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Note

β-D-Fructofuranosyl α-D-*arabino*-hexopyranosid-2-ulose (2-ketosucrose)*

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During the isolation of β-D-fructofuranosyl α-D-*ribo*-hexopyranosid-3-ulose (**1**; 3-ketosucrose) from the cultures of bacterial oxidation of sucrose by *Agrobacterium tumefaciens*[1,2], a clarified concentrate was observed to contain three major components, each containing a ketose residue, when examined by paper chromatography with the urea–phosphate reagent[3]. Chromatography of this mixture on a column of cellulose[4] afforded D-fructose, followed by 3-ketosucrose (**1**)[1,2], and finally an appreciable quantity of a product subsequently identified as 2-ketosucrose (**2**). Thus, reduction of **2** with aqueous sodium borodeuteride gave [2-^{2}H]sucrose (**3**) as the sole product, characterised as the octa-acetate (**4**) from the ^{1}H- and ^{13}C-n.m.r. (Table I) spectra. The absence of H-2 coupling in the proton spectrum of **4** was revealed by the appearance of H-1 as a singlet at τ 4.16 and H-3 as a doublet at τ 4.22 with $J_{3,4}$ 9.5 Hz. The stereoselective reduction of **2**, presumably *via* axial attack by the deuteride ion to give an equatorial 2-hydroxyl group, was also observed in the reduction of isopropyl 3,4,6-tri-*O*-acetyl-α-D-*arabino*-hexopyranosid-2-ulose[5a] and its related 4,6-*O*-benzylidene derivative[5b].

In the ^{13}C-n.m.r. spectrum of **2**, the resonance due to the 2-carbonyl group was at lowest field (183.2 p.p.m. downfield from Me_4Si). The electron-withdrawing effect of the carbonyl group in **2** on the β carbon atoms was reflected in the signals for C-1 and C-3, which had shifted 1.4 and 1.7 p.p.m., respectively, downfield when compared with the same carbon atoms in sucrose (**5**). The signals for C-3′, C-4′, and C-5′ of **2** also showed downfield shifts of 0.4–0.6 p.p.m. when compared with sucrose (**5**), whereas that for C-2′ was shifted 1.2 p.p.m. upfield. The assignment of the signals in the ^{13}C-n.m.r. spectrum of sucrose is of importance in chemical studies. Assignments can be made by replacement of a selected proton by a deuteron[2], the signal of the attached ^{13}C nucleus either disappearing[7] or being converted into a triplet[8]

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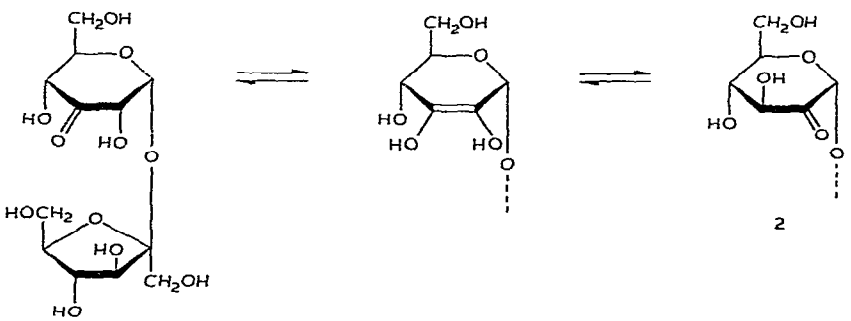
TABLE I

^{13}C-CHEMICAL SHIFTS IN P.P.M. DOWNFIELD FROM Me_4Si[a]

Atom	2 *(D_2O)*	3 *(D_2O)*	4 *($CDCl_3$)*	5 *(D_2O)*	6 *($CDCl_3$)*
C-2′	103.26	104.56	104.16	104.46	104.16
C-1	94.36	93.06	90.04	92.96	90.11
C-5′	82.52	82.29	79.20	82.12	79.27
C-3′	77.91	77.48	75.86	77.30	75.86
C-4′	75.37	75.00	74.99	74.83	75.19
C-2	183.19	—	—	71.82	70.37
C-3	75.03	73.47	69.70	73.36	69.77
C-5	68.94	73.36	68.63	73.16	68.63
C-4	68.27	70.19	68.37	70.01	68.37
C-6′	65.66	63.30	63.75	63.12	63.68
C-1′	63.66	62.36	63.01	62.18	62.95
C-6	61.31	61.09	61.88	60.98	61.88

[a]D.S.S. was used as internal standard in D_2O solution, and the above values were obtained by subtracting 1.7 from the print-out values.



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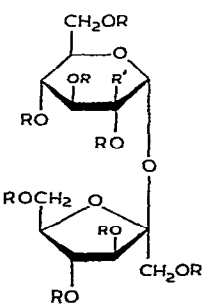

3 R = H, R′ = D
4 R = Ac, R′ = D
5 R = R′ = H
6 R = Ac, R′ = H

at 0.1–0.5 p.p.m. higher field[9–12]. The spectra of sucrose octa-acetate (**6**) and its [2-^{2}H] derivative (**4**) were identical, apart from the absence of the C-2 signal at 70.4 p.p.m. in the latter, thereby confirming the assignment[2] of this signal in **6**. Similarly, the signal for C-2 in sucrose is now unequivocally assigned at 71.8 p.p.m. downfield from Me_4Si in D_2O, in accord with the results of Pfeffer *et al.*[13].

In the light of the known specificity[1] of the microbiological oxidation of sucrose to 3-ketosucrose (**1**), it appears likely that 2-ketosucrose (**2**) arises *via* an enolisation process[14]. Defaye *et al.*[15] noted the conversion of methyl α-D-*arabino*-hexopyranosid-2-ulose and its *ribo* isomer in the presence of sodium hydroxide into the 3-ulose *via* the 2,3-enediol anion. Furthermore, they postulated the formation of a hexa-coordinated magnesium complex from the reaction of two of the enolate anions and a magnesium cation. Significantly, Mg^{2+} was present in our growth medium[2], which would lead to the formation of a 2,3-enolate complex and thence, by its dissociation, give both **1** and **2**. The generation of a 2,3-enediol from a hexapyranosid-3-ulose was also demonstrated[5b] by the isolation of the enediol diacetate after acetylation with acetic anhydride–pyridine.

EXPERIMENTAL

General. — A clarified concentrate of the culture medium containing 3-ketosucrose (**1**) was supplied by M.R.E. (Porton Down, Salisbury, Great Britain). Descending paper chromatography was used as before[2]; t.l.c. of sugars was performed on 0.2-mm plates of cellulose (Koch–Light), using urea phosphate[3] for the detection of ketoses. Acetate derivatives were examined by t.l.c. on silica gel (60 F_{254}, Merck) with 10% ethanolic sulphuric acid for detection. ^{13}C-N.m.r. spectra were recorded on a Bruker WP-60/DS spectrometer, as previously described[6].

β-D-Fructofuranosyl α-D-arabino-hexopyranosid-2-ulose (**2**). — The clarified culture medium (1 L) was concentrated to one-tenth volume by rotary evaporation at 30°. Slow addition of 95% ethanol (2 vol.) with stirring then gave a precipitate which was removed by centrifugation. The supernatant solution was concentrated to one-fifth volume and absolute ethanol (5 vol.) was added. After standing overnight at 4°, the precipitate was removed by centrifugation and the supernatant solution was concentrated, to give a light-brown syrup (4.5 g). T.l.c. and paper chromatography revealed three components containing ketose. This crude product was eluted from a column (50 × 4.5 cm) of dry cellulose powder (Whatman)[4] with butanone–acetone–water (3:1:0.6) at a flow rate of 10 ml/min. Fractions (10 ml) were collected, and monitored by paper chromatography[2] and t.l.c. D-Fructose (1.6 g) was eluted first, followed by 3-ketosucrose (**1**, 0.9 g), which had $[\alpha]_D$ +124° (*c* 0.2, water), and finally **2** was obtained as a chromatographically pure syrup (0.6 g), $[\alpha]_D$ +19.6° (*c* 0.25, water), R_{FRU} 0.4.

[2-^{2}*H*]*Sucrose octa-acetate* (**4**). — A solution of **2** (0.17 g) in water (6 ml) at 0° was treated with sodium borodeuteride (15 mg) for 15 min. The mixture was stirred overnight at 0–5°, and excess of borodeuteride was decomposed with acetic

acid. The solution was concentrated to a syrup, and the residue was treated with acetic anhydride and pyridine, to give the octa-acetate **4** (0.31 g, 84%), m.p. 67°, $[\alpha]_D$ +58.2° (*c* 0.57, chloroform) (Found: C, 49.5; H, 5.6. $C_{28}H_{37}DO_{19}$ calc.: C, 49.4; H, 5.7%). ^{1}H-N.m.r. data (C_6D_6: 220 MHz): τ 4.16 (s, 1 H, H-1), 4.22 (d, 1 H, $J_{3,4}$ 9.5 Hz, H-3), 4.71 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 4.33 (d, 1 H, $J_{3',4'}$ 5.5 Hz, H-3'), 4.5 (t, 1 H, $J_{4',5'}$ 5.5 Hz, H-4'), and 8.1–8.4 (OAc).

De-esterification of **4** (0.29 g) with 0.1M sodium methoxide gave [2-^{2}H]sucrose (**3**; 0.13 g, 95%), $[\alpha]_D$ +62.4° (*c* 0.2, water) (Found: C, 42.5; H, 6.5. $C_{12}H_{21}DO_{11}$ calc.: C, 42.0; H, 6.2%).

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