ORGANIC LETTERS

2003
Vol. 5, No. 8
1213−1215

Valanimycin Biosynthesis: Investigations of the Mechanism of Isobutylhydroxylamine Incorporation

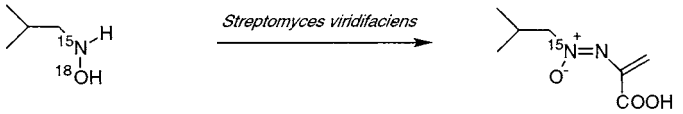
Tao Tao, Lawrence B. Alemany, and Ronald J. Parry*

Department of Chemistry MS65, Rice University, 6100 Main St., Houston, Texas 77005

parry@rice.edu

Received January 20, 2003

ABSTRACT



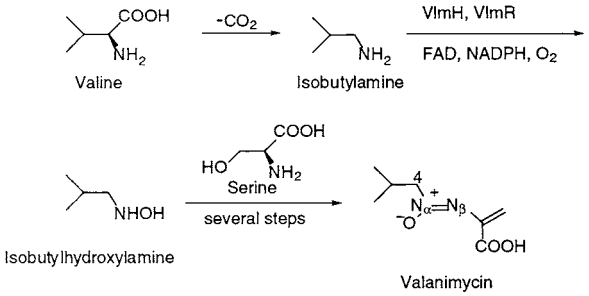
Valanimycin

The incorporation of [^{15}N, ^{18}O]-isobutylhydroxylamine into the antibiotic valanimycin by *Streptomyces viridifaciens* has been shown to proceed with loss of the ^{18}O label, thereby demonstrating that the azoxy oxygen atom of valanimycin is not derived from the oxygen atom of isobutylhydroxylamine.

The antibiotic valanimycin is a naturally occurring azoxy compound produced by *Streptomyces viridifaciens* MG456-hF10. In addition to antibacterial activity, valanimycin exhibits potent cytotoxic activity against in vitro cell cultures of mouse leukemia L1210, P388/S (doxorubicin-sensitive), and P388/ADR (doxorubicin-resistant).[1] Other naturally occurring azoxy compounds produced by bacteria include the carcinogen elaiomycin,[2−4] antifungal agents maniwamycins A and B,[5] nematocidal compounds jietacins A and B,[6] and the antifungal agent azoxybacilin.[7]

Relatively little is known about the biosynthesis of naturally occurring azoxy compounds. Investigations of elaiomycin biosynthesis revealed that its carbon and nitrogen skeleton are derived from *n*-octylamine, L-serine, and C-2 of acetate,[8,9] while studies of valanimycin showed it to be derived from L-serine and L-valine, with the latter amino acid being incorporated via the intermediacy of isobutylamine and isobutylhydroxylamine (Scheme 1).[10] Furthermore, the two nitrogen atoms of the valanimycin azoxy group were shown to be derived from the nitrogen atoms of serine and isobutylhydroxylamine,[10] and the conversion of isobutyl-

Scheme 1. Valanimycin Biosynthetic Pathway



(1) Yamato, M.; Iinuma, H.; Naganawa, H.; Yamagishi, Y.; Hamada, M.; Masuda, T.; Umezawa, H.; Abe, Y.; Hori, M. *J. Antibiot.* **1986**, *39*, 184−191.

(2) Stevens, C. L.; Gillis, B. T.; French, J. C.; Haskell, T. H. *J. Am. Chem. Soc.* **1956**, *78*, 3229−3230.

(3) Stevens, C. L.; Gillis, B. T.; French, J. C.; Haskell, T. H. *J. Am. Chem. Soc.* **1958**, *80*, 6088−6092.

(4) Stevens, C. L.; Gillis, B. T.; Haskell, T. H. *J. Am. Chem. Soc.* **1959**, *81*, 1435−1437.

(5) Takahashi, Y.; Nakayama, M.; Watanabe, I.; Deushi, T.; Ishiwata, H.; Shiratsuchi, M.; Otani, G. *J. Antibiot.* **1989**, *42*, 1541−1546.

(6) Imamura, N.; Kuga, H.; Otoguro, K.; Tanaka, H.; Omura, S. *J. Antibiot.* **1989**, *42*, 156−158.

(7) Fujiu, M.; Sawairi, S.; Shimada, H.; Takaya, H. Aoki, Y.; Okuda, T.; Yokose, K. *J. Antibiot.* **1994**, *47*, 833−835.

(8) Parry, R. J.; Rao, H. S. P.; Mueller, J. *J. Am. Chem. Soc.* **1982**, *104*, 339−340.

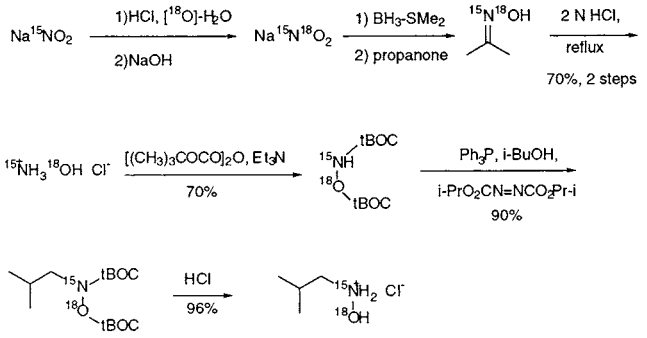
(9) Parry, R. J.; Mueller, J. V. *J. Am. Chem. Soc.* **1984**, *106*, 5764−5765.

10.1021/ol0340989 CCC: $25.00
Published on Web 03/19/2003

amine to isobutylhydroxylamine was found to be catalyzed by a two-component, flavin monooxygenase (Scheme 1).[11,12] In more recent studies, the valanimycin biosynthetic gene cluster has been cloned from *S. viridifaciens* and analyzed.[13] The analysis revealed a number of unusual features of valanimycin biosynthesis, but it has not yet clarified the mechanism of azoxy group formation. To limit the number of possible mechanisms for azoxy group formation and thereby assist future studies, we have chosen to investigate the fate of the oxygen atom of isobutylhydroxylamine during its conversion to valanimycin. The results of this investigation are described here.

The fate of the isobutylhydroxylamine oxygen was investigated by means of precursor incorporation experiments with [^{15}N, ^{18}O]-isobutylhydroxylamine. This doubly labeled precursor was synthesized as outlined in Scheme 2. [^{15}N, ^{18}O]-

Scheme 2. Synthesis of [^{15}N, ^{18}O]-Isobutylhydroxylamine Hydrochloride



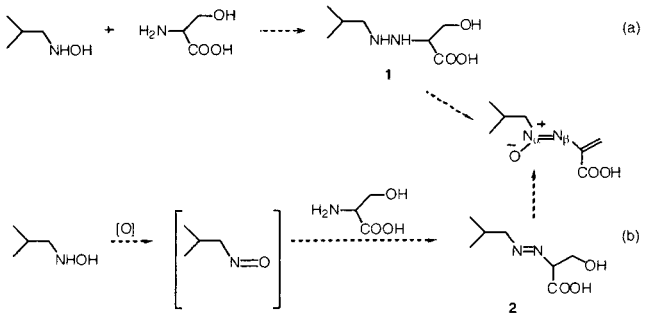
Hydroxylamine hydrochloride was synthesized from sodium [^{15}N]-nitrite (99 atom % ^{15}N) and [^{18}O]-water (95 atom % ^{18}O) by the method of Van Etten and Rajendran.[14] Analysis of the intermediate acetoxime by chemical ionization mass spectrometry (CIMS) indicated that the ratio of [^{15}N, ^{18}O]- to [^{15}N, ^{16}O]-labeled oxime was about 3:1. The doubly labeled hydroxylamine obtained from the oxime was converted into the corresponding *N*,*O*-di-*tert*-butoxycarbonyl derivative, whose ^{18}O content was determined by both CIMS and ^{15}N NMR spectroscopy. The intensities of the M + 1 peaks for the [^{15}N, ^{18}O] and [^{15}N, ^{16}O] species in the mass spectrum indicated that their ratio was about 3:1. The ^{15}N NMR spectrum ($CDCl_3$, 50.7 MHz, [^{15}N]-glycine external reference at 0 ppm) exhibited two ^{15}N signals, one at 127.02 ppm generated by the [^{15}N]-labeled compound and a second at 126.94 ppm, resulting from an ^{18}O-isotope-induced shift[15] on the ^{15}N resonance position in the doubly labeled hydroxylamine derivative. The intensities of the two ^{15}N signals were consistent with the mass spectral data and indicated that the ratio of [^{15}N, ^{18}O] to [^{15}N, ^{16}O] species was 3:1. The protected hydroxylamine derivative was next converted into the *N*,*O*-di-*tert*-butoxycarbonyl derivative of isobutylhydroxylamine by means of a Mitsunobu reaction.[16] The ratio of the [^{15}N, ^{18}O]- to [^{15}N, ^{16}O]-labeled forms of the protected isobutylhydroxylamine determined by CIMS and ^{15}N NMR was approximately the same as that of the parent hydroxylamine. Finally, acid-catalyzed removal of the protecting groups proceeded in high yield to produce the desired [^{15}N, ^{18}O]-isobutylhydroxylamine hydrochloride. An ^{18}O-induced isotope shift could not be observed in the ^{15}N NMR spectrum of the isobutylhydroxylamine hydrochloride due the broad nature of the ^{15}N resonance, but a CIMS analysis indicated that the ratio of [^{15}N, ^{18}O]- to [^{15}N, ^{16}O]-labeled isobutylhydroxylamine was about 2.8:1.

The doubly labeled isobutylhydroxylamine was administered to 1 L of a 17-h-old culture of *S. viridifaciens* concurrently with unlabeled L-alanine to increase the level of valanimycin production.[17] After an additional 19 h, valanimycin was isolated from the fermentation broth and converted into its ammonia adduct in the usual manner.[1] The ammonia adduct was purified by medium-pressure reverse-phase chromatography and the purified compound analyzed by CIMS and by ^{15}N and ^{13}C NMR spectroscopy. Mass spectral analysis indicated the presence of an approximately 1:1 mixture of [^{14}N]- and [^{15}N]-labeled species, with no evidence for the presence of [^{15}N, ^{18}O]-labeled species. The ^{15}N NMR spectrum (D_2O, 50.7 MHz, [^{15}N]-glycine reference), which was consistent with the CIMS spectrum, exhibited a sharp ^{15}N resonance at 314.49 ppm corresponding to the α-nitrogen atom of the azoxy group, with no evidence for an upfield, ^{18}O-shifted ^{15}N resonance signal. The presence of ^{15}N in the α-position of the valanimycin ammonia adduct was confirmed by the ^{13}C NMR spectrum (D_2O, 125 MHz) in which the C-4 resonance appeared as an overlapping singlet (79.627 ppm) and doublet (79.605 ppm, J_{CN} = 9.4 Hz[10]) whose relative intensities indicated approximately 50% ^{15}N enrichment. This level of enrichment is similar to that previously observed in precursor incorporation experiments with labeled forms of isobutylhydroxylamine.[10]

The results of the preceding experiments indicate that the oxygen atom of the valanimycin azoxy group is not derived from the hydroxyl group of isobutylhydroxylamine. At least two mechanisms for azoxy group formation can be envisioned that are compatible with this observation. One mechanism would proceed by condensation of isobutylhydroxylamine with serine to produce the hydrazine **1**, which could then be converted to valanimycin (Scheme 3a). Some evidence in favor of this mechanism has previously been obtained by precursor incorporation experiments with doubly labeled forms of **1**.[18] However, the incorporation levels observed with **1** were very low (<1%) compared to those

(10) Parry, R. J.; Li, Y.; Lii, F.-L. *J. Am. Chem. Soc*. **1992**, *114*, 10062−10064.
(11) Parry, R. J.; Li, W.; Cooper, H. N. *J. Bacteriol*. **1997**, *179*, 409−416.
(12) Parry, R. J.; Li, W. *J. Biol. Chem*. **1997**, *272*, 23303−23311.
(13) Garg, R. P.; Ma, Y.; Hoyt, J. C.; Parry, R. J. *Mol. Microbiol*. **2002**, *46*, 505−517.
(14) Rajendran, G.; Van Etten, R. L. *Inorg. Chem*. **1986**, *25*, 876−878.
(15) Rajendran, G.; Santini, R. E.; Van Etten, R. L. *J. Am. Chem. Soc*. **1987**, *109*, 4357−4362.
(16) Knight, D. W.; Leese, M. P. *Tetrahedron Lett*. **2001**, *42*, 2593−2595.
(17) Yamato, M.; Takeuchi, T.; Umezawa, H.; Sakata, N.; Hiyashi, H.; Hori, M. *J. Antibiot*. **1986**, *39*, 1263−1269.
(18) Parry, R. J.; Li, W. *Chem. Commun*. **1994**, 995−996.

Scheme 3. Hypothetical Valanimycin Biosynthetic Pathways



observed with other precursors,[10] suggesting that this compound may not be a true intermediate. A second compatible mechanism would proceed by oxidation of isobutylhydroxylamine to the corresponding nitroso derivative, which could condense with serine while still confined within an enzyme active site to produce the azo compound **2** (Scheme 3b). Additional investigations will be required to determine whether either of these two mechanisms actually plays a role in valanimycin biosynthesis.

Acknowledgment. We thank the General Medical Sciences Institute of the NIH (Grant GM53818) and the Robert A. Welch Foundation (Grant C-0729) for financial support of these investigations. We also thank Dr. T. Takeuchi for a culture of *S. viridifaciens*.

Supporting Information Available: Experimental procedures and spectroscopic data for compounds shown in Scheme 2 and for the isolation and characterization of the valanimycin ammonia adduct. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0340989