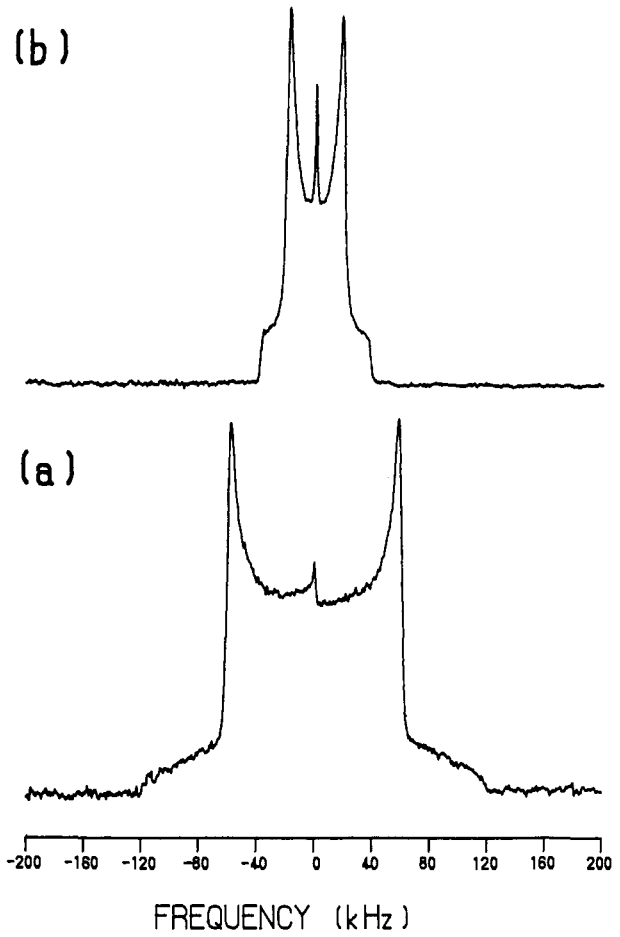

Figure 2. Deuterium NMR spectra of 200 mg of the Schiff base *N*-butyl-*1,1'-d*$_2$-2,4,6-octatrienylideneimine (a) in the crystalline phase at 30 °C and (b) in the smectic phase at 63 °C. Spectra were obtained at a field of 7.1 T (^{2}H frequency 46.3 MHz) by using a quadrupole echo pulse sequence with a 46-μs echo delay and a 2.5-μs $\pi/2$ pulse. Recycle delays were 20 s for the crystalline sample and 0.4 s for the liquid crystal. Line broadening of 1 kHz was applied to the former and 100 Hz to the latter spectrum; total transients accumulated were 1440 for (a) and 4096 for (b).

of their simple molecular structure, the polyene Schiff bases are therefore likely to be of some importance for the understanding of liquid crystalline phases in general. Polyenes and polyene Schiff bases are also of considerable theoretical interest in themselves, as oligomeric models for conjugated polymers, and are central to the mechanism of vision[8] and to the operation of the proton pump bacteriorhodopsin;[9] the existence of readily accessible anisotropic phases should facilitate the determination of the structures and the understanding of the spectroscopy of such compounds. Such studies are in progress.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, Grant number 19107G7, for partial support of this research. Support was also obtained from the National Institutes of Health (GM-39071) and the National Science Foundation Materials Research Initiative (DMR-8706432). We thank Wei-Jyun Chien for assistance with the spectroscopy, Pei Tang for her help with the figures, and P. J. Herley for assistance with the DSC.

Supplementary Material Available: DSC traces for the Schiff bases of 2,4,6-octatrienal with *n*-alkylamines of chain length 3–10, respectively (3 pages). Ordering information is given on any current masthead page.

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Biosynthesis of the Antibiotic Thiostrepton. Methylation of Tryptophan in the Formation of the Quinaldic Acid Moiety by Transfer of the Methionine Methyl Group with Net Retention of Configuration

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Thiostrepton (**1**),[1] first isolated from *Streptomyces azureus*,[2] is the parent of a family of highly modified, sulfur-rich polypeptide antibiotics, which also includes the micrococcins,[3] the siomycins,[4] the thiopeptins,[5] and nosiheptide.[6] These compounds inhibit protein synthesis in gram-positive bacteria;[7] however, their limited solubility has so far prevented development for clinical use, although nosiheptide is used commercially as a growth promotant for poultry.[8] As part of our interest in this family of compounds,[9,10] we have examined the biosynthesis of **1**.

Following unequivocal assignment of all the signals in the ^{13}C NMR spectrum of **1**[11,12] (Tables I and II, Supplementary Material), feeding experiments with ^{13}C-labeled precursors in *S. laurentii* gave the results summarized in Figure 1 (see Tables I and II, Supplementary Material). As expected, based on precedent,[9,13] both the butyrine and the dehydroalanine moieties arise from the corresponding β-hydroxyamino acids, threonine and serine, the thiostreptine moiety is formed from isoleucine, and the thiazole rings each originate from a molecule of cysteine and the carboxyl group of an adjacent amino acid. Two molecules of serine, connected through their carbon atoms 3, and the carboxyl group of an adjacent cysteine give rise to the piperidine ring. Finally, the quinaldic acid moiety is formed from L-tryptophan. The latter accounts for all the carbon atoms except C12, which is contributed by methionine. The transformation of tryptophan may involve a ring expansion similar to that leading to the formation of the quinine type alkaloids.[14] The methyl group, C12, would thus be attached to the carbon originating from C2 of the indole ring of tryptophan, and the question arises whether tryptophan is first methylated at C2 and then transformed into the quinoline system or whether methylation is a later step in the biosynthesis.

We therefore synthesized D,L-2-methyl-[3'-^{13}C]tryptophan (99% ^{13}C) in analogy to the method of Weygand and Linden[15] and fed

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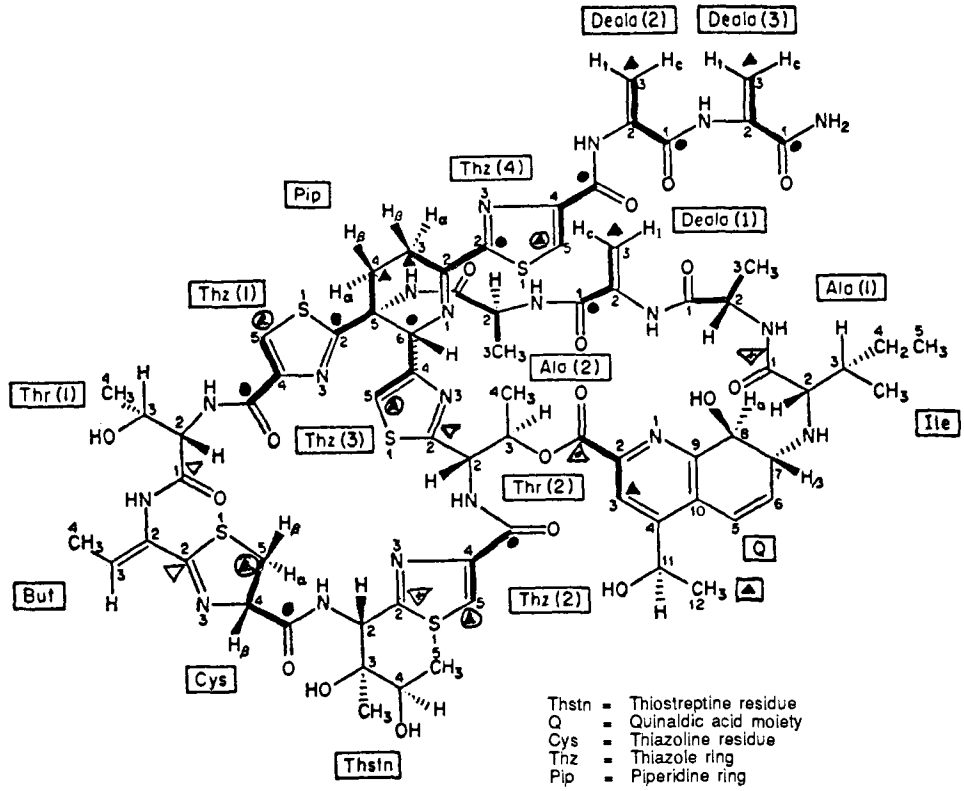


Enrichment from:

D,L-[3-^{13}C]cysteine	○	L-[methyl-^{13}C]methionine	□
D,L-[1-^{13}C]serine	•	L-[1′,^{13}C]tryptophan	△
D,L-[3-^{13}C]serine	▲	D,L-[1-^{13}C]isoleucine	+
D,L-[1-^{13}C]threonine	▽		

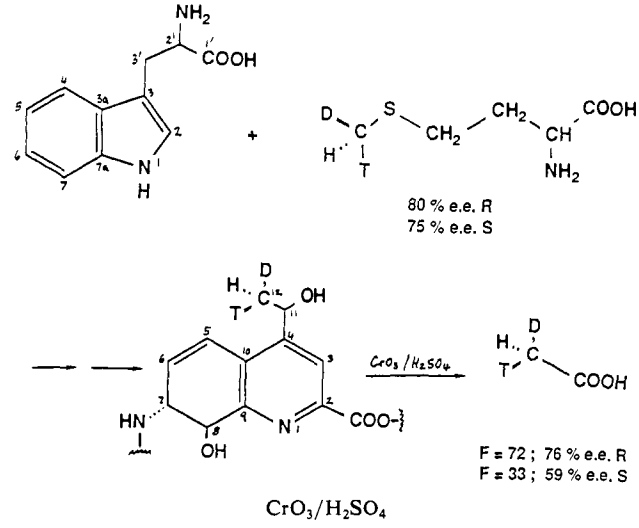
^{13}C-^{13}C coupling from L-[1,2-^{13}C$_2$]- or L-[2,3-^{13}C]serine ▬

Figure 1. Structure of thiostrepton and its labeling pattern from various ^{13}C-labeled amino acids.

it to cultures of *S. laurentii* (100 mg to 1 L). ^{13}C NMR analysis of the resulting **1** (100 mg) revealed a single enhanced signal at 122.26 ppm (~40% ^{13}C enrichment) corresponding to C3 of the quinaldic acid, indicating efficient and specific incorporation of 2-methyltryptophan (**2**). In further support of the notion that **2** is an intermediate in the biosynthesis of **1** we were able to demonstrate its formation and presence in **1**-producing cultures of *S. laurentii*. Trapping experiments with D,L-2-methyl-[3′-^{13}C]tryptophan (200 mg/L) showed 5–10% dilution of the isotope in the reisolated material, butanol extraction of the mycelia followed by derivatization and GC-MS revealed the presence of **2**, with concentrations highest just prior to the appearance of **1**, and cell-free extracts of 36 h old mycelia of *S. laurentii* catalyzed the formation of tritiated **2** from tryptophan and [*methyl*-^{3}H]-AdoMet. All these results strongly point to methylation of tryptophan as the first step in the formation of the quinaldic acid moiety of **1**.

To determine the steric course of tryptophan methylation at C-2 we fed (*methyl-R*)- and (*methyl-S*)-[*methyl*-^{2}H$_1$,^{3}H]-methionine[16] to cultures of *S. laurentii* and subjected the resulting **1** to Kuhn–Roth oxidation[17] to give acetic acid which was analyzed for the chirality of its methyl group.[18-20] As summarized in

Scheme I



Scheme I the methyl group is clearly transferred with net retention of configuration, contrary to most[21,22] but not all[23] methionine-dependent methylations. This is not the result of a process in-

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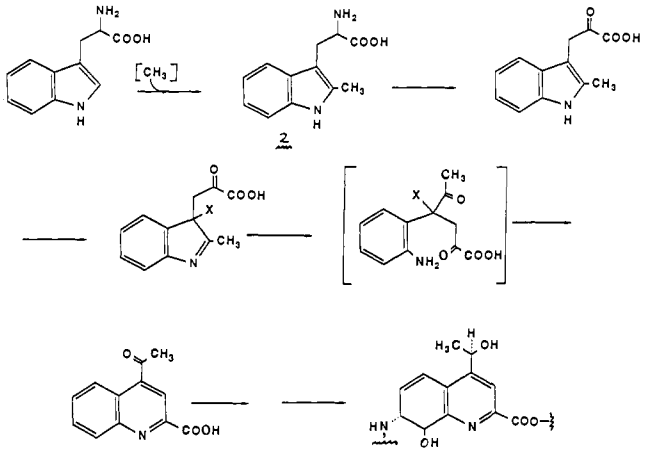
Scheme II



quinaldic acid moiety of **1**

volving an intermediate methylene group, as in sterol side-chain methylation,[21] because L-[*methyl*-^{13}C,$^{2}H_3$]methionine is incorporated with complete retention of all three deuterium atoms (data not shown). No change in methyl group configuration takes place after the initial transfer to C2 of the indole ring since in another series of experiments (*methyl-R*)-[*methyl*-$^{2}H_1$,^{3}H]methionine[24] gave **2** carrying an *R* methyl group (acetic acid from Kuhn-Roth oxidation: $F = 69$; 65% ee *R*) and the *S* isomer gave **2** carrying an *S* methyl group ($F = 31$; 66% ee *S*). Whether the unusual retention stereochemistry reflects the transient methylation of a site on the enzyme, e.g., a cobalamin cofactor,[25] in the process or results from initial transfer of the methyl group to a different site on the substrate followed by intramolecular migration remains to be established.

The further conversion of **2** into the quinaldic acid moiety of **1** may involve either (a) cleavage of the N1/C7a bond and connection of the side-chain nitrogen to C7a or (b) cleavage of the N1/C2 bond and connection of C2′ to N1. This issue was decided in favor of option (b) by feeding L-[*indole*-^{15}N,1′,2′-$^{13}C_2$]tryptophan.[26] The resulting **1** showed ^{13}C enrichment in the carboxyl group and C2 of the quinaldic acid moiety and one-bond coupling of these two signals to each other. In addition, the QC2 signal at 143.56 ppm displayed 3.02 Hz one-bond coupling to ^{15}N, and the QCO showed a two-bond coupling of 8.08 Hz to the ^{15}N,[28] indicating that C2′ of tryptophan has been intramolecularly connected to the ^{15}N-labeled indole nitrogen. A mechanistically reasonable pathway for the transformation of the indole to the quinoline system, which is consistent with the experimental data, is portrayed in Scheme II. This process has chemical precedent in the hypochlorite-catalyzed conversion of **2** into 4-acetylquinoline.[29]

Acknowledgment. We are indebted to Dr. Edith W. Miles, Bethesda, for a generous gift of tryptophan synthase, to E. R. Squibb and Co. for supplying thiostrepton, to Drs. Koji Kobayashi and Motomasa Kobayashi for the chiral methionine samples, and to Kyungok Lee and Lynda Callicotte for chirality analyses of acetate samples. This work was supported by NIH research Grants AI 20264 and GM 32333 and by NIH (RR02231)-US-DOE/OHER grants to the Stable Isotope Program at Los Alamos.

Supplementary Material Available: Table I, ^{13}C enrichments in **1** derived from ^{13}C-labeled amino acids, and Table II, ^{13}C–^{13}C coupling patterns in **1** derived from L-[1,2-$^{13}C_2$]- and L-[2,3-$^{13}C_2$]serine (3 pages). Ordering information is given on any current masthead page.

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{Tris(pyrazolyl)hydroborato}magnesium and -aluminum Alkyl Derivatives: Alkyl Exchange with Methyl Iodide and Enolate Formation with Acetone

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Organometallic derivatives of the *s*- and *p*-block elements, e.g., Li, Mg, and Al, are extremely important reagents in both organic and organometallic chemistry,[1] and significant efforts have been directed to determine the mechanisms of their reactions.[2] For example, studies have shown that, in addition to the conventional view of Grignard reactions with ketones as simple nucleophilic additions, an additional pathway involves single electron-transfer processes and the formation of radicals.[3] However, mechanistic studies of Grignard reagents are complicated by the complexity of the species present in solution.[3,4] Mechanistic investigations of the reactions of *s*- and *p*-block organometallic complexes would be aided by the synthesis of well-defined monomeric derivatives, L_nM-R. This paper describes the use of {η^3-tris(pyrazolyl)-hydroborato} ligands[5,6] to prepare alkyl derivatives of Mg and Al, in which chelation of the three nitrogen atom donors would be expected to provide a sterically demanding ligand environment[7]

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