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pH Profile for the mutarotation of 6-thio-D-fructose*

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6-Thio-D-fructose has been reported to exist as a pyranose[1], and not to undergo mutarotation in aqueous solution of neutral pH upon dissolution of the crystalline material, which is the β-D anomer[2]. However, recent n.m.r. data[3] have shown that 10–15% of α-D anomer is present in solution, and thus one might expect this thio sugar to show mutarotation upon dissolution. In view of the very high rate of ring opening of 6-thio-D-fructose[4], and the observation that, for 5-thio-D-glucose, base-catalyzed mutarotation is 500 times faster than ring opening[4], it seemed likely that base-catalyzed mutarotation of 6-thio-D-fructose would also be much faster than ring opening, and thus that, at neutral pH, mutarotation would occur too rapidly to be observed. To verify this hypothesis, we have measured the mutarotation of 6-thio-D-fructose over the pH range 1.5–5.45, and the resulting values are shown in

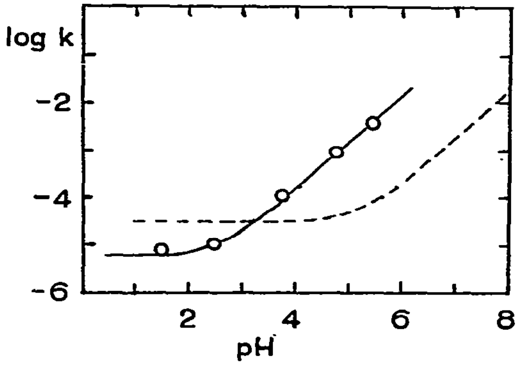

Fig. 1. pH Profile for mutarotation rates of 6-thio-D-fructose (circles). The dotted line shows published rates for ring opening[4].

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*Dedicated to Professor Stephen J. Angyal on the occasion of his retirement.

Fig. 1, together with the ring-opening rates previously published for 6-thio-D-fructose which, in the base-catalyzed region, are for the equilibrium mixture of anomers. These experiments showed that the specific rotation, $[\alpha]_D^{25}$, of the freshly dissolved β-D anomer is —210°, and that at anomeric equilibrium, the value is —185° (previously reported[2] as —194°, and with an error in sign in ref. 3). As the n.m.r. data indicate the presence of 10–15% of α-D anomer at equilibrium, the calculated range for the specific rotation of the α-D anomer is —43 to +27°. A more accurate value could be obtained by separating the two anomers by a chromatographic technique at pH 2 and 0°, where the half-life for interconversion is several days.

The mutarotation data in Fig. 1 were fitted to the equation:

$$k_{\mathrm{mut}} = k_0 + k_{\mathrm{B}}[\mathrm{OH}^-],$$

giving $k_0 = 6.3 \pm 0.9 \times 10^{-6}\ \mathrm{s}^{-1}$ and $k_{\mathrm{B}} = 1.5 \pm 0.1 \times 10^6\ \mathrm{M}^{-1}\mathrm{s}^{-1}$. The comparable values for ring opening[4] were $29 \times 10^{-6}\ \mathrm{s}^{-1}$ and $0.0172 \times 10^6\ \mathrm{M}^{-1}\mathrm{s}^{-1}$, and thus base-catalyzed mutarotation is 87 times faster than ring opening. For 5-thio-D-glucose, this ratio[4] was 500. At pH 7, the half-time for mutarotation of 6-thio-D-fructose should thus be 4.6 s, and it is clear why no mutarotation was previously observed at neutral pH.

We have previously proposed that, for 5-thio-D-glucose, the mechanism of mutarotation involves a non-covalently bonded intermediate in which a free thiolate group is in intimate contact with the aldehyde carbon atom[4]. This intermediate undergoes rotation of the aldehyde group and ring-closure much more rapidly than it undergoes the other internal rotations that separate the thiolate group from the aldehyde. A similar situation appears to apply for 6-thio-D-fructose, except that the group undergoing rapid rotation is the hydroxymethyl–carbonyl group (carbon atoms 1 and 2). It seems probable that all sugars having sulfur in the ring will show base-catalyzed mutarotation that is several orders of magnitude faster than ring opening.

The apparent difference of a factor of 4 in the pH-independent k_0 values for ring opening and mutarotation probably results from scatter in measurements of the ring-opening rate at low pH. We thus assume that these rates are essentially equal, and that, at low pH (as previously postulated[4]), the closed but not covalently bonded intermediate is rapidly protonated by H^+, so that mutarotation always involves ring opening.

Interestingly, 6-thio-D-fructose shows the same behavior as other sugars having sulfur in the ring with respect to ring opening and mutarotation, even though both processes are much faster than for other such thio sugars (both base-catalyzed and pH-independent rates of ring opening and mutarotation are 50 times as high for 6-thio-D-fructose as for 5-thio-D-glucose, and base-catalyzed mutarotation is 17 times faster than for D-fructose[4]). These data reinforce our previous conclusion that the unique property of 6-thio-D-fructose is a higher equilibrium constant for converting the ring form having an ionized, anomeric hydroxyl group into the closed, but non-covalently bonded, intermediate[4].

EXPERIMENTAL

6-Thio-D-fructose was prepared as previously reported[2]. Mutarotations were measured for a solution (17 mg/mL) in 30mM buffer at the desired pH with a Perkin–Elmer Model 141 spectropolarimeter. Reactions were conducted in a 1.0-dm, water-jacketed cell at 25°. Mutarotation rates were calculated from least-squares fits to the equation: $(\alpha_0 - \alpha_t) = (\alpha_0 - \alpha_\infty)(1 - e^{-k_{mut}t})$, where α_0, α_t, and α_∞ are the observed optical rotations at zero time, at t, and at equilibrium.

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