the formation of the pyruvate adduct of TDP and the substitution process in which a proton replaces CO_2 to generate HETDP should be stereospecific[17] as is the case for the E1 subunit of pyruvate dehydrogenase.[5,14] The stereochemistry of these processes in enzymic and nonenzymic systems is the subject of ongoing studies.

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Supplementary Material Available: Listing of crystal data and tables of final atomic positional and thermal parameters for HETI (1 page). Ordering information is given on any current masthead page.

(17) Kluger, R. and Gish, G. In *Thiamin Pyrophosphate Enzymes*; Schellenberger, A., Schowen, R. L., Eds.; CRC Press: Boca Raton, FL, in press.

Streptonigrin Biosynthesis. 8. Evidence for the Involvement of a New Shikimate Pathway Product and a New Route to Quinolines[1]

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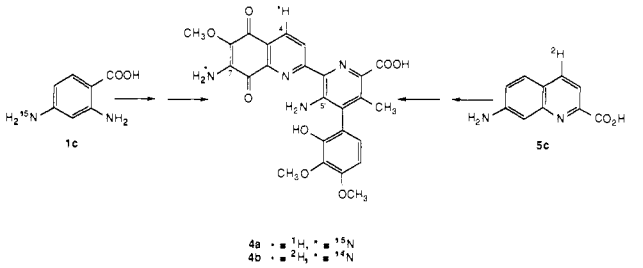
We have previously reported data[3,4] suggesting that a 4-aminoanthranilic acid (**1**), D-erythrose-4-phosphate (**2**), and β-methyltryptophan[5] (**3**) are the key precursors in biosynthesis of the anticancer antibiotic streptonigrin (**4**). As shown in Scheme I, these can be combined in sequences that lead either to a 7-aminoquinoline-2-carboxylic acid **5** (pathway A) or to a β-carboline **6** (pathway B) as the pivotal intermediate. We now report that pathway A is operative with 4-aminoanthranilic acid (**1a**) and 7-aminoquinoline-2-carboxylic acid (**5a**) as intermediates and that all three A-ring oxygenations occur at a later stage.

A fermentation in the presence of $^{18}O_2$ gas had yielded streptonigrin, labeled—among other positions—at C-5 and C-6 but not at C-8, suggesting that the C-8 oxygen was retained from a prearomatic precursor and that the hydroxylated compounds **1b** and **5b** were likely intermediates.[6] However, we recognized that because C-8 is the carbonyl of a vinylogous ester, an oxygen atom may have been introduced by a metabolic oxidation but subsequently lost by exchange,[7,8] either with the fermentation medium or during extractive workup. Indeed, when samples of authentic streptonigrin were stirred overnight in solutions of $THF/H_2{}^{18}O$ at pH 5.0 and at pH 10.5 and then reisolated and analyzed by ^{13}C NMR, it was found that ^{18}O had been incorporated to the extent of 15% and 30%, respectively, exclusively at C-8. Thus, neither the exact origin of the C-8 oxygen nor the oxidation level of the putative aromatic precursor(s) were certain at this point.

[4-^{15}N]4-Aminoanthranilic acid (**1c**) was then synthesized in three steps (22% overall yield)[9] utilizing $H^{15}NO_3$ (99% enriched).[10] A sample of the sodium salt of **1c** in 0.05 M pH 8.5 phosphate buffer was added under sterile conditions to shaken fermentations[11] of *Streptomyces flocculus*. Pulse feedings of 61.1 mg/15 mL, 20.8 mg/10 mL, 20.4 mg/10 mL, and 20.2 mg/10 mL were divided among the flasks (3 × 500 mL) at 24, 36, 48, and 60 h, respectively. At the termination of the fermentation, standard workup afforded 27.6 mg of pure **4a**. The ^{15}N NMR spectrum of **4a**[12] exhibited a single resonance at 73.6 ppm[13] that is attributable to the C-7 amine nitrogen of **4**.[5] Although the specific enrichment could not be calculated because of NOE due to proton decoupling, no resonance was detectable for the unenriched C-5′ amine nitrogen.[14]

[4-^{2}H]7-Aminoquinoline-2-carboxylic acid (**5c**) was next synthesized from the quinoline **7**[15] as shown in Scheme II. Reductive removal of chloride from **8** with deuterium gas afforded the labeled ester **9**, and mild hydrolysis gave the amino acid **5c** in 25% overall yield.

The sodium salt of **5c** was fed by dividing pulses of 47.0, 44.5, 38.2, and 42.4 mg, each in 15 mL of buffer, among three 500-mL cultures at 28, 38, 48, and 58 h after inoculation, respectively. Standard workup afforded 20.6 mg of pure **4b** which was analyzed by ^{2}H NMR.[16] A singlet at δ 8.23[17] was observed corresponding



to a deuterium label at C-4. By comparison with the natural abundance deuterium signal for solvent Me_2SO (also employed as internal chemical shift reference), incorporation was determined to be 1.4%.

On the basis of these data it appears that streptonigrin is biosynthesized via pathway A with R = H (Scheme I), and unless there is a metabolic grid, it is unlikely that **1b** is also an intermediate. The evidence suggests that compound **1a** represents a new metabolite of the shikimate pathway,[19] while the involvement of **5a** reveals a fundamentally new biosynthetic pathway to the quinoline ring system.[20] This may be viewed (Scheme III) as

(1) Presented by WRE at the 41st Northwest Regional ACS Meeting, Portland, OR, June 16-18, 1986.
(2) Career Development Awardee of the National Cancer Institute (CA00880), 1979-1984.
(3) Gould, S. J.; Cane, D. E. *J. Am. Chem. Soc.* **1982**, *104*, 343.
(4) Gerwick, W. H.; Gould, S. J.; Fonouni, H. *Tetrahedron Lett.* **1983**, 5445.
(5) Gould, S. J.; Chang, C. C.; Darling, D. S.; Roberts, J. D.; Squillacote, M. *J. Am. Chem. Soc.* **1980**, *102*, 1707.
(6) Erickson, W. R.; Gould, S. J. *J. Am. Chem. Soc.* **1985**, *107*, 5831.
(7) Loss of ^{18}O at a quinone carbonyl was also found in naphthyridinomycin: Palaniswamy, V. A.; Gould, S. J., unpublished results.
(8) Snyder, D. C.; Rapoport, H. *Biochemistry* **1970**, *9*, 2033.
(9) Huntress, E. H.; Shriner, R. L. *Organic Syntheses*; Wiley: New York, 1943; Collect. Vol. II, p 459. Wallis, E. S.; Lane, J. F. *Org. React.* **1946**, 3, 267.
(10) Obtained from Stohler/KOR Isotopes.
(11) Gould, S. J.; Chang, C. C. *J. Am. Chem. Soc.* **1980**, *102*, 1702.
(12) Spectrum taken on 22.0 mg in 0.4 mL of Me_2SO-d_6 with a Bruker AM 400 spectrometer at 40.5 MHz (sweep width = 2778 Hz, data points = 4K zero filled to 8K, Hz/pt = 0.68, acquisition time = 0.737 s, pulse width = 38°, line broadening = 2.0 s, relaxation delay = 1.2 s, no. of scans = 22 305).
(13) Relative to external [^{15}N]aniline, 56.5 ppm, obtained from MSD Isotopes.
(14) In earlier work both the C-7 amine and C-5′ amine peaks were of equal intensity in a natural abundance ^{15}N NMR spectrum. See ref 5. The C-7 amine nitrogen was also observed in the enriched and natural abundance samples by using a refocused decoupled INEPT sequence. This gave a signal-to-noise ratio approximately 3 times greater than that of the standard experiment described in ref 12. The C-5 amine nitrogen was not observed in this case, presumably due to rapid proton exchange eliminating the possibility of efficient polarization transfer.
(15) Heindel, N. D.; Bechara, I. S.; Lemke, T. F.; Fish, V. B. *J. Org. Chem.* **1967**, *32*, 4155.
(16) Spectrum taken on 10.0 mg in 0.4 mL of Me_2SO with a Bruker AM 400 spectrometer at 61.4 MHz (sweep width = 639 Hz, data points = 2K zero filled to 8K, Hz/pt = 0.16, acquisition time = 1.604 s, pulse width = 45°, no. of scans = 33 993).
(17) The previous assignments of H-3 and H-4 were inadvertently reversed.[18] The correct assignments were confirmed by a 2-D $^{13}C/^1H$ HETCOR experiment on authentic **4**.
(18) Gould, S. J.; Weinreb, S. M. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer-Verlag: New York, 1982; Vol. 41, pp 77-114.
(19) For other recently discovered metabolites of the shikimate pathway, see: Hillis, L. R.; Gould, S. J. *J. Am. Chem. Soc.* **1985**, *107*, 4593. Rinehart, K. L., Jr.; Potgieter, M.; Wright, D. A. *J. Am. Chem. Soc.* **1982**, *104*, 2649. Becker, A. M.; Herlt, A. J.; Hilton, G. L.; Kibby, J. J.; Rickards, R. W. *J. Antibiot.* **1983**, *36*, 1323. Rinehart, K. L., Jr.; Potgieter, M.; Delaware, D. L.; Seto, H. *J. Am. Chem. Soc.* **1983**, *103*, 2099.

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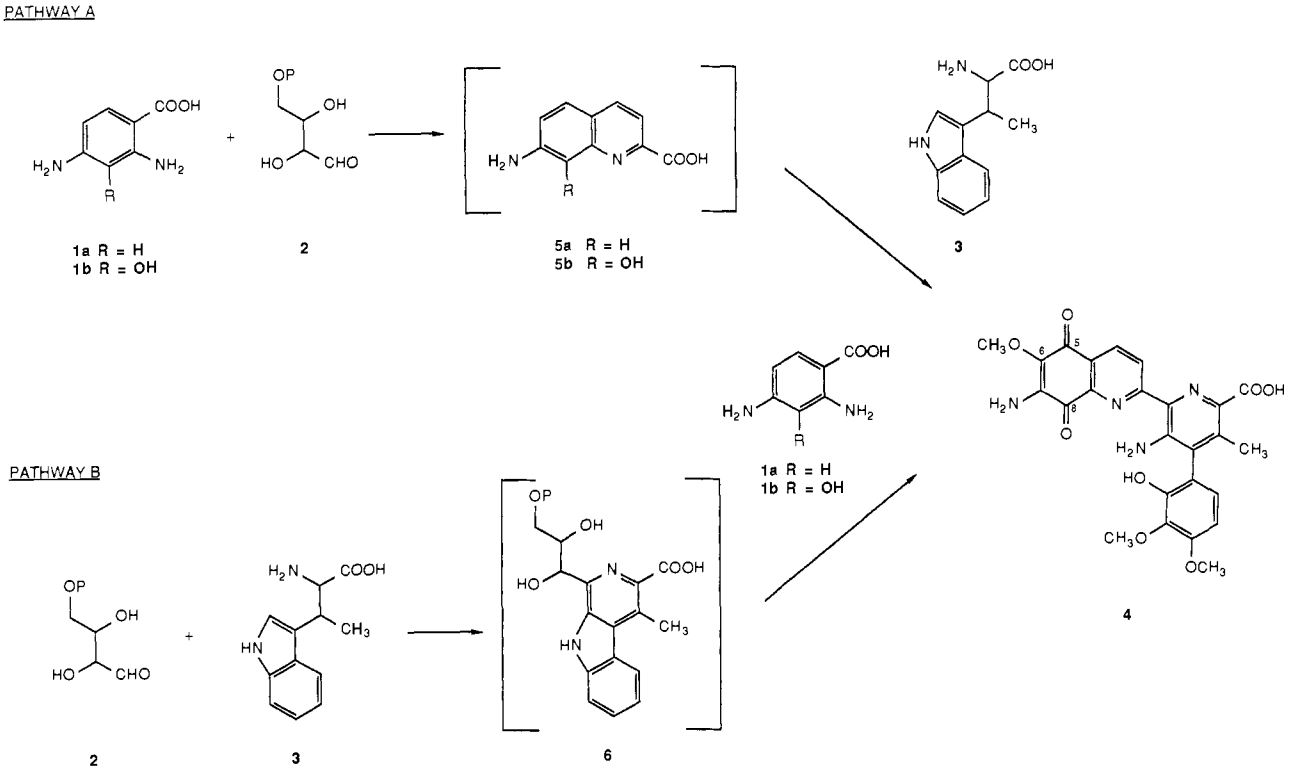
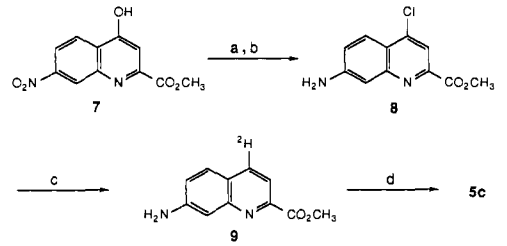
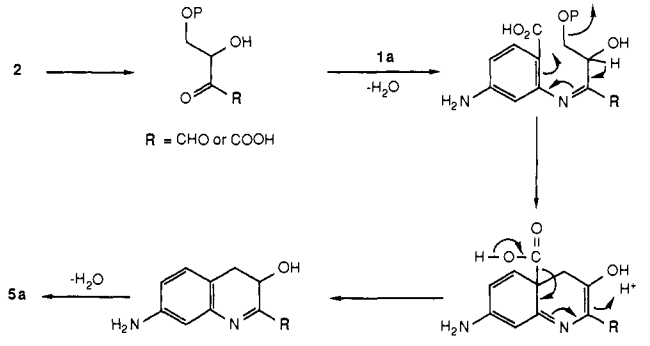
Scheme I



Scheme II



Scheme III



analogous to the biosynthesis of tryptophan from anthranilic acid and ribose diphosphate.[21]

In order to further probe this remarkable pathway, testing of **1b**, 7-amino-8-hydroxyquinoline-2-carboxylic acid (**5b**), and 7-amino-5-hydroxyquinoline-2-carboxylic acid as potential later intermediates is currently under investigation.

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(20) For examples of quinoline biosynthesis from anthranilic acid and "acetate", see: Herbert, R. B. *The Biosynthesis of Secondary Metabolites*; Chapman and Hall: New York, 1981; pp 131-132. For examples from tryptophan metabolism, see: Luckner, M. *Secondary Metabolism in Microorganisms, Plants, and Animals*; Springer-Verlag: New York, 1984; pp 404-406.

(21) Lehninger, A. L. *Biochemistry*; Worth: New York, 1975; p 709.

Intermediates in Nucleophilic Aromatic Substitution

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Bimolecular nucleophilic aromatic substitution by anions in polar hydroxylic solvents is generally written as rate-limiting formation of a Meisenheimer, or σ, complex,[1,2] but π-complexes[3] are also postulated reaction intermediates.[4]

Unexpectedly, reported rate constants for formation of Meisenheimer complexes from OH^- and a nitroarene or quinazoline

(1) (a) Bunnett, J. F. *Q. Rev., Chem. Soc.* **1958**, *12*, 1. (b) Buncel, E.; Norris, A. R.; Russell, K. F. *Ibid.* **1968**, *22*, 123.

(2) Strauss, M. J. *Chem. Rev.* **1970**, *70*, 667. Terrier, F. *Ibid.* **1982**, *82*, 78.

(3) (a) Miller, R. E.; Wynne-Jones, W. F. K. *J. Chem. Soc.* **1959**, 2377. (b) Allen, C. R.; Brook, A. J.; Caldin, E. F. *Ibid.* **1961**, 2171 and references cited therein.

(4) Tamaru, K.; Ichikawa, M. *Catalysis by Electron Donor-Acceptor Complexes*; Wiley: New York, 1975; p 53.

0002-7863/87/1509-0621$01.50/0