Revised Structures for the Kinamycin Antibiotics: 5-Diazobenzo[*b*]fluorenes Rather Than Benzo[*b*]carbazole Cyanamides

Steven J. Gould,* Nuria Tamayo, Chris R. Melville, and Martha C. Cone

Department of Chemistry
Oregon State University
Corvallis, Oregon 97331-4003

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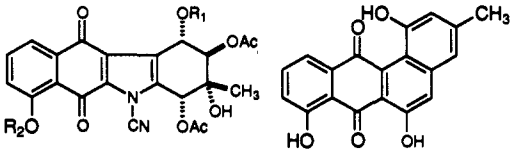
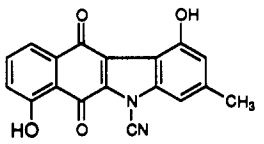
The kinamycin antibiotics, first reported in 1970,[1] were isolated from *Streptomyces murayamaensis*, and they were characterized as benzo[*b*]carbazoloquinone cyanamides (e.g., **1** for kinamycin D, KD) on the basis of chemical, spectroscopic, and X-ray crystallographic data.[2-5] In 1989[6] and 1992[7] the first additional organisms to produce kinamycins were reported. We have studied the biosynthesis of the kinamycins extensively and have established that they are polyketides derived from the benz[*a*]anthraquinone dehydrorabelomycin, **2**.[8-11] A number of intermediates between **2** and **1** have been isolated and characterized, including a purple metabolite that we had characterized as **3**, and had named prekinamycin.[12,13] Results from synthetic studies from other laboratories have led us to reexamine these structures. We now report a revised structure for the skeleton of the kinamycins.



1, $R_1 = R_2 = H$
4, $R_1 = Ac$, $R_2 = p$-BrPhCO

2



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^{1}H NMR spectroscopic data had been used to identify the substitution patterns of the naphthoquinone system and the highly oxygenated D-ring of the kinamycins.[5] The unusual cyanamide moiety had been assigned from the infrared absorbance (ca. 2150 cm^{-1}) and from detection of ammonia upon subjecting the kinamycins to hydrolytic conditions.[5] An X-ray diffraction study of a crystal of kinamycin C *p*-bromobenzoate, **4**, appeared to confirm these features.[4] When we initiated our biosynthetic studies, we were able to observe and assign ^{13}C NMR resonances for all carbons except the cyanamide, which was expected to occur at ca. δ 110–120.[8,14] This resonance was subsequently found at δ 78 by generating a sample of kinamycin D from a fermentation using $(^{15}NH_4)_2SO_4$ as the sole nitrogen source.[11] The unusual chemical shift could not be readily explained, but was attributed to possible electronic effects of the indoloquinone.

Numerous researchers have been attempting to synthesize members of the kinamycin family.[15-20] A set of *N*-cyanoindoles has been prepared, and the IR absorbances (2237–2245 cm^{-1}) and ^{13}C NMR resonances (δ 105–108) agreed poorly with data from the kinamycins.[17] Echavarren's group has recently reported[21] the synthesis of **3**, and spectroscopic data did not match the natural product. Advanced intermediates in this synthesis already possessing a cyanamide moiety exhibited ^{13}C NMR spectra containing a resonance at approximately δ 112,[22] rather than one in the region of δ 78. Furthermore, these compounds, as well as synthetic **3**, exhibited IR absorbances consistently in the region of 2250 cm^{-1}.[23,24] Reconsideration of the cyanamide moiety was in order, and since the structural solution for **4** had given R = 8.9%,[4] it was possible that the original crystallographic data set may not have distinguished $R_2NC{\equiv}N$ from $R_2CN{\equiv}N$ or $R_2NN{\equiv}C$ (charges not shown). The diazo alternative was clearly possible: ^{13}C NMR resonances of a number of diazo compounds have been observed in the δ 60–80 region, including 9-diazofluorene at δ 63.2.[25]

Although only small quantities of prekinamycin had been available from fermentations of *S. murayamaensis*, substantial quantities recently became available from a mutant blocked in the kinamycin pathway that was found to overproduce prekinamycin (ca. 100 mg/L).[26] We tried unsuccessfully to obtain a crystal of prekinamycin, or of a simple derivative, suitable for X-ray diffraction; however, treatment of prekinamycin with $Rh_2(OAc)_4$[27] in methanol yielded a single main product that proved to be the fluorene **5**.[28] A key problem in the data set for prekinamycin had been the lack of any observable long-range ^{13}C–^{1}H couplings directly linking the naphthoquinone A/B-rings with the phenolic D-ring. Now, however, a $^3J_{CH}$ coupling was observed between the new olefinic hydrogen (δ 7.25) and the semiquinone phenolic carbon (δ 150.2), and an NOE was observed between this hydrogen and the hydrogen para to the D-ring phenol (δ 6.92). All other predictable $^3J_{CH}$'s were also observed, including one between H-10 and the semiquinone carbonyl (δ 198.0). Concurrently, the (+)-α-methylbutyrate of kinamycin D was prepared using the acid anhydride and $ZnCl_2$ catalysis[28] and crystallized by diffusion of water into a DMF solution. A single crystal of this derivative (orthorhombic space gorup $P2_12_12_1$)

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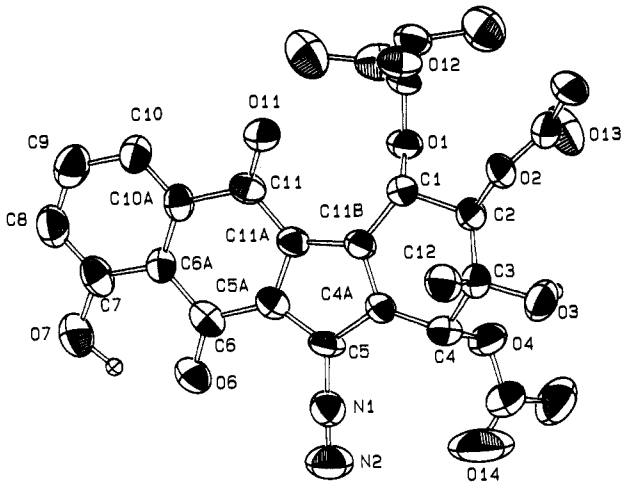
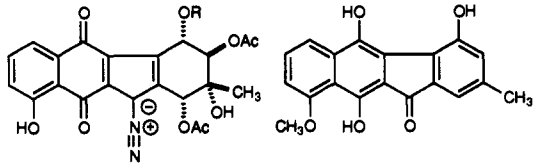


Figure 1. ORTEP drawing from the single-crystal X-ray structure determination of **6**. Hydrogens have been omitted for clarity.

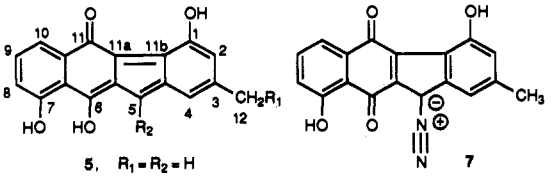
was analyzed by X-ray diffraction, and the direct methods program SHELXS (TEXSAN[29] crystallographic software package) was used to determine the positions of all non-hydrogen atoms, based on 2577 unique observations. Final cycle anisotropic refinement with a DIFABS absorption correction[30] gave R = 5.18% and R_w = 5.63% for the diazo structure **6** (see Figure 1 for ORTEP plot), which was significantly better than the values obtained for the cyanamide (5.69 and 6.35%, respectively) or the isonitrile (5.73 and 6.41%, respectively). Furthermore, when the data set was solved for a cyanamide structure, the calculated temperature factor for the putative cyanamide carbon was anomalously low compared to those of the remaining non-hydrogen atoms, indicating that more electron density exists at this position than would be accounted for by carbon.



6, R = (+)-α-methylbutryryl
8, R = H

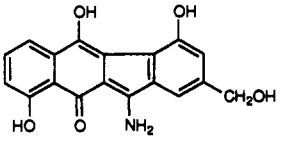
9

From these results, it is clear that prekinamycin is the diazo compound **7**, of which all the kinamycins are derivatives (e.g., KD = **8**). Naturally-occurring diazo compounds have been very rare.[31-33] The benzo[*b*]fluorene skeleton has been unknown among natural products, but we recently reported the structure of kinafluorenone, **9**, from another *S. murayamaensis* blocked mutant.[34] In addition, examination of an HPLC/photodiode array data base of extracts of our blocked mutants has now revealed the presence of **5**.[35] While we had suggested formation of the benzo[*b*]fluorene skeleton to be a shunt pathway from kinamycin biosynthesis, it now must be considered an essential feature. During the past year, two other *Streptomyces* species have been reported to produce benzo[*b*]fluorene derivatives, including two with an amino group at C-5 (e.g., **10**).[36-38] Either 12-deoxy-**10**, 7-*O*-demethyl-**9**, or **5** may prove to be intermediates in kinamycin biosynthesis. This is currently under investigation.



5, $R_1 = R_2 = H$

7



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Supplementary Material Available: Tables I–V containing relevant details of the X-ray crystallographic studies, atomic parameters, bond distances, bond angles, and anisotropic thermal parameters (8 pages); Table VI containing structure factor amplitudes (18 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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