Deuterium Isotope Effect on Shifts of ^{13}C Magnetic Resonance Signals of Sugars: Signal Assignment Studies[1]

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The ^{13}C chemical shifts of nuclei in several sugars undergo an isotope effect of 0 to -0.10 p.p.m. when a proton in the β position is substituted by a deuteron. The effect may be used for signal assignments in crowded spectra if it is taken into account that lower values are observed for ^{13}C nuclei which are attached to more electronegative groups. The β effect coupled with the elimination of the α-carbon signal have been used to assign signals in c.m.r. spectra of the α- and β-anomers of D-glucose, D-mannose, D-allose, and D-galactose.

Les déplacements chimiques du ^{13}C de plusieurs noyaux dans différents sucres subissent un effet isotopique de 0 à -0.10 p.p.m. lorsqu'un proton en position β est remplacé par un deutérium. Cet effet peut être utilisé lors de l'attribution des signaux d'un spectre complexe si l'on tient compte du fait que l'on observe les valeurs les plus basses pour les noyaux ^{13}C attachés aux groupes les plus électronégatifs. L'effet β en même temps que l'élimination du signal α-carbon, a été utilisée pour attribuer les signaux des spectres r.m.c. de l'αβ-D-glucose, de l'αβ-D-mannose, de l'αβ-D-allose et de l'αβ-D-galactose.
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Signals of carbon-13 magnetic resonance (c.m.r.) spectra can be assigned following replacement of a given proton by a deuteron. The signal of the attached ^{13}C nucleus either disappears (1) or is converted to a triplet (2) at 0.1–0.5 p.p.m. higher field (3–6). Progressively smaller upfield shifts are observed for signals of the β-carbon (~0.1 p.p.m.; (3, 5–7)) and γ-carbon (~0.01 p.p.m.; (6)) nuclei. $^{13}C—C—^{2}H$ (5, 7) and $^{13}C—C—C—^{2}H$ (6) coupling can also occur.

In the carbohydrate field the $^{13}C—^{2}H$ effect, in which the ^{13}C signal is eliminated, has been used to assign c.m.r. signals (8). The current results show that the $^{13}C—C—^{2}H$ effect can also be used for assignment of certain sugar signals. For example, C-5 signals of hexose derivatives are identifiable using 6-$^{2}H_2$ derivatives which are more easily prepared than 5-^{2}H derivatives (9). The upfield shift of the C-5 signal is ~0.12 p.p.m. (measured to an accuracy of ±0.01 p.p.m.) (Table 1). The values were determined using mixtures of deuterated and undeuterated material in D_2O using conditions that do not detect differences of shift of <0.02 p.p.m. Assignments of C-5 signals for α- and β-anomers of D-mannose and D-galactose agree with previous ones obtained in H_2O (10, 11). Although shifts of C-5 signals of αβ-D-glucose in D_2O differ a little from those described in H_2O (8), the assignments essentially agree (see Experimental section).

The upfield shifts due to the $^{13}C—C—^{2}H$ effect in a number of monodeuterated hexoses were determined and recorded in Table 1. Their magnitude varied from approximately 0.01 to 0.10 p.p.m. per deuteron which is less than 0.09–0.15 p.p.m. observed with compounds having less electronegative substituents on the β-carbon (3, 5). (In contrast with nonoxygenated derivatives, $^{13}C—C—^{2}H$ and $^{13}C—C—C—^{2}H$ coupling (5–7) were not detected.) Similarly, an inverse relationship between the size of the shift and the deshielding on the ^{13}C nucleus was observed. The C-1 resonances of αβ-D-glucose-2-^{2}H and αβ-D-mannose-2-^{2}H are shifted 0–0.02 p.p.m. upfield, compared with the C-3 resonances of the methyl 2,3-di-*O*-methyl derivatives of α-D-glucopyranoside-4-^{2}H and α-D-galactopyranoside-4-^{2}H (0.04 p.p.m.), and the other β-CHOH derivatives (0.05–0.10 p.p.m.). No variation was noted on going from an axial to an equatorial ^{2}H nucleus.

It is possible, therefore, using the α- and β-carbon effects to assign the ^{13}C nuclei in spectra containing signals that are very close together, such as those of αβ-D-glucose and αβ-D-allose. The signal assignments obtained for αβ-D-glucose, αβ-D-mannose, and β-D-allose in D_2O agree essentially with those made by Perlin and co-workers (8, 10) and somewhat less with

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TABLE 1. Upfield displacement of $^{13}C—C—^{2}H$ signals of sugars* compared with those of corresponding $^{13}C—C—^{1}H$ resonances in p.p.m.

Sugar	Displacement
αβ-D-Glucose-2-^{2}H	C_β-3 (0.06), C_α-3 (0.05); C_α-1, C_β-1 (0–0.02)
αβ-D-Glucose-3-^{2}H	C_β-2 (0.05), C_β-4 (0.06); C_α-2, C_α-4 obscured
αβ-D-Glucose-5-^{2}H	C_α-4, C_α-6, C_β-6 (0.06); C_α-4 obscured
αβ-D-Glucose-6-$^{2}H_2$	C_α-5, C_β-5 (0.12)
αβ-D-Mannose-6-$^{2}H_2$	C_β-5 (0.11), C_α-5 (0.13)
αβ-D-Galactose-6-$^{2}H_2$	C_α-5 (0.12), C_β-5 (0.10)
αβ-D-Galactose-4-^{2}H	C_β-5 (0.04), C_β-3 (0.06), C_α-5 (0.06), C_α-3 (0.10)
αβ-D-Allose-3-^{2}H	C_α-2, C_β-2 (0.09); C_α-4, C_β-4 (0.08)
αβ-D-Mannose-2-^{2}H	C_α-3, C_β-3 (0.06); C_α-1, C_β-1 (0–0.02)
Methyl 2,3-Di-*O*-methyl-α-D-galactopyranoside-4-^{2}H	C-3 (0.04), C-5 (0.08)
Methyl 2,3-Di-*O*-methyl-α-D-glucopyranoside-4-^{2}H	C-3 (0.04), C-5 (0.07)

*α and β subscripts refer to configuration of hexose.

those of Dorman and Roberts (11), who both used H_2O as solvent. In the case of αβ-D-galactose in D_2O, the present assignments of the six low-field signals agree with those of both groups of workers. However, the next four signals at δ_C 69.96, 69.81, 69.40, and 69.01 should be given different assignments of C_α-4, C_α-3, C_β-4, and C_α-2. Examination of the c.m.r. spectra of αβ-D-galactose-4-^{2}H and unmutarotated α-D-galactose served to assign the C_α-4 and C_β-4 signals. The remaining C_α-3 and C_α-2 signals were distinguishable since the spectrum of αβ-D-galactose mixed with its 4-^{2}H derivative contained C_α-3 as two signals with a 0.10 p.p.m. shift difference. Repetition of these experiments using aqueous solutions indicated a similar order of signals.

Three deuterated compounds were prepared for spectral examination. Methyl 6-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-glucopyranoside (12) was oxidized with dimethyl sulfoxide – phosphorus pentoxide to the 4-keto derivative. Reduction with sodium borodeuteride followed by debenzylation gave a mixture containing methyl 2,3-di-*O*-benzyl-β-D-galactopyranoside-4-^{2}H. It was hydrogenolyzed using palladium on charcoal and the product hydrolyzed with acid. Chromatography on a column of cellulose provided the required galactose-4-^{2}H. In another reaction sequence methyl 2,3-di-*O*-methyl-α-D-glucopyranoside (13) was converted to its 6-benzoate, which was oxidized by dimethyl sulfoxide – acetic anhydride to the 4-keto derivative. Sodium borodeuteride reduction gave the 6-*O*-benzoyl-D-galacto isomer as the predominant one and a portion was debenzoylated to give required methyl 2,3-di-*O*-methyl-α-D-galactopyranoside-4-^{2}H. Another portion of the 6-*O*-benzoyl derivative was converted to its 4-*O*-*p*-toluenesulfonyl derivative which was treated with sodium benzoate in refluxing *N*,*N*-dimethylformamide to give methyl 4,6-di-*O*-benzoyl-2,3-di-*O*-methyl-α-D-glucopyranoside-4-^{2}H. Debenzoylation gave methyl 2,3-di-*O*-methyl-α-D-glucopyranoside-4-^{2}H.

Experimental

Carbon magnetic resonance spectra were obtained using a Varian XL-100-15 spectrometer with Fourier transform on D_2O solutions (0.5 ml) of compound (80 mg) contained in a coaxial cylinder within a 12 mm diameter × 8 in. tube maintained at 33°. The spectral width was 500 Hz (the total spectrum being thus obtained by combining two portions); the acquisition time 4 s, and the pulse width 117 μs, representing a 90° pulse. The number of transients used was approximately 18 000. In order to determine the extent of the $^{13}C—C—^{2}H$ shift, spectra of deuterated and undeuterated sugar were obtained and then a mixture of deuterated and undeuterated compound was prepared and examined. The latter spectrum was expanded by a factor of two which facilitated manual measurement of the shift difference. Chemical shifts are in p.p.m., based on the downfield difference of the resonance signal and that of external tetramethylsilane. Dioxane (δ_C 67.22) was used as internal standard.

Preparation of Deuterated Derivatives

α-D-Glucose-3-^{2}H, β-D-allose-3-^{2}H (8), α-D-glucose-2-^{2}H, α-D-mannose-2-^{2}H (14), and α-D-glucose-5-^{2}H (15) were prepared as described. The preparations of other deuterated derivatives are presented below.

α-D-*Glucose-6-*$^{2}H_2$

1,2-*O*-Isopropylidene-α-D-glucofuranose-6-$^{2}H_2$ was prepared from 1,2-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone using the same procedure as described for preparation of the 5,6,6′-$^{2}H_3$ derivative from 1,2-*O*-isopropylidene-α-D-xylohexofuranurono-6,3-lactone-5-ulose (8). Hydrolysis with 0.05 *M* H_2SO_4 at 100° for 1 h gave α-D-glucose-6-$^{2}H_2$ (from MeOH) with m.p. 144–145° and $[\alpha]_D^{25}$ +103 → +49° (*c*, 0.4 H_2O; constant value).

Anal. Calcd. for $C_6DH_{11}O_6$: C, 39.56; H + D, 7.74. Found: C, 39.3; H + D, 7.8.

α-D-*Galactose-6-*$^2H_2$

D-Galacturonic acid (0.40 g) was treated with refluxing 3% methanolic hydrogen chloride (10 ml) for 3 h, the solution neutralized (Ag_2CO_3), filtered, and evaporated to a sirup. Dissolution in 0.1 *M* $NaOCH_3$ in methanol (5 ml) was carried out and sodium borodeuteride (0.10 g) added. After 18 h the solution was evaporated, Na^+ removed with a suspension of Amberlite 1R120 (H^+ form) in water, and boric acid was removed as trimethyl borate by repeated evaporations of methanol. The resulting methyl galactosides were hydrolyzed in 0.5 *M* sulfuric acid (5 ml) at 100° for 6 h, the solution neutralized ($BaCO_3$), filtered, and the filtrate deionized with mixed bed resin. The residue obtained on evaporation crystallized from methanol giving α-D-galactose-6-$^2H_2$ with m.p. 159–161° and $[\alpha]_D^{25}$ +134 → +79° (*c*, 0.3 H_2O; constant value).

Anal. Calcd. for $C_6D_2H_{10}O_6$: C, 39.56; H + D, 7.74. Found: C, 39.6; H + D, 7.6.

α-D-*Mannose-6-*$^2H_2$

Using a procedure similar to that described above β-D-mannofuranurono-γ-lactone (200 mg; supplied by ICN-K&K Laboratories) was converted to α-D-mannose-6-$^2H_2$ (80 mg from EtOH) with m.p. 122° and $[\alpha]_D^{25}$ +25 → +15° (*c*, 0.3 H_2O; constant value).

Anal. Calcd. for $C_6D_2H_{10}O_6$: C, 39.56; H + D, 7.74. Found: C, 39.2; H + D, 7.8.

α-D-*Galactose-4-*^2H

Methyl 6-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-glucopyranoside (1.90 g) was dissolved in dimethyl sulfoxide (50 ml) containing phosphorus pentoxide (7 g). After 1 week chloroform was added to the reaction mixture which was washed with water and evaporated. Thin-layer chromatography on silica gel (solvent, chloroform) showed the presence (spray, 50% sulfuric acid) of unchanged material and a faster moving spot corresponding to a ketone. After longer reaction times the relative amount of the faster spot did not increase. Use of the α-anomer as starting material was unsuccessful. The mixture was fractionated on a column of silicic acid (eluant, chloroform, 2:hexane, 3) to give the ketone (0.15 g) and a mixed fraction (1.12 g), from which the benzoate (0.35 g) crystallized. The mother liquor was evaporated and treated in methanol (10 ml) at −20° with sodium borodeuteride (0.20 g). After 1 h the product partitioned between water and chloroform. To the chloroform layer was added 0.1 *M* sodium methoxide in methanol (10 ml) in order to remove the benzoate group. The product was fractionated on a column of silicic acid, using as eluants chloroform followed by chloroform containing 2% methanol. Crystallization of the eluate from ether–hexane gave methyl 2,3-di-*O*-benzyl-β-D-glucopyranoside (0.27 g) and 0.13 g of sirupy material. The original sirupy ketone fraction (0.15 g) was reduced with $NaBD_4$ in a fashion similar to that described above, the product debenzoylated and combined with the above sirupy material (0.13 g). Hydrogenolytic debenzylation of the product with 5% palladium on charcoal in acetic acid gave a mixture of methyl β-D-glucopyranoside and methyl β-D-galactopyranoside (0.14 g). Hydrolysis with 0.5 *M* H_2SO_4 (5 ml) for 6 h at 100° gave galactose and glucose which were separated on a column of cellulose (eluant, *n*-butyl alcohol containing 5% water) providing a fraction containing galactose (60 mg). Crystallization from methanol gave α-D-galactose-4-^2H (36 mg) with m.p. 160–161° and $[\alpha]_D^{25}$ +127 → +76° (*c*, 0.1 H_2O; constant value).

Anal. Calcd. for $C_6DH_{11}O_6$: C, 39.68; H + D, 7.23. Found: C, 39.5; H + D, 7.4.

Methyl 2,3-Di-O-methyl Derivatives of α-D-*Galactopyranoside-4-*^2H *and* α-D-*Glucopyranoside-4-*^2H

Using benzoyl chloride (1.1 mol/mol) in pyridine as reagent methyl 2,3-di-*O*-methyl-α-D-glucopyranoside (3.60 g) was converted to its sirupy 6-benzoate (6.31 g). The product gave a single spot on t.l.c. (solvent, chloroform containing 4% methanol) and had $[\alpha]_D^{25}$ +108° (*c*, 0.5 EtOH); p.m.r. data (dimethyl sulfoxide-$^2H_6$; tetramethylsilane as external standard), δ 5.74 (*J* = 5 Hz, secondary OH).

Anal. Calcd. for $C_{16}H_{22}O_7$: C, 58.88; H, 6.80. Found: C, 58.4; H, 6.6.

The benzoate was dissolved in dimethyl sulfoxide (30 ml) containing acetic anhydride (20 ml). After 18 h the mixture was partitioned between chloroform and water and the material in the chloroform layer chromatographed on a column of silicic acid (eluant, chloroform, 1:hexane, 1). The product (5.9 g) gave two spots on t.l.c. (solvent, chloroform containing 3% methanol), the slower of which corresponded to the ketone. The mixture was reduced with sodium borodeuteride (for procedure see the above reduction of the di-*O*-benzyl derivative) and the product chromatographed on a column of silicic acid (eluant, chloroform) to give methyl 6-*O*-benzoyl-2,3-di-*O*-α-D-galactopyranoside-4-^2H which contained a trace of the α-D-glucopyranoside isomer.

Debenzoylation of a portion gave methyl 2,3-di-*O*-methyl-α-D-galactopyranoside-4-^2H, which had a c.m.r. spectrum that would be anticipated from that of authentic undeuterated material (16). Trace signals were present which corresponded to the deuterated gluco-derivative.

Anal. Calcd. for $C_9DH_{17}O_6$: C, 48.42; H + D, 8.58. Found: C, 48.0; H + D, 8.4.

The deuterated 6-benzoate fraction (1.32 g) was dissolved in pyridine (5 ml) containing *p*-toluenesulfonyl chloride (1.5 g). After 4 days at 37° the solution was added to water and the precipitate, which crystallized, was recrystallized from ethanol. The resulting methyl 6-*O*-benzoyl-2,3-di-*O*-methyl-4-*O*-*p*-toluenesulfonyl-α-D-galactopyranoside-4-^2H had m.p. 143–144° and $[\alpha]_D^{25}$ +86° (*c*, 0.8 $CHCl_3$).

Anal. Calcd. for $C_{23}DH_{27}O_9S$: C, 57.37; H + D, 6.07. Found: C, 57.3; H + D, 6.1.

The *p*-toluenesulfonate (0.30 g) was treated with refluxing *N*,*N*-dimethylformamide (60 ml) containing sodium benzoate (2.0 g). After 20 h the reaction mixture was partitioned between chloroform and water. The chloroform layer was evaporated and the residue crystallized from ether–hexane to give methyl 4,6-di-*O*-benzoyl-2,3-di-*O*-methyl-α-D-glucopyranoside-4-^2H (0.16 g) with m.p. 120–121° and $[\alpha]_D^{25}$ +92° (*c*, 0.5 EtOH); p.m.r. (dimethyl sulfoxide-$^2H_6$; tetramethylsilane as external standard); δ 7.85–8.42 (10 aromatic protons); 5.34 (*J* = 3.6 Hz, H-1), 3.68, 3.72, 3.74 (3 OCH_3's).

Anal. Calcd. for $C_{23}DH_{25}O_8$: C, 64.02; H + D, 6.31. Found: C, 63.8; H + D, 6.3.

Debenzoylation of the dibenzoate (0.12 g) with methanolic sodium methoxide gave methyl 2,3-di-*O*-methyl-α-D-glucopyranoside-4-^{2}H (52 mg from ether) with m.p. 77–78° and $[\alpha]_D^{25}$ +150° (*c*, 0.3 H_2O).

Anal. Calcd. for $C_9DH_{17}O_6$: C, 48.42; H + D, 8.58. Found: C, 48.2; H + D, 8.3.

Signal Assignments

Chemical shifts described below are those obtained in this laboratory.

αβ-D-*Mannose*. Assignments based on results of refs. 10 and 11 in H_2O: 94.60 (C_α-1), 94.24 (C_β-1), 76.75 (C_β-5), 73.65 (C_β-3), 73.02 (C_α-5), 71.83 (C_β-2), 71.31 (C_α-2), 70.83 (C_α-3), 67.50 (C_α-4), 67.22 (C_β-4), 61.63 ($C_{\alpha\beta}$-6), which are confirmed by the ^{13}C—C—^{2}H method.

αβ-D-*Glucose*. Assignments based on results of reference 10 in H_2O and present ^{13}C—C—^{2}H shift results which serve to distinguish C_β-5 from C_β-3, and C_α-2 from C_α-5 in D_2O: 96.50 (C_β-1), 92.70 (C_α-1), 76.59 (C_β-5), 76.43 (C_β-3), 74.80 (C_β-2), 73.45 (C_α-3), 72.14 (C_α-2), 72.10 (C_α-5), 70.36 (C_α-4), 70.28 (C_β-4), 61.47 (C_β-6), 61.31 (C_α-6).

αβ-D-*Galactose*. Assignments for the six lowest field signals of refs. 10 and 11 in H_2O agree with each other and those obtained by the ^{13}C—C—^{2}H method using the 6-^{2}H derivative: 97.02 (C_β-1), 92.90 (C_α-1), 75.71 (C_β-5), 73.45 (C_β-3), 72.54 (C_β-2), 71.07 (C_α-5), 69.96 (C_α-4), 69.81 (C_α-3), 69.40 (C_β-4), 69.01 (C_α-2), 61.81 (C_α-6), 61.63 (C_β-6). Signals at δ_C 69.96–69.01 were assigned using αβ-D-galactose-4-^{2}H (see theoretical section).

αβ-D-*Allose*. The results from the β-anomer were from ref. 10 in H_2O and confirmed (these disagreed with those of ref. 11). The partial results of ref. 11 for the α-anomer were confirmed and C_α-2 and C_α-5 assignments (120.9 or 121.4, respectively in ref. 11) made by the ^{13}C—C—^{2}H method: 93.97 (C_β-1), 93.37 (C_α-1), 74.17 (C_β-5), 72.26 (C_α-3), 71.87 (C_β-2), 71.75 (C_β-3), 67.62 (C_α-2), 67.50 (C_α-5), 67.42 (C_β-4), 66.71 (C_α-4), 61.83 (C_β-6), 61.35 (C_α-6). The spectrum of αβ-D-allose-3-^{2}H does not contain the C_β-3 signal but the C_β-2 signal is shifted upfield to occupy the approximate position of the C_β-3 signal of αβ-D-allose.

Methyl 2,3-Di-O-methyl-α-D-glucopyranoside. Assignment based on D-glucose in H_2O (10): 97.22 (C-1), 82.94 (C-3), 80.56 (C-2), 72.14 (C-5), 69.72 (C-4), 61.11 (C-6), 60.63, 58.73, 55.48 (3 OCH_3's).

Methyl 2,3-Di-O-methyl-α-D-galactopyranoside. Assignment based on above assignments for α-D-galactose and the ^{13}C—C—^{2}H method: 97.14 (C-1), 78.70 (C-3), 77.06 (C-2), 71.07 (C-5), 69.72 (C-4), 61.90 (C-6), 58.06, 56.43, 55.48 (3 OCH_3's).

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