The Biosynthesis of Sparsomycin. Elucidation of the Origins of the Carbon Skeleton

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Sparsomycin (**1**) (Scheme I) is a unique antibiotic isolated from the fermentation broth of *Streptomyces sparsogenes* var. *sparsogenes*.[1] The structure of sparsomycin was assigned in 1970,[2] and two syntheses of the antibiotic have been reported.[3] Sparsomycin exhibits antibiotic activity against a variety of gram-negative and gram-positive bacteria, and it shows potent antitumor activity against KB human epidermoid carcinoma cells in vitro.[4] The biological activity of sparsomycin is apparently the result of its ability to inhibit the peptide bond-forming step of protein biosynthesis.[5] The results of an investigation of the biosynthesis of this interesting antibiotic will now be reported.

The initial investigations of sparsomycin biosynthesis focused upon the origin of the left-hand portion of the molecule (C-1′–C-5′), which contains the unusual monooxo–dithioacetal group. Administration of (*methyl*-^{13}C)-L-methionine to *S. sparsogenes* produced the antibiotic labeled at both C-4′ and C-5′ (Table I, expt 1), thereby demonstrating that both of these carbon atoms are derived from the methionine methyl group. Another significant observation derived from this experiment was the fact that the C-methyl group (C-1) of sparsomycin was *not* labeled by the methionine. The next experiment utilized (3-^{13}C)-DL-cysteine synthesized by the method of Gasparini et al.[6] Administration of this precursor to *S. sparsogenes* yielded sparsomycin that showed the expected enrichment at C-3′ (Table I, expt 2), but, surprisingly, enrichment was also present at C-4′. We hypothesized that the enrichment at C-4′ might be due to the conversion of (3-^{13}C)-cysteine to (3-^{13}C)-serine followed by formation of (*methylene*-^{13}C)-5,10-methylenetetrahydrofolate from the labeled serine via the action of serine transhydroxymethylase. The label from methylenetetrahydrofolate could then be incorporated into C-4′ of sparsomycin.[7] To evaluate this hypothesis, (3-^{13}C)-DL-serine[8] was administered to *S. sparsogenes*. The resulting sparsomycin showed no enrichment (Table I, expt 3) in its ^{13}C NMR spectrum. It therefore appears that the incorporation of label from C-3 of cysteine into C-4′ of sparsomycin does not proceed via the intermediacy of (3-^{13}C)-serine. It is also noteworthy that the (3-^{13}C)-serine did not label sparsomycin at C-3′ as would be expected from the conversion of the labeled serine into cysteine. The reason for the apparent lack of conversion of serine into cysteine is presently unclear.

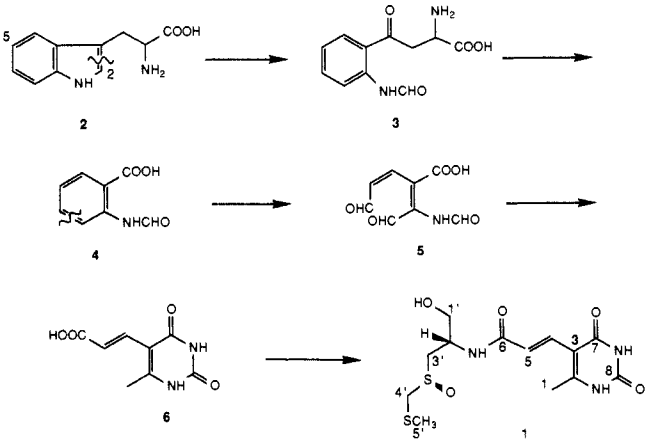
The results from the incorporation experiment using labeled methionine suggested that *S*-methylcysteine might be an intermediate in sparsomycin biosynthesis. Since the configuration at C-2′ of the antibiotic corresponds to that of D-cysteine, both L- and D-(*methyl*-^{13}C)-*S*-methylcysteine were evaluated as precursors. The synthesis of these compounds was easily accomplished by reduction of L- and D-cystine with sodium in liquid ammonia followed by alkylation with (^{13}C)-methyl iodide. Administration of each of these substances to *S. sparsogenes* revealed that they are both specific precursors of sparsomycin, with the D-isomer being the more efficient precursor (Table I, expt 4 and 5). Since

Table I. Precursor Incorporation Experiments with *S. sparsogenes*

expt no.	precursor (^{3}H/^{14}C)	% incorpn[a]	labeling pattern (^{3}H/^{14}C)
1	(*methyl*-^{13}C)-L-methionine	2.7 (C-4′) 6.7 (C-5′)	C-4′, C-5′
2	(3-^{13}C)-DL-cysteine	1.0 (C-3′) 0.6 (C-4′)	C-3′, C-4′
3	(3-^{13}C)-DL-serine	no visible enrichment	
4	(*methyl*-^{13}C)-*S*-methyl-L-cysteine	0.3	C-4′
5	(*methyl*-^{13}C)-*S*-methyl-D-cysteine	0.6	C-4′
6	[5-^{3}H]-L-tryptophan	0.17	
7	[5-^{3}H,U-^{14}C]-L-tryptophan (5.45)	0.16	(7.96)
8	(2-^{13}C)-DL-tryptophan	22	C-8
9	(5-^{2}H)-DL-tryptophan	6	C-5

[a] The incorporation figures for the experiments utilizing stable isotopes were obtained from the appropriate NMR spectra.

Scheme I



no label appeared at C-5′ of the antibiotic, we can presume that both of these amino acids were incorporated into the antibiotic intact. The biosynthesis of the monooxo–dithioacetal moiety of sparsomycin therefore appears to be accomplished by the introduction of sulfur into the *S*-methyl group of *S*-methyl-D-cysteine. The mechanism of this conversion may prove to be related to the mechanism of sulfur introduction observed in the biosynthesis of biotin, lipoic acid, and penicillin.[9]

One of the most intriguing questions to be answered with respect to sparsomycin biosynthesis concerns the origin of the modified uracil moiety present in the antibiotic. After some negative preliminary experiments examining this question, it occurred to us that the uracil moiety of sparsomycin could be derived from tryptophan via the pathway shown in Scheme I. The pathway begins with the well-known[10] oxidative cleavage of tryptophan (**2**) to *N*-formylkynurenine (**3**). Further oxidation of **3** could then yield *N*-formylanthranilic acid (**4**). Oxidative cleavage of **4** to **5** followed by several additional steps would finally generate the uracil moiety (**6**) found in sparsomycin. The hypothesis predicts that C-8 of sparsomycin should be derived from C-2 of tryptophan, while C-5 of the antibiotic should be derived from C-5 of tryptophan.

The pathway shown in Scheme I was first tested by administration of [5-^{3}H]-L-tryptophan to *S. sparsogenes*. We were pleased to observe a relatively high incorporation of this amino acid into the antibiotic (Table I, expt 6). A double-label experiment was then carried out to verify that tryptophan was serving as a specific precursor. [5-^{3}H,U-^{14}C]-L-Tryptophan was added to the fermentation broth, and the tritium-to-carbon-14 ratio of the resulting labeled antibiotic was determined (Table I, expt 7). The hypothesis outlined in Scheme I predicts complete retention of tritium and retention of eight carbon atoms of tryptophan. Since the

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precursor had a ratio of 5.45, the antibiotic should have a ratio of 7.49, if eight carbons are retained. An alternative version of the pathway could proceed via anthranilic acid. In this case, C-2 of the indole ring would be lost and only seven carbon atoms of tryptophan retained. The theoretical value for the ratio in this instance would be 8.56. The experimental value (7.96) falls between the two theoretical values, and so it does not allow one to decide whether seven or eight carbon atoms of tryptophan have been incorporated into sparsomycin. The results of expt 7 also do not prove that tryptophan has been incorporated into the antibiotic in the manner predicted by Scheme I. Nevertheless, the data clearly demonstrate that tryptophan is a specific precursor of sparsomycin. The validity of the hypothesis outlined in the scheme was proven in two ways. First, (2-^{13}C)-DL-tryptophan was synthesized by a combination of literature procedures[11] and administered to the producing organism. The results of this experiment were exceedingly gratifying, as a very strong enrichment appeared at C-8 of the antibiotic (Table I, expt 8). Second, the incorporation of (5-$^{2}H_1$)-DL-tryptophan[12] into sparsomycin was examined. The deuterium NMR spectrum of the antibiotic isolated in this experiment exhibited a high deuterium enrichment at 7.17 ppm demonstrating that C-5 of sparsomycin is derived from C-5 of tryptophan (Table I, expt 9).[13] We can therefore conclude that the uracil moiety of sparsomycin is biosynthesized from tryptophan in the unprecedented manner shown in Scheme I.

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Registry No. 1, 1404-64-4; Met, 63-68-3; Cys, 52-90-4; *S*-methyl-Cys, 1187-84-4; Trp, 73-22-3.

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Synthesis of Inositol Phosphates

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The discovery that D-*myo*-inositol 1,4,5-tris(phosphate) (1,4,5-IP$_3$, **3b**) is involved in signal transduction in animal cells has led to intense interest in the biosynthesis, function, and metabolism of inositol phosphates.[1] To date, at least 15 inositol phosphates have been isolated from natural sources, including another putative "second messenger", D-*myo*-inositol-1,3,4,5-tetrakis(phosphate) (1,3,4,5-IP$_4$, **6**).[2] Research on the biological function of these compounds would be greatly aided by the availability of adequate amounts of synthetic inositol phosphates which are isomerically pure and/or radioactively labeled. Although a number of inositol poly(phosphates)[3-6] have been prepared by total synthesis, access to this family of compounds has generally been limited by difficulties in phosphorylation[7] and cumbersome routes to protected precursors. Phosphorylation of inositol derivatives is complicated by three well-known problems: (a) the relative unreactivity of conventional phosphorus(V) reagents, such as diphenylphosphorochloridate, toward the secondary alcohols of inositol;[3a,b,4a] (b) the tendency of the resulting phosphate triester intermediates to form cyclic phosphates;[6,8] and (c) the propensity of phosphate monoester groups to migrate to neighboring hydroxyl groups under acidic conditions via similar cyclic intermediates.[4a,9] Since chlorophosphites are remarkably more reactive than the corresponding phosphorus(V) phosphorylating agents,[10] we hoped that use of phosphitylating agents would alleviate the reactivity problem. We hypothesized that phosphorus(III) intermediates resulting from phosphitylation would also not suffer from cyclization or migration problems. Although a few reports of preparation of phosphate monoesters via phosphitylation[3c,d,11,12] have appeared recently, the full power of this methodology is not illustrated by these papers. We describe herein[13] (a) the use of the simple reagent dimethyl chlorophosphite[14] for the preparation of phosphate monoesters from alcohols, (b) the first successful bisphosphorylation of a cis vicinal diol, (c) a short synthesis of 1,4,5-IP$_3$ which uses a regioselective "partial" phosphoitylation as the key step, and (d) the first syntheses of 1,3,4,5-IP$_4$ and another naturally occurring inositol poly(phosphate), 1,4,5,6-IP$_4$ (**9**).[15,16]

D,L-1,4-Dibenzoyl-*myo*-inositol (**1**), which has both cis and trans vicinal diols, was selected as a convenient,[17] but challenging, model substrate for evaluation of our phosphitylation strategy. Treatment of tetraol **1** with excess dimethoxychlorophosphite and diisopropylethylamine followed by oxidation with hydrogen peroxide gave exclusively tetrakis(dimethyl phosphate) **2a**. This material was conveniently isolated in 73% yield by direct crystallization from the reaction mixture. Removal of the phosphate methyl groups was cleanly effected by exposure to bromotrimethylsilane[19] or hydrogen bromide in acetic acid. The resulting tetrakis(dihydrogen phosphate) was isolated by removing the volatile byproducts and precipitating the lithium salt from water at pH 10 with ethanol. Subsequent hydrolysis of the benzoates with lithium hydroxide gave the octalithium salt of 1,2,4,5-IP$_4$ (**3a**) in addition to about 10% of a mixture of inositol tris(phosphates)[20] which were

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