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Structure and solution equilibria of D-glucose and D-mannose sulfite adducts

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Abstract

An X-ray crystallographic study has confirmed that the potassium bisulfite adducts of D-glucose and D-mannose have open-chain structures with *R* and *S* configurations respectively at C-1. NMR studies have shown that each sugar gives rise to two bisulfite compounds, and solution-state structures and conformations of these isomers have been deduced from analysis of ^{1}H NMR spectra. ^{13}C NMR data for the four adducts are given. Furanose forms of the D-glucose and D-mannose have been detected in the equilibrium solutions. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Aldose bisulfite compounds; X-Ray structure; NMR spectroscopy; Conformational analysis; Furanose forms of aldoses

1. Introduction

The reactions of reducing sugars with sulfites have been studied intermittently over many years for a variety of reasons.[1–3] Conditions employed may be divided into those at low (< 40 °C) and high (100–130 °C) temperatures. The low-temperature reactions, concerned with the 'free' (directly titratable with iodine) and the 'combined' (titratable after prior treatment with alkali) sulfite equilibrium and its relevance to preservative action,[4] have generally used lower proportions of sulfite in contrast to the high-temperature reactions, concerned with inhibition of non-enzymic browning, where sulfite–sugar molar ratios up to 3:1 have been used.[5] However the distinction is not rigid, since the question arises about the extent to which compounds formed at low temperature are precursors in high-temperature reactions.

The addition of sulfite to a solution of a reducing sugar introduces more equilibria into discussions of the variety of the forms of the sugar present in solution (see Scheme 1). It has been generally accepted on the basis of presumptive evidence that the addition compound, illustrated with D-glucose, has an open-chain structure.[3] The absence from the infrared spectrum of a band attributable to a C–O–C bond expected from a cyclic structure has been noted.[3] Also the progressive lowering of the optical rotation of solutions of glucose and other sugars with additions of sulfite has been accounted for by the formation of open-chain compounds.[3] Assignment of the configuration at C-1 of a series of bisulfite addition compounds was made on the basis of the change of optical rotation of solutions in which alkali-catalysed hydrolysis was proceed-

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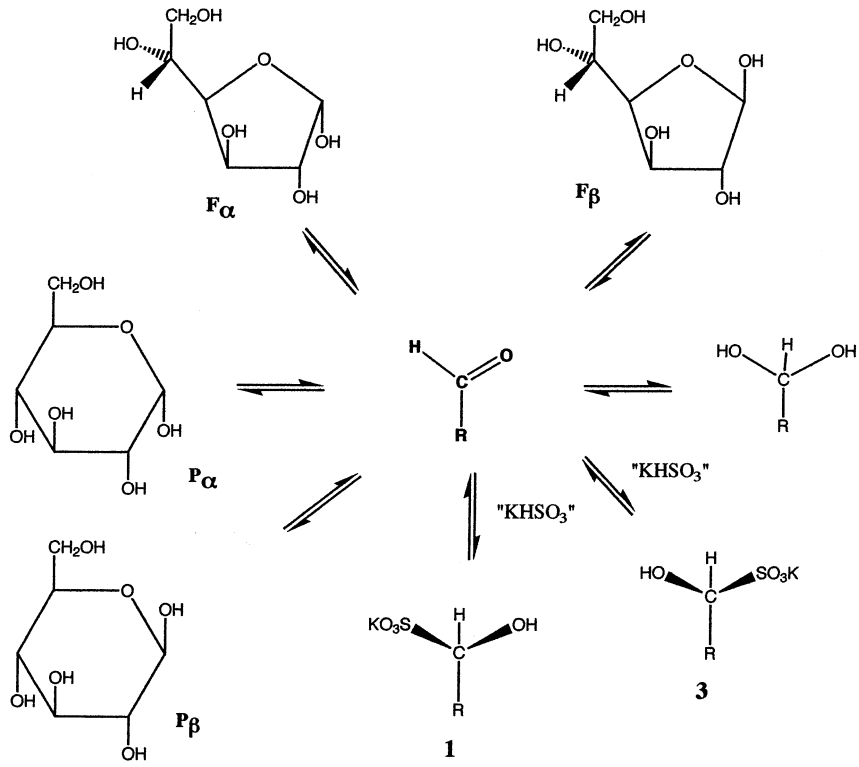

Scheme 1. Equilibria involved for a solution of D-glucose and potassium bisulfite. F_α is α-D-glucofuranose; P_α is α-D-glucopyranose; R, D-arabinit-1-yl.

ing, assuming that the hydrolysis causes inversion of the configuration at C-1.[6] In this paper are presented the structures of the crystalline potassium bisulfite addition compounds from D-glucose and D-mannose as determined by X-ray diffraction, together with a nuclear magnetic resonance study of the formation of the two isomers from each sugar and an analysis of the ^{1}H NMR spectra leading to the solution-state structures and conformations of these products.

2. Results and discussion

Solid-state structures.—X-Ray structure determinations were carried out on the crystalline potassium bisulfite compounds from D-glucose and D-mannose, compounds **1** and **2**, respectively. The molecular structures of **1** and **2** with atomic numbering are shown in Figs. 1 and 2, respectively. As previously reported,[7] **1** crystallises with one molecule of water per molecule of bisulfite compound whereas **2** crystallises with two independent molecules per asymmetric unit. Atomic

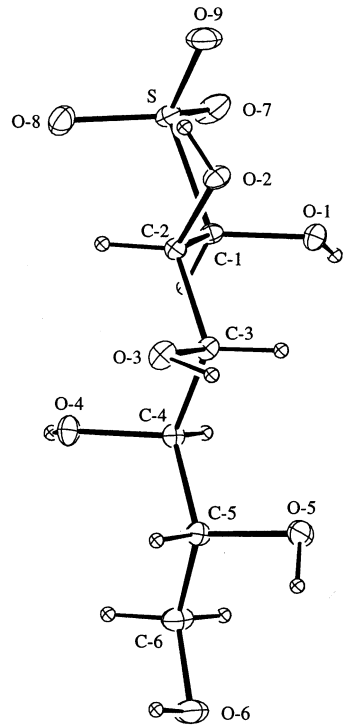

Fig. 1. ORTEP plot of potassium (1*R*)-D-glucit-1-ylsulfonate, showing atomic notation and thermal ellipsoids.

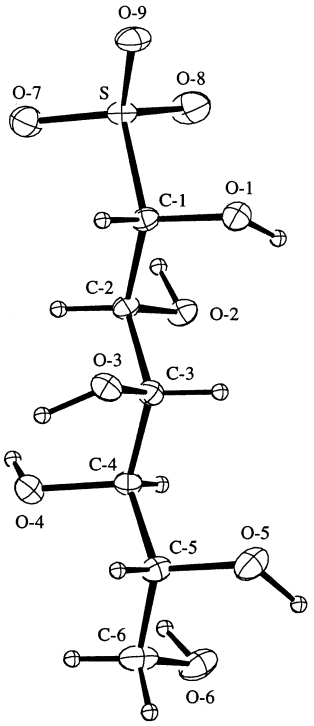

Fig. 2. ORTEP plot of potassium (1*S*)-D-mannit-1-ylsulfonate, showing atomic notation and thermal ellipsoids.

Table 1
Non-hydrogen atomic parameters for **1**

	x	*y*	*z*	B_{eq}
K	0.6315(1)	0.7344(1)	0.8090(1)	2.64(1)
S	0.8188(1)	0.9961(1)	0.6761(1)	2.09(2)
O-1	0.8797(3)	0.7361(2)	0.6972(1)	2.09(4)
O-2	0.3809(4)	0.7958(2)	0.6964(1)	2.17(4)
O-3	0.2328(4)	0.6279(2)	0.6017(1)	2.41(5)
O-4	0.5576(4)	0.7040(2)	0.5063(1)	2.50(5)
O-5	0.5908(5)	0.3748(2)	0.5805(1)	3.11(6)
O-6	0.6685(5)	0.3066(2)	0.4467(1)	3.57(6)
O-7	1.0757(4)	1.0120(2)	0.6890(1)	3.57(6)
O-8	0.7357(5)	1.0791(2)	0.6234(1)	3.20(5)
O-9	0.6746(5)	1.0146(2)	0.7333(1)	3.70(6)
C-1	0.7837(5)	0.8217(3)	0.6504(1)	1.70(5)
C-2	0.5185(5)	0.7868(3)	0.6388(1)	1.69(5)
C-3	0.4840(5)	0.6416(3)	0.6152(1)	1.69(5)
C-4	0.6260(5)	0.6099(3)	0.5546(1)	1.76(5)
C-5	0.5681(5)	0.4700(3)	0.5292(1)	2.05(6)
C-6	0.7325(7)	0.4314(3)	0.4751(2)	2.95(7)
OW	0.9979(5)	0.4110(3)	0.6617(1)	3.90(6)

E.s.d. in parentheses. B_{eq} (Å^2) is the isotropic equivalent of the anisotropic temperature factor.

parameters and selected torsional angles are given in Tables 1–4. Both compounds are open chain, confirming earlier conclusions.[3,7,8] For **1**, the configuration at C-1 is *R* and for **2**, it is *S*. The compounds may therefore be named potassium (1*R*)-D-glucityl-1-sulfonate (**1**) and potassium (1*S*)-D-mannityl-1-sulfonate (**2**).

Table 2
Selected torsional angles (°) for **1**

O-9–S–C-1–C-2	57.2(2)	O-1–C-1–C-2–O-2	57.4(2)
S–C-1–C-2–C-3	178.3(2)	O-2–C-2–C-3–O-3	63.1(2)
C-1–C-2–C-3–C-4	−56.4(3)	O-3–C-3–C-4–O-4	60.9(3)
C-2–C-3–C-4–C-5	−174.4(2)	O-4–C-4–C-5–O-5	−169.1(2)
C-3–C-4–C-5–C-6	−172.0(3)	O-5–C-5–C-6–O-6	64.5(3)
O-9–S–C-1–O-1	−64.3(2)		

E.s.d. in parentheses.

Table 3
Non-hydrogen atomic parameters for **2**

	x	*y*	*z*	B_{eq}
K-1	0.2501(1)	0.0000	0.4919(1)	2.65(2)
K-20	0.2353(1)	0.5426(2)	0.4838(1)	3.45(2)
SA	0.5140(1)	0.2515(1)	0.6490(1)	2.03(2)
O-1A	0.6748(2)	0.1454(3)	0.8242(2)	2.32(6)
O-2A	0.3990(2)	0.1240(3)	0.8706(2)	2.16(6)
O-3A	0.6372(2)	0.3645(3)	1.0304(2)	2.27(6)
O-4A	0.3746(2)	0.3871(3)	1.0876(2)	2.27(6)
O-5A	0.5983(3)	0.1272(4)	1.2434(3)	3.22(8)
O-6A	0.3415(3)	0.1126(4)	1.3008(3)	3.34(8)
O-7A	0.4186(3)	0.3682(4)	0.6335(3)	3.20(8)
O-8A	0.4553(3)	0.1115(4)	0.6268(3)	3.63(8)
O-9A	0.6258(3)	0.2741(4)	0.5869(2)	2.92(7)
C-1A	0.5820(3)	0.2559(5)	0.8023(3)	1.87(7)
C-2A	0.4766(3)	0.2515(5)	0.8848(3)	1.82(7)
C-3A	0.5446(3)	0.2503(5)	1.0123(3)	1.89(8)
C-4A	0.4460(3)	0.2548(5)	1.0996(3)	1.91(8)
C-5A	0.5130(3)	0.2479(5)	1.2269(3)	2.26(8)
C-6A	0.4138(4)	0.2435(6)	1.3119(3)	3.23(11)
SB	−0.0230(1)	0.2395(2)	0.3520(1)	2.27(2)
O-1B	−0.1644(2)	0.1211(3)	0.1677(2)	2.40(6)
O-2B	0.1186(2)	0.1363(3)	0.1398(2)	2.39(6)
O-3B	−0.1162(2)	0.3790(3)	−0.0334(2)	2.41(6)
O-4B	0.1585(2)	0.3647(4)	−0.0670(2)	2.55(7)
O-5B	−0.0874(3)	0.1503(4)	−0.2565(3)	3.45(8)
O-6B	0.1616(3)	0.1112(4)	−0.3042(3)	3.41(8)
O-7B	0.0843(5)	0.3384(6)	0.3708(3)	7.10(15)
O-8B	0.0149(4)	0.0958(4)	0.3848(3)	4.54(10)
O-9B	−0.1379(4)	0.2812(5)	0.4051(3)	5.45(12)
C-1B	−0.0843(3)	0.2421(4)	0.1956(3)	1.95(8)
C-2B	0.0252(3)	0.2488(5)	0.1170(3)	1.86(8)
C-3B	−0.0381(3)	0.2518(5)	−0.0122(3)	1.88(8)
C-4B	0.0655(3)	0.2521(5)	−0.0952(3)	1.94(8)
C-5B	0.0029(3)	0.2640(5)	−0.2247(3)	2.31(8)
C-6B	0.1052(4)	0.2518(6)	−0.3083(3)	2.70(9)

E.s.d. in parentheses. B_{eq} (Å^2) is the isotropic equivalent of the anisotropic temperature factor.

Table 4
Selected torsional angles (°) for **2**

O-9A–SA–C-1A–C-2A	173.3(3)	O-9B–SB–C-1B–C-2B	161.7(3)
SA–C-1A–C-2A–C-3A	178.0(3)	SB–C-1B–C-2B–C-3B	−179.5(3)
C-1A–C-2A–C-3A–C-4A	176.7(3)	C-1B–C-2B–C-3B–C-4B	−177.6(3)
C-2A–C-3A–C-4A–C-5A	177.8(3)	C-2B–C-3B–C-4B–C-5B	−176.7(3)
C-3A–C-4A–C-5A–C-6A	−176.6(4)	C-3B–C-4B–C-5B–C-6B	−175.2(4)
O-9A–SA–C-1A–O-1A	−60.7(3)	O-9B–SB–C-1B–O-1B	−74.7(3)
O-1A–C-1A–C-2A–O-2A	−63.1(4)	O-1B–C-1B–C-2B–O-2B	−67.6(4)
O-2A–C-2A–C-3A–O-3A	172.7(3)	O-2B–C-2B–C-3B–O-3B	−171.6(3)
O-3A–C-3A–C-4A–O-4A	60.1(4)	O-3B–C-3B–C-4B–O-4B	67.2(3)
O-4A–C-4A–C-5A–O-5A	−173.5(3)	O-4B–C-4B–C-5B–O-5B	178.1(3)
O-5A–C-5A–C-6A–O-6A	−56.1(4)	O-5B–C-5B–C-6B–O-6B	−54.5(4)

E.s.d. in parentheses.

The manno isomer adopts the planar zigzag conformation with the sulfonate group extending the zigzag, O-2 being gauche to O-1 and S, and the C-6 hydroxyl group is gauche to O-5 and C-4. This structure is similar to that of 2-deoxy-2-*S*-ethyl-D-mannose diethyl dithioacetal in which the sulfur atom on C-2 is gauche to the two S atoms on C-1 and with the C-6 hydroxyl group gauche to the C-5 oxygen and to C-4.[9] This conformation around C-5 and C-6 is also shown by D-mannitol.[10]

For the gluco isomer, twisting of the planar zigzag conformation about C-2–C-3 gives a sickle conformation in the region C-1 to C-4. The twisting about C-2–C-3 relieves the O-1, O-3 and O-2, O-4 interactions that would exist in the planar zigzag conformation for this isomer. The C-6 hydroxyl group is gauche to H-5 and O-5, an arrangement found in D-glucitol.[11]

Solution-state studies.—^{1}H NMR spectra of D_2O solutions of D-glucose and of D-mannose plus potassium hydrogen sulfite revealed the formation of two products from each sugar. In each case, the identity of one product as the crystalline bisulfite compound was determined by recording the NMR spectra of solutions of the crystalline compounds in a mixture of D_2O and acetic acid. In the case of D-glucose, the equilibrium ratio of the second isomer, **3** to **1** is 1:1.9 and for D-mannose, the ratio of the second isomer, **4** to **2** is 1:10.8.

We have been able to show that the solution-state structures of **1** to **4** are all open chain, by detailed analysis of the NMR spectra (see Table 5). For each compound, it was necessary to eliminate the possibility of a five- or six-membered ring form. For **1**, the magnitude of $J_{3,4}$ eliminated the possibility of a pyranose form and the large magnitude of $J_{2,3}$ eliminated both α- and β-furanose structures, $J_{2,3}$ being 3.6 Hz for methyl α-D-glucofuranoside and 0.6 Hz for methyl β-D-glucofuranoside.[12] For **3**, $J_{2,3}$ and $J_{3,4}$ are inconsistent with a pyranose structure, and a furanose structure is not consistent with the value of $J_{1,2}$, which is considerably larger than the value of $J_{1,2}$ for ethyl 1-thio-α-D-glucofuranoside, 4.00 Hz. For both **2** and **4**, the large values of $J_{2,3}$ are inconsistent with either a pyranose or furanose structure. The four isomers **1** to **4** therefore have open-chain structures in solution.

A complete analysis of the ^{1}H NMR spectra of **1** and **2** was made possible by utilising the known stability of **1** and **2** when dissolved in a mixture of water and acetic acid, a procedure that was used in optical rotation determinations.[7] For isomers **3** and **4**, which have not been isolated from the reaction mixtures, superposition of signals due to the two pyranose forms of the reducing sugar precluded assignments except for H-1 to H-3. We have simplified the assignment problem in the case of **3** by using D-glucose-6,6′-d_2 (**5**). The spectrum of **5** is significantly simpler than that of D-glucose, and the signals of H-4 and H-5 of **3a** (the 6,6′-d_2 analog of **3**) could be readily assigned.

Solution conformations.—The NMR parameters of **1** can be rationalised in terms of a major contribution from that conformation found in the crystalline compound. The low-

field shifts of H-2 and H-3 are accounted for by the known[13] deshielding effect of an opposing oxygen atom: H-2 is opposed by one of the sulfite oxygens, as well as by O-4, and H-3 is opposed by O-1 and O-5. The spin coupling constants are largely accounted for by this conformation, although the magnitude of $J_{2,3}$ indicates a contribution from a conformation in which H-2 is gauche to H-3, probably the sickle conformation with torsional angle C-1–C-2–C-3–C-4 $+60°$. In the zigzag conformation of this isomer, O-2 and O-4 are opposed by either O-1 or S, making this conformation unfavourable. For the minor gluco isomer, **3**, spin coupling constants are accounted for by the zigzag conformation in which H-1 and H-2 are antiperiplanar, although O-2 is opposed by both O-4 and an oxygen on S. An anticlockwise 120° rotation around C-2, C-3 relieves the O-2, O-4 interaction, but introduces another, between O-1 and O-3.

For the major manno isomer, **2**, spin coupling constants are accounted for by the same zigzag conformation that occurs in the crystals. Atom H-1 is antiperiplanar to O-2 and H-2 is antiperiplanar to O-1, resulting in small magnitude $J_{1,2}$, as is found for β-mannopyranose[14] where the same geometry holds. The large value of $J_{2,3}$ may be accounted for by the antiperiplanar orientation of O-1 and H-2 and of O-4 and H-3. A similar geometry occurs in α-D-galactopyranose, resulting in a large value for $J_{2,3}$.[15] A similar zigzag conformation for the minor manno isomer, **4**, accounts for $J_{2,3}$ and $J_{3,4}$ but is less satisfactory for $J_{1,2}$. Also, O-1 and O-3 are now opposed. Rotation around C-1–C-2 such that H-1 is antiperiplanar to O-2 relieves this opposition, but places H-1 gauche to H-2. A mixture of these two conformers would account for the magnitude of $J_{1,2}$.

Minor components.—The presence of the furanose forms of D-glucose in solution has not been established by ^{1}H NMR, however we observe a 3.9 Hz doublet at 5.51 ppm in the D-glucose–potassium bisulfite reaction mixture. If this doublet is due to H-1 of α-D-glucofuranose, taking the spectrum of 5-O-methyl-D-glucose as a guide, we should expect to see also a singlet of equal intensity for H-1 of β-D-glucofuranose at ~ 0.26 ppm to higher field.[16] As this corresponds to the chemical shift of H-1 of α-D-glucopyranose, the resonance of H-1 of β-D-glucofuranose is hidden. We have verified that the 5.51 ppm doublet is in fact due to α-D-glucofuranose by recording the ^{13}C NMR spectrum of the D-glucose–potassium bisulfite reaction mixture. Two low intensity signals, at 103.16 and 97.56 ppm were easily detected. These correspond to two resonances observed in an ultra-high resolution ^{13}C spectrum of D-glucose which were assigned to C-1 of β-D-glucofuranose and α-D-

Table 5
^{1}H NMR chemical shifts (δ, ppm) and coupling constants (J, Hz)

Compound	Solvent	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
1	$D_2O+DOAc$	4.559	4.240	4.047	3.703	3.810	3.855	3.679
1a	D_2O	4.558	4.227	4.026	3.688	3.795		
3a	D_2O	4.496	4.036	4.170	3.820	3.782		
2	$D_2O+DOAc$	4.746	4.221	3.892	3.859	3.788	3.894	3.703
2	D_2O	4.743	4.205					
4	D_2O	4.661	4.075	4.201				
		$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
1	$D_2O+DOAc$	2.67	6.43	2.16	8.17	2.90	6.22	11.68
1a	D_2O	2.58	6.53	2.16	8.31			
3a	D_2O	7.73	2.37	3.44	7.44			
2	$D_2O+DOAc$	0.93	9.57	1.05	9.56	2.85	6.20	11.77
2	D_2O	0.90	9.60					
4	D_2O	5.14	7.90	1.34				

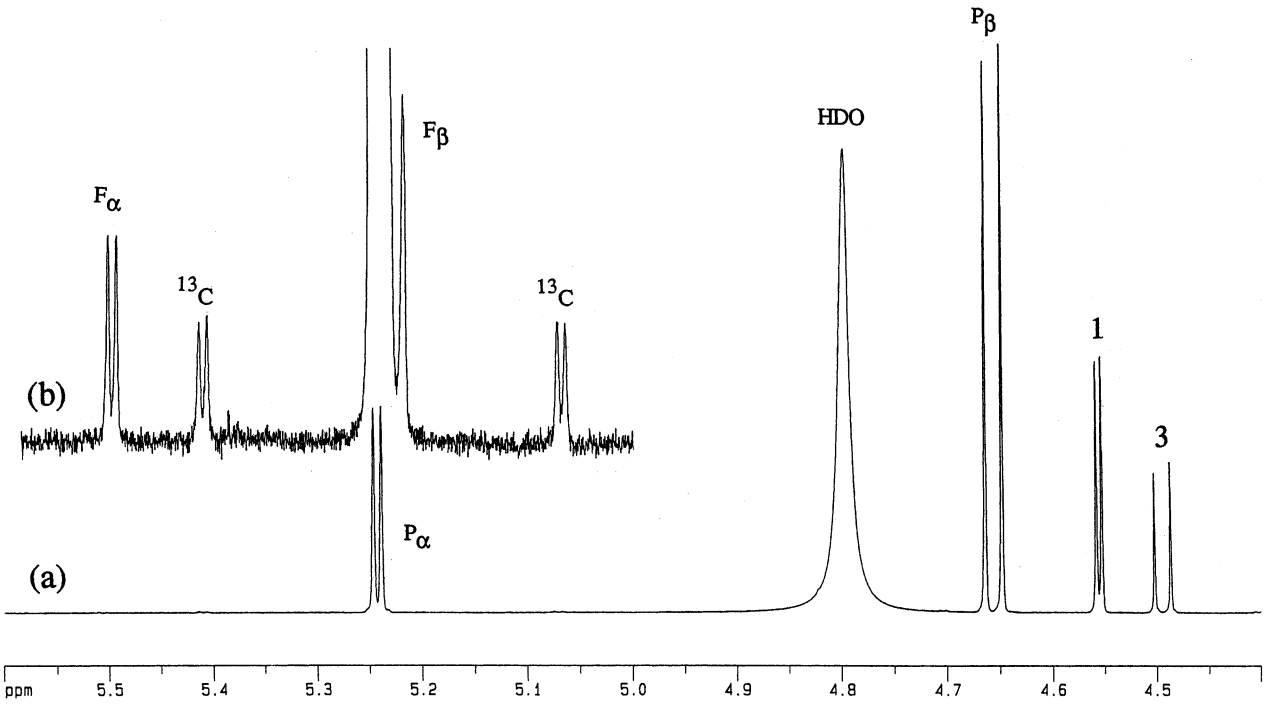

Fig. 3. (a) Anomeric signals for a ^{1}H NMR spectrum of a D-glucose/potassium bisulfite equilibrium solution at 20 °C. (b) Higher amplitude spectrum for the region 5.0–5.6 ppm at 50 °C. See Scheme 1 for notations.

Table 6
^{13}C NMR chemical shifts (δ, ppm)

Compound	C-1	C-2	C-3	C-4	C-5	C-6
1	83.00	71.41	71.00	71.34	71.85	63.63
3	84.15	73.25	69.18	73.93	72.08	63.34
α-D-Glucopyranose	92.88	72.29	73.56	70.48	72.32	61.45
β-D-Glucopyranose	96.68	74.95	76.53	70.44	76.68	61.57
2	82.54	69.40	69.15	69.69	71.71	64.11
4	84.59	72.59	70.09	70.42	71.67	63.94
α-D-Mannopyranose	94.85	71.50	71.05	67.67	73.21	61.76
β-D-Mannopyranose	94.48	72.04	73.83	67.42	76.91	61.76

glucofuranose, respectively.[17] A $^{13}C/^{1}H$ correlation experiment verified these assignments. Increasing the glucose–bisulfite sample temperature to 50 °C resulted in the appearance of the signal due to H-1 of β-D-glucofuranose at lower frequency than H-1 of α-D-glucopyranose, confirming the presence of both furanose forms (see Fig. 3). Similarly, a D-mannose–bisulfite mixture showed the presence of two doublets at 5.276 ppm ($J_{1,2}$, 5.16 Hz) and 5.256 ppm ($J_{1,2}$, 5.04 Hz) ppm, relative intensities $\sim$3:1 which we assign to the α- and β-furanose forms of D-mannose. Approximate relaxation times (T_1) for the α- and β-furanose isomers are 7 and 3.5 sec, consistent with 1,2-trans and 1,2-cis configurations, respectively.[18] In contrast to the common order of furanose chemical shifts for which H-1 in 1,2-cis furanose structures is at lower field than H-1 in 1,2-trans furanose structures,[12,19] the higher field shift of H-1 in β-D-mannofuranose compared to H-1 in α-D-mannofuranose is consistent with the relative chemical shifts for H-1 in the corresponding methyl glycosides.[12]

^{13}C chemical shifts of **1**–**4** and of the pyranose forms of D-glucose and D-mannose are listed in Table 6. Values for **1** have been recorded elsewhere.[20]

Preferential formation of **2** from a mixture of D-glucose and D-mannose was demonstrated by ^{1}H NMR analysis of a D_2O solution of a mixture of D-glucose, D-mannose, and potassium metabisulfite in molar ratio 1:1:0.5. The ratio of bisulfite adducts of D-glucose and D-mannose was 1.0:4.1. This preference for the formation of D-mannose adducts results from the smaller number of unfavourable non-bonded interactions in **2** and **4** compared to **1** and **3**. It has been shown elsewhere[21] that the sodium analogue of **2** may be utilised for the isolation of D-mannose from spent liquors generated by conifer woodpulping processes. We have verified that as a result of the excellent crystallising properties of **2** as well as the favoured formation of **2** compared to **1**, D-mannose may be isolated (as its bisulfite adduct) from a 2:1 mixture of D-glucose and D-mannose. This isolation procedure has allowed the preparation of **2** from the mixture of D-glucose and D-mannose formed by heating an aqueous solution of D-glucose and molybdenum trioxide.[22]

Earlier studies on the equilibria in sugar–bisulfite solutions involved determination of free and combined bisulfite. In this work, we have made use of ^{1}H NMR spectroscopy to monitor the formation of bisulfite adducts by determining the concentrations of α- and β-aldopyranoses as well as the bisulfite adducts, utilising integrated intensities of the anomeric hydrogens as a function of time. Representing the equilibria as in Eq. (1) (Section 3), if [S] is the (total) sugar concentration, [B] is the bisulfite concentration, and [P] is the sugar bisulfite concentration, the equilibrium constant, K, is given by $[P]/[S]^2$, if the initial molar concentrations of aldose and bisulfite are equal. Table 7 presents K, k_1 and k_2 values for **1** to **4** as well as the values for the combined bisulfite adducts.

Table 7
Rate and equilibrium constants

Product	$k_1 \times 10^5$ (mol s^{-1})	$k_2 \times 10^5$ (mol^2 s^{-1})	K (mol^{-1})
1	6.49	5.59	1.16
3	7.20	12.03	0.60
2	14.95	2.03	7.34
4	9.31	10.71	0.87
1+3	13.05	7.42	1.76
2+4	24.96	3.04	8.21

3. Experimental

Materials.—Compounds **1** and **2** were prepared using literature procedures.[7] The following procedure was found to be more satisfactory for the preparation of **1**: A mixture containing D-glucose (9.00 g), $K_2S_2O_5$ (5.60 g), and water (10 mL) was warmed to give a homogeneous solution. The cooled solution was seeded with crystals of **1** and left for 14 h at 22 °C. The crystalline product was collected, washed with 1:1 MeOH–water, followed by 4:1 MeOH–water and finally with MeOH to give **1** (8.80 g) after air drying. A further 2.26 g of **1** was obtained from the original filtrate plus the first washings.

Crystals for X-ray diffraction were obtained from EtOH and water mixtures.

Crystallography.—Crystal data for **1**.—$C_6H_{13}KO_9S{\cdot}H_2O$, M 318.3, orthorhombic, space group $P2_12_12_1$, a 5.546(1), b 9.946(1), c 20.950(3) Å, V 1155.6(3) Å^3, D_{calcd} 1.83 g cm^{-3}, Z 4, μ_{Cu} 62.31 cm^{-1}. Crystal size, $0.07 \times 0.09 \times 0.32$ mm; $2\theta_{max}$, 140°; min/max transmission factors, 0.36 and 0.70. The number of reflections was 1242 considered observed out of 1299 unique data. Final residuals R, R_w were 0.027, 0.039 for the observed data. The enantiomer was confirmed as correct, the alternative giving R 0.049.

Crystal data for **2**.—$C_6H_{13}KO_9S$, M 300.3, monoclinic, space group $P2_1$, a 10.219(2), b 9.314(1), c 11.551(3) Å, β 98.13(1)°, V 1088.4(4) Å^3, D_{calcd} 1.83 g cm^{-3}, Z 4, μ_{Cu} 65.08 cm^{-1}. Crystal size, $0.03 \times 0.20 \times 0.21$ mm; $2\theta_{max}$, 140°; min/max transmission factors, 0.31 and 0.80. The number of reflections was 2073 considered observed out of 2194 unique data, with R_{merge} 0.019 for 107 pairs of equivalent $hk0$ reflections. Final residuals R, R_w were 0.029, 0.049 for the observed data. The enantiomer was confirmed as correct, the alternative giving R 0.035.

Structure determination.—Reflection data were measured with an Enraf–Nonius CAD-4 diffractometer in $\theta/2\theta$ scan mode using

graphite monochromatised copper radiation (λ 1.5418 Å). Data were corrected for absorption using the analytical method of de Meulenaer and Tompa.[23] Reflexions with $I > 3\sigma(I)$ were considered observed. The structures were determined by direct phasing and Fourier methods. Hydroxyl and water hydrogens were located in difference Fouriers, the remainder were included in calculated positions and all were assigned thermal parameters equal to those of the atom to which they were bonded. Positional and anisotropic thermal parameters for the non-hydrogen atoms were refined using full-matrix least-squares. Reflection weights used were $1/\sigma^2(F_o)$, with $\sigma(F_o)$ being derived from $\sigma(I_o) = [\sigma^2(I_o) + (0.04I_o)^2]^{1/2}$. The weighted residual is defined as $R_w = (\Sigma w\Delta^2/\Sigma wF_o^2)^{1/2}$. Atomic scattering factors and anomalous dispersion parameters were from International Tables for X-ray Crystallography.[24] Structure solutions were by SIR92[25] and refinements used BLOCKLS, a local version of ORFLS.[26] ORTEP-II[27] running on a Macintosh IIcx was used for the structural diagrams, and a DEC Alpha-AXP workstation was used for calculations.

Nuclear magnetic resonance measurements.—A Bruker DMX-500 NMR spectrometer was used for ^{1}H, ^{13}C, and ^{1}H/^{13}C measurements. For quantitative measurements, spin–lattice relaxation times were estimated using the inversion-recovery process to arrive at the null-time for the slowest relaxing anomeric hydrogen and the time between pulses was set at five times the derived T_1 value.

Using 1 mL volumetric flasks, solutions were prepared by dissolving 90 mg of aldose (exchanged by freeze-drying with three 1 mL portions of D_2O) in D_2O, adding a solution of $K_2S_2O_5$ (55.5 mg) in D_2O and making the mixture up to the mark with D_2O. Spectra were recorded at 22 °C, until a steady state was reached, $\sim$25 h.

^{1}H spectra of **1** and **2** were obtained using solutions of **1** and **2** (40 mg) in mixtures of D_2O (0.55 mL), DOAc (0.05 mL) and 1,4-dioxane (0.6 μL). The chemical shift of 1,4-dioxane was taken as 3.767 ppm (determined for a solution of 1,4-dioxane in D_2O containing sodium 4,4-dimethyl-4-silatetradeuteriopentanoate (TSP) (δ 0.000 ppm)). Equilibrium bisulfite solutions of D-glucose, D-glucose-6,6-d_2 (Aldrich), and D-mannose were prepared by carrying out three D_2O exchanges on the reducing sugars (54 mg) using a small freeze-drying apparatus, adding D_2O (0.55 mL), potassium metabisulfite (40 mg) and dioxane (0.6 μL). Assignments were made using ^{1}H/^{1}H correlation spectra.

For ^{13}C spectra, shifts are reported relative to internal 1,4-dioxane taken as 67.40 ppm. The chemical shift of TSP in D_2O containing 1,4-dioxane (set at 76.40 ppm) was −2.03 ppm. Assignments were made using published values for the reducing sugars[28] and ^{1}H/^{13}C correlation spectra. For the minor D-mannose bisulfite adduct, **4**, C-5 was assigned by comparison with the shift of C-5 of **2**, leaving C-4 as the remaining carbon to be assigned.

Rate measurements: theory.—The kinetics of the reversible scheme:

$$S + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} P \tag{1}$$

where S is the sugar, B is bisulfite and P is the product may be written:

$$-\frac{d[S]}{dt} = -\frac{d[B]}{dt} = \frac{d[P]}{dt} = k_1[S][B] - k_2[P] \tag{2}$$

At equilibrium:

$$K = \frac{k_1}{k_2} = \frac{[P]_e}{[S]_e[B]_e} \tag{3}$$

For a reaction scheme in which two products are formed:

$$S + B \underset{k_3}{\overset{k_1}{\rightleftharpoons}} P_1 \tag{4}$$

$$S + B \underset{k_4}{\overset{k_2}{\rightleftharpoons}} P_2 \tag{5}$$

and the kinetic equations are now:

$$-\frac{d[S]}{dt} = -\frac{d[B]}{dt} = (k_1 + k_3)[S][B] - k_2[P_1] - k_4[P_2] \tag{6}$$

$$\frac{d[P_1]}{dt} = k_1[S][B] - k_2[P_1] \tag{7}$$

$$\frac{d[P_2]}{dt} = k_3[S][B] - k_4[P_2] \tag{8}$$

and two equilibrium constants may be defined:

$$K_1 = \frac{k_1}{k_2} = \frac{[P_1]_e}{[S]_e[B]_e} \quad (9)$$

$$K_2 = \frac{k_3}{k_4} = \frac{[P_2]_e}{[S]_e[B]_e} \quad (10)$$

Given measurements of the concentrations of sugar and products with time, it is possible to fit the kinetic models of Eqs. (2), (6)–(8) to the data. The differential equations were numerically integrated by a fourth order Runge–Kutta method implemented in Microsoft EXCEL V8 (Microsoft Corporation, USA) running on a Pentium II, 330 MHz personal computer.[29] The equilibrium constant was determined from the steady state concentrations and used to calculate k_2 and k_4 from k_1 and k_3. NMR data taken at intervals during the reaction were normalised to the total organic known to be present. The single-product model was fitted to the data with parameters $[S]_0$, $[B]_0$, and k_1, and the two-product model was fitted with parameters $[S]_0$, $[B]_0$, k_1, and k_3, where $[S]_0$ and $[B]_0$ are the initial concentrations of the sugar and bisulfite respectively. Fitting was performed with the EXCEL add-in, SOLVER, by minimising the sum of squares of the differences between calculated concentrations and measured concentrations. SOLVER uses the Generalised Reduced Gradient (GRG2) nonlinear optimisation code developed by Lasdon and Waren.

Integrated equations for the equilibrium (1) have been presented.[30]

Isolation of **2**.—A mixture containing D-glucose (2.00 g), D-mannose (1.00 g), $K_2S_2O_5$ (0.74 g) and water (2 mL) was heated in a hot water bath (80 °C) to give a clear solution. The cooled solution was seeded with **2** and left for 20 h at 22 °C. The crystalline product was collected, washed twice with 1:1 methanol–water, followed by methanol to give **2** (0.92 g, 56%), identified by ^{1}H NMR.

A mixture of D-glucose (3.00 g), MoO_3 (100 mg), and water (12 mL) in a stoppered flask was heated in a boiling water bath for 3.5 h. The deeply coloured reaction mixture was filtered through Celite, passed down a short column of Amberlite IRA 400 (HCO_3^-) ion exchange resin, treated with decolourising charcoal, filtered through Celite then concentrated under vacuum to a net weight of 4 g. Analysis by HPLC showed a 2:1 glucose–mannose ratio. After adding water (1 mL) and $K_2S_2O_5$ (0.74 g) to the syrupy products, the mixture was warmed to give a clear solution. After seeding the cooled solution with crystals of **2**, the mixture was kept at 22 °C for 14 h. The crystalline product was collected as above to give **2** (0.21 g). Second and third crops of **2** gave a total yield of 0.75 g, each crop identified by ^{1}H NMR.

4. Supplementary material

Full crystallographic details, excluding structure factors, have been deposited with the Cambridge Crystallographic Data Centre. Copies of these data may be obtained, free of charge from, The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

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