Simplified ^{13}C Spectral Assignments Using a Graphical Method to Present ^{13}C Spectra Recorded under Conditions of Proton Off-resonance Spin Decoupling

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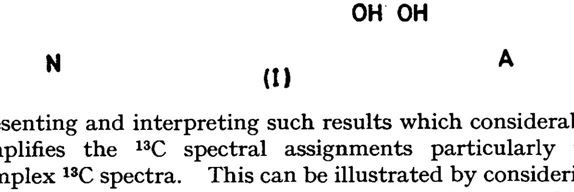
Summary In the ^{13}C spectrum of nicotinamide adenine dinucleotide (NAD^+) the adenine and nicotinamide ring carbons are assigned by a graphical method of presenting the proton off-resonance decoupled ^{13}C spectra as a function of proton irradiation frequency; this method is particularly applicable to closely spaced ^{13}C signals.

OFF-RESONANCE C–{H} spin-decoupling is an established method of assigning ^{13}C absorptions l ɩecting them with the proton signals from directly n of anisole ha l protons of phonate.[15] We known assignment.[1,2] We report a g salt (1) was de method for



(I)

presenting and interpreting such results which considerably simplifies the ^{13}C spectral assignments particularly for complex ^{13}C spectra. This can be illustrated by considering the assignment of the adenine and nicotinamide ring carbons in nicotinamide adenine dinucleotide (NAD^+) (I). The Gyrocode spin decoupler of the Varian XL100 was used to irradiate selectively at constant power at various single frequencies in the range of the proton resonances and the corresponding ^{13}C spectra, containing several partially decoupled multiplets, were accumulated using the Fourier transform technique. Figure 1 shows the low field part of the ^{13}C spectrum of NAD^+ (0·4M-aqueous solution, pH 1) recorded under such conditions and also under conditions of proton noise decoupling. The high repetition rate of the pulses (0·5 s) was chosen for the off-resonance selectively decoupled spectra so that carbon nuclei with long relaxation times (those with no directly bonded protons) would either not be observed or appear with low intensities. These simplified spectra, Figure 1 (ii) and (iii), only feature carbons N-4, A-8, A-2, N-6, N-2, and N-5 as strong ^{13}C absorptions.

From the off-resonance selective decoupling experiments indicated in Figure 1, it is obvious that there is considerable difficulty in measuring the various residual splittings in the spectra because of the extensive overlap of the absorption lines. However, if the peak frequencies of the partially decoupled ^{13}C spectra are plotted against the proton irradiating frequencies as shown in Figure 2, one can readily identify the connected proton and carbon nuclei. Ernst[2] has derived the relationship between the residual splitting (J_R) and the coupling constant (J_{CH}) which is given by the expression (1). At constant power, $\gamma H_2/2\pi$,

$$\gamma H_2/2\pi = J_{CH}\Delta f/J_R \tag{1}$$

the residual coupling J_R should vary linearly with Δf, the separation of the irradiation proton frequency from the correct proton frequency of the nucleus under consideration. Furthermore, for a series of carbon atoms where the J_{CH}

values ar similar.

r, the gradients of the lines should also be ne can easily draw the lines connecting

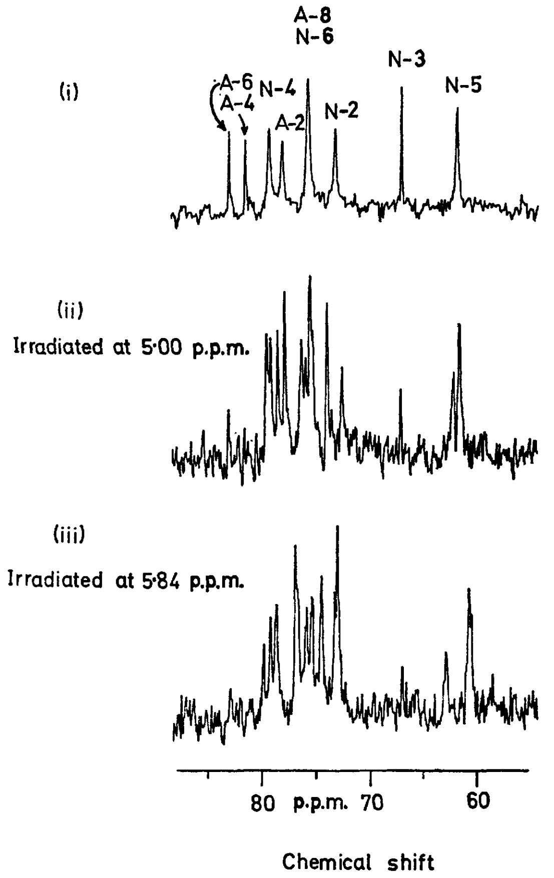


FIGURE 1. (i) ^{1}H *Noise decoupled* ^{13}C *spectrum at* 25·2 MHz *of the aromatic region of* NAD$^+$. *The carbon atoms of the adenine ring are denoted by* A *and of the nicotinamide ring by* N. (ii) ^{1}H *Off-resonance decoupled* ^{13}C *spectrum at* 25·2 MHz *of* NAD, *irradiated at* 5·00 *p.p.m. downfield from dioxan in the* ^{1}H *spectrum.* (iii) ^{1}H *Off-resonance decoupled* ^{13}C *spectrum at* 25·2 MHz *of* NAD$^+$, *irradiated at* 5·84 *p.p.m. downfield from dioxan in the* ^{1}H *spectrum. Spectral conditions:* 500 *pulses,* $\pi/2$ *pulse, repetition rate* (i) 4 *s,* (ii), (iii) 0·5 s.

all the points as shown in Figure 2. The straight lines will intersect at the chemical shifts of the connected ^{13}C and ^{1}H nuclei since the intersection corresponds to the optimum spin decoupling of the ^{13}C nucleus: these chemical shifts are indicated on the axes of Figure 2. The ^{1}H assignments are known from H–H decoupling studies and from considerations of chemical shifts in model compounds[3] and hence one can unambiguously assign the connected carbon nuclei. These assignments are indicated in Figure 1 (i) and the measured chemical shifts are: N-4, 79·4; A-2, 78·3; N-6, 75·9; A-8, 75·9; N-2, 73·4, and N-5, 62·2 p.p.m. downfield from a dioxan internal reference.

The remaining ^{13}C assignments in NAD$^+$ made on the basis of ionisation studies, chemical shift comparisons with model compounds and C–{H} heteronuclear decoupling studies are: C=O, 99·0; A-6, 83·3; A-4, 81·8; N-3, 67·5; A-5, 51·9; N-1′, 33·5; A-1′, 21·7; N-4′, 20·4; A-4′, 17·4; N-2′, 11·1; A-2′, 8·1; N-3′, 4·2: A-3′, 3·7; N-5′, −1·1, and A-5′, −1·1 p.p.m. (from a dioxan internal reference, downfield shifts denoted as positive).

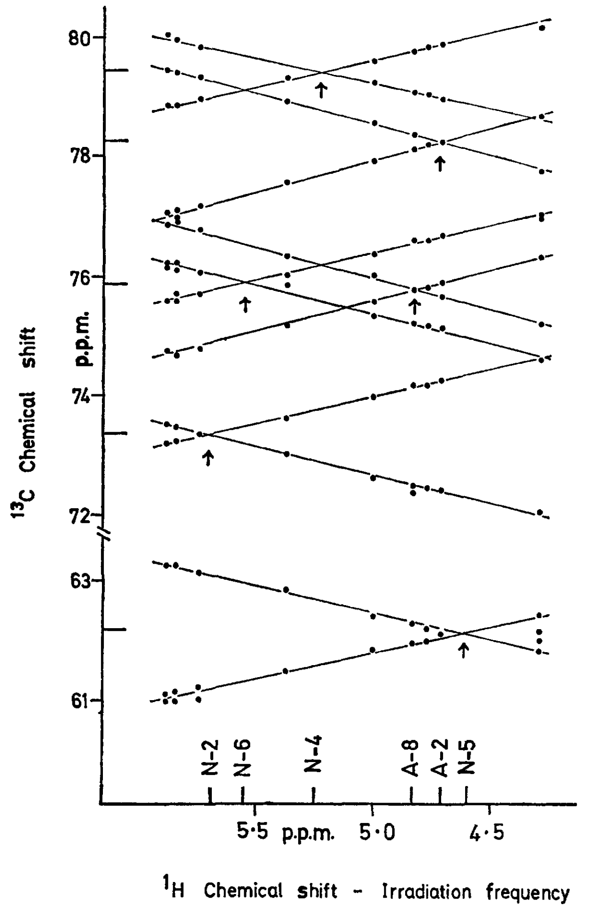


FIGURE 2. *Plot of peak frequencies in the* ^{1}H *off-resonance selectively decoupled* ^{13}C *spectra of* NAD$^+$ *as a function of position of irradiation in the* ^{1}H *spectrum, expressed in p.p.m. downfield from internal dioxan. The positions of the peaks in the* ^{1}H *noise decoupled* ^{13}C *spectrum are shown by lines on the ordinate and the position of the proton peaks by lines on the abscissa. The arrows* ↑ *indicate the point of collapse of the* ^{13}C *doublet and the connection between a given* ^{13}C *peak and the assigned proton peak. The errors in the position measurements of the* ^{13}C *peaks are indicated by the size of the points except near the cross-over positions where the errors are larger* ($\pm$0·15 *p.p.m.). Small doublet splittings are observed on some of the signals from long-range* CH *spin coupling interactions.*

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