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2,5-ANHYDRO-1-DEOXY-D-LYXITOL, 2,5-ANHYDRO-1-DEOXY-D-MANNITOL, AND 2,5-ANHYDRO-1-DEOXY-D-TALITOL. SYNTHESIS AND ENZYMIC STUDIES*

WILLIAM L. DILLS, JR, THOMAS R. COVEY, PAUL SINGER, SHARON NEAL, AND MARK S. RAPPAPORT
Division of Nutritional Sciences and Section of Biochemistry, Cell and Molecular Biology, Division of Biological Sciences, Cornell University, Ithaca, NY 14853 (U.S.A.)

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ABSTRACT

2,5-Anhydro-1-deoxy-D-mannitol (**4**), 2,5-anhydro-1-deoxy-D-talitol (**8**), and 2,5-anhydro-1-deoxy-D-lyxitol (**12**) were prepared by Raney-nickel desulfurization of 2,5-anhydro-D-mannose dihexyl dithioacetal (**3**), 2,5-anhydro-D-talose dihexyl dithioacetal (**7**), and 2,5-anhydro-D-lyxose diethyl dithioacetal (**11**), respectively, in 64% yield based on starting thioacetal. Compounds **3** and **7** were prepared in 42% yield, by the acid-catalyzed condensation of 2,5-anhydro-D-mannose and 2,5-anhydro-D-talose, respectively, with hexanethiol. Compound **11** was prepared in 66% yield, by treatment of D-lyxose diethyl dithioacetal with *p*-toluenesulfonyl chloride–pyridine. Compounds **4**, **8**, and **12** were examined as substrates and inhibitors for yeast hexokinase and bovine-liver fructokinase. Hexokinase and fructokinase phosphorylated **4** with K_m values of 25 and 1.94mM, and V_{max} (**4**)/V_{max} (D-fructose) 0.007 and 0.13, respectively. Fructokinase was competitively inhibited by **8** and **12**, with K_i values of 17 and 99mM, respectively.

INTRODUCTION

The biochemical effects of 2,5-anhydro sugars and their derivatives have been of recent interest. 2,5-Anhydro-D-mannitol, 2,5-anhydro-D-mannose (**2**), and 2,5-anhydro-D-glucitol have been shown to be substrates for fructokinase and hexokinase[1,2]. The mono- and di-phosphate esters of these compounds have been studied as substrates and inhibitors of phosphofructokinase[3–5], aldolase[6], and fructose-1,6-diphosphatase[7]. In addition, 2,5-anhydro-D-mannitol has been shown to inhibit glycolysis in Krebs 2 ascites-carcinoma cells[8] and bovine spermatozoa[9], D-fructose-stimulated lysine incorporation in rat testes slices[10], and D-glucose synthesis in rat hepatocytes[11]. Our ongoing metabolic studies of D-fructose analogs required several 2,5-anhydro-1-deoxypolyols. Herein, we report convenient syntheses of 2,5-anhydro-

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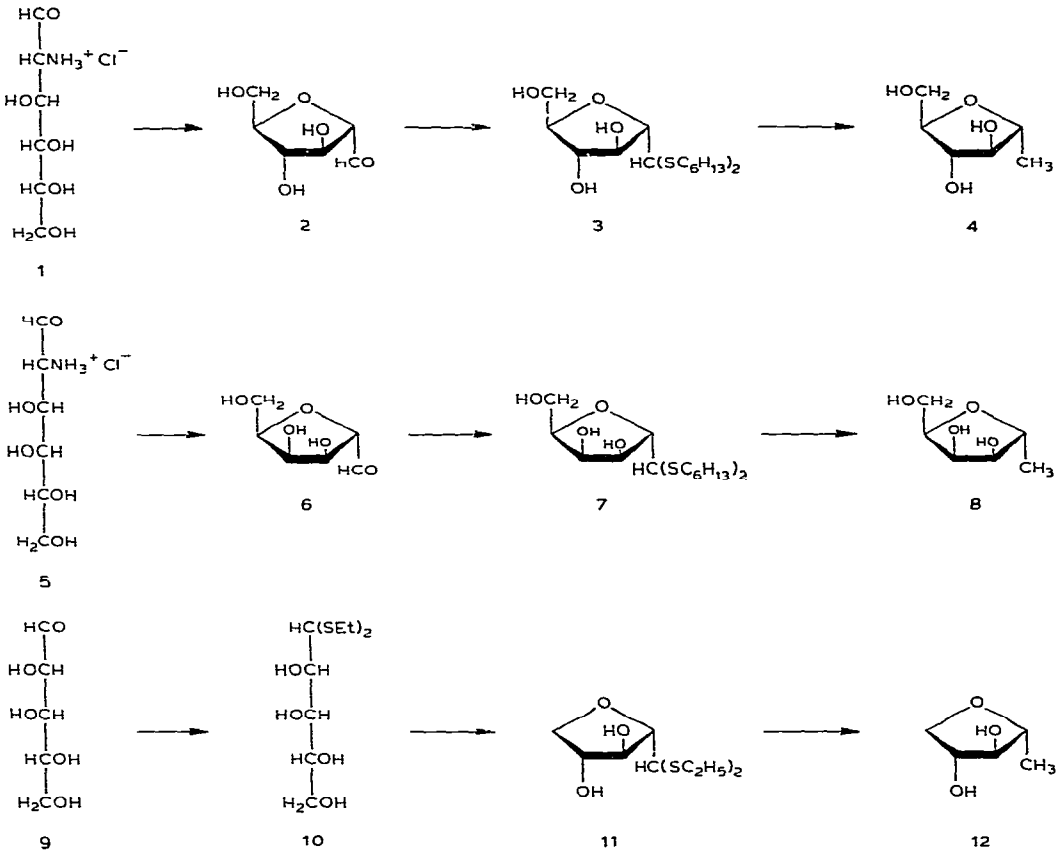

1-deoxy-D-mannitol (**4**), 2,5-anhydro-1-deoxy-D-talitol (**8**), and 2,5-anhydro-1-deoxy-D-lyxitol (**12**), and their interactions with hexokinase and fructokinase.

RESULTS AND DISCUSSION

Synthesis and characterization of **7**, **8**, *and* **12**. — The ready availability of dialkyl dithioacetals of 2,5-anhydro-D-mannose[12] (**3**) and -D-lyxose[13] suggested that such compounds could be used as precursors for the synthesis of the 2,5-anhydro-1-deoxypolyols **4**, **8**, and **12**. Compounds **2** and **6** were not isolated in purified form, but were converted immediately to the respective dihexyl dithioacetals **3** and **7**, thus avoiding some of the stability problems associated with the isolation of the free anhydro *aldehydo*-sugars[14]. Treatment with hexanethiol was found to give an overall yield of the dialkyl dithioacetals **3** and **7** that was higher than that obtained by analogous treatment with ethanethiol. The mixtures resulting from the desulfurization reactions generally contained a major component and one or more

TABLE I

PHOSPHORYLATION OF VARIOUS SUBSTRATES BY YEAST HEXOKINASE AND BY BOVINE LIVER D-FRUCTOKINASE[a]

Substrate	*Hexokinase interaction*		*D-Fructokinase interaction*		
	K_m (mM)	V_{max} (*relative to* D-*glucose*)	K_m (mM)	V_{max} (*relative to* D-*fructose*)	K_i (mM)[b]
D-Fructose	1.20 ± 0.28	1.51 ± 0.29	0.46 ± 0.13	1.00 ± 0.20	
1-Deoxy-D-fructose	40.8 ± 9.0	0.024 ± 0.002	Not phosphorylated		4.6 ± 1.6
2,5-Anhydro-D-mannitol	5.1 ± 2.8	0.79 ± 0.41	1.09 ± 0.10	0.45 ± 0.04	
2,5-Anhydro-1-deoxy-D-mannitol (4)	57.9 ± 28.8	0.035 ± 0.015	19.4 ± 2.9	0.13 ± 0.03	
D-Tagatose	Not tested		1.07 ± 0.07	0.59 ± 0.04	
1-Deoxy-D-tagatose	Not tested		Not phosphorylated		40.2 ± 6.0
2,5-Anhydro-D-talitol	Not tested		3.31 ± 0.38	0.88 ± 0.06	
2,5-Anhydro-1-deoxy-D-talitol (8)	None[c]		Not phosphorylated		17.3 ± 3.4
2,5-Anhydro-1-deoxy-D-lyxitol (12)	None[c]		Not phosphorylated		99 ± 8

[a]Values for kinetic parameters are the average ± s.e.m. for several measurements. [b]Inhibition was competitive with respect to D-fructose. [c]Analog was neither a substrate nor an inhibitor at concentrations up to 10mM.

minor contaminants. The purified products were isolated in good yield by ion-exchange chromatography on the calcium form of a cation-exchange resin[15].

Compounds **4**, **8**, and **12** were characterized by ^{1}H-n.m.r. spectroscopy. In each case, the major component showed the expected doublet for CH_3-1 adjacent to the single methine H-2. Enzymic interactions reported below also support the structure assignments.

Enzymic interactions of **4**, **8**, *and* **12**. — Table I shows the kinetic constants for the interactions of **4**, **8**, and related compounds with yeast hexokinase and bovine-liver fructokinase. Compound **4** was a poor substrate for yeast hexokinase with a K_m of 57.9mM and a V_{max} of 0.023 relative to that obtained for D-fructose. In this regard, **4** is similar to 1-deoxy-D-fructose, which is also a poor hexokinase substrate[16]. Since both D-fructose and 2,5-anhydro-D-mannitol are good hexokinase substrates[1,16], the primary hydroxyl group would appear to play an important role in the binding of D-fructose and D-fructose analogs to the enzyme. Neither **8** nor **12** was inhibitor or substrate for hexokinase. Compound **4** was also phosphorylated readily by bovine-liver fructokinase A K_m value of 19.4mM was obtained, and the maximal velocity was 13% of that obtained for the phosphorylation of D-fructose. The K_m value is higher than the K_m value of 1.09mM obtained with 2,5-anhydro-D-mannitol. Compounds **8** and **12** were competitive inhibitors of fructokinase activity, having K_i values of 17.3 and 99mM, respectively. Under analogous assay conditions, the K_i values for 1-deoxy-D-fructose and 1-deoxy-D-tagatose were 4.6 and 40.2mM, respectively. The ability of **4**, **8**, and **12** to interact with fructokinase is consistent with the suggested structural requirements for binding of substrates to fructokinase[1,2], as well as the proposed structures of the compounds. In all cases, however, the lack of a hydroxyl group resulted in a decreased binding of the analog to the enzyme, as judged by the K_m and K_i values[1,2] (see Table I). This suggests that OH-1 may play a role in substrate binding as well as serving as a phosphate receptor.

Possible use of **4**, **8**, *and* **12** *as antimetabolites*. — The synthesis of **4**, **8**, and **12** was undertaken with the ultimate goal that such compounds would be able to serve as antimetabolites. Three possible types of antimetabolite effects are possible with D-fructose analogs, based on their interactions with fructokinase and hexokinase: (a) An analog which either inhibits hexokinase or which is phosphorylated by hexokinase and accumulates intracellularly as a phosphorylated metabolite is a potential glycolytic inhibitor. (b) An analog which inhibits fructokinase has the potential to influence D-fructose tolerance and results in changes in tissue utilization patterns for D-fructose. (c) An analog which is phosphorylated by fructokinase and which accumulates intracellularly as a phosphorylated metabolite will probably inhibit hepatic D-glucose synthesis. This type of interaction has recently been reported for 2,5-anhydro-D-mannitol[11].

Compounds **8** and **12** are neither substrates nor inhibitors for hexokinase, and consequently have little potential as glycolytic inhibitors. Both **8** and **12** do inhibit fructokinase in a manner similar to 1-deoxy-D-fructose[2] and 1-deoxy-D-tagatose, suggesting that any of these four analogs might serve to impair D-fructose

clearance *in vivo*. 1-Deoxy-D-fructose appears to have the greatest affinity in this regard, in light of its low K_i value of 4.6mM (Table I).

Compound **4** was a poor hexokinase substrate, suggesting little potential as a glycolytic inhibitor. It was also a relatively poor substrate for fructokinase. Despite its slow phosphorylation by fructokinase, **4** has some potential as an inhibitor of hepatic D-glucose synthesis. In this regard, **4** will probably be more specific than 2,5-anhydro-D-mannitol, because this latter compound is also a good hexokinase substrate[1] and a glycolytic inhibitor[8,9].

EXPERIMENTAL

Materials. — 2-Amino-2-deoxy-D-glucose hydrochloride (**1**), yeast hexokinase (EC 2.7.1.1.), rabbit muscle pyruvate kinase (EC 2.7.1.40), bovine heart lactate dehydrogenase (EC 1.1.1.27), and 2-amino-2-deoxy-D-galactose hydrochloride (**5**) were obtained from Sigma Chemical Co. (St. Louis, MO 63178). *p*-Toluenesulfonyl chloride, ethanethiol, and hexanethiol were obtained from Aldrich Chemical Co. (Metuchen, NJ 08840). Raney nickel was generated from powdered Raney nickel–aluminum alloy obtained from Apache Chemical Co., (Seward, IL 61077) by previously described methods[16,17]. Ion-exchange resins were obtained from Bio-Rad Laboratories (Rockville Centre, NY, 11571). Bovine liver fructokinase (EC 2.7.1.3) was purified, as described for the rat liver enzyme[2], by DEAE-cellulose chromatography. 2,5-Anhydro-D-mannitol was prepared by the method of Horton and Philips[18], 2,5-anhydro-D-talitol by that of Defaye[19], 1-deoxy-D-tagatose by that of Dills and Covey[20], and 1-deoxy-D-fructose by that of Dills and Meyer[16]. All other chemicals were standard laboratory reagents.

General methods. — Melting points were determined with an Electrothermal melting-point apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN 37921 Optical rotations were determined with a Perkin–Elmer Model 141 polarimeter. T.l.c. was performed on Eastman silica-gel plates (without fluorescent indicator) which had been previously treated with 80mM sodium acetate in 90% aqueous ethanol, and dried before use. Solvent systems were 4:1 (v/v) acetone–2-propanol (Solvent *A*) and 4:1 (v/v) 2-propanol–water (Solvent *B*). Spray reagents for identification were acidic ninhydrin for amino sugars[21], alkaline methanolic triphenyltetrazolium chloride for reducing sugars[21], *N* bromosuccinimide–fluorescein for sulfur-containing compounds[22], and alkaline permanganate for all sugars and polyalcohols[23]. All evaporations were performed at 25–45° in a rotary evaporator, ^{1}H-n.m.r. data were recorded with a Varian CFT-20 spectrophotometer operating at 80 MHz.

Fractionation of sugar derivatives by chromatography was performed as described by Angyal *et al.*[15]. Samples (1.5 g) were applied to a column (110 × 2.5 cm) of cation-exchange resin (Bio-Rad AG 50W-4X, 200–400 mesh, Ca^{2+}) and eluted with distilled water at a flow rate of 1.4 mL/min.

2,5-Anhydro-D-mannose (**2**). — The method of Horton and Philips[18] was used

to convert **1** into **2**, the procedure being followed until the crude product from 10.8 g (50 mmol) of **1** was isolated. This product [R_F 0.34 (*A*) and 0.62 (*B*)] was used directly in the next reaction.

2,5-Anhydro-D-*mannose dihexyl dithioacetal* (**3**). — Product (**2**) from the preceding reaction was dissolved in distilled water (50 mL), and the solution cooled to 0° in an ice–salt bath. Hexanethiol (14.2 g, 120 mmol) was added with vigorous stirring, followed by concentrated hydrochloric acid (20 mL). The reaction flask was securely stoppered, allowed to warm to room temperature, and the mixture stirred for 18 h. At this time, it was neutralized with lead carbonate, filtered, and the lead salts were rinsed with 50% aqueous ethanol (100 mL). The combined filtrate and washings were evaporated under reduced pressure to give a syrup, which was taken up in chloroform (50 mL). The solution was washed three times with distilled water (50 mL) and evaporated to give chromatographically pure **3** [R_F 0.92 (*A*) and 0.81 (*B*)], which was obtained as a thick syrup. This product was used directly in the next reaction without further characterization.

2,5-Anhydro-1-deoxy-D-*mannitol* (**4**). — Syrupy **3**, dissolved in 70% aqueous ethanol, (250 mL) was added to Raney nickel (prepared from 200 g of nickel–aluminum alloy) in 70% aqueous ethanol (250 mL). The reaction was allowed to proceed for 24 h at room temperature, and then filtered through glass-fiber, filter paper (*caution*: the catalyst is pyrophoric and should be kept moist). The catalyst was suspended in 0.1M hydrochloric acid (250 mL) and filtered off. This same washing-filtration procedure was repeated twice with distilled water (250 mL). The combined filtrates and washings were concentrated to 30 mL and neutralized by titration with AG 1-X8, (OH^-) anion-exchange resin. After filtration, the solution was concentrated and partially desalted by repeated additions and filtrations with absolute ethanol. The final desalting was done on a column (2.5 × 30 cm) of AG 501-X8 mixed-bead, ion-exchange resin eluted with distilled water (1 L). Evaporation gave a syrup (5 g; 59% based on **1**) that contained a major product and three minor contaminants as judged by t.l.c. The syrup was purified, as described under General methods, by ion-exchange chromatography. The major product, consisting of 92% of the applied sample, was eluted at 280 mL [R_F 0.71 (*A*), 0.67 (*B*)]. Following concentration, it crystallized, m.p. 72–73°, $[\alpha]_D^{20}$ +45.0° (*c* 1.21, methanol); ^{1}H-n.m.r.: δ 3.80–3.69 (H-2–H-6), 1.33, and 1.26 (CH_3), consistent with identity as **4**. Two other fractions were obtained from the column: the first, which was eluted at 406 mL, contained two components [R_F 0.40, 0.60 (*A*); 0.65, 0.71 (*B*)], and the second, which was eluted at 583 mL, was homogeneous [R_F 0.27 (*A*), 0.71 (*B*)]. Neither fraction showed the expected methyl doublet and they were not further characterized.

Anal. Calc. for $C_6H_{12}O_4$: C, 48.64; H, 8.16. Found. C, 48.79; H, 8.18.

2,5-Anhydro-D-*talose* (**6**). — This compound was prepared in a manner analogous[19] to the synthesis of **2**. A solution of **5** (2.5 g, 11.5 mmol) in distilled water (40 mL) was treated as described by Horton and Philips[18] for the synthesis of **2**. The resulting, amorphous **6** [R_F 0.25 (*A*), 0.36 (*B*)] isolated by lyophilization was used directly for the next reaction.

*2,5-Anhydro-*D*-talose dihexyl dithioacetal* (**7**) — This compound was prepared in a manner exactly analogous to that described for **3** with an appropriate reduction in scale. Compound **7** was obtained as an amber syrup after evaporation of the chloroform solution [R_F 0.90 (*A*), 0.81 (*B*)] and was used directly for the next reaction

*2,5-Anhydro-1-deoxy-*D*-talitol* (**8**). — Syrupy product (**7**), isolated from the reaction just described, was desulfurized in a manner analogous to that used to convert **3** to **4** with an appropriate reduction in scale. Compound **8** (1.0 g, 58% from **2**) was isolated as a major component with one minor contaminant. Ion-exchange chromatography gave the major product [R_F 0.43 (*A*), 0.67 (*B*)], which was eluted at 400 mL. Compound **8**, the major component, isolated in this manner, crystallized, m.p. 74–76°, $[\alpha]_D^{20}$ +28.35° (*c* 0.57, methanol); ^{1}H-n.m.r.: δ 3.95–3.80 (H-2 to 6), 1.32, and 1.25 (CH_3), consistent with the proposed structure. The minor contaminant, which was eluted at 624 mL [R_F 0.30 (*A*), 0.70 (*B*)], was not further characterized.

Anal. Calc. for $C_6H_{12}O_4$: C, 48.64, H, 8.16. Found: C, 48.52; H, 8.09.

D-lyxose diethyl dithioacetal (**10**) — D-Lyxose (**9**) (9.7 g, 66 mmol) was dissolved in cold, concentrated hydrochloric acid (10 mL). Ethanethiol (12 mL) was added and the reaction allowed to proceed with vigorous stirring for 12 h. The acid was neutralized with lead carbonate, the mixture filtered, and the residue rinsed with 70% ethanol. The filtrate and washings were concentrated to a small volume and de-ionized by passage through a column (2.5 × 30 cm) of AG 501-X8 mixed-bed resin, eluted with 70% ethanol. Evaporation gave a syrup which crystallized. Two recrystallizations from absolute ethanol gave **10** (6.6 g, 26 mmol, 39% from **9**), m.p. 101–103°; R_F 0.78 (*A*), 0.79 (*B*).

*2,5-Anhydro-*D*-lyxose diethyl dithioacetal* (**11**). — The synthesis of **11** was accomplished by a variation of published procedures[13,24]. A solution of **10** (3.0 g, 11.7 mmol) was dissolved in pyridine (12.6 mL, previously dried with potassium hydroxide). The solution was cooled to −5°. Dry, recrystallized[25] *p*-toluenesulfonyl chloride (2.64 g, 13.9 mmol) was added, and the mixture was incubated for 2 h at 0°, and for 24 h at 23°. Dry acetic anhydride (12.6 mL) was added and the reaction stirred for an additional 24 h. The mixture was poured into cold water (50 mL) and extracted twice with chloroform (50 mL each). The combined chloroform extracts were washed with 0.1M hydrochloric acid (50 mL), saturated aqueous sodium hydrogencarbonate (50 mL), and twice with water (50 mL each). The chloroform layer was dried (sodium sulfate) and evaporated to give a syrup, which was taken up in methanol and *O*-deacetylated with a catalytic amount (100 mg) of sodium methoxide[26]. After evaporation, the product [**1**; R_F 0.85 (*A*), 0.88 (*B*)] was used directly for the next reaction.

*2,5-Anhydro-*D*-1-deoxy-lyxitol* (**12**). — Syrupy **11** was dissolved in 50% ethanol (100 mL) and desulfurized as described for **4** with an appropriate reduction in scale. After the work-up, the product (0.9 g, 60% from **10**) was purified by ion-exchange chromatography as described earlier. Compound **12**, eluted at 314 mL [R_F 0.79 (*A*), 0.75 (*B*)], was obtained as a clear syrup, $[\alpha]_D^{20}$ +2.27° (*c* 3.53, methanol); ^{1}H-n.m.r.: δ 3.95–3.80 (H-2 to -5), 1.32, and 1.29 (CH_3), consistent with the

proposed structure. The major product was followed by two minor fractions at 394 mL [R_F 0.52 (*A*), 0.55 (*B*)] and 578 mL (R_F 0.67 (*A*), 0.7 (*B*)], which did not show the expected doublet for the methyl group and were not further characterized.

Anal. Calc. for $C_5H_{10}O_3$: C, 50.84; H, 8.53. Found: C, 49.95; H, 8.20.

Enzyme assay. — The kinase assays contained, in a final volume of 1 mL, 80mM Tris · HCl (pH 7.4), 10mM 2-mercaptoethanol, 50mM potassium chloride, 10mM magnesium acetate, 5mM ATP, mM sodium phosphoenolpyruvate, 0.2mM NADH, lactate dehydrogenase (50 μg), pyruvate kinase (20 μg), and appropriate amounts of hexokinase, fructokinase, inhibitors, and substrates. The reaction was initiated by the addition of substrate. All enzyme reactions were run at 23°. Changes in absorbance at 340 nm were monitored using a Gilford 250 spectrophotometer attached to a Gilford 6051 recorder. Initial rates were used for all kinetic evaluations. Rate data for enzyme–substrate interaction were analyzed by direct linear plots[27], and enzyme–inhibitor interactions by the method of Dixon[28]. The values shown have been averaged from five or more measurements ± s.e.m.

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