Studies on 1-Deoxy-D-fructose, 1-Deoxy-D-glucitol, and 1-Deoxy-D-mannitol as Antimetabolites†

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ABSTRACT: 1-Deoxy-D-fructose was synthesized in 27% yield from D-glucosamine in a three-step procedure involving Raney nickel desulfurization and oxidative deamination with 3,5-di-*tert*-butyl-1,2-benzoquinone applied to appropriate intermediates. 1-Deoxyfructose and its reduction products, 1-deoxyglucitol and 1-deoxymannitol, were tested as substrates and antimetabolites. For sheep liver glucitol dehydrogenase, the K_m is 53 mM for 1-deoxyglucitol and 89 mM for 1-deoxymannitol with maximal velocities 33 and 18%, respectively, of that with glucitol as substrate. These results require substantial revision of the long-accepted polyol substrate structural requirements for this enzyme which have been reported to include a 1-hydroxy group and a *cis*-2,4-dihydroxy configuration. K_m is 614 and 280 mM for yeast and muscle hexokinases, respectively, acting on 1-deoxyfructose; maximal velocities are 2 and 5% of those obtained with fructose. 1-Deoxyfructose 6-phosphate is a competitive inhibitor of phosphoglucose isomerase with a K_i of 1.1 mM; this is about the same as K_m for the natural substrates. It is also an effective inhibitor of phosphofructokinase but does not alter the cooperativity of the enzyme interaction with fructose 6-phosphate nor exhibit cooperativity in its own interaction therewith. These results suggest that the 1-hydroxy group is not crucial for binding but does play a role in the cooperative interactions of this allosteric protein. At equivalent concentrations, 1-deoxyfructose is somewhat better than 2-deoxyglucose as an inhibitor of erythrocyte glycolysis; the 1-deoxypolyols are ineffective. All three 1-deoxy compounds are readily, though incompletely, absorbed from the intestine of mice; most of the absorbed dose appears in the urine unchanged within 24 h. Whether given by oral or intraperitoneal routes, 2 to 6% of administered deoxypolyol or deoxyketose appears in the urine as ketose or polyol, respectively. No acute toxic effects or growth retardation are noted for any of the 1-deoxy analogues when given to mice at levels where 2-deoxyglucose has such effects. The properties of these 1-deoxy sugar analogues recommend them for further studies of enzyme mechanisms, for metabolic studies, and for testing as therapeutic agents against such organisms as certain mammalian parasites with heavy reliance on glycolysis.

Despite the importance of fructose in mammalian metabolism (for a review, see Nikkila and Huttunen, 1972) few studies have been made on the metabolism of fructose analogues. In this paper we report a new synthesis of 1-deoxyfructose and some metabolic studies on this compound and its two reduction products. The 1-deoxy analogue of fructose was selected with the hope that its 6-phosphate ester would inhibit phosphofructokinase (EC 2.7.1.11). Because of the importance of phosphofructokinase in the regulation of glycolysis (see review by Mansour, 1972), this compound would have potential as a glycolytic inhibitor.

Materials and Methods

2-Deoxy-D-glucose was obtained from Sigma, 3,5-di-*tert*-butylcatechol from Aldrich Chemical Company, and nickel-aluminum alloy from W. R. Grace Company. All other substances, cofactors, and chemicals were standard laboratory reagents.

Raney nickel catalyst was prepared from powdered nickel–aluminum alloy by the method of Mozingo (1955). Since the products of the various desulfurizations were not base sensitive, the freshly generated catalyst was washed only eight times. In order to avoid mixtures of partially desulfurized products (Jones and Mitchell, 1958; Dills, 1973), it was necessary to maintain the temperature of the catalyst below 20 °C during its preparation and to use it one or two days after its generation.

3,5-Di-*tert*-butyl-1,2-benzoquinone was prepared from 3,5-di-*tert*-butylcatechol by the method of Flaig *et al.* (1955).

Glucose and mannose diethyl dithioacetals were prepared by the method Fischer (1894). Glucosamine diethyl dithioacetal hydrochloride was prepared by the method of Hough and Taha (1957).

Sheep liver glucitol[1] dehydrogenase (EC 1.1.1.14), yeast phosphoglucose isomerase (EC 5.3.1.9), and the yeast hexokinase (EC 2.7.1.1) used in the assays were obtained from Boehringer Mannheim. Rabbit muscle phosphofructokinase and rabbit muscle lactate dehydrogenase (EC 1.1.1.27) were obtained from Sigma. For the preparation of 1-deoxyfructose-6-P,[2] Sigma type III yeast hexokinase was used. The auxiliary enzymes used in the assays were obtained from either Boehringer Mannheim or Sigma. Rat skeletal muscle hexokinase, isoenzyme II, was prepared by the method of Grossbard

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[1] Glucitol and 1-deoxymannitol are frequently encountered in the literature as sorbitol and rhamnitol, respectively.

[2] Abbreviations used: Tris, tris(hydroxymethyl)aminomethane; NAD^+, oxidized nicotinamide adenine dinucleotide; $NADP^+$, oxidized nicotinamide adenine dinucleotide phosphate; NADH, reduced nicotinamide adenine dinucleotide; ATP, adenosine 5′-triphosphate; DEAE, diethylaminoethyl; DO, deoxy; G-1-P, glucose 1-phosphate; G-6-P, glucose 6-phosphate; F-6-P, fructose 6-phosphate; F-1,6-diP, fructose 1,6-diphosphate.

and Schimke (1966).

Thin-layer chromatography was done on Eastman silica gel plates without fluorescent indicator. Plates were activated for 30 min at 100 °C before use. The solvent used was acetone–chloroform–methanol (8:1:1, v/v/v). Spray reagents were aqueous, alkaline permanganate for polyols (Pacsu et al., 1949), alkaline methanolic triphenyltetrazolium chloride for reducing sugars (Waldi, 1965; spray 145), and acidic ninhydrin for aminosugar derivatives (Waldi, 1965; spray 108). 1-Deoxymannitol and 1-deoxyglucitol could not be distinguished by this method.

Descending paper chromatography was carried out on Whatman No. 1 paper using methanol–formic acid–water (16:3:1) to separate phosphate esters (Bandurski and Axelrod, 1951) and 1-butanol–pyridine–water (10:3:3) to separate deoxypolyols (Coxon and Fletcher, 1964). Phosphate esters were visualized by the phosphomolybdate spray of Hanes and Isherwood (1949) and polyols as above.

Phosphate analyses were carried out according to Leloir and Cardini (1957) using the ashing procedure of Ames (1966). Analyses for C–S–C linkages were carried out by the method of Schneider (1964). Reducing sugar was determined by the method of Fairbridge et al. (1951).

Glucitol dehydrogenase reactions were run in 70 mM Tris-HCl, pH 7.4, containing 0.5% bovine serum albumin, and 0.2 mM NAD^+ or NADH. Hexokinase reaction mixtures contained 80 mM Tris-HCl, pH 8.0, 10 mM 2-mercaptoethanol, 50 mM KCl, 10 mM magnesium acetate, 5 mM ATP, 1 mM phosphoenolpyruvate, 0.2 mM NADH, 50 μg of lactate dehydrogenase/ml, and 20 μg of pyruvate kinase (EC 2.7.1.40)/ml. Phosphoglucose isomerase reaction mixtures contained 80 mM Tris-HCl, pH 8.0, 0.2 mM $NADP^+$, and 136 μg of yeast glucose-6-phosphate dehydrogenase (EC 1.1.1.49)/ml. Phosphofructokinase reaction mixtures contained 60 mM Tris-HCl, pH 7.2, 10 mM 2-mercaptoethanol, 1 mM magnesium acetate, 10 mM NH_4HCO_3, 1 mM ATP, 0.2 mM NADH, 100 μg of aldolase (EC 4.1.2.13)/ml, and 7 μg of a mixture of triosephosphate isomerase (EC 5.3.1.1) and α-glycerolphosphate dehydrogenase (EC 1.1.1.8)/ml. All enzyme reactions were conducted at 30 °C. Absorbance changes at 340 nm were monitored with a Beckman DB spectrophotometer attached to a Photovolt Varicord 43 recorder. Initial rates were used for the determinations of kinetic constants.

Rate data for substrate–enzyme interactions were plotted by the method of Lineweaver and Burk (1934); or for the case of fructose-6-P interaction with phosphofructokinase, a Hill plot after Kemp (1969) was used. On such plots (Dills, 1973) ten data points evenly spaced over as wide a range of substrate concentration as practical were employed for the new compounds tested here. Five or more data points were used for comparable plots with the classical substrates of the enzymes studied. For studies of inhibitor–enzyme interactions data were plotted by the method of Dixon (1953) or using a Hill plot (Figure 1); five evenly spaced inhibitor concentrations at three or four different substrate concentrations were analyzed (Dills, 1973). K_m and maximum velocity values were obtained from the raw data with a weighted linear least-squares regression program using a Hewlett-Packard 9820 A calculator, and where dispersions of the data are given these are standard deviations.

Erythrocytes were obtained from New Zealand white rabbits and were washed four times in 10 volumes of buffer. Incubations were carried out in a shaking water bath at 37 °C in stoppered 10-ml Erlenmeyer flasks. The incubation buffer

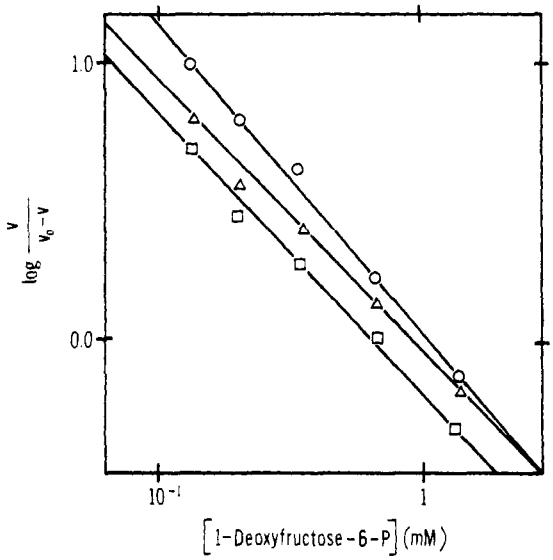


FIGURE 1: Hill plot (Monod et al., 1963) for the inhibition of phosphofructokinase by 1-deoxyfructose-6-P. Assay mixtures contained designated concentrations of 1-deoxyfructose-6-P in the presence of 0.1 (□), 0.2 (△), and 0.3 mM (○) fructose-6-P.

contained 20 mM morpholinopropanesulfonic acid, pH 7.5, 1 mM K_2HPO_4, 0.8% NaCl, and 0.1% bovine serum albumin. Three milliliters of buffer was added to each flask chilled on ice followed by 0.8 ml of packed erythrocytes. The inhibitors were added in 0.1 ml of distilled water. A 30-min incubation followed. Then 0.1 ml of 400 mM glucose was added and incubation was continued. Lactate analyses on aliquots of incubated erythrocytes were by the method of Hohorst (1965).

Mice used were 14 or 40 to 45 day old Charles River albino males obtained from Canadian Breeding Laboratory, St. Constant, Quebec, Canada. They were allowed free access to Purina Mouse Chow and either drinking water or experimental solutions. Injections were made intraperitoneally with a designated amount of solute in 0.3 ml of 0.9% aqueous NaCl. Compounds administered through drinking water were in 0.1% aqueous sodium benzoate titrated to pH 3.5 with citric acid. Urine and feces were collected in metabolic cages with an assembly to separate them. Urine was diluted with an equal volume of 0.1% sodium benzoate titrated to pH 3.5 with citric acid. Feces were extracted with 10 volumes of this same solution for 16 h at 25 °C. Deoxypolyol and 1-deoxyfructose were determined using the thin-layer chromatographic system described above. Chromatograms were prepared by spotting the urine or fecal extract in question and graded amounts of the compound being measured. One microliter of a control urine or fecal extract obtained from the same mice from before the initiation of the experiment was spotted with each standard spot. After development and visualization the amount of compound was estimated by comparing the size and intensity of the extract spot with the standards. Each determination was repeated three or more times using amounts of extracts containing between 2.5 and 10.0 μg of the compound being considered.

Preparation of 1-Deoxy-D-mannitol and 1-Deoxy-D-glucitol.[1] These deoxypolyols were prepared with an adaption of the method used by Richtmyer and Hudson (1950) to prepare 1-deoxymannitol. The amount of Raney nickel catalyst prepared from 453 g of nickel–aluminum alloy and 25 g (89 mmol) of either D-mannose or D-glucose diethyl dithioacetal were used in a single preparation. This ratio of starting mate-

rials was found to give the greatest yields of products with negligible contamination by partially desulfurized compounds. Yields of 9.5 g (58 mmol, 65% of theoretical) were obtained.

1-Deoxymannitol melted at 122–123 °C in agreement with values reported in the literature (Richtmyer and Hudson, 1950; Gatzi and Reichstein, 1938) and sulfur analysis showed no C–S–C bonds as opposed to two for starting mannose diethyl dithioacetal. 3,4:5,6-Diisopropylidene-1-deoxy-D-mannitol was prepared by the method of Bukhari et al. (1963). This compound melted at 64–65 °C and had an $[\alpha]^{23}D$ of +16.1° (*c* 1.5 in methanol) compared with literature values of 64–66° and −16° (*c* 1.5 in methanol) for the L isomer (Bukhari et al., 1963).

1-Deoxyglucitol melted at 128–129 °C in agreement with reported values (Gatzi and Reichstein, 1938; DeBelder and Weigel, 1964)[3] and sulfur analysis showed no C–S–C bonds as opposed to one for 1-deoxy-1-ethylthioglucitol (Dills, 1973) and two for starting glucose diethyl dithioacetal. 2,3,4,5,6-Penta-*O*-acetyl-1-deoxyglucitol prepared by the method of Gatzi and Reichstein (1938) had a melting point of 104–105 °C and an $[\alpha]^{23}D$ of +20.7° (*c* 1.56 in methanol) in agreement with their values.

Preparation of 1-Deoxy-D-fructose. 1,2-Dideoxy-2-amino-D-glucitol was prepared by the desulfurization of 25 g (77 mmol) of D-glucosamine diethyl dithioacetal hydrochloride with the amount of Raney nickel catalyst prepared from 453 g of nickel–aluminum alloy. The reaction was run in 1 l. of 50% aqueous ethanol for 16 h at room temperature. The product was separated from spent catalyst by washing once with a liter of 0.1 M HCl and three times with a liter of distilled water each time. The combined reaction liquor and washings were filtered through glass fiber filter paper, concentrated to 100 ml, and applied to a strong cation exchange column (Bio-Rad AG50-X8, H^+ form, 2.5 × 28.0 cm). The column was washed with water until no chloride could be detected. The product was eluted with 200 ml of 1 M NH_4OH. Concentration gave 6.5 g (39 mmol) of product (51% of theory) which crystallized slowly upon standing at room temperature. Recrystallization from 2-propanol–methanol resulted in a product that melted at 120–121 °C. 1,2-Dideoxy-2-aminoglucitol was found to decompose readily when heated and all steps of its synthesis and work-up were carried out below 60 °C.

1-Deoxyfructose was prepared from 1,2-dideoxy-2-aminoglucitol by the general method of Corey and Achiwa (1969). 3,5-Di-*tert*-butyl-1,2-benzoquinone (25.4 g, 116 mmol) and the aminopolyol (18.2 g, 110 mmol) were dissolved in 250 ml of absolute methanol. After 90 min of stirring at room temperature, 250 ml of tetrahydrofuran, 100 ml of distilled water, and enough solid oxalic acid to lower the pH of the solution to 4.0 were added. Stirring was continued for 16 h, after which 500 ml of ethyl ether and 500 ml of distilled water were added. The mixture was shaken in a separatory funnel and the water layer was removed. The ether layer was washed twice with 100 ml of distilled water each time. The combined water layers were concentrated to 100 ml under vacuum at 40 °C, washed with 100 ml of ethyl ether, and applied to a strong-cation-exchange column (Bio-Rad AG50W-X8, H^+ form, 1.5 × 21 cm). Distilled water was passed through the column until the effluent was no longer acidic. Acidic fractions were combined, concentrated under vacuum at 40 °C to 50 ml, and placed on an anion-exchange column (DEAE-cellulose, 1.5 × 20 cm) which was washed with distilled water. Fractions containing 1-deoxyfructose as detected by thin-layer chromatography were combined and concentrated to a colorless syrup. After drying 11.0 g (67 mmol) of syrupy 1-deoxyfructose was obtained representing a yield of 69%. The proton magnetic resonance spectrum of 1-deoxyfructose in D_2O showed the expected large singlet for the methyl ketone protons at τ 8.5 ppm and a mixture of peaks for H–C–O protons between τ 6.0 and 6.5 ppm. 1-Deoxyfructose prepared in this manner had an $[\alpha]^{23}D$ of −81° (*c* 1.5 in distilled water) which compares favorably with values reported in the literature (Haylock et al., 1971; Ishizu et al., 1967).

1-Deoxyfructose was characterized by sodium borohydride reduction which gave a yield of 91% of a mixture of two products which were separated on a 1.1 × 43 cm cellulose column by the method of Coxon and Fletcher (1964). Evaporation of solvent gave crystalline 1-deoxymannitol and 1-deoxyglucitol identical in physical properties and mobility on paper chromatography with the same compounds prepared as described elsewhere in this paper.

1-Deoxyfructose can be stored as a syrup or as an aqueous solution in 50 mM Tris-HCl, pH 7.4, for several months at −20 °C without significant color development. Exposure to acid or base, or heating above 40 °C causes the appearance of yellow material. For most of the experiments reported here 1-deoxyfructose was generated as needed from crystalline 1,2-dideoxy-2-aminoglucitol.

Preparation of 1-Deoxyfructose-6-P. 1-Deoxyfructose-6-P was prepared by enzymatic phosphorylation of the free sugar by a method similar in essential detail to that described by Bar-Tana and Cleland (1974). After the reaction was complete the mixture was filtered through 10 g of Bio-Rad AG50W-X8 strong-cation-exchange resin (H^+ form) which was washed with 25 ml of distilled water. The filtrate and wash were concentrated and applied to a 1.1 × 45 cm column of Bio-Rad AGl-X2 (borate form) which was eluted with an ammonium borate gradient as described by Lefebvre et al. (1964). The amorphous ammonium salt was obtained upon concentration and removal of ammonium borate by repeated distillation from methanol. 1-Deoxyfructose-6-P prepared by this method gave a single spot on paper chromatography and contained no inorganic phosphate. Phosphate analysis gave 12.1% phosphorus (expected on the basis of $C_6H_{16}NO_8P$:12.1%) and contained 1 mol of reducing sugar per mol of phosphate.

TABLE I: Kinetic Constants of Glucitol Dehydrogenase with Various Substrates.

Substrate	K_m (mM)	Rel. V_{max}
Glucitol	8.5 ± 1.6	1.00 ± 0.22[a]
Mannitol	1980 ± 900	0.10 ± 0.12[a]
1-Deoxyglucitol	53 ± 4	0.33 ± 0.12[a]
1-Deoxymannitol	89 ± 12	0.18 ± 0.11[a]
Fructose	185 ± 54	1.00 ± 0.30[b]
L-Sorbose	351 ± 178	0.51 ± 0.22[b]
1-Deoxyfructose	587 ± 98	0.06 ± 0.02[b]

[a] Relative to glucitol. [b] Relative to fructose.

Results

Kinetic Interactions of Fructose Analogues with Enzymes. Kinetic constants for sheep liver glucitol dehydrogenase are given in Table I. Both deoxypolyols are moderately active as

[3] Crystallization of 1-deoxyglucitol generally occurred within a few minutes after seeding. Initially, crystallization occurred spontaneously only after a period of seven months and several early preparations required the introduction of seed crystals.

TABLE II: Kinetic Constants for Hexokinases with Fructose and 1-Deoxyfructose.

Enzyme and Hexose	K_m (mM)	V_{max}[a]
Yeast hexokinase		
Fructose	8.7 ± 2.4	1.00
1-Deoxyfructose	614 ± 62	0.02
Rat skeletal muscle hexokinase		
Fructose	8.2 ± 4.1	1.00
1-Deoxyfructose	280 ± 99	0.05

[a] Relative to fructose.

TABLE III: Inhibition of Glycolysis in Rabbit Erythrocytes by 1-Deoxyfructose and Its Reduced Forms.

Inhibitor Added	Lactate Production[b]	Inhibition (%)
None[a]	1.3	
None	7.3	
25 mM 2-deoxyglucose	5.2	29
50 mM 2-deoxyglucose	4.5	38
25 mM 1-deoxyfructose	4.1	42
50 mM 1-deoxyfructose	3.1	56
25 mM 1-deoxyglucitol	7.0	4
50 mM 1-deoxyglucitol	6.7	8
25 mM 1-deoxymannitol	7.1	3
50 mM 1-deoxymannitol	6.7	8

[a] No glucose added as substrate. [b] In μmol $h^{-t0(ml}$ of packed cells)$^{-1}$.

substrates compared with the natural substrate glucitol. The product from both deoxypolyols was identified as 1-deoxyfructose by thin-layer chromatography. The observation that 1-deoxyglucitol is oxidized is in direct contradiction to the results reported by McCorkindale and Edson (1954). The possibilities exist that their preparation of 1-deoxyglucitol contained inhibitory impurities such as heavy metal ions or that the rat liver enzyme which they used differs in steric requirements from that of sheep liver. In the direction of ketose reduction 1-deoxyfructose was found to be a poor substrate relative to L-sorbose and the natural substrate, fructose; this confirms the results of Barnett and Atkins (1972).

Table II shows the kinetic constants for hexokinases from yeast and muscle. 1-Deoxyfructose is slowly phosphorylated by hexokinases from both sources, although it is an inferior substrate to fructose. Barnett and Atkins (1972) reported negligible activity of hexokinase toward 1-deoxyfructose; however, their observation was based on experiments at low substrate concentration with an insensitive assay procedure. Neither deoxypolyol is a substrate for hexokinase.

1-Deoxyfructose-6-P is a competitive inhibitor for fructose-6-P isomerization by phosphoglucose isomerase. The K_i value determined from a Dixon plot is 1.1 mM. This value is close to the K_m for fructose-6-P of 0.71 mM determined by a Lineweaver–Burk plot and literature values of 0.87 mM for the K_m of glucose-6-P and 1.9 mM for the K_i of 2-deoxyglucose-6-P (Bessell and Thomas, 1973).

At pH 7.4, 1.5 mM 1-deoxyfructose-6-P inhibited phosphofructokinase activity greater than 40% at fructose-6-P concentrations ranging from 0.018 to 0.60 mM. Under our assay conditions (pH 7.2, 1 mM ATP, 10 mM NH_4^+) Hill coefficients of about 2 were obtained for phosphofructokinase activity toward fructose-6-P in the presence and absence of 1.5 mM 1-deoxyfructose-6-P. Figure 1 shows a Hill plot for 1-deoxyfructose-6-P inhibition of enzyme activity. The slopes obtained at three different fructose-6-P concentrations are all about 1.

TABLE IV: Appearance of Deoxypolyol and 1-Deoxyfructose in Mouse Urine Following Injections of Fructose Antimetabolites.

Antimetabolite[a]	Collection Period (h)	Deoxypolyol[b] (%)	1-Deoxyfructose[b] (%)
1-Deoxyglucitol	0–5	60 ± 10	2 ± 1
	5–24	15 ± 5	4 ± 2
1-Deoxymannitol	0–5	70 ± 15	2 ± 1
	5–24	5 ± 2	<1
1-Deoxyfructose	0–5	2 ± 1	47 ± 10
	5–24	2 ± 1	27 ± 8

[a] Antimetabolites were administered at the rate of 3 g per kg of body weight. [b] Data are expressed as percent of total dose of administered antimetabolite. Plus or minus values reflect the range of several determinations on single samples.

Inhibition of Glycolysis by Fructose Analogues. 1-Deoxyfructose, 2-deoxyglucose, 1-deoxyglucitol, and 1-deoxymannitol did not serve as substrates for lactate production by rabbit erythrocytes. Table III shows the results of experiments in which these compounds were tested as inhibitors of lactate production from glucose. 1-Deoxyfructose inhibited lactate production to a greater extent than the known glycolytic inhibitor 2-deoxyglucose. Neither deoxypolyol produced any significant inhibition.

Disposition of Fructose Analogues in Vivo. Groups of 3–4 mice were injected daily with the various fructose derivatives at dosages of up to 100 mg per kg per day over periods ranging from 1 to 21 days. Mice treated in this manner showed neither weight nor behavior differences when compared with saline-injected controls (Dills, 1973). In one experiment (Table IV) the appearance of deoxypolyols and 1-deoxyfructose in the urine was monitored after a single injection of 3 g per kg. None of the compounds was detected in the feces of the animals. There was significant interconversion between deoxyfructose and the deoxypolyols probably due to the action of liver glucitol dehydrogenase. Fructose, glucitol, and mannitol have all been shown to enter liver cells (Cahill et al., 1958) and their 1-deoxy derivatives could be expected to as well. Liver has high levels of glucitol dehydrogenase (Schmidt and Schmidt, 1960). The compounds are retained in the system for varying periods with 1-deoxymannitol for the shortest and 1-deoxyfructose for the longest period of time. It appears that all three compounds are largely excreted within 24 h.

Table V shows the results typical of similar experiments using the oral route of administration to groups of 3–4 mice for each compound. Drinking solutions contained 25 mg of compound per ml which corresponded to a dose of about 3 g per kg per day. Mice treated for up to 21 days showed neither weight nor behavioral differences compared with controls (Dills, 1973). The appearance of deoxyfructose and deoxypolyol was monitored in the urine and feces of the experimental mice. Measurements were made over three sequential 24-h periods following an initial 6-h equilibration period. Similar percentages were obtained over each 24-h period. The urinary excretion of the experimental compounds reflects interconversion of deoxyfructose and deoxypolyol as observed in the previous experiment. In addition, it can be seen from Table V that deoxyfructose is absorbed from the intestinal tract of mice to the extent of 72% as opposed to 36 and 30% for deoxyglucitol

and deoxymannitol, respectively. These absorption patterns are consistent with previous results with the natural analogues of these substances (Olmsted, 1953; Mehnert et al., 1959).

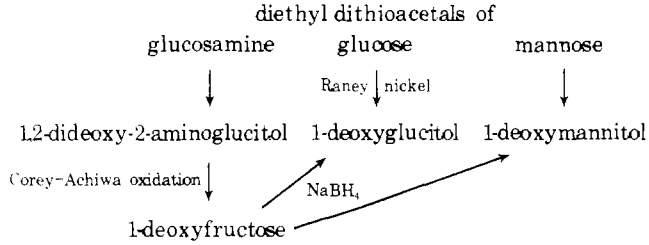
Recoveries of material listed in Tables IV and V are somewhat short of 100%. This probably represents the imprecision of intake and analytical measurements, but we cannot eliminate the possibility of some modest level of metabolism or retention of the compounds in the animals.

Since no effects of large amounts of fructose derivatives were noted on mature mice, these compounds were administered at about 1 g per kg per day dosage schedule by the oral route to precociously weaned male mice for a 21-day period starting at 14 days of age. No effects were noted on growth rate at this stage of rapid development when compared with controls (Dills, 1973).

Discussion

Scheme I presents the paths developed for synthesis of useful quantities of the three fructose analogues studied in this work.

SCHEME I: Synthetic Paths.



Although the direct synthesis of the free deoxypolyols by Raney nickel desulfurization is not a new route to this type of compound, to our knowledge ours is the first report of its application to synthesis of 1-deoxyglucitol. In these syntheses and the corresponding step in the procedure for 1-deoxyfructose, partially desulfurized contaminants are troublesome to remove; proper attention to Raney nickel preparation and proportions used prevents significant contamination.

Our synthesis of 1-deoxyfructose is superior to other published methods. The overall yield of 1-deoxyfructose from starting glucosamine hydrochloride is 26–27%. This compares with the overall yields of 8–9% from mannose accomplished by Dills (1973), 3–4% from glucose (Dills, 1973) via the general route of Wolfrom et al. (1942), 18–19% from fructose by the method of Haylock et al. (1971; Pacsu et al., 1939), and 5–6% from mannitol by the method of Ishizu et al. (1967; Zervas and Papadimitriou, 1940; Fletcher and Diehl, 1952). The yield from arabinose by intermediates described by Wolfrom et al. (1941) is not likely to be over 10% (see Dills, 1973). Our pathway consists of three simple steps and is suitable for the expeditious production of the compound in quantities sufficient for extensive biological testing.

Our results and those of Haas and Schlimmer (1972) indicate the utility of the oxidation method of Corey and Achiwa (1969) to carbohydrate chemistry. This procedure allows preparations of free ketoses and aldoses in a single step where the corresponding amino derivatives are available.

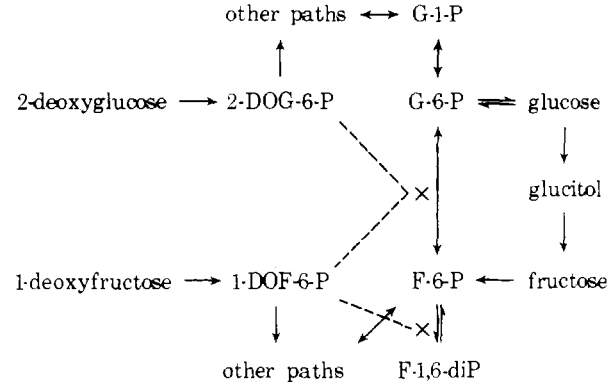
Scheme II illustrates the fact that 1-deoxyfructose and 2-deoxyglucose, unlike glucose and fructose, can not be interconverted and, therefore, should have different metabolic fates and interact with different spectra of enzymes. The potential of 1-deoxyfructose and its reduced analogues as antimetabolites or as research tools is of interest compared with 2-deoxyglucose. The latter has long been known as a potent glycolytic inhibitor, although its use on a clinical level is limited by its toxic side effects (see review by Brown, 1962). The potential

TABLE V: Appearance of Deoxypolyol and 1-Deoxyfructose in Mouse Urine and Feces during a 24-Hour Period of Continuous Ingestion of Fructose Antimetabolites.

Antimetabolite[a]	Sample	Deoxypolyol[b] (%)	1-Deoxyfructose[b] (%)
1-Deoxyglucitol	Urine	33 ± 7	3 ± 1
	Feces	58 ± 9	0
1-Deoxymannitol	Urine	28 ± 9	2 ± 1
	Feces	58 ± 12	0
1-Deoxyfructose	Urine	2 ± 1	70 ± 10
	Feces	0	14 ± 5

[a] Total intake was about 3 g per kg of body weight per 24 h. [b] Data are expressed as in Table IV.

SCHEME II: Metabolic Paths and Effects.



of the 1-deoxypolyols as economical and possibly organ specific precursors of 1-deoxyfructose in vivo represents an added dimension for such studies. An understanding of the interaction of fructose analogues with some of the key enzymes of carbohydrate metabolism is important to the appreciation of the possible uses of these compounds.

The biochemistry of acyclic polyols has been thoroughly reviewed by Touster and Shaw (1962) who updated the classic discussion by Edson (1953) of the structural requirements of polyol dehydrogenases. Our results require that substantial revision be made in the accepted structural requirements of substrates for liver glucitol dehydrogenase as summarized in these reviews. The activity of the dehydrogenase which we noted toward the two deoxypolyols disproves the postulated requirement for a hydroxyl on carbon one which is based on the report by McCorkindale and Edson (1954) of the inactivity of the enzyme toward 1-deoxyglucitol. The suitability of 1-deoxymannitol as a substrate strongly contradicts the supposed *cis*-2,4-dihydroxy requirement for enzyme activity (Edson, 1953; Smith, 1962). Other evidence contrary to the postulated requirement exists (Touster and Shaw, 1962; Smith, 1962); this evidence is consistent with the data for mannitol in Table I. It is not clear why absence of the hydroxyl group in position one which causes 1-deoxyglucitol to be somewhat less suitable than glucitol can have such a marked effect in the opposite direction in the case of 1-deoxymannitol. A possibility is that rotation about the C2–C3 bond to allow polyols of the mannitol configuration to assume a pseudo *cis*-2,4-dihydroxy alignment for binding to the enzyme is relatively unproductive in the case of mannitol because the hydroxy group on C1 is brought into an unfavorable steric interaction with some group on the enzyme. Decreased binding of the ketoses was observed for both fructose and deoxyfructose relative to the corresponding polyols. This may reflect optimal active site accommodation

to a hydroxyl on C-2 (L or D configuration), to an open chain structure, or to a tetrahedral geometry about C-2.

The fact that 1-deoxyfructose was a poor substrate for hexokinases is consistent with the observations of Sols and Crane (1954) who found that other hexose analogues without a hydroxyl on carbon one were poor substrates.

In agreement with the results of Bar-Tana and Cleland (1974) we observed that 1-deoxyfructose-6-P is an effective inhibitor of phosphofructokinase. The difference in the Hill coefficients for fructose-6-P as a substrate, about 2, and 1-deoxyfructose-6-P as an inhibitor (about 1, Figure 1) suggests that the 1-OH is not crucial for binding to the enzyme (in essential agreement with Bar-Tana and Cleland, 1974) but is crucial for cooperative interactions of the protein. Thus, the 1-deoxy compound is analogous to a "minimal substrate" as discussed by Koshland (1970); this property may explain why, unlike some substrate analogues capable of inhibiting allosteric enzymes, 1-deoxyfructose-6-P fails to act as an activator at very low concentrations of substrate (Dills, 1973). In this sense one might term it a "minimal inhibitor". Further study of phosphofructokinase interactions with derivatives at carbon one of fructose-6-P may prove fruitful.

In view of the interaction of 1-deoxyfructose with hexokinase and of its phosphate ester with phosphofructokinase, administration of 1-deoxfructose has the potential to result in inhibition of glycolysis at sites other than those found for 2-deoxyglucose. The latter is phosphorylated by hexokinase (Sols and Crane, 1954) and its 6-phosphate is an inhibitor of phosphoglucose isomerase (Bessell and Thomas, 1973). Its major glycolytic inhibitory capacity appears to be due to the inhibition of glucose uptake by 2-deoxyglucose 6-phosphate (Kipnis and Cori, 1959; Helmreich and Eisen, 1959). 1-Deoxyfructose may have similar effects in the glycolytic sequence and inhibit at the phosphofructokinase step as well. Our results show that the fructose analogue is as good or better than 2-deoxyglucose as a glycolytic inhibitor (Table III).

The 1-deoxypolyols, which have potential as economical, stable precursors of 1-deoxyfructose, have little effect on glycolysis in erythrocytes; this is not unexpected in view of the inability of these cells to take up polyol to any appreciable extent (LeFevre and Davies, 1951). In the whole animal certain tissues such as liver can absorb and metabolize polyols. 1-Deoxypolyols might serve as precursors of 1-deoxyfructose in such tissues and selectively influence their metabolism or leave them to give general systemic effects. The deoxypolyols might have special effects in tissues where glucose–sorbitol–fructose interconversions play important roles.

The whole animal data show that all three 1-deoxy compounds studied are absorbed in significant amounts. No acute toxic effects are evident. Although cleared into the urine at reasonable rates, they appear to persist long enough in the body to be able to be metabolized and have some metabolic consequences. In fact, there is evidence of some interconversion of these compounds; however, they do not appear to reach metabolic equilibrium. Renal clearance appears to be rapid compared with either rates of cellular uptake or reaction with intracellular enzyme systems or both. The rapid clearance of 1-deoxyfructose probably reflects, at least in part, its slow phosphorylation by hexokinase. 5-Thio-D-glucose, another slowly phosphorylated hexose analogue (Chen and Whistler, 1975), also is cleared rapidly (Pitts et al., 1975). 2-Deoxyglucose, on the other hand, is readily phosphorylated and accumulates intracellularly (Kipnis and Cori, 1959; Helmreich and Eisen, 1959). Its clearance into the urine is more limited (Landau et al., 1958; Ely, 1954) and its effects more toxic to the organism (Ely, 1954; Landau and Lubs, 1958; Laszlo et al., 1960).

The ability of the fructose analogues to be absorbed and interconverted in vivo and to interact with key enzymes of sugar metabolism and their demonstrated as well as theoretical potential as metabolic inhibitors support further study of their utility in studies (a) of systemic carbohydrate metabolism, (b) of metabolism of tumor tissue, (c) of effects on specialized metabolism such as that of reproductive tissues, and (d) of treatment of parasitic infections where the agent has a high reliance on glycolysis. The apparent low toxicity to the mammalian host might be an advantage in the latter case. In this regard 2-deoxyglucose, although a potent glycolytic inhibitor and a carcinostatic agent, has limited clinical usefulness due to toxic side effects (see review by Brown, 1962).

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