Isoprekinamycin Is a Diazobenzo[*a*]fluorene Rather than a Diazobenzo[*b*]fluorene

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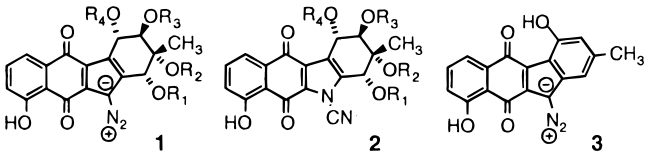
Received May 12, 2000

The discovery[1,2] that the kinamycin antitumor antibiotics possess the diazobenzo[*b*]fluorene (**1**) rather than the *N*-cyanobenzo[*b*]carbazole ring (**2**) system has stimulated considerable interest in the biosynthesis,[3] mechanism of action,[4] and chemical synthesis of this novel class of natural products.[5,6]

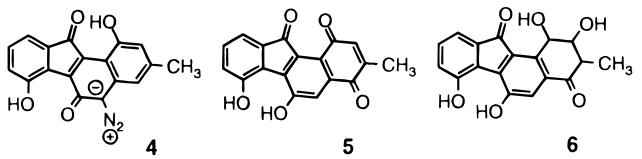


1 **2** **3**

Recently, Hauser and Zhou[5] reported the total synthesis of the structure **3** assigned to a metabolite which had been given the laboratory designation CpdA[7] and was suspected to be a precursor to the kinamycins.[8] Hauser and Zhou's synthetic compound was found to differ from natural CpdA, which had been assigned the trivial name prekinamycin, but to be identical to another metabolite which had been given the laboratory designation CpdB.[8] As a result, the structure **3** and the name prekinamycin were reassigned to CpdB and the structure of metabolite CpdA became a mystery.

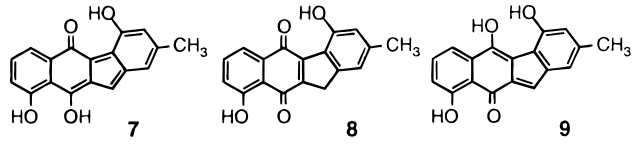
We report, herein, a solution to this mystery which establishes that CpdA possesses the diazobenzo[*a*]fluorene structure **4** to which we assign the name isoprekinamycin. Hence, CpdA is related to the recently discovered fluostatins A (**5**) and B (**6**).[9]

The recognition that CpdB possessed structure **3** prompted us to reexamine critically all of the evidence which had led to the conclusion that CpdA possessed this diazobenzo[*b*]fluorene structure to see if any clues as to the true identity of CpdA might



4 **5** **6**

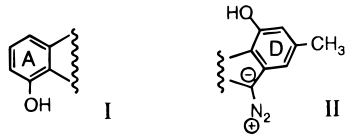
be unearthed. In the course of the revision of the structures of the kinamycins from the *N*-cyanobenzo[*b*]carbazole (**2**) to the diazobenzo[*b*]fluorene (**1**) structure, chemical evidence regarding the structure, involving deazotization of CpdA, had been obtained. In particular, treatment of CpdA with $Rh_2(OAc)_4$ in methanol gave a product which exhibited spectroscopic characteristics compatible with structure **7**.[1] Further critical analysis of this result, however, has led us to question the accuracy of **7** for the structure of the deazotization product of CpdA.



7 **8** **9**

In particular, the tautomers of **7**, **8**, and **9**, were rejected as possible structures because they are incompatible with nOe and HMBC NMR results. The fact that **8** and **9** each incorporate two benzenoid rings whereas **7** incorporates just one benzenoid ring suggested, intuitively, that tautomer **7** might be expected to be the least favorable of the three. Detailed ab initio calculations[10] confirmed our suspicions in that structure **7** was predicted to be much higher in energy than either structures **8** or **9** (by 27.8 and 25.2 kcal/mol respectively). Thus, it was concluded that the CpdA deazotization product was not a benzo[*b*]fluorene, and the suspicion arose that CpdA also did not possess this ring system.

An HMBC spectrum of CpdA diacetate[11] revealed that it incorporated the partial structures I and II below. The remainder of the structure consisted of two carbonyls and two aromatic carbons. How these fragments were combined to form the final structure of CpdA was unclear.



I II

Any combination of these partial structures must fit the criteria that both phenolic hydroxyls are capable of intramolecular hydrogen bonding and a carbonyl must be in the ring adjacent to ring A (based on a weak HMBC correlation between H10 and the carbonyl at 192.48 ppm in the diacetate). Because the ^{13}C NMR shift of 192.48 ppm suggests that this carbonyl is contained within a five-membered ring, the possible structures should have a 6,5,6,6 ring system, rather than the 6,6,5,6 ring system observed for the kinamycins. Distinguishing between the various possibilities by direct NMR experiments, however, is hampered by the lack of protons in rings B and C which would allow for the connection of rings A and D. A further challenge to NMR studies of unmodified CpdA is the poor solubility of this compound.[11]

Fortunately, a derivative produced by treating CpdA with sodium borohydride provided the necessary connectivities. Sodium borohydride reduced a carbonyl and deazotized CpdA to produce a tetrahydroxy compound, which was converted to the tetraacetate derivative **10** and analyzed by both 1D and 2D

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(1) Gould, S. J.; Tamayo, N.; Melville, C. R.; Cone, M. C. *J. Am. Chem. Soc.* **1994**, *116*, 2207−2208.
(2) Mithani, S.; Weeratunga, G.; Taylor, N. J.; Dmitrienko, G. I. *J. Am. Chem. Soc.* **1994**, *116*, 2209−2210.
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(4) Arya, D. P.; Jebaratnam, D. J. *J. Org. Chem.* **1995**, *60*, 3268−3269.
(5) Hauser, F.; Zhou, M. J. *J. Org Chem.* **1996**, *61*, 5722−5723.
(6) (a) Williams, W.; Sun, X.; Jebaratnam, D. J. *J. Org. Chem.* **1997**, *62*, 4364−4369. (b) Mohri, S.; Stefjnovic, M.; Snieckus, V. *J. Org. Chem.* **1997**, *62*, 7072−7073. (c) DeFrutos, O; Echevarren, A. M. *Tetrahedron Lett.* **1997**, *38*, 7941−7942.

(7) The terms CpdA and CpdB refer to two isomeric natural products isolated from *S. murayamaensis* and are applied to these compounds regardless of the structures assigned to them.[8] The term prekinamycin, previously applied to structure **2**,[11] is now applied to structure **3**, and the term isoprekinamycin is now applied to the isomeric structure **4**. The work by Hauser and Zhou[5] and by Gould and co-workers[8] leads to the conclusion that CpdB possesses the prekinamycin structure **3**, and the work described herein leads to the conclusion that CpdA must possess the isoprekinamycin structure **4**.

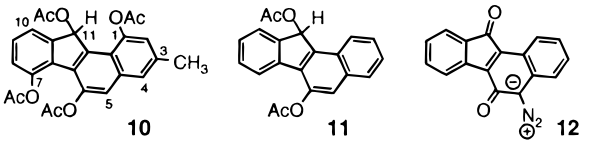
(8) Gould, S. J.; Chen, J.; Cone, M. C.; Gore, M. P.; Melville, C. R.; Tamayo, N. *J. Org. Chem.* **1996**, *61*, 5720−5721.
(9) Akiyama, T.; Nakamura, K. T.; Takahashi, Y.; Naganawa, H.; Muraoka, Y.; Aoyagi, T. *J. Antibiot.* **1998**, *51*, 586−588.

(10) For full details see the Supporting Information.

(11) Seaton, P. J.; Gould, S. J. *J. Antibiot.* **1989**, *42*, 189−197.

10.1021/ja001631w CCC: $19.00
Published on Web 08/11/2000

(HSQC, HMBC) NMR spectroscopy. The two newly introduced hydrogens, appearing at 7.16 and 7.50 ppm, proved critical to assembling the structure. The proton at 7.16 ppm was attached to a carbon at 76.08 ppm, revealing the site of ketone reduction. This hydrogen showed HMBC correlations to ring A carbons 6b, 10, and 10a, indicating the placement of the secondary alcohol center in ring B. An additional HMBC correlation from H10 to the carbon at 76.08 ppm confirmed the placement of the alcohol at C11. The 7.50 ppm proton had HMBC correlations with C4, C4a, and C11b, indicating that this hydrogen, H5, had replaced the diazo group. Further HMBC correlations from H5 to 144.22 ppm and to the acetyl carbonyl at 169.92 ppm established C6 as a phenolic carbon. Additional correlations in the HMBC spectrum allowed for complete assignment of the tetraacetate which was determined to have structure **10**.[10] To provide further support for the assignments of the carbons adjacent to the newly introduced hydrogens, compound **10** was prepared with sodium borodeuteride in methanol-d_4. In deuterated **10**, H11 was replaced almost completely, while H5 was deuterated to ~30%. The ^{13}C NMR spectrum of this sample showed isotope induced shifts for the signals at 145.81, 144.22, 137.42, and 136.70 ppm, positioning these carbons adjacent to C11 and C5, confirming the assignments based on the HMBC spectrum.



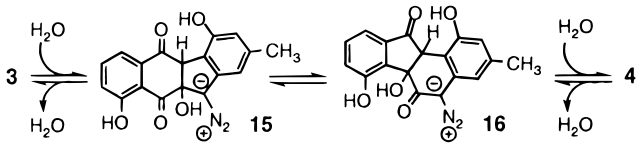
The structure of this reduction product is compatible only with the structure **4** from among several possibilities being considered for CpdA. Both phenolic hydrogens can participate in hydrogen bonds with neighboring carbonyls and the carbonyl in the five-membered B ring is adjacent to H10. This structural assignment was further bolstered by comparison of the spectroscopic properties of **10** and CpdA with the model benzo[*a*]fluorene systems **11** and **12** which were prepared by unambiguous synthesis for this purpose.[12]

The ^{13}C NMR spectrum of the **11** in CD_2Cl_2 exhibits a signal at 74.37 ppm corresponding to C11 as compared to 76.08 ppm for the analogous ring carbon in **10**. The C11−H in **11** appears as a singlet at 7.23 ppm as compared to 7.16 ppm in the proton NMR spectrum of **10**. The ^{13}C NMR characteristics of model **12** are in excellent agreement with the corresponding structural features of CpdA. In particular, the signal assignable to the diazo group appears at 82.64 ppm in **12** and at 83.71 ppm in CpdA diacetate. The carbonyl at 196.77 in the spectrum of **12** assigned to the five-membered ring ketone has a counterpart at 192.48 ppm in the spectrum of CpdA diacetate. In addition, the diazo group gives rise to a band at 2105 cm-1 in the IR spectrum of **12** as compared with a band at 2119 cm-1 in the spectrum of CpdA diacetate.

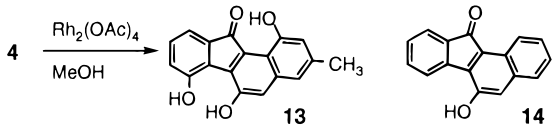
The assignment of structure **4** to CpdA is also in good agreement with the chemical evidence obtained in the course of the earlier structural revision of prekinamycin.[1] That is, the product of rhodium acetate induced deazotization of CpdA is **13**, ratherthan the benzo[*b*]fluorene system **7** as previously reported.

(12) Laufer, R. S.; Dmitrienko, G. I. Manuscript submitted to *Org. Lett.*

Scheme 1. Proposed Interconversion of **3** and **4**



Structure **13** is expected to represent a perfectly stable system and its spectroscopic properties agree very well with those of the synthetic model system **14**.[12] For example, in the ^{1}H NMR spectrum of **13**, a singlet appears at 7.25 ppm and shows a 2-bond correlation with a ^{13}C signal at 150.2 ppm, whereas in the model **14**, a corresponding proton signal is seen at 7.45 ppm and a ^{13}C signal at 152.3 ppm is observed.



The co-occurrence of closely related benzo[*a*]fluorene (isoprekinamycin) and benzo[*b*]fluorene (prekinamycin) natural products in the same *Streptomyces* species suggests that some biochemical mechanism for interconversion of these ring systems is present.[13,14] Ab initio calculations suggest that these ring systems are comparable in stability with the diazobenzo[*b*]fluorene ring system (prekinamycin) being 2.4 kcal/mol more stable than the diazobenzo[*a*]flourene system (isoprekinamycin).[10] Thus, a substantial amount of each of these systems is expected to be present at equilibrium.

Examination of the structural relationship between **3** and **4** suggests that interconversion might occur by reversible hydration of **3** to form **15** (Scheme 1), 1,2-carbon shift to give **16**, and dehydration to generate **4**. Such a rearrangement has chemical precedence in the base-induced ring contraction of certain hydroxyquinones to form indenone carboxylic acids.[15]

In conclusion, the assignment of the isoprekinamycin structure (**4**) to CpdA suggests that mechanisms exist in *S. murayamaensis* for the interconversion of benzo[*b*]fluorenes and benzo[*a*]fluorenes. Furthermore, it is reasonable to speculate that benzo[*b*]-fluorene precursors to the novel fluostatins exist in *Streptomyces* sp. TA-3391, the fluostatin producer. Studies aimed at effecting biomimetic conversion of benzo[*b*]fluorenes into benzo[*a*]fluorenes are in progress in order to make fluostatin-like systems accessible via the extensive methodology developed in the context of synthesis of kinamycin-related compounds.

Acknowledgment. Funding for this research was provided by NIH (GM 31715; S.J.G. and P.J.P.) and the NSERC of Canada (G.I.D). The Bruker DRX600 spectrometer was purchased with financial assistance from NSF (CHE-9413692) and the Keck Foundation.

Supporting Information Available: A procedure for the preparation of **10**, detailed spectroscopic data for **10**, and full details of ab initio calculations related to compounds **3**, **4**, **7**, **8**, and **9** (PDF). This material is avalailable free of charge via the Internet at http://pubs.acs.org.

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(13) The isotopic labeling pattern for CpdA formed from [1,2-$^{13}C_2$]acetate is consistent with the benzo[*a*]fluorene ring system arising from a benzo[*b*]-fluorene intermediate.

(14) The biosynthesis of CpdA using [1,2-$^{13}C_2$]acetate was performed as described previously for the kinamycins. Sato, Y.; Gould, S. J. *J. Am. Chem. Soc*. **1986**, *108*, 4625−4631.

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