Note

Elucidation of the structure of "dibenzylidene fructose" by physical methods and use of a shift reagent†

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For several years, we[1–3] have been interested in studying the mechanism of action of the allosteric enzyme phosphofructokinase, which catalyzes the phosphorylation of D-fructose 6-phosphate to D-fructose 1,6-diphosphate. In order to assess the relative importance of various structural features present in this substrate, we have embarked on a program to synthesize deoxy analogs of D-fructose 6-phosphate. In such an undertaking, use of selective blocking techniques is essential, and one of these is the condensation of D-fructose with benzaldehyde. However, the structure of the product of this reaction has not been established unequivocally.

Brigl and Schinle[4] were the first to treat D-fructose with an excess of benzaldehyde in the presence of zinc chloride, obtaining a solid product (**1**), which they identified as "dibenzylidene fructose". By using a series of chemical conversions and a modicum of intuition, Brigl and Widmaier[5] assigned the partial structure 2,3:4,5-di-*O*-benzylidene-D-fructopyranose to **1**. Because of the equivocal nature of the experimental approach used by those workers, and the availability of more sophisticated methods for structural determination, we undertook to re-investigate this compound. We now report the structure of product **1** as deduced from its spectral properties together with those of its monoacetate, **2**. Application of the shift reagent tris[2,2,6,6-tetramethyl-3,5-heptanedionato]europium [Eu(thd)$_3$] to **1** permitted assignment of the configuration at its anomeric center.

The mass spectra of **1** and its monoacetate **2** exhibit molecular ions at the proper mass number for a monomeric, dibenzylidene adduct of a hexulose. Neglecting stereochemical considerations, over 50 isomeric structures can be envisaged for the monomeric product **1**. The i.r. spectrum of **1** shows a band at $\sim$3500 cm^{-1}, which indicates the presence of a free hydroxyl group, and a band for a carbonyl group is

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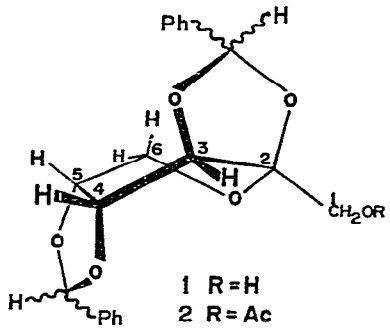

1 R=H
2 R=Ac

absent; these observations eliminate a number of possibilities from further consideration. As expected, the i.r. spectrum of **2** is transparent in the hydroxyl region, and contains an ester carbonyl absorption at 1750 cm^{-1}.

Major fragments in the mass spectra of **1** and **2** indicate the loss of a chain-terminal, oxygenated carbon atom as $-CH_2OR$. Thus, **1** must have a tricyclic structure and a free hydroxyl group on either C-1 or C-6, a condition met by only seven of the structures originally considered.

Additional evidence for the primary nature of the free hydroxyl group of **1** was obtained from the n.m.r. spectrum of **1** in methyl sulfoxide-d_6; the hydroxyl resonance of **1** appeared downfield (5.3 p.p.m.) as a doublet of doublets, owing to slightly unequal coupling to the diastereotopic methylene protons[6], and slowly disappeared because of exchange with deuterons of the solvent. As only C-1 and C-6 are attached to two protons, the location of the free hydroxyl group at one or other of these two atoms is certain.

Foster *et al.*[7] have shown that the benzylidene protons of structures containing the 2-phenyl-1,3-dioxolane ring resonate at lower field (typically, 5.33–5.76 δ) than the analogous protons of 2-phenyl-1,3-dioxane rings. The benzylidene signals of **1** were observed at 5.67 and 5.85 δ, indicating the presence of two 5-membered, acetal rings in this compound.

These findings are consistent with only two isomeric structures, namely, 1,2:3,4-di-*O*-benzylidene-D-fructofuranose and 2,3:4,5-di-*O*-benzylidene-D-fructopyranose. Acetylation of the free hydroxyl group in **1** is accompanied by a substantial, downfield shift of two signals that are observed as an AB pattern in the 100-MHz n.m.r. spectrum of **2** in chloroform-*d* (see Fig. 1); the AB portion of an ABX subspectrum remains at higher field. Whereas H-5 would be expected to couple with H-6 and H-6′, H-1 and H-1′ are insulated from further coupling by the adjacent, quaternary carbon atom (namely, C-2). It is thus apparent that the lowfield, AB pattern represents H-1 and H-1′, and that the upfield, AB(X) pattern corresponds to H-6 and H-6′. Therefore, the structure 1,2:3,4-di-*O*-benzylidene-D-fructofuranose can be excluded, and the earlier, partial structure[5] assigned to **1** is verified.

The stereochemistry at every carbon atom, except the anomeric cent erand the benzylidene groups, is established by the configurational relationships[8] in the parent

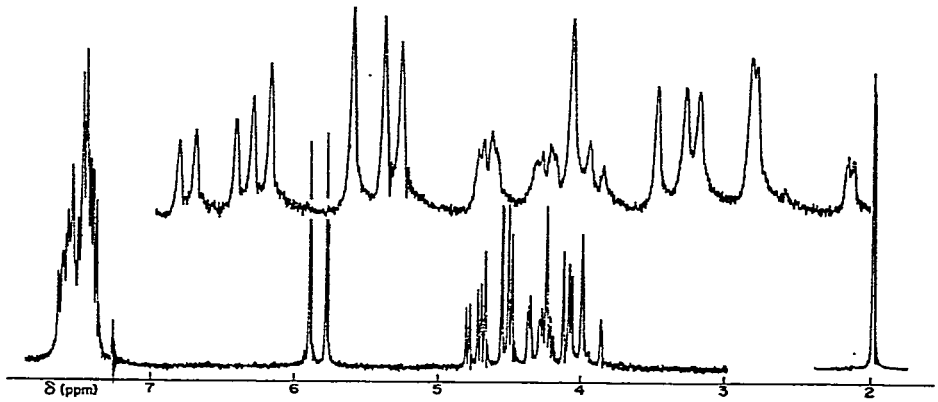

Fig. 1. The 100-MHz n.m.r. spectrum of compound **2** in chloroform-*d*. The upper trace is an expansion of signals from the methylene and methine protons attached to the sugar skeleton.

sugar. Examination of a Dreiding model of the β-anomer of 2,3:4,5-di-*O*-benzylidene-D-fructopyranose reveals that the separation between O-1 and H-3 is somewhat less than the distance between O-1 and the benzylidene proton. On the other hand, in the α-anomer, these distances are almost identical. DeMarco *et al.*[9] have demonstrated an inverse dependence of n.m.r. signal shifts induced by lanthanide complex on such O–H distances. Although **1** contains six potential sites for binding of the (Lewis acid) lanthanide complex[10] tris[2,2,6,6-tetramethyl-3,5-heptanedionato]europium [$Eu(thd)_3$], it has been shown[10–12] that coordination to the hydroxyl group is more efficient than to other oxygen-containing functions. Accordingly, a solution of **1** in carbon tetrachloride was treated with successively increased amounts of a saturated solution of $Eu(thd)_3$ in the same solvent, and the n.m.r. spectrum was determined at each concentration of the shift reagent. A two-proton signal corresponding to H-1 and H-1′ moved downfield at the highest rate, followed by the H-3 doublet (respectively 2.5 and 1.4 times the rate of shift observed for the benzylidene proton of the 2,3-acetal ring). This observation indicated that H-3 is closer than the 2,3-benzylidene proton to the paramagnetic center, and permitted assignment of the β-D configuration to **1**.

Taken together, the data obtained from the i.r., mass, and n.m.r. spectra establish unequivocally that **1** is 2,3:4,5-di-*O*-benzylidene-β-D-fructopyranose. The skew conformation S_4^6 depicted for **1** (and **2**) is that adopted by the Dreiding model; in this conformation, the dihedral angles between H-3 and H-4 is ~60°, and that between H-4 and H-5 is <20°, consistent with the small (2 Hz) and large (8 Hz) values (see Experimental section) of $J_{3,4}$ and $J_{4,5}$, respectively. The S_4^6 conformation has also been determined, by p.m.r. spectroscopy, to be the favored conformation of analogous acetal derivatives of aldoses[13] and ketoses[14] having 1,2:3,4-di-*O*-alkylidene-β-D-*arabino* stereochemistry[15].

EXPERIMENTAL

Mass spectra were recorded with a Varian M-66 cycloidal, mass spectrometer at an inlet temperature of 145° and an ionizing potential of 70 eV. Infrared spectra

were recorded with a Beckman IR-10 infrared spectrometer for potassium bromide pellets of the compounds. N.m.r. spectra were recorded with a Varian HA-100 n.m.r. spectrometer (frequency-sweep mode) at the ambient temperature (35°) of the probe, with tetramethylsilane (3% v/v) as the internal standard and lock signal.

2,3:4,5-Di-O-benzylidene-β-D-fructopyranose (**1**). — D-Fructose was treated with benzaldehyde in the presence of zinc chloride at room temperature, as described by Brigl and Schinle[4]. Recrystallization of the product, three times from carbon tetrachloride and twice from methanol, gave pure **1**, m.p. 160° (identical with that reported[4]); $[\alpha]_D^{20}$ −25.5° (in chloroform); ν_{max}^{KBr} 3500 (broad, OH), 1465 (medium), 1415 (medium), 1075 (broad, multiple, R–O–R), 760 (sharp, Ph), and 700 cm^{-1} (sharp, Ph); n.m.r. data (100 MHz, CCl_4): 1.73 δ (1-proton multiplet, OH; exchanges rapidly with D_2O), 3.59–3.74 (2-proton multiplet, H-1 and H-1'), 3.73 (1-proton multiplet, H-6'), 3.91 (1-proton doublet of doublets, H-6, $J_{5,6}$ 2 Hz, $J_{6,6'}$ 13 Hz), 4.14 (1-proton multiplet, H-5), 4.46 (1-proton doublet, H-3, $J_{3,4}$ 2.7 Hz), 4.63 (1-proton doublet of doublets, H-4, $J_{3,4}$ 2.7 Hz, $J_{4,5}$ 8.0 Hz), 5.66 (1-proton singlet, 4,5-benzylidene H), 5.80 (1-proton singlet, 2,3-benzylidene H), and 7.21–7.53 (10-proton multiplet, phenyl protons); (60 MHz, *p*-dioxane): δ 5.67, 5.85 (1-proton singlets, benzylidene protons on 1,3-dioxolane rings); mass-spectral data (intensity expressed as % of base peak): *m/e* 356 (15, $M^+\cdot$), 355 (15, $M^+\cdot - H\cdot$), 325 (25, $M^+\cdot - \cdot CH_2OH$), 105, (100, $PhCO^+$), 77 (60, Ph^+), and 31 (15, $^+CH_2OH$).

Anal. Calc. for $C_{20}H_{20}O_6$: C; 67.41; H, 5.62. Found: C, 67.22; H, 5.71.

1-O-Acetyl-2,3:4,5-di-O-benzylidene-β-D-fructopyranose (**2**). — Compound **1** was acetylated with pyridine–acetic anhydride under the usual conditions. Recrystallization from absolute ethanol gave **2**, m.p. 145° (lit.[4] m.p. 145–146°); $[\alpha]_D^{20}$–42.2° (in chloroform); ν_{max}^{KBr} 2960 (medium, CH), 1750 (sharp, C=O), 1460 (medium), 1410 (medium), 1080 (broad, R–O–R), 760 (sharp, Ph), and 710 cm^{-1} (sharp, Ph); n.m.r. data (100 MHz, $CDCl_3$): 1.99 δ (3-proton singlet, OAc), 3.92 (1-proton, A portion of ABX, H-6', $J_{5,6'}$, 0.6 Hz, $J_{6,6'}$ 13.3 Hz), 4.14 (1-proton, B portion of ABX, H-6, $J_{5,6}$ 2.0 Hz, $J_{6,6'}$ 13.3 Hz), 4.19 (1-proton, A of AB, H-1', $J_{1,1'}$ 11.8 Hz), 4.31 (1-proton multiplet, H-5), 4.48 (1-proton doublet, H-3, $J_{3,4}$ 2.4 Hz), 4.60 (1-proton, B of AB, H-1, $J_{1,1'}$ 11.8 Hz), 4.74 (1-proton doublet of doublets, H-4, $J_{3,4}$ 2.4 Hz, $J_{4,5}$ 7.9 Hz), 5.78 (1-proton singlet, benzylidene), 5.90 (1-proton singlet, benzylidene), 7.33–7.66 (10-proton multiplet, phenyl protons); mass spectral data (intensity expressed as % of base peak): *m/e* 398 (10, $M^+\cdot$), 397 (20, $M^+\cdot - H\cdot$), 325 (10, $M^+\cdot - \cdot CH_2OAc$), 105 (100, $PhCO^+$), 77 (35, Ph^+), 73 (20, $^+CH_2OAc$), and 43 (55, CH_3CO^+).

Anal. Calc. for $C_{22}H_{22}O_7$: C, 66.33; H, 5.53. Found: C, 66.49; H, 5.41.

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