THE SYNTHESIS OF 5-DEOXY-5-*S*-ETHYL-D-THREO-PENTULOSE[1]

J. K. N. JONES AND D. L. MITCHELL

ABSTRACT

The microbiological oxidation of 1-deoxy-1-*S*-ethyl-D-arabitol by *Acetobacter suboxydans* has yielded syrupy 5-deoxy-5-*S*-ethyl-D-*threo*-pentulose from which a crystalline phenylosazone identical with the one prepared from 5-deoxy-5-*S*-ethyl-D-*xylo*-pentose was obtained. A syrupy 3,4-*O*-isopropylidene-5-deoxy-5-*S*-ethyl-D-*threo*-pentulose and its crystalline 2,5-dichlorophenylhydrazone were prepared.

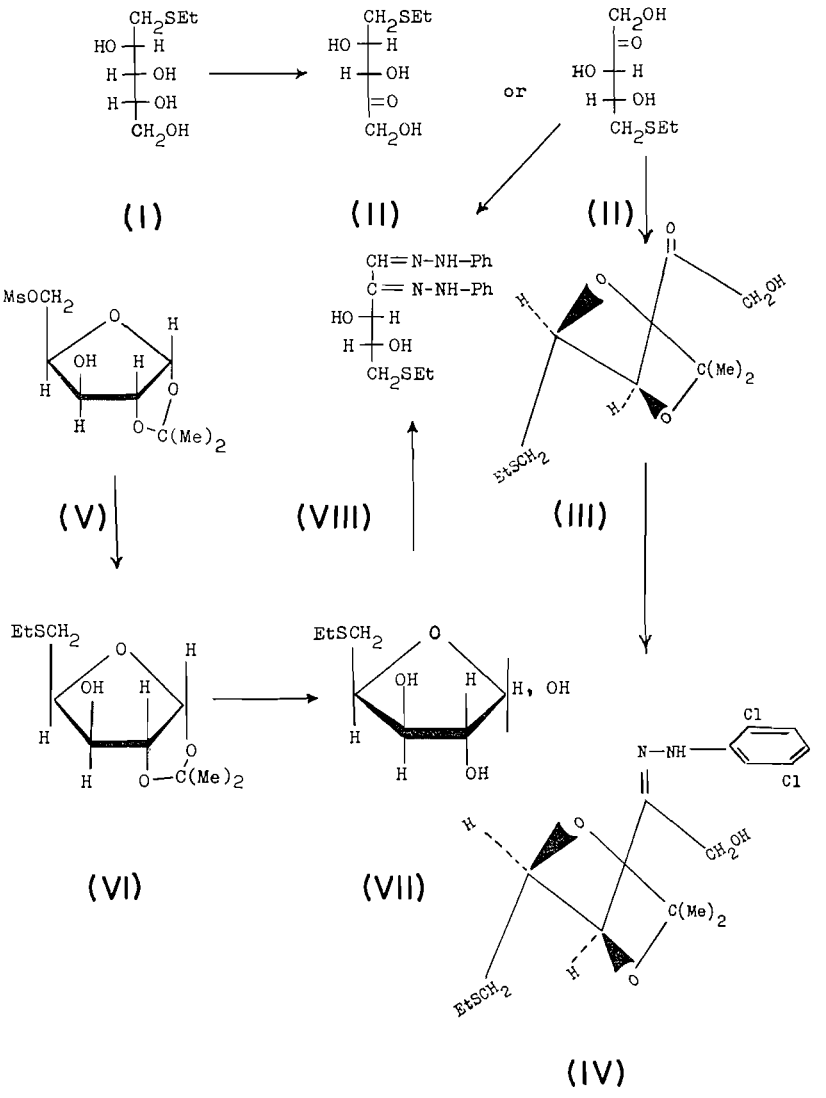
INTRODUCTION

The microbiological oxidation of terminal substituted polyhydric alcohols by *Acetobacter suboxydans* has been the subject of investigation in several laboratories (1, 2, 3). Recently, we reported that 1-deoxy-1-*S*-ethyl-D-glucitol was oxidized to 6-deoxy-6-*S*-ethyl-L-sorbose (3), and in this communication we report the microbiological oxidation of a structurally similar polyol, 1-deoxy-1-*S*-ethyl-D-arabitol (I), to 5-deoxy-5-*S*-ethyl-D-*threo*-pentulose (II). The ω-deoxy analogue, 5-deoxy-D-*threo*-pentulose, $[\alpha]_D$ −5° in water, has been prepared by an aldolase-catalyzed condensation between acetaldehyde and dihydroxyacetone monophosphate (4); it was also most likely obtained when 1-deoxy-D-arabitol was oxidized by *A. suboxydans* (1).

When the culture medium contained sorbitol (0.4%), 1-deoxy-1-*S*-ethyl-D-arabitol was completely oxidized after 10 days to a strongly reducing compound. Copper reducing values previously published also support this conclusion (3). However, when sorbitol was not included in the medium, oxidation proceeded at a slower rate and was incomplete after 8 days. The oxidation product (II), $[\alpha]_D$ −41° in ethanol, which was obtained as a chromatographically pure syrup, revealed a strong absorption band in the carbonyl stretching frequency at 1710 cm^{-1}, while the corresponding 5-deoxy-5-*S*-ethyl-(D-*xylo*- and L-*arabino*-pentoses) possessed only a trace of carbonyl absorption and must therefore exist almost entirely as furanoses. Periodate oxidation of (II) at pH 3.7 followed by an estimation of the liberated formaldehyde (0.6 mole) indicated that the reducing compound had a primary alcohol group and that the site of enzymic oxidation was not at carbon 1. It appeared that periodate oxidation took place mainly between carbons 1 and 2. However, cleavage between carbons 2 and 3, or carbons 3 and 4, is possible although it could lead to an intermediate (i.e. glycollic acid) which is very stable to further attack by periodate ion (5). If enzymic oxidation occurred at a site other than the first carbon atom, the formation of a furanol ring system would be prohibited and the molecule would most likely exist in a zigzag conformation. The hydroxyl groups at carbons 3 and 4 would then be favorably situated for scission by periodate ion and also for ketal formation. Reaction of the ketose (II) with acidified acetone led to the formation of a strongly reducing *O*-isopropylidene derivative which possessed strong absorption at the carbonyl and carbon–hydrogen stretching frequencies. Considerable reduction of the absorption peak in the hydroxyl region of this compound was observed when its spectrum was compared with that of the ketose (II). Periodate oxidation of the *O*-isopropylidene derivative at pH 7 liberated formaldehyde (0.6 mole), which indicated

[1]*Manuscript received May 19, 1959.*
Contribution from the Department of Chemistry, Queen's University, Kingston, Ontario.

that carbons 1 and 2 were not involved in ketal formation. Reaction of the *O*-isopropylidene derivative (III) with 2,5-dichlorophenylhydrazine produced a small amount of the crystalline 2,5-dichlorophenylhydrazone (IV) which gave absorptions in the infrared corresponding to hydroxyl, —C=N—, and ketal groups (6). This evidence indicates that the biochemical oxidation product is the acyclic 2-pentulose, 5-deoxy-5-*S*-ethyl-D-*threo*-pentulose (II) and that the derived ketal is 3,4-*O*-isopropylidene-5-deoxy-5-*S*-ethyl-D-*threo*-pentulose (III). These conclusions are also consistent with the well-known enzyme specificity for oxidations at pH 6.



Independent proof of the structure (II) was obtained through the synthesis of 5-deoxy-5-*S*-ethyl-(D-*xylo*- (VII) and L-*arabino*-pentose) via the 1,2-*O*-isopropylidene-5-*O*-toluene-*p*- (or methane-) sulphonyl ester (e.g. (V) → (VI)). The thioethyl aldoses (VII) displayed physical properties different from the thioethyl ketose (II). However, 5-deoxy-5-*S*-ethyl-

D-*xylo*-pentose gave a phenylosazone (VIII) identical with that which was prepared from (II) but which differed from the phenylosazone of 5-deoxy-5-*S*-ethyl-L-*arabino*-pentose. Therefore, the hydroxyls at carbons 3 and 4 of (II) possess the D-*threo*-configuration.

EXPERIMENTAL

Solutions were concentrated under reduced pressure (ca. 15 mm) and at 40° C or less. Melting points were uncorrected and optical rotations were determined in water unless otherwise stated. Paper chromatography was carried out by the descending method on Whatman No. 1 filter paper using the following solvent systems (v/v): (*a*) ethyl acetate – acetic acid – formic acid – water (18:3:1:4); (*b*) ethyl acetate – acetic acid – water (9:2:2); (*c*) butan-1-ol–ethanol–water (9:3:3); and (*d*) butan-1-ol–pyridine–water (10:3:3). Non-reducing compounds were detected on paper chromatograms with an alkaline silver nitrate spray reagent (7) and reducing compounds were detected with *p*-anisidine hydrochloride reagent (8). The rate of movement of the compound on paper chromatograms is quoted relative to that of rhamnose (R_{Rh} value). Infrared absorptions were measured as solutions in chloroform or as a powder suspended in a potassium bromide pellet on a Perkin–Elmer Model 21 spectrophotometer and wave numbers are given to within ± 10 cm^{-1}.

*5-Deoxy-5-S-ethyl-*D*-threo-pentulose*

A broth consisting of 1-deoxy-1-*S*-ethyl-D-arabitol (3) (0.42 g), sorbitol (0.2 g), yeast extract* (0.1 g), and potassium dihydrogen phosphate (0.01 g) in tap water (50 ml) was autoclaved (15 p.s.i., 30 minutes), cooled, inoculated with *Acetobacter suboxydans* (A.T.C.C. No. 621), and stored at 28° C for 10 days. Paper chromatographic examination of the medium then indicated the formation of a single major reducing constituent, besides sorbose, which gave an orange-red (apricot) color with the *p*-anisidine hydrochloride reagent. Chromatographic comparison with 5-deoxy-5-*S*-ethyl-L-*arabino*-pentose and 5-deoxy-5-*S*-ethyl-D-*xylo*-pentose, which both gave bright pink colors with the same reagent, indicated that it was a different compound because its rate of movement was slower in solvent systems (*c*) and (*d*). The rates of movement were practically identical in solvent systems (*a*) and (*b*). With the orcinol – trichloroacetic acid spray reagent no color reaction was produced but with the silver nitrate reagent a very rapid formation of silver resulted.

The broth was poured with stirring into ethanol (150 ml) and the cell debris was separated by filtration. After concentration of the filtrate, the oxidation product was separated on Whatman 3 MM filter paper and was obtained as a chromatographically pure syrup (0.20 g, 67%). The syrup was soluble in water, ethanol, acetone, and chloroform. A specimen which was dried *in vacuo* for 4 days was dissolved in chloroform (3% w:v) and its absorption measured in the infrared. A strong, sharp peak in the carbonyl stretching frequency at 1710 cm^{-1} was observed; $[\alpha]_D$ $-41° \pm 2°$ (*c*, 0.9, ethanol). After 3 weeks' storage at room temperature, the syrup was re-examined by paper chromatography, and revealed streaking in the vicinity of the constituent, presumably due to the development of isomers via keto $\rightleftarrows$ enol tautomerism. Some time later a second reducing constituent appeared with a rate of movement approximately that of rhamnose (solvent *a*).

In another experiment, *Acetobacter suboxydans* was grown on the same medium described above except that sorbitol was not included. After 8 days' oxidation, the

**Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.*

substrate and oxidized product appeared to be present on paper chromatograms in a ratio of 1:1.

TABLE I

Paper chromatographic values

Solvent system	(*c*)	(*d*)
R_{Rh} value of:		
5-Deoxy-5-*S*-ethyl-L-*arabino*-pentose	2.11*	1.98*
5-Deoxy-5-*S*-ethyl-D-*threo*-pentulose	2.02	1.86
5-Deoxy-5-*S*-ethyl-D-*xylo*-pentose	2.14	2.00

*Mean of two measurements.

*5-Deoxy-5-S-ethyl-*D*-threo-pentulose Phenylosazone*

A portion of the syrupy 5-deoxy-5-*S*-ethyl-D-*threo*-pentulose (ca. 50 mg) was dissolved in a mixture of phenylhydrazine (0.1 ml), acetic acid (0.2 ml), and water (2 ml) and the solution was heated to 80° C for 2 hours. The derived phenylosazone was collected after the solution had been cooled and diluted with water. It was recrystallized from a small volume of ethanol and the product was washed with cold (−60° C) ethanol. Several recrystallizations gave a product of constant melting point 158–159° C (decomp.) which possessed an infrared absorption spectrum identical with that of the phenylosazone derived from 5-deoxy-5-*S*-ethyl-D-*xylo*-pentose. Mixed melting point 155–156° C, $[\alpha]_D^{20} -29° \pm 4°$ (*c*, 1.2, pyridine). Anal. Calc. for $C_{19}H_{24}N_4O_2S$: C, 60.0; H, 6.7. Found: C, 59.5; H, 6.4.

*3,4-O-Isopropylidene-5-deoxy-5-S-ethyl-*D*-threo-pentulose*

Syrupy 5-deoxy-5-*S*-ethyl-D-*threo*-pentulose (ca. 75 mg) was dissolved in acetone (50 ml) containing concentrated sulphuric acid (1 drop) and the solution was shaken for 18 hours. After neutralization with barium carbonate, filtration, and concentration of the filtrate, the resulting syrup was examined by paper chromatography and infrared spectroscopy. A compound which moved much faster than the original material and which had R_{Rh} 2.7 (solvent (*a*)) was detected on the chromatogram as an orange-colored spot (*p*-anisidine hydrochloride); it also gave an immediate reaction with the silver nitrate – sodium hydroxide reagent. Traces of acetone were removed by repeated codistillation with chloroform, and a chloroform solution of the *O*-isopropylidene derivative was examined in the infrared. It possessed a medium weak absorption band at 3350 cm^{-1} and a medium strong absorption band at 1709 cm^{-1}. When the syrup was examined chromatographically in high concentration, a trace of 5-deoxy-5-*S*-ethyl-D-*threo*-pentulose was detected, $[\alpha]_D$ 21.5° (*c*, 0.6, ethanol).

*3,4-O-Isopropylidene-5-deoxy-5-S-ethyl-*D*-threo-pentulose 2,5-Dichlorophenylhydrazone*

A solution of the syrupy *O*-isopropylidene derivative (30 mg) and 2,5-dichlorophenylhydrazine (30 mg) in methanol (10 ml) were heated under reflux for 3 hours. After removal of the solvent, the light brown syrup was redissolved in *n*-hexane (5 ml), the solution was chilled in a solid carbon dioxide – ethanol mixture, and crystallization was induced by rubbing the walls of the flask with a spatula. The white powder (10 mg), m.p. 89–91° C, absorbed strongly in the infrared at 1595 cm^{-1} and the carbonyl absorption at 1710 cm^{-1} was present as a very weak peak barely detectable over the background fluctuation. Absorptions were also recorded at 1458 (m), 1378 (vs), 1250 (s), 1234 (vs), 1200 (s), 1160 (vs), 1087 (vs), 1033 (vs), and 794 (s) cm^{-1}. Anal. Calc. for $C_{16}H_{22}Cl_2N_2O_3S$: C, 48.8; H, 5.6. Found: C, 48.5; H, 5.4.

*1,2-O-Isopropylidene-5-deoxy-5-S-ethyl-*D*-xylo-pentose*

1,2-*O*-Isopropylidene-5-*O*-methanesulphonyl-D-xylose (1.08 g, m.p. 136–137° C (9)) and freshly prepared sodium thioethylate (0.70 g) in acetone (25 ml) were heated to 100° C in a pressure bottle for 2 hours. The solution was cooled and concentrated, and the acetone removed by several codistillations with water. The aqueous solution was extracted thrice with ether and the ethereal extracts were shaken once with ice cold, dilute sulphuric acid, twice with water, and were finally dried (Na_2SO_4). The ethereal extract was concentrated and the residual pale yellow syrup was dissolved in a small volume of *n*-hexane and the solution was cooled in a solid carbon dioxide – ethanol mixture. Crystallization was induced by rubbing the walls of the vessel with a spatula. The product (0.4 g, 42%) was collected and washed thoroughly with cold (−60° C) *n*-hexane. The product had m.p. 64–65° C, and $[\alpha]_D$ −58° (*c*, 0.9, ethanol).

The reported constants were: m.p. 66.5–67.5° C, $[\alpha]_D$ −57.5° (ethanol) (10).

A test portion of the product was hydrolyzed with acetic acid for 3 hours under reflux. Removal of the solvents and acetic acid left a syrup which was soluble in chloroform. Examination of this solution in the infrared revealed only a trace of absorption in the vicinity of 1710 cm^{-1}.

*5-Deoxy-5-S-ethyl-*D*-threo-pentose Phenylosazone*

A solution of 1,2-*O*-isopropylidene-5-deoxy-5-*S*-ethyl-D-*xylo*-pentose (100 mg) in 50% aqueous alcohol (10 ml) and glacial acetic acid (5 drops) was hydrolyzed by heating for 3 hours under reflux. Water was added and the ethanol and acetone were removed by several codistillations with water. Phenylhydrazine (0.2 ml) and acetic acid (0.2 ml) were added and the phenylosazone was prepared according to the method previously described. The bright yellow product after recrystallization from a small volume of ethanol followed by washing with cold (−60° C) ethanol melted at 155–156° C, $[\alpha]_D^{20}$ −28°±4° (*c*, 1.1, pyridine). Anal. Calc. for $C_{19}H_{24}N_4O_2S$: C, 60.0; H, 6.7; S, 8.4. Found: C, 59.9; H, 6.3; S, 8.3.

*1,2-O-Isopropylidene-5-deoxy-5-S-ethyl-*L*-arabino-pentose*

1,2-*O*-Isopropylidene-5-*O*-toluene-*p*-sulphonyl-L-arabinose (1.09 g, m.p. 129–130° C (11)) and freshly prepared sodium thioethylate (0.5 g) in acetone (25 ml) were heated at 100° C in a pressure flask for 2 hours. Water was added and the acetone was removed by several codistillations with water. The aqueous solution was then extracted twice with chloroform and the chloroform extracts were washed successively with cold, dilute sulphuric acid and then with water. The dried (Na_2SO_4) solution was concentrated to a crystalline mass which was readily recrystallized from a small volume of *n*-hexane to yield needles (0.61 g, 82%) melting at 88° C, $[\alpha]_D$ 1.3° (*c*, 0.8, ethanol). Anal. Calc. for $C_{10}H_{18}O_4S$: C, 51.3; H, 7.7; S, 13.7. Found: C, 51.2; H, 7.8; S, 13.8.

*5-Deoxy-5-S-ethyl-*L*-arabino-pentose*

1,2-*O*-Isopropylidene-5-deoxy-5-*S*-ethyl-L-*arabino*-pentose (0.46 g) was hydrolyzed for 3 hours with Amberlite IR-120 (H) resin (20 cc, wet volume) suspended in 50% aqueous ethanol (25 ml). The solution was filtered through a charcoal pad, concentrated, and the resulting syrup was redissolved in ethyl acetate. The solution was clarified by percolation under gravity through a fine porosity sintered glass funnel. Concentration yielded a colorless syrup (0.38 g) which revealed a single reducing constituent by paper chromatography and possessed no carbonyl absorption in the infrared; $[\alpha]_D$ −32°±2° (*c*, 0.9, ethanol).

Further hydrolysis with 0.05 *N* hydrochloric acid for 1.5 hours at 80° C, followed by deionization on Duolite A-4 (OH) resin, yielded a colorless syrup with $[\alpha]_D$ −29.5° (*c*, 3.3, ethanol). Since the optical rotation of the product was not significantly changed by the second acid hydrolysis, hydrolysis by the resin was considered complete.

*5-Deoxy-5-S-ethyl-*L*-erythro-pentose Phenylosazone*

The crystalline phenylosazone was prepared from syrupy 5-deoxy-5-*S*-ethyl-L-*arabino*-pentose (66 mg) in the usual way to yield a product that melted at 157° C after recrystallization from aqueous ethanol. The infrared absorption spectrum differs in minor features from 5-deoxy-5-*S*-ethyl-D-*threo*-pentose phenylosazone; the major difference was the absence of a peak at 817 cm^{-1} in the 5-deoxy-5-*S*-ethyl-L-*erythro*-derivative. The mixed melting point with both specimens of 5-deoxy-5-*S*-ethyl-D-*threo*-pentose phenylosazone was 120–135° C; $[\alpha]_D^{20}$ +19°±4° (*c*, 0.9, pyridine). Anal. Calc. for $C_{19}H_{29}N_4O_2S$: C, 60.0; H, 6.7; S, 8.4. Found: C, 59.5; H, 6.8; S, 8.7.

Determination of the Formaldehyde Liberated

Samples (0.5–1.5 mg; accurately weighed) were dissolved in buffer solution or 40% aqueous pyridine containing 0.3 *M* sodium metaperiodate solution (0.5 ml) and were made up to 10 ml. At intervals, aliquot samples (1 ml) were withdrawn and the formaldehyde was estimated by the chromotropic acid method (12). A trial experiment indicated that pyridine did not interfere in the estimation.

TABLE II

Liberation of formaldehyde during periodate oxidation

Compound	pH	Time (hours) 0.08	0.25	0.50	1.0	20
5-Deoxy-5-*S*-ethyl-D-*threo*-pentulose	3.7[a]	0.60[c]		0.63	0.60	
	40% pyridine		0.64			0.59
3,4-*O*-Isopropylidene-5-deoxy-5-*S*-ethyl-D-*threo*-pentulose	7.6[b]	0.33				0.61
	40% pyridine		0.38			0.43

[a, b] 0.5 *M* acetate buffer.

[c] Moles of formaldehyde liberated per mole of substrate.

ACKNOWLEDGMENTS

The authors wish to thank Dr. N. K. Richtmyer of the National Institute of Arthritis and Metabolic Diseases, National Institute of Health, Bethesda, Maryland, for cultures, and Dr. D. H. Ball for generous specimens of 1,2-*O*-isopropylidene-5-*O*-methane-sulphonyl-D-xylose and 1,2-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-L-arabinose. One of us (D.L.M.) thanks the Ontario Research Foundation for a scholarship.

REFERENCES

1. Bollenback, G. N. and Underkofler, L. A. J. Am. Chem. Soc. **72**, 741 (1950).
2. Richtmyer, N. K., Stewart, L. C., and Hudson, C. S. J. Am. Chem. Soc. **72**, 4934 (1950).
3. Hough, L., Jones, J. K. N., and Mitchell, D. L. Can. J. Chem. **37**, 725 (1959).
4. Gorin, P. A. J., Hough, L., and Jones, J. K. N. J. Chem. Soc. 2140 (1953).
5. Fleury, P., Courtois, J., Perles, R., and Le Dizet, L. Bull. soc. chim. France, 347 (1954).
6. Barker, S. A., Bourne, E. J., Pinkard, R. M., and Whiffen, D. H. J. Chem. Soc. 807 (1959).
7. Trevelyan, W. E., Proctor, D. P., and Harrison, J. S. Nature, **166**, 444 (1950).
8. Hough, L., Jones, J. K. N., and Wadman, W. H. J. Chem. Soc. 1702 (1950).
9. Ball, D. H. Unpublished work. Department of Chemistry, Queen's University, Kingston, Ontario.
10. Raymond, A. L. J. Biol. Chem. **107**, 88 (1934).
11. Levene, P. A. and Compton, J. J. Biol. Chem. **116**, 189 (1936).
12. O'Dea, J. F. and Gibbons, R. A. Biochem. J. **55**, 580 (1953).