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Registry No. $Zn(phen)Cl_2$, 14049-94-6; $Zn(phen)_2^{2+}$, 16561-56-1; Δ-$Zn(phen)_3^{2+}$, 82660-10-4; Λ-$Zn(phen)_3^{2+}$, 82660-11-5.

Supplementary Material Available: Photographs (Figure 1S) for the gel electrophoretic data given in Figure 1 (2 pages). Ordering information is given on any current masthead page.

Biosynthesis of the Antitumor Antibiotic Naphthyridinomycin

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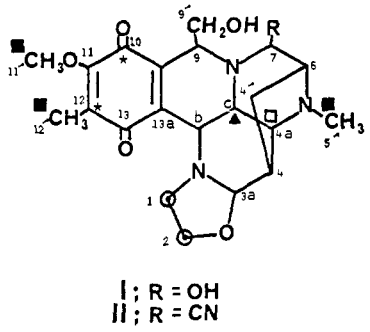
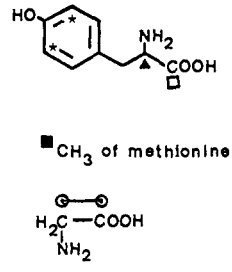
Naphthyridinomycin (I), a novel heterocyclic quinone antibiotic,



I; R = OH
II; R = CN



■ CH_3 of methionine

is produced in liquid shake culture by *Streptomyces lusitanus* NRRL 8034.[1,2] The structure of naphthyridinomycin was established by X-ray crystallography.[3] This compound has been shown to be a potent antimicrobial and antitumor agent.[4] Mechanism of action studies have shown that in bacteria naphthyridinomycin primarily inhibits DNA synthesis,[5] due to the binding of the antibiotic to dG·dC base pairs of the DNA.[6] A major impediment to our biosynthetic studies was the instability of naphthyridinomycin in concentrated organic or aqueous solvents. This problem was resolved by converting naphthyridinomycin to cyanonaphthyridinomycin (II).[4] This latter compound is a stable crystalline derivative suitable for chemical or spectral studies. Cyanonapthyridinomycin, therefore, was utilized to demonstrate that tyrosine, the *S*-methyl group of methionine, glycine, and ornithine are efficiently incorporated into the antibiotic.

Radioisotope-labeled substrates were fed to cultures of *S. lusitanus* growing in 100 mL of a complex production medium in stoppered 500-mL Erlenmeyer flasks as described previously.[6] After 72 h of growth at 25 °C, the labeled substrates were added, and the culture was incubated an additional 24 h.[7] The filtered broth from the 96-h culture was treated with sodium cyanide to generate cyanonaphthyridinomycin and the antibiotic extracted with methylene chloride. The bright orange compound was successively purified as described in the legend of Table I to yield ~20 mg/L.

Table I. Incorporation of ^{14}C-Labeled Substrates into Cyanonaphthyridinomycin

substrate	% incorporation[a]
[U-^{14}C]-L-tyrosine	1.8[b]
[1-^{14}C]-L-tyrosine	1.2
[*methyl*-^{14}C]-L-methionine	2.5
[3-^{14}C]-DL-dihydroxyphenylalanine	<0.1
[2-^{14}C]glycine	2.1
[1-^{14}C]-DL-ornithine	1.5
[1-^{14}C]-DL-glutamate	<0.1
[U-^{14}C]-D-glucose	<0.1
[1-^{14}C]acetate	<0.1
[2-^{14}C]acetate	<0.1

[a] Per 2 mg produced in 100 mL of broth. [b] Each antibiotic extract was purified successively on an Alumina V column (80:20 benzene:chloroform), silica gel preparative plates (60:20:30 acetone:isopropanol:dichloroethane) and ODS reverse-phase plates (80:20 methanol:water) or until constant specific activity was reached.

Of the potential precursors tested, [U-^{14}C]- and [1-^{14}C]-L-tyrosine, [*methyl*-^{14}C]-L-methionine, [2-^{14}C]glycine, and [1-^{14}C]-DL-ornithine (Table I) were well incorporated into cyanonaphthyridinomycin. To obtain further information on the incorporation of tyrosine and methionine, we degraded cyanonaphthyridinomycin samples labeled from [U-^{14}C]-L-tyrosine and [*methyl*-^{14}C]-L-methionine by Kuhn–Roth oxidation and Schmidt degradation[8] to yield carbons 12 and 12′ of the antibiotic. Approximately one-ninth (12%) of the label that [U-^{14}C]-L-tyrosine contributes to the antiobiotic was found at C-12, suggesting that all nine carbons of tyrosine were incorporated. The methyl group from methionine labeled C-12′ (37%) and presumably the other two-thirds of the label was incorporated at the *O*- and *N*-methyl positions. [3-^{14}C]-DL-dihydroxylphenylalanine (DOPA) was not detectably incorporated into the antibiotic, suggesting that the oxygen functionality at C-10 was being introduced in a subsequent step in the biosynthesis. None of the other substrates listed in Table I were detectably incorporated.

To confirm the incorporation of tyrosine and methionine and to examine the role of glycine in the biosynthesis, in additional studies we utilized ^{13}C-enriched precursors and ^{13}C NMR. The ^{13}C NMR spectrum of cyanonaphthyridinomycin (Table II) was assigned in part on the basis of the previous assignments of the ^{13}C NMR spectrum of the saframycins[10,11] and in part by utilizing established NMR pulsing techniques to discriminate quaternary, methine, methylene, and methyl carbons.[12] Assignments of the protons in the ^1H NMR spectrum of cyanonaphthyridinomycin and 9′-acetylcyanonaphthyridinomycin were completed, and this information was used to assign the carbon resonance signals of the proton-bearing carbons.[9]

As expected from the degradation studies, [*methyl*-^{13}C]-DL-methionine enriched only the *O*-, *N*-, and *C*-methyl groups of the antibiotic (Table II). [1-^{13}C]- [2-^{13}C]-DL-tyrosine enriched carbons 4a and 13c, respectively. The quinone ring of cyano-

(1) Kluepfel, D.; Baker, H. A.; Piattoni, G.; Sehgel, S. N.; Sidorowicz, A.; Singh, K.; Vezina, C. *J. Antibiot.* **1975**, *28*, 497.
(2) Kluepfel, D.; Sehgel, N.; Vezina, C. U.S. Patent 4003902, January 18, 1977.
(3) Sygusch, J.; Brisse, F.; Hanessian, S.; Kluepfel, D. *Tetrahedron Lett.* **1974**, 4021.
(4) Zmijewski, M. J., Jr.; Goebel, M. *J. Antibiot.* **1982**, *35*, 524.
(5) Singh, K.; Sun, S.; Kluepfel, D. *Dev. Ind. Microbiol.* **1976**, *17*, 209.
(6) Zmijewski, M. J., Jr.; Miller-Hatch, K.; Goebel, M. *Antimicrob. Agents Chemother.* **1982**, *21*, 787.
(7) Each radioisotope-labeled substrate (10–20 μCi) was added to one 100-mL cultures in 500-mL Erlenmeyer flasks. Stable isotope-labeled precursors were added to between eight and ten 100-mL cultures. [1-^{13}C]-DL-tyrosine (100 mg), [2-^{13}C]-DL-tyrosine (100 mg), [3,5-$^{13}C_2$]tyrosine (100 mg), [*methyl*-^{13}C]-DL-methionine (60 mg), and [1,2-$^{13}C_2$]serine (80 mg) were added. All labeled compounds were enriched with 90% or greater ^{13}C.
(8) Simon, H.; Floss, H. G. "Bestimmung der Isotopen Vesterling in Markierten Verbindugen"; Springer-Verlag: West Berlin, 1967, pp 12, 23.
(9) ^1H and ^{13}C NMR spectra were recorded on a JEOL FX-270 NMR spectrometer at 270 and 67.8 MHz, respectively. The ^{13}C NMR spectrum of cyanonaphthyridinomycin consisted of 21 resolved resonances. Acetylation at C-9′ revealed that C-9′ and C-2 were overlapping in the original spectrum. Even in the acetylated antibiotic, these carbons were split by less than 0.1 ppm. The proton signals in the ^1H NMR spectrum of the acetylated compound were resolved well enough to carry out selective homonuclear decoupling. This aided in the assignment of the proton spectrum. Selective ^1H–^{13}C decoupling was employed to assign the carbon signals in the ^{13}C NMR spectrum of the acetylated compound.
(10) Arai, T.; Takahashi, K.; Nakahara, S.; Kubo, A. *Experientia* **1980**, *36*, 1025.
(11) Arai, T.; Takahashi, K., Ishiguro, K.; Yazawa, K. *J. Antibiot.* **1980**, *33*, 951.
(12) Burum, D. P.; Ernst, R. R. *J. Magn. Reson.* **1980**, *39*, 163. Doddrell, D. M.; Pegg, D. T. *J. Am. Chem. Soc.* **1980**, *102*, 6388.

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Table II. ^{13}C NMR Assignments of Cyanonaphthyridinomycin and Enrichments from Labeled Precursors

		I_e/I_u[b]			
carbon no.	δ[a]	[1-^{13}C]tyrosine	[2-^{13}C]tyrosine	[3,5-$^{13}C_2$]tyrosine	[*methyl*-^{13}C]-methionine
1	50.1	1.2	0.9	0.8	1.1
2	61.4[e]	1.8	1.0	1.0	1.8
3a	93.1	1.1	0.8	0.8	0.9
4	35.1	0.8	0.8	0.9	1.0
4a	60.2	*31.0*	1.0	1.1	1.4
4′	28.9	1.3	0.9	0.9	1.1
6	62.3	1.0	0.9	1.0	1.1
7	54.1[d]	1.2	1.0	1.0	1.0
9	56.9[d]	1.2	0.9	0.9	1.1
9′	61.4[e]	1.8	1.0	1.0	1.8
9a	141.0	0.9	0.7	0.5	0.9
10	181.1	0.9	0.8	*10.0*[c,d]	0.9
11	155.8	0.1	0.5	0.4	0.8
12	128.2	0.8	0.9	*9.6*[c,d]	1.2
13	186.4	0.5	0.6	0.5	0.5
13a	142.7	0.8	0.6	0.6	0.7
13b	48.0	1.0	0.7	0.7	0.9
13c	53.2	0.8	*13.8*	1.0	1.0
5′	41.3	1.1	0.8	0.8	*16.4*
11′	61.1	1.8	0.7	0.7	*11.5*
12′	8.8	1.1	0.8	0.7	*17.7*
C=N	117.2	1.0	1.0	1.0	1.0

[a] Chemical shifts in ppm are downfield from Me_4Si in $CDCl_3$. Spectra were recorded on a JEOL FX 270 multinuclear spectrometer at 67.8 MHz. [b] $I_{enriched}/I_{unenriched}$. Intensity of each peak was normalized by dividing it by the intensity of the carbon signal due to the CN group. [c] Carbon-carbon spin-spin two-bond coupling of 5.86 Hz was observed. [d] These assignments may be reversed. [e] These carbons overlapped in the spectrum of cyanonaphthyridinomycin, but acetylation of the alcohol functionality at C-9′ separated these carbon resonance signals.

naphthyridinomycin was labeled from [3,5-$^{13}C_2$]-L-tyrosine[13] at carbons 10 and 12. Each enriched carbon resonance signal was actually made up of two signals [$^2J_{CC}$ = 5.86 Hz] due to a small two-bond ^{13}C–^{13}C spin–spin coupling between carbons 10 and 12.[14] These studies confirm that the entire carbon skeleton of tyrosine is incorporated into the antibiotic.

[1,2-$^{13}C_2$]Glycine was found to be incorporated intact only into C-1 and C-2 of cyanonaphthyridinomycin [$^1J_{CC}$ = 31 Hz, Figure 1]. No other coupled carbons in the spectrum were detected. Both center singlets of carbons 1 and 2 were also enriched about 2.5-fold over natural abundance (Figure 1). This would indicate that the carbons of glycine were being metabolized in part prior to incorporation. The metabolism of glycine was confirmed by examination of the relative enrichment of other carbon signals of the [1,2-$^{13}C_2$]glycine labeled antibiotic. The other enriched carbon signals were the *O*-, *N*-, and *C*-methyl groups (I_e/I_u = 4.2, 4.9, and 5.0, respectively).[15] The incorporation of glycine into the one carbon pool is undoubtedly due to the presence of a glycine cleavage complex in this organism.[16] This reversible enzyme complex generates NH_3 and CO_2 from glycine and transfers the C-2 of this amino acid to tetrahydrofolate to form methylene tetrahydrofolate.[17] The lack of nonspecific incorporation of glycine into any of the other carbons of the antibiotic and the absence of incorporation of either acetate or glucose (Table I) would suggest that glycine is the immediate precursor for carbons 1 and 2.

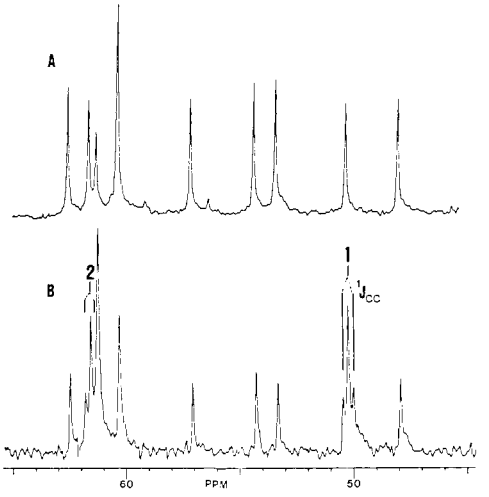


Figure 1. Partial ^{13}C NMR spectra of cyanonaphthyridinomycin: (A) natural abundance; (B) enriched with [1,2-$^{13}C_2$]glycine.

The novel biosynthetic origin of naphthyridinomycin is summarized in I. Tyrosine, but not DOPA, is incorporated into the antibiotic. All three methyl carbons arise either directly from methionine or indirectly from C-2 of glycine, and an intact glycine carbon skeleton is the precursor for C-1 and C-2 of naphthyridinomycin. It would be reasonable to speculate that the five-carbon non-tyrosine-derived portion of the antibiotic originates from ornithine. The absence of incorporation of glutamate is surprising since this amino acid is the precursor for ornithine. This may, however, just indicate separate pools for exogenous and endogenous glutamate in this organism. While naphthyridinomycin, cyanonaphthyridinomycin, and saframycin S (III) and A (IV) probably share a common mechanism of action, our results would indicate that the two antibiotic groups have different biogenetic origins. Tyrosine is also incorporated into the saframycins,[18] but on the basis of the structure of these antibiotics,

(13) Prepared according to Viswanatra and Hruby Viswanatra, V.; Hruby, V. J. *J. Org. Chem.* **1980**, *45*, 2010.

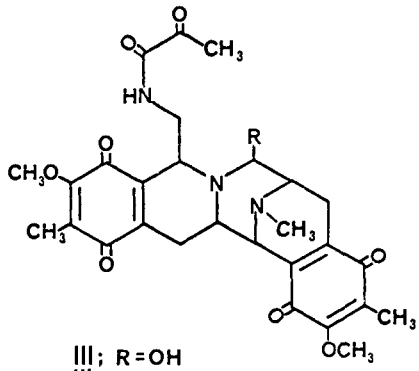
(14) Levy, G. C.; Lichter, R. L.; Nelson, G. L. "Carbon-13 Nuclear Magnetic Resonance Spectroscopy", 2nd ed.; Wiley: New York, 1980; p 129.

(15) Acetylation of this enriched antibiotic at C-9′ did not adequately separate the signals for this enriched carbon from that of C-2. However, no other enriched carbon signals were observed in the spectrum aside from the methyl carbon signals.

(16) Newman, E. B.; Magasanik, B. *Biochim. Biophys. Acta* **1963**, *78*, 437. Kochi, H.; Kikuchi, G. *J. Biochem.* **1974**, *75*, 1113.

(17) The lower incorporation of glycine compared to that for methionine into the methyl carbons is also indicative of the conversion of the methylene–THF to methyl–THF and then transfer to homocysteine to synthesize methionine. The C-2 of glycine is, therefore, indirectly incorporated via methionine. This reversible enzyme system would also be responsible for the generation of glycines containing only one ^{13}C-enriched carbon either at C-1 or C-2 or the amino acid.

(18) Ishiguro, K.; Takahashi, K.; Yazawa, K.; Sakiyama, S.; Arai, T. *J. Biol. Chem.* **1981**, *256*, 2162.



III; R = OH
IV; R = CN

two tyrosine units must condense to generate the basic ring system of this group. Additional studies on naphthyridinomycin are underway to determine the orientation of glycine's incorporation into C-1 and C-2, the role of ornithine in the biosynthesis, and the source of carbons 9 and 9′.

Acknowledgment. We thank Dr. L. Hurley, University of Texas, for [*methyl*-^{13}C]-DL-methionine and helpful discussions. This work was supported in part by a grant from the National Institutes of Health (RR05738 and AI16771) to Milton J. Zmijewski and a grant from the National Science Foundation to Victor J. Hruby. The multinuclear JEOL FX-270 NMR spectrometer was obtained through a grant from the National Science Foundation (PCM-7922984) to the Department of Medicinal Chemistry, University of Utah.

Registry No. I, 54913-26-7; L-tyrosine, 60-18-4; L-methionine, 63-68-3; glycine, 56-40-6; L-ornithine, 70-26-8.

Composition of Elemental Sulfur in Solution: Equilibrium of S_6, S_7, and S_8 at Ambient Temperatures

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We report that S_8 is partially transformed to S_6 and S_7 at ambient temperatures by its dissolution in polar solvents such as methanol or acetonitrile. In time, an equilibrium is established in which ~1% of the sulfur is present as the smaller rings. In view of the much greater reactivity of S_6[1] and S_7[2] compared to S_8, the discovery of this equilibrium and processes that facilitate its attainment are potentially of value in defining and controlling the chemistry of sulfur. In particular, we may now ask whether reactions of sulfur proceed through S_6, S_7, or intermediates that lead to these compounds.

The chemistry of common sulfur at ambient temperatures is usually regarded as that of S_8. Consequently, we were surprised to find S_6 and S_7 present with S_8 in the mixtures described above, as determined by high-pressure liquid chromatography (HPLC).[3]

Table I. Equilibration of Sulfur in Methanol - Cyclohexane or Acetonitrile

	product distribution,[a] equilibrated mixtures		
reactant	S_6	S_7	S_8
S_6	0.32	0.76	98.9
S_7	0.30	0.77	98.9
S_8	0.30	0.78	98.9

[a] Wt % S_6, S_7, or S_8; quantities by HPLC;[6,7] reaction solvent 80% methanol–20% cyclohexane (similar results were obtained with acetonitrile); sulfur concentration 0.12 mg/mL; period elapsed from solution preparation to analysis, 1 day (S_7 and S_8 equilibrations), 3 days (S_6 equilibration). Rates of equilibration were variable.

We also observed that S_6 and S_7 in methanol at room temperature revert to solutions containing the three rings. To determine whether these mixtures result from an equilibration process, we compared the quantities of S_6, S_7, and S_8 produced from decompositions of the individual compounds. Solvents used for the equilibration reactions were methanol–cyclohexane or acetonitrile. The HPLC analysis (eluant 80% methanol–20% cyclohexane) is based on a scheme for separation of sulfur allotropes reported by Steudel and co-workers.[6,7] The data, presented in Table I, show that the three sulfur compounds yield, within experimental uncertainties, the same final mixture of S_6 (0.30 ± 0.05%), S_7 (0.8 ± 0.1%), and S_8 (98.9%).

A study of the variation of allotropic composition with total concentration provides additional evidence for the existence of the equilibrium and an independent demonstration of the number of sulfur atoms in the molecule associated with a given HPLC peak. Thus for the equilibrium $8S_n \rightleftarrows nS_8$, a plot of ln $([S_n]/[S_8])$ vs. ln $[S_8]$ is expected to be linear with a slope of $(n/8) - 1$. We find $n = 6.08 \pm 0.13$ for the "S_6" peak and $n = 7.07 \pm 0.10$ for "S_7". The values summarize data in acetonitrile and methanol–cyclohexane solutions that were originally enriched in S_6 or S_8.

There is a significant variation in the ability of solvents to promote the equilibration of S_6, S_7, and S_8. At room temperature, methanol, acetonitrile, and dimethyl sulfoxide dissolve S_8 to yield solutions in which appreciable quantities (>0.2%) of S_6 and S_7 are detected within minutes to hours. In contrast, solutions of S_8 in cyclohexane at room temperature show little conversion to S_6 or S_7 over weeks, although the smaller rings do appear within hours to days when these solutions are heated above 100 °C.

In Bartlett's study of the conversion of S_6 to S_8 in aromatic solvents, several polar catalysts were reported.[8,9] We find that S_6 and S_7 are much less stable in methanol than in cyclohexane and that partial conversion of these rings toward the equilibrium mixture occurs during HPLC analysis under our conditions[11] with methanol–cyclohexane as eluant. This chromatography routine

(1) Bartlett, P. D.; Cox, E. F.; Davis, R. E. *J. Am. Chem. Soc.* **1961**, *83*, 103–109. In reactions with triphenylphosphine S_6 is 2×10^4 more reactive than S_8 as determined by extrapolation to comparable conditions.

(2) Steudel, R. "Chemistry of the Non-Metals"; W. de Gruyter: New York, 1977; p 208. S_7 is suggested to be more reactive than S_6.

(3) Identification of the S_6 and S_7 peaks follow from their retention volumes being within 1% of those of authentic samples[4] prepared by the method of Schmidt.[5] Mass spectra (MS) of the "S_7" fraction isolated from an equilibrated S_8 source confirmed the identification. The "S_6" peak gave only the MS of S_8, presumably as the result of rapid transformation of S_6 to S_8 on concentration.

(4) Data for the authentic reference samples are given in the following. S_6: found, S, 99.4%; mol wt calcd for S_6 192, found 192 (mass spectrum) and 200 (vapor-pressure osmometry, benzene). S_7: found, S, 99.9%; mol wt calcd for S_7 224, found 224 (mass spectrum) and 225 (vapor-pressure osmometry, benzene). Recrystallization of Fisher (sublimed) sulfur from carbon disulfide or evaporation of a cyclohexane extract of Baker and Adamson (precipitated) sulfur yielded samples of S_8 with less than 0.1% S_6 and S_7.

(5) Schmidt, M.; Block, B.; Block, H. D.; Köpf, H.; Wilhelm, E. *Angew. Chem., Int. Ed. Engl.* **1968**, *7*, 632–633.

(6) Steudel, R.; Mausle, H. J.; Rosenbauer, D.; Mockel, H.; Freyholdt, T. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 394–395.

(7) We used a Zorbax ODS (octadecylsilane) column, 250 mm × 6.2 mm i.d. The compounds were detected at 254 nm. The reported quantities are corrected for differences in extinction coefficients of S_6, S_7, and S_8 at that wavelength.

(8) Bartlett, P. D.; Lohaus, G.; Weis, C. D. *J. Am. Chem. Soc.* **1958**, *80*, 5064–5069.

(9) $NaAlO_2(SiO_2)_7 \cdot 2H_2O$:[10] surface area 313 m^2/g; pore volume 188 Å^3 was found to facilitate the approach to equilibrium. Bartlett et al.[8] found a non-acid alumina promoted the conversion of S_6 to S_8.

(10) Mahler, W.; Forshey, W. O., Jr. U.S. Patent 4213949.

(11) S_7 was stable in methanol during chromatography under the reported conditions.[6] Catalysts in minute amounts promote the conversion of S_6 to S_8.[12]

(12) Bartlett, P. D.; Colter, A. K.; Davis, R. E.; Roderick, W. R. *J. Am. Chem. Soc.* **1961**, *83*, 109–114.

0002-7863/82/1504-4971$01.25/0