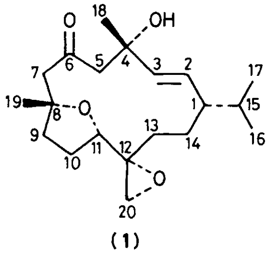
A Sensitive and Convenient Method for the Determination of Multiplicities of Lines in ^{13}C N.M.R. Spectra

Toshiaki Nishida,[a] Curt Enzell,[a] and James Keeler*[b]

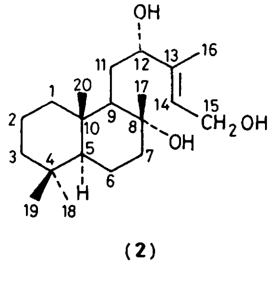
[a] *Research Department, Swedish Tobacco Company, P.O. Box 17007, S-104 62, Stockholm, Sweden*
[b] *University Chemical Laboratory, Lensfield Rd., Cambridge CB2 1EW, U.K.*

Multiplicity determination from an optimized heteronuclear *J*-spectrum is shown to have several significant advantages over spectral editing techniques; despite the experiment being two-dimensional it is of comparable sensitivity with equivalent one-dimensional techniques and involves little extra data processing.

Assignment of a ^{13}C n.m.r. spectrum usually requires a knowledge of the multiplicity of each line in the spectrum. In the past this information was generally obtained from coherent off-resonance decoupled spectra,[1] but such spectra have low sensitivity and are fraught with a variety of well known problems. Recently spectral 'editing' methods have become popular.[2—10] These aim to produce a set of subspectra in which only lines associated with a particular multiplicity appear. Editing methods, while being a considerable improvement over off-resonance decoupling, are also subject to various problems.[11,12] In this communication we demonstrate that a two-dimensional heteronuclear *J*-spectrum[13] is a very convenient and sensitive method of determining multiplicities. Two-dimensional experiments are often considered to have poor sensitivity and long data processing times, but this need not necessarily be the case. The two-dimensional experiment described in this communication has sensitivity comparable to one-dimensional methods. Furthermore, it is easier to imple-



(1)



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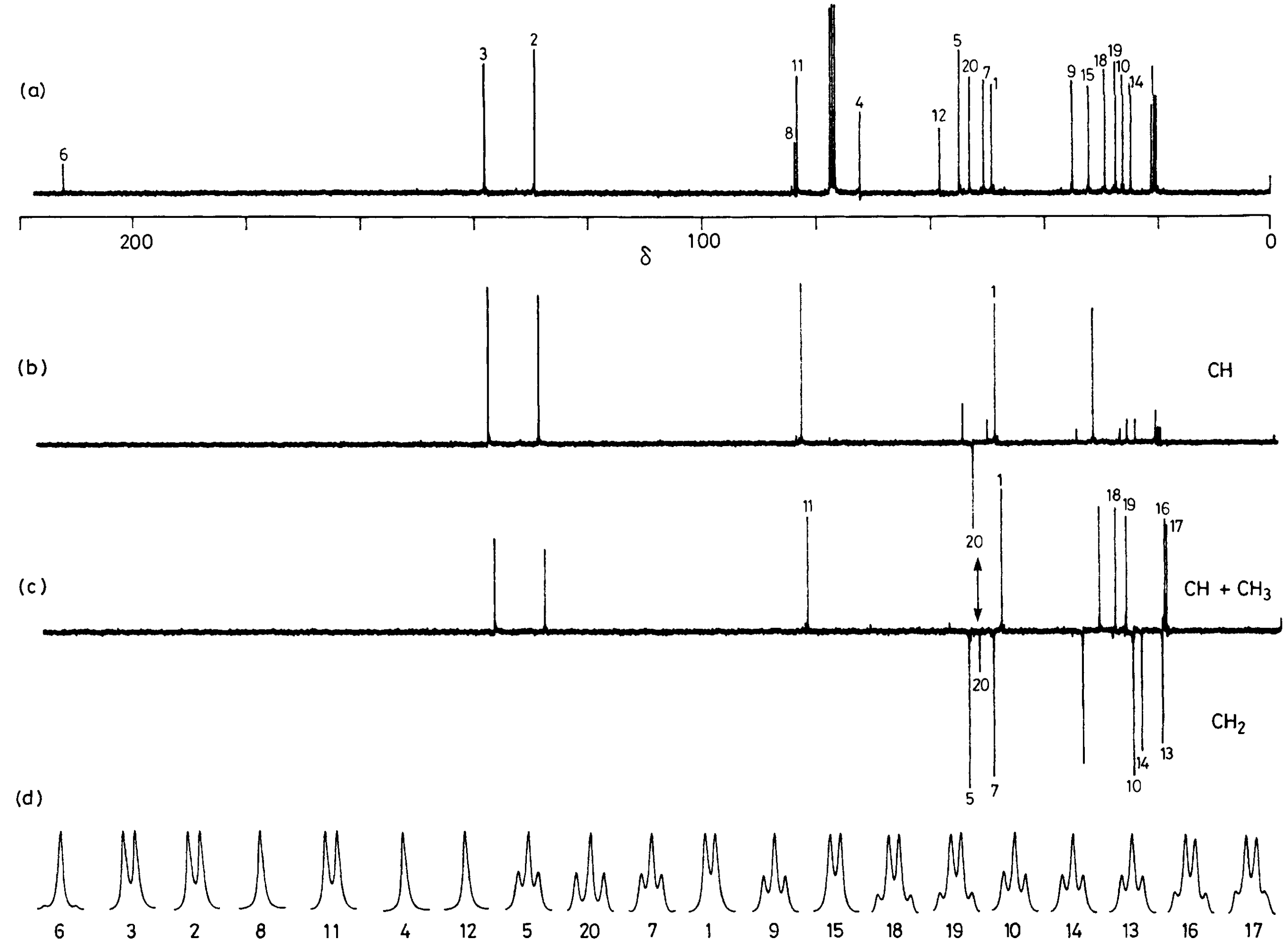

Figure 1. (a) ^{13}C N.m.r. spectrum of the terpene 1,2-epoxide (**1**). (b) INEPT CH only subspectrum. (c) CH,CH_3 up–CH_2 down INEPT subspectrum. C-20 has an abnormally large one-bond C–H coupling constant, and appears anomalously inverted in both subspectra. Note also that other methylene carbons appear weakly in the CH subspectrum. (d) Cross-sections, corresponding to each ^{13}C line, through the two-dimensional heteronuclear *J*-spectrum, recorded as described in the text. The multiplicities of all the lines can be determined at a glance and without ambiguity. All spectra recorded on a Varian XL300 spectrometer. Five increments of t_1 were used to record the two-dimensional spectrum. Data acquisition times approximately 9 h for both spectra. Sample concentration 0.05 M.

ment and is less prone to artefacts than commonly used editing methods.

The problems with editing methods, and some possible solutions, have been described.[11,12] Briefly, they are two fold. First, the pulse sequences contain delays whose optimum values depend critically on the size of the one bond carbon–proton couplings. Second, the application of carefully calibrated pulses to the decoupler channel is required. Failure to set the delays correctly, or to calibrate the pulses properly, results in poor editing and consequently confusion can very easily arise as to the real multiplicities of the lines. There is also a reduction in sensitivity. Furthermore, serious ambiguities often arise when the spectrum contains degenerate resonances, since it is difficult to distinguish poor editing from degenerate shifts.

In a heteronuclear *J*-spectrum the ^{13}C shifts are displayed in the f_2 dimension and the associated carbon–proton multiplet appears in the f_1 dimension. The multiplicity is found by simply inspecting this f_1 multiplet (*e.g.* a methine gives a doublet, a methyl gives a quartet). Because the carbon–proton coupling is so large only very low resolution is needed in the f_1 dimension, thus, as was shown elsewhere,[14] only *five* to *seven* increments of t_1 need to be recorded. This very restricted sampling gives a sensitivity comparable to the conventional one-dimensional spectrum,[15] while maintaining sufficient resolution to distinguish between the multiplet types.

The spectra of the novel diterpene 1,2-epoxide (**1**),[16] Figure 1, illustrate the problems we encountered with variations in one bond carbon–proton couplings when using INEPT editing. The coupling constant of the epoxide carbon is between 170—175 Hz, considerably larger than the couplings in the rest of the molecule. Figure 1 shows the INEPT-edited spectra intended to produce a CH only and a CH,CH_3 up–CH_2 down subspectrum. Poor editing is demonstrated by the appearance of C-20 as an inverted line in both spectra; it is in fact a CH_2 group. Other carbon atoms, such as C-5 and C-7 also show poor editing. In this spectrum the delays were optimized empirically to a value corresponding to a coupling of 138 Hz, but this is fairly arbitrary given the wide range of one bond couplings in the molecule. Figure 1 also shows the cross-sections (along f_1) from the *J*-spectrum. From these the multiplicity of all the lines can be determined without

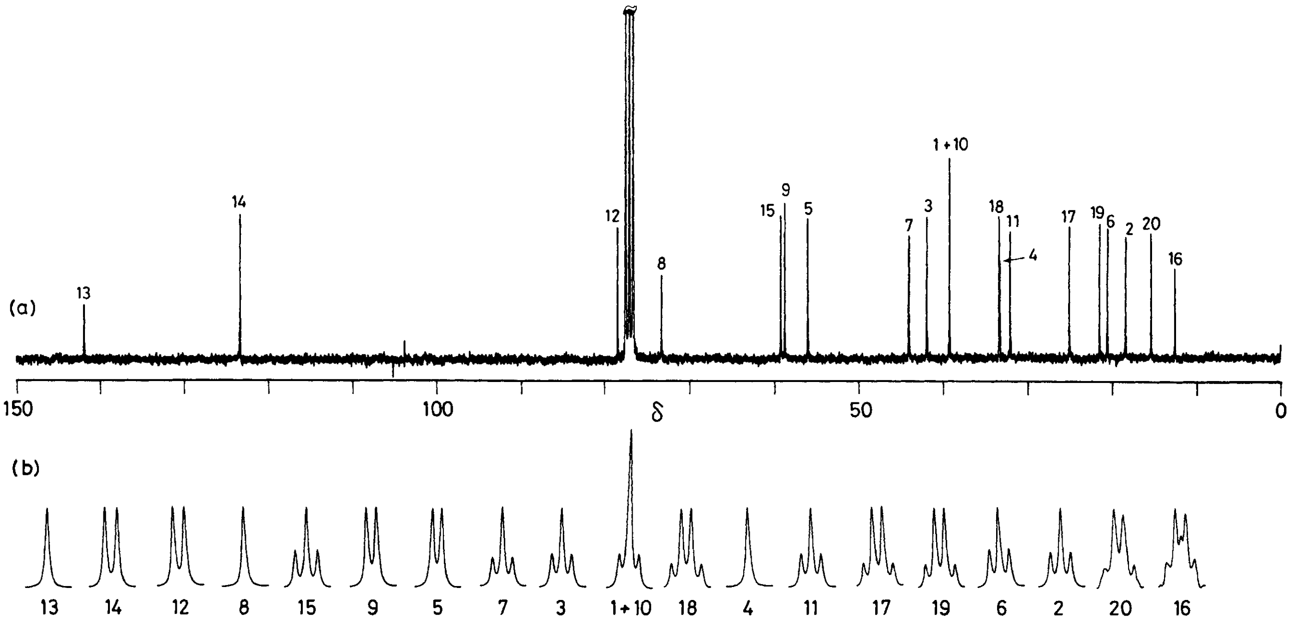

Figure 2. (a) ^{13}C N.m.r. spectrum of the labdanic diterpenoid (**2**). (b) Cross-sections, corresponding to each ^{13}C line, through the two-dimensional *J*-spectrum. C-1 and C-10 have degenerate shifts and their multiplicities would be difficult to distinguish in an edited spectrum. The corresponding cross-section is an anomalous triplet, clearly indicating a singlet overlapping a triplet. Seven increments of t_1 were used to record the two-dimensional spectrum. Data acquisition time 12 h. Sample concentration 0.04 M.

ambiguity; the C-20 resonance shows up as a triplet. The *J*-spectrum and the INEPT-edited spectra were recorded in comparable times.

The spectra of the labdanic diterpenoid (**2**),[17] Figure 2, illustrate that overlap of carbon lines with different multiplicities is not a problem with the *J*-spectrum method. The C-1 and C-10 resonances are degenerate, corresponding to methylene and quaternary carbons. As quaternary carbons do not appear in INEPT-edited spectra it would be difficult to detect this degeneracy, although some alternative editing methods do reveal quaternary carbons (*e.g.* SEMUT[10]). In any case it is difficult to distinguish degenerate shifts from poor editing. The sections through the *J*-spectrum show clearly that this shift is associated with two carbons atoms as the apparent multiplet structure is a highly abnormal triplet with a strong central line. It is clearly a singlet overlapping a triplet. A doublet overlapping a singlet is ruled out because of the wide spacing of the outer lines.

Further examples have been encountered, especially in some organometallic compounds where the one-bond carbon–proton couplings are subject to unpredictable variations.[18]

Data processing of the two-dimensional spectra was not time-consuming as only interferograms (time domain functions of t_1) corresponding to lines in the carbon spectrum were computed. Owing to the very restricted sampling in t_1, Fourier transformation of these interferograms produces 'sinc wiggle' artefacts in the spectra which can confuse the interpretation. For the spectra shown here, these artefacts were removed by a data processing method described previously.[14] An alternative, if such software is not available, is to use strong Gaussian apodization.

With regard to experimental convenience we routinely find the *J*-spectrum methods of multiplicity determination to be superior to spectral editing. Furthermore, it gives results which are unambiguous since there are no critical delays to set and decoupler pulse calibration is not required. Despite being two-dimensional the experiment has comparable sensitivity with one-dimensional methods and does not need excessive data processing time.

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