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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

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Table I. Peptide Fragments of Emerimicin IV

a'	b	c
Ac-Phe-Aib-Aib-Aib-Val-Gly-Leu-Aib-Aib	Hyp-Gln-Iva	Hyp-Aib-Phol
Ac-Phe-Aib-Aib-Aib-Val-Gly-Leu-Aib-Aib[a,d]		
Ac-Phe-Aib-Aib-Aib[d]		
Phe-Aib-Aib-Aib-Val-Gly-Leu[d,e]		
Phe-Aib[c]		
Aib-Aib-Aib-Val-Gly-Leu-Aib-Aib[d]		
Aib-Aib[b,c]		
Aib-Aib-Val-Gly[d]		
Val-Gly[d]		
Gly-Leu-Aib-Aib[d]		
Gly-Leu-Aib[c]		
Leu-Aib-Aib[c,d]		
	Hyp-Gln-Iva[d]	
	Glu-(α)-Iva[b-d]	
		Hyp-Aib[b-d]
		Hyp-Aib-Phol[a,c]
		Aib-Phol[a,b]

[a] Identified by HREIMS on emerimicin IV. By [b] GC–HREIMS, [c] GC–FIMS, or [d] FDMS on mixtures of peptides or their derivatives. FDMS cannot, of course, assign sequences from protonated molecular ions alone and the FDMS sequences shown here are assigned from the partial structures of a', c, and Glu-Iva determined by other means (HREIMS or GC–HREIMS). The sequence Hyp-Gln-Iva (rather than Gln-Iva-Hyp) is assigned by analogy to the compound Hyp-Gln-Iva isolated and identified from antiamoebin I hydrolysis, as well as the general observation of preferential acidic cleavage of tertiary amide bonds involving hydroxyproline. [e] Isolated on Sephadex LH-20.

($C_{44}H_{70}N_9O_{10}$), also found in the HREIMS of antiamoebin I.[1b]

Ac-Phe-Aib-Aib-Aib-(Val or Iva)-Gly-Leu-Aib-Aib
a

The sequence of amino acids was extended by partial hydrolysis of emerimicin IV in 12 N hydrochloric acid–glacial acetic acid (1:1, room temperature, 72 h). In one experiment a pure heptapeptide was isolated (Table I) from a Sephadex LH-20 column and hydrolyzed to its amino acids, identified as their *N*-trifluoroacetyl *n*-butyl ester derivatives as in the ratio 1 Phe:1 Leu:1 Gly:1 Val:3 Aib, which clearly indicated that Val (rather than Iva) is attached to Aib and Gly in a, giving a' (Table I).

In other experiments the resulting mixture of peptides was analyzed by FDMS (including recognition of unique O:N ratios for Glu, Hyp, and Phol and unique C:H ratios for Phe and Phol, extension of structures from smaller peptides, etc.) or their derivatives (*N*-trifluoroacetyl *n*-butyl esters) were analyzed by GC–HREIMS and GC–FIMS. A summary of the oligopeptides identified is found in Table I. Overlapping some of these oligopeptides confirms the sequence a', while other oligopeptides assign the sequences b and c (-Hyp-Gln-Iva- and -Hyp-Aib-Phol, respectively).

Sequences a', b, and c account for all the amino acids of emerimicin IV. Thus, structure **1** is assigned, which differs from that assigned antiamoebin I[1b] only in that the Pro unit (between Aib and Phol) of antiamoebin I (**3**) is lacking in **1** and in that the Iva unit of **3** between Aib and Gly is replaced by Val in **1**.

The minor component, emerimicin III, is very similar to emerimicin IV. Its ^1H NMR spectrum shows an acetyl group at 1.83 ppm. Analysis of its hydrolysis products (6 N HCl, 110 ± 1 °C, 24 h) by FDMS and HRFDMS reveals that emerimicin III contains Ala in addition to the amino acids (Aib, Iva, Gly, Val, Leu, Hyp, Phe, Glu) and Phol present in emerimicin IV, while analysis by GC and GC–EIMS of their *N*-trifluoroacetyl *n*-butyl ester derivatives on a 6 ft × ⅛ in. column of 0.75% EGA on 80–100 Chrom WAW[9] indicated that emerimicin III could differ from emerimicin IV in replacement of an Aib unit by Ala. That this is the case is shown by the FDMS of emerimicin III, which contains an M + Na ion at *m/e* 1581. Thus, the molecular weight of emerimicin III is 1558, 14 amu less than the molecular weight of emerimicin IV. The position of Ala and sequence of amino acids in emerimicin III was established by mass spectrometry. The EIMS of emerimicin III was nearly identical with that of emerimicin IV except for a few ions; HREIMS of these ions (e.g., 316.1648, $C_{17}H_{22}N_3O_3$, -Hyp-Ala-Phol − $1H_2O$; 231.1131, $C_{13}H_{15}N_2O_2$, -CO-Ala-Phol − $1H_2O$) indicated that in emerimicin III Phol is attached through Ala to the rest of the molecule. Based on the mass spectral data and biogenetic considerations structure **2** is assigned to emerimicin III.

All the optically active amino acids from emerimicins III and IV were found to have the L configuration based on comparison of the retention times of their *N*-trifluoroacetyl methyl ester derivatives with those of authentic samples on a 12 ft × ⅛ in. column of 10% *N*-lauroyl-*N'-tert*-butyl-L-valinamide on 60–80 Chrom WAW.[1b,10]

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