. 1.

Experimental

A. Synthesis of L-Threitan (III). (a) Butyl L-Tartrate.—A mixture of L-tartaric acid (10 g.), 1-butanol (50 ml.) and sulfuric acid (2 ml.) was refluxed for nine hours, cooled and poured into water. The butanol layer was extracted with water to remove mineral acid, diluted with ether (100 ml.) and dried ($MgSO_4$). Filtration and removal of ether and excess butanol left butyl L-tartrate, a colorless liquid, b.p. (bath temp.) 160–165° (0.5 mm.), $n^{21}D$ 1.4450, d^{25} 1.11, m.p. 20–25°, $[\alpha]^{25}D$ +9.5° (no solvent used).

Replacing 1-butanol by 1-pentanol in the above reaction afforded amyl L-tartrate as a pale yellow fairly mobile liquid (12.5 g.), b.p. (bath temp.) 153° (0.5 mm.), $n^{21}D$ 1.4480, d^{25} 1.07, $[\alpha]^{25}D$ +8.7° (no solvent used).

(b) L-Threitol.—(i) A solution of butyl L-tartrate (2.04 g.) in tetrahydrofuran (15 ml. dried and distilled over P_2O_5) was added dropwise to a solution of lithium aluminum hydride (1.0 g.) in dry tetrahydrofuran (20 ml.) with cooling.[13] After 15 minutes when the hydroxamic acid test[17,18] for esters was negative the reaction mixture was poured slowly with stirring into water (60 ml.). The precipitate was centrifuged, washed with water and the filtrate concentrated *in vacuo* almost to dryness. The residue was dissolved in 50% aqueous methanol (40 ml.) and small pieces of Dry Ice added to precipitate lithium carbonate. Filtration of the solution followed by concentration *in vacuo* afforded L-threitol as a colorless glassy solid (0.7 g.) which failed to crystallize. The product was therefore dissolved in 50% sulfuric acid (4 ml.) and shaken with benzaldehyde (3 ml.) whereupon the dibenzylidene L-threitol separated almost immediately. After one hour the reaction mixture was diluted with water, filtered, and washed thoroughly with water, followed by a small amount of cold methanol to remove residual benzaldehyde. Recrystallization from toluene gave dibenzylidene L-threitol as long needles (0.75 g.), m.p. 224°, $[\alpha]^{25}D$ +81° in acetone (*c* 0.5); Ness, *et al.*, quote m.p. 219–223° for this compound.[14]

Anal. Calcd. for $C_{18}H_{18}O_4$: C, 72.5; H, 6.1. Found: C, 72.4; H, 6.0.

The dibenzylidene L-threitol (0.32 g.) was boiled for 30 minutes with a mixture of *N* sulfuric acid (10 ml.) and ethanol (5 ml.) to regenerate the L-threitol. The solution was evaporated *in vacuo* to remove as much ethanol as possible and then extracted with ether to remove benzaldehyde. The aqueous layer was neutralized ("Duolite" A_4) and evaporated to dryness *in vacuo* to give crystalline L-threitol (0.1 g.), m.p. 88° (after recrystallization from ethanol), $[\alpha]^{27}D$ −4.0° in water (*c* 7.0) and $[\alpha]^{22}D$ +13° in ethanol (*c* 2.0). Bertrand[19] quotes m.p. 88–89° and $[\alpha]D$ −4.5° in water, while Ness, *et al.*, quote m.p. 87–88°, $[\alpha]D$ +11° in ethanol[14] for this compound.

Anal. Calcd. for $C_4H_{10}O_4$: C, 39.4; H, 8.3. Found: C, 39.45; H, 8.5.

(ii) A solution of butyl L-tartrate (5 g.) in tetrahydrofuran (25 ml.), was added slowly with stirring to a solution of lithium aluminum hydride (3 g.) in a mixture of tetrahydrofuran (75 ml.) and ether (25 ml.). As in the first experiment, no precipitation took place during this or any subsequent stage of the reduction. The mixture was boiled for four hours, cooled and poured slowly with stirring into water (150 ml.). After adding acetic acid (10 ml.), the mixture was evaporated *in vacuo* to dryness. To the dry residue, acetic anhydride (100 ml.) was added and the mixture was heated for one hour on the boiling water-bath and then boiled for two hours under reflux (oil-bath), to effect simultaneous cleavage and acetylation of the metal complex. After removing most of the acetic anhydride by distillation *in vacuo*, the residue was cooled and shaken with water (100 ml.) and sufficient hydrochloric acid to dissolve the inorganic salts. The acetylated L-threitol was extracted with chloroform and the chloroform extract washed with water to remove mineral acid, and dried ($MgSO_4$). Removal of solvent afforded tetraacetyl-L-threitol (5.1 g.), which distilled as a fairly mobile colorless liquid, b.p. (bath temp.) 145° (0.05 mm.), $n^{26}D$ 1.4380, $[\alpha]^{25}D$ −32° in ethanol (*c* 4.0). It could not be induced to crystallize.

Treatment of the tetraacetyl-L-threitol with a mixture of *N* sodium hydroxide (75 ml.) and ethanol (50 ml.), followed by passage through a cation exchange resin ("Amberlite" IR 120) and removal of solvent, yielded L-threitol (2.18 g.), which crystallized spontaneously, m.p. 88°, after crystallization from methanol. In other experiments the deacetylation of tetraacetyl L-threitol was carried out by Zemplén's method.[20]

Several experiments showed that amyl L-tartrate could replace butyl L-tartrate equally well in the above reaction with lithium aluminum hydride to give L-threitol.

When L-threitol (50 mg.) was treated with *p*-toluenesulfonyl chloride (4.5 moles) in dry pyridine (3 ml.) for four days at room temperature, the crystalline product, isolated in the usual way by pouring the reaction mixture into water, proved to be a mixture of two derivatives. The principal product was ditosyl L-threitol which readily crystallized from ethanol or ethanol–petroleum in the form of needles, m.p. 92° (yield 65 mg.).

Anal. Calcd. for $C_{18}H_{22}O_8S_2$: C, 50.2; H, 5.2; S, 14.9. Found: C, 50.1; H, 5.1; S, 14.4.

The second component, believed to be a monotosyl derivative, was less soluble in ethanol than the ditosyl derivative and separated from ethanol in the form of short prisms, m.p. 120° (yield 10 mg.). Inasmuch as the yields of these two derivatives were so low and could not be improved by varying the conditions, neither is recommended for the identification of L-threitol. It is of some interest, particularly from a stereochemical point of view, to note that erythritol readily yields a tetratosyl derivative in excellent yield.[21]

(c) L-Threitan (III).—L-Threitol (2.25 g.) was heated in a sealed tube for 24 hours at 120° with a mixture of water (2.2 g.) and sulfuric acid (2.2 g.). The solution was diluted with water (15–20 ml.), neutralized ("Duolite" A_4 anion exchange resin) and evaporated *in vacuo* to dryness. Distillation of the residue yielded L-threitan (1.3 g.), b.p. 180–185° (bath temp.) (17 mm.), m.p. 60–61°, unchanged after recrystallization from dioxane–ether, $[\alpha]^{25}D$ −5° in water (*c* 6.8). L-Threitan is very hygroscopic and quickly changes to a sirup when exposed to the air.

Anal. Calcd. for $C_4H_8O_3$: C, 46.2; H, 7.8. Found: C, 46.1; H, 7.7.

L-Threitan di-*p*-nitrobenzoate, prepared in the usual way by the action of *p*-nitrobenzoyl chloride (1.1 moles) on L-threitan in dry pyridine, had m.p. 191–192° after crystallization from acetone, ethanol, benzene or chloroform–petroleum ether.

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Anal. Calcd. for $C_{18}H_{14}N_2O_9$: C, 53.7; H, 3.5; N, 7.0. Found: C, 53.8; H, 3.6; N, 7.0.

B. Synthesis of Erythritan (IV).—A solution of erythritol (3 g.) in a mixture of sulfuric acid (3 g.) and water (3 g.) was boiled gently under reflux for 15 hours. The erythritan, isolated in the same manner as L-threitan, was a colorless, hygroscopic liquid, b.p. (bath temperature) 160–165° (0.17 mm.), $n^{24}D$ 1.4370 (yield 1.3 g.).[15]

Anal. Calcd. for $C_4H_8O_3$: C, 46.2; H, 7.8. Found: C, 46.0; H, 7.8.

Erythritan di-*p*-nitrobenzoate, prepared in the usual way, separated from acetone in the form of needles, m.p. 173–174°.

Anal. Calcd. for $C_{18}H_{14}N_2O_9$: C, 53.7; H, 3.5; N, 7.0. Found: C, 53.9; H, 3.6; N, 7.1.

C. Oxidation with Periodate.—The compounds were oxidized with sodium periodate and with periodic acid in aqueous solution under the conditions previously used.[12] The results of the oxidations with 0.1 *N* sodium periodate are given in Table I. Oxidation with 0.1 *N* periodic acid, which proceeded according to the results recorded in Table II and represented graphically in Fig. 1, illustrates clearly the marked effect of the stereochemical arrangement of adjacent hydroxyl groups on the rate of oxidation by periodate.

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[CONTRIBUTION FROM THE NORTHERN REGIONAL RESEARCH LABORATORY[1]]

Dextran Triacetates

BY ALLENE JEANES AND C. A. WILHAM

RECEIVED JUNE 2, 1952

A simple, non-degradative method is described for preparing dextran triacetates from dextrans in the form of either the hydrated gum or the dry powder. This method has been applied to the acetylation of nine bacterial dextrans differing widely in chemical and physical characteristics. Intrinsic viscosities and specific rotations of the dextran triacetates show close relationship to the corresponding data for the respective dextrans as well as to the periodate oxidation data for these dextrans. This proves that differences among the dextrans are due to fundamental structural characteristics which are carried over into the triacetates. The degradation temperatures of these dextran triacetates and the film-forming ability of one of them are shown to correlate with their chemical structures.

This report describes the preparation and some of the properties of triacetates of nine bacterial dextrans. The particular dextrans were chosen for this purpose because of their widely different characteristics. These triacetates are being employed at this Laboratory in a program on chemical and physical characterization of many dextrans and in comparative studies on the α-1,6-glucopyranosidic linkage in starch and in dextrans.[2] The rapidly expanding interest in the structure,[3-5] properties[2,6] and application in medicine[7] of dextrans makes it desirable to confirm and extend, through study of a derivative such as the triacetate, differences previously established only in the polysaccharides. The observations reported here also augment the already recognized industrial potentialities of dextran acetates[8] by making available for the first time undegraded products of favorable solubility characteristics.

Previously reported methods for preparation of dextran acetates resulted in incomplete acetylation[9,10] or in products which appeared to be fractionated[10,11] or degraded.[11]

Experimental

Materials and Analytical Methods.—All the dextrans used in this study were prepared in a manner similar to that already described for dextran from *Leuconostoc mesenteroides* NRRL B-512.[2] They contained no more than about 0.05% ash, 0.01% nitrogen, 0.001% phosphorus and no detectable fructose.

Formamide was a neutral fraction obtained from commercial material by distillation *in vacuo*. *sym*-Tetrachloroethane was washed free of acid, dried and distilled before use.

Acetyl determinations were made on 150-mg. samples (80 mesh, unless the preparation had been freeze-dried) by a modified Eberstadt method as described by Murray, Staud and Gray.[12] The values are reproducible to ±0.2% acetyl.

Viscosity measurements were made in Ostwald–Cannon–Fenske No. 100 tubes.

All samples for analytical measurements were equilibrated with atmospheric moisture in the room in which the samples were weighed and where a constant relative humidity of 61% at 21° was maintained. The dextran triacetates were found to have moisture contents of 3 to 4% by drying samples *in vacuo* over phosphorus pentoxide for 4 hours at 100°. All calculations were made on a dry basis.

Acetylation in Formamide.—An adaptation of the method of Carson and Maclay was employed,[13] under conditions so mild as to preclude the likelihood of degradation. Air-dry dextran in a fluffy, homogeneously reactive state[2] was used, or gum dextran which had been precipitated from aqueous solution by addition of ethanol to 50% by volume and from which excess aqueous ethanol had been expressed. Five grams (dry basis) of the dextran was mixed with 75 ml. of ice-cold formamide and solution was completed at room temperature. To this stirred solution was added slowly 75 ml. of pyridine and then 65 ml. of acetic anhydride was added over a period of about 1.5 to 3 hours. The reaction mixture was maintained at room temperature by cooling, if necessary. In most cases addition of acetic anhydride caused a finely divided precipitate to form. However, acetylation mixtures of dextrans from B-1299 and B-1355 and of fraction C from B-742, remained homogeneous, and that from B-523 was heterogeneous throughout the acetylation procedure. The reaction mixture was stirred for 4 hours at room temperature and allowed to stand 20 hours.

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

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