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Note

The effect of *O*-deuteration upon the proton-decoupled, ^{13}C-n.m.r. spectra of carbohydrates*†

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C-Deuteration at specific positions of a carbohydrate molecule is probably the most definitive tool for the assignment of carbon resonances, as the signals of carbon atoms bonded to deuterium generally become very weak[1,2]. However, synthetic difficulties often limit the utility of this approach.

In a recent communication[3], we reported the catalytic *C*-protium–*C*-deuterium exchange of carbohydrate derivatives by use of deuterated Raney nickel and deuterium oxide. This simple exchange technique causes *C*-deuteration at carbon atoms that are bonded to hydroxyl groups; the process is useful not only for the synthesis of deuterated carbohydrates, but also for the assignment of ^{1}H- and ^{13}C-n.m.r. signals, as it permits the introduction of deuterium into existing molecular structures, generally without loss of configuration[3]. In combination with the β-deuterium isotope-effect on ^{13}C-n.m.r. signals reported by Gorin[4], the scope of this method for the assignment of carbon resonances is extended. However, it cannot be applied directly to free aldoses, ketoses, or other compounds having reducible substituents; for example, methyl 4,6-*O*-benzylidene-α-D-glucopyranoside is rapidly converted into methyl α-D-glucopyranoside plus toluene by Raney nickel and water, even at room temperature.

DISCUSSION

[$^{2}H_6$]Dimethyl sulfoxide has been widely used as a solvent for carbohydrates, because of their great solubility in this solvent and because anomerization is greatly

*A description of the preparation of the deuterated compounds was presented at the 2nd. Joint CIC–ACS Conference, Montreal, Canada, June 1, 1977.

†After the preparation of this manuscript, a preliminary report [D. Gagnaire and M. Vincendon, *J. Chem. Soc. Chem. Commun.*, (1977) 509] appeared in which small chemical-shift differences (at 68 MHz) of carbon atoms bonded to OH and O^{2}H groups of two partially *O*-deuterated carbohydrates were reported. As some exchange between hydroxyl groups occurs, it appears preferable to measure the "light" and *O*-deuterated forms separately.

lessened[5,6]. Generally, the ^{13}C-n.m.r. spectra measured in [$^2H_6$]dimethyl sulfoxide are similar to those obtained in deuterium oxide. When the proton-decoupled, ^{13}C-n.m.r. spectra of carbohydrates measured in water* or [$^2H_6$]dimethyl sulfoxide were compared to those of the same compounds in which the protium of the hydroxyl groups had previously been exchanged by use of deuterium oxide, a small, but definite, upfield displacement of the resonances of the carbon atoms bonded to free hydroxyl groups was observed. The other carbon resonances were, if at all, affected to a much smaller extent (see Tables I and II). Deuteration of the hydroxyl groups was effected simply by evaporation of deuterium oxide from the sample prior to dissolution in deuterium oxide or [$^2H_6$]dimethyl sulfoxide.

TABLE I

THE EFFECT OF *O*-DEUTERIUM EXCHANGE ON THE PROTON-DECOUPLED, ^{13}C-N.M.R. SPECTRA[a] OF SOME CARBOHYDRATES

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*Me*
α-D-Glucopyranose	93.04[b]	73.17	73.96	71.41	72.71	62.09	—
	0.10[c]	0.17	0.17	0.13	0.05	0.12	
β-D-Glucopyranose	97.66	77.68	77.54	71.15	77.46	62.09	—
	0.10	0.19	0.15	0.13	0.05	0.12	
α-D-Mannopyranose	94.82	72.19	71.46	68.22	73.80	62.35	
	0.10	0.15	0.12	0.14	0.05	0.12	
β-D-Mannopyranose	94.82	72.39	74.59	67.88	77.73	62.35	—
	0.10	0.13	0.16	0.13	0.05	0.12	
Methyl α-D-xylopyranoside	100.96	72.79	74.29	70.79	62.53	—	55.40
	0.03	0.14	0.17	0.14	0.05	—	0.00
Methyl α-D-glucopyranoside	100.53	72.57	74.37	71.26	73.37	61.94	55.30
	0.02	0.14	0.19	0.14	0.05	0.12	0.00
Methyl β-D-glucopyranoside	104.74	74.23	77.59	70.97	77.59	61.97	56.78
	0.05	0.13	0.19	0.13	0.05	0.12	0.01
Methyl α-D-mannopyranoside	101.81	70.04	71.89	67.93	74.58	62.15	54.78
	0.03	0.14	0.17	0.14	0.05	0.12	0.00
Methyl β-D-galactopyranoside	105.29	71.49	74.30	69.14	75.95	61.49	56.11
	0.01	0.11	0.14	0.10	0.03	0.10	0.02
α,α-Trehalose	93.95	72.44	73.73	71.01	73.25	61.65	—
	0.00	0.10	0.16	0.12	0.01	0.10	—
1,2-*O*-Isopropylidene-	105.32	85.58	74.24	80.95	69.39	64.53	—
α-D-glucofuranose	0.00	0.03	0.11	0.03	0.11	0.14	—
(isopropylidene)	27.43	111.28	26.92	—	—	—	—
	0.02	0.00	0.02	—	—	—	—
Sucrose	92.63	72.50	73.77	70.81	73.69	61.45	—
(α-D-glucopyranosyl)	0.05	0.12	0.15	0.12	0.05	0.15	—
(β-D-fructofuranosyl)	63.00	104.91	78.08	75.25	83.41	63.00	—
	0.10	0.03	0.17	0.15	0.05	0.10	—

[a]Chemical shifts are in p.p.m. from Me_4Si, using 1,4-dioxane (67.22 p.p.m.) as the internal reference standard; solvent: sulfoxide [$^2H_6$]dimethyl. [b]Chemical shift of OH form. [c]Upfield displacement, in p.p.m., on *O*-deuterium exchange.

*OH forms were measured in H_2O, and O^{2}H forms in ^2H_2O.

TABLE II

THE EFFECT OF *O*-DEUTERIUM EXCHANGE ON THE PROTON-DECOUPLED, ^{13}C-N.M.R. SPECTRA[a] OF SOME CARBOHYDRATES

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*Me*
α-D-Glucopyranose	92.76[b]	72.22	73.58	70.39	72.04	61.58	—
	0.11[c]	0.17	0.19	0.12	0.01	0.13	—
β-D-Glucopyranose	96.58	74.92	76.53	70.39	76.44	61.58	—
	0.10	0.18	0.13	0.12	0.02	0.13	—
α-D-Mannopyranose	94.71	71.46	70.05	67.64	72.99	61.80	—
	0.12	0.16	0.15	0.14	0.05	0.13	—
β-D-Mannopyranose	94.35	71.97	73.82	67.37	76.68	61.80	—
	0.10	0.17	0.15	0.15	0.03	0.13	—
Methyl α-D-xylopyranoside[d]	100.18	72.07	74.08	70.15	61.65	—	55.80
	0.01	0.12	0.18	0.13	0.03	—	0.01
Methyl α-D-glucopyranoside	99.88	72.05	73.97	70.42	72.25	61.48	55.72
	0.02	0.11	0.16	0.11	0.02	0.12	0.00
Methyl α-D-mannopyranoside	101.48	70.71	71.42	67.54	73.22	61.74	55.36
	0.01	0.12	0.16	0.12	0.04	0.11	0.00
Methyl β-D-glucopyranoside	103.87	73.84	76.63	70.45	76.54	61.64	57.83
	0.00	0.11	0.13	0.11	0.04	0.11	0.00
Methyl β-D-galactopyranoside	104.47	71.50	73.64	69.43	75.68	61.72	57.78
	0.01	0.12	0.15	0.14	0.05	0.13	0.02
α,α-Trehalose	93.83	71.79	73.37	70.48	72.79	61.44	—
	0.00	0.13	0.17	0.10	0.04	0.11	—
1,2-*O*-Isopropylidene-	105.22	85.03	74.30	80.37	69.16	64.26	—
α-D-glucofuranose	0.01	0.00	0.10	0.00	0.11	0.15	—
(isopropylidene)	26.34	113.00	25.91	—	—	—	—
	0.00	0.01	0.00	—	—	—	—
Sucrose	92.7	71.79	73.41	70.03	73.03	61.04	—
(α-D-glucopyranosyl)	0.01	0.11	0.19	0.14	0.04	0.17	—
(β-D-fructofuranosyl)	63.09	104.25	77.34	74.84	82.01	62.25	—
	0.12	0.00	0.12	0.14	0.03	0.10	—

[a]Chemical shifts are in p.p.m. from Me_4Si, using 1,4-dioxane (67.22 p.p.m.) as the internal reference standard; solvent: for OH forms, water; for O^2H forms, deuterium oxide. [b]Chemical shift of OH form. [c]Upfield displacement, in p.p.m., on *O*-deuterium exchange. [d]Concentration: 0.5g/ml of solvent.

The upfield displacements of the ^{13}C resonances of carbon atoms bonded to hydroxyl groups was 0.1–0.2 p.p.m., whereas the displacement of the other signals was 0.00–0.05 p.p.m. It appears that this upfield displacement is similar to the β-deuterium isotope-effect[4], with the difference that the α-carbon atom has been replaced by the hydroxyl oxygen atom. In general, the upfield-signal displacements agree with assignments previously reported[2,4,7].

The observed upfield displacements of the ^{13}C-n.m.r. signals of methyl α-D-glucopyranoside upon *O*-deuteration (see Tables I and II) suggest that the assignments reported[2,7] for the signals of C-2 and C-5 should be reversed. The original assignments had been partly based on the comparison of the spectrum of methyl α-D-glucopyranoside with that of α-D-glucopyranose. Indeed, the ^{13}C-n.m.r. spectra of methyl

TABLE III

PROTON-DECOUPLED, 20-MHZ, ^{13}C-N.M.R. SPECTRA OF 10, INDIVIDUALLY PREPARED, AQUEOUS SOLUTIONS OF METHYL α-D-GLUCOPYRANOSIDE (1.00 G IN 1.0 mL OF H_2O CONTAINING 5%, BY VOL., OF 1,4-DIOXANE)

Sample	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*Me*
1	1997.9	1441.4	1479.7	1408.7	1445.3	1229.8	1114.3
2	1997.8	1441.5	1479.7	1408.7	1445.3	1229.8	1114.4
3	1997.9	1441.5	1479.7	1408.8	1445.3	1229.9	1114.4
4	1997.9	1441.4	1479.7	1408.6	1445.2	1229.8	1114.3
5	1997.9	1441.5	1479.6	1408.7	1445.3	1229.9	1114.3
6	1997.9	1441.6	1479.8	1408.8	1445.3	1229.9	1114.3
7	1998.0	1441.5	1479.7	1408.6	1445.3	1229.8	1114.4
8	1997.9	1441.4	1479.7	1408.7	1445.2	1229.9	1114.3
9	1997.9	1441.4	1479.7	1408.7	1445.3	1229.9	1114.3
10	1997.9	1441.4	1479.7	1408.6	1445.3	1229.9	1114.3
Standard deviation (in Hz)	±0.047	±0.070	±0.047	±0.074	±0.042	±0.052	±0.048
Standard deviation (in p.p.m.)	±0.002	±0.003	±0.002	±0.004	±0.002	±0.003	±0.002

[a]The chemical shifts are in Hz from Me_4Si, using 1,4-dioxane (1344.4 Hz) as the internal reference standard.

α-D-glucopyranoside-5-^2H and methyl α-D-glucopyranoside-5,6,6-$^2H_3$ (in which the C-5 resonances, and, in the latter, the C-6 resonance also have been eliminated), confirm the reversal of the original assignment; these compounds were prepared by a method reported recently[3].

Solutions of "light" and *O*-deuterated methyl α-D-glucopyranoside in [$^2H_6$]dimethyl sulfoxide were mixed, to determine whether superposition of their individual spectra could be observed, as exchange is generally assumed to be slow in this solvent[5,6]; however, only an averaged spectrum was obtained. This appears to be due to some exchange between the hydroxyl groups, a supposition supported by the observation that the individual hydroxyl-proton resonances of methyl α-D-glucopyranoside and methyl α-D-glucopyranoside-2,3,4,6,6-$^2H_5$ (for preparation, see ref. 3) in [$^2H_6$]dimethyl sulfoxide solution were rapidly diminished upon the addition[8] of small proportions of [$^2H_4$]methanol.

As small differences in chemical shifts were measured, it was important to compare solutions of identical concentration. 1,4-Dioxane (5% by volume of solvent) was used as the internal reference standard[4] at 67.22 p.p.m. from Me_4Si. The reproducibility of the measurements of the chemical shift is good, as is shown by the spectra (see Table III) of ten, individually prepared solutions of methyl α-D-glucopyranoside. The spectra of the "light" and *O*-deuterated forms of methyl α-D-glucopyranoside and methyl β-D-galactopyranoside were also measured simultaneously, by placing the solutions of the OH forms in water (5% of 1,4-dioxane) in 10-mm n.m.r.-tubes and the solutions of the O^2H forms in deuterium oxide (5% of 1,4-dioxane) in

concentric, 5-mm n.m.r.-tubes. The order of the OH and O^2H forms was also reversed. In all cases, only a single, sharp 1,4-dioxane signal was observed, indicating that the chemical shift of 1,4-dioxane is not (or only slightly) affected by deuterium oxide. Those carbon atoms of the glycosides bonded to hydroxyl groups gave two signals, caused by the OH and O^2H forms, whereas the other carbon atoms gave single, sharp signals.

It also appears that the upfield displacement of the ^{13}C-n.m.r. signals (upon *O*-deuteration) is not restricted solely to carbon atoms bonded directly to hydroxyl groups (geminal), but that it can also be observed — to a much smaller extent — for carbon atoms vicinal to hydroxyl groups. Thus, the signals of C-5 of the pyranoses and pyranosides generally show a small, upfield displacement. In addition, the signals of C-3 of the pyranoses and pyranosides, and C-2 and C-3 of the pyranoses, usually show a greater displacement than the signals of other hydroxyl-bonded carbon atoms. On this basis, the original assignments[7] for the C-2 and C-3 signals of methyl α-D-mannopyranoside should probably be reversed, as shown in Tables I and II.

EXPERIMENTAL

General. — Natural-abundance, proton-decoupled, ^{13}C-n.m.r. spectra were measured with a Varian CFT-20 instrument at 20 MHz. The acquisition time was 2.047 s, the pulse width 5μs, and the spectrum width 2 kHz, using 8192 data points. The chemical shifts, taken from the computer print-out, are from Me_4Si, using 1,4-dioxane (5%, v/v) as the internal reference standard at[4] 67.22 p.p.m. (1344.4 Hz).

The solutions in [$^2H_6$]dimethyl sulfoxide were measured in 10-mm tubes, using the solvent deuterium as the internal lock. The water and deuterium oxide solutions were measured in 10-mm tubes containing a concentric, 5-mm tube with deuterium oxide in it.

The reproducibility of the chemical shifts was determined by measuring the spectra of ten, individually prepared solutions of methyl α-D-glucopyranoside (1 g) in 19:1 water–1,4-dioxane (1 mL). The solutions in [$^2H_6$]dimethyl sulfoxide had a concentration of 150 mg/mL of 19:1 (v/v) water–1,4-dioxane, and the aqueous (H_2O and ^2H_2O) solutions had a concentration of 1.00 g/mL of 19:1 (v/v) water–1,4-dioxane. The melting points and optical rotations reported are those of the corresponding "light" compounds.

O-Deuterium exchanges. — The compounds (see Tables I and II) were dissolved in deuterium oxide (50 mL/g), and the solutions were evaporated to dryness prior to dissolution in [$^2H_6$]dimethyl sulfoxide or deuterium oxide.

Anomerization of D-*glucose and* D-*mannose.* — The chemical-shift data for α- and β-D-gluco- and -manno-pyranose were obtained from the spectra of their anomeric mixtures. As anomerization is slow[5,6] in [$^2H_6$]dimethyl sulfoxide solutions, the sugar (300 mg) was first dissolved either in water (15 mL), for the OH forms, or deuterium oxide (15 mL), for the O^2H forms. The solutions were heated, and then evaporated to dryness prior to dissolution in [$^2H_6$]dimethyl sulfoxide (2.0 mL). The relative

intensities of the ^{13}C-n.m.r. signals of both anomers of the sugars showed that the OH and O^{2}H forms had the same composition.

1,2-O-Isopropylidene-α-D-glucofuranose-5,6,6-$^2H_3$ (**1**). — 1,2-*O*-Isopropylidene-α-D-glucofuranose (10 g) was dissolved in deuterium oxide (30 mL), and the solution was evaporated to dryness. Then, deuterium oxide (200 mL) and deuterated Raney nickel[3] (50 mL, settled volume) were added, and the mixture was boiled overnight under reflux. The nickel was filtered off, the filtrate evaporated to dryness, and the solid residue recrystallized from ethyl acetate, to give 1,2-*O*-isopropylidene-α-D-glucofuranose-5,6,6-$^2H_3$; yield 4.5 g (45%), m.p. 160–161°, $[\alpha]_D^{13}$ −12° (*c* 5, water); lit.[9] m.p. 161°, $[\alpha]_D$ −11.8° (*c* 8, water).

Methyl α-D-glucopyranoside-5,6,6-$^2H_3$ (**2**). — Compound **1** (4 g) was dissolved in methanol (100 mL) containing anhydrous hydrogen chloride (1% by wt.), and the solution was boiled overnight under reflux. Then an excess of silver carbonate was added, and the mixture was stirred until neutral. The silver salts were filtered off (Celite), and the filtrate was evaporated to dryness. Two recrystallizations from anhydrous ethanol gave methyl α-D-glucopyranoside-5,6,6-$^2H_3$, yield 2.1 g (60%), m.p. 167°, $[\alpha]_D^{23}$ +153° (*c* 2, water); lit.[10] m.p. 167–168°, $[\alpha]_D$ +157° (*c* 2, water). This compound was also synthesized from 1,2-*O*-isopropylidene-α-D-glucofuranose-5,6,6-$^2H_3$ prepared by reduction of 1,2-*O*-isopropylidene-α-D-*xylo*-hexofuranurono-6,3-lactone-5-ulose[11].

Methyl α-D-glucopyranoside-5-^2H (**3**). — Methyl α-D-glucopyranoside-5,6,6-$^2H_3$ (**2**; 2 g), water, and Raney nickel (W. R. Grace and Co., No. 28) were boiled under reflux overnight. Then, the nickel was filtered off while the mixture was still hot, the filtrate was evaporated to dryness, and the solid residue was recrystallized twice from anhydrous ethanol, to give methyl α-D-glucopyranoside-5-^2H, yield 1.5 g (75%), m.p. 165–166°, $[\alpha]_D^{23}$ +152° (*c* 2, water); lit.[10] m.p. 167–168°, $[\alpha]_D$ +157° (*c* 2, water).

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