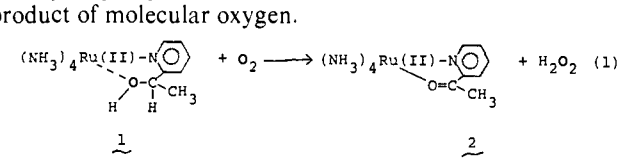
(4) All new compounds were characterized by complete (NMR, IR, mass spectrum (electron and/or chemical ionization)) spectroscopic analysis. Yields refer to isolated and chromatographically purified products.
(5) Aplysistatin numbering (see 1) is used throughout the text.
(6) Rathke, M. W.; Lindert, A. *J. Am. Chem. Soc.* **1971**, *93*, 2318. Sharpless, K. B.; Lauer, R. F.; Teranishi, A. Y. *Ibid.* **1973**, *95*, 6137.
(7) Cornforth, J. W.; Cornforth, R. H.; Mathew, K. K. *J. Chem. Soc.* **1959**, 2539.
(8) Rigby, W. *J. Chem. Soc.* **1950**, 1907.
(9) (a) House, H. O.; Crumrine, D. S.; Teranishi, A. Y.; Olmstead, H. D. *J. Am. Chem. Soc.* **1973**, *95*, 3310. Auerbach, R. A.; Crumrine, D. S.; Ellison, D. L.; House, H. O. *Org. Synth.* **54**, 49. It is interesting to note that enolates of the analogous α-alkoxy[9b,c] and α-dialkylamino[9c] esters and α-aryloxy-[9d] and α-phenylthio[9e] carboxylates do add efficiently to aldehydes and ketones in the absence of strongly chelating metals: (b) Glass, R. S.; Deardorff, D. R. Abstracts of Papers, 175th National Meeting of the American Chemical Society, Anaheim, Calif., March 1978; American Chemical Society: Washington, D.C., 1978; ORGN 81. (c) Touzin, A. M. *Tetrahedron Lett.* **1975**, 1477. (d) Adam, W.; Fick, H.-H. *J. Org. Chem.* **1978**, *43*, 772, 4574. (e) Kosugi, H.; Uda, H.; Yamagiwa, S. *J. Chem. Soc., Chem. Commun.* **1975**, 192.
(10) The stereochemical assignments for the two diastereomers of **9** (and therefore for **10** vs. **12**, **11** vs. **13**, **19**, **20**, and **21**) are not yet firm. Experiments are in progress to establish them conclusively and will be reported soon.
(11) Yajima, H.; Kawasaki, K.; Kinomura, Y.; Oshima, T.; Kimoto, S.; Okamoto, M. *Chem. Pharm. Bull.* **1968**, *16*, 1342.
(12) Barton, D. H. R.; Magnus, P. D.; Smith, G.; Streckert, G.; Zurr, D. *J. Chem. Soc., Perkin Trans 1* **1972**, 542.
(13) We are currently in the process of assessing the synthetic generality of this type of cyclization reaction.
(14) The racemic synthetic material (mp 179–181 °C from hexanes–acetone (lit.[1] mp 173–175 °C)) gave identical NMR and mass spectra and a nearly identical IR (KBr) spectrum with those of a natural sample kindly provided by Professor Pettit.
(15) A second, more or less independent synthetic approach to aplysistatin (**1**) which focuses upon the formation of the C_{12}–O^5 ether linkage to complete the oxepane construction has led to a synthesis of 3-norbromo-4,4-nordimethylaplysistatin.[16] This work is currently being extended to aplysistatin itself and the details will be reported in due course.
(16) Experimental work of A. J. Caruso.
(17) This crystalline (mp 138–140 °C) epimer gave a satisfactory combustion analysis for C, H, Br.

Thomas R. Hoye,* Mark J. Kurth
Department of Chemistry, University of Minnesota
Minneapolis, Minnesota 55455
Received March 21, 1979

Oxidation of Ruthenium Coordinated Alcohols by Molecular Oxygen to Ketones and Hydrogen Peroxide

Sir:

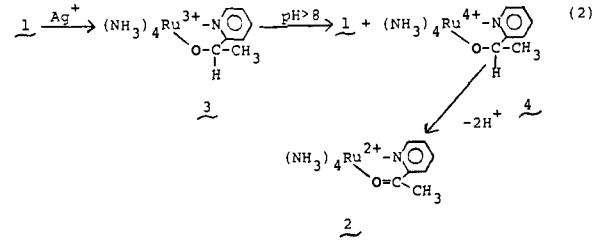
Selective oxidations of organic substrates mediated by metal complexes which would allow the formation of H_2O_2 instead of water (the usual final product of oxygen reduction) would be of great importance. This concept is demonstrated in the work described herein using ruthenium(II) complexes. Previously, ruthenium(II) complexes have been utilized to facilitate oxidation of coordinated imines and primary or secondary amines by molecular oxygen.[1-6] However, the mechanism of these oxidations and the fate of the molecular oxygen have only been speculated upon. We have observed that Ru(II)–alcohol complexes can be oxidized by O_2 to H_2O_2 and the corresponding Ru(IV)–alcohol complexes which then undergo metal-ligand redox leading to a Ru(II)–ketone complex (eq 1). Hydrogen peroxide has been identified as a reduction product of molecular oxygen.



1 2

Our initial study has centered on the oxidation of 2-(1′-hydroxyethyl)pyridine coordinated to Ru(II) as a bidentate ligand (eq 1). The complex **1** is prepared by substitution of the aquo ligands in *cis*-$(NH_3)_4Ru^{II}(OH_2)_2$ by 2-(1′-hydroxyethyl)pyridine in deoxygenated aqueous solution at 25 °C and pH 5. In analogy to other pyridine–Ru(II) complexes the absorption of **1** at 4100 Å (ϵ 3900) confirms coordination of the pyridine ring. Coordination of the alcohol group can be verified by the observed chemical shift in the ^{1}H NMR from δ_{CH_3} 1.51 in free 2-(1′-hydroxyethyl)pyridine to δ_{CH_3} 1.54 in $(NH_3)_5Ru^{II}$[2-(1′-hydroxyethyl)pyridine][7] where only the pyridine nitrogen is coordinated, to δ_{CH_3} 1.63 in **1**. The oxidation of the hydroxyethyl group in **1** to the acetyl group in **2** has been achieved via the Ru(IV) analogue of **1** which was generated by two independent routes as discussed below.

Disproportionation of Ru(III) Complex. Rudd and Taube have shown that $(NH_3)_5Ru^{III}py$ ($E°_{Ru(III)/Ru(II)} = +0.30$ V) disproportionates in solutions of pH >8 to $(NH_3)_5Ru^{II}py$ and the Ru(IV) analogue.[8] To determine if Ru(IV) could function as an oxidant, we performed an analogous disproportionation reaction with **3** (eq 2). Complex **3** was prepared in deoxygen-



ated acidic solution by oxidation of **1** with AgTFA. The pale yellow solution obtained after filtration of Ag^0 is stable indefinitely toward conversion into **2.** Upon raising the pH above 8, **3** disproportionates to **1** and **4.** The deep blue color characteristic of **2** (λ_{max} 6220 Å)[6] forms slowly, reaching a value of 72% of theoretical after 24 h at pH 11. Concurrently, formation of **1** can be verified by its absorption at 4100 Å. Similarly, the NMR of the reaction solution shows the presence of **1** and **2** in roughly similar amounts as observed in the visible spectra.[9]

The chemical shift in **2**,[10] δ_{CH_3} 2.92, relative to that in free 2-acetylpyridine, δ_{CH_3} 2.72, reveals that the ketone group in **2** is coordinated.

The disproportionation of $(NH_3)_5Ru^{III}py$ can be reversed by lowering the pH.[8] However, disproportionation of **3** can only be partially reversed owing to the formation of **2** from **4** produced in the disproportionation. For example, in a solution of **3**, [**3**] = 3.08×10^{-2} M at pH 10, 30% **2** is produced after 1 h. Upon lowering the pH at this time, the visible spectrum due to **2** is unchanged and the presence of **1** is apparent. These facts demand that **3** is only partially recovered and indicate that **4** has undergone an irreversible reaction, the ligand oxidation indicated in eq 2. Thus it has been demonstrated that Ru(IV) can be efficiently generated by Ru(III) disproportionation and that Ru(IV) can function as an oxidant for coordinated alcohols.

Reaction of 1 with O_2. The product profile obtained in reactions of **1** with O_2 is pH dependent (Table I). At pH 1 (expt 1) 5% **2** is produced in 2 h, an amount which does not increase with time. The other product of the reaction is **3**,[11] which is formed in analogy to the reaction of many other ammine–Ru(II) complexes with O_2.[12]

Reaction of **1** with O_2 at pH >7, however, is much different. For example, at pH 10, in an initial rapid reaction **1** is converted into **3** or **4.** This result alone is in contrast to the reactivity of the similar complex, $(NH_3)_5Ru^{II}py$, which is completely oxidized to its Ru(III) analogue in ~30 min[13] at pH 1 but only extremely slowly (hours) at pH 10. We attribute this difference to deprotonation of the hydroxy group in **3** or **4** leading to formation of Ru(III) or Ru(IV) alkoxide. This alkoxide formation should stabilize these higher oxidation states and lower the $E°_{Ru(III)/Ru(II)}$ of **3** below that of $(NH_3)_5Ru^{III}py$ ($E°_{Ru(III)/Ru(II)} = 0.30$ V).[14] A lower $E°$ should facilitate a faster reaction of **1** with O_2.[15] A similar

0002-7863/79/1501-5067$01.00/0

Table I. Reaction of $1 + O_2 \rightarrow 2$[a]

expt	[1] × 10^{-2} M	pH	t, h	% 2 (based on 1)[b]
1	1.02	1.0	1	5
	1.02	1.0	20	5
2	1.02	4.0	20	5
3	3.07	7.0	20	6
4	1.03	7.0	1	4
	1.03	7.0	2	5
	1.03	7.0	20	6
5	0.77	9.0	20	48
6	1.03	9.0	0.25	20
	1.03	9.0	2.5	39
	1.03	9.0	20	50
7	1.53	9.0	20	61
8	3.07	9.0	20	66
9	1.53	10.0	1.5	34
			20	70

[a] Reaction was run at 25 °C in H_2O using 1 atm of O_2. [b] Determined by monitoring λ = 6220 Å. Since **2** is slowly oxidized by O_2, visible spectra samples were prepared by acidification with deoxygenated 0.01 M HTFA and treatment with zinc amalgam for 30 min.

deprotonation of a hydroxy ligand upon oxidation of $[(bpy)_2pyRuOH]^{2+}$ to $[(bpy)_2pyRu{=}O]^{2+}$ serves dramatically to lower the Ru(IV)/Ru(III) potential in this complex (+0.99 V vs. SCE) as compared with a similar complex which does not possess ligands which can deprotonate, e.g., $[(bpy)_2RuCl_2]^{2+}$, whose Ru(IV)/Ru(III) potential is +1.98 V vs. SCE.[16]

At the time **1** is oxidized by O_2 to **3** or **4**, 44% H_2O_2 based on **1** can be detected.[17] Subsequently, **2** is formed slowly. The amount of **2** increases with higher pH (expt 4-9), concentration of **1** (expt 5-8), and time (expt 4, 6, 9). The dramatic difference between the results obtained at low and high pH can be understood in terms of Scheme I.

In acidic solution, reaction of **1** with O_2 forms **4** and H_2O_2 (step a).[18] Complex **4** can then react in one of two ways: (1) with a molecule of **1** to form two molecules of **3** (step b), or (2) internal alcohol oxidation to form **2** (step c).[19]

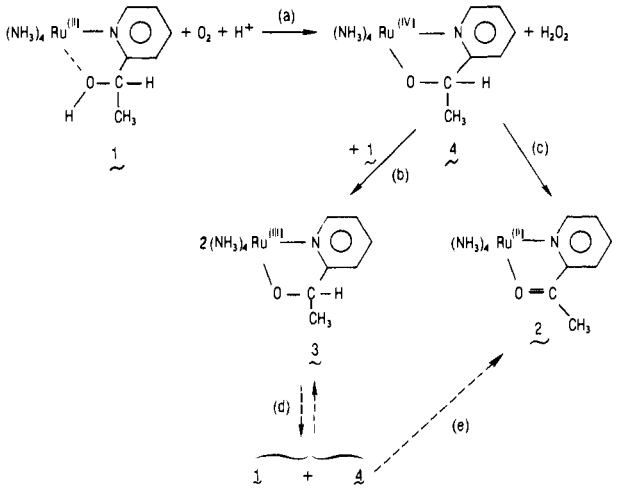
In acidic solution, **3** is the predominant product (Table I) and is stable under these conditions. However, in basic solution, **3** disproportionates to give **1** and **4** (step d). Complex **1** can react with O_2 (step a) and reenter the cycle. Complex **4** can undergo internal redox reaction to give **2** (step e). Thus, in basic solution, **3** can be recycled because of disproportionation which facilitates formation of **2** in high yield.

Scheme I is supported by several facts. The dramatic effect of pH on the yield of **2** (Table I) reflects the fact that disproportionation (step d) of **3** to **4** (precursor to **2**) is pH dependent.[8] Furthermore, the observed increase in the yield of **2** with the initial concentration of **1** and the observed second-order kinetics for disproportionation of similar Ru(III) complexes[8] is again consistent with this scheme. This work supports the suggestion of Diamond et al.[1] concerning the intermediacy of Ru(IV) in the oxidation of coordinated primary and secondary amines. A similar reaction scheme can be invoked to explain the observations of Guengerich et al.[5] on the conversion of Ru(III)-imine complexes into Ru(II)-nitrile complexes in the presence and absence of O_2.

These results clearly demonstrate that, when a two-electron oxidative pathway is provided (i.e., Ru(IV) → Ru(II)), the alcohol oxidation proceeds smoothly. On the other hand, the inability of Ru(III) complexes to form **2** suggests that a one-electron path is not feasible. The chemistry of the copper center in galactose oxidase as discussed by Hamilton[20] exhibits many features similar to those of this ruthenium system. In galactose oxidase a high-valent species, Cu(III) (compare with Ru(IV) complex, **4**), performs a two-electron oxidation of a primary alcohol, giving the corresponding aldehyde (compare with 2-acetylpyridine in Scheme I) and Cu(I) (compare with Ru(II) complex, **1**). A two-electron reduction of O_2 regenerates Cu(III) with the concomitant formation of H_2O_2 (compare step a in Scheme I). In both systems intermediate oxidation state complexes, i.e., Cu(II) in galactose oxidase or Ru(III) in **3**, are inactive for reaction with O_2 or alcohol. Although $E°$ values for complexes **1**, **3**, or **4** are not known, the reactivities discussed in this work suggest that $E°_{4/1} \geq E°_{3/1}$ and $E°_{3/1} \leq 0.30$ V. These estimates roughly coincide with the copper counterparts in the galactose oxidase system.[20]

It is our intention to apply the principals discussed in this work to the generation of H_2O_2 and potent two-electron, specific oxidants from other metal complexes and O_2.

Scheme I



[a] Solid lines refer to reactions taking place at low pH; broken lines represent additional reactions taking place at high pH.

Acknowledgment. The authors thank Mr. Peter Kudyba for his technical assistance.

References and Notes

(1) S. E. Diamond, G. M. Tom, and H. Taube, *J. Am. Chem. Soc.*, **97**, 2661 (1975).
(2) B. C. Lane, J. E. Lester, and F. Basolo, *Chem. Commun.*, 1616 (1971); D. F. Mahoney and J. K. Beattie, *Inorg. Chem.*, **12**, 2561 (1973).
(3) W. R. McWhinnie, J. D. Miller, J. B. Watts, and D. Y. Wadden, *Chem. Commun.*, 629 (1971); W. R. McWhinnie, J. D. Miller, J. B. Watts, and D. Y. Wadden, *Inorg. Chim. Acta*, 7, 461 (1973).
(4) F. R. Keene, D. J. Salmon, and T. J. Meyer, *J. Am. Chem. Soc.*, **98**, 1884 (1976).
(5) C. P. Guengerich and K. Schug, *J. Am. Chem. Soc.*, **99**, 3298 (1977).
(6) V. E. Alvarez, R. J. Allen, T. Matsubara, and P. Ford, *J. Am. Chem. Soc.*, **96**, 7686 (1974).
(7) Formed in reaction of $(NH_3)_5Ru^{2+}$–OH_2 and 2-(1′-hydroxyethyl)pyridine at pH 5. In acidic solution the alcohol group rapidly displaces an NH_3 forming complex **1**.
(8) DeF. P. Rudd and H. Taube, *Inorg. Chem.*, **10**, 1543 (1971).
(9) Upon admission of O_2 to solutions of **1**, all resonances in the NMR become very broad. This is likely due to the formation of paramagnetic Ru(III) and Ru(IV) (one and two unpaired electrons, respectively) which serve to broaden the resonances of diamagnetic species such as **1** and **2**. For this reason, quantitative integration of the appearance of **2** is not possible.
(10) Prepared as described in ref 8.
(11) Although **3** does not have any distinctive visible characteristics, its presence is verified as follows. After a reaction for 2 h at pH 1 with O_2, the solution is degassed and the pH raised to 11. The rate and amount of **2** formed (correcting for 5% **2** formed in reaction with O_2) is identical with that for disproportionation of **3**.
(12) P. Ford, DeF. P. Rudd, G. Gaunder, and H. Taube, *J. Am. Chem. Soc.*, **90**, 1187 (1968).
(13) In this reaction, roughly 65% of the theoretical amount of H_2O_2 is found.
(14) H. S. Lim, D. J. Barclay, and F. C. Anson, *Inorg. Chem.*, 11, 1460 (1972).
(15) For example, $(NH_3)_6Ru^{2+}$ ($E°_{Ru(III)/Ru(II)} = 0.05$ V) reacts in seconds with O_2 to produce Ru(III) and $\frac{1}{2}H_2O_2$: J. R. Pladiewicz, T. J. Meyer, J. A. Broomhead, and H. Taube, *Inorg. Chem.*, **12**, 639 (1973). These workers were the first to suggest formation of Ru(IV) from reaction of Ru(II) with O_2.
(16) B. A. Moyer and T. J. Meyer, *J. Am. Chem. Soc.*, *100*, 3601 (1978).

(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.
(20) G. A. Hamilton, P. K. Adolf, J. deJessey, G. C. DuBois, G. R. Dyrkacz, and R. D. Libby, *J. Am. Chem. Soc.*, **100**, 1899 (1978).

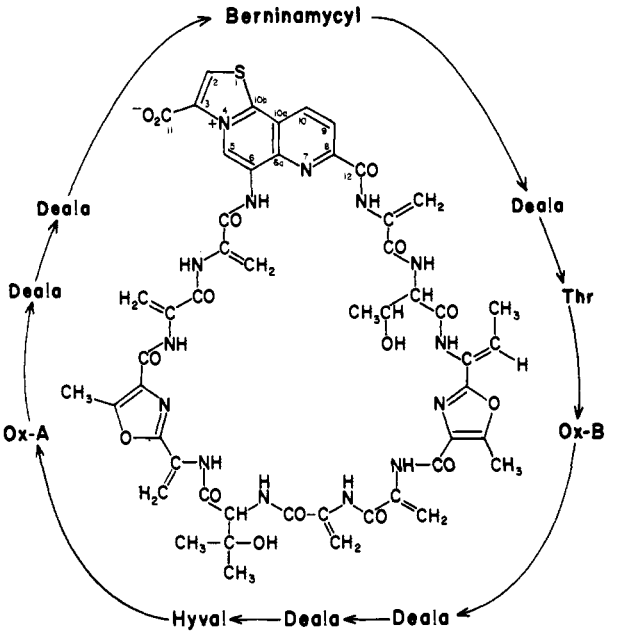
Benjamin S. Tovrog,* Steven E. Diamond,* Frank Mares*
Allied Chemical Corporation, Corporate Research Center
Morristown, New Jersey 07960
Received April 9, 1979

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



1

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.

0002-7863/79/1501-5069$01.00/0