(32) The NMR spectrum of synthetic **1** was identical with the spectrum of an authentic (natural) sample. We thank Dr. W. J. McGahren for the comparison spectrum.
(33) More involved explanations are possible. For example, epimerization might have occurred at the distal α-carbon during the conversion of **10** to its proximal α-carbanion with $(i\text{-Pr})_2\text{NLi}$. However, we have shown that similar reactions with optically active 2-octyl-*NNO*-azoxymethane *do not* result in significant racemization.[15] Moreover, as pointed out by a referee, epimerization at the distal α-carbon (epimerization at the hydroxyl-bearing, distal β-carbon is unlikey) would afford a mixture of diastereomers. If such epimerization occurred at the most sensitive step (**10**→**11** requires the most strongly basic conditions, see above), then a mixture of (*S,S*)-**11** and (2*S*,3*R*)-**11** would have been generated. We feel that it is unlikely that the (2*S*,3*R*) diastereomer would have survived the repetitive TLC purifications applied to **12**, **13**, and synthetic **1**.
(34) This report is Alkane Diazotates, 24; for part 23, see ref 16.
(35) Fellow of the A. P. Sloan Foundation.
(36) Postdoctoral Fellow on leave from Sumitomo Chemical Co.

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Berninamycin. 3. Total Structure of Berninamycin A[1,2]

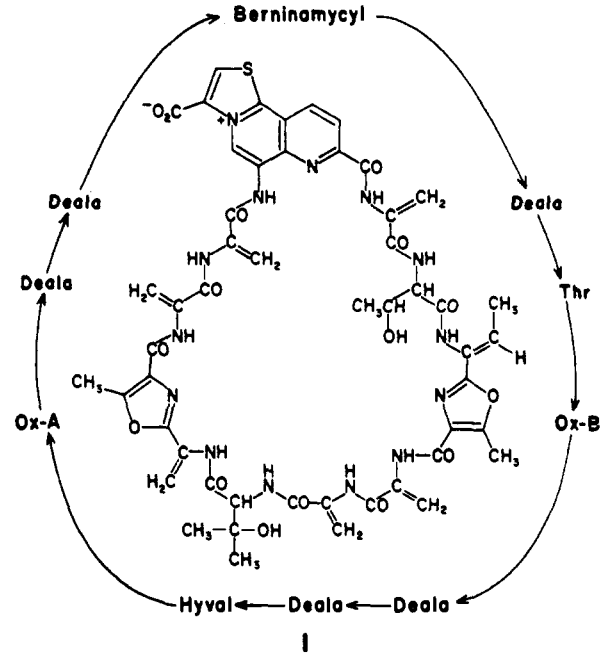
Sir:

In earlier reports[1-3] from this laboratory we have described the results of initial structural studies on the novel, sulfur-containing antibiotic berninamycin A, which is a potent inhibitor of bacterial protein synthesis. Degradation products obtained from acidic hydrolysis, methanolysis, and acetolysis of berninamycin A allowed the assignment of the structural subunits shown in the top row of Figure 1,[2] which account for the total composition of the antibiotic. In the present communication, we assign the total structure of berninamycin A as **1**, based upon new compounds obtained by trifluoroacetolysis of the intact antibiotic and its sodium borohydride-reduced and catalytically hydrogenated derivatives.

Treatment of berninamycin A with trifluoroacetic acid at room temperature for 18 h afforded three major compounds (Figure 2). The least polar compound was identified as the previously reported **2**.[2] A second compound (mp 109–110 °C; $C_{15}H_{20}N_4O_6$)[4a] was assigned structure **3**. As previously discussed,[2] the residues (Deala, Thr, Hyval, Ox-A, Ox-B, Berninamycyl) which comprise berninamycin A have unique ^{1}H NMR resonances which allow their identification in degradation products formed from the intact antibiotic. The ^{1}H NMR spectrum of **3** contains the resonances assignable[2] to the Hyval (1.40 ppm, s, 3 H; 1.50, s, 3 H; 5.49, d, 7 Hz, 1 H) and Ox-A (2.63, s, 3 H; 2.04, s, 3 H) residues and to a pyruvyl unit (2.42 ppm, s, 3 H).

The pyruvyl residue (which results from cleavage of a Deala residue)[2] can only occupy the N-terminal position, and a structure including the sequence Ox-A→Hyval is eliminated by subunit a of Figure 1. Thus, the expected structure for the second trifluoroacetolysis product would be pyruvyl→Hyval→Ox-A→NH_2 (**4**), a structural isomer of **3**. The 1,3-tetrahydrooxazine ring of **3** results from intramolecular addition of the hydroxyl group of Hyval to the enamine of Ox-A in **4** during trifluoroacetolysis. Combination of the sequence of **4** with subunit a allows the assignment of c (Figure 1) as a sequence in the intact antibiotic.

The most polar compound from trifluoroacetolysis of **1** is assigned structure **5** (mp 153 °C dec; $C_{27}H_{26}N_8O_8S$).[4a] The ^{1}H NMR spectrum of **5** has resonances assignable[2] to Thr, Ox-B, Deala, and Berninamycyl (Figure 1). These residues,



1

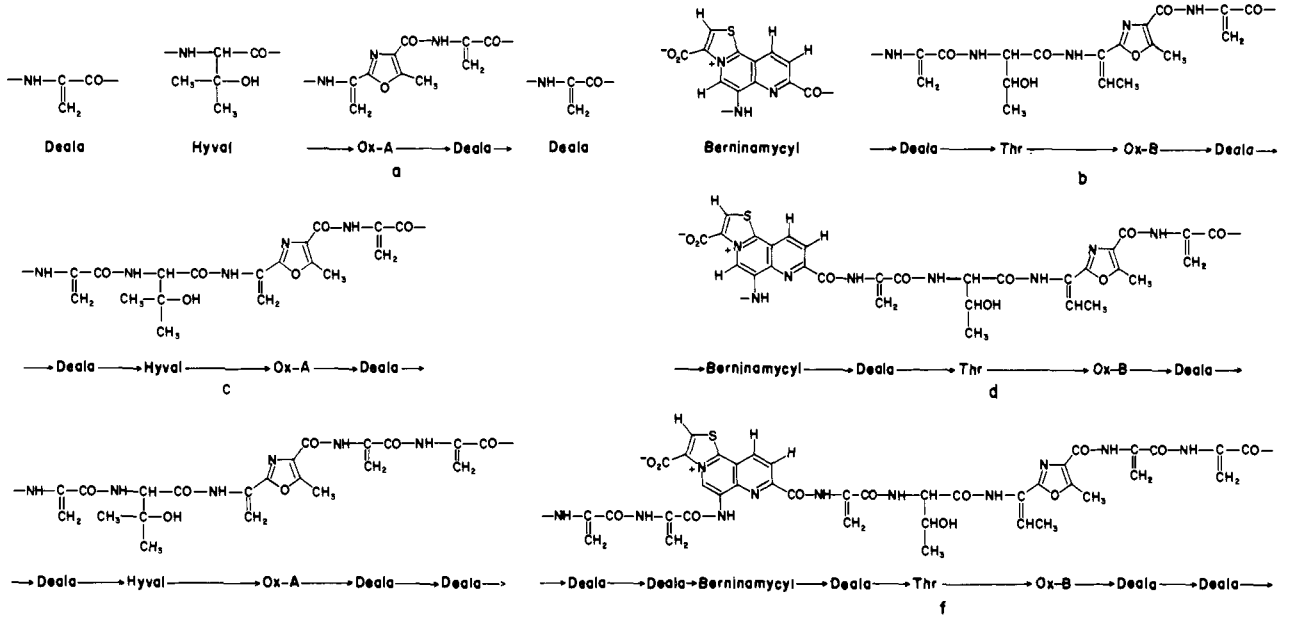


Figure 1. Subunit sequences found in berninamycin A. Subunits shown in the top line were established earlier.[2]

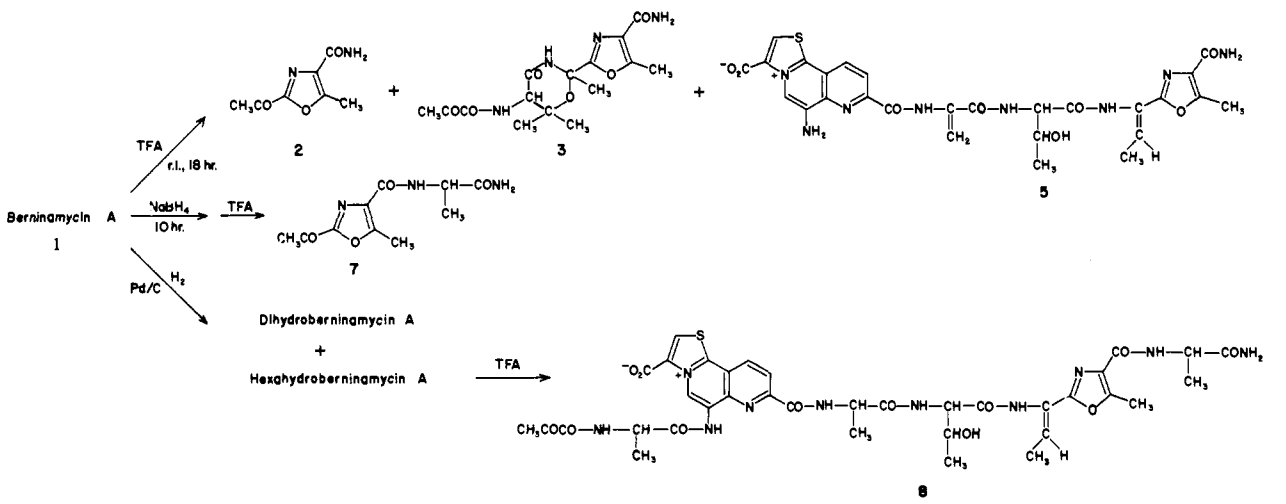


Figure 2. Products obtained by trifluoroacetolysis of berninamycin A and its reduction products.

joined by peptide bonds, account for the formula $C_{27}H_{23}N_7O_8S$; adding NH_2 for a C-terminal primary carboxamide (in keeping with previous results) and H for an N-terminal primary amine gives the empirical formula obtained by HRMS ($C_{27}H_{26}N_8O_8S$). Since sequence b (Figure 1) was previously assigned, there are only two possible structures for the trifluoroacetolysis product, Berninamycyl→Deala→Thr→Ox-B→NH_2 (**5**) and Thr→Ox-B→Deala→Berninamycyl→NH_2 (**6**). Structure **6** can be ruled out, since its primary aliphatic amino group should afford a strong positive ninhydrin test and should have a pK_a near 10 (cf. threonine, pK_a 10.43), whereas the trifluoroacetolysis product **5** gives only a weak color reaction with ninhydrin and has no pK_a above 2. Structure **5** is also in accord with mass spectral peaks at *m/e* 422 and 341, representing sequential losses of Ox-B-NH_2 and Thr via CO–NH cleavage. The structure of **5** indicates that d (Figure 1) is a sequence in the intact antibiotic.

In an attempt to obtain larger degradation fragments, a sample of berninamycin A was reduced with sodium borohydride for 10 h in order to convert some of the dehydroalanine residues to saturated alanine residues, which would not be expected to cleave under the trifluoroacetolysis conditions. Trifluoroacetolysis yielded one major compound (**7**; mp 151–153 °C; $C_{10}H_{13}N_3O_4$),[4a] the amide of a compound previously isolated[2] as the 2,4-dinitrophenylhydrazone of its corresponding methyl ester. Compound **7** extends subunit c to e in Figure 1. Subunits d and e would, in fact, account for the entire berninamycin structure if the C-terminal Deala residue of d were different from the N-terminal Deala residue of e.

In an attempt to settle that point by obtaining still larger degradation fragments, a sample of berninamycin A was catalytically hydrogenated over palladium-on-charcoal to give two products, identified as dihydroberninamycin A (mp 268–272 °C dec; $C_{51}H_{52}N_{14}O_{16}S$)[4b] and hexahydroberninamycin A (mp 275–280 °C; $C_{51}H_{56}N_{14}O_{16}S$).[4b] Hydrolysis of samples of each followed by quantitative amino acid analysis[2] verified that the former contained 1 (new) equiv of alanine and the latter three. Treatment of hexahydroberninamycin A with trifluoroacetic acid afforded **8** (mp 178–182 °C dec; $C_{36}H_{40}N_{10}O_{12}S$).[4b,c] The ^1H NMR spectrum of **8** indicated three alanyl (Ala) units plus Thr, Ox-B, Berninamycyl, and pyruvyl residues. Joining these residues by peptide bonds and assuming a C-terminal primary carboxamide (as in other trifluoroacetolysis products) accounts for the observed formula, $C_{36}H_{40}N_{10}O_{12}S$.

In light of known sequence d (Figure 1) only three structures are possible: pyruvyl→Ala→Berninamycyl→Ala→Thr→Ox-B→Ala→NH_2 (**8**), pyruvyl→Ala→Ala→Berninamycyl→Ala→Thr→Ox-B→NH_2 (**9**), and pyruvyl→Berninamycyl→Ala→Thr→Ox-B→Ala→Ala→NH_2 (**10**). Structure **10** would be derived from the sequence →Deala→Berninamycyl→Deala→Thr→Ox-B→Deala→Deala→Deala→ and can be eliminated, since that sequence contains three adjacent C-terminal Deala residues which, when overlapped with e and its two adjacent C-terminal Deala residues, would require a total of six Deala residues in berninamycin A instead of the five observed.[2]

To differentiate between structures **8** and **9** a sample of the trifluoroacetolysis product was treated with methanolic hydrogen chloride. The ^1H NMR spectrum of the major product ($C_{34}H_{36}N_8O_{12}S$),[4c] obtained in low yield as an oil, indicated[2] that it was a methyl ester and retained the N-terminal pyruvyl group but contained only 2 equiv of alanine. Since it is impossible to lose a C-terminal alanine from **9**, the structure of the trifluoroacetolysis product is **8**, allowing the assignment of subunit f in Figure 1.

Subunit f accounts for all the residues in berninamycin A except for Hyval and Ox-A, which are known to be arranged Hyval→Ox-A (as in c and e). To complete the structure of the antibiotic the C-terminal group of Hyval→Ox-A must be attached to the N-terminal group of f and the C-terminal group of f to the N-terminal group of Hyval→Ox-A, yielding structure **1**, in which the terminal Deala units of e and f overlap.

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References and Notes

(1) (a) Presented in part at the 170th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1975; cf. Abstract No. ORGN 20. (b) Taken in part from the Ph.D. Thesis of J. M. Liesch, University of Illinois, Urbana, 1975.
(2) Paper 2: J. M. Liesch, D. S. Millington, R. C. Pandey, F. Reusser, and K. L. Rinehart, Jr., *J. Am. Chem. Soc.*, **98**, 8237–8249 (1976).
(3) J. M. Liesch, J. A. McMillan, R. C. Pandey, I. C. Paul, K. L. Rinehart, Jr., and F. Reusser, *ibid.*, **98**, 299–300 (1976).
(4) In accord with the formula indicated were: (a) high resolution mass spectra; (b) microanalyses; (c) field desorption mass spectrometry.
(5) University of Illinois Fellow, 1971–1973; Mobil Foundation Fellow, 1973–1974; Uniroyal Fellow, 1974–1975.

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