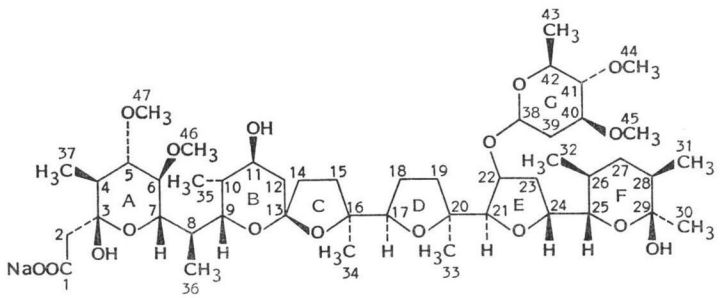
# NATURAL ABUNDANCE TWO-DIMENSIONAL DOUBLE-QUANTUM $^{13}C$ NMR SPECTROSCOPY OF MADURAMICIN, A POLYETHER IONOPHORE ANTIBIOTIC AND COCCIDIOSTAT

Sir:

Maduramicin is a polyether carboxylic ionophore produced by the prokaryotic Gram-positive bacterium *Actinomadura yumaensis* (formerly classified incorrectly as *Nocardia* X-14868[1]). It is a potent antibiotic with useful activity as a coccidiostat. Maduramicin was discovered and characterized independently by two different commercial laboratories: Hoffmann-La Roche Inc. designated the compound X-14868A[1,2], while American Cyanamid Company coined the generic name maduramicin. The chemical structure of maduramicin was determined earlier by single-crystal X-ray diffraction (ref 2 and internal report, Lederle Laboratories, American Cyanamid Company, Pearl River, NY, June 16, 1981), and it is shown as structure **I** with conventional carbon numbering and ring identification. We report here the complete assignment of the $^{13}C$ NMR spectrum for maduramicin determined at natural isotopic abundance by two-dimensional double-quantum spectroscopy[3,4].

$^{13}C$ NMR assignments for several polyether antibiotics are already available in the literature[5~9]. However, most of the reported assignments are based on empirical rules, comparison with model compounds, $^{13}C$ labelling, and chemical modification. Recent advances in NMR spectroscopy have opened up new possibilities for completely independent assignment of complex $^{13}C$ NMR spectra. Unambiguous assignments of the spectra of these important antibiotics are essential for the identification of isomers and derivatives, and for the study of their biosynthesis. In addition, the accuracy of earlier empirical assignments can be evaluated.

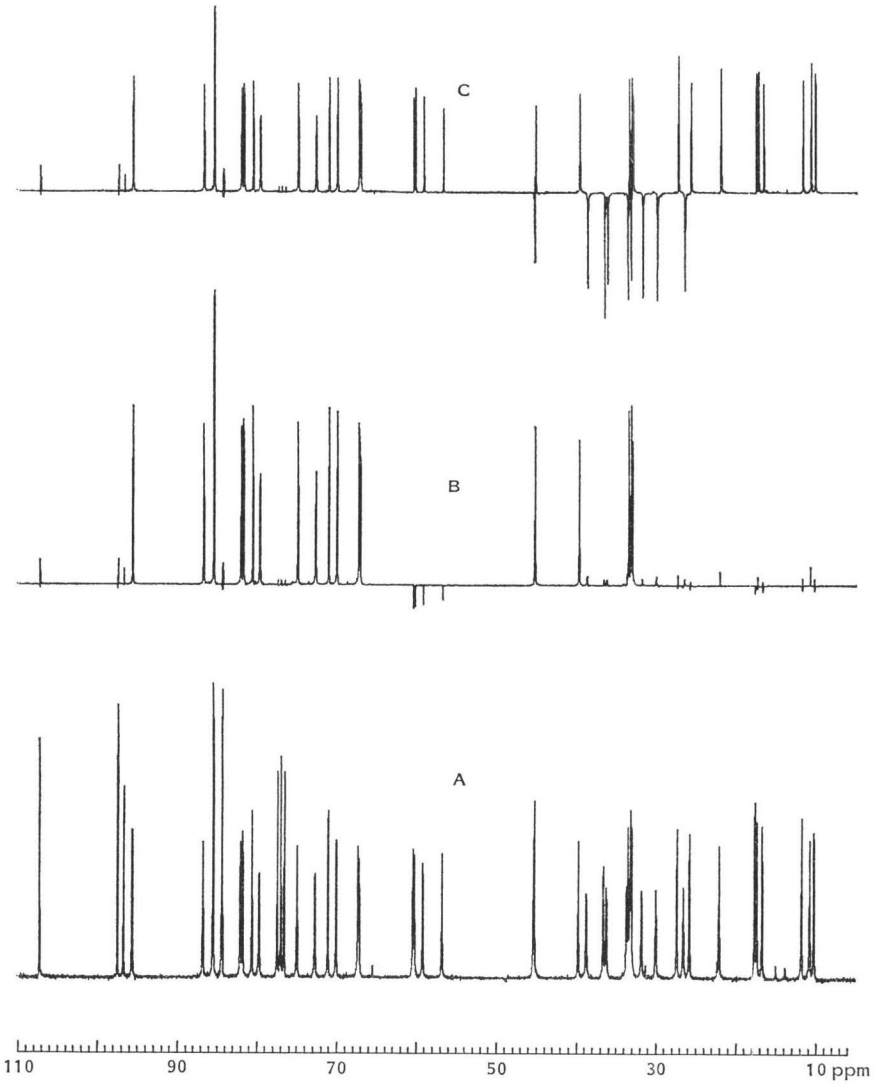
Maduramicin contains forty-seven carbon atoms in a seven-ring system and separate resonances for all of them are observed in the conventional one-dimensional single-quantum $^{13}C$ NMR spectrum, as shown in Fig. 1-A. Except for the carboxylate resonance at 179.14 ppm, all of the $^{13}C$ signals fall within a range of 108 ppm. Consequently, the spectrum is crowded with closely spaced lines that are difficult to assign empirically. It has become common practice to simplify $^{13}C$ NMR spectra by resolving them into subspectra according to the number of attached hydrogens[10]. In polarization-transfer experiments known by the acronym "DEPT", the methyl and methyne carbons are distinguished from the methylene and quaternary carbons by their characteristic modulations in spin-echos. A heteronuclear double-quantum *J*-ordered state is generated, followed by a polarization-transfer step which yields the desired *J*-ordered single-quantum state[11]. Accordingly, the resolved subspectra for maduramicin are shown in Figs. 1-B and 1-C. The subspectra indicate 13 methyl resonances, 9 methylenes, 19 methynes and 6 quaternaries, in full agreement with the X-ray structure. A provisional assignment was worked out based on the empirical rules available in the literature[5~9], and using the results of our own C-13 and O-18 labelling experiments (H. Tsou, *et al.*, to be published). Nevertheless, definitive assignments were not possible for several closely-spaced resonances, and there remained a need for an independent NMR experiment capable of unambiguously defining the carbon skeleton of the molecule. Conventional heteronuclear correlation spectroscopy did not turn out to be useful because of the complex nature of the $^{1}H$ NMR



Maduramicin (I)

Fig. 1.

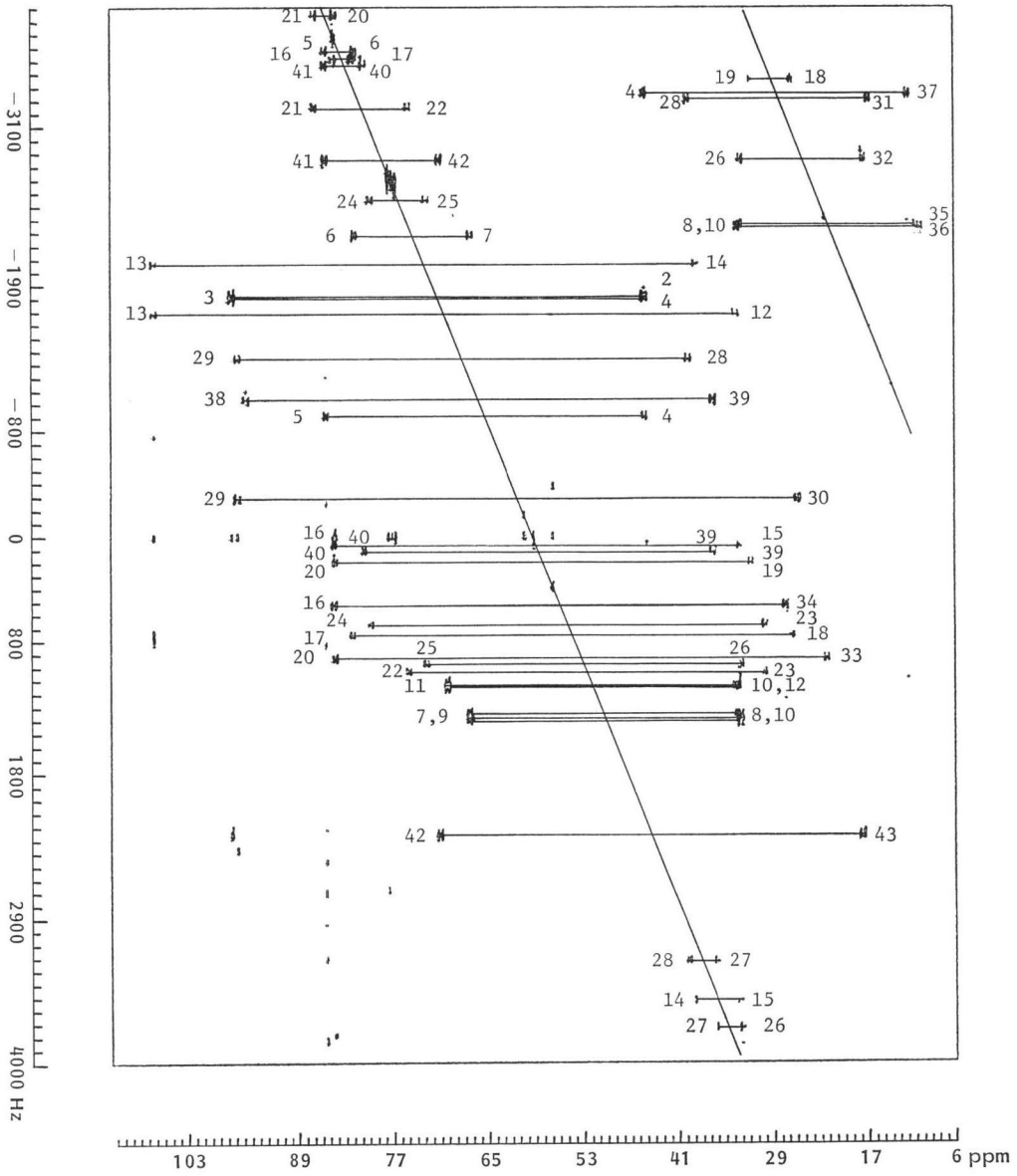
A) Conventional single-quantum $^{13}C$ NMR spectrum of maduramicin, B) methyne resonances isolated in the "DEPT" experiment, C) protonated-carbon resonances isolated in a "DEPT" experiment: The methylene resonances are inverted relative to the methyl and methyne signals.




spectrum. Consequently, we resorted to homonuclear multiple-quantum spectroscopy to aid in the assignment of the carbon spectrum.

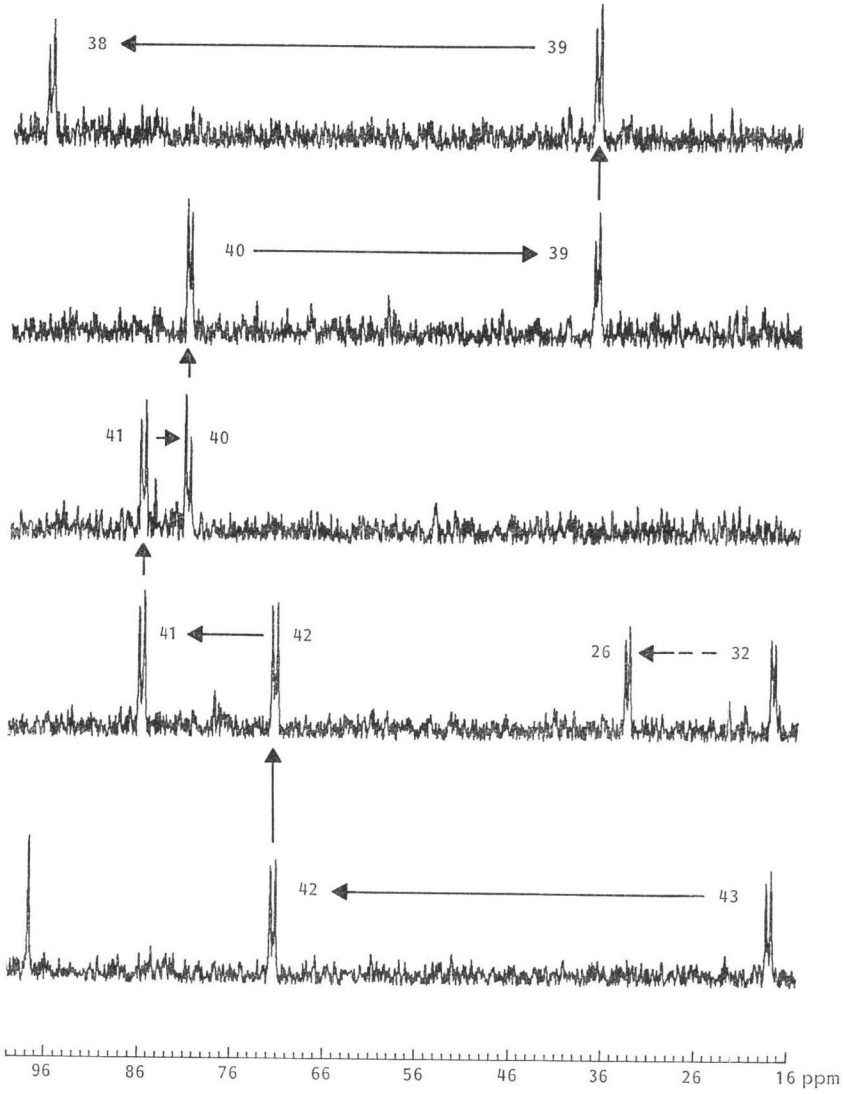
The "INADEQUATE" experiment introduced by FREEMAN, *et al.*[8], allows the assignment of carbon resonances for unbroken chains through the observation of the $^{13}C$-$^{13}C$ double-quantum coherences. Two-dimensional double-quantum spectroscopy[4] allows the carbon-carbon connectivities to be identified. However, for materials at natural isotopic abundance, the sensitivity of the experiment is very low: only one molecule in ten thousand has the required isotopic composition. For large molecules, such as maduramicin (MW 938), the two-dimensional "INADEQUATE" experiment is feasible only at high magnetic field strengths and with large samples. Applications of this technique to smaller molecules have been described[12], and ROBINSON and TURNER[13] have assigned the $^{13}C$ NMR spectrum for the polyether antibiotic monensin (MW 691) using two-dimensional correlation spectroscopy. To the best of our knowledge, maduramicin is the largest molecule to date to be

Fig. 2. Two-dimensional $^{13}C$ "INADEQUATE" experiment for maduramicin at 75 MHz. Using a 0.6 M solution in $CDCl_3$, shown as a contour plot. The connectivities for 42 carbon atoms are shown as horizontal lines connecting pairs of doublets centered on axes of slope = −2.




studied at natural abundance by this difficult technique. A useful two-dimensional "INADEQUATE" spectrum of maduramicin in a *ca.* 0.6 M solution, was obtained in an aquisition time of 110 hours. The single-quantum coherences were almost completely suppressed and the $^{13}C$-$^{13}C$ double-quantum coherences yielded a two-dimensional spectrum comprised of doublets due to the homonuclear coupling constant of *ca.* 38 Hz, as shown in Figs. 2 and 3. The carbon-carbon connectivities for the entire 42-carbon skeleton were unambiguously established. These connectivities are indicated in Fig. 2 by horizontal lines joining the double-quantum doublets, which are centered about an axis of slope = −2. The break in this axis is due to deliberate aliasing of some resonances to maximize digital resolution. Of the remaining five carbons, the car-

Fig. 3. A series of one-dimensional slices isolated from the two-dimensional "INADEQUATE" spectrum to illustrate the carbon-carbon connectivity of the sugar ring (ring G) of maduramicin.




boxyl is already established at 179.14 ppm and the four isolated methoxy carbons were assigned empirically. To illustrate more clearly the interpretation of the two-dimensional "INADEQUATE" experiment, a series of one dimensional slices were extracted, as shown in Fig. 3. The carbon-carbon connectivity for the sugar ring (ring G) is clearly demonstrated, as is the signal-to-noise ratio for the experiment.

The complete $^{13}C$ chemical-shift assignments for maduramicin are given in Table 1. The shifts are referenced to tetramethylsilane at 0 ppm, although they were measured relative to deuteriochloroform at 77.00 ppm. The resonances at 45.49 and 45.53 ppm were only partly resolved in the "INADEQUATE" experiment due to their close spacing. However, their assignments were confirmed independently in the "DEPT" experiment since the resonance at 45.53 ppm belongs to a methylene group, while the one at 45.49 ppm is due to a methyne.

It is noteworthy that our provisional assignments for the backbone carbons based on empirical rules[5~9] were in error for six pairs of carbons. Consequently, the two-dimensional "INADEQUATE" experiment was shown to be a useful

Table 1. Complete $^{13}C$ chemical shift assignments for maduramicin, a polyether ionophore antibiotic. The shifts are reported in ppm relative to TMS.

| Carbon | Shift | Carbon | Shift | Carbon | Shift |
|---|---|---|---|---|---|
| 1 | 179.14 | 17 | 82.34 | 33 | 22.41 |
| 2 | 45.53 | 18 | 26.88 | 34 | 27.62 |
| 3 | 97.69 | 19 | 32.11 | 35 | 10.49 |
| 4 | 45.49 | 20 | 84.51 | 36 | 10.95 |
| 5 | 85.67 | 21 | 86.88 | 37 | 11.99 |
| 6 | 82.01 | 22 | 75.25 | 38 | 95.87 |
| 7 | 67.45 | 23 | 30.21 | 39 | 36.90 |
| 8 | 33.40 | 24 | 79.92 | 40 | 80.88 |
| 9 | 67.61 | 25 | 72.99 | 41 | 85.72 |
| 10 | 33.78 | 26 | 33.31 | 42 | 71.34 |
| 11 | 70.32 | 27 | 36.54 | 43 | 17.91 |
| 12 | 33.94 | 28 | 40.00 | 44 | 60.69 |
| 13 | 107.52 | 29 | 97.03 | 45 | 57.05 |
| 14 | 39.00 | 30 | 26.11 | 46 | 59.48 |
| 15 | 33.56 | 31 | 16.96 | 47 | 60.54 |
| 16 | 84.67 | 32 | 17.61 | | |

and powerful technique for assigning the resonances of a large molecule where unambiguous empirical assignments were not possible. The inherent difficulty of the technique is more than offset by the accuracy of the information provided. The $^{13}C$ NMR chemical shift assignments for maduramicin listed in Table 1 have provided a strong basis for NMR studies of the biosynthesis of this antibiotic. Preparations of maduramicin derived from isotopically labeled precursors such as $^{13}C$ and $^{18}O$ enriched acetate, propionate and methionine, as well as molecular $^{18}O_2$, have been studied. The biochemical origin of the carbon and oxygen atoms in maduramicin has been deduced (H. Tsou, *et al.* to be published).

The $^{13}C$ NMR spectra reported here were obtained using a Bruker CXP 300 spectrometer equipped with a standard 10 mm saddle-coil probe-head tuned to 75.47 MHz. The two-dimensional "INADEQUATE" spectrum, shown in Fig. 2, was obtained in 110 hours in an 8,192 × 256 point data array, corresponding to frequency dimensions of ±4 kHz × ±4 kHz. For each of the 256 incremental delays, the NMR transient was time-averaged over six cycles of a 128-step phase-cycling sequence. Thus, a total of 768 × 256 transients were acquired in the complete experiment. The sample was made by dissolving 2 g of maduramicin in 3.5 ml of deuteriochloroform and transferring an aliquot to a 10 mm NMR tube to a sample length of 3 cm. Broad-band proton decoupling was used with a power level of 4 W to minimize sample-heating. A slight concentration dependence of the chemical shifts was observed, and at low concentrations, one pair of closely spaced peaks was unresolved.

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