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APPLICATION OF A LONG-RANGE HETERONUCLEAR COSY EXPERIMENT TO CARBON NMR ASSIGNMENTS. KINAMYCIN D

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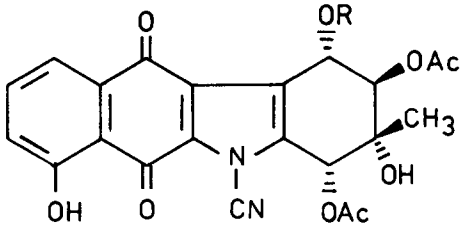
Abstract: A simple 2D NMR experiment that reveals long-range J_{CH}'s as aids in assigning quaternary carbons and its application to the assignments for kinamycin D are described.

The kinamycins, a group of antibiotics[2,3] produced by *Streptomyces murayamaensis* ATCC 21414, differ from each other only in the hydroxyl acetylation pattern. Represented by kinamycin D, 1, the benzo[b]carbazole skeleton and the N-cyano moiety are both highly unusual among natural products. The kinamycin structures were determined in part by chemical and spectroscopic means,[4] and that of kinamycin C, 2, was completed by an x-ray crystallographic study[5] of the phenolic p-bromobenzoate. This also established the absolute stereochemistry. These antibiotics are strongly active against gram-positive bacteria but less so against gram-negative organisms; 2 showed weak anti-tumor activity.[3]



1 R=H

2 R=Ac

As a necessary step in our study of the biosynthesis of kinamycin D (see following paper), fully assigned proton and carbon NMR spectra were needed. Ōmura[4] had previously assigned the proton spectrum, and had tentatively[6] assigned the carbon spectrum. Using standard NMR experiments and a new long range heteronuclear COSY experiment we have now assigned all of the carbon-13 resonances.

The ^{1}H NMR assignments of the A- and D-rings were first confirmed using a ^{1}H-^{1}H COSY experiment.[7] A DEPT experiment then identified the multiplicity of each ^{13}C NMR resonance, and in conjunction with a standard $^{1}H/^{13}C$ HETCOR spectrum C-5, -6, -7, -8, -1', -2', -3', -4', the acetates, and the C-2' methyl could all be assigned.[8]

We recently described a pulse sequence for a $^{1}H/^{2}H$ COSY experiment that allows the unambiguous detection of deuterium enriched sites through the small proton-deuterium geminal and vicinal couplings.[9,10] This sequence can, in principle, be used to detect any small magnitude heteronuclear spin couplings.[11] By using the ^{13}C and ^{1}H frequencies for the pulses, the long-range $^{1}H/^{13}C$ COSY spectrum shown in Figure 1 was obtained. The intense matrices[12] reveal all the one-bond proton-carbon couplings, while the weak cross-peaks are due to long-range couplings.[13] Although long-range couplings could be detected in a one-dimensional gated decoupled spectrum, ambiguity would likely remain if a carbon resonance were coupled to

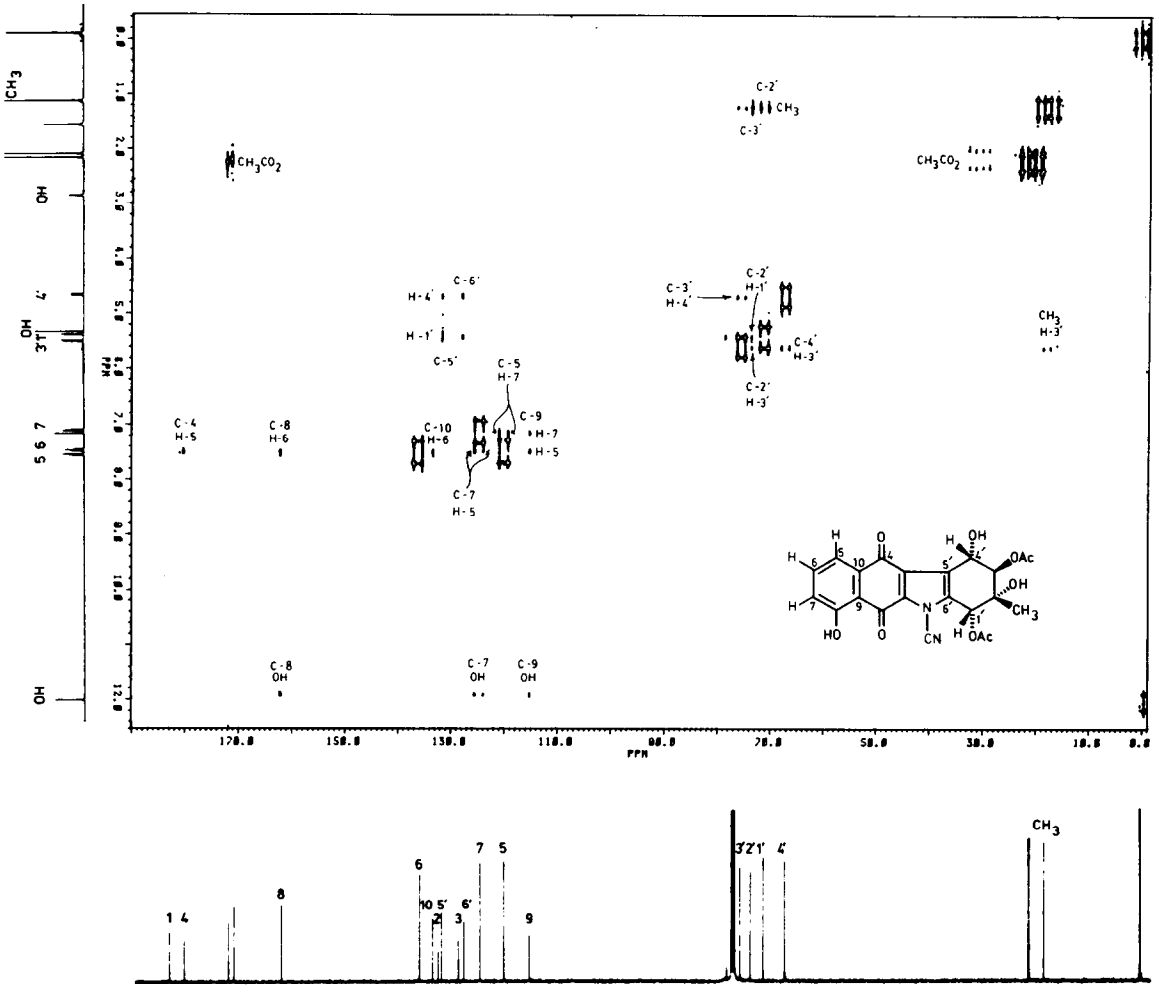


Figure 1. Contour plot of a long-range $^{13}C/^{1}H$ HETCOSY experiment for kinamycin D. Spectral acquisition parameters: 19231 Hz sweep width in the F_2 dimension; 512 spectra (128 scans each) were accumulated with 0.192 msec increments across the interval 0.192 to 98.496 msec. Resolution was 9.4 Hz/pt in the F_2 dimension and 5.1 Hz/pt in the F_1 dimension. Assignments of the long-range cross peaks are noted on the plot.

more than one proton resonance. The second dimension provided in the experiment described here edits such data into an unambiguous form. Table 1 lists all of the CNMR assignments.

Table 1. ^{13}C and ^{1}H-Chemical Shift Assignments.

Carbon Position	^{13}C-Chemical Shifts	Carbon Position	^{13}C-Chemical Shifts	Proton Position	^{1}H-Chemical Shifts (J_{HH} in Hz)
1	δ 183.6	7	δ 124.3	OH- at C-8	δ 12.13
4	180.8	5	120.3	H-5	7.68 t J=8
$CH_3\underline{C}OO$-	172.3	9	115.6	H-6	7.58 d J=8
$CH_3\underline{C}OO$-	171.2	3'	75.7	H-7	7.22 d J=8
8	162.4	2'	73.7	H-3'	5.59 d J=8
6	136.3	1'	71.3	H-1'	5.48 s
10	133.8	4'	67.3	OH- at C-4'	5.43 s
2	132.8	$\underline{C}H_3COO$-	21.2	H-4'	4.78 d J=8
5'	132.1	$\underline{C}H_3COO$-	20.9	OH- at C-2'	3.10 bs
3	129.0	CH_3-	18.3	CH_3COO-	2.26 s
6'	127.8			CH_3COO-	2.19 s
				CH_3-	1.22 s

The three-bond proton-carbon couplings, typical of aromatic systems, detected between H-5 (δ7.68) and the resonances at δ180.8 and δ115.6 unequivocally assign these to C-4 and C-9, respectively. The long-range couplings detected between H-6 (δ7.58) and the resonances at δ162.4 and δ133.8 confirm the lower field resonance as due to C-8 and now assigns the latter resonance to C-10. As expected H-5 shows long-range coupling to δ124.3 (C-7) and H-7 shows long-range coupling δ120.3 (C-5). Interestingly, the hydrogen-bonded phenolic proton (δ12.13) shows long-range couplings to δ124.3 (C-7), δ162.4 (C-8), and δ115.6 (C-9), the last of these supporting the new assignment mentioned above.

Both two- and three-bond proton-carbon couplings can be expected in aliphatic systems. The protons on C-1', -3', -4', and the C-2' methyl all show long-range couplings consistent with the previous assignments (see Table 1). In addition, H-1' (δ5.48) and H-4' (δ4.78) both show long-range couplings to δ127.8 and to δ132.1. Thus, these resonances can now be assigned to C-6' and C-5'.[14] By difference, C-2 and C-3 resonate at δ129.0 and δ132.8; by analogy with mitomycin C[15] and with the mitocenes[16] the former can be assigned to C-3 and the latter to C-2. The new 2D NMR experiment reported here should prove generally useful for ^{13}C NMR assignments.[17]

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12. A CH_3 results in a 2 x 4 matrix, a CH_2 results in a 2 x 3 matrix, and a CH results in a 2 x 2 matrix.
13. Due to the resolution used a quaternary carbon appears as a single cross-peak, while a CH results in a doublet, and a CH_2 and CH_3 would result in a triplet and a quartet, respectively.
14. By analogy to tryptophan (J.H. Bradburg and R.S. Norton, *Biochem. Biophys. Acta*, 1973, 32B, 10) and to mitosenes (ref. 15), C-5' was assigned to δ127.8 and C-6' to δ132.1. However, the results of biosynthetically incorporating [^{13}C]-labeled acetates require the reversal of these two assignments (see following paper).
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