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Preliminary communication

Applications of catalytic, hydrogen–deuterium exchange in ^{13}C-n.m.r. spectroscopy

FELIPE BALZA, NATSUKO CYR, GORDON K. HAMER, ARTHUR S. PERLIN,
Department of Chemistry, McGill University, Montreal (Canada)
HANS J. KOCH, and RONALD S. STUART
Merck Frosst Laboratories, Kirkland, Quebec (Canada)
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Deuteration is used extensively to facilitate the analysis of ^{13}C-n.m.r. spectra; thus, replacement (on a carbon atom) of a proton by deuterium may effectively remove the signal of the α-carbon atom from the spectrum[1,2] and shift the signal of a β-carbon atom[3], thereby identifying these signals, or it can demonstrate the presence or absence of coupling between the proton and various carbon atoms in the molecule. The introduction of a deuterium atom is often an involved process: in the sugar series[4], for example, it commonly requires synthesis of a suitably protected derivative that can be oxidized regioselectively, and the product reduced stereoselectively with a deuteride.

Recently, it has been shown by Koch and Stuart[5] that carbon-attached hydrogen atoms of hydroxymethyl groups of various carbohydrate derivatives undergo ready ^{1}H–^{2}H exchange in deuterium oxide in the presence of Raney nickel catalyst. We have utilized this reaction with a number of compounds in conjunction with ^{13}C-n.m.r. studies, and have found it to be a remarkably convenient aid in the assignment of signals and measurement of ^{13}C–^{1}H coupling. Some examples are summarized here.

As already reported[5], methyl α-D-glucopyranoside is fully deuterated at carbon atoms 2, 3, 4, and 6, to give **1**, by treatment with deuterium oxide under reflux for 18 h in the presence of Raney nickel catalyst prewashed with deuterium oxide*. The ^{1}H-coupled ^{13}C spectrum of **1** shows a signal for C-5 as a doublet of doublets ($^{1}J_{C\text{-}5-H\text{-}5}$ 142 Hz, $^{3}J_{C\text{-}5-H\text{-}1}$ 6.5 Hz), whereas, prior to deuteration, the signal is a complex multiplet. Hence, the simplified splitting-pattern for **1** furnishes a value of 6.5 Hz for vicinal coupling between

*The use of $^{3}H_2O$ instead of $^{2}H_2O$ affords, as would be expected, *C*-tritiated methyl α-D-glucopyranoside; *e.g.*, the uptake of the tracer by the glucoside (0.67 g) when heated for 24 h under reflux in $^{3}H_2O$ (5 mL; 4×10^5 dpm/g) containing a suspension of Raney nickel (4 g, wet wt.), corresponded to ~2.5 atoms of tritium/molecule. Judging by ^{13}C-n.m.r. measurements of ^{2}H-exchange rates for the glucoside, it is likely that the tritium-exchange had occurred mainly with H-2, H-4, and H-6(*S*).

an sp^3-hybridized, ^{13}C atom and an *anti*-oriented ^1H, *through oxygen* ($^{13}C–O–C–^1H$ pathway), information that had not previously been accessible[6]. A similar application involves the determination of coupling between ^{13}C-4 and H-1 and H-2 of methyl α-D-mannofuranoside as an aid in the conformational analysis of this glycoside: catalytic exchange introduced deuterium selectively at carbon atoms 3, 5, and 6, to give **2**, for which the C-4 signal (a complex, multiplet structure for the initial compound) was a doublet of doublets ($^1J_{C4-H4}$ 144 Hz, $^3J_{C4-H1\ (or\ H2)}$ 3 Hz). Selective ^1H-decoupling showed that the smaller splitting was due to H-1. In these circumstances, not only was the C-4 signal simplified but, owing to replacement of most of the other protons, it was easier to carry out the selective, ^1H-decoupling experiment.

An example of how the exchange reaction has been utilized in assigning ^{13}C resonances is afforded by its application to methyl 3,6-anhydro-α-D-galactopyranoside. Only H-2 and H-4 of this anhydride would be expected to exchange. After treatment of this compound with D_2O–Ni for 2 h, to give **3**, the ^1H-decoupled spectrum (see Fig. 1) shows that one of those protons is far more rapidly exchangeable than the other; *i.e.*, there is only one, relatively weak, signal (~0.4 C, ~60% deuteration), at 70.4 p.p.m. This signal must be due to C-2, because the C-1 signal (the only one readily assignable initially) exhibits a partial upfield-displacement (–0.05 p.p.m.), attributable[3,7] to the β-isotope-effect of the 2-deuterium atom. Because the signal at 82.2 p.p.m. also appears as two components ($\Delta\delta$ = –0.07 p.p.m.), it may be assigned to C-3 (*i.e.*, to the other carbon atom β to the deuterium atom). Prolonged exchange-treatment (24 h) of **3** caused virtual disappearance of the C-2 signal and, in addition, of the signal at 71.3 p.p.m.; accordingly, the latter must be that of C-4 (which bears the second hydroxyl group). As conventional, off-resonance decoupling confirmed that the signal at 70.2 p.p.m. (triplet) is that of C-6, the remaining signal (at 78.5 p.p.m.) must be that of C-5.

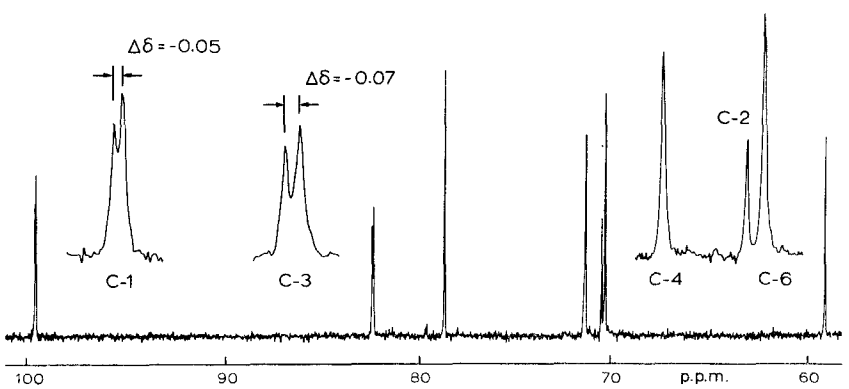
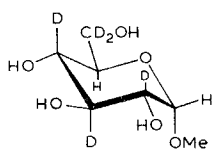


Fig. 1. ^{13}C-N.m.r. spectrum (^1H-decoupled), at 22.63 MHz, of methyl 3,6-anhydro-α-D-galactopyranoside (**3**) that is 60%-deuterated at C-2. [Spectrum recorded for D_2O solution (250 mg/mL) at 35°; chemical shifts given in p.p.m. relative to external Me_4Si. Portions of the spectrum are shown on an expanded scale (inset, C-1 and C-3, x 10); inset, C-4, C-2, and C-6, x 5)].

With ketopyranoses, the C-1 and C-6 signals are frequently close together. As found for methyl α-L-sorbopyranoside, however, deuteration afforded a ready distinction between these two signals, because the protons on C-1, but not those on C-6, are readily exchangeable (see formula **4**). This experiment confirmed earlier assignments[8] for C-1 and C-6.

Applications to higher saccharides are also feasible. Following a 24-h, $^1H-^2H$-exchange treatment, the spectrum of deuterated methyl β-maltoside consisted of six signals, attributable to the four carbon atoms engaged with the oxygen atoms in the pyranoid rings and in the two glycosidic bonds, namely, C-1, C-5, C-1′, and C-5′, and C-4′ and CH_3 (see **5**), *plus* a residual signal due to slow exchange of H-2′. The analogous product (**6**) was obtained from methyl β-cellobioside. In this way, it was found that earlier assignments made for[9] C-3′ and C-5′ and for[9,10] C-2 and C-5 of the maltoside, and also for C-3 and C-5 and for C-3′ and C-5′ of the cellobioside[9,10] should be reversed. In a related application, deuteration of cyclohexaamylose (**7**) permitted a distinction between the closely spaced C-2 and C-5 signals, as only C-2 bears an exchangeable proton. When the latter was replaced (in addition to H-6,6′ and ~50% of H-3) by treatment with D_2O–Ni for 24 h, it was found that the earlier assignments[10] of 73.7 and 73.5 p.p.m. for the C-2 and C-5 signals, respectively, should be reversed*. Glycogen and soluble starch were resistant to deuterium exchange under the same conditions. The ready availability, through exchange, of such extensively deuterated oligosaccharides as **5**, **6**, and **7** is proving to be of value for conformational analysis involv-



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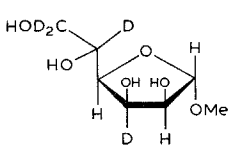

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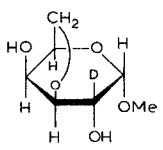

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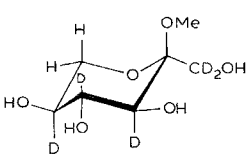

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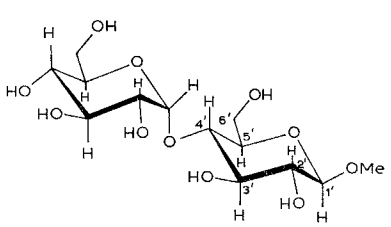

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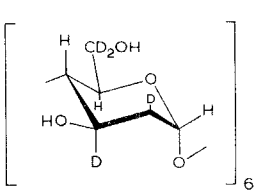
6 (β-D for 1′→4)



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*The fact that deuterium was introduced at C-3 of **7** is of particular interest to a consideration of stereochemical aspects of these $^1H-^2H$ exchange-reactions. X-Ray crystallography has shown[11] that the C-3–H-3 bond is on the inner surface of the torus, and this might have been expected to render it relatively inaccessible to catalyst particles. Another observation related to the geometry of the catalytic exchange concerns the two 6-protons of methyl α-D-glucopyranoside. During the formation of **1**, H-6(*S*) is replaced 3–4 times as rapidly as H-6(*R*), a process readily examined[12] by the $^1H-^1H$ coupling-pattern with non-exchanged H-5.

ing measurements of inter-residue $^{13}C-^{1}H$ coupling[13,14], because the ^{1}H-coupled spectra of the deuterated molecules are relatively less complex.

Although the replacement of ^{1}H by ^{2}H in the examples reported by Koch and Stuart[5] (and those already cited) takes place primarily with retention of the configuration of the carbon atom of the hydroxymethyl group, inversion is also frequently observed. However, inversion is a much slower process than deuteration, and it is likely to become prominent only during prolonged exchange-treatments. In some instances, isomerization is relatively facile, as with methyl α-D-galactopyranoside, exchange at C-4 of which is accompanied by 32% inversion thereat in 24 h. Similarly, in ensuring a high percentage of incorporation of deuterium at C-2 and C-4 of **3**, ready inversion at C-2 (20% in 24 h) was also promoted**. These isomerizing side-reactions are the subject of further studies to be described, but the fact of their occurrence means that some applications of the deuterium-exchange could suffer interference to a greater or lesser extent.

In general, it may prove adequate, or, indeed, preferable, to effect only partial deuteration, as illustrated with **3**. Because this would require a relatively short exchange-period, complications due to the accompanying formation of diastereoisomers would be minimized. Clearly, each compound must be treated as an individual case. However, as the experimental procedure is simple and the yield of product is very high, the examination of a given compound could conveniently embody two or three successive, short exchange-treatments, with the product of each being checked by ^{13}C-n.m.r. spectroscopy.

Purification of the products of the deuteration reaction with an ion-exchange resin is strongly advisable, to ensure removal of paramagnetic metal ions that may cause broadening of the ^{13}C signals.

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