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Note

^{1}H- and ^{13}C-N.m.r. spectroscopy of α-D-glucose and α-D-mannose with boron(III) oxide as shift reagent

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N.m.r. spectroscopy has proved extremely fruitful for studying the configuration and conformation of many carbohydrates and their derivatives. The reactions of boric acid with diols in solution have been used for almost a century to examine structural differences among carbohydrates. However, studies of sugars reacting with B_2O_3 in non-aqueous solvents have not hitherto been reported.

The hydroxyl-proton resonances of sugars in dimethyl sulfoxide are usually well resolved peaks, but the 100-MHz spectra of sugars generally show very poor separation of the ring-proton signals. Most ^{1}H-n.m.r. work on the ring-proton signals have been made on sugars substituted by such groups as nitro[1], halogen[2], thio[3], *O*-acetyl[4–7], benzylidene[8], benzoyloxy[9,10], *O*-methyl[11], and *O*-isopropylidene[12,13].

Lanthanide shift-reagents[14,15] have proved useful for improving n.m.r.-spectral dispersion of carbohydrates[16–20]. Lanthanide salts can also be used to effect small induced shifts for carbohydrates in $(CD_3)_2SO$ and other polar sovents.

Solvents effective for mixtures of B_2O_3 and sugars are dimethyl sulfoxide, *N*,*N*-dimethylformamide, morpholine, and hexamethylphosphoric triamide. The present study used dimethyl sulfoxide and *N*,*N*-dimethylformamide, and the spectra of those sugar–B_2O_3 mixtures investigated were substantially the same in the two solvents.

Most spectra were measured at 100 MHz. The normal spectrum of α-D-glucose in $(CD_3)_2SO$ (Fig. 1a) shows the hydroxyl proton signals well separated (4.2–6.2 p.p.m.); the ring-proton signals were in the range 2.9–3.7 p.p.m. except for the H-1 quasi-triplet at 4.9 p.p.m. The hydroxyl-proton signals in $(CD_3)_2SO$, whose chemical shifts are independent of concentration in the range 5–20% (w/v), have already been assigned by Casu *et al.*[21].

The chemical shifts of hydroxy, methine, and methylene protons of α-D-glucose in $(CD_3)_2SO$ were found to depend on the molar ratio of B_2O_3 to glucose. Addition of 0.25 mol of B_2O_3 to glucose markedly affected the spectra in the 4.5–

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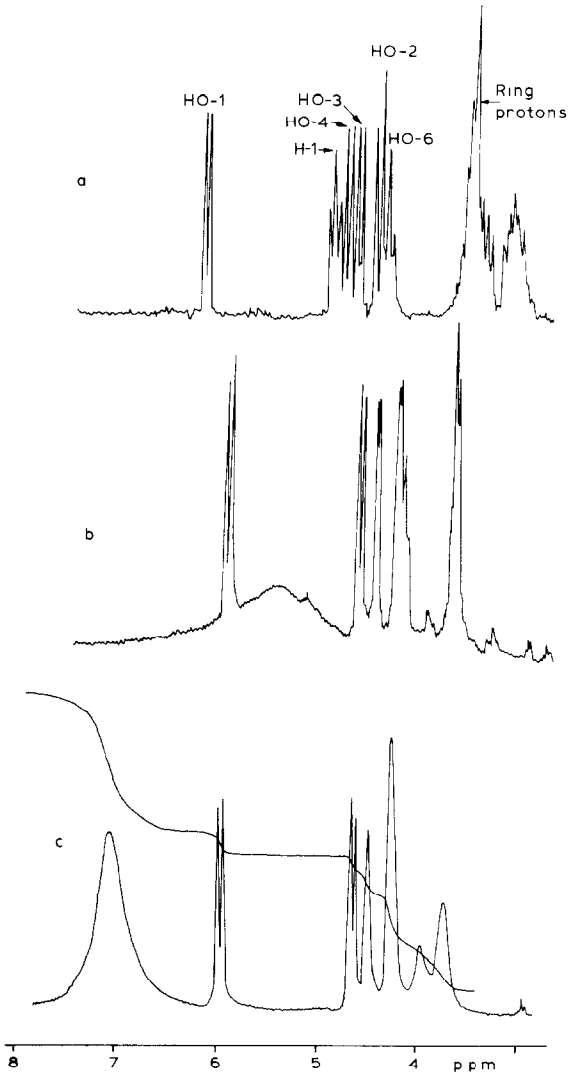

Fig. 1. (a) Normal spectrum of α-D-glucose (10%, w/v) in $(CD_3)_2SO$; (b) spectrum of 10% (w/v) α-D-glucose containing B_2O_3. ($[B_2O_3]$/[glucose] = 1:1); (c) spectrum of α-D-glucose containing B_2O_3. ($[B_2O_3]$/[glucose] = 2:1).

5.5-p.p.m. region and the signals of ring protons were moved slightly downfield. An equimolar amount of B_2O_3 caused significant changes, with dispersion of the ring-proton signals of α-D-glucose, as shown in Fig. 1b. Two molar equivalents of B_2O_3 to glucose collapsed all hydroxyl resonances into a broad peak at 7.1 p.p.m. (Fig. 1c). This broad peak shifts farther downfield and sharpens with addition of more B_2O_3.

Under the conditions of Fig. 1c, but with a doubled molar amount of B_2O_3, the peak for -OH · · · B_2O_3 was observed at 7.7 p.p.m. and all ring-proton signals were slightly broadened, because of an increase in the viscosity of the solution. The

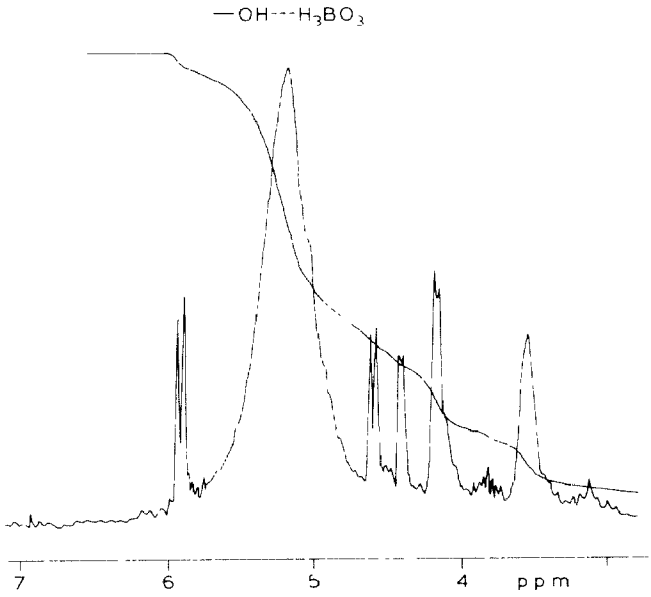

Fig. 2. Spectrum of α-D-glucose (10% w/v) and H_3BO_3 in $(CD_3)_2SO$ ([H_3BO_3]/[glucose] = 3).

OH · · · B_2O_3 peak broadened considerably with dilution of the 2.5:1 B_2O_3:D-glucose mixture from 10% (w/v) to 1% (w/v), and it was scarcely observed at the lowest concentration. Such a significant dilution-shift suggests that there is no covalent bonding between the sugar and B_2O_3. However, a large hydroxyl-proton peak is observed (4.7–5.6 p.p.m.) on addition of 3 mol of H_3BO_3 to 1 mol of α-D-glucose in $(CD_3)_2SO$ (Fig. 2), and the formation of an ester linkage between the sugar and reagent is possible.

The signal assignments in Fig. 1c were verified by integration and by ^{1}H-decoupling and ^{13}C–^{1}H coupling. The absence of protons in B_2O_3 is advantageous for the quantitative determination through spectral integrals of hydroxyl groups of sugars in anhydrous systems.

The H-1 resonance is a readily identifiable, low-field doublet at 5.91 p.p.m., showing $J_{1,2}$ 4.15 Hz. Operating with a 89.6-MHz spectrometer, irradiation of the H-1 doublet collapsed a doublet at 4.59 p.p.m. to a singlet, and the latter was thus assigned to H-2. However, no coupling between H-2 and H-3 was observed. This absence of coupling between H-2 and H-3 suggests that these protons are *trans*-disposed, with a dihedral angle of ~90°. Assignments of H-3 and H-4 were therefore made by correlation with the characteristic C-3 and C-4 signals in the ^{13}C-n.m.r. spectrum. The ^{13}C-n.m.r. spectrum obtained under the experimental conditions of Fig. 1c was substantially in agreement with that reported[22] for α-D-glucose in H_2O. Table I gives the ^{13}C-chemical shifts and ^{13}C–^{1}H coupling constants, which were confirmed by selective proton decoupling. The signal at 4.39 p.p.m. was thus definitely assigned to H-3 and that at 4.17 p.p.m. to H-4. The signals of H-4 and H-5 overlapped, so that certain first-order couplings were of questionable validity. In

TABLE I

^{13}C–^{1}H COUPLING CONSTANTS AND ^{13}C-CHEMICAL SHIFTS FOR 10% (w/v) SOLUTIONS OF α-D-GLUCOSE AND B_2O_3 IN $(CD_3)_2SO$ ($[B_3O_3]$/[GLUCOSE] = 2:1)

Parameter	*C-1,H-1*	*C-2,H-2*	*C-3,H-3*	*C-4,H-4*	*C-5,H-5*	*C-6,H-6,6'*
Coupling constants (Hz)	185.55	166.01	160.70	148.73	147.02	150 44
^{13}C-Chemical shifts (p.p.m.)	102.02	84.16	74.49	71.16	74.23	62.31
^{13}C-Chemical shifts[22] (p.p.m.) of α-D-glucopyranose in H_2O	92.5	73.3	72.0	70.1	71.8	61.3

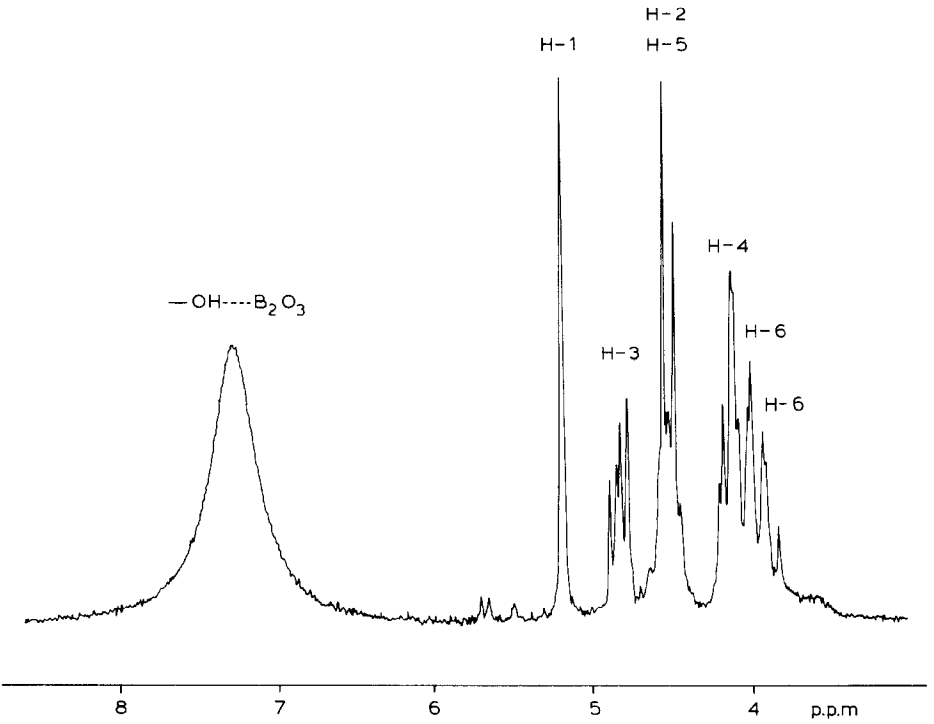

Fig. 3. Spectrum of α-D-mannose (5%, w/v) and B_2O_3 in $(CD_3)_2SO$. ($[B_2O_3]$/[mannose] = 5 1)

addition, the H-5, H-6, and H-6′ signals were sufficiently strongly coupled for the H-4 signal to be broadened by "virtual coupling" effects.

There are some differences in the 4.0–3.5-p.p.m. region between the spectra in Fig. 1c and in Fig. 2. The highest-field peak in Fig. 2 may be attributable to H-6 and H-6′.

Analysis of ring-proton resonances of α-D-mannose with B_2O_3 by spin-decoupling experiments was also performed. The spectrum (Fig. 3) shows a sharp singlet for H-1 at 4.82 p.p.m., separated from other proton signals and indicating

that H-1 and H-2 are oriented at ~90° dihedral angles. Consequently, the H-2 peak (4.51 p.p.m.) is a doublet being coupled only to H-3. By irradiation of the H-2 doublet, a doublet of doublets centered at 4.83 p.p.m. collapsed to a simpler pattern, so that this signal was assigned to H-3. Irradiation of H-3 collapsed the H-2 doublet to a singlet, and a four-line pattern centered at 4.13 p.p.m. was simplified; this resonance was thus assigned to H-4. Irradiation of H-4 altered peaks overlapping the H-2 signal, and these resonances were assigned to H-5. Residual peaks at 4.01, 3.99, 3.91 and 3.82 p.p.m. may be attributable to H-6 and H-6', as the AB portion of an ABX system.

EXPERIMENTAL

Materials. — The following compounds were obtained from commercial sources: α-D-glucose and α-D-mannose from Nakarai Chemicals, Ltd., Kyoto; B_2O_3, H_3BO_3, and D_3BO_3 from E. Merck; and $(CD_3)_2SO$ and $(CD_3)_2NCDO$ from Aldrich. B_2O_3 was finely pulverized with a pestle in an alumina mortar in a dry atmosphere, and was dried over P_2O_5 under vacuum for 48 h at 115°.

Spectra. — ^{1}H-N.m.r. and ^{13}C-n.m.r. spectra were recorded at 89.6 MHz with a JEOL Model FX-90Q instrument. ^{1}H-N.m.r. spectra at 100 MHz were also recorded with a JEOL Model 4H-100 spectrometer. Chemical shifts (p.p.m.) are referenced to tetramethylsilane.

A definite amount of B_2O_3 was weighed into a vial containing the sugar dissolved in the solvent, and the air in the vial was swept out with dry nitrogen. The vial was placed in a bath at 40–60° and vibrated effectively for 4–5 h until the B_2O_3 dissolved. The resultant, clear solution was then transferred to an n.m.r. sample tube. The solubility of B_2O_3 in the sugar solution is dependent on the concentration of dissolved sugar. Anhydrous B_2O_3 was not sufficiently soluble in diluted solutions of the sugar. Samples for n.m.r. measurements were best obtained by dissolving B_2O_3 in a concentrated solution of the sugar, and then diluting with solvent. In some instances, B_2O_3 and the sugar could be introduced together into the solvent, in which case, care had to be taken to avoid high temperatures and consequent dehydration of the sugar and coloration.

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