Streptonigrin Biosynthesis. 7. Incorporation of Oxygen from $^{18}O_2$: Evidence for an Oxidative β-Carboline Cleavage

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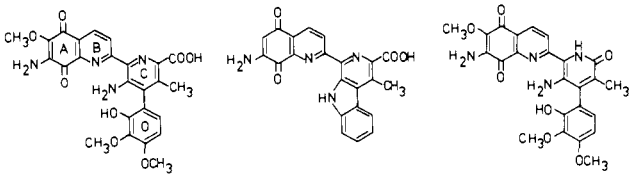
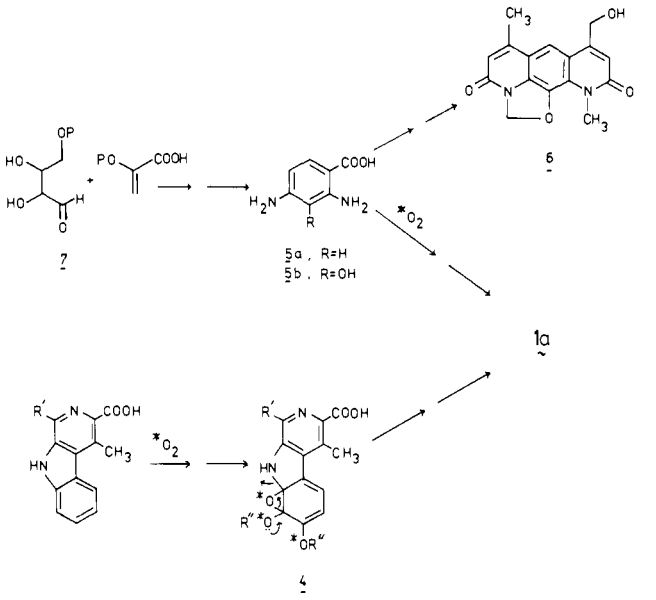
Some years ago we reported results[2] on the biosynthesis of streptonigrin (**1**), a potent anticancer antibiotic obtained from



1 **2** **3**

Streptomyces flocculus, which indicated that the 4-phenylpicolinic acid moiety was derived by cleavage of a β-carboline intermediate. Two mechanisms were presented, illustrative of chemically rational possibilities, that involved oxidation of the D ring. In one the C-8′ phenolic oxygen was derived from O_2 while in the other it was derived from H_2O. Subsequently, we characterized lavendamycin, **2**,[3] from *S. lavendulae*, clearly a member of the streptonigrin family, and containing the putative β-carboline moiety. Recently, Rickards et al.[4,5] reported the structure of streptonigrone (**3**) and proposed a third mechanism for the β-carboline cleavage that was formally nonoxidative and involved a covalent hydration[6] at C-8′ followed by a 1,6-elimination of the original indole nitrogen. By this mechanism the C-8′ phenolic oxygen would be derived from H_2O. This paper prompts us to report data that clearly indicate the oxygen in question is derived from molecular oxygen rather than from water.

A seed culture of *S. flocculus* was prepared[7] and used to inoculate four production broths[7] (250 mL in 1-L Erlenmeyer flasks). These were connected in series via two sterile filters to a closed system containing a burette which was refillable with $^{18}O_2$



7 **5a**, R=H **5b**, R=OH **6** **1a** **4**

gas (50% enriched, obtained from Cambridge Isotopes, Inc.), a small aquarium pump, and a CO_2 trap (aqueous KOH). Air was circulated at 2 L/min while the fermentation flasks were shaken at 29 °C and 225 rpm. After 92 h a total of 6 L of O_2 had been consumed.[8] The fermentation was terminated and the broths worked up in the usual fashion to give 10 mg of pure **1a**.

The 100.6-MHz ^{13}C NMR spectrum of **1a** in Me_2SO-d_6 was taken under standardized conditions, and eight isotope-shifted peaks were observed. These are shown in Figure 1, along with the C-8 and COOH resonances for comparison. Since we had previously assigned the complete ^{13}C NMR spectrum of **1**,[9] the data revealed that not only were all three methoxyl oxygens derived from O_2 but that the C-8′ phenolic oxygen and the C-5 quinone oxygen were also derived from molecular oxygen. Therefore, the oxygenation and the cleavage of the intermediate β-carboline leading to **1** were entirely oxidative processes. Our original proposal[2] involving an arene oxide **4** would fulfill these requirements; while other oxidative mechanisms may also be imagined, any process requiring H_2O as the source of any of the D-ring

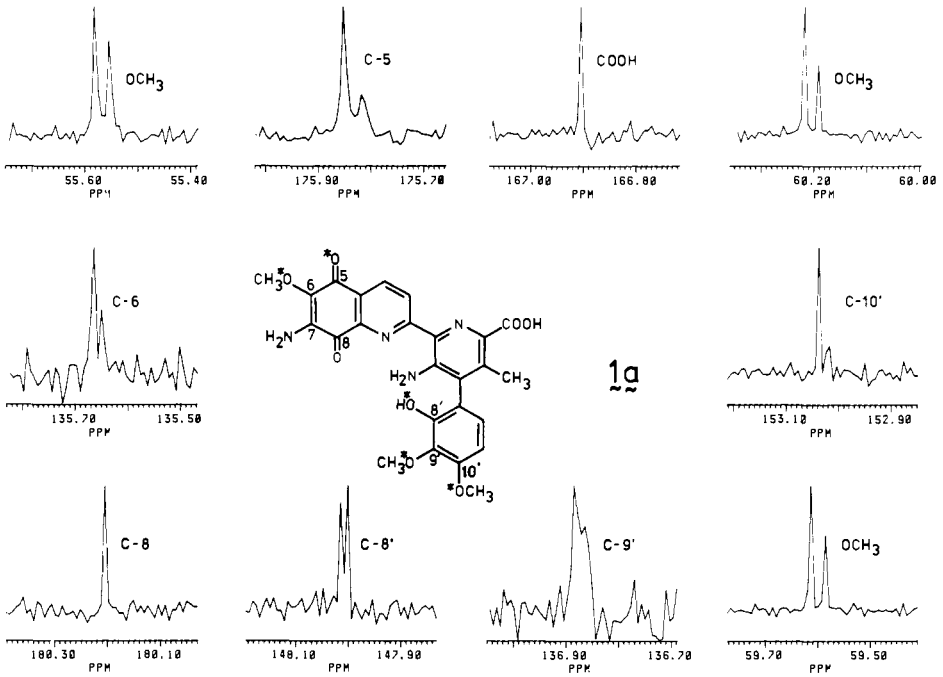


Figure 1. Portions of the ^{13}C NMR spectrum of **1a** (10 mg in 0.5 mL of Me_2SO-d_6) taken on a Bruker AM 400 spectrometer at 100.6 MHz (SW = 21 739 Hz, SI = TD = 64K, Hz/Pt = 0.66, AQ = 1.507 s, PW = 36°, LB = 0, GB = 0.9, NS = 42298). The relevant carbons are indicated. The three *O*-methyl carbons cannot be specifically assigned, but all three show isotope-shifted resonances.

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oxygens would be inconsistent with our results.

The experiment described has also shed light on the nature of the A-ring precursor. We had previously suggested[3] 4-amino-anthranilic acid (**5a**) for this role in the biosynthesis of **1** and **2**, as well as of nybomycin (**6**).[10] With the apparent lack of ^{18}O incorporation at C-8, it would appear that this oxygen was retained from the prearomatic precursor erythrose 4-phosphate (**7**),[11] such that 4-amino-3-hydroxyanthranilic acid (**5b**) may be the actual intermediate. This is currently under investigation.

Acknowledgment. Strains of *S. flocculus* were originally obtained from Dr. John DeZeeuw of Pfizer and Co., Inc., Groton, CT. This work was supported by Public Health Service Grant GM31715 to S.J.G. The multinuclear Bruker AM 400 NMR spectrometer was purchased in part through grants from the National Science Foundation (CHE-8216190) and from the M.J. Murdock Charitable Trust to Oregon State University.

(1) Career Development Awardee of the National Cancer Institute (CA00880), 1979–1984.

(2) Gould, S. J.; Chang, C. C.; Darling, D. S.; Roberts, J. D.; Squillacote, M. *J. Am. Chem. Soc.* **1980**, *102*, 1707. Gould, S. J.; Chang, C. C. *Ibid.* **1978**, *100*, 1624.

(3) Doyle, T. W.; Balitz, D. M.; Grulich, R. E.; Nettleton, D. E.; Gould, S. J.; Tann, C.; Moews, A. E. *Tetrahedron Lett.* **1981**, *22*, 4595.

(4) Herlt, A. J.; Rickards, R. W.; Wu, J.-P. *J. Antibiot.* **1985**, *38*, 516.

(5) Previous ^1H NMR assignments for H-11′ and H-12′ of streptonigrin have been equivocal. We have found that in TFA-d_1, the upfield doublet (δ 7.02) of the AB quartet slowly exchanges and the downfield doublet (δ 7.07) collapses to a singlet. Thus, the upfield resonance is due to H-11′ and the downfield resonance is due to H-12′. This is presumably the relationship in streptonigrone, as well.

(6) Albert, A. *Adv. Heterocycl. Chem.* **1976**, *20*, 117.

(7) Gould, S. J.; Chang, C. C. *J. Am. Chem. Soc.* **1980**, *102*, 1702.

(8) For the first 24 h of the fermentation the burette was charged with $^{16}O_2$ and 0.5 L was consumed. The burette was then filled with the enriched $^{18}O_2$ and over the next 56 h 4.5 L were consumed. For the last 12 h $^{16}O_2$ was again utilized and 1.0 L was taken up.

(9) Gould, S. J.; Cane, D. E. *J. Am. Chem. Soc.* **1982**, *104*, 343.

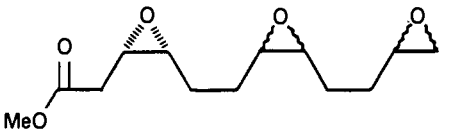
(10) Nazdan, A. M.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1976**, *98*, 5012.

(11) Gerwick, W. J.; Gould, S. J.; Fonouni, H. *Tetrahedron Lett.* **1983**, *24*, 5445.

Additions and Corrections

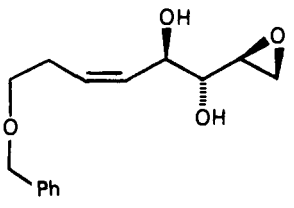
Total Synthesis of Elfamycins: Aurodox and Efrotomycin. 1. Strategy and Construction of Key Intermediates [*J. Am. Chem. Soc.* **1985**, *107*, 1691–1694]. R. E. DOLLE and K. C. NICOLAOU*

Page 1692: Formula i should read



i

Page 1693: Formulae **24** and **25** should be read



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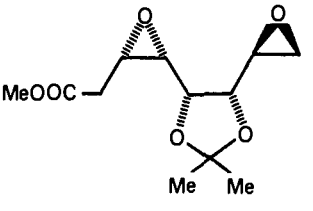

25

Molecular Structure of 1,4,5,8-Tetramethylnaphthalene and In-Plane Molecular Rotation of Some Methyl-Substituted Naphthalenes in Solids [*J. Am. Chem. Soc.* **1985**, *107*, 2341–2346]. FUMIO IMASHIRO,* KIYONORI TAKEGOSHI, A. SAIKA,* ZENEI TAIRA, and YUTAKA ASAHI

Reference to related study on the crystal structure of 1,4,5,8-tetramethylnaphthalene was inadvertently omitted. See: Shiner, C. S.; Noordik, J.; Fisher, A. M.; Eckley, D. M.; Bodenhamer, J.; Haltiwanger, R. C. *Acta Crystallogr.* **1984**, *C40*, 540-542.

Page 2344, column 1, line 7: 0.08 should be 0.008.

Stable Simple Enols. 11. Equilibrium Constants for the 1-Alkyl-2,2-dimesitylethenol/1-Alkyl-2,2-dimesitylethanone Systems in Hexane. The Predominance of Steric Effects on K_{enol} Values [*J. Am. Chem. Soc.* **1985**, *107*, 3669–3676]. DAVID A. NUGIEL and ZVI RAPPOPORT

Page 3672: Compound **19** should be compound **18a**.

Pages 3673 and 3674: Figure 2 and 3 (but not their captions) should be exchanged.

Organoboron Compounds in Organic Synthesis. 1. Asymmetric Hydroboration [*J. Am. Chem. Soc.* **1985**, *107*, 4549–4551]. S. MASAMUNE,* B. KIM, J. S. PETERSEN, T. SATO, S. J. VEENSTRA, and T. IMAI

Page 4549: In the 12th line of the first paragraph "**1a** and **1b** of C_2 symmetry" should read "**1a** and **1b** of D_2 symmetry".