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Note

3-Deoxy-D-*erythro*-hexulose: a convenient synthesis and its interaction with the enzymes of fructose metabolism

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As part of our ongoing studies of the interactions of fructose analogs with the enzymes[1–3] and tissues[1,4–8] of fructose metabolism, we had need of 3-deoxy-D-*erythro*-hexulose (3-deoxy-D-fructose, **4**). Compound **4** has previously been synthesized by two methods[9,10]. The recent synthesis of 1,2:4,5-di-*O*-isopropylidene-3-deoxy-β-D-*erythro*-2-hexulopyranose (**3**) by the reduction of 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose (**2**) (ref. 11) makes possible a convenient synthesis of **4** directly from fructose (**1**). Herein the details of this synthesis are reported along with data concerning the interaction of **4** with the enzymes of mammalian fructose metabolism. The synthesis is shown in Scheme 1.

Compound **2** was synthesized from **1** by the method of Brady[12] and reduced to **3** by the method of Rasmussen *et al.*[11] Compound **3** was readily hydrolyzed to **4** in aqueous acid. After chromatography and recrystallization of the crude product, **4** was

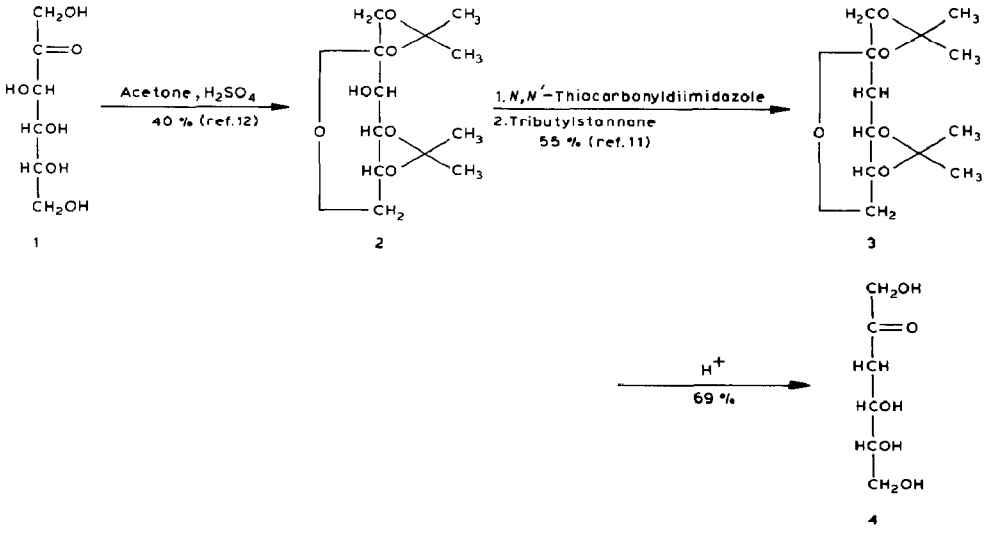

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TABLE I

Interaction of 3-deoxy-D-*erythro*-hexulose with the enzymes of fructose metabolism

Enzyme	*Fructose*			*3-Deoxy*-D-erythro*hexulose*	
	K_m(mM)	V_{max}	*Ref.*	K_m(mM)	V_{max}
L-Iditol Dehydrogenase (EC 1.1.1.14)	185	1.00[a]	1	182 ± 18	0.83 ± 0.11[a]
Fructokinase (EC 2.7.1.4)	0.46	1.00[a]	2	67 ± 20	0.97 ± 0.13[a]
Hexokinase (EC 2.7.1.1)	1.20	1.51[b]	2	750 ± 200	0.36 ± 0.18[b]

[a] Relative to D-fructose. [b] Relative to D-glucose.

obtained in a yield of 69% from **3** (15% from **1**). For comparison, the overall yield of **4** was about 21% over three steps from **1** by the method of Thiem *et al.* (ref. 10 and references therein) and less than 15% over three steps from 2-amino-2-deoxy-D-glucose by the method of Kuhn *et al.* (ref. 11 and references therein).

Compound **4** was phosphorylated by yeast hexokinase and by bovine liver fructokinase (Table I). In both cases the interaction was weak as compared to that with fructose. This result is consistent with the importance of the 3-hydroxyl established for binding and phosphorylation of substrates by hexokinase[13,14] and by fructokinase[15]. In this regard it should be noted that **4** does not interact with the phosphoenolpyruvate-dependent fructose-specific phosphotransferase system of *Rhodopseudomonas sphaeroides*[16]. Compound **4** was readily reduced by sheep liver L-iditol dehydrogenase (Table I), reflecting the lack of importance of the 3-hydroxyl for substrate binding to this enzyme[17–19].

The slow phosphorylation of **4** makes it unlikely that it will serve as a gluconeogenic, glycogenolytic, or glycolytic inhibitor in the manner observed for such other fructose analogs as 2,5-anhydro-D-mannitol[6,7,20–24] or 2,5-anhydro-D-mannose[24–26]. On the other hand, preliminary experiments have indicated that **4** stimulates gluconeogenesis from xylitol but not from dihydroxyacetone in hepatocytes from fasted rats[27]. It is suggested that **4**, being a good L-iditol dehydrogenase substrate as noted in Table I, allows rapid reoxidation of cytosolic NADH, thereby enhancing the rate of xylitol oxidation.

EXPERIMENTAL

General. — Tributylstannane was obtained from Alfa Products (Danvers, MA 01923). *N,N'*-Thiocarbonyldiimidazole was obtained from Aldrich Chemical Co. (Metuchen, NJ 08840). The ion-exchange resins were obtained from Bio-Rad Laboratories (Rockville Centre, NY 11571). Yeast hexokinase (EC 2.7.1.1), sheep liver L-iditol dehydrogenase (EC 1.1.1.14), and all coupling enzymes were obtained from Sigma Chemical Co. (St. Louis, MO 63178). Bovine liver fructokinase was purified by the

method of Raushel and Cleland[15] as modified[3]. All other chemicals were standard laboratory reagents.

Descending paper chromatography was performed on Whatman No. 1 paper using 4:1:1 (v/v) butanol: acetic acid: water[9]. Spots were visualized with alkaline potassium permanganate[28]. Ion-exchange chromatography[29] was performed on a column of Bio-Rad AG 50W-X4 [Ca^{2+}] (200–400 mesh) resin, which was eluted with distilled water at a flow rate of 1.4 mL/min. Enzyme assays for kinases[1-3] and L-iditol dehydrogenase[1] have been previously described.

1,2:4,5-Di-*O*-isopropylidene-β-D-fructopyranose (**2**) was synthesized by the method of Brady[12] and 3-deoxy-1,2:4,5-di-*O*-isopropylidene-β-D-*erythro*hexulose (**3**) by that of Rasmussen *et al.*[11]

Preparation of 3-deoxy-D-erythro-*hexulose* (**4**). Typically 0.80 g (3.3 mmol) of **3** were suspended in 50 mL of 0.10M HCl and kept for 48 h at 70° with constant stirring. The reaction was neutralized by the addition of Bio-Rad AG 3-X4A [OH^-] ion exchange resin, filtered, concentrated to 20 mL, and purified by ion-exchange chromatography[2,29]. Compound **4** eluted at a volume of 340 mL. Two minor components eluting at 400 mL and 450 mL were not further characterized. Following lyophilization, **4** was obtained in crystalline form. After recrystallization from absolute ethanol, the product (0.37 g; 2.3 mmol; 69% from **3**; 15% from **1**) had a melting point of 113–114° and $[\alpha]_D^{23}$ of −44° (*c* 1.0, water) [Lit. 112–114°, −43.4° (*c* 1, water)[9] and −48° (*c* 4.2, water)[10]]; R_f 0.41 (paper, homogeneous); R_f fructose 0.24.

Anal. Calc. for $C_6H_{12}O_5$: C, 43.90; H, 7.32. Found: C, 43.78; H, 7.29.

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