presence of covalent hydrates for the ground- and excited-state chemistry of bipy and other aromatic N heterocycles and their coordination complexes cannot be ignored.

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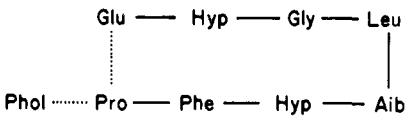
Structure of Antiamoebin I from High Resolution Field Desorption and Gas Chromatographic Mass Spectrometry Studies

Sir:

The antibiotic antiamoebin was reported in 1968[2a] to be produced by *Emericellopsis poonensis* Thirum., *E. synnematicola* Mathur and Thirum., and *Cephalosporium pimprina* Thirum., and to be active against protozoa and helminths.[2b] Subsequently, its structure was assigned as **1**, a cyclic octapeptide linked to phenylalaninol.[3] Oleic, linoleic, and palmitic acids were also indicated to be a part of the molecule.[4] We have now shown that antiamoebin is a mixture of two closely related compounds, antiamoebins I (~98%) and II (~2%), separable by countercurrent distribution in the system $CHCl_3$-C_6H_6-MeOH-H_2O, 30:30:46:14,[3] in which we find $K_I = 5.7$ and $K_{II} = 2.1$, and that the structures of these antibiotics are actually quite different from **1**.

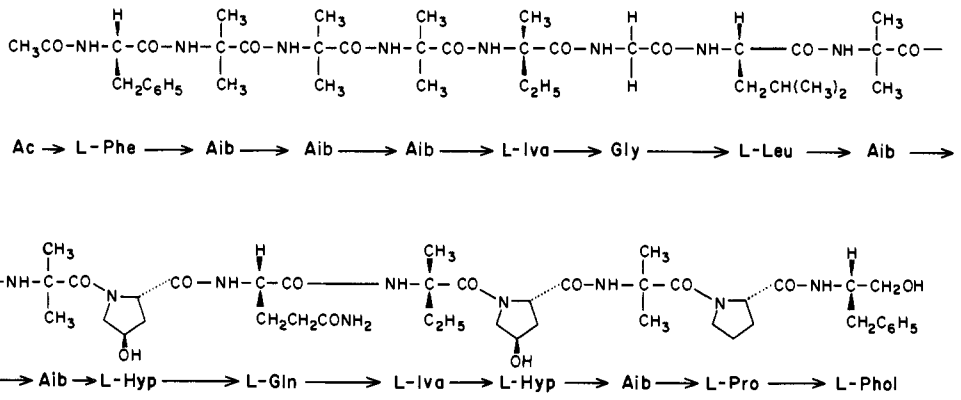


1

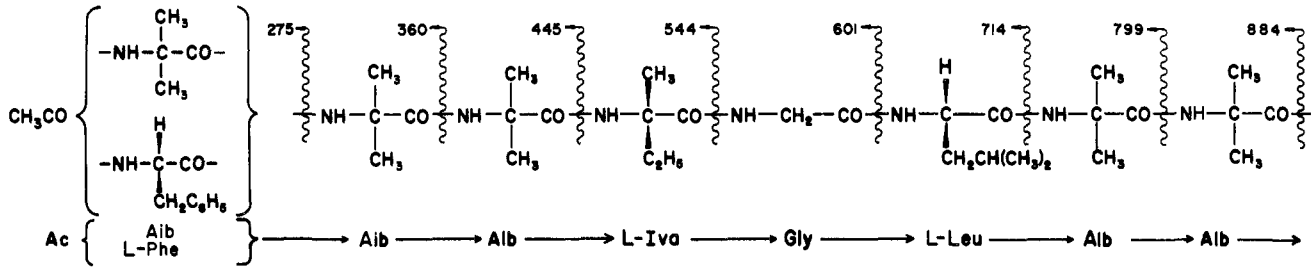
We assign here structure **2** to antiamoebin I, employing gas chromatography–high resolution electron impact mass spectrometry (GC-HREIMS) and high resolution field desorption mass spectrometry (HRFDMS) as principal structural tools. We also propose the name peptaibophol (defined as peptide antibiotics containing phenylalaninol and several moles of α-aminoisobutyric acid as well as other amino acids) for this class of antibiotics, which we have recently shown includes emerimicins II, III, and IV,[5] zervamicins I and II,[5b] and alamethicins I and II,[6] as well as antiamoebins I and II. Two other antibiotics, suzukacillin[7] and samarosporin,[8] also belong to this class, as does (probably) stilbellin.[9]

Antiamoebin I, mp 194–196 °C (MeOH-H_2O), $[\alpha]^{25}_D$ +17.8° (*c* 2.1, MeOH), gives microanalyses agreeing with the molecular formula $C_{82}H_{127}N_{17}O_{20}\cdot 2H_2O$ (mol wt (anhydrous), 1669), while the cationated molecular ion[10] is found at *m/e* 1692 (M + Na) in the field desorption mass spectrum (FDMS).[11] Acetylation of antiamoebin I with acetic anhydride–pyridine gave antiamoebin I triacetate, whose FDMS showed ions at *m/e* 1818.9596 (matched vs. the peak at *m/e* 1720.9514 ($C_{34}H_{18}F_{56}N_3O_6P_3$) in the FDMS of bis(dodecafluoroheptoxy)tetrakis(octafluoropentoxy)cyclotriphosphazene),[11c] 1758, and 1698, corresponding to $C_{88}H_{133}N_{17}O_{23}Na$ (M + Na), M + Na − HOAc, and M + Na − 2HOAc, respectively. Cationated molecular ions (M + Na) for the tripropionate and tributyrate were found by FDMS at 1860 and 1902, respectively, while those for the lithium and potassium conjugates of the triacetate were found at *m/e* 1802 and 1834, respectively.[10]

Hydrolysis of antiamoebin I with 6 N hydrochloric acid gave a mixture of amino acids but no fatty acids. Analysis of the hydrolysis mixture was carried out by FDMS followed by HRFDMS (employing a variety of reference standards described elsewhere),[6] which gave M + H ions at *m/e* 76.0409 ($C_2H_6NO_2$, Gly), 104.0714 ($C_4H_{10}NO_2$, Aib, α-aminoisobutyric acid;[12,13] cf. seq.), 116.0721 ($C_5H_{10}NO_2$, Pro), 118.0883 ($C_5H_{12}NO_2$, Iva, isovaline, α-amino-α-methylbutyric acid;[14,15] cf. seq.), 132.0654 ($C_5H_{10}NO_3$, Hyp), 132.1012 ($C_6H_{14}NO_2$, Leu), 148.0604 ($C_5H_{10}NO_4$, Glu), 152.1078 ($C_9H_{14}NO$, Phol, phenylalaninol; cf. seq.), and 166.0849 ($C_9H_{12}NO_2$, Phe); by amino acid analysis (Beckman/Spinco, Model 120), which indicated 6–7 mol of Aib, 2



2



a

mol of Hyp, and 1 mol each of Gly, Leu, Pro, Phe, Glu, and Phol;[10a] and by GC–HREIMS of the *n*-butyl and methyl ester derivatives of the *N*-trifluoroacetylamino acids, which indicated 5–7 mol of Aib, 2 mol each of Iva and Hyp, and 1 mol each of Gly, Leu, Phol, Pro, Phe, and Glu (all identified by comparison of retention times and fragmentation patterns with those of authentic samples). Combining these results indicates the composition 5–7 Aib, 2 Iva, 2 Hyp, and 1 each of Gly, Leu, Phol, Pro, Phe, and Glu.

Analysis of the *N*-trifluoroacetylamino acid methyl esters on a column of 10% *N*-lauroyl-*N'*-*tert*-butyl-L-valinamide on 60–80 mesh Chrom WAW,[16,17] employing somewhat modified conditions,[5] indicated that all of the optically active amino acids have the L configuration. Phol was isolated and also found to have the L configuration:[18] $[\alpha]^{23}_D$ −23.5° (*c* 2.47, MeOH) (lit. $[\alpha]^{24}_D$ −23.3° (*c* 2.01, MeOH)].[19]

Antiamoebin I does not react with reagents for amino (N terminal) or carboxyl (C terminal) groups. Thus, either it is a cyclic peptide in which there are no N-terminal or C-terminal amino acids (and the γ-carboxyl group of Glu and the amino group of Phol are present in amide linkages) or it is a linear peptide in which the N-terminal and C-terminal groups occur derivatized. Antiamoebin I analyzes for one $-CONH_2$ group on hydrolysis to ammonia, and its ^1H NMR spectrum shows a singlet at 1.91 ppm (3 H, s) assignable to an acetyl methyl group. The presence of acetyl, primary amide, and three esterifiable hydroxyl groups and the mol wt 1669 argue that antiamoebin I is a linear peptide, as in **3**, composed of 1 mol each of Gly, Leu, Pro, Glu, Phe, and Phol, 2 each of Iva and Hyp, and 6 of Aib. In agreement with this structure, 17 amide carbonyl carbons can be observed in the ^{13}C NMR spectrum (DMSO-d_6) of antiamoebin I: at 175.9, 175.7, 175.6, 175.2, 174.9, 173.5, 173.2, 173.0, 172.9, 172.5, 172.2, 171.8 (2), 171.7, 170.8, and 170.5 (2) ppm.

$$CH_3CO\text{-}(aa)_x\text{-}Glu\text{-}(aa)_y\text{-}Phol$$
$$|$$
$$(aa)_zNH_2$$

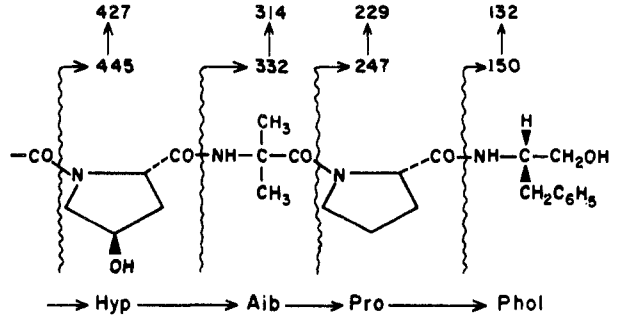
3, $x + y + z = 14$

Dehydration of antiamoebin I with ethylene chlorophosphite in triethyl phosphite followed by reduction ($Na\text{-}NH_3$–MeOH),[20] hydrolysis (6 N HCl, 110 °C, 24 h), derivatization (*N*-trifluoroacetyl *n*-butyl ester), and analysis of the derivatives by GC and GC–EIMS did not show any glutamic acid but showed the presence of ornithine in the mixture. This clearly indicated that Glu is present as Gln in antiamoebin I. Structure **3** can then be written as **4**.

$$CH_3CO\text{-}(aa)_x\text{-}Gln\text{-}(aa)_y\text{-}Phol$$

4, $x + y = 14$

The order of linkage of the amino acids was established by mass spectrometry. The HREIMS of antiamoebin I shows characteristic CO–N cleavage peaks (the major peaks in the spectrum) at *m/e* 884.5327 ($C_{44}H_{70}N_9O_{10}$), 799.4770 ($C_{40}H_{63}N_8O_9$), 714.4209 ($C_{36}H_{56}N_7O_8$), 601.3374 ($C_{30}H_{45}N_6O_7$), 544.3163 ($C_{28}H_{42}N_5O_6$), 445.2471 ($C_{23}H_{33}N_4O_5$), 360.1968 ($C_{19}H_{26}N_3O_4$), and 275.1217 ($C_{15}H_{19}N_2O_3$) for the N-terminal sequence, and a weak CO–N cleavage peak at 190.0850 ($C_{11}H_{12}NO_2$), thus establishing the partial structure a. Similar, generally smaller peaks at 445.2453 ($C_{23}H_{33}N_4O_5$), 427.2374 ($C_{23}H_{31}N_4O_4$), 332.1951 ($C_{18}H_{26}N_3O_3$), 314.1893 ($C_{18}H_{24}N_3O_2$), 247.1431 ($C_{14}H_{19}N_2O_2$), 229.1341 ($C_{14}H_{17}N_2O$), 150.0915 ($C_9H_{12}NO$), and 132.0814 ($C_9H_{10}N$) establish the C-terminal sequence and the partial structure b. The peaks at *m/e* 884 and 799 are also present in the FDMS of antiamoebin I and its acetate.



b

Additional and confirmatory peptide sequences come from partial hydrolysis of antiamoebin I: (i) in 12 N hydrochloric acid–acetic acid (1:1), 25 °C, 72 h; (ii) in 6 N hydrochloric acid in methanol, 70 °C, 6 h. Each mixture of oligopeptides formed was analyzed in two ways. First, it was converted to the corresponding mixture of *N*-trifluoroacetyl *n*-butyl (or methyl) ester derivatives and analyzed by GC–HREIMS and GC–FIMS (FI, field ionization); peptides identified from their molecular formulas[21] and fragmentation patterns in this way are marked with an asterisk (*). Second, the mixture of peptides was analyzed by HRFDMS;[22] peptides identified from their protonated molecular ions in this way are marked with a dagger (†). A partial list of the peptides found (Ac-Phe-Aib-Aib-Aib-Iva† (562.3234, $C_{28}H_{44}N_5O_7$), Ac-Phe-Aib-Aib-Aib† (463.2499, $C_{23}H_{35}N_4O_6$), Phe-Aib*, Phe-Aib-Aib*, Aib-Aib*, Aib-Iva*, Iva-Gly-Leu*, Gly-Leu-Aib-Aib† (359.2283, $C_{16}H_{31}N_4O_5$), Gly-Leu*, Gly-Leu-Aib*, Leu-Aib*, Leu-Aib-Aib*† (302.2054, $C_{14}H_{28}N_3O_4$), Hyp-Gln-Iva† (359.1921, $C_{15}H_{27}N_4O_6$), Glu(α)-Iva*, Hyp-Aib-Pro*, Pro-Phol*† (249.1625, $C_{14}H_{21}N_2O_2$)) confirms a and b and establishes c. Thus, only one structure for antiamoebin I is allowed: a-c-b (**2**).[22]

Hyp-Gln-Iva

c

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(21) Molecular formulas determined by HRFDMS suggest the amino acid compositions for the oligopeptides, though they cannot, of course, sequence the amino acids in the peptides. The sequences shown for the peptides marked with a dagger are obtained by combining the HRFD data with the partial structures a and b, with the exception of the tripeptide Hyp-Gln-Iva, whose sequence was assigned from GC–MS data. That key tripeptide has also been isolated under the same hydrolysis conditions (II) and characterized by IR and ^{1}H NMR spectra, and its sequence has been established by direct probe HREIMS.
(22) Although no peptides were observed containing Aib-Hyp and Iva-Hyp linkages, the molecular formula of antiamoebin I allows no additional amino acids and partial structures a, b, and c account for the entire molecule.

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Structures of the Peptide Antibiotics Emerimicins III and IV[1,2]

Sir:

The emerimicins, produced by *Emericellopsis microspora* in the presence of *trans*-4-*n*-propyl-L-proline (propylproline), are peptide antibiotics with moderate activity against gram-positive bacteria and against protozoa.[3] In the present report we assign structures **1** to emerimicin IV, the principal component, and **2** to emerimicin III, employing mainly field desorption mass spectrometry (FDMS), gas chromatography–high resolution electron impact mass spectrometry (GC-HREIMS) and gas chromatography–field ionization mass spectrometry (GC-FIMS). We also relate the structures of these antibiotics to that of antiamoebin I (**3**),[1b] another member of this class of antibiotics for which we propose the name peptaibophols.[4]

Emerimicin IV (mp >200 °C dec, pure by HPLC) has mol wt 1572, established by its field desorption mass spectrum (FDMS)[5] after cation exchange,[6] which shows M + Na and M + K ions at *m/e* 1595 and 1611, respectively. This was confirmed by the FDMS of cationated emerimicin IV triacetate (obtained from emerimicin IV by acetylation with acetic anhydride–pyridine at room temperature and purification on silica gel), which shows ions at *m/e* 1721 (M + Na), 1661 (M + Na − HOAc), and 1601 (M + Na − 2HOAc), and at 1737 (M + K), 1677 (M + K − HOAc), and 1617 (M + K − 2HOAc).

On vigorous acidic hydrolysis (6 N aqueous HCl, 110 ± 1 °C, 24 h) emerimicin IV gave glycine (Gly), α-aminoisobutyric acid (Aib), valine (Val) and/or isovaline (Iva), hydroxyproline (Hyp) and/or leucine (Leu), glutamic acid (Glu), phenylalaninol (Phol), and phenylalanine (Phe), all identified from their molecular ions in an FDMS[1b] of the hydrolysate. Quantitation of the amino acids on an amino acid analyzer (Beckman/Spinco, Model 120) indicated the molar ratio 7.0 ± 0.3 Aib:1 Gly:1 Val:1 Leu:1 Phol:2 Hyp:1 Phe:1 Glu, while gas chromatographic and GC-EIMS analysis[1b] of the *N*-trifluoroacetyl derivatives of the *n*-butyl esters indicated the molar ratio 6.5 ± 1.3 Aib:1 Iva:1 Gly:1 Val:1 Leu:1 Phol:2 Hyp:1 Phe:1 Glu.

Emerimicin IV contains no carboxyl, carbalkoxyl, or primary amino group but analyzes for one $-CONH_2$ group per mole, while the ^{1}H NMR spectrum shows an acetyl (acetamido) methyl group at 1.88 ppm.[1b] With an acetyl group, a terminal amide, 1 mol each of Gly, Val, Iva, Leu, Phe, Glu, and Phol, 2 mol of Hyp, and 6–8 mol of Aib, the mol wt 1572 indicates that emerimicin IV has the molecular formula $C_{77}H_{120}N_{16}O_{19}$ and contains 6 mol of Aib. Microanalyses reported earlier[3] agree with this formula, as the dihydrate, while the ^{13}C NMR spectrum of emerimicin IV contains the expected 16 carbonyl carbons, located at 175.9, 175.7, 175.0, 174.9, 173.8, 173.7, 173.2, 172.4, 172.2, 172.1, 171.9, 171.7, 171.5, 170.3 (2), and 170.0 ppm.

Emerimicin IV, like antiamoebin I,[1b,8] on dehydration (ethylene chlorophosphite/triethyl phosphite, 100 ± 1 °C, 24 h), reduction ($Na-NH_3$–MeOH), and hydrolysis (6 N HCl, 110 ± 1 °C, 24 h) followed by derivatization (*N*-trifluoroacetyl *n*-butyl esters) did not give any Glu but gave a new amino acid identified as ornithine (Orn) by GC and GC–MS. Thus, the primary amide group is present in Gln in emerimicin IV and the general structure **4** can be written.

$$\text{Ac-(aa)}_x\text{-Gln-(aa)}_y\text{-Phol}$$
$$\mathbf{4},\ x + y = 13$$

The HREIMS of emerimicin IV and its triacetate contain peaks (all intense save that at *m/e* 190) for the sequence a at 190.0865 ($C_{11}H_{12}NO_2$), 275.1396 ($C_{15}H_{19}N_2O_3$), 360.1926 ($C_{19}H_{26}N_3O_4$), 445.2448 ($C_{23}H_{33}N_4O_5$), 544.3133 ($C_{28}H_{42}N_5O_6$), 601.3344 ($C_{30}H_{45}N_6O_7$), 714.4177 ($C_{36}H_{56}N_7O_8$), 799.4682 ($C_{40}H_{63}N_8O_9$), and 884.5242

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 (emerimicin IV):	Ac-L-	Phe-	Aib-	Aib-	Aib-	L-*Val*-	Gly-	L-	Leu-	Aib-	Aib-	L-Hyp-	L-Gln-	L-Iva-	L-Hyp-	*Aib*-L-Phol
2 (emerimicin III):	Ac-L-	Phe-	Aib-	Aib-	Aib-	L-*Val*-	Gly-	L-	Leu-	Aib-	Aib-	L-Hyp-	L-Gln-	L-Iva-	L-Hyp-	L-*Ala*-L-Phol
3 (antiamoebin I):	Ac-L-	Phe-	Aib-	Aib-	Aib-	L-*Iva*-	Gly-	L-	Leu-	Aib-	Aib-	L-Hyp-	L-Gln-	L-Iva-	L-Hyp-	*Aib*-L-*Pro*-L-Phol