Communications to the editor

STRUCTURE OF THE PEPTIDE ANTIBIOTIC ANTIAMOEBIN II[1)]

Sir:

In a recent communication[2)] we reported that antiamoebin,[3)] which is produced by *Emericellopsis poonensis* THIRUM., *E. synnematicola* MATHUR and THIRUM., and *Cephalosporium pimprina* THIRUM., has membrane modifying properties similar to alamethicin[4)] and suzukacillin[5)] and 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),[6)] in which we find K_I=5.7 and K_{II}=2.1. Their antibacterial spectra (Table 1) show both antibiotics to have mild inhibitory activity against gram-positive bacteria, with antiamoebin I being slightly more active. The structure of antiamoebin I was reported earlier[2)] as **1**. In the present communication we assign structure **2** to the minor component, antiamoebin II.

Antiamoebin II is a white crystalline compound, mp 182~186°C after crystallization from methanol-water, $[\alpha]_D^{25}$+16.1° (*c* 2.1, MeOH). The FDMS technique[1b)] recently developed in this laboratory of cationization using alkali metal salts indicated that 1,653 is the molecular weight of antiamoebin II: by adding a methanolic solution of sodium chloride the molecular ion moved up by 23 amu to *m/e* 1,676 and by adding potassium chloride it appeared at *m/e* 1,692. Microanalyses agreed with the molecular formula $C_{82}H_{127}N_{17}O_{19}\cdot 2H_2O$ (1,653+$2H_2O$).

Vigorous hydrolysis of antiamoebin II (6 N HCl, 110±1°C, 24 hours) yielded a mixture which when analyzed by FDMS, showed M+H ions at *m/e* 76 (glycine, Gly, *cf. seq.*), 104 (α-aminoisobutyric acid, Aib), 116 (proline, Pro), 118 (isovaline, α-amino-α-methyl butyric acid, Iva), 132 (hydroxyproline and/or leucine, Hyp and/or Leu), 148 (glutamic acid, Glu), 152 (phenylalaninol, Phol), and 166 (phenylalanine, Phe). Quantitation of these hydrolysis products by gas chromatography (GC) as their N-TFA *n*-butyl ester derivatives[1,7)] and comparison of the GC chromatograms of antiamoebins I and II clearly revealed (Table 2) that the difference between the two antibiotics lies in the replacement of one of the two Hyp units of antiamoebin I by a mole of Pro in antiamoebin II, *i.e.*, that antiamoebin II contains 2 Pro and 1 Hyp units, whereas antiamoebin I has 1 Pro and 2 Hyp units. This replacement agrees with the 16 amu difference in

Fig. 1.

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     1   2   3   4    5   6     7   8   9     10    11    12   13   14   15    16
1:  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

     1   2   3   4    5   6     7   8   9     10    11    12   13   14   15    16
2:  Ac-L-Phe-Aib-Aib-Aib-L-Iva-Gly-L-Leu-Aib-Aib-L-Hyp-L-Gln-L-Iva-L-Pro-Aib-L-Pro-L-Phol
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Table 1. *In vitro* antibacterial spectra of antiamoebins I and II

Test organism (strain)	Minimum inhibitory concentration (μg/ml)*	
	Antiamoebin I	Antiamoebin II
Staphylococcus aureus 284 (UC 76)	50	100
Streptococcus hemolyticus (UC 152)	25	50
Streptococcus faecalis (UC 694)	100	400
Escherichia coli (UC 311)	>400	>400
Proteus vulgaris (UC 232)	>400	>400
Klebsiella pneumoniae (UC 53)	>400	>400
Salmonella schottmuelleri (UC 126)	>400	>400
Pseudomonas aeruginosa (UC 95)	>400	>400
Streptococcus pneumoniae (UC 41)	50	50

* Tested in suspension, by the agar dilution method, by Mr. G. E. ZURENKO, The Upjohn Co.

Table 2. Amino acid hydrolysis products* from antiamoebins I and II

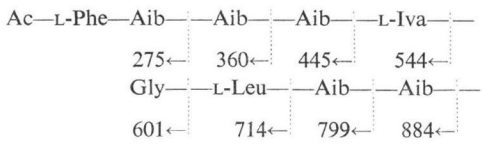
Hydrolysis products	Antiamoebin I	Antiamoebin II
Aib	6	6
Iva	2	2
Gly	1	1
Leu	1	1
Pro	1	2
Hyp	2	1
Glu	1	1
Phe	1	1
Phol	1	1

* Analyzed by GC as N-TFA *n*-butyl ester derivatives on a glass column, 6 ft×2 mm i. d., filled with 3% OV-17 on 100~120 GCQ, flow 36 ml/min, temperature program 20°C/min (80~300°C).

the molecular weights of antiamoebins I and II observed by FDMS. Analysis of the N-TFA methyl ester derivatives of the total acid hydrolyzate of antiamoebin on a column of 10% N-lauroyl-N′-*tert*-butyl-L-valinamide on 60~80 mesh Chrom W AW[2,7] indicated all the amino acids to have the L configuration.

The IR spectrum (KBr) of antiamoebin II showed bands at 3440, 1660, and 1540 cm^{-1} (amide) and the ^1H NMR spectrum (DMSO-d_6) a characteristic acetyl signal at 1.84 ppm (s, 3H). Since, like antiamoebin I, it analyzes for one $-CONH_2$ group per mole, antiamoebin II must also be linear, with N-terminal acetyl, Gln, and C-terminal Phol units.

The electron impact (EI) mass spectrum of antiamoebin II showed characteristic ions at *m/e* 884, 799, 714, 601, 544, 445, 360, and 275, indicating partial structure **a** in antiamoebin II,



a

the same as in antiamoebin I. Antiamoebin II was next partially hydrolyzed to a mixture of oligopeptides [glac. HOAc - 12 N HCl (1:1), 24°C, 72 hours] and part of the product was analyzed by FDMS and high resolution (HR) FDMS. The ions observed were assigned as follows: *m/e* 619 (Ac-Phe-Aib-Aib-Aib-Iva-Gly+H), 562.3249 ($C_{28}H_{44}N_5O_7$, Δ 0.9 mmu, Ac-Phe-Aib-Aib-Aib-Iva+H), 431.2682 ($C_{23}H_{35}N_4O_4$, Δ 2.4 mmu, Pro-Aib-Pro-Phol−H), 359.1921 ($C_{15}H_{27}N_4O_6$, Δ −0.9 mmu, Hyp-Gln-Iva+H), 332.1966 ($C_{18}H_{26}N_3O_3$, Δ −0.8 mmu, Aib-Pro-Phol−H), 302.2068 ($C_{14}H_{28}N_3O_4$, Δ −1.1 mmu, Leu-Aib-Aib+H), 249.1625 ($C_{14}H_{21}N_2O_2$, Δ 2.2 mmu, Pro-Phol+H), 201.1247 ($C_9H_{17}N_2O_3$, Δ 0.8 mmu, Pro-Aib+H). The HRFDMS data thus confirmed sequence **a** by the ions at *m/e* 619, 562, and 302. More importantly, the ions at *m/e* 431, 332, 249, and 201 established the unit **b**, thus locating the Pro unit in antiamoebin II which replaces the Hyp unit of antiamoebin I. Finally, the ion at *m/e* 359 establishes the remaining three amino acids in a tripeptide unit, Hyp-Gln-Iva. There were no FDMS ions corresponding to Hyp-Aib.

Pro—Aib—Pro—Phol

b

A portion of the oligopeptide mixture from partial acid hydrolysis was also derivatized as described earlier[1,7] to N-TFA *n*-butyl ester derivatives and analyzed by GC and GC–MS. Again, no Hyp-Aib was observed, but a peak due to Pro-Aib was present.

Based on the above evidence and on biogenetic considerations structure **2** is assigned to antiamoebin II.

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References

1) (a) Paper 5 in the series Peptaibophol Antibiotics. (b) Paper 4: RINEHART, Jr., K. L.; J. C. COOK, Jr., H. MENG, K. L. OLSON & R. C. PANDEY: Mass spectrometric determination of molecular formulas for membrane-modifying antibiotics. Nature 269: 832~833, 1977
2) PANDEY, R. C.; H. MENG, J. C. COOK, Jr. & K. L. RINEHART, Jr.: Structure of antiamoebin I from high resolution field desorption and gas chromatographic mass spectrometry studies. J. Am. Chem. Soc. 99: 5203~5205, 1977
3) THIRUMALACHAR, M. J.: Antiamoebin, a new antiprotozoal-anthelminthic antibiotic. I. Production and biological studies. Hindustan Antibiot. Bull. 10: 287~289, 1968
4) MUELLER, P. & D. O. RUDIN: Action potential induced in biomolecular lipid membranes. Nature 217: 713~719, 1968
5) BOHEIM, G.; K. JANKO, D. LEIBFRITZ, T. OOKA, W. A. KÖNIG & G. JUNG: Structural and membrane modifying properties of suzukacillin, a peptide antibiotic related to alamethicin. Part B. Pore formation in black lipid films. Biochim. Biophys. Acta 433: 182~199, 1976
6) VAIDYA, M. G ; P. V. DESHMUKH & S. N. CHARI: Amino acid sequence in antiamoebin. Hindustan Antibiot. Bull. 11: 81~89, 1968
7) PANDEY, R.C.; J.C. COOK, Jr. & K.L. RINEHART, Jr.: High resolution and field desorption mass spectrometry studies and revised structures of alamethicins I and II. J. Am. Chem. Soc. 99: 8469~8483, 1977