Total Assignment of the Carbon-13 N.M.R. Spectrum of Monensin by Two-Dimensional Correlation Spectroscopy

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The assignment of the complex carbon-13 n.m.r. spectrum of the polyether ionophore antibiotic monensin is shown to be over-determined by two simple two-dimensional experiments.

The polyether ionophore antibiotics have as a common structural feature a long substituted carbon backbone constructed *in vivo* by an undisclosed pathway from simple building blocks which include acetate, propionate, and butyrate.[1] Similar carbon chains can also be recognised in many related classes of antibiotic including the macrolides, the

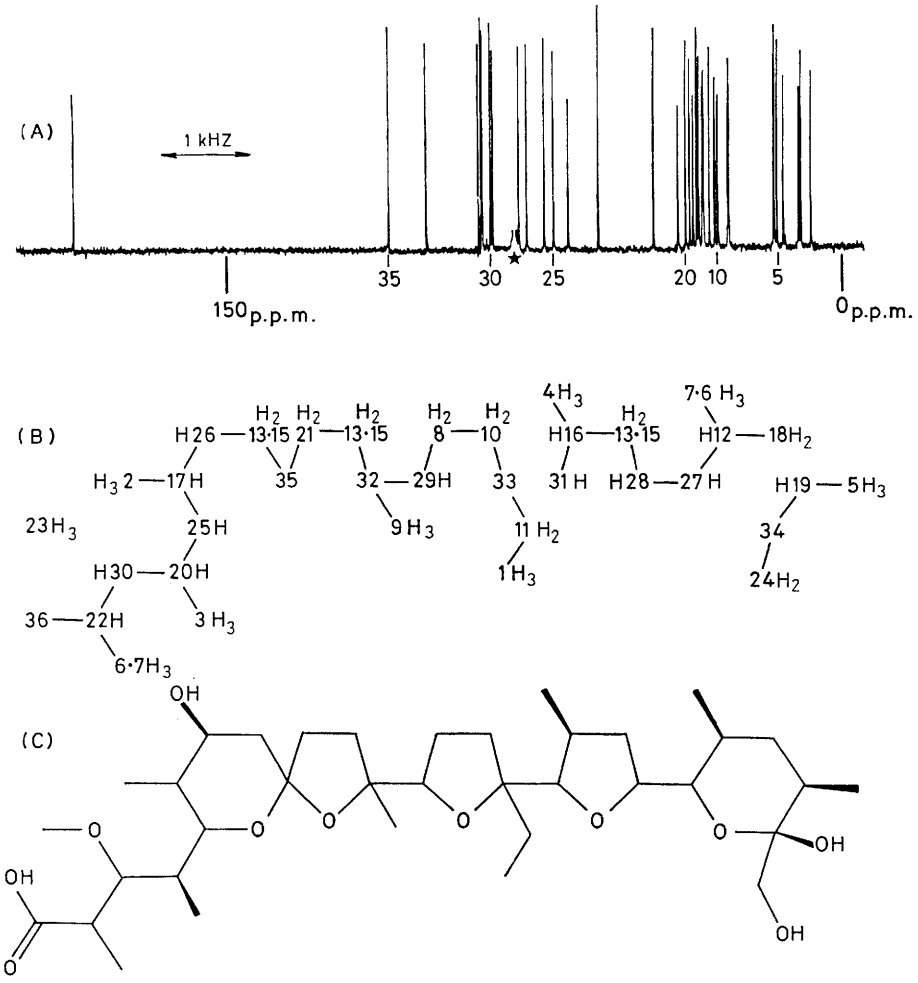

Figure 1. (A) The proton-decoupled carbon-13 n.m.r. spectrum of monensin (**1**) at 50.3 MHz. Lines are numbered in order of increasing frequency. * = chloroform. (B) Carbon–proton and carbon–carbon bonds identified by two-dimensional spectroscopy (see text). (C) The known structure of monensin; comparison with the information of part (B) gives the complete assignment of the spectrum (A).

polyene macrolides, and the ansamycins. Unambiguous ^{13}C n.m.r. spectral data on such compounds are invaluable for structural comparisons within a class, for biosynthetic investigations, and for conformational studies related to biological activity. Such data, however, are obtained only with great difficulty and for this reason this area has recently attracted considerable attention.[2] We report here the first assignment of the ^{13}C n.m.r. spectrum of monensin (**1**) (Figure 1), a polyether ionophore antibiotic produced[3] by *Streptomyces cinnamonensis*, using a two-dimensional correlation technique that should be of general interest and find wide application in this field.

Typically, the assignment of an n.m.r. spectrum relies on the evaluation of information from a number of sources: in particular chemical shifts, longitudinal relaxation times, proton-coupled multiplicities, comparison with model compounds, and chemical modification. Such information is often ambiguous and results only in a tentative or probable assignment. This applies equally to proton and carbon-13 n.m.r. spectra, so that, although selective decoupling and two-dimensional shift correlation[4] allow the spectrum of one nucleus to be assigned from the spectrum of a coupled nucleus for which the assignment is known, any ambiguities are perpetuated. For the carbon-13 n.m.r. spectra of the majority of organic molecules, however, only two pieces of information are required to give an absolute assignment: the number of protons bound to each resonant carbon nucleus and the pattern of carbon–carbon bonds.

It is usually possible to obtain the proton-coupled multiplicities of carbon resonances by off-resonance decoupling if the spectrum is reasonably simple. Overlapping lines in more complex spectra require more subtle techniques: for example, the distinction of resonances with singlet or triplet structure from those of doublets or quartets by their characteristic modulations in spin-echo experiments.[5] The most general form of this experiment, a complete two-dimensional analysis of the spin echo generated by a 180° refocussing pulse, allows the separation of the effects of chemical shift and of proton coupling into two frequency dimensions so that the detailed multiplet structure of all resolvable lines in the *proton-decoupled* spectrum may be obtained unambiguously in a simple experiment.[6] The spectrum of monensin shown in Figure 2 correlates chemical shifts with proton coupled multiplicities in this way, and identifies all the carbon–proton bonds in the molecules.

The pattern of carbon–carbon bonds may also be obtained by conventional methods; the steadily increasing sensitivity of commercial spectrometers now allows the routine observation of carbon-13 satellites in natural-abundance carbon-13 spectra. Resonances may then be identified as those of directly bonded carbon atoms if they have identical splittings in the satellite spectrum. This method has recently been used to

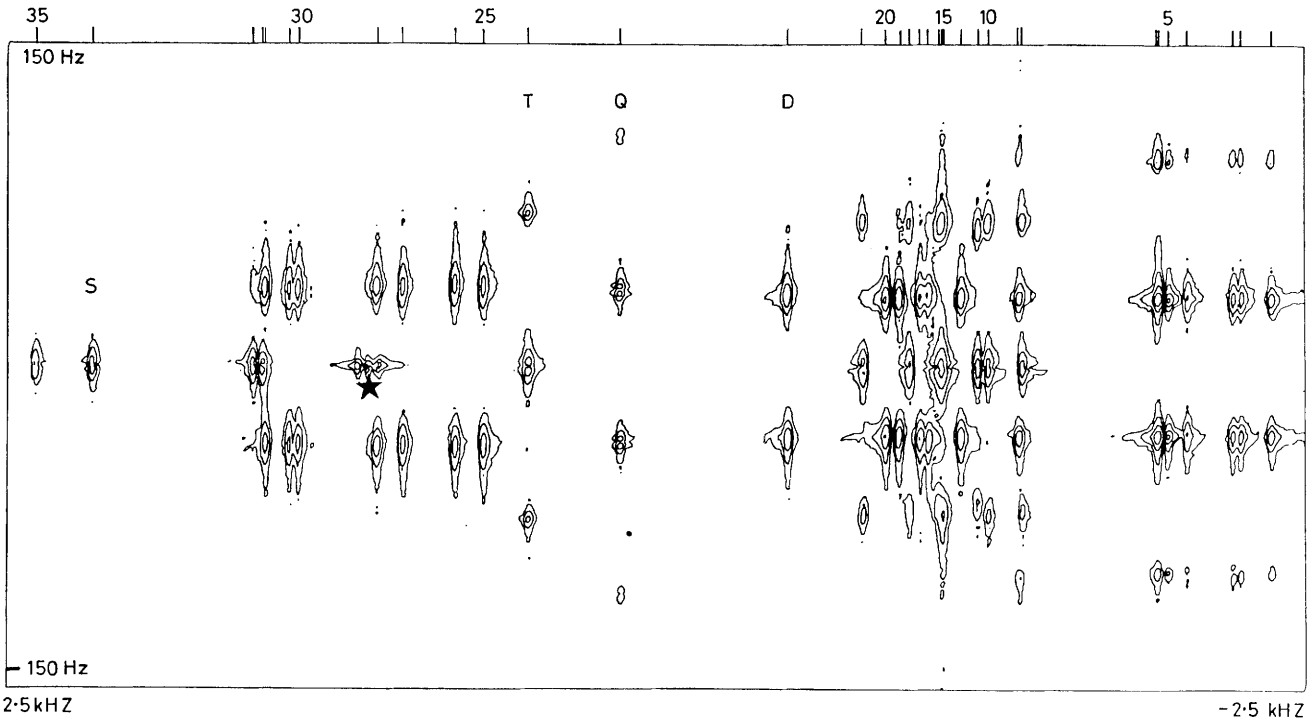

Figure 2. A two-dimensional spectrum with carbon-13 chemical shifts (horizontal axis) correlated with proton-coupled multiplet structure (vertical dimension). The number of protons bonded to each carbon atom is easily determined from the multiplicity; examples of a singlet, doublet, triplet, and quartet are indicated. * = deuteriochloroform.

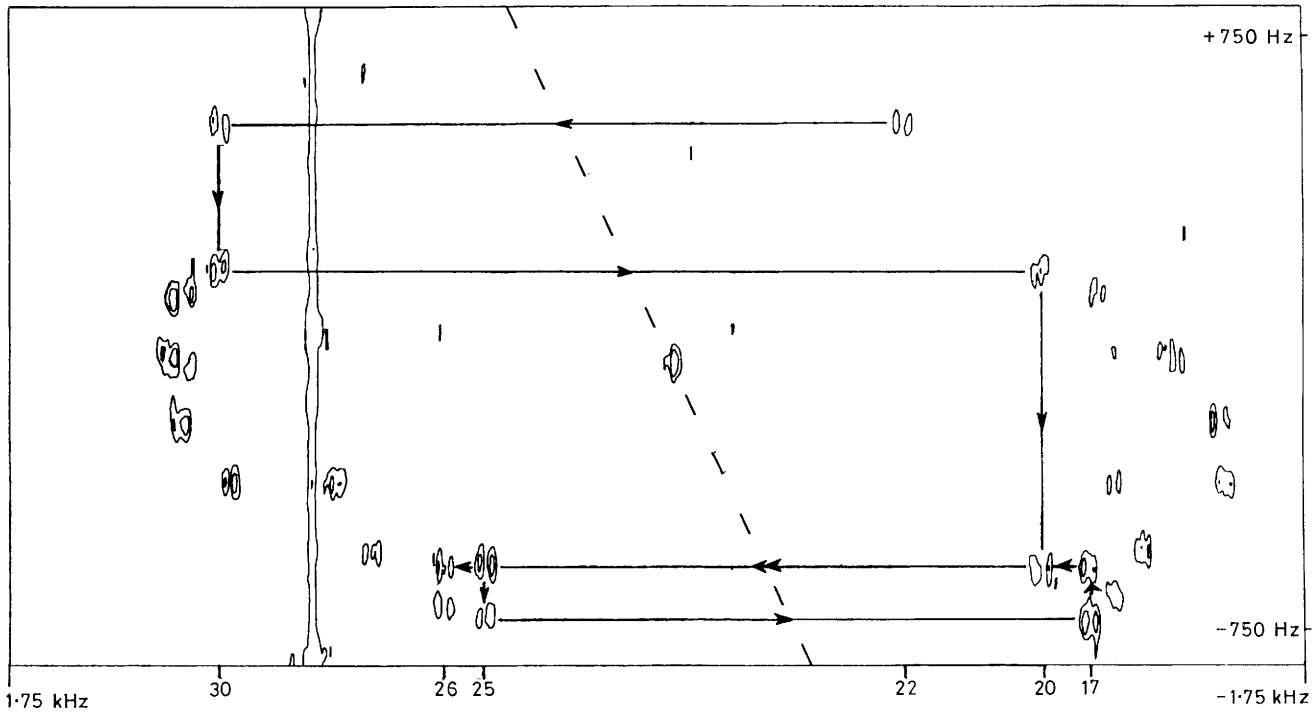

Figure 3. Part of the carbon-13 autocorrelation spectrum of monensin; singlets generated by isolated carbon-13 nuclei have been suppressed leaving only the weak carbon-13 satellite doublets. The chloroform singlet (vertical band) is incompletely suppressed because of its long relaxation time. Carbon-13 chemical shifts appear horizontally, and directly bound carbon atoms are represented by pairs of doublets with a common frequency in the second (vertical) dimension. Frequencies in the second dimension are sums of resonance frequencies with respect to the transmitter and correlated peaks are therefore equidistant from the major diagonal (dashed) in the horizontal dimension. The chain of carbon atoms represented by peaks 22–30–20–25–17–26 is indicated to illustrate the method of interpretation.

assign the spectrum of patchoulol.[7] For more complex spectra, the narrow range of one-bond carbon–carbon coupling constants would lead to ambiguities and direct correlation of carbon resonances becomes necessary. Approximately 1 in 10^4 molecules will contain a pair of directly bonded carbon-13 nuclei in any given site. Such nuclei form AB or AX groups and so have common energy levels which provide the basis for correlating resonance frequencies in two-dimensional experiments.[8] As a refinement, all signals other than those from doublets in the characteristic range of one-bond carbon–carbon scalar couplings may be suppressed.[9] A standard two-dimensional autocorrelation experiment using either multiple quantum or spin-echo pulse sequences will then yield only those peaks which represent directly bonded nuclei.[10] Related experiments have been demonstrated by Bax *et al.*[11]

Part of a spectrum obtained by method A of reference 10 is shown in Figure 3. Conventional n.m.r. frequencies appear in the horizontal dimension while peaks representing correlated nuclei have frequencies in the vertical dimension which are sums of their frequencies with respect to the transmitter. Directly bonded carbon atoms therefore have identical resonance frequencies in the second (vertical) dimension which are equidistant from the major diagonal of the spectrum in the horizontal or conventional frequency dimension. A few of the bonds represented in this section of the spectrum have been picked out: a chain formed by the resonances 22–30–20–25–17–26 is clearly visible. Examination of the complete spectrum positively identified all the carbon–carbon bonds in monensin, excepting only two which give rise to strongly coupled AB systems and are effectively suppressed.

The information obtained from the spectrum of Figure 2 concerning carbon–proton bonds, and that of Figure 3 concerning carbon–carbon bonds, has been assembled in part B of Figure 1. Part A of Figure 1 shows the normal carbon-13 spectrum and it should be noted that the three triplets 13—15, and the two quartets 6—7, are not resolved in these spectra. However, comparison of the bonding information with the known structure of monensin, given in part C of Figure 1 leaves no doubt over the assignment. For molecules of unknown structure, the pattern of bonds can be sufficient to provide a complete structural determination, with the total assignment as a by-product.[12]

Carbon-13 spectra were obtained at 50.3 MHz using a Bruker CXP 200 spectrometer. Noise-modulated proton decoupling of *ca.* 5 W was used.

The 'two-dimensional *J* spectrum' (Figure 2) was obtained in an overnight run with 512 points in each acquisition and 128 increments of t_1 corresponding to frequency dimensions of ± 150 Hz $\times$ ± 2.5 kHz. Each signal was time-averaged over 12 sixteen-step cycles.[13] The sample was a 10 mm tube containing *ca.* 0.5 g of monensin in 2 ml of deuteriochloroform in a standard saddle coil probe.

The carbon-13 autocorrelation spectrum (Figure 3) was obtained in 60 h using 1024 points for each acquisition and 128 increments of t_1 corresponding to frequency dimensions of ± 3.3 kHz $\times$ ± 2.5 kHz. Each signal was time-averaged over 48 thirty-two-step cycles.[10] The sample was a 10 mm tube containing *ca.* 1.0 g of monensin in 2 ml of chloroform in a home-built double-tuned solenoid. Slight concentration-dependent changes in chemical shifts were observed, but the order of lines remained unchanged in the range 50 mg to 1 g ml^{-1}.

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