Journal of

The Chemical Society,

Chemical Communications

NUMBER 15/1977 3 AUGUST

Easy Identification of Hydroxy-bearing Carbon Atoms in ^{13}C Nuclear Magnetic Resonance Spectroscopy: a New Method for Signal Assignment in Carbohydrates

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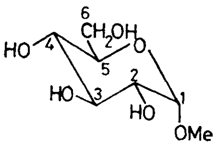
Summary By observing the isotopic chemical shifts when OH groups are replaced by OD under conditions of low exchange rate, it is possible to differentiate hydroxy-bearing carbon atoms in carbohydrates using ^{13}C n.m.r. spectroscopy at 62·8 MHz.

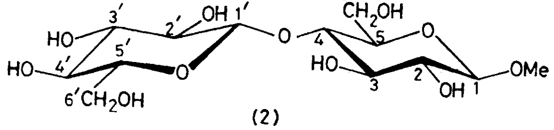
FULL and reliable assignment of the ^{13}C n.m.r. spectra of classical mono- and poly-saccharides is the first step towards utilizing ^{13}C n.m.r. spectroscopy as a routine analytical technique for carbohydrates.

Conventional methods of assignment are based on comparison between similar compounds, particularly by selective substitution. Thus, substitution of a hydroxy-group induces a substantial downfield shift of the directly bonded carbon atom, and a smaller shift upfield of the adjacent carbon atom (β-effect).[1] However, many errors have arisen through exclusive use of such chemical-shift methods, and two unambiguous methods are now in use, namely: selective heteronuclear spin-decoupling[2] and selective spin-labelling, either by ^{13}C or by deuterium.[3,4] In the carbohydrate field, the initial assignment of the proton spectrum is often very difficult for free sugars, and restricts the scope of the heteronuclear decoupling approach. In the second method, an isotopic β-effect is observed (replacement of ^{13}C–C–H by ^{13}C–C–D) allowing identification of neighbouring carbon atoms.

Here we show how a similar β isotopic effect, achieved by partial deuteriation of hydroxy-groups, allows unambiguous identification of hydroxy-bearing carbon atoms (replacement of ^{13}C–O–H by ^{13}C–O–D). For this purpose, three experimental conditions are necessary: (a) operation with a high-field spectrometer (^{13}C at 62·8 MHz), as the isotopic effect is small ($\Delta\delta$ *ca.* $-0{\cdot}08$ p.p.m.); (b) operation under conditions of low exchange rate between hydroxy-groups (for instance in dimethyl sulphoxide at 20 °C); and (c) utilisation of a partially deuteriated mixture. The chemical shifts of carbon atoms are often quite sensitive to the experimental conditions, and comparison between two different spectra (OH and then OD) is, in fact, quite difficult.



(1)



(2)

As a typical example, methyl α-D-glucopyranoside (**1**) was chosen as all the carbon atoms have been reliably assigned (through ^{13}C labelling at C-1 and use of ^{3}J coupling-con-

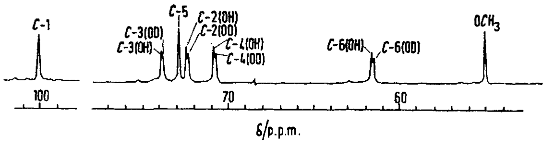


FIGURE 1. ^{13}C n.m.r. spectrum at 62·8 MHz of methyl α-D-glucopyranoside (**1**) containing OD and OH in a 1 : 2 ratio; 30% solution in $(CD_3)_2SO$, 25 °C, 2000 scans, proton-noise decoupled.

stants).[3] Figure 1 shows the spectrum of (**1**) having OH and OD groups in a 2 : 1 ratio. Isotopic chemical shifts are seen for C-2, C-3, C-4, and C-6, thus permitting direct and clear identification of C-5. Raising the temperature causes the doublets to collapse at *ca.* 80 °C allowing, in principle, the direct measurement of the exchange rate for each of the hydroxy-groups.

When disaccharides are examined under the same experimental conditions, the signals of the hydroxy-bearing carbon atoms are not usually resolved as doublets, but the differences in intensity of the signals are large enough for unambiguous identification. Figure 2a shows, as an example, the ^{13}C n.m.r. spectrum of methyl β-D-cellobioside (**2**) containing 2/3 OH and 1/3 OD groups, where the two C-5 atoms could be identified by their intensities. In fact a control experiment with the undeuteriated hydroxy compound under the same experimental conditions is necessary to ensure that no other sources of intensity differences are involved. Better results were obtained by lowering temperature to 14 °C: Figure 2b shows doublets for hydroxy-bearing carbon signals of (**2**). In this example, the assignment of carbon atoms has also been confirmed by ^{1}H–^{13}C heteronuclear spin-decoupling experiments, as the signal assignments for C-3′ and C-5′ were reversed in a previous report.[5]

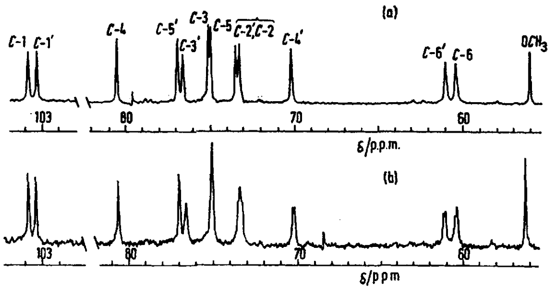


FIGURE 2. (a) Spectrum of methyl β-D-cellobioside (**2**) under the same experimental conditions as described for Figure 1. Primed numbers denote carbon atoms of the unsubstituted D-glucopyranosyl group. (b) Spectrum of methyl β-D-cellobioside (OH : OD 1 : 1) at 14 °C.

The method used here allows unambiguous assignment of hydroxy-bearing carbon atoms, and is now in current use in our laboratory for more complex examples such as cycloamyloses, amylose, and other polysaccharides. In these cases, a 1 : 1 mixture of OH and OD derivatives gives the best experimental results.[6]

Although only carbohydrates have been used as examples to test this technique, it is clear that using this isotopic effect provides a new, simple, and very general method for assignment of any hydroxy compound having carbon atoms resonating in the range 60—80 p.p.m.

We thank F. W. Wehrli and B. Casu for helpful discussions and Professor Derek Horton for his suggestions in the formulation of this manuscript.

(*Received*, 5*th April* 1977; *Com.* 332.)

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