the formation of a turbid solution containing only lamella upon dispersing DODAC *below 35 °C*.[4] Negatively stained electron micrographs of DODAC vesicles, sonicated at *50 °C* (phase transition temperature of DODAC vesicles were determined to be in the range of 30 to 50 °C),[8] clearly indicated the presence of single-walled vesicles whose diameters are in the range of 1000 to 1500 Å, and which are identical with those obtained by Deguchi and Mino[2] under identical conditions. The size of these vesicles were also confirmed by their appearance in the void volume on gel filtration on Sepharose 2-B.[9] Addition of cholesterol to DODAC decreased substantially the size of DODAC vesicles.[9]

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(17) In the presence of cholesterol, rates of OH^- permeabilities become measurable.[9]

Chieu D. Tran, Patricia L. Klahn
Alejandro Romero, Janos H. Fendler*
Department of Chemistry, Texas A&M University
College Station, Texas 77843
Received November 4, 1977

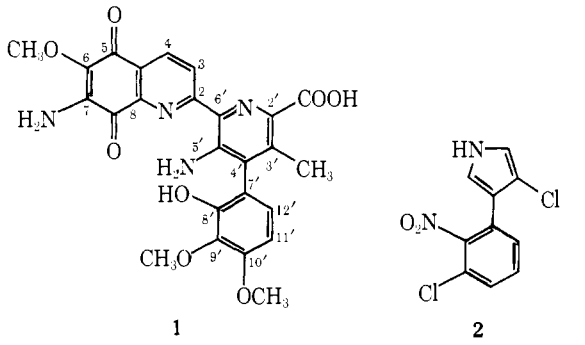
Studies of Nitrogen Metabolism Using ^{13}C NMR Spectroscopy. 1. Streptonigrin Biosynthesis

Sir:

We describe herein the use of ^{15}N-^{13}C couplings in ^{13}C NMR spectroscopy as an efficient tool for the study of nitrogen metabolism. Since there is no long-lived radioactive nitrogen isotope, the study of secondary nitrogen metabolism has lagged far behind those of carbon and hydrogen. While the fate of nitrogen-15 enriched precursors in secondary metabolism has been determined in a few instances by mass spectrometry,[1] the presence of more than one nitrogen in a metabolite leads to ambiguous results unless useful fragmentation patterns can be clearly defined; frequently some chemical degradation is necessary. Bycroft and co-workers have reported[2] the use of ^{15}N NMR in the study of penicillin biosynthesis using a high-producing *Penicillium* strain that afforded a percent incorporation of precursor unusually high even for microbial metabolites.

In the last few years homonuclear couplings (^{13}C-^{13}C)[3] and heteronuclear couplings (^{2}H-^{13}C)[4] in ^{13}C NMR spectra have been used to study biosynthetic pathways. They provide relatively direct indications of bond-breaking and bond-forming reactions without recourse to time-consuming and often impractical chemical degradations. The investigation of ^{15}N-^{13}C couplings seemed a logical extension which might simplify the study of nitrogen metabolism. Rinehart and co-workers have recently used this technique to determine the origin of the carbamate residue in geldanamycin.[5]

We recently reported[6] that in the biosynthesis of streptonigrin (**1**),[7] an antibiotic produced by *Streptomyces flocculus* ATCC 13257, the 4-phenylpicolinic acid moiety is derived from tryptophan, suggesting a new pathway for the formation of pyridine rings[8] and a new metabolism of tryptophan.[9] This apparent biosynthesis required cleavage of the intact indole ring at a C-N bond. Only one example of such a cleavage has previously been demonstrated; the biosynthesis



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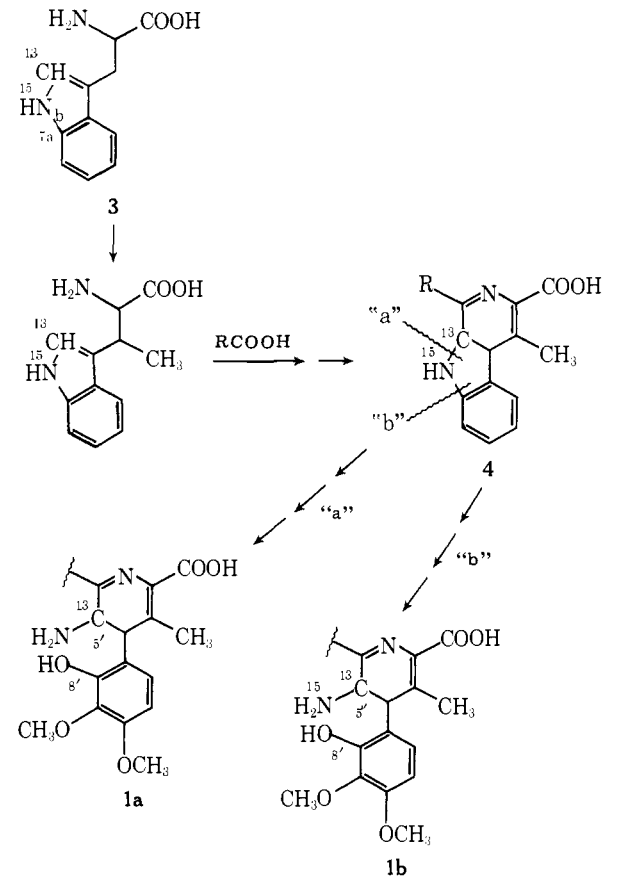
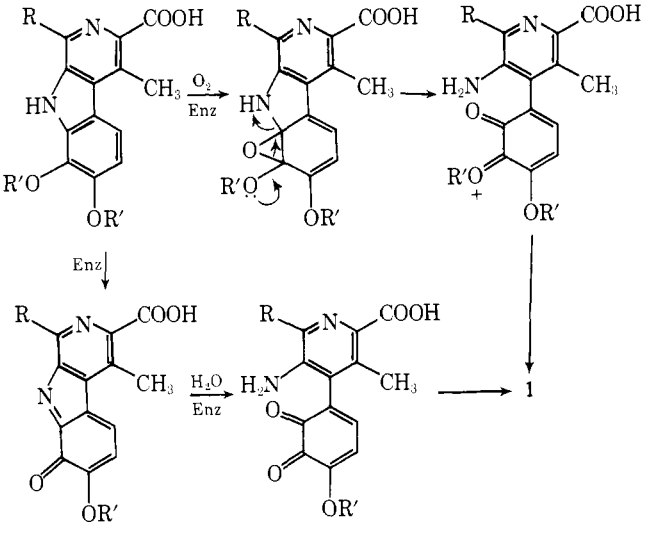
Scheme I



Scheme II[a]



[a] R' = H or CH_3. The exact timing of the methylations has not yet been determined.

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Table I. Assignment of the ^{13}C NMR Spectrum of Streptonigrin in Me_2SO-d_6 and Comparison with the Spectrum Taken in Pyridine-d_5[17] (Experimental Parameters Given in Ref 16)

Me_2SO	Pyridine	Assignment
180.165	181.1	8
175.850	176.9	5
166.842	168.3	COOH
159.748	161.0	7,8a
153.059	154.2	
147.989	150.0	6′
145.615	147.1	5′
143.997	144.9	10′
141.354	141.9	2′
136.931	138.6	8′
136.149	137.8	3′
135.717	137.1	4′
134.422	135.9	
133.910	135.3	
133.287	133.5	4
129.487	130.7	9′
126.601	127.4	
125.927	126.2	3
124.578	125.4	12′
114.788	116.4	7′
104.458	105.1	11′
60.253	60.5	9′,10′-OCH_3
59.632	60.1	
55.614	56.1	6-OCH_3
16.856	17.8	CH_3

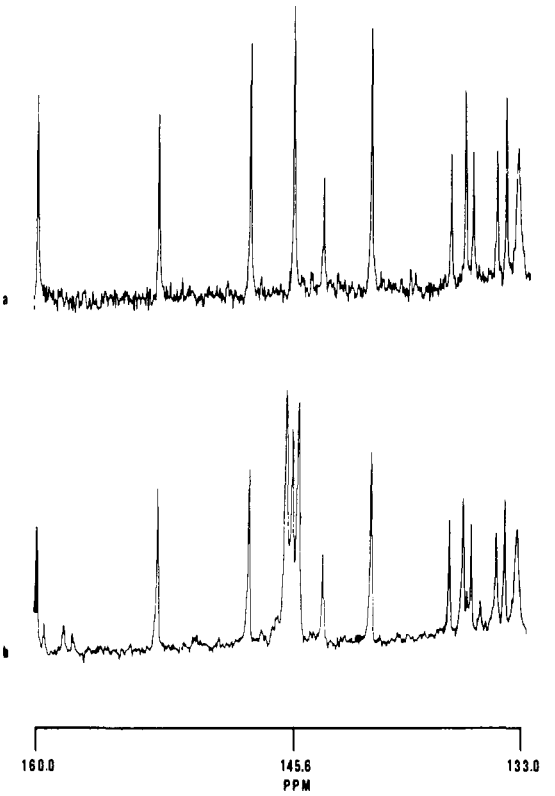

Figure 1. (a) A portion of the ^{13}C NMR spectrum of authentic streptonigrin (50 mg in 0.5 mL of Me_2SO-d_6, 8618 transients). (b) A portion of the spectrum of streptonigrin produced in the presence of [2-^{13}C-$^{15}N_b$]tryptophan (50 mg in 0.5 mL of Me_2SO-d_6, 34444 transients).

of pyrrolnitrin **2** involves cleavage of the tryptophan N_b-C_2 bond to give an aromatic amine that is finally oxidized to the nitro group.[10] An analogous mechanism for the biosynthesis of streptonigrin (shown in Scheme I, pathway a with isotopic labels) seemed unnecessarily cumbersome since it would require replacement of one nitrogen at C-8′ by hydroxyl and the introduction of a new nitrogen at C-5′. The alternative, cleavage of the N_b-C_{7a} bond (Scheme I, pathway b), while unprecedented appeared much more attractive. The extensive oxygenation of the phenyl ring allows for a number of reasonable mechanisms involving oxygen or hydroxyl participation in the cleavage process. Two such possibilities are shown in Scheme II.

In order to determine how the indole ring is cleaved, and, specifically, to test our hypothesis that the N_b-C_{7a} bond of tryptophan is broken, we have synthesized [2-^{13}C-$^{15}N_b$]-DL-tryptophan **3**[11] and have fed it to cultures of *S. flocculus*. The labeled tryptophan was synthesized by the acetamidomalonate route[12] using doubly labeled indole[13] prepared by the procedure of Leete and Wemple.[14] As shown in Scheme I, pathway a would give **1a** resulting in loss of the ^{15}N and a simple enhancement of the C-5′ signal in the ^{13}C NMR, whereas in pathway b both the ^{15}N and ^{13}C would be retained; in this case the C-5′ signal of **1b** would appear as a doublet due to the ^{15}N coupling.

A portion of the doubly labeled tryptophan (48 mg, 0.09 mmol), mixed with [β-^{14}C]-DL-tryptophan (2.8×10^7 dpm, 33 mCi/mmol), was fed in the usual fashion[6] to producing mycelia of *S. flocculus* in eight 2-L Erlenmeyer flasks, each containing 500 mL of fermentation broth.[15] Workup 2 days later afforded 82 mg of pure streptonigrin. Determination of the specific radioactivity of labeled **1** (6.4×10^6 dpm/mmol) indicated a 3.5% incorporation of tryptophan. A portion of the natural abundance proton noise decoupled FT ^{13}C NMR spectrum of streptonigrin in Me_2SO-d_6[16] is given in Figure 1a, and the assignments[17] are given in Table I. As shown in Figure 1b of the biosynthetically enriched sample, the signal at 145.6 ppm, assigned to C-5′, is flanked by a doublet (J = 14.6 Hz) resulting from the enriching ^{13}C being bonded to an adjacent ^{15}N.[18] Comparing the integral of the natural abundance singlet with that of the doublet indicates a 3.2-fold enrichment. No other signal in the ^{13}C NMR spectrum has been altered.

Our results clearly show that both isotope labels have been retained and that the C-N bond between them has remained intact during streptonigrin biosynthesis. Thus, the metabolic fate of tryptophan in *S. flocculus* is even more unusual that we had previously indicated.[6]

The use of ^{15}N-^{13}C couplings in ^{13}C NMR spectroscopy clearly provides a means for studying pathways of nitrogen metabolism that would otherwise present formidable technical difficulties. We are currently investigating the use of this technique to study other problems in the biosynthesis of nitrogen-containing metabolites.

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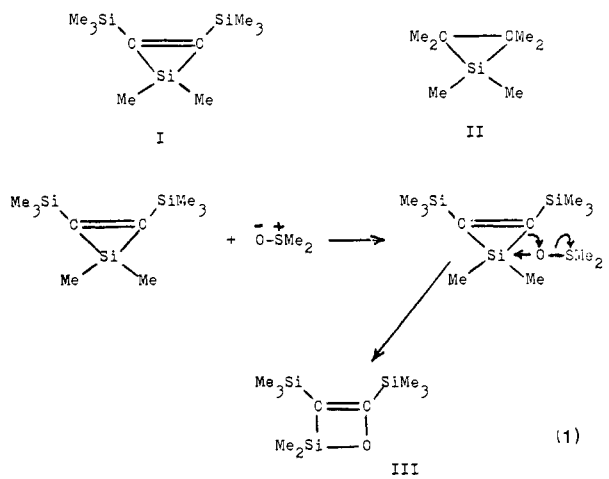
(18) ^{15}N-^{13}C couplings are typically on the order of 5-15 Hz: R. L. Lichter in "Determination of Organic Structures by Physical Methods", Vol. 4, F. C. Nachod and J. J. Zuckerman, Ed., Academic Press, New York, N.Y., 1971, p 221.

Steven J. Gould,* Chiu Chin Chang
School of Pharmacy
Section of Medicinal Chemistry and Pharmacognosy
University of Connecticut, Storrs, Connecticut 06268
Received December 7, 1977

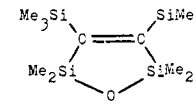
Reactions of 1,1-Dimethyl-2,3-bis(trimethylsilyl)-1-silirene and Hexamethylsilirane with Dimethyl Sulfoxide. Insertion of Dimethylsilanone into the Silirene and Silirane Rings

Sir:

In a continuation of our recent investigations of the highly strained and hyperreactive silirene and silirane ring systems,[1] we have examined their reactions with dimethyl sulfoxide. In view of the propensity of both silirenes and siliranes to undergo attack at the ring silicon atom by nucleophilic reagents, we expected that the reaction of dimethyl sulfoxide with the SiC_2 cyclics I and II would follow the course shown for I in eq 1. The 1-oxa-2-silacyclobutene III would be a new ring system of some interest, one which we have sought without success as a product of the autoxidation of I. The actual course of the reaction of silirene I with dimethyl sulfoxide, however, proceeded differently, although the initial step could well be that shown in eq 1. The reaction is of particular significance and interest in that dimethylsilanone (or dimethylsilylene oxide), $Me_2Si{=}O$, appears to be an intermediate.



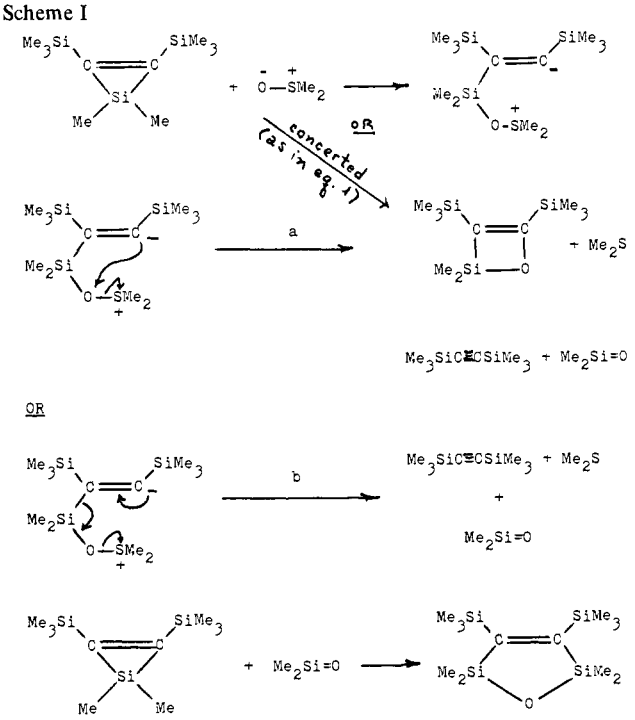
In one such reaction, 0.5 mmol of dimethyl sulfoxide was added slowly, under argon to a cold (0 °C) solution of silirene I (1.54 mmol) in 1 mL of dry benzene in a flame-dried, 10 mL, one-necked flask. The mixture was stirred and kept at 0 °C for 5 min and then was allowed to warm to room temperature.[2] After 1 h, gas-liquid chromatographic (GLC) analysis showed the presence of dimethyl sulfide, bis(trimethylsilyl)acetylene (94%, based on Me_2SO), and the unsaturated, cyclic siloxane IV in 66% yield (assuming that it requires 2 mol of I to produce 1 mol of IV). The latter, a solid of mp 74.5-75.5 °C, was


IV

identified by combustion analysis and by comparison of its IR and NMR spectra with those of an authentic sample obtained by autoxidation of 1,1,2,2-tetramethyl-3,4-bis(trimethylsilyl)-1,2-disilacyclobut-3-ene.[1b,3] The formation of these products can be rationalized as shown in Scheme I. Whether a cyclic intermediate is involved (path a) or not (path b), in either case, a dimethylsilanone, $Me_2Si{=}O$, intermediate is generated and this then inserts into the Si—C bond of the strained, highly reactive silirene to give IV.

If $Me_2Si{=}O$ is an actual intermediate, other substrates which are known to react with this species might be added to the reaction mixture to intercept it.[4] Accordingly, a Me_2SO-I reaction (4 mmol of Me_2SO, 1.7 mmol of I) was carried out in the presence of dimethyldimethoxysilane. The silirene was added slowly to a solution of Me_2SO in dimethyldimethoxysilane (4 mL) to give an exothermic reaction in which the following products were formed: dimethyl sulfide, bis(trimethylsilylacetylene (86%), and *sym*-tetramethyldimethoxydisiloxane (81%), n^{25}_D 1.3815 (lit.[8] n^{25}_D 1.3811), ^1H NMR (CCl_4)

Scheme I



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