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Note

Effect of deuteration on the ^{13}C–^{1}H nuclear Overhauser effect of methyl α-D-glucopyranoside*

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Monosaccharides having a ring proton replaced by a deuteron give ^{13}C-n.m.r. spectra that differ from those of undeuterated sugars. Under routine conditions for acquisition of sugar spectra, the ^{13}C signal of the appended carbon atom "disappears"[1], as originally observed in the spectra, obtained without proton decoupling of monosubstituted benzenes[2]. The effect on the resonances of adjacent carbon atoms is less pronounced, but they generally undergo a small displacement, which is frequently upfield[3] by 0.04–0.12 p.p.m. However, a downfield shift can also occur, as in the case of [$^{2}H_6$]acetone[4].

The disappearance of the deuterated carbon resonances of monosubstituted benzenes is due to two factors, (a) their long spin–lattice relaxation times (T_l), which require pulse-delay intervals far in excess of those usually used for the acquisition of the resonances of protonated carbon atoms, and (b) the occurrence of ^{13}C–^{2}H coupling. In the case of carbohydrate spectra, obtained under conditions of proton decoupling, one question remains unresolved. This is the extent of diminution of signals, if any, that could arise from the lack of ^{13}C–^{1}H nuclear Overhauser effect (NOE). In low-molecular-weight molecules, this has a value close to the maximum of 2.98 (or NOE factor $\eta = 1.98$). To measure the NOE, a series of partly deuterated methyl α-D-glucopyranosides were examined.

Measurements[5] of T_l values of carbon atoms of these derivatives were carried out on solutions in deuterium oxide or water. In some experiments, the determinations were facilitated by reduction of the complex deuterated resonances to singlets under conditions of simultaneous proton- and deuterium-decoupling[6,7]. As can be seen from Table I, T_l values of deuterated carbon atoms of the (1-^{2}H), (3-^{2}H), (5-^{2}H), (6-$^{2}H_2$), (5,6-$^{2}H_3$), and (2,3,4,6-$^{2}H_5$) derivatives are from 4.2 to 15.5 s, sufficiently long to suppress signals in ^{13}C-n.m.r. spectra, obtained with conventional acquisition-times of 0.4–0.8 s.

According to theoretical considerations, the initial rate of build-up of NOE

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TABLE I

T_1 VALUES OF CARBON ATOMS OF VARIOUS DEUTERATED DERIVATIVES OF METHYL α-D-GLUCOPYRANOSIDE IN WATER OR DEUTERIUM OXIDE SOLUTION[a]

Derivative	*Solvent*	*Decoupled nuclei*	T_1 *value of each carbon atom(s)*[a,b,c]						
			C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCH₃*[c]
(1-^2H)	H_2O	^1H	$\underline{5.3}$	0.9	0.9	0.9	0.9	0.5	3.1
	H_2O	$^1H,^2H$	$\underline{7.7}$	0.9	0.8	0.7	0.9	0.5	2.8
	D_2O	^1H	$\underline{10.3}$	0.8	0.8	0.8	0.8	0.5	2.7
	D_2O	$^1H,^2H$	$\underline{8.7}$	1.0	1.9	1.0	0.9	0.55	3.1
(3-^2H)	H_2O	^1H	0.8	0.9	$\underline{6.6}$	0.9	0.9	0.5	3.3
	H_2O	$^1H,^2H$	1.0	1.0	$\underline{7.2}$	1.0	1.0	0.6	3.3
	D_2O	^1H	0.9	1.0	$\underline{8.2}$	1.0	1.0	0.5	2.7
(5-^2H)	H_2O	^1H	0.8	0.9	0.9	0.8	$\underline{4.1}$	0.5	3.0
(6-^2H)	D_2O	^1H	0.9	0.9	0.9	0.9	0.9	$\underline{0.8}$	2.2
(6-$^2H_2$)	H_2O	^1H	0.9	0.8	0.8	0.8	0.8	$\underline{4.8}$	2.5
	D_2O	^1H	0.9	0.9	0.9	0.9	0.9	$\underline{5.0}$	2.5
	D_2O	$^1H,^2H$	0.8	0.8	0.85	0.9	0.85	$\underline{5.0}$	2.5
($5,6$-$^2H_3$)	H_2O	^1H	1.1	1.2	1.3	1.2	$\underline{7.3}$	$\underline{6.6}$	3.5
	H_2O	$^1H,^2H$	1.3	1.2	1.3	1.2	$\underline{9.2}$	$\underline{7.6}$	3.9
	D_2O	^1H	0.95	1.0	1.0	1.0	$\underline{7.6}$	$\underline{7.2}$	2.9
($2,3,4,6$-$^2H_5$)	H_2O	^1H	1.3		$\underline{9.7}$	$\underline{10.3}$	1.6	$\underline{5.6}$	3.4
	H_2O	$^1H,^2H$	1.5	$\underline{11.5}$	$\underline{12.9}$	$\underline{11.9}$	1.55	$\underline{8.8}$	4.4
	D_2O	^1H	1.0	$\underline{9.2}$	$\underline{12.2}$	$\underline{9.5}$	1.1	$\underline{12.4}$	2.8
	D_2O	$^1H,^2H$	1.0	$\underline{10.3}$	$\underline{15.5}$	$\underline{12.0}$	1.0	$\underline{7.0}$	3.55

[a] Obtained under conditions of ^1H decoupling and simultaneous ^1H and ^2H decoupling. [b] Underlined T_1 values are those of carbon atoms linked to a deuterium atom. [c] Signal assignments of C-1 to C-6 according to reference 3

depends on the inverse sixth power of the internuclear distance between the ^{13}C nucleus and the adjacent proton. In order to obtain experimental η values, several compounds having quaternary carbon atoms have been examined. These include sucrose, whose quaternary C-2 of the D-fructofuranosyl unit has η ~2, a value close to the maximum values found for protonated carbon atoms[8]. The related quaternary C-2 of inulin, a (2→6)-linked α-D-fructofuranan, has η 0.69, a value that is low, owing to the limited, segmental motion of the polymer, but which differs little from the values[9] of monoprotonated carbon atoms of C-2, -4, and -5. However, in the case of the quaternary carboxyl group of the barium salt of methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranoside, the carbon atom is not affected by a proton on the adjacent acetal carbon atoms, and a value of η 0.4 was observed, lower than that of η 2 of the other carbon atoms[10]. These data, obtained for carbohydrates, were analogous to values obtained for pyrene, which were η 1.25 for quaternary atoms with protons on adjacent carbon atoms and η 0.4 for those buried in the interior of the molecule, and which have no geminal protons (see ref. 11).

By use of the aforementioned T_1 values, it was possible to calculate experimental parameters for accurate determination of η values of the carbon atoms of the deuterated methyl α-D-glucopyranosides. A comparison was made of the signal integral-ratios, obtained under proton coupled and decoupled conditions[12,13]. The results, as recorded in Table II, show that carbon atoms attached to deuterons, and

TABLE II

η VALUES OF CARBON ATOMS OF VARIOUS DEUTERATED DERIVATIVES OF METHYL α-D-GLUCOPYRANOSIDE IN WATER OR DEUTERIUM OXIDE SOLUTION

Derivative	*Solvent*	*η Value of each carbon atom*[a]						
		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCH_3*
(1-^{2}H)	H_2O	$\underline{0.78}$	2.02[b]	1.93	1.87	2.02[b]	1.70	2.09
	D_2O	$\underline{0.42}$	1.79	1.63	1.92	2.03	1.76	1.81
(3-^{2}H)	H_2O	1.92	1.90	$\underline{0.93}$	2.10	1.64	1.94	1.69
	D_2O	1.80	1.99[b]	$\underline{0.69}$	2.02	1.99[b]	1.93	
(5-^{2}H)	H_2O	1.71		1.98	1.89	$\underline{1.11}$	2.20	2.03
	D_2O	1.89	1.92	2.02	2.15	$\underline{1.17}$	2.27	1.98
(6-^{2}H)	H_2O	1.59	1.85	1.99	1.94	1.97	$\underline{1.60}$	1.62
	D_2O	1.93	2.00	1.99	2.30	2.01	$\underline{1.95}$	1.85
(6-$^2H_2$)	H_2O	1.62	1.97[b]	1.91	2.19	1.97[b]	$\underline{0.63}$	1.62
	D_2O	1.92	1.86[b]	1.85	1.83	1.86[b]	$\underline{0.38}$	1.92
(5,6-$^2H_3$)	H_2O	1.82	1.68	1.50	1.63	$\underline{0.75}$	$\underline{0.26}$	1.57
	D_2O	2.00	1.72		1.84	$\underline{0.94}$	$\underline{0.20}$	1.95
(2,3,4,6-$^2H_5$)	H_2O	2.32	←Average, $\underline{0.93}$→[b]			—	$\underline{0.60}$	1.66
	D_2O	1.93	$\underline{0.64}$	$\underline{0.24}$	$\underline{0.28}$	2.25	$\underline{0.31}$	1.77
			←Average, $\underline{0.39}$→[b]					

[a]Obtained under conditions of ^{1}H decoupling only; η values of carbon atoms linked to a deuterium atom are underlined. [b]These η values are the average of two or more overlapping resonances. Problems were often experienced in integrating C-2 and -5 signals because of their proximity.

TABLE III

COMPARISON OF η VALUES OF DEUTERATED CARBON ATOMS OF VARIOUS DEUTERATED DERIVATIVES OF METHYL α-D-GLUCOPYRANOSIDE IN THE OH AND O^{2}H FORMS IN VARIOUS SOLVENTS

Derivative	*Observed atom*	*η Value[a] of carbon atoms for solutions in*			
		H_2O	*D_2O*	*$(^2H_6)Me_2SO$*	
				OH Form	*O^{2}H Form*
(1-^2H)	C-1	0.78	0.42	1.16	0.62
(3-^2H)	C-3	0.93	0.69	1.00	0.88
(5-^2H)	C-5	1.11	1.17	1.15	1.17
(6-^2H)	C-6	0.63	0.38	1.00	0.50
$(5,6\text{-}^2H_3)$	C-5	0.75	0.91	0.98	1.04
	C-6	0.26	0.20	0.72	0.43
$(2,3,4,6\text{-}^2H_5)$	C-6	0.60	0.31	1.04	0.52

[a]Obtained under conditions of ^{1}H decoupling

TABLE IV

T_1 VALUES OF CARBON ATOMS OF PARTLY DEUTERATED DERIVATIVES OF METHYL α-D-GLUCOPYRANOSIDE AS OH AND O^{2}H FORMS, IN $(^2H_6)$DIMETHYL SULFOXIDE SOLUTION

Derivative	*Form in $(^2H_6)$-Me_2SO*	T_1 *value of each carbon atom*[a]						
		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCH_3*
(1-^2H)	OH	3.0	0.4	0.4	0.4	0.4	0.2	1.2
	O^{2}H	3.0	0.4	0.4	0.4	0.4	0.2	1.2
(3-^2H)	O^{2}H	0.3	0.3	2.7	0.3	0.3	0.2	1.3
(5-^2H)	O^{2}H	0.3	0.4	0.4	0.4	1.9	0.2	1.2
	OH	0.3	0.3	0.3	0.3	0.3	1.8	1.2
(6-^2H_2)	O^{2}H	0.3	0.3	0.3	0.3	0.3	2.0	1.0
$(5,6\text{-}^2H_3)$	O^{2}H	0.3		0.3	0.3		2.2	1.1
$(2,3,4,6\text{-}^2H_5)$	O^{2}H	0.4	4.9	4.6	5.1	0.4	3.1	1.0

[a]TISM measurements were performed with ^{1}H decoupling only. Underlined T_1 values are those of carbon atoms linked to a deuterium atom

which have no appended protons, experience a considerable lack of NOE. The η values of ring carbon atoms range from 0.42 to 1.17, values that are lower than those obtained for sucrose. With glucosides dideuterated at C-6, η 0.38 was observed for C-6 of the (6-^2H_2) derivative, and $\eta = 0.20$ for C-6 of the $(5,6\text{-}^2H_3)$ derivative, in which neighboring protons are more remote.

Not surprisingly, an increase in η values of most of the deuterated resonances was observed when the solvent was changed from deuterium oxide to water. Such an effect could be due either to the influence of the solvent protons, as reported for solutions of carbon tetrachloride and carbon disulfide in alkanes[14], or to the protons of appended hydroxyl groups (or both). Increases in η values were observed for deuterated carbon atoms bearing hydroxyl groups in the (3-^{2}H), (6-$^2H_2$), (5,6-$^2H_3$), and (2,3,4,6-$^2H_5$) derivatives, but not with the nonhydroxylated C-5 of the (5-^{2}H) derivative (Table III). This effect is apparently due to the presence of protons of OH groups in place of deuterons of O^{2}H groups, rather than to intermolecular effects. This conclusion was confirmed by comparison of the η values of appropriate deuterated carbon atoms of the CHO^{2}H and CHOH forms in ($^2H_6$)dimethyl sulfoxide [a solvent that gave rise to conveniently short T_l values (Table IV)], since a comparable increase of NOE was observed, except in the case of C-5 of the (5-^{2}H) derivative.

An apparently anomalous result was obtained with methyl α-D-(1-^{2}H)glucopyranoside whose η value for C-1 increased from 0.42 to 0.78 on changing the solvent from deuterium oxide to water, despite the lack of an OH-1 group. However, the suggested intermolecular solvent effect gave, at most, only a minor contribution, since in ($^2H_6$)dimethyl sulfoxide a similar increase from 0.62 to 1.16 occurred on going from the O^{2}H to the OH derivative. It appears that C-1 could be subject to NOE from protons of more-distant groups.

Complete ^{13}C-n.m.r. spectra of deuterated compounds can be obtained when a pulse delay is used in excess of the T_l value of the deuterated carbon atom, and when the amount of sample is sufficient to compensate for the lack of NOE. If decoupled spectra are desired, a system having simultaneous proton and deuterium coupling is required. Maximum signal to noise ratios are obtained using the OH form of the glycoside in ($^2H_6$)dimethyl sulfoxide solution.

EXPERIMENTAL

T_l and η values were measured on degassed, 20% solutions of deuterated methyl α-D-glucopyranosides, with a Varian XL-100 n.m.r. spectrometer, coupled to a Nicolet 1180 data-acquisition system. The inversion-recovery method, as modified by Freeman and Hill[5], was used for estimation of T_l, and is called TISM in the Nicolet Ft n.m.r. computer programs. Values were obtained under conditions of ^{1}H decoupling, or simultaneous ^{1}H and ^{2}H decoupling. The latter effect was obtained by linking a Varian model V3512-1 100-MHz, noise-decoupler by a matching network with standard XL-100 decoupler and decoupling coils. A ^{19}F lock was used when irradiating simultaneously at 100 and 15 MHz. Decoupling powers were: $\gamma H_H/2\pi = 1980$ Hz and $\gamma H_D/2\pi = 570$ Hz. The system was constructed with the aid of information provided by McInnes[6] and principally the circuit diagram of Roznyatovskii *et al.*[7].

NOE values were determined by comparison of signal integrals obtained as follows[12,13]. For the maximized NOE spectrum, the decoupler was operated continuously in order to obtain maximum NOE. For the other spectrum, the decoupler was operated only during the acquisition time. In each set of spectra, a 90° pulse

(18.8 μs) was used with a delay between pulses of at least five times the longest T_1 value of the resonances in the spectrum. The parameters in each run, such as the number of transients, line broadening, etc., were identical.

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