Identification of Prekinamycin in Extracts of *Streptomyces murayamaensis*

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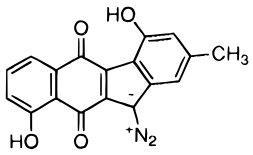
The kinamycin antibiotics, first isolated from *Streptomyces murayamaensis*,[1−5] were originally reported as *N*-cyanobenzo[*b*]carbazoles. Among the newer kinamycins, we named one ("Cpd A") as prekinamycin,[6,7] since it only lacked the oxidative elaboration of one ring. This structure was synthesized,[8] but its IR and NMR data did not match those of Cpd A. Subsequently, we[9] and Dimitrienko[10] revised the structures of the kinamycins, showing them to be 5-diazobenzo[*b*]fluorenes (e.g. prekinamycin, **1**, and kinamycin D, **2**). We then synthesized **3**[11,12] and **4**[13,14] from tetramethyl benzo[*b*]fluorenone **5**[15,16] and demonstrated the existence of intermediates **3** (kinoscurinone)[11] and **4** (stealthin C)[13] in kinamycin biosynthesis.



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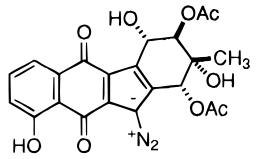

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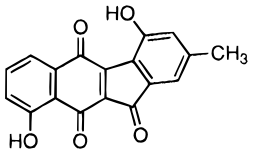

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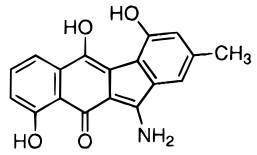

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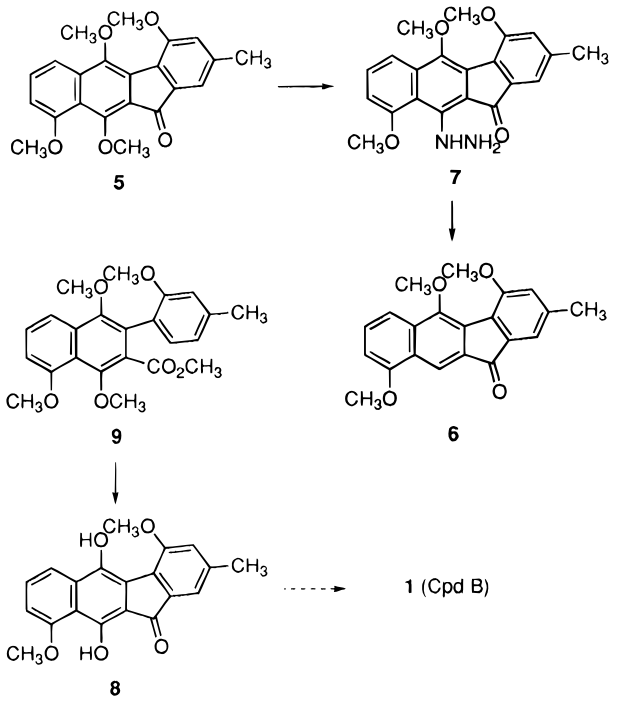
Scheme 1



Fluorenone **5** was next chosen as the starting point for synthesis of prekinamycin. It was treated with anhydrous hydrazine in ethanol, yielding a product that was then treated with either ceric ammonium nitrate or silver(I) oxide (Scheme 1). In each case the product contained only three methoxy groups and its structure was unexpectedly shown to be fluorene **6**. Thus, rather than forming the expected hydrazone, hydrazine had added at C-6 of **5**, followed by elimination of methanol, to give **7**. We next prepared the unprotected hydroquinone **8** by treating ester **9**[15] with polyphosphoric acid. This compound could also be prepared from **5** with the same reagent. Independently, Hauser *et al.* had prepared this same compound by a different route and carried it forward to complete the synthesis of **1**.[17] We have since determined that synthetic **1** actually matches a different *S. murayamaensis* metabolite from that previously thought to be prekinamycin, as described below.

Treatment of Cpd A with $Rh_2(OAc)_4$ in methanol had yielded the unsubstituted fluorene **10**.[9] Methylation afforded two monomethyl ethers, **11** and **12**, with their regiochemistry readily identified by observable NOE's, as shown. While structures **10**−**12** are secure and had led us to assign **1** to Cpd A, small differences in IR and ^{1}H NMR values between Cpd A and synthetic prekinamycin once again placed the structure of the former in doubt. Comparison of the two by HPLC with photodiode array detection[12] confirmed that they were different.

While Cpd A was clearly not prekinamycin, comparison of the HPLC retention time and UV/vis spectrum of synthetic prekinamycin with our library of *S. murayamaensis* compounds and extracts did yield a perfect

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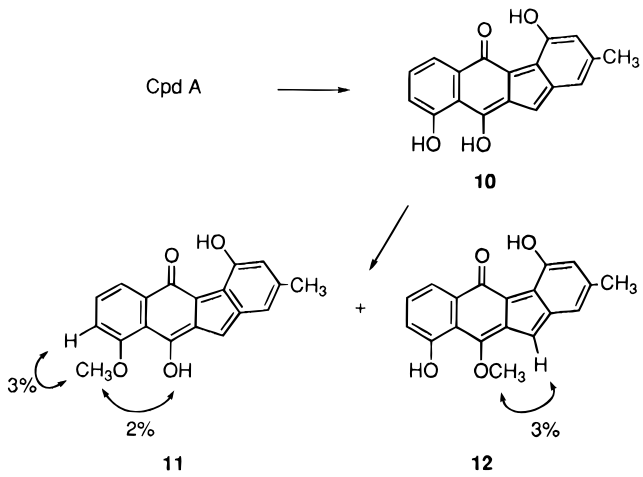

match between synthetic prekinamycin and another metabolite ("Cpd B"). Cpd B had been observed in extracts of *S. murayamaensis* mutant MC2 that had been grown in a 7% farina-based medium[18] at 28 °C, as well as in extracts of the wild strain grown in a galactose/asparagine-based medium[19] at 35 °C. The UV/vis spectra of the two compounds were identical. HPLC coinjection of the two gave a single peak. Cpd B was then purified by flash silica gel chromatography (CH_2Cl_2) from a fermentation of mutant MC2, and its ^1H NMR spectrum was the same as that of the synthetic material. Thus, although the structure of Cpd A is now unclear,[20] Cpd B has structure **1**, which belongs to prekinamycin. Future efforts will test its intermediacy in kinamycin biosynthesis.

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Supporting Information Available: Experimental procedures and characterization data (5 pages).

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