Note

The preparation of 1-deoxy-1-halogeno-D-fructoses

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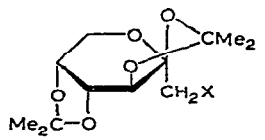
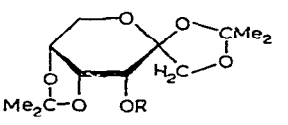
1-Deoxy-1-halogeno-D-fructoses are of potential interest as active-site-directed alkylating agents for enzymes and membrane carriers. Several haloketones recently prepared as substrate-analogue alkylating agents for enzymes, such as the haloacetol phosphates[1] and bromopyruvic acid[2], are too reactive, and react non-specifically with thiol groups away from the active site. The tendency of 1-deoxy-1-halogeno-D-fructoses to exist in the ring form may be expected to mask the reactivity of these haloketones until they are adsorbed onto an enzyme active-site in the *keto* form. This note describes the preparation of 1-deoxy-1-fluoro-, 1-chloro-1-deoxy-, and 1-bromo-1-deoxy-D-fructose, and preliminary tests of these compounds as substrates and inactivators of hexokinase and sorbitol dehydrogenase. Brain[3] and yeast[4] hexokinase are considered to utilise the furanoid form of D-fructose as substrate, whereas sorbitol dehydrogenase must be expected to use the acyclic form of D-fructose.

Richardson[5] has pointed out that the hexulopyranose 1-sulphonates should be resistant to nucleophilic attack by ionic reagents because of the dipolar interaction of the ring and anomeric oxygen bonds with the transition-state dipole. As predicted, 2,3:4,5-di-*O*-isopropylidene-1-*O*-methanesulphonyl-β-D-fructopyranose (**1**) offered resistance to attack by halide ions, but a slow reaction was observed in *N,N*-dimethylformamide at 144°. The reaction was most successful with chloride ion, the reaction with tetrabutylammonium fluoride was very slow, and the yield of bromo sugar was limited even when pyridine was added to the reaction mixture to minimize acid-catalysed decomposition. After chromatographic separation of the halogenated compounds from unchanged starting material, the isopropylidene groups were removed and the syrupy 1-deoxy-1-halogenofructoses further purified by paper chromatography.



1 X = OMs 3 X = Br
2 X = Cl 4 X = F



5 R = H
6 R = Ms

While this work was in progress, a preparation of 1-chloro-1-deoxy-D-fructose was reported[6], using triphenylphosphine in carbon tetrachloride, which is more convenient than that reported here.

Attempts to prepare the corresponding 3-deoxy-3-halogeno-D-fructoses by displacement of 3-*O*-methanesulphonyl (**6**) or 3-*O*-chlorosulphonyl derivatives of 1,2:4,5-di-*O*-isopropylidene-β-D-*ribo*-hexulopyranose (**5**) were unsuccessful.

Preliminary tests showed that 1-deoxy-D-fructose and the halogenated analogues were neither inactivators of, nor substrates for, yeast hexokinase. The second observation was unexpected because D-fructose itself is quite a good substrate. Since hydrogen and fluorine atoms are smaller than a hydroxyl group, this implies that HO-1 of D-fructose is important for either the binding or the orientation of the enzyme and substrate, or is in some other way involved in the reaction. 1-Bromo-1-deoxy-D-fructose was hydrolysed at a significant rate at pH 7.5, and its interaction with the enzyme could not be rigorously tested.

1-Deoxy-D-fructose and the 1-deoxy-1-halogeno-D-fructoses were similarly found to be neither inhibitors, inactivators, nor good substrates of sorbitol dehydrogenase in the presence of NADH. 1-Deoxy-D-fructose and 1-deoxy-1-fluoro-D-fructose appeared to be extremely poor substrates. 1-Chloro-1-deoxy and 1-bromo-1-deoxy-D-fructose, but not 1-deoxy-1-fluoro-D-fructose, were slowly hydrolysed to fructose at pH 7.4 and 25°, as shown by reaction with sorbitol dehydrogenase. By contrast, in the absence of NADH, bromopyruvic acid rapidly and completely inactivated the enzyme. The lack of inactivation by the chloro- and bromo-fructose analogues appears to justify the assumption that the predominance of the ring form in such a compound reduces the amount of non-specific alkylation, but in this case no specific active-site-directed alkylation occurred.

EXPERIMENTAL

General methods. — Optical rotations were measured on a Perkin–Elmer 141 polarimeter. Melting points are uncorrected. Thin-layer chromatography (t.l.c.) was performed on Silica Gel F_{254} with ethyl acetate–light petroleum (b.p. 40–60°) (3:2), and 10% sulphuric acid in water for detection. Preparative chromatography was performed with a column (46 × 2.5 cm) of Merck Silica Gel (0.05–2 mm mesh, 110 g) and ethyl acetate–light petroleum (b.p. 40–60°) as eluant. Paper chromatography was effected on Whatman No. 1 or 3MM paper with 1-butanol–ethanol–water (4:1:5, upper layer) as eluant. Mass spectra were determined by the Physico-chemical Measurements Unit, Harwell.

2,3:4,5-Di-O-isopropylidene-1-O-methanesulphonyl-β-D-fructopyranose (**1**). — Methanesulphonyl chloride (10 ml) was added to 2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranose[7] (10 g, m.p. 94–95°) in anhydrous pyridine at −70°. The reaction mixture was allowed to reach room temperature, and after 90 min it was poured into ice–water. After 30 min, the product was filtered off and recrystallised from ethanol

to give **1** (11.3 g), m.p. 121–122°, $[\alpha]_D^{22}$ −19.4° (*c* 0.17, chloroform) (Found: C, 45.9; H, 6.3; S, 10.2. $C_{13}H_{22}O_8S$ calc.: C, 45.9; H, 6.5; S, 9.4%).

1-Chloro-1-deoxy-2,3;4,5-di-O-*isopropylidene-β*-D-*fructopyranose* (**2**). — A solution of **1** (8 g) in anhydrous *N,N*-dimethylformamide (50 ml) and anhydrous pyridine (1 ml) containing lithium chloride (8 g) was stirred for 70 h at 144°, cooled, and then poured into chloroform and water. The aqueous layer was extracted with chloroform (total 150 ml), and the combined chloroform solutions were washed with 3M hydrochloric acid, aqueous sodium hydrogen carbonate, and water, and dried with sodium sulphate. T.l.c. showed two spots, the slower of which corresponded to starting material. Separation by column chromatography and recrystallization from ether–light petroleum (b.p. 40–60°) gave **2** (3.1 g), m.p. 53–53.5°, $[\alpha]_D^{22}$ −29.5° (*c* 0.19, chloroform); lit.[6] m.p. 53–53.5°. P.m.r. spectra were consistent with the structure proposed.

1-Bromo-1-deoxy-2,3:4,5-di-O-*isopropylidene-β*-D-*fructopyranose* (**3**). — A solution of **1** (5 g) in anhydrous *N,N*-dimethylformamide (15 ml) and anhydrous pyridine (0.2 ml) containing lithium bromide (5 g) was heated at 144° for 4 h. The cooled mixture was worked up as described above, and the products were separated by column chromatography to give **3** (300 mg), m.p. 46–47° (from ether–light petroleum), $[\alpha]_D^{23}$ −34.4° (*c* 0.48, chloroform) (Found: C, 44.8; H, 6.0; Br, 24.5. $C_{12}H_{19}Br_5O_5$ calc.: C, 44.6; H, 5.9; Br, 24.8%).

1-Deoxy-1-fluoro-2,3:4,5-di-O-*isopropylidene-β*-D-*fructopyranose* (**4**). — A solution of **1** (3 g) in *N,N*-dimethylformamide (15 ml) and anhydrous pyridine (0.1 ml) containing tetrabutylammonium fluoride (13 g) was stirred for 14 days at 144°, cooled, and then worked up as above. T.l.c. showed three spots. In addition to **1** and **4**, a small amount of 2,3:4,5-di-*O*-isopropylidene-*β*-D-fructopyranose was present. After column chromatography, **4** (210 mg) was isolated as a syrup which failed to crystallize but gave a single spot on t.l.c.; it had $[\alpha]_D^{24}$ −22.2° (*c* 0.47, chloroform) (Found: F, 5.1. $C_{12}H_{19}FO_5$ calc.: F, 7.2%).

The ^{19}F n.m.r. spectrum showed the expected triplet due to CH_2F, J_{HF} 47.5 Hz, and the p.m.r. spectrum was consistent with the structure proposed. Mass spectrometry showed a molecular-ion peak at *m/e* 247.0974 (calc. for $C_{11}H_{16}FO_5$: 247.0981) and a peak at 189 corresponding to $M-(CH_3)_2CO$. The spectrum also contained peaks corresponding to traces of the chloro and bromo analogues, which may account for the low fluorine analysis. The presence of the other halogen atoms is difficult to explain unless they were present in the hydrofluoric acid from which the tetrabutylammonium fluoride was made. Traces in this reagent would be concentrated by the more rapid reaction of these halides with the methanesulphonyl derivative.

1-Deoxy-1-halogeno-D-*fructoses*. — Each di-*O*-isopropylidenefructose (**2**, **3**, and **4**; 1 g) was dissolved in ethanol (5 ml) and water (15 ml) and stirred at 60–70° with Amberlite IR-120(H^+) resin for 3 h. The cooled and filtered solution was extracted with ether (which was discarded) and evaporated to dryness, below 30° and usually by freeze-drying. If necessary, the halogeno sugars were further purified by preparative paper chromatography. They did not crystallize but gave only one spot on paper

chromatography, except for the bromo sugar which spontaneously decomposes to D-fructose. 1-Deoxy-1-fluoro-D-fructose (R_{Fru} 1.49) had $[\alpha]_D^{22}$ −75° (*c* 3.6, water) (Found: F, 9.0. $C_6H_{11}FO_5$ calc.: F, 10.4%). 1-Chloro-1-deoxy-D-fructose (R_{Fru} 2.05) had $[\alpha]_D^{22}$ −63° (*c* 0.25, water); lit.[6] $[\alpha]_D$ −53.3° (*c* 1.38, water). 1-Bromo-1-deoxy-D-fructose (R_{Fru} 2.21) had $[\alpha]_D^{22}$ −55.1° (*c* 2.43, water); the rotation was obtained on a sample purified by paper chromatography.

D-arabino-*Hexulose phenylosazone.* — D-Fructose or the 1-deoxy-1-halogeno-D-fructoses (40 mg) were each dissolved in water (1 ml) containing phenylhydrazine hydrochloride (80 mg), sodium acetate (120 mg), and sodium metabisulphite (40 mg), and heated in a boiling water bath. Crystals of the phenylosazone appeared after the following approximate times: from D-fructose, 2 min; 1-bromo-1-deoxy-D-fructose, 3 min; 1-chloro-1-deoxy-D-fructose, 4 min; and 1-deoxy-1-fluoro-D-fructose, 4 min. In each case, the yield was good and, after recrystallization from ethanol, each product had a m.p. in the range 202–205°; lit.[8] m.p. 207°.

*1,2:4,5-Di-O-isopropylidene-3-O-methanesulphonyl-β-*D-ribo-*hexulopyranose* (**6**). 1,2:4,5-Di-*O*-isopropylidene-β-D-*ribo*-hexulopyranose[9] (R_F 0.28, 1 g) was dissolved in dry pyridine (5 ml) and methanesulphonyl chloride (1 ml) was added dropwise at room temperature. After 28 h at room temperature, the mixture was poured into ice–water and extracted with chloroform. The chloroform layer was worked up in the usual way to give **6** (0.97 g), m.p. 103–104° (from ethanol), $[\alpha]_D^{22}$ −111° (*c* 0.27, chloroform), R_F 0.38 (Found: C, 46.5; H, 6.3; S, 9.65. $C_{13}H_{22}O_8S$ calc.: C, 45.9; H, 6.5; S, 9.4%). Attempts to displace the methanesulphonyl group with lithium chloride in *N,N*-dimethylformamide were unsuccessful.

*3-Chlorosulphonyl-1,2:4,5-di-*O*-isopropylidene-β-*D-ribo-*hexulopyranose.* — A solution of 1,2:4,5-di-*O*-isopropylidene-β-D-*ribo*-hexulopyranose (1 g) in chloroform (10 ml) and dry pyridine (8 ml) was treated with sulphuryl chloride (5.2 ml), dropwise with magnetic stirring at 0°. After a further 1.5 h, chloroform (50 ml) was added and the solution was poured into 20% sulphuric acid (100 ml) at 0°. Pyridine salts were filtered off, and the chloroform layer was washed with saturated aqueous sodium hydrogen carbonate and water, and then dried over sodium sulphate. T.l.c. showed one spot, R_F 0.46. Removal of the solvent gave a product which was recrystallized from ether–light petroleum to give the title compound (0.27 g), m.p. 74°. The substance was very unstable at room temperature. Attempts to displace the chlorosulphonyl group with lithium chloride in *N,N*-dimethylformamide were unsuccessful.

Tests for substrate activity with hexokinase. — 10mM D-Fructose (0.5 ml), or the deoxyhalogenofructoses or 1-deoxy-D-fructose, with 5% potassium chloride (0.5 ml), 12mM ATP (1 ml) and 0.2M magnesium chloride (0.1 ml) were made up to a total volume of 5 ml at pH 7.5 using carbonate-free water and sodium hydroxide in a Radiometer pH-stat. The reaction vessel was maintained carbonate-free by the passage of CO_2-free nitrogen through the solution, and the uptake of alkali was negligible. Hexokinase (Miles–Seravac) (10 μl, 2 units) was then added and the rate of addition of 0.01M sodium hydroxide measured. The rate of production of acid was 0.12 μmole/min at 22°, using D-fructose, and was less than 0.01 times this rate with all

the other substrates. After 30-min incubation with the deoxyhalogenofructoses, D-fructose (10mM, 0.5 ml) was added. The rate of acid production was identical to that in the absence of the halogenated sugars.

Tests for substrate activity with sorbitol dehydrogenase. — 80mM D-Fructose (0.2 ml), or 1-deoxy-D-fructose or the deoxyhalogenofructoses, 15mM NADH (0.05 ml), and 0.2M Tris-chloride buffer (pH 7.4, 2.9 ml) were placed in a cuvette and balanced, in a Unicam SP 1800 at 25°, against a similar cuvette from which the sugar had been omitted. Sorbitol dehydrogenase (Boehringer) (0.05 ml, 10 μg of enzyme protein) was added to each cuvette and the difference in absorption was measured. The rate of oxidation of NADH was 6.4 nmoles/min with D-fructose as the substrate. 1-Deoxy-D-fructose (0.015 nmole/min) and 1-deoxy-1-fluoro-D-fructose (0.007 nmole/min) gave an extremely slow reaction. This could have been due to traces of impurity. Both 1-chloro-1-deoxy- and 1-bromo-1-deoxy-D-fructose were inactive at first, but slowly an oxidation was observed, after ~2 h the rate of oxidation had reached 0.7 nmole/min for the chloro compound; after 30 min the rate had reached 6.0 nmoles/min for the bromo compound. This oxidation was ascribed to the formation of fructose from the halogenated fructoses at this pH. For the bromo compound, this was confirmed by paper chromatography.

Tests for inhibition and inactivation of sorbitol dehydrogenase. — 80mM 1-Deoxy-D-fructose or the deoxyhalogenofructoses were incubated with NADH, buffer, and enzyme, as described above, and 80mM D-fructose (0.2 ml) was added. The rate of NADH oxidation was the same both in the presence and absence of the fructose derivatives. When the enzyme was incubated in the presence of 5mM 1-chloro-1-deoxy-D-fructose for 30 min in the absence of NADH, there was no inactivation. When it was incubated with 5mM bromopyruvic acid for 30 min at 25°, the enzyme was inactivated completely.

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