determined by its energy gap dependence.[16] Table I shows that the electron transfer in **1** is faster than **2** by about a factor of 5 both in THF and in DMF. The difference is probably attributable to the favorable orientation of **1** for the electron transfer, since the donor and acceptor are separated by almost the same distance and the same number of bonds (seven bonds) in **1** and **2**. However, there is some possibility[17] that the σ-orbitals of the spacer in **1** are much more involved in electron transfer by super exchange mechanism than is **2**. Quantitative evaluation of electron-transfer rates by such a mechanism is difficult at this stage, and further interpretation must await theoretical calculations of the interaction matrix element[18] for electron transfer. Compounds **1** and **2** clearly demonstrate that the relative orientation between the porphyrin and quinone is a factor in the photodriven charge separation reaction.

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Biosynthesis of Sinefungin: On the Mode of Incorporation of L-Ornithine

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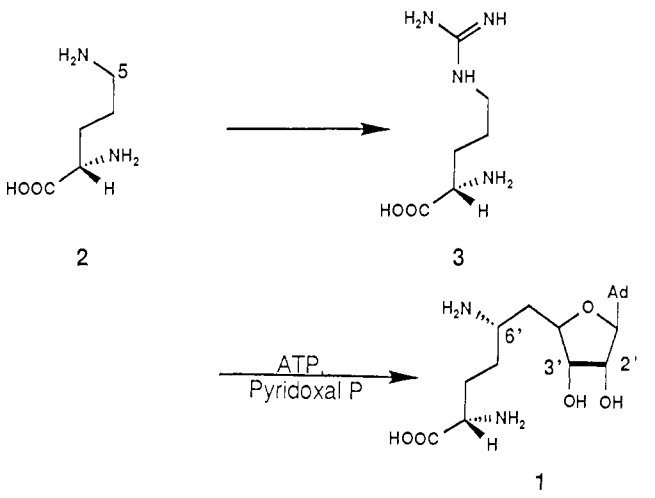
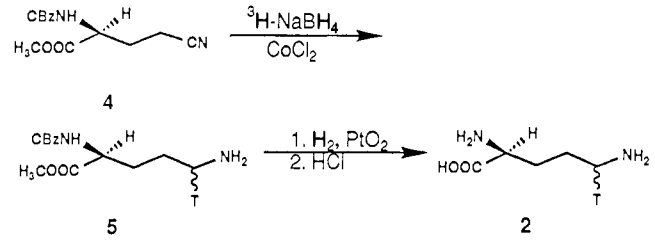
Sinefungin (**1**) is a nucleoside antibiotic isolated from the fermentation broth of *Streptomyces griseolus* and *Streptomyces incarnatus*.[1,2] Sinefungin exhibits antifungal[1] and antiviral[3] activity as well as potent activity against a number of protozoal parasites.[4] It is also a powerful inhibitor of *S*-adenosylmethionine dependent methyltransferases.[5] The biosynthesis of sinefungin was first scrutinized by Berry and Abbott.[6] These authors administered a number of ^{14}C-labeled compounds to *S. griseolus*, and on the basis of the observed incorporation levels, they suggested that sinefungin is formed by the condensation of L-ornithine (**2**) with adenosine. More recently, the biosynthesis of sinefungin has been examined in cell-free extracts of *S. incarnatus* by Robert-Gero and co-workers.[7] The results of these investigations suggested that sinefungin is biosynthesized from L-arginine (**3**) and ATP, with a requirement for pyridoxal phosphate (Scheme I). We now present the results of our own studies on the mode of incorporation of L-ornithine into sinefungin. These studies shed additional light on the mechanism of sinefungin biosynthesis.

Scheme I



Scheme II



Preliminary incorporation experiments were carried out by administration of [U-^{14}C]-L-ornithine and [U-^{14}C]-L-arginine to *S. griseolus*. Since the incorporation levels were similar (data not shown), additional experiments were conducted with L-ornithine, which is known to be the precursor of L-arginine in vivo.[8] (5-^{13}C)-L-Ornithine was synthesized from (^{13}C)KCN by using a procedure developed to prepare the corresponding ^{14}C-labeled compound.[9] Administration of this precursor to *S. griseolus* yielded sinefungin that exhibited clear enrichment at the expected position of the antibiotic (Table I, experiment 1). Ornithine is thereby shown to be a specific precursor of sinefungin. We next examined the origin of the amino group present at C-6′ of the antibiotic. (5-^{15}N,5-^{13}C)-L-Ornithine synthesized from (^{15}N, ^{13}C)KCN was supplied to the sinefungin fermentation and the isolated antibiotic examined by ^{13}C NMR spectrometry. Since the enriched signal for C-6′ appeared as a doublet, one can conclude that the amino group present at C-6′ is derived from the δ-amino group of ornithine (Table I, experiment 2).

The most novel feature of sinefungin biosynthesis is the formation of a carbon–carbon single bond between C-5 of ornithine and C-5′ of an adenylyl moiety. The stereochemistry of C–C bond formation is therefore a matter of some interest. This problem was first approached by utilizing (5*RS*)-[5-^{3}H]-L-ornithine as a sinefungin precursor. The labeled ornithine was synthesized by reduction of the nitrile ester **4**[9] with sodium borotritide in the presence of cobalt chloride[10] to yield the tritiated amine **5**. The labeled amine was then converted into tritiated L-ornithine by hydrogenolysis of the CBz group and hydrolytic removal of the methyl ester (Scheme II). Administration of the tritiated L-ornithine to *S. griseolus* in conjunction with [U-^{14}C]-L-ornithine

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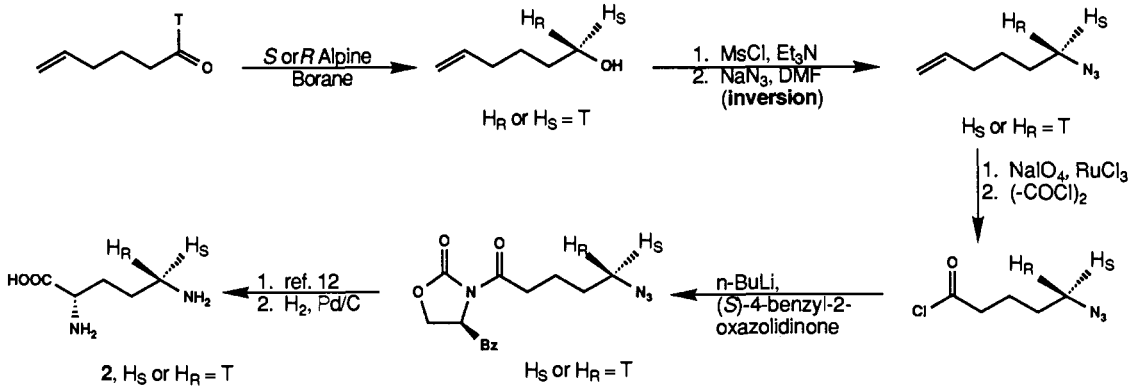
Table I. Incorporation of Labeled L-Ornithine (**2**) into Sinefungin (**1**)

expt no.	precursor ($^3H/^{14}C$)	% incorpn or enrichment	$^3H/^{14}C$ in sinefungin	labeling pattern in sinefungin
1	(5-^{13}C)-**2**	4.0		C-6′
2	(5-^{15}N,5-^{13}C)-**2**	6.7		C-6′,J_{CN} = 4.3 Hz
3	(5*RS*)-[5-^{3}H,U-^{14}C]-**2** (4.55)	0.12	2.34	51.4% ^{3}H retention
4	(5*R*)-[5-^{3}H,5-^{14}C]-**2** (3.95)	4.1	3.50	88.6% ^{3}H retention
5	(5*S*)-[5-^{3}H,5-^{14}C]-**2** (4.80)	7.2	0.75	15.6% ^{3}H retention

Scheme III



yielded radioactive sinefungin that was rigorously purified by chromatography, conversion to its 2′,3′-*O*-isopropylidene derivative followed by additional chromatography, and cyclization of the 2′,3′-*O*-isopropylidene derivative to the corresponding δ-lactam in refluxing methanol. The isotopic ratio in the lactam derivative indicated that the tritiated ornithine had been converted into sinefungin with approximately 50% tritium loss (Table I, experiment 3). This observation indicates that one hydrogen atom is stereospecifically removed from C-5 of ornithine as the result of C–C bond formation. The stereochemistry of hydrogen removal was elucidated by means of precursor incorporation experiments with (5*R*)- and (5*S*)-[5-^{3}H]-L-ornithine. Since no efficient synthesis of these compounds has been previously reported,[11] we devised a new synthesis based upon the Evans electrophilic azidation reaction.[12] The synthesis[13] (Scheme III) proceeds via (1*R*)- and (1*S*)-[1-^{3}H]-5-hexenol, which are prepared from [1-^{3}H]-5-hexenal by reduction with *S*- and *R*-Alpine-Borane, respectively.[14] The stereochemistry of the *R*-Alpine-Borane reduction was verified by reduction of (1-$^2H_1$)-5-hexenal to (1*S*)-(1-$^2H_1$)-5-hexenol, esterification of this alcohol with (−)-camphanyl chloride, catalytic reduction of the terminal double bond, and NMR analysis of the chirality according to Gerlach.[15] Administration of the two stereospecifically tritiated forms of L-ornithine to *S. griseolus* in combination with [5-^{14}C]-L-ornithine yielded two samples of radioactive antibiotic whose tritium to carbon-14 ratios after derivatization and purification clearly show that ornithine is converted into sinefungin with loss of the 5-*pro*-*S* hydrogen atom (Table I, experiments 4 and 5).[16] Since the absolute configuration at C-6′ of sinefungin is *S*,[17] it follows that C–C bond formation at C-5 of ornithine occurs with overall *inversion* of configuration.

The mechanistic information obtained from the preceding experiments is largely consistent with observations[7] indicating that sinefungin is formed from L-arginine and ATP by a pyridoxal phosphate requiring enzyme. The stereochemistry of C–C bond formation at C-5 of ornithine is, however, something of a surprise. The majority of enzymatic reactions involving pyridoxal phosphate occur on one face of the substrate–cofactor complex, leading to an overall *retention* of stereochemistry.[18] Important exceptions to this rule include the α-amino acid racemases, *meso*-diaminopimelate decarboxylase, and 5-aminolaevulinic acid synthase.[18] It appears that sinefungin synthase can now be added to this list of stereochemically deviant enzymes. Nevertheless, the deeper mechanistic significance of these exceptions to the more common stereochemical pattern remains obscure.

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Registry No. Sinefungin, 58944-73-3; L-ornithine, 70-26-8.

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