A Method of Assigning ^{13}C Nuclear Magnetic Resonance Spectra Using Europium(III) Ion-induced Pseudocontact Shifts and C–H Heteronuclear Spin Decoupling Techniques

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Summary We report a novel method for making ^{13}C n.m.r. assignments using lanthanide ion-induced pseudocontact shifts in the ^{1}H spectrum to separate out the different absorptions and thus to facilitate the use of C–H selective heteronuclear spin decoupling experiments.

IN order to assign unambiguously ^{13}C n.m.r. spectra, it is often necessary to resort to C–H heteronuclear double resonance techniques. If an unequivocal assignment of the proton spectrum of a molecule is available then by selective irradiation of the proton absorption signals they can be related to the carbon signals from the directly bonded carbon atoms by observing the complete collapse of their ^{13}C multiplets. However, for many molecules the proton spectrum contains overlapping multiplets resulting from different protons which have similar chemical shifts. We now report a method for making ^{13}C assignments for such molecules in which lanthanide ion-induced pseudocontact shifts[1,2] in the ^{1}H spectrum separate out the different absorptions and thus facilitate the use of the C–H heteronuclear double resonance technique.

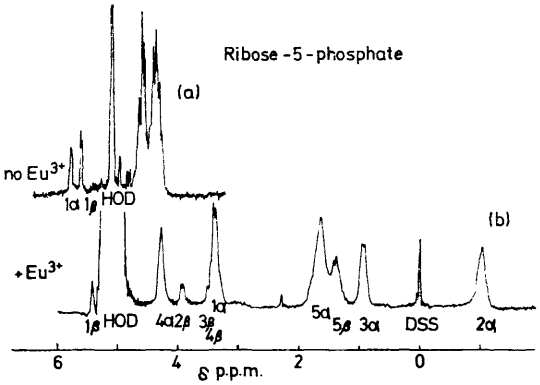

FIGURE 1. ^{1}H *Resonance spectrum of ribose-5-phosphate at* 100 MHz *in the absence and presence of* Eu^{3+} *ion. The addition of the lanthanide ions alters the proportions of the two anomers in favour of the α-form as can be seen by comparing the intensities of the* α-2-H *and* β-2-H *peaks which suggests that the α-anomer binds* Eu^{3+} *more strongly.*[4]

In the ^{1}H resonance spectrum of ribose-5-phosphate at 100 MHz [Figure 1 (a)] the absorption signals for the 1-H protons from the α- and β-anomers are clearly resolved from the other bands in the spectrum. The α-1-H proton has the larger coupling constant and appears downfield from β-1-H by 0·17 p.p.m.[3] Approximately 55% of the ribose-5-phosphate exists as the α-anomer. By selective irradiation at the α-1-H signal it is possible to collapse the corresponding ^{13}C doublet and thus assign it unambiguously. The selective proton irradiation is carried out at the centre of the ^{13}CH satellite doublet bands in the ^{1}H spectrum which coincides almost exactly with the ^{12}CH proton frequency because isotopic shifts in the proton spectra are small. When α-1-H is selectively irradiated only the α-C(1) carbon doublet is completely decoupled, the remaining ^{13}C doublets showing only partial collapse; β-C(1) can be assigned similarly. The chemical shifts of the ten other protons in the two anomers of ribose-5-phosphate are very similar and it is impossible to make further ^{13}C assignments on the basis of selective proton decoupling experiments. However, the C-4 and C-5 carbon signals can be characterised by the P–C doublet splittings and it is possible to distinguish between them because the C-5 signals show large C–H triplet splittings in the undecoupled ^{13}C spectrum, whereas the C-4 signals have large C–H doublet splittings. The anomeric assignment of C-4 and C-5 is not possible.

Figure 1 (b) shows the ^{1}H spectrum at 100 MHz of an aqueous solution of ribose-5-phosphate (0·7 M) in the presence of Eu^{3+} ions (0·97M at pH 1·1). The protons are now differently shielded because of the substantial pseudocontact shifts induced by the presence of the paramagnetic europium ions. Many of the proton signals are completely resolved thus facilitating selective proton irradiation experiments. By conventional H–H homonuclear spin decoupling experiments it is possible to assign all the protons unambiguously, and then by selectively irradiating the assigned protons and observing the collapse of the corresponding ^{13}C signals the latter can also be assigned.

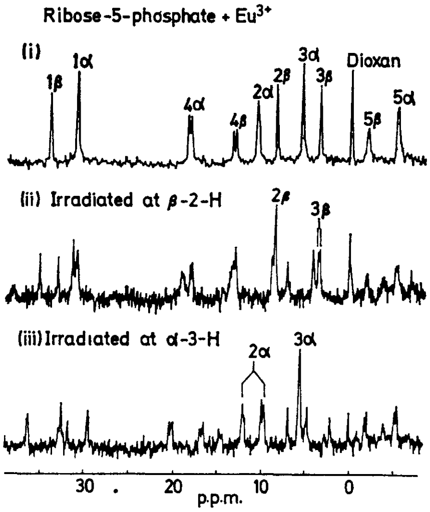

FIGURE 2. ^{13}C *Resonance spectrum at* 25·2 MHz *of* 0·6M-*ribose-5-phosphate and* 0·72M-Eu^{3+} (pH 1·1) *under conditions of:* (i) *proton noise decoupling;* (ii) *selective irradiation of* β-2-H *frequency;* (iii) *selective irradiation at* α-3-H *frequency. The* ^{13}C *spectra were accumulated using the Fourier Transform technique* (*Varian* XL100).

Figure 2 shows the ^{13}C resonance spectrum at 25·2 MHz of ribose-5-phosphate (0·6M) in the presence of Eu^{3+} ions (0·72M at pH 1·1) recorded under different conditions of proton decoupling. In the noise-decoupled spectrum at high Eu^{3+} concentration the signals from the α- and β-anomers can be clearly distinguished by their different intensity. The remaining signals C-2 and C-3 which could not be assigned previously can be assigned by selectively irradiating at the now well separated α-2-H, β-2-H, α-3-H, and β-3-H signals. Figures 2 (ii) and (iii) show the collapse of the corresponding carbon signals when β-2-H and α-3-H are selectively irradiated. The magnitudes of the residual coupling of the other carbon signals give an internal check on all the assignments. For example the residual splitting of α-C(1) is much smaller than that of β-C(1) when selectively irradiated at β-2-H frequency because the resonance frequency of β-2-H is nearer to that of α-1-H than to that of β-1-H.

Examination of the ^{13}C spectra over a range of Eu^{3+} concentrations enables one to extrapolate the results to the ^{13}C spectrum in the absence of Eu^{3+} ions. The pseudocontact shifts cause several ^{13}C nuclei to reverse their signal positions. The assignments for ribose-5-phosphate (0·9M at pH 1·1) in the absence of Eu are: β-C(1) 34·60; α-C(1), 29·72; α-C(4) 15·63; β-C(4) 14·56; β-C(2) 8·57; α-C(2) 4·09; β-C(3), 3·85; α-C(3), 3·45; β-C(5), −0·40 and α-C(5), −1·12 p.p.m. from dioxan internal reference.

Thus the combination of lanthanide ion-induced pseudocontact proton shifts and selective C–H heteronuclear decoupling measurements can provide unequivocal ^{13}C spectral assignments.

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