(17) The H_2O_2 was determined by use of the iron(II) phenanthroline method as described previously (see ref 15).
(18) Thermodynamically the two-electron reaction of **1** with O_2 (reaction a, Scheme I) is favored ($E^\circ_{O_2/H_2O_2} = 0.2$ V, $E^\circ_{O_2/HO_2} = -0.4$ V at pH 9) (see ref 20). However, we do not have experimental evidence confirming that the reaction of **1** with O_2 gives **4** directly. An alternate possibility involves formation of **3** and $HO_2\cdot$ (or $O_2^{-}\cdot$) and subsequent reaction of $HO_2\cdot$ ($O_2^{-}\cdot$) with another molecule of **1** to give **3** and H_2O_2.
(19) In independent experiments, oxidation of **1** by an equivalent quantity of H_2O_2 yielded only small quantities of **2** (~5% after 2 h). Similarly, after 1 h only negligible quantities (<1%) of **2** were obtained in a reaction of **3** with $HO_2\cdot$ (**3**/KO_2 = 1; pH 2). These results strongly suggest that step c in Scheme I is the dominant pathway for production of **2** in acidic solution.
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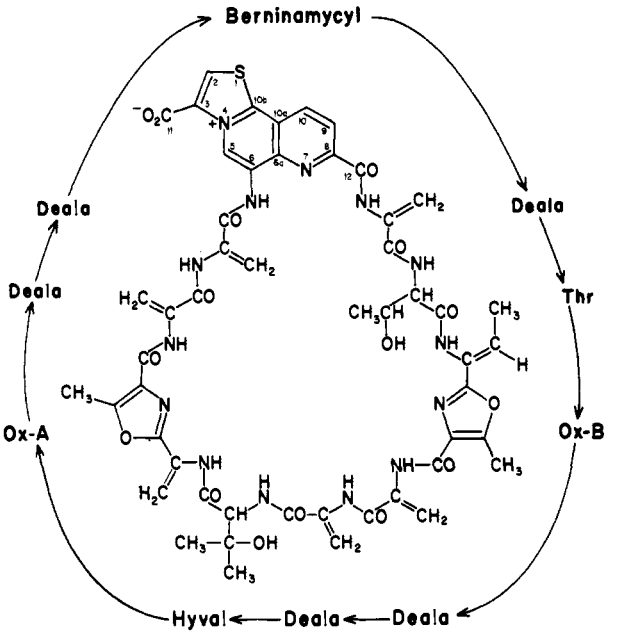
Berninamycin Biosynthesis. 1. Origin of the Dehydroalanine Residues

Sir:

Dehydro amino acid residues are a common feature of many microbial peptides. In particular, dehydroalanine occurs in a number of peptide antibiotics, inter alia, in nisin,[1] alternariolide,[2] subtilin,[3] siomycin,[4] thiostrepton,[5] nosiheptide,[6] and thiopeptin.[7] Dehydro amino acids have also frequently been implicated as biochemical reaction intermediates. It has been suggested, for example, that dehydrocysteine- and dehydrovaline-containing peptides, produced from dehydrogenation of the relevant precursors, could be intermediates of penicillin and cephalosporin biosynthesis.[8] In addition, enzyme-bound dehydroalanine has been proposed as an intermediate in the desulfuration of cysteine catalyzed by *S*-alkyl-L-cysteine lyase,[9] in the dehydration of serine by serine dehydrolase,[10] and in the metabolism of *O*-acetylserine.[11]

The biochemical origins of the peptide antibiotic dehydro amino acid residues have received little attention. There is some experimental evidence suggesting that the lanthionine and β-methyllanthionine residues found in nisin are derived, in part, from serine and threonine, respectively, and it was proposed that dehydro amino acids play a role as reaction intermediates.[12] In addition, it was suggested that the dehydroalanine and dehydrobutyrine residues present in nisin may also be produced by dehydration of the relevant precursors. Furthermore, Bycroft[13] has postulated that the biosynthesis of dehydroalanine residues found in peptide antibiotics results from either the dehydration of serine or the dehydrogenation of alanine, but until now no experimental evidence has provided a sensible choice between the two possibilities. The present communication describes results which demonstrate that dehydroalanine residues arise by dehydration of serine, at least in berninamycin A (**1**), a polypeptide antibiotic produced by



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Streptomyces bernensis[14] which has been shown to inhibit protein synthesis at the ribosome level.[15] The structure assigned in this laboratory[16-18] contains five dehydroalanine residues; in addition, other dehydro amino acids are involved in the oxazole A and B units and the berninamycinic acid residue contains a dehydrocysteine unit and yet another potential dehydroalanine unit.

Incubation of *S. bernensis* in the presence of DL-[1-^{14}C]- or L-[U-^{14}C]serine produced heavily labeled berninamycin (Table I), with incorporation from L-serine ~1.5 times that of DL-serine, demonstrating that the L isomer is preferentially utilized for berninamycin biosynthesis. On the other hand, similar experiments using DL-[1-^{14}C]alanine indicated only a very small incorporation of label from that precursor ($^1/_{100}$th the incorporation of L-serine) into the antibiotic, showing that alanine is not an effective precursor.

Samples of berninamycin produced by incubating *S. bernensis* with ^{14}C-labeled serine were degraded, as described previously, to berninamycinic acid[17] and pyruvic acid dinitrophenylhydrazone,[17] with dehydroalanine residues being isolated as the latter derivatives. The specific activities of the fragments isolated are reported in Table I.

Table I. Incorporations of ^{14}C-Labeled Amino Acids into Berninamycin and Subunits[a]

	DL-[1-^{14}C]serine added		L-[U-^{14}C]serine added		DL-[1-^{14}C]alanine added		L-[U-^{14}C]cysteine added	
	48 h	72 h	48 h	72 h	48 h	72 h	48 h	72 h
incorporation into berninamycin, %[b]	2.8	1.9	4.2	2.6	0.042	0.018	1.0	0.27
specific activity of berninamycin[c]	0.497	0.384	0.755	0.369	ND	ND	0.138	0.031
specific activity of pyruvic acid dinitrophenylhydrazone[c]	0.056	0.056	0.074	0.061	ND	ND	0.0012	0.0013
specific activity of berninamycinic acid[c]	0.151	0.129	0.159	0.110	ND	ND	0.128	0.039

[a] The labeled amino acids were added to 50 mL of *S. bernensis* cultures in 500-mL Erlenmeyer flasks. The culture medium contained glucose, 1%; L-glutamic acid, 0.2%; K_2HPO_4, 0.1%; $MgSO_4\cdot H_2O$, $FeSO_4\cdot 7H_2O$, $ZnSO_4\cdot 7H_2O$, $CaCl_2\cdot 2H_2O$, 0.0025%; and distilled water to 50 mL, with a final pH of 7.2-7.3. This was inoculated using 0.5 mL of a 24-h culture of *S. bernensis* grown in Pharmamedia, 2.5%; glucose, 2.5%; distilled water to 50 mL, with a final pH of 7.2. The cultures were incubated on a rotary shaker at 30 °C and 250 rpm for 96 h. Berninamycin was isolated by the method previously described.[14] Final purification was achieved by TLC over silica gel (CH_2Cl_2-CH_3OH, 12:1). [b] The incorporation values have been adjusted for berninamycin recovery. There was some variation in the exact percent incorporation observed in individual experiments, related to the yield of berninamycin. In a series of strictly parallel experiments, the ratio of incorporation/mole berninamycin for L/DL-serine was 1.80 for 48-h addition and 1.73 for 72-h addition. [c] Carrier berninamycin was added prior to degradation. Specific activities are expressed in terms of μCi/mmol; ND = not determined.

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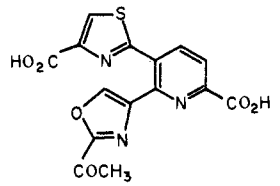
Less randomization of label was found when either the DL or L ^{14}C-labeled serine was added at 72 h, and in both of these 72-h runs the label in berninamycin was accounted for totally by the ^{14}C present in berninamycinic acid and the five pyruvic acid dinitrophenylhydrazone units isolated from each antibiotic molecule. When ^{14}C-labeled serine was added to the cultures at 48 h, somewhat less of the total label was associated with dehydroalanine and berninamycinic acid, probably owing to the metabolic conversion of serine into the other amino acid residues.

These results support the argument that dehydroalanine is synthesized via dehydration of serine rather than dehydrogenation of alanine. However, present results do not determine whether this occurs at the individual amino acid level or following incorporation into a peptide structure.

The specific activity of berninamycinic acid is approximately twice that of the dehydroalanine residues (pyruvic acid dinitrophenylhydrazone), suggesting that two serine residues are incorporated into this unit. One serine residue presumably labels C-5, C-6, and C-6a of berninamycinic acid, which would accord with the recent suggestion[19] that berninamycinic acid arises in sulfomycin from an oxazole precursor (**2**).[20] When



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L-[U-^{14}C]cysteine was incubated with *S. bernensis*, berninamycin was labeled, but, following degradation, almost no label was found in pyruvic acid dinitrophenylhydrazone (dehydroalanine), excluding the possibility that dehydroalanine is derived from cysteine. Essentially all of the label was in the berninamycinic acid residue, presumably in the thiazole (dehydrocysteine) portion (C-2, C-3, C-11). These results suggest that one of the serine units found in berninamycinic acid had been converted into cysteine and incorporated into berninamycin as described.

The observation that dehydroalanine is derived from dehydration of serine argues for a similar origin of other unsaturated residues in berninamycin as well as of the dehydroalanine residues in other antibiotics.[1-7] Thus, it can be postulated that oxazole A is biosynthesized from one threonine and one serine residue and oxazole B from two threonine residues. These possibilities are under investigation, as are the identities of the carbons labeled by serine and cysteine in berninamycinic acid.

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A New Bisannulation Reagent Easily Prepared from a Butadiene Telomer, and Its Application to Steroid Synthesis

Sir:

We introduce 1,7-octadien-3-one (**4**) as an easily available and very convenient bisannulation reagent. Annulation reaction to form fused six-membered cyclic ketones using alkyl vinyl ketones or their equivalents is well known as the Robinson annulation[1] and has wide application, particularly for stepwise construction of polycyclic compounds such as steroids and certain terpenoids.[2] One important offshoot of the Robinson procedure is the construction of two fused six-membered cyclic ketones from one reagent, instead of repeating the common Robinson cyclization twice. This new method of cyclization is called bisannulation, and few compounds have been recommended for this purpose. According to Danishefsy,[3] the bisannulation reagent is a synthetic equivalent to 7-octene-2,6-dione. In other words, the bisannulation reagent must have a terminal enone or its equivalent in one side and a masked ketone to generate a 1,5-diketone system in the other end of the molecule. As the masked ketone, Stork's isoxazole[4] and Danishefsky's 6-vinyl-2-picoline[3,5] are well known. The usefulness of the bisannulation reagent is determined by easy accessibility of the reagent itself and the facile procedure of unmasking. For example, the isoxazole ring is unmasked by hydrogenation and base-catalyzed hydrolysis. Birch reduction, followed by acid-catalyzed hydrolysis is the method of converting the picoline into 1,5-dione.

The new bisannulation reagent, 1,7-octadien-3-one, that we now introduce, is very easily prepared in high yield from butadiene. After initial Michael reaction at the enone moiety, the terminal double bond is converted into the desired methyl ketone in one step by palladium-catalyzed oxidation in high yield under mild conditions as shown below.[6] Thus, this compound is the most cheaply and easily available, convenient bisannulation reagent.

We are actively working on synthetic uses of butadiene telomers[7] easily prepared in one step by the palladium-catalyzed reactions of butadiene with various nucleophiles.[8] Reaction of butadiene with acetic acid catalyzed by $Pd(OAc)_2$ and PPh_3 affords 3-acetoxy-1,7-octadiene (**1**) and 1-acetoxy-2,7-octadiene (**2**) in high yield.[9] The acetate **2** can be rearranged to **1** by the palladium catalyst. The acetate **1** was hydrolyzed to the alcohol **3**, which was dehydrogenated by gas-phase reaction catalyzed by Cu/Zn alloy[10] at 360 °C to give the desired enone **4** in high yield (Scheme I): bp 31 °C (4 mmHg), semicarbazide mp 180-182 °C.

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