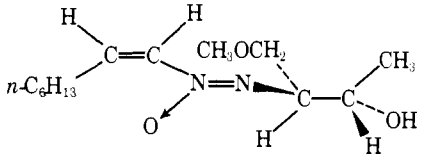
Communications to the Editor

The Synthesis of Elaiomycin, a Naturally Occurring Azoxyalkene

Sir:

Two of the five naturally occurring azoxy compounds, macrozamin[1] and cycasin,[2] are glycosides of "methylazoxymethanol", which has been prepared (as the acetate) from azoxymethane.[3] However, syntheses of the mutually related, more complicated, proximal[4] α,β-(*cis*)-unsaturated azoxyalkenes, elaiomycin (**1**)[5] and LL-BH872α (**2**),[6] require a general synthesis of azoxyalkanes and specific methods for the configurationally controlled introduction of unsaturation.[7] Despite a recent quickening of interest in azoxyalkanes,[8,9] routes to **1** or **2** have remained obscure, although the biological significance of **1**[10] (an antibiotic and a carcinogen) and **2**[6a] (an antifungal agent) makes syntheses highly desirable.[11]



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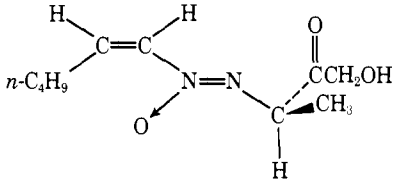

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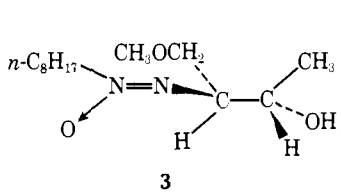
We are therefore pleased to report the total synthesis of **1** from D-threonine by a three-phase synthetic approach: (A) construction of the distal moiety of **1**, including the azoxy function; (B) elaboration of a proximal *trans*-octenyl group; (C) isomerization to the *cis*-octenyl group. This approach was based on key synthetic methods discovered in our laboratory.[12-16] A detailed description follows.



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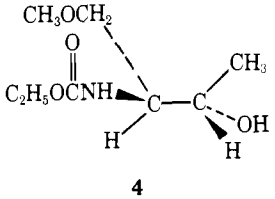

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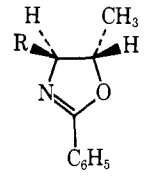
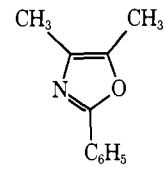
(A) The distal group.[12-14] As in the synthesis of dihydroelaiomycin, **3**,[14] D-threonine was converted to pivotal urethane **4** by extension of Stevens' method for synthesis of the corresponding amine. The hydrochloride salt of D-threonine ethyl ester was reacted with ethyl benzimidate to yield oxazoline **5** (52%). Reduction of **5** ($LiAlH_4$, 94%) gave **6**,[17] which was converted (92%) to methyl ether **7** using NaH/CH_3I in THF.[18] Ether **7** was identical with a sample prepared from **6**-OTs and $NaOCH_3/CH_3OH$,[5d,14] but the yield was higher in the NaH procedure, and oxazole **8** (a by-product of the methoxide procedure) was not formed. Hydrolysis of **7** (refluxing 6 N HCl, 5 h, then 25 °C, 12 h) gave benzoic acid (96%) and the aqueous amine-hydrochloride, which was neutralized (Na_2CO_3) and converted in situ ($ClCOOC_2H_5$, 93%) to **4**, identical with a previously prepared sample.[14,19]



5, R = $COOC_2H_5$
6, R = CH_2OH
7, R = CH_2OCH_3



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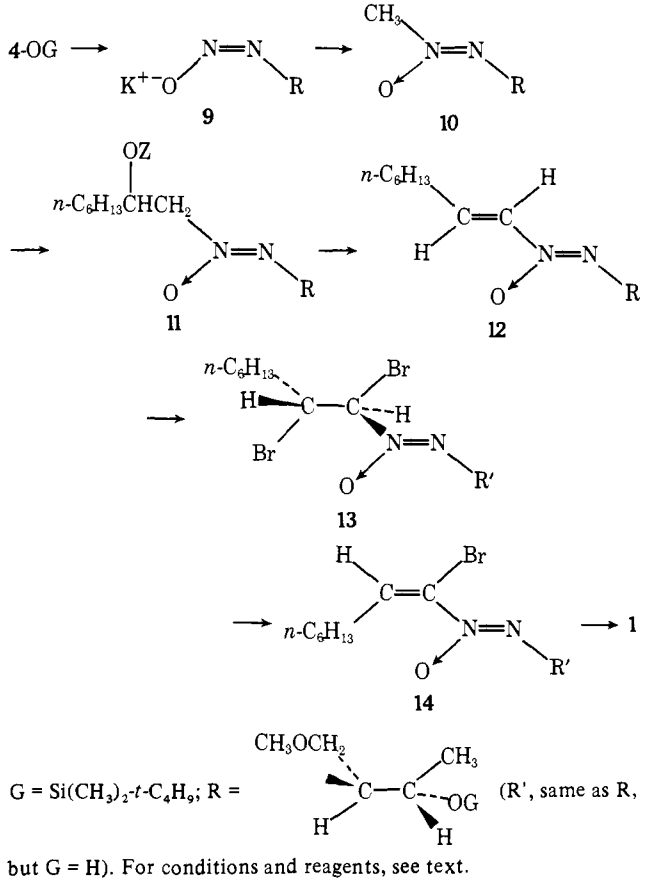
Treatment of **4** with t-$C_4H_9(CH_3)_2SiCl$ (imidazole, DMF, 25 °C, 17 h)[20] quantitatively afforded protected urethane **4**-OG (see Chart I), Its NMR spectrum resembled that of **4**,[14] but showed δ 0.87 (s, 9 H, t-C_4H_9) and 0.03 (s, 6 H, $Si(CH_3)_2$). Quantitative conversion of **4**-OG to the *N*-nitrosourethane ($\Delta\delta^{CCl_4}$ OCH_2CH_3 = 0.42)[21] with ethereal N_2O_4 was followed by cleavage to diazotate **9** using KOt-C_4H_9 in ether.[14,21,22] Treatment of an HMPA solution of **9** with excess CH_3I (25 °C, 12 h, 29% based on nitrosourethane) afforded azoxyalkane **10**, which was purified by repetitive TLC[23] (3:1 hexane/ether): NMR δ 4.03 (s, 3 H, $CH_3N(O)=N$);[24,25] IR (neat) 1500 cm^{-1} (azoxy);[12] exact mass ($M^+ - 15$), calcd 261.1633, found 261.1648.

(B) Elaboration of the *trans*-octenyl moiety.[15] Azoxyalkane **10** was converted to its proximal α-carbanion ($(i\text{-}Pr)_2NLi$, THF, 0-5 °C, 30 min),[15] which was quenched with excess

Chart I



G = $Si(CH_3)_2$-t-C_4H_9; R = (R', same as R, but G = H). For conditions and reagents, see text.

n-heptaldehyde (0–5 °C, 1 h) quantitatively affording crude azoxy alcohol **11** (Z = H): NMR, δ 4.06 (m, 3 H, C*H*N=N(O)CH_2);[12,24] IR (neat), 3450 (OH), 1490 (azoxy) cm^{-1}. Without purification, this was converted (CH_3SO_2Cl, pyridine, 25 °C, 23 h, 94%) to mesylate **11** (Z = SO_2CH_3); NMR δ 2.86 (s, 3 H, CH_3SO_3).[24] The crude mesylate, under reflux in toluene containing excess triethylamine (20 h), gave protected *trans*-elaiomycin, **12,** which was purified by repetitive TLC (9:1 hexane/ether). The yield of **12** was 13% from **11** (Z = H): NMR δ 6.90 (m, 2 H, vinyl), 4.23 (m, 2 H, distal α-H + SiOC*H*), 3.58 (m, 2 H, CH_2OCH$_3$), 3.28 (s, 3 H, OCH_3), 2.25 (m, 2 H, allyl), (1.40 (m, C_5H_{11}) + 1.11 (d, *J* = 6 Hz, CHCH_3) + 0.91 (s, *t*-C_4H_9), total ~23 H), 0.08 (s, 6 H, Si(CH_3)$_2$); IR (neat) 1640 (C=C), 1460 (azoxy), 950 (trans-disubstituted C=C) cm^{-1}. The spectral properties of **12** coincide with corresponding data for **3**,[14] *trans*-CH_3CH=CHN(O)=N-2-C_8H_{17},[15] and *trans*-*n*-C_6H_{13}-CH=CHN(O)=N-2-C_4H_9.[26,27]

(C) Isomerization.[16] Bromine (CCl_4, 25 °C, 30 min, 100%) added to **12** yielding the corresponding *erythro*-dibromide, whence deprotection[20] (CH_3COOH:H_2O:THF, 3:1:1, 25 °C, 18 h, 95%) gave *erthyro*-dibromoelaiomycin, **13,** which was purified by repetitive TLC (3:1 hexane/ether): NMR, δ 5.96 (d, *J* = 11 Hz, 1 H, proximal α-H), 4.65 (m, 1 H, proximal β-H), 4.11 (m, 2 H, distal α-H + HOC*H*), 3.58 (m, 2 H, CH_2OCH$_3$), 3.28 (s, 3 H, OCH_3), 2.21 (br s, 1 H, O*H*), 1.71–0.65 (m, residual alkyl); IR (neat), 3450 (OH), 1495 (azoxy) cm^{-1}.[27] For comparison, the α- and β-proximal protons of *erythro*-CH_3CHBrCHBrN(O)=N-2-C_8H_{17} appear at δ 5.85 (d, *J* = 11 Hz) and 4.73 (m); its distal α-H appears at δ 4.00 (m).[16]

Anti elimination of HBr from **13** (DBU, 25 °C, 30 min, 75%)[28] gave crude α-bromoelaiomycin, **14:** NMR (CCl_4, Me_4Si), δ 5.92 (t, *J* = 8 Hz, 1 H, vinyl);[24] IR (neat), 3400 (OH), 1620 (C=C), 1460 (azoxy) cm^{-1}. For comparison, the vinyl proton of *E*-CH_3CH=CBrN(O)=N-2-C_8H_{17} appears at δ 5.96 (q, *J* = 7.5 Hz).[16] Crude **14** was debrominated with powdered zinc (Mallinkrodt AR grade ether,[29] containing 4 vol % of 30 wt % aqueous CH_3COOH, 25 °C, 24 h, 52%); repetitive TLC (3:1 hexane/ether) afforded elaiomycin, **1,** as well as unreacted **14.**[30]

Synthetic **1** contained a trace of carbonyl impurity (1740 cm^{-1}), but its IR spectrum was otherwise identical with the published spectrum[5a] of natural **1,** including bands at 3450 (OH), 1650 (C=C), 1455 (azoxy), and 785 (cis disubstituted C=C ?) cm^{-1}. The UV spectrum gave $\lambda_{max}^{CH_3OH}$ 235, ε 1.0 × 10^4 (lit.[5a,b] 237.5, 1.1 × 10^4). The NMR spectrum (CCl_4, Me_4Si) was persuasive: δ 6.83 ("d", J ~ 9 Hz, 1 H, proximal α-H),[31] 5.70 (q, *J* ~ 9 Hz, 1 H, proximal β-H), 4.17 (m, 2 H, distal α-H + C*H*OH), 3.58 (m, 2 H, CH_2OCH$_3$), 3.33 (s, 3 H, OCH_3), 2.70 (m, 2 H, allyl), 2.13 (m, 1 H, O*H*), 1.78–0.60 (m, residual alkyl). Both natural **1** and **2** exhibit vinyl doublets, *J* = 9 Hz, at δ 6.83,[6a] and **2** exhibits a quartet at δ 5.83, *J* = 9 Hz.[6a] In *cis*-CH_3CH=CHN(O)=N-2-C_8H_{17}, the corresponding vinyl signals appear at δ 6.70 ("d", *J* = 9 Hz) and 5.73 (quintet, *J* = 8 Hz).[16] Other NMR signals of synthetic **1** are in accord with structural expectation.[6a,14,32]

Reduction of synthetic **1** (5% Rh/Al_2O_3, 1 atm of H_2, CH_3OH, 1 h) gave dihydroelaiomycin, identical in NMR spectrum[14] and TLC behavior with an authentic sample produced via alkylation of **9** (G = tetrahydropyranyl) with *n*-$C_8H_{17}I$.[14]

Synthetic **1** had $[\alpha]^{24}_D$ +24.0° (*c* 2.8, ethanol), 62.5% of the rotation of natural **1**.[5a] It is possible that the apparent loss of optical activity is due to the presence of a trace of highly levorotatory impurity in the synthetic **1**.[31] Alternatively, a dextrorotatory impurity may have been present in natural **1**.[5a,33]

The overall yield for the 18-step conversion of D-threonine to **1** was only 0.55%, but we have not optimized the key low-yield steps **9** → **10** and **11**-OMs → **12,** so that an enhanced yield should be attainable. This initial synthesis of elaiomycin employs strategies which are applicable to **2** and synthetic analogues. Moreover, the crucial sequences substantially broaden the scope of azoxyalkane chemistry.[34]

Acknowledgments. We thank the Public Health Service (Grant CA-14912 from the National Cancer Institute) and the National Science Foundation for financial support. Helpful discussions with Professors P. F. Hudrlik and R. R. Ruden were much appreciated.

References and Notes

(1) B. Lythgoe and N. V. Riggs, *J. Chem. Soc.*, 2716 (1949); B. W. Langley, B. Lythgoe, and N. V. Riggs, *Chem. Ind. (London)*, 75 (1951); B. W. Langley, B. Lythgoe, and N. V. Riggs, *J. Chem. Soc.*, 2309 (1951).
(2) N. V. Riggs, *Chem. Ind. (London)*, 926 (1956); B. Korsch and N. V. Riggs, *Tetrahedron Lett.*, 523 (1964).
(3) H. Matsumoto, T. Nagahama, and H. O. Larson, *Biochem. J.*, **95,** 13c (1965).
(4) In $R_1N(O)$=NR_2, we refer to R_1, or any subfunction of R_1, as *proximal,* and to R_2, or any subfunction of R_2, as *distal.* The reference point is the *N*-oxide.
(5) (a) T. H. Haskell, A. Ryder, and Q. R. Bartz, *Antibiot. Chemother. (Washington, D.C.)*, **4,** 141 (1954); (b) C. L. Stevens, B. T. Gillis, J. C. French, and T. H. Haskell, *J. Am. Chem. Soc.*, **78,** 3229 (1956); (c) *ibid.*, **80,** 6088 (1958); (d) C. L. Stevens, B. T. Gillis, and T. H. Haskell, *ibid.*, **81,** 1435 (1959); (e) K. G. Taylor and T. Riehl, *ibid.*, **94,** 250 (1972).
(6) (a) W. J. McGahren and M. P. Kunstmann, *J. Am. Chem. Soc.*, **91,** 2808 (1969); **92,** 1587 (1970); (c) *J. Org. Chem.*, **37,** 902 (1972); see, also, ref 5e.
(7) The fifth naturally occurring azoxy compound is *p*-carboxyphenylazoxycyanide: A. Gasco, A. Serafino, V. Mortarini, and E. Menziani, *Tetrahedron Lett.*, 3431 (1974).
(8) K. G. Taylor, M.-S. Chi, and M. S. Clark, Jr., *J. Org. Chem.*, **41,** 1131 (1976); K. G. Taylor, S. R. Isaac, and M. S. Clark, Jr., *Ibid.*, **41,** 1135 (1976); K. G. Taylor and M. S. Clark, Jr., *ibid.*, **41,** 1141 (1976); K. G. Taylor, S. R. Isaac, and J. L. Swigert, *ibid.*, **41,** 1146 (1976); V. Nelson, A. Serianz, and P. Kovacic, *ibid.*, **41,** 1751 (1976); F. R. Sullivan, E. Luck, and P. Kovacic, *J. Org. Chem.*, **39,** 2967 (1974).
(9) For a collection of reviews: S. Patai, Ed., "The Chemistry of the Hydrazo, Azo, and Azoxy Groups", Parts 1 and 2, Wiley, New York, N.Y., 1975.
(10) R. Schoental, *Nature (London)*, **221,** 765 (1969); A. G. Karlson, *Antibiot. Chemother. (Washington, D.C.)*, **12,** 446 (1962); J. Ehrlich et al., *ibid.*, **4,** 338 (1954).
(11) Macrozamin and cycasin are also biologically potent (as toxins and carcinogens): D. W. E. Smith, *Science*, **152,** 1273 (1966); G. L. Laquer and M. Spatz, *Cancer Res.*, **28,** 2262 (1968); M. Spatz, *Ann. N.Y. Acad. Sci.*, **163,** 848 (1969); H. Druckrey, R. Preussmann, and S. Ivanković, *ibid.*, **163,** 676 (1969); R. Preussmann, H. Druckrey, S. Ivanković, and A. v. Hodenberg, *ibid.*, **163,** 697 (1969).
(12) R. A. Moss, M. J. Landon, K. M. Luchter, and A. Mamantov, *J. Am. Chem. Soc.*, **94,** 4392 (1972); R. A. Moss and M. J. Landon, *Tetrahedron Lett.*, 3897 (1969).
(13) R. A. Moss and G. M. Love, *J. Am. Chem. Soc.*, **95,** 3070 (1973).
(14) R. A. Moss and T. B. K. Lee, *J. Chem. Soc., Perkin Trans. 1,* 2778 (1973). Although drawn here as D-dihydroelaiomycin, previously synthesized **3** was the L enantiomer.
(15) R. A. Moss and G. M. Love, *Tetrahedron Lett.*, 4701 (1973).
(16) R. A. Moss and M. Matsuo, *Synthesis*, 726 (1976).
(17) Conversion of threonine to **6** is fully described in ref 14.
(18) U. E. Diner, F. Sweet, and R. K. Brown, *Can. J. Chem.*, **44,** 1591 (1966).
(19) Urethane **4** from D-threonine had $[\alpha]^{24}_D$ +29.6° (*c* 1.15, $CHCl_3$); the enantiomer, prepared from L-threonine, had $[\alpha]^{25}_D$ −23.9° (*c* 1.10, $CHCl_3$).[14]
(20) E. J. Corey and A. Venkateswarlu, *J. Am. Chem. Soc.*, **94,** 6190 (1972).
(21) R. A. Moss, *Tetrahedron Lett.*, 711 (1966).
(22) R. A. Moss, *J. Org. Chem.*, **31,** 1082 (1966).
(23) All preparative TLC used EM Laboratories silica gel 60 F-254, 2.0-mm plates.
(24) Characteristic bands are cited for most spectra; other spectral features were in accord with structure. Citations of NMR multiplet positions refer to the multiplet's center or most prominant feature. The NMR solvent was CCl_4, $CHCl_3$, unless otherwise noted.
(25) The corresponding resonance of 2-C_8H_{17}N=N(O)CH_3 appears at δ 3.94.[15] Despite TLC homogeneity, **10** was not pure: singlets appeared at δ 3.32 and 3.00, perhaps due to the isomeric *N*-methyl-*N*-nitrosoamine.
(26) R. A. Moss and R. C. Nahas, unpublished work.
(27) Despite TLC homogeneity, two separately prepared and purified samples gave C, H microanalyses which were ~1% high in C; residual traces of hexane acquired during extensive TLC may have been responsible. All intermediates after **9** were oils, and difficult to purify, but their spectra leave little structural uncertainty.
(28) H. Oediger and F. Möller, *Angew. Chem.*, **79,** 53 (1967).
(29) The ether must contain a radical inhibitor.[16]
(30) The reaction was stopped prior to completion to avoid overreduction of **1.**
(31) A trace of *trans*-**1** (<5%) may be present as evidenced by a minor absorption at δ 6.90; cf. **12.**

(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,

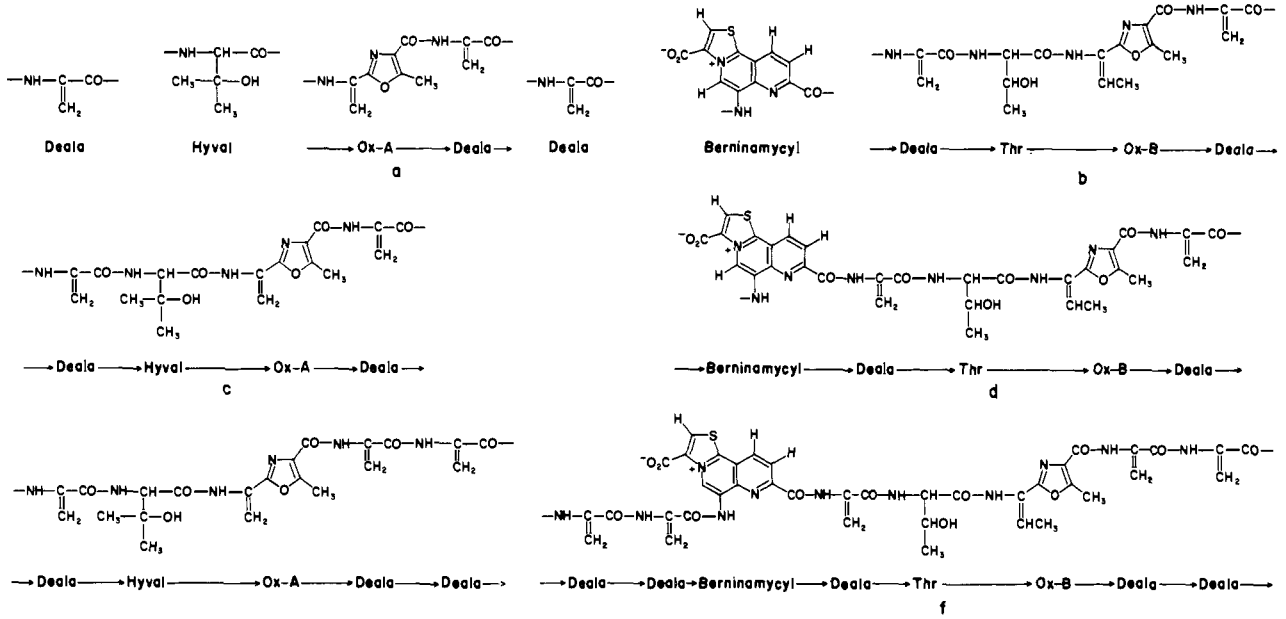
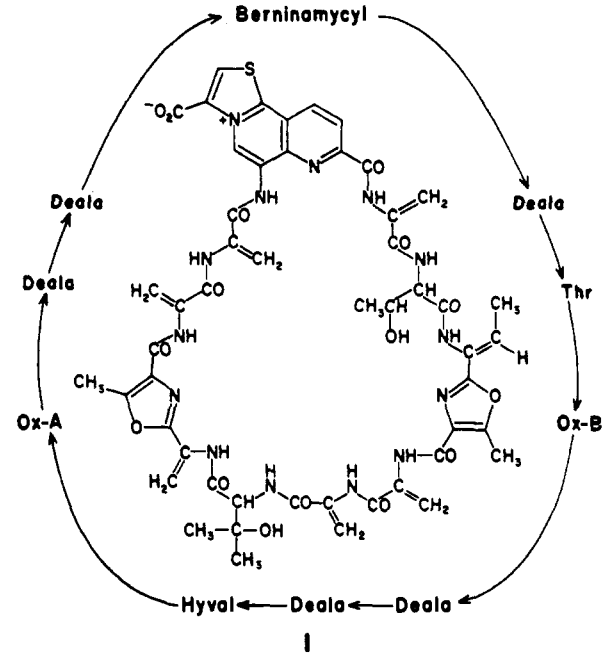


Figure 1. Subunit sequences found in berninamycin A. Subunits shown in the top line were established earlier.[2]



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