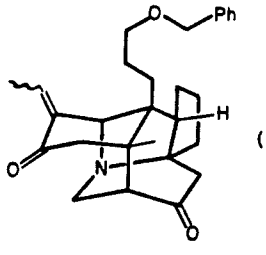
mations ensues, including dehydration, deketalization, and intramolecular Michael addition. The product, pentacyclic diketone **14** (mixture of *E* and *Z* double-bond isomers), is obtained in 69% overall yield, based on enone **12**.

The final methyl group of methyl homodaphniphyllate is added by reaction of **14** with lithium dimethylcopper; the resulting enolate is trapped with diethyl phosphochloridate in THF at 25 °C in the presence of triethylamine to obtain enol phosphate **15** (88%). Treatment of **15** with LDA in a mixture of THF and hexamethylphosphoric triamide, followed by addition of diethyl phosphochloridate, gives bis(enol phosphate) **16** (73%). Reduction of **16** with lithium and *tert*-butyl alcohol in ethylamine gives unsaturated alcohol **17**. Oxidation of the primary alcohol (Jones reagent, 100%) and Fischer esterification (6 N HCl, refluxing methanol, 16 h, 75%) afford methyl dehydrohomodaphniphyllate (**18**).



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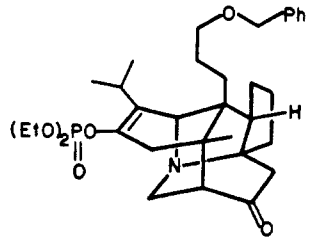

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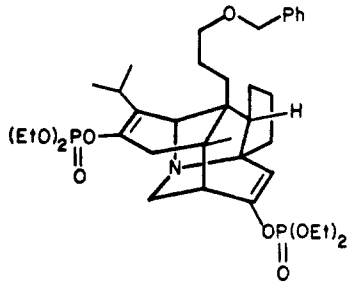

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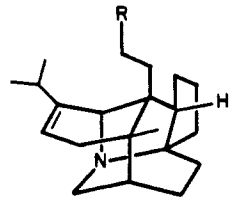
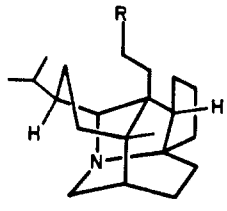
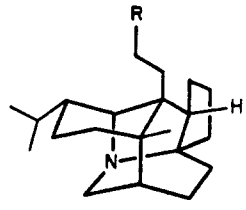


17: R = CH_2OH
18: R = CO_2Me



19: R = CH_2OH
20: R = CO_2Me



21: R = CH_2OH
2: R = CO_2Me

Reduction of the double bond with proper introduction of the final stereocenter has been a demanding task. The double bond is exceedingly unreactive to normal conditions for hydrogenation. Eventually, we were able to accomplish complete reduction using 1800 psi of H_2 over rhodium on alumina in methylcyclohexane solvent[10] at 130 °C for 24 h. The product formed under these conditions has the correct constitution and is similar spectrally with methyl homodaphniphyllate. However, it is clearly different, and has been assigned the diastereomeric structure **20**, even though molecular models indicate that the "bottom" face of the double bond in **18** is much more hindered than the top. We assume that double-bond rearrangement is the problem, and that isomerization occurs to the less hindered exocyclic position. In the exocyclic isomer of **18**, hydrogenation from the bottom face of the double bond is not unexpected.

At this point it was clear that intramolecular assistance by the functionalized side chain was desirable. With unsaturated alcohol **17** we evaluated the possible use of a cationic rhodium complex that had shown promise for intramolecular hydrogenation of relatively hindered double bonds.[11] However, the drastic conditions required to achieve any hydrogenation in our system caused general degradation of both the substrate and the catalyst, giving none of the desired reduction product. Success was finally realized with Pearlman's catalyst (palladium hydroxide on carbon);[12] reduction of alcohol **17** over this catalyst (1600 psi of H_2, 120 °C, 24 h, ethanol) gives alcohols **19** and **21** in a ratio of 1:1 (100%). Oxidation of the mixture (Jones reagent) and Fischer esterification (6 N HCl, refluxing methanol) give an easily separable 1:1 mixture of amino ester **20** and methyl homodaphniphyllate (**2**), identical by TLC mobility and 500-MHz ^1H NMR spectroscopy with an authentic sample.

Acknowledgment. This work was supported by a grant from the National Science Foundation (CHE-84-18437). We are especially indebted to Professor Y. Hirata for assisting us in obtaining a sample of codaphniphylline and to Professor S. Yamamura for supplying us with an authentic specimen of methyl homodaphniphyllate.

(10) The unsaturated amino ester is not soluble in methylcyclohexane. Thus, toluene is actually employed as solvent. The entire solvent is rapidly hydrogenated, giving methylcyclohexane. After the initial loss of pressure in this reduction, the hydrogenation bomb is repressurized to 1800 psi to complete the reduction.

(11) Evans, D. A.; Morrisey, M. M. *J. Am. Chem. Soc.* **1984**, *106*, 3866.
(12) (a) Pearlman, W. M. *Tetrahedron Lett.* **1967**, 1663. (b) The catalyst used was obtained from the Aldrich Chemical Co., catalog no. 21,291-1.

Incorporation of 3′-Methyltyrosine and 5′-Methyl-DOPA into Naphthyridinomycin

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Our studies[2] and those of Zmijewski[3,4] on the biosynthesis of naphthyridinomycin (**1**), an anticancer antibiotic produced by *Streptomyces lusitanus*,[5,6] have identified the primary precursors to the entire skeleton except for C-9 and C-9′. With five such precursors (tyrosine, ornithine, serine, methionine, and the piece yet to be identified) and numerous peripheral modifications, the number of chemically—and biochemically—rational pathways that could be envisioned for the formation of **1** is extraordinarily large. Nonetheless, we report here a few simple experiments that define the first steps of the biosynthetic pathway.

Since tyrosine is a precursor while dihydroxyphenylalanine (DOPA) is not,[3] we reasoned that ring methylation might procede hydroxylation. To test this, 3′-methyl-[2-^{13}C]tyrosine (**2**) was synthesized in two steps from 3-methyl-4-methoxybenzyl chloride (**3**)[7] and diethyl acetamido[2-^{13}C]malonate[8] in 73% yield (based on labeled malonate) (Scheme I).

Three 200-mL production broths were inoculated in standard fashion with a seed culture of *S. lusitanus*.[2] The labeled **2** (50

(1) Career Development Awardee of the National Cancer Institute (CA00880), 1979-1984.
(2) Zmijewski, M. J., Jr.; Palaniswamy, V. A.; Gould, S. J. *J. Chem. Soc., Chem. Commun.* **1985**, 1261.
(3) Zmijewski, M. J., Jr.; Mikolajczak, M.; Viswanatha, V.; Hruby, V. J. *J. Am. Chem. Soc.* **1982**, *104*, 4969.
(4) Zmijewski, M. J., Jr. *J. Antibiot.* **1985**, *38*, 819.
(5) Kluepfel, D.; Baker, H. A.; Piattoni, G.; Sehgal, S. N.; Sidorowicz, A.; Singh, K.; Vezina, C. *J. Antibiot.* **1975**, *28*, 497.
(6) Kluepfel, D.; Sehgal, S. N.; Vezina, C. U.S. Patent 4 003 902, Jan 18, 1977.
(7) Jorgensen, E. C.; Wiley, R. A. *J. Pharm. Sci.* **1963**, *52*, 122.
(8) Available from Aldrich Chemical Co. (99 atom % ^{13}C).

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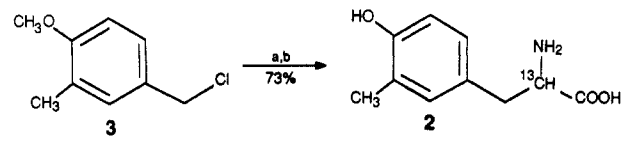
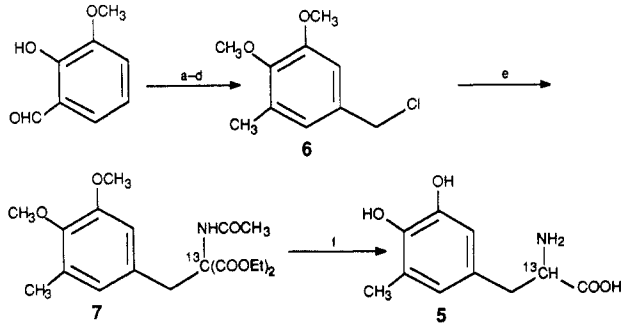
Scheme I[a]



[a] (a) NaOEt, $AcNH^{13}CH(COOEt)_2$, EtOH; (b) 47% HI, HOAc, reflux.

Scheme II[a]



[a] (a) 10% Pd/C, HOAc, H_2; (b) 37% HCHO, KOH, H_2O; (c) CH_2N_2, CH_3OH; (d) HCl, Et_2O; (e) NaOEt, $AcNH^{13}CH(COOEt)$, EtOH; (f) 47% HI, HOAc, reflux.

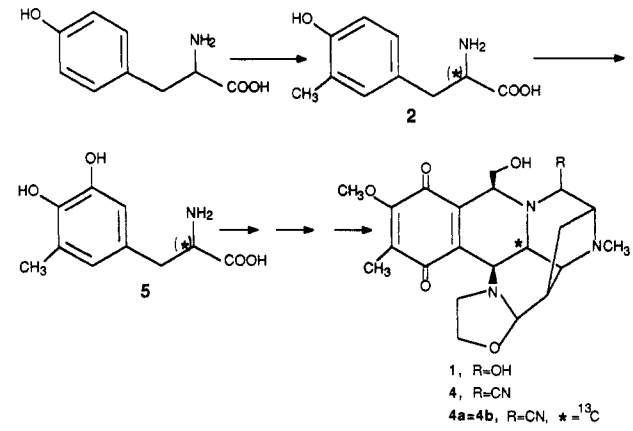
mg, 0.25 mmol) was dissolved in 10 mL of water and divided into two equal portions, and each was divided equally among the three broths at 60 and 72 h after inoculation. After an additional 24 h, the fermentation was worked up in the usual manner[3] to yield 3.1 mg of crystalline cyanonaphthyridinomycin (**4a**). This was diluted with 5.0 mg of unlabeled **4** and recrystallized again.

The 100.6-MHz ^{13}C NMR spectrum of **4a** in $CDCl_3$ revealed an intense resonance for C-13c (53.2 ppm). When normalized to the resonance for C-6 (62.3 ppm) and corrected for dilution with authentic **4**, a 16.8% enrichment in ^{13}C was determined. On the basis of the quantity of **2** fed and that of **4a** produced (determined by UV at 270 nm during the purification), a 1.7% incorporation of **2** had been obtained.

Since formation of the isoquinoline ring could be envisioned to occur by a biochemical Friedel–Crafts reaction, the presence of an hydroxyl ortho to the ring closure seemed likely. To test this, 5′-methyl-[2-^{13}C]DOPA (**5**) was synthesized. 5′-Methyl-DOPA has previously been synthesized in 3% overall yield and what would be a 14% yield for steps after a ^{13}C label could be reasonably introduced.[9] To circumvent this poor yield we adapted the procedure of Knabe et al.[10] using *o*-vanillin as starting material. This was converted in four steps to the benzyl chloride **6** (35% overall yield). Coupling of **6** with diethyl acetamido[2-^{13}C]-malonate[8] yielded 66% of adduct **7**, and hydrolysis with 47–51% HI and glacial acetic acid at reflux provided **5** in 93% yield (Scheme II). A portion of **5** (57 mg, 0.27 mmol) was fed to *S. lusitanus* (5 × 200 mL production broths) as described above and subsequent workup and purification afforded 10.1 mg of **4b**. This, too, was diluted with authentic **4** and recrystallized again. The ^{13}C NMR spectrum obtained from this sample was nearly identical with that obtained from **4a**. In this case a 12.4% enrichment at C-13c was determined, indicating a 2.1% incorporation of **5**.

These results provide strong evidence that **2** and **5**, previously unknown as natural products, are intermediates in the biosynthesis of **1**, and the early stages of the pathway may now reasonably

Scheme III



be formulated as shown in Scheme III.

Acknowledgment. Dr. Milton Zmijewski, Jr., now at the Eli Lilly Co., originally provided the culture of *S. lusitanus* and authentic samples of cyanonaphthyridinomycin. This work was supported by Public Health Service Grants GM 31715 and GM 32110 to S.J.G. The NMR spectra were obtained on a Bruker AM 400 spectrometer purchased in part with grants from the National Science Foundation (CHE-8216190) and from the M. J. Murdock Charitable Trust to Oregon State University.

(9) Cromartie, R. I. R.; Harley-Mason, J. *J. Chem. Soc.* **1952**, 1052.
(10) Knabe, J.; Mathews, H.; Schepers, A. *Arch. Pharm. (Weinheim, Ger.)* **1963**, *296*, 650.
(11) Characterized by IR and ^1H and ^{13}C NMR spectra and comparison with natural abundance **6**. Elemental analysis for **6** at natural abundance. Anal. Calcd for $C_{19}H_{27}NO_7$: C, 59.83; H, 7.13; N, 3.67. Found: C, 59.61; H, 7.20; N, 3.68.

9,18-Diphenyltetrabenz[*a*,*c*,*h*,*j*]anthracene: A Remarkably Twisted Polycyclic Aromatic Hydrocarbon

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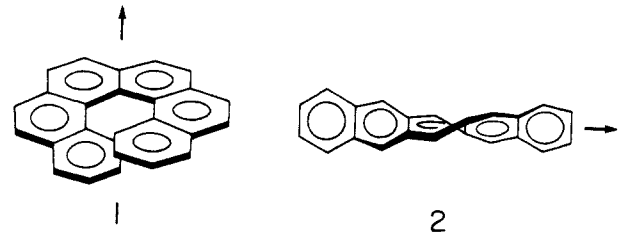
Helical organic molecules have long attracted the attention of synthetic chemists, and their efforts have been rewarded with the successful preparation and characterization of a variety of twisted compounds.[1] Among the most pleasing constructions are the helicenes (e.g., **1**), spiral polycyclic aromatic hydrocarbons in which



1 2

the axis of the molecular helix is roughly perpendicular to the mean planes of the constituent rings. However, one also can imagine a twisted hydrocarbon ribbon (**2**) in which the helix propagates in a direction parallel to the aromatic ring planes. Since simple

(1) Meurer, K. P.; Vogtle, F. *Top. Curr. Chem.* **1985**, *127*, 1–76 and references cited.

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