Carbohydrate Research, 67 (1978) 479–483

Note

Parameters of ^{17}O-n.m.r. signals of some isotope-enriched monosaccharide derivatives*

PHILIP A. J. GORIN AND MYTOSK MAZUREK

Prairie Regional Laboratory, National Research Council of Canada, Saskatoon, Saskatchewan S7N 0W9 (Canada)

(Received February 10th, 1978; accepted for publication, March 4th, 1978)

It is of interest to assess ^{17}O-n.m.r. spectroscopy as an analytical tool in carbohydrate chemistry, but no reports exist on chemical-shift ranges and line widths, the important factors in determining the resolution of a n.m.r. spectrum. Values have been found for many mono- and di-oxygenated organic compounds having the natural-^{17}O abundance of 0.037% by using continuous-wave[1–4] and Fourier-transform techniques[5,6]. However, the line widths of 45 to 2000 Hz (6 to 260 p.p.m.) were large, in resolution terms, compared with the chemical-shift range of ~600 p.p.m. for oxygen atoms linked to carbon atoms. The line width is due to quadrupole relaxation and the tumbling time of the molecule[7], and decreases with increased temperature and increased speed of tumbling[8].

The line widths of signals of monosaccharides specifically labelled with ^{17}O at levels up to 10 atom-percent, and dissolved in 2,6-lutidine, were so large at room temperature that they were indistinguishable from baseline roll: this appears consistent with their molecular size, which is larger than those of compounds previously examined. Signals were better defined at 100°, when their line widths were 230–600 Hz (see Table I). Although ^{17}O–H decoupling was not possible as the methyl protons of the solvent were used for locking, none of the hydroxy compounds gave such complex signals as the 1:2:1 triplet observed for ^{17}O in acetone[9].

Even at 100°, the line widths of 240–520 Hz were large in comparison with the chemical-shift range of signals of ^{17}O nuclei of primary alcohols (**2**, **11**; Table I), secondary alcohols of linear molecules (**3**, **4**), five-membered rings (**6**, **7**), and equatorial or axial groups in six-membered rings. Significant deshielding occurs, however, with O-1 of 2,3,4,6-tetra-*O*-methyl-α-D-mannopyranose (**5**), as with acetals[4]. In only one case was the shift of the signal of a hydroxyl ^{17}O nucleus affected by the presence of a vicinal substituent. The O-2 atom of D-mannose-*2*-^{17}O diethyl dithioacetal (**3**) was deshielded by the two thioacetal groups, contrasting with O-2 of methyl α-D-mannopyranoside-*2*-^{17}O (**8**), which is unaffected by the vicinal hemiacetal grouping.

* NRCC No. 16683.

TABLE I

CHEMICAL SHIFTS AND HALF-HEIGHT LINE-WIDTHS OF ^{17}O RESONANCES AT VARIOUS TEMPERATURES

Number	*Labelled compound*	*80°*		*100°*	
		Chemical shift (p.p.m.)	*Line width (Hz)*[a]	*Chemical shift (p.p.m.)*[b]	*Line width (Hz)*
Hydroxyl derivatives					
1	$H_2{}^{17}O$	+1	100	−2	80
2	D-Glucitol-*1*-^{17}O	−8	520	−4	280[c]
3	D-Mannose-*2*-^{17}O diethyl dithioacetal	+17	800	+17	520
4	1,2-*O*-Isopropylidene-α-D-glucofuranose-*5*-^{17}O	—	—	−4	260
5	2,3,4,6-Tetra-*O*-methyl-α-D-mannose-*1*-^{17}O[d]	—	—	+40	260
6	1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose-*3*-^{17}O	—	—	+5	310
7	1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose-*3*-^{17}O	+2	400	+4	270
8	Methyl α-D-mannopyranoside-*2*-^{17}O	+2	640	+2	480
9	Methyl β-D-allopyranoside-*3*-^{17}O	—	—	−8	400
10	Methyl α-D-glucopyranoside-*3*-^{17}O[e]	—	—	+3 to +6	—
11	Methyl α-L-glucopyranoside-*6*-^{17}O[e]	—	—	−6	240
Ether derivatives					
12	Methyl α-D-glucopyranoside-*5*-^{17}O[e]	—	—	+48	500
13	Methyl 3-*O*-methyl-β-D-allopyranoside-*3*-^{17}O	—	—	−17	600
14	Acetate of 13	—	—	−17	600
15	Hexa-*O*-methyl-D-glucitol-*1*-^{17}O	−22	330	−25	230
Acetate derivatives					
16	Hexaacetate of 2	+154	720	+149	450
17	Pentaacetate of 3	—	800	+149	520
18	Triacetate of 4	—	—	+164	460
19	3-Acetate of 7	—	540	+162	340

[a]1 p.p.m. = 13.56 Hz at 2.3 T. [b]Convention of expressing chemical shift as described in the Experimental section, paragraph 2. [c]Line width of 180 Hz at 125°. [d]Dissolved in anhydrous 1,4-dioxane. [e]Dissolved in 2,6-lutidine containing *N,N*-dimethylformamide.

Replacement of hydroxyl groups by methoxyl groups resulted[4] in a shielding of 21 p.p.m.* for the primary O-1 of D-glucitol-*1*-^{17}O (**2** and **15**), and 9 p.p.m. for the axial O-3 of methyl β-D-allopyranoside-*3*-^{17}O (**9** and **13**). Also in agreement with previous work[4] is the strong deshielding of 120–168 p.p.m. occurring on *O*-acetylation.

Synthesis of some oxygenated derivatives is difficult, but it appears possible to predict the ^{17}O-n.m.r. spectra of certain simple monosaccharides. For example, glucose should give a single signal at ~ -6 to $+6$ p.p.m. corresponding to O-2, O-3, O-4, and O-6, and the deshielded O-1 and O-5 nuclei should give another composite signal, at $\sim +40$ to $+48$ p.p.m., by analogy with these nuclei in 2,3,4,6-tetra-*O*-methyl-α-D-mannose-*1*-^{17}O (**5**) and methyl α-D-glucopyranoside-*5*-^{17}O (**12**), respectively. Methyl α-D-glucopyranoside should give three ^{17}O signals, as, on methylation of the OH group, the O-1 nucleus would be more shielded, so that its signal would lie between the resonance of O-5 and those of O-2, -3, -4, and -6.

In terms of resolution of the signals of ^{17}O-n.m.r. spectra of monosaccharides, the method suffers in comparison with those wherein proton, carbon-13, and deuterium are the nuclei observed. Furthermore, compounds containing 2 atom-percent of ^{17}O are needed, and many of these are difficult to synthesize by known methods.

EXPERIMENTAL

General. — Oxygen-17 n.m.r. spectra were recorded with a Varian XL-100-15 n.m.r. spectrometer equipped with a Gyrocode Observe Accessory to provide the 13.56-MHz observe frequency. All spectra were obtained by using the Fourier-transform mode with a Computer Alternating Pulse Sequence (CAPS) data-acquisition method, to lower baseline roll in the transformed spectrum.

The acquisition time was 0.02 s and the transients were ~100,000. Under these conditions, 250 acquisition points were used, and the spectral width was 10 kHz. In terms of sensitivity, at 33° a recognizable signal was obtainable by using 1 transient with $H_2{}^{17}O$ (0.1 mL; 10 atom-percent) in 2,6-lutidine (2 mL) contained in a 12-mm diameter tube fitted with a vortex plug. Chemical shifts are expressed in p.p.m. relative to the ^{17}O resonance of 1,4-dioxane in 2,6-lutidine, determined in separate experiments. Under these conditions, recognizable signals could be obtained at 80° upward by using a 200-mg sample of carbohydrate having 2 atom-percent of ^{17}O in one position.

Preparation of ^{17}O*-labelled compounds.* — Samples of $H_2{}^{17}O$ containing 2 and 10 atom-percent of ^{17}O were obtained from Stohler Isotope Chemicals. Oxygen-17 was introduced into carbohydrates by exchange of that in $H_2{}^{17}O$ with ^{16}O in appropriate aldehydes, ketones, or reducing sugars. When the exchanged product was not desired, it was, in most cases, reduced with sodium borohydride as indicated, to

*For the convention adopted for chemical shifts, see the Experimental section, paragraph 2.

give alditols listed in Table I. The incorporation of ^{17}O was measured by mass spectrometry in the chemical ionization mode.

D-*Glucitol*-1-^{17}O (**2**) *and its hexamethyl ether* (**15**) *and hexaacetate* (**16**). — A solution of D-glucose (300 mg) in $H_2{}^{17}O$ (10 atom-percent; 0.5 mL) was heated for 18 h at 100°, and then sodium borohydride (50 mg) was carefully added, with cooling. Compound **2** was isolated in the usual way, and one-third of it was methylated by the method of Kuhn *et al.*[10], giving **15**; the rest was acetylated with Ac_2O–pyridine to afford compound **16**.

D-*Mannose*-2-^{17}O *diethyl dithioacetal* (**3**) *and its pentaacetate* (**17**). — A solution of D-*arabino*-hexosulose 1-(diethyl dithioacetal)[11] (400 mg) in 1,4-dioxane (0.8 mL) containing $H_2{}^{17}O$ (2 atom-percent; 0.4 mL) was kept for 2 h at 80°. Sodium borohydride (60 mg) was added, and, after reduction was complete, the solution was processed; examination by g.l.c. [4:1 (v/v); chloroform–ethanol; spray: 50% aqueous sulfuric acid] then showed two spots, one being present in only a very small proportion. Compound **3** (305 mg), m.p. and mixed m.p. 130–132°, was obtained by crystallization from aqueous ethanol. Its pentaacetate (**17**) was obtained by acetylation with acetic anhydride–pyridine.

2,3,4,6-Tetra-O-*methyl*-α-D-*mannose*-1-^{17}O (**5**). — A solution of the unlabelled sugar (100 mg) in $H_2{}^{17}O$ (10 atom-percent: 0.2 mL) was kept overnight at 100°. Dry 1,4-dioxane (2 mL) was added, and the solution was evaporated. The residue was dissolved in dry 1,4-dioxane (2 mL), and the solution, which contained mainly the 1-labelled, α-D anomer, was used in spectral determinations.

1,2-O-*Isopropylidene*-α-D-*glucofuranose*-5-^{17}O (**4**) *and methyl* α-D-*gluco-pyranoside*-5-^{17}O (**12**). — 1,2-*O*-Isopropylidene-α-D-*xylo*-hexofuranurono-6,3-lactone-5-ulose[12] (300 mg) was equilibrated (at O-5) in 1,4-dioxane (0.6 mL) containing $H_2{}^{17}O$ (0.3 mL; 2 atom-percent) for 2 h at 80°. Sodium borohydride (74 mg) was then added, and the resulting **4** (223 g) was obtained crystalline from ethyl acetate. Its triacetate (**18**) was prepared by acetylation with Ac_2O–pyridine, and, from **18**, compound **12** was obtained by refluxing in 3% methanolic hydrogen chloride for 2 h. An improved isotope yield was obtained by addition of 0.3 g of carrier. For complete dissolution, 20% of *N*,*N*-dimethylformamide was added to the n.m.r. solvent, namely, 2,6-lutidine.

1,2:5,6-Di-O-*isopropylidene*-α-D-*allofuranose*-3-^{17}O (**7**), *its 3-acetate* (**19**), *and 1,2:5,6-di*-O-*isopropylidene*-α-D-*glucofuranose*-3-^{17}O (**6**). — A solution of 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-2-ulose[13] (1.0 g) in 1,4-dioxane (2 mL) containing $H_2{}^{17}O$ (1 mL: 10 atom-percent) was heated for 30 min at 80°, and cooled. Sodium borohydride (0.2 g) was added, and, following isolation, the mixed di-*O*-isopropylidene derivatives were fractionated on a column of silicic acid, using chloroform as the eluant. The first fraction was crystallized from ether–heptane, giving compound **7** (0.26 g). The 3-acetate (**19**) of **7** was obtained by acetylation with Ac_2O–pyridine. To the second fraction, which was a mixture, was added 0.8 g of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose and the product was recrystallized twice from ether–heptane to give **6**.

Methyl α-D-mannopyranoside-2-^{17}O (**8**). — Compound **8** was obtained from D-mannose-*2-^{17}O* diethyl dithioacetal by the method outlined by Pacsu[14].

Methyl β-D-allopyranoside-3-^{17}O (**9**), *its 3-methyl ether* (**13**), *and methyl α-D-glucopyranoside-3-^{17}O* (**10**). — Compounds **9**, **13**, and **10** were prepared by the action of refluxing methanolic hydrogen chloride (3%) on **7**, its 3-methyl ether (prepared from **7** by the action of MeI–Ag_2O), and **6**. Acetylation of **13** with Ac_2O–pyridine gave the 2,4,6-triacetate (**14**).

Methyl α-L-glucopyranoside-6-^{17}O (**11**). — Methyl α-D-*glycero*-L-*gluco*-heptopyranoside[15] (300 mg) was oxidized with 1 molar equivalent of sodium metaperiodate in water (10 mL) at 4°. After 18 h, the solution was de-ionized with resins, and evaporated to a syrup; this was equilibrated in $H_2{}^{17}O$ (0.5 mL; 2 atom-percent) for 1 h at 100°. Sodium borohydride (60 mg) was added, and, after processing in the usual way, compound **11** was crystallized from ethanol.

REFERENCES

1 H. E. Weaver, B. M. Tolbert, and R. C. LaForce, *J. Chem. Phys.*, 23 (1955) 1956–1957.
2 S. S. Dharmatti, K. J. S. Rao, and R. Vijayaraghavan, *Nuovo Cimento*, 11 (1959) 656–669.
3 H. A. Christ, *Helv. Phys. Acta*, 33 (1960) 572–576.
4 H. A. Christ, P. Diehl, H. R. Schneider, and H. Dahn, *Helv. Chim. Acta*, 44 (1961) 865–880.
5 C. Delseth and J.-P. Kintzinger, *Helv. Chim. Acta*, 59 (1976) 466–475.
6 D. Canet, C. Goulon-Ginet, and J. P. Marchal, *J. Org. Magn. Reson.*, 22 (1976) 537–542.
7 H. A. Christ and P. Diehl, *Helv. Phys. Acta*, 36 (1963) 170–182.
8 S. W. Rabideau and J. A. Jackson, *J. Chem. Phys.* 41 (1964) 3405–3410.
9 J. Reuben, A. Tzalmona, and D. Samuel, *Proc. Chem. Soc.*, (1962) 353.
10 R. Kuhn, H. Trischmann, and I. Löw, *Angew. Chem.*, 67 (1955) 32.
11 S. Bayne, *Methods Carbohydr. Chem.*, 2 (1963) 421–423.
12 W. Mackie and A. S. Perlin, *Can. J. Chem.*, 43 (1965) 2921–2924.
13 K. Onodera and N. Kashimura, *Methods Carbohydr. Chem.*, 6 (1972) 331–336.
14 E. Pacsu, *Methods Carbohydr. Chem.*, 2 (1963) 354–367.
15 H. S. Isbell and H. L. Frush, *J. Res. Natl. Bur. Stand.*, 24 (1940) 125–151.