Note

The interconversion of the D-tetroses in pyridine

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The Lobry de Bruyn–Alberda van Ekenstein transformation of saccharides is known to be an important reaction involving the epimerization of aldoses and ketoses and their mutual isomerization. Much attention has been paid to the mechanism of the transformation and to the preparative value of the reaction; the work performed prior to 1958 has been reviewed[1].

Transformation of the appropriate aldose is a convenient method for preparing ketoses[2–5], and supplements the biochemical oxidation of alditols[6], the diazomethane synthesis[7], and the oxidation of alditols by mercury(II) acetate[8].

The enediol mechanism of transformation was first advanced by Wohl and Neuberg[9], and verified by using isotopes of hydrogen[10,11]. Recently, Isbell and coworkers have developed a method for measuring the enolization of sugars[12–14]. The lack of suitable analytical methods was the limiting factor in the study of the kinetics of the transformation. The selective dehydration of D-fructose to 5-(hydroxymethyl)-2-furaldehyde in the presence of D-glucose and D-mannose has been used in studying the transformation of these hexoses[15]; a similar analytical method has been developed for the pentoses[16]. A polarographic method for examining the electrochemical properties of trioses[17,18] and tetroses[19] was found convenient for studying the kinetics of transformation and dehydration of trioses[20]. In the present work, the polarographic method[19] for studying the transformation of tetroses was employed.

A very convenient method for the preparation of L-*glycero*-tetrulose is the biochemical oxidation of erythritol by bacteria; this method produces only the L-isomer[21]. D-*glycero*-Tetrulose, which is of considerable interest to biochemists, has been prepared by the diazomethane synthesis[23] and by transformation of D-erythrose[24], but only in poor yield.

The treatment of tetroses with alkali can lead to various types of reaction. The aldol reaction of D-erythrose occurs in the presence of lime-water, and D-*gluco*-L-*glycero*-3-octulose was isolated from the reaction mixture[25]. For the preparation of D-*glycero*-tetrulose, we chose pyridine; being a tertiary amine, it neither reacts with monosaccharides nor catalyzes the aldol reaction[26,27].

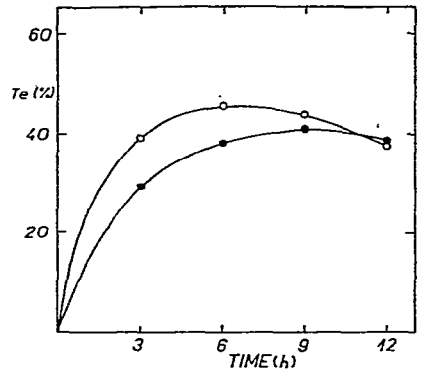


Fig. 1. The formation, with time, of D-*glycero*-tetrulose (Te) from the D-aldotetroses in boiling pyridine; (D-erythrose, O; D-threose, ●).

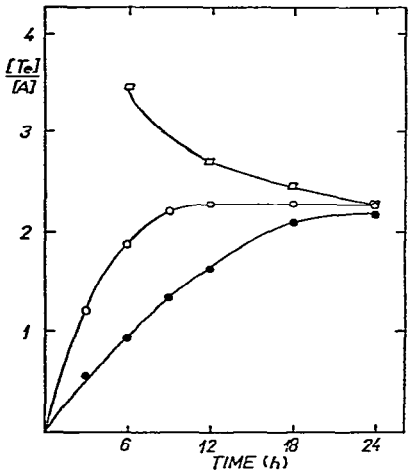
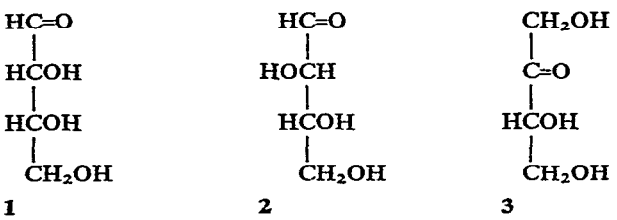


Fig. 2. Change, with time, in ratio of concentration of D-*glycero*-tetrulose (Te) to that of the D-aldotetroses (A) in boiling pyridine (the starting tetrose: D-erythrose, O; D-threose, ●; and D-*glycero*-tetrulose, □).

D-Erythrose (**1**) and D-threose (**2**) are both transformed in boiling pyridine into D-*glycero*-tetrulose (**3**); the rate of transformation of D-erythrose is the greater (see Fig. 1). The equilibrium constant of the reaction tetrulose↔aldotetroses was



1 **2** **3**

found to be 2.2 (see Fig. 2). The value of the equilibrium constant for the transformation of trioses (1,3-dihydroxy-2-propanone↔DL-glyceraldehyde) was found[20] to be 5. The total concentration of tetroses decreases during the course of the transformation owing to side reactions.

D-*glycero*-Tetrulose prepared by this method has the same magnitude (but opposite sign) of optical rotation as L-*glycero*-tetrulose prepared by the biochemical oxidation of erythritol. It is obvious that, during the transformation carried out under these conditions, only the 1,2-enediol is formed; the formation of the 2,3-enediol would decrease the value of the optical activity[3].

EXPERIMENTAL

Melting points were determined on a Kofler micro-hot-stage. Solutions were evaporated under diminished pressure at 30–40°.

D-Erythrose was prepared by oxidation of D-glucose with lead tetraacetate[28], or by hydrolysis of crystalline 2,4-*O*-ethylidene-D-erythrose[29]. D-Threose was obtained by oxidation of D-galactose with lead tetraacetate[30]. L-*glycero*-Tetrulose was prepared by biochemical oxidation of erythritol with *Acetobacter suboxidans* B. S. 1784 (Czechoslovak Collection of Micro-organisms)[31]. Anhydrous pyridine was prepared as described by Reichstein and Bosshard[32].

Descending paper chromatography was performed on Whatman No. 1 paper with (*a*) 12:1:1 (v/v) ethyl acetate–acetic acid–4% aqueous boric acid (D-threose, R_T unity; D-*glycero*-tetrulose, R_T 1.23; D-erythrose, R_T 2.22); (*b*) 9:1:1 (v/v) ethyl acetate–acetic acid–4% aqueous boric acid; (*c*) 6:5:2 (v/v) cyclohexanol–pyridine–water saturated with boric acid and containing phenol (1 g/100 ml); the chromatographic paper was soaked with 1% aqueous boric acid (threitol, R_T unity; erythritol, R_T 1.14); (*d*) 7:2 (v/v) chloroform–acetic acid containing water (1.5 ml/100 ml) (D-threose, R_T unity; D-*glycero*-tetrulose, R_T 1.31; D-erythrose, R_T 1.36). Detection was effected with aniline hydrogen phthalate[33] and potassium periodate–benzidine[34].

Preparative chromatography was performed on columns (90 × 4.5 cm) of Whatman cellulose or microcrystalline cellulose[35].

Analytical control of the transformation. — A solution (0.1M) of the tetrose in anhydrous pyridine was de-aerated with a stream of dry nitrogen; it was then boiled under reflux while a gentle stream of nitrogen was bubbled through. Simultaneous determination of D-*glycero*-tetrulose and D-aldotetroses in the mixture was conducted by the polarographic method (in 0.3M isobutylamine buffer) according to a published method[19]. The formation of D-*glycero*-tetrulose from the D-aldotetroses is shown in Fig. 1. The equilibrium constant was determined from the ratio of the concentrations of D-*glycero*-tetrulose and D-aldotetroses (see Fig. 2). The course of the transformation was also monitored by paper chromatography (solvents *a* and *d*) and the presence of all three of the tetroses was found, regardless of the tetrose started with.

Preparative separation[36]. — A solution of D-erythrose (2.02 g) in anhydrous pyridine (168 ml) was boiled under reflux in a stream of nitrogen for 5 h. Pyridine was

removed under diminished pressure, and the mixture was chromatographed on a cellulose column with solvent *b*. The composition of separate fractions was checked by paper chromatography (solvent *a*), boric acid being removed by evaporation with methanol. Besides unreacted D-erythrose (0.623 g, 30.8%), D-*glycero*-tetrulose (0.78 g, 38.6%) was isolated. D-Threose was present only in traces, and could not be isolated.

Identification of tetroses. — The tetroses were identified by optical rotation, and by the sign of their Cotton effect[37]; by conversion into their crystalline, substituted phenylhydrazones; and by paper chromatography (solvent *c*) of the tetritols resulting from reduction with sodium borohydride.

D-Erythrose[29] was isolated as a syrup, $[\alpha]_D^{24}$ −40.6° (*c* 1.0, water); its (*p*-nitrophenyl)hydrazone (a new derivative) was prepared by refluxing a solution of D-erythrose and (*p*-nitrophenyl)hydrazine in 96% ethanol for 10 min; m.p. 212–214° (from ethanol–ethyl acetate).

Anal. Calc. for $C_{10}H_{13}N_3O_5$: C, 47.06; H, 5.13; N, 16.46. Found: C, 46.99; H, 5.26; N, 16.30.

Erythritol was formed by reduction.

L-*glycero*-Tetrulose[21] was obtained as a syrup, $[\alpha]_D^{20}$ +11.5° (*c* 1.0, water); it showed a positive Cotton effect; its (*o*-nitrophenyl)hydrazone[22] had m.p. 151–153° (from ethanol) and $[\alpha]_D^{20}$ +51° (*c* 2.0, ethanol). Erythritol and L-threitol were identified as reduction products.

D-*glycero*-Tetrulose[23] was isolated as a syrup, $[\alpha]_D^{20}$ −11.3° (*c* 1.0, water), that showed a negative Cotton effect; its (*o*-nitrophenyl)hydrazone had m.p. 152–153° (from ethanol), $[\alpha]_D^{20}$ −52° (*c* 2.0, ethanol). The value reported[24] for the m.p., namely, 116–117° (from water), is too low.

Anal. Calc. for $C_{10}H_{13}N_3O_5$: C, 47.06; H, 5.13; N, 16.46. Found: C, 47.26; H, 5.31; N, 16.55.

Erythritol and D-threitol were formed by reduction.

D-Threose was obtained as a syrup, $[\alpha]_D^{20}$ −13.0° (*c* 1.0, water); its (2,5-dichlorophenyl)hydrazone[30] had m.p. 108–109°. D-Threitol was formed by reduction.

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