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Application of Long Range $^{1}H/^{13}C$ Heteronuclear Correlation Spectroscopy (LR HETCOSY) to Structure Elucidation: The Structure of Murayaquinone

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Abstract: The structure of a polyketide-derived, o-phenanthraquinone streptomyces metabolite has been deduced from interpretation of a 2D heteronuclear correlation experiment that reveals long range ^{1}H-^{13}C couplings.

Streptomyces murayamaensis has previously been shown to produce the structurally unusual kinamycins A - D,[2] possessing a benzo[b]tetrahydrocarbazole skeleton.[3] In the course of our studies of kinamycin biosynthesis,[4] we observed two additional colored metabolites upon TLC analysis (silica gel) of crude extracts of S. murayamaensis fermentations. One of these, giving a pink spot on TLC, has now been characterized, primarily by the application of a simple 2D NMR experiment (LR HETCOSY) that reveals long-range ^{1}H-^{13}C spin couplings.[5,6] This experiment allowed the assignment of all the quaternary ^{13}C resonances and defined the carbon-carbon connectivity for the structure of murayaquinone, 1, possessing the rare ortho-phenanthraquinone skeleton.

S. murayamaensis was grown as previously described, and the broth and mycelial mat were extracted with benzene.[4] The extracts were dried (Na_2SO_4) and concentrated in vacuo to yield a gum (900 mg from 1.2 L) that was taken up in $CHCl_3$ and chromatographed on a column (2.2 x 33 cm) of Silicar CC-4 (Mallinkrodt 7086) prepared and eluted with $CHCl_3$. The pink band was collected separately (104 mg) and further purified by HPLC (two Partisil PXS 10/25 columns in series, iso-octane:THF = 9:1, 3.0 mL/min at 1600 psi, detection by refractive index). After removing the solvent, crystallization from CH_2Cl_2 yielded 9 mg of 1 : mp 108-109°C.[7]

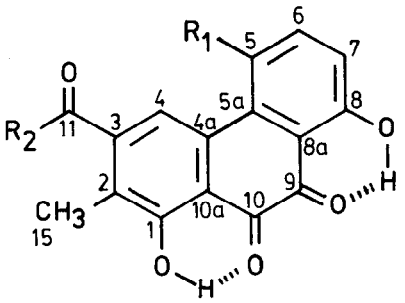
The infrared spectrum contained absorbances at 3400 (H-bonded OH), 1700 (aryl

$\underset{\sim}{1}$: R_1 = H, R_2 = $CH_2^{12}CH_2^{13}CH_3^{14}$

$\underset{\sim}{2}$: R_1 = H, R_2 = $CH_2CH_2CH(CH_3)_2$

$\underset{\sim}{3}$: R_1 = OH, R_2 = "

$\underset{\sim}{4}$: R_1 = H R_2 = CH_3



ketone) and 1615 (quinone) cm^{-1}. A single quinone absorbance indicated a similar environment for each of these carbonyls. The ^1H NMR and ^1H COSY spectra (400.13 MHz) indicated one methyl group and one n-propyl group on quaternary unsaturated carbons, two hydrogen-bonded phenols, an isolated aromatic proton (penta-substituted ring) and a grouping of three aromatic protons indicative of 1,2,3-substitution. Analysis of the ^{13}C NMR and ^{13}C DEPT spectra (100.6 MHz) confirmed the two CH_3's, two CH_2's, and four CH's, as well as revealed eleven unsaturated quaternary carbons. Thus, the data suggested a tricyclic quinone with methyl and butyryl side chains. These substituents were inferred to be ortho to each other on the assumption that the biogenesis of $\underset{\sim}{1}$ was from a straight-chain polyketide precursor. With the required hydrogen-bonding of phenols to both quinone carbonyls, four anthraquinones and four phenanthraquinones remained as possible structures.

All but one of these eight structures could be eliminated by data from the LR HETCOSY spectrum (Figure 1). The cross-peak matrices (2 x 4, 2 x 3, and 2 x 2) identify sets of directly bonded carbons and hydrogens. The remaining cross peaks were due to long range ^1H-^{13}C coupling. Thus, the phenolic proton at 12.27 ppm is coupled to a methine carbon and two quaternary carbons, while the phenolic proton at 12.73 ppm shows coupling to three quaternary carbons. These data implied that the lone aromatic hydrogen (7.31 ppm) must be para to the latter phenol and meta to the methyl group (coupling between 2.20 and 126.2 ppm).

Four cross peaks with the aromatic hydrogen singlet at 7.31 ppm were observed. A cross peak from 126.2 ppm ($^3J_{CH}$) demonstrated its meta relationship to the methyl group, thus confirming its para relationship to the 12.73 ppm phenol. A second cross peak, from 205.4 ppm, placed the butyryl side chain on the adjacent ring carbon, confirming the biogenetic assumption (vide infra), and a cross peak from 114.8 ppm confirmed this as the ring fusion next to the quinone. The most important cross peak was that from 135.0 ppm. Since this is not due to a quinone carbonyl, $\underset{\sim}{1}$ must have a phenanthrene skeleton, and the structure shown fulfills all the requirements. All remaining cross peaks fully support the unique structure $\underset{\sim}{1}$.

Only three polyketide-derived phenanthrenes ($\underset{\sim}{2}^8$, $\underset{\sim}{3}^9$, and $\underset{\sim}{4}^{10}$) have been previously characterized. The present example demonstrates how effective the LR HETCOSY can be in assigning quaternary carbons and mapping carbon-carbon connectivities.

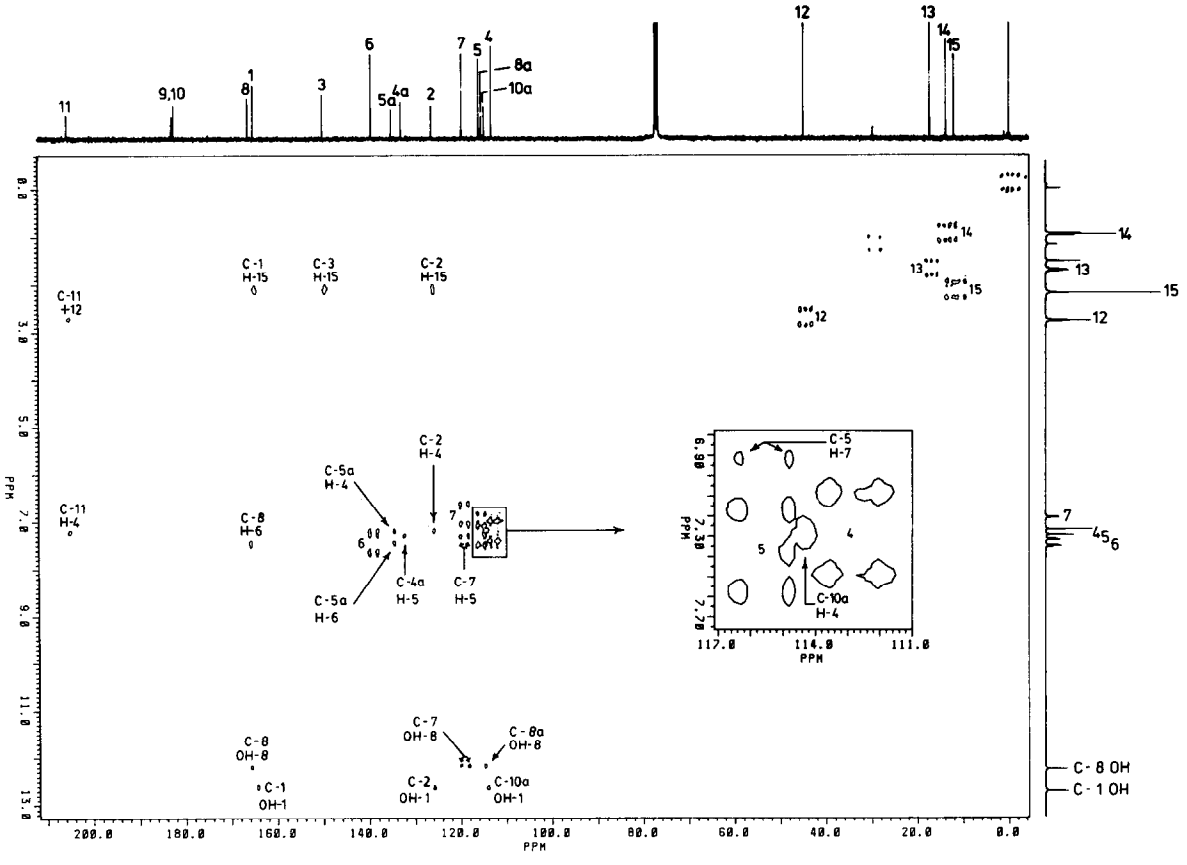


Figure 1. Contour plot of $^{1}H/^{13}C$ LR HETCOSY spectrum of 1. Spectral aquisition parameters: 21739 Hz sweep width in the F_2 dimension; 256 spectra (128 scans each) were accumulated with a 1.0 sec relaxation delay and 0.179 msec increments across the interval 8.0 to 53.722 msec. Resolution was 21.2 Hz/pt in the F_2 dimension and 10.9 Hz/pt in the F_1 dimension.

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