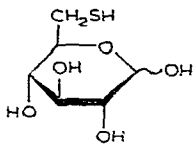
Carbohydrate Research, 49 (1976) 479–481

Note

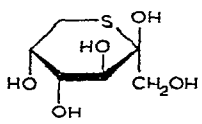
6-Thio-β-D-fructopyranose*

MAREK CHMIELEWSKI, MING-SHENG CHEN, AND ROY L. WHISTLER
Department of Biochemistry, Purdue University, Lafayette, Indiana 47907 (U. S. A.)
(Received December 1st, 1975; accepted for publication, January 30th, 1975)

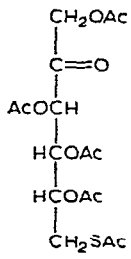
In continuing our work on the synthesis and biochemistry of sugars having sulphur replacing the ring oxygen atom, we had need for a substantial quantity of 6-thio-D-fructopyranose. In earlier work[1], this sugar was chemically synthesised from D-fructose by a rather long and low-yielding route. In seeking an alternative route, the conversion 6-thio-D-glucose (**1**) → 6-thio-D-fructopyranose (**2**) by D-glucose (xylose) isomerase was investigated. The natural substrate for isomerase is D-xylose or D-xylulose, but the enzyme can catalyze the D-glucose⇌D-fructose interconversion. Through adaptive mutation, microorganisms are now available that can produce an isomerase which effectively catalyzes this interconversion.



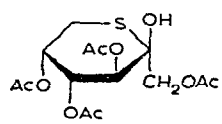
1



2



3



4

D-Glucose isomerase can utilise 6-thio-D-glucose, but not 6-thio-D-fructose, as a substrate; hence, the conversion of 6-thio-D-glucose into 6-thio-D-fructose is probably quantitative since, when the reaction was complete, 6-thio-D-glucose was not detectable (chromatography) and 6-thio-D-fructose was isolated in 90% yield. There was probably some loss during chromatography and general work-up, and by partial conversion of 6-thio-D-glucose into disulphide.

At equilibrium, the isomerase-catalysed interconversion of D-glucose and D-fructose involves approximately equal quantities of each sugar[2].

*Dedicated to the memory of Professor Edward J. Bourne.

The Dische method[3], which is normally used for the determination of D-fructose, was not applicable in the present reaction because of interference by 6-thio-D-glucose.

Because of the stability of the pyranose ring in 6-thio-D-fructose, it is not surprising to find that this compound is not a substrate for yeast hexokinase. However, like normal D-fructose, 6-thio-D-fructose is phosphorylated by rat-liver fructokinase (K_m 0.666mM; *cf.* 0.303mM for D-fructose). The turnover numbers are 4.1 and 16.5 μmol/min/mg of protein, respectively, which suggests that substitution of sulphur for oxygen in the sugar ring reduces the affinity of the analogue for the enzyme.

Treatment of 6-thio-D-fructopyranose (**2**) with acetic anhydride–zinc chloride gave the acyclic penta-acetate **3**, whereas the pyranose tetra-acetate **4** was the product when acetic anhydride–pyridine was used.

EXPERIMENTAL

General. — Purity of products was determined by t.l.c. on Silica Gel G (Merck) and detection effected by charring with sulphuric acid. Column chromatography was performed on silica gel (60–200 mesh, J. T. Baker Chemical Co.). Melting points were determined with a Fisher–Johns apparatus and are corrected.

Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter. N.m.r. spectra were recorded for solutions in chloroform-*d* (internal Me_4Si) or deuterium oxide (internal *tert*-butyl alcohol) with a Varian T-60A spectrometer.

Yeast hexokinase (EC 2.7.1.1), rabbit skeletal muscle pyruvate kinase (EC 2.7.1.40), lactate dehydrogenase (EC 1.1.1.27), adenosine-5′-triphosphate, phospho(enol)pyruvate, and β-NADH were purchased from Sigma Chemical Company. Fructokinase (EC 2.7.1.4) was purified by the method of Adelman *et al.*[4]. D-Glucose isomerase (EC 5.3.1.18) was a gift from CPC International, Inc. All chemicals used in the organic synthesis were obtained commercially, and were used without further purification, unless specified.

6-Thio-D-glucose (**1**). — Compound **1** was prepared as previously described[5,6], except that the tosyl displacement was conducted in *N*,*N*-dimethylformamide[7] which reduced the reaction time to 45 min. The syrupy product was lightly straw-coloured, and contained a trace of disulphide which increased rapidly if the syrup was exposed to air.

6-Thio-β-D-fructopyranose (**2**). — Isomerase[8] was added to a 50% aqueous solution of **1** at pH 8 and 60° in an atmosphere of nitrogen. After 48 h, the mixture was passed through a mixed-bed ion-exchange column and concentrated to dryness. The residue was dried and extracted with methanol, and the extract was concentrated onto silica gel (60–200 mesh). The syrup was then eluted from a column of silica gel with chloroform–methanol (8:2), and crystallisation of the product from ethanol–chloroform at −20° gave **2** (90%), m.p. 63–64°, $[\alpha]_D^{25}$ −194° (*c* 1, water) (no mutarotation). N.m.r. data (D_2O): δ 2.74 (dd, $J_{5,6ax}$ 4.2 Hz, H-6ax), 3.31 (dd, $J_{5,6eq}$ 1.8 Hz, H-6eq), 3.87 (s, H-1,1′), 3.90 (dd, $J_{3,4}$ 7.9, $J_{4,5}$ 2.5 Hz, H-4), 4.11

(d, H-3), and 4.41 (m, H-5). These data indicate that **2** has a pyranose ring in the $^2C_5$ conformation.

Anal. Calc. for $C_6H_{12}O_5S$: C, 36.83; H, 6.61; S, 16.34. Found: C, 36.93; H, 6.48; S, 16.44.

When a solution of **1** in saturated, aqueous calcium hydroxide was stored at 55° for 40 h under nitrogen, purification, as described above, gave **2** (20%) together with numerous other products (t.l.c.).

1,3,4,5-Tetra-O-*acetyl-6*-S-*acetyl-6-thio*-keto-D-*fructose* (**3**). — A mixture of **2** (0.4 g), acetic anhydride (4 ml), and zinc chloride (0.06 g) was stirred at 25° under nitrogen for 20 h, then poured into ice and water, and extracted with ether. The extract was washed with water, aqueous sodium hydrogen carbonate, and water, dried, and concentrated. Crystallization of the residue from ethanol gave **3** (49%), m.p. 90–91°, $[\alpha]_D$ +47° (*c* 1, chloroform). The n.m.r. data ($CDCl_3$) indicated the presence of an AcS and 4AcO groups, and the values $J_{3,4}$ 2.1, $J_{4,5}$ 8.2, $J_{5,6}$ 3.7, and $J_{5,6'}$ 6.2 Hz indicated an open-chain form.

Anal. Calc. for $C_{16}H_{22}O_{10}S$: C, 47.3; H, 5.5; S, 7.9. Found: C, 47.4; H, 5.7; S, 8.1.

1,3,4,5-Tetra-O-*acetyl-6-thio-β*-D-*fructopyranose* (**4**). — Compound **2** (0.2 g) was treated with acetic anhydride (1 ml) and pyridine (1 ml) for 20 h. The mixture was concentrated under diminished pressure and the crude product was purified by chromatography to give **4** (0.12 g), m.p. 155–156° (from ethanol), $[\alpha]_D^{25}$ −150° (*c* 1.6, chloroform). N.m.r. data ($CDCl_3$): δ 2.08, 2.18, 2.22, and 2.24 (4 s, 4 Ac); 2.8–3.8 (m, 4 H); 4.4 (s, 2 H); 5.7 (m, 2 H).

Anal. Calc. for $C_{14}H_{20}O_9S$: C, 46.1; H, 5.5; S, 8.8. Found: C, 46.1; H, 5.4; S, 8.9.

Enzymic assay. — Hexokinase was assayed by the procedure of Joshi and Jagannathan[8], and fructokinase by a spectrophotometric assay[4]. Assays were made at 25° and at pH 7.5. Protein concentration was determined by the procedure of Lowry *et al.*[9].

ACKNOWLEDGMENTS

This paper was supported, in part, by Grant No. AM 18482 of the National Institutes of Health.

Journal Paper No. 6110 of the Purdue Agricultural Experiment Station.

REFERENCES

1 M. S. Feather and R. L. Whistler, *J. Org. Chem.*, 28 (1963) 1567–1569.
2 Y. Takasaki, Y. Kosugi, and A. Kanbayashi, in D. Perlman (Ed.), *Fermentation Advances*, Academic Press, New York, 1969, pp. 561–589.
3 Z. Dische and E. Borenfreund, *J. Biol. Chem.*, 192 (1951) 583–587.
4 R. C. Adelman, F. J. Ballard, and S. Weinhouse, *J. Biol. Chem.*, 242 (1967) 3360–3365.
5 H. Ohle and W. Mertens, *Ber.*, 68 (1935) 2176–2187.
6 M. Akagi, S. Tejima, and M. Haga, *Chem. Pharm. Bull.*, 10 (1962) 562–566.
7 J. M. Cox and L. N. Owen, *J. Chem. Soc., C*, (1967) 1121–1130.
8 M. D. Joshi and V. Jagannathan, *Arch. Biochem. Biophys.*, 125 (1968) 460–467.
9 O. H. Lowry, N. J. Rosenbrough, A. L. Farr, and R. N. Randall, *J. Biol. Chem.*, 193 (1951) 265–275.