THE STRUCTURE OF ARPHAMENINES A AND B

Sir:

In a previous communication[1], we reported on the isolation and physicochemical and biological properties of arphamenines A and B, new aminopeptidase B inhibitors which enhance immune responses. In this communication, the structure determination of arphamenines is reported.

Arphamenine A (**I-a**) was obtained as its monohydrochloride, and its molecular formula was established as $C_{16}H_{24}N_4O_3 \cdot HCl$ by elemental analysis and field desorption mass spectrometry [*m/z* 321 (M+1)] as reported in a previous paper[1]. The UV spectrum suggested the presence of phenyl group [$\lambda_{max}^{H_2O}$ 257 nm (ε 180)], which was confirmed by ^1H NMR spectrometry (*vide post*). **I-a** showed positive ninhydrin and SAKAGUCHI reactions. The potentiometric titration showed the presence of three dissociable groups at *pKa* 3.7, 7.6 and >12.0. They can be assigned to the carboxyl, amino and guanidino groups, respectively. The ^1H NMR spectrum (in D_2O) of **I-a** revealed the presence of two carbon chains, $-CH_2-CH_2-CH_2-\underset{|}{C}H-$ and $-CH_2-\underset{|}{C}H-CH_2-$, in addition to a monosubstituted benzene (Table 1). The ^{13}C NMR spectrum showed a signal at δ 206.5, which appears to be a keto-function, in addition to the 15 signals of which were characterized as shown in Table 2. From the results described above, all atoms in the molecule of **I-a** were characterized, and **I-a** was shown to be a linear compound having each one of carboxyl, amino, guanidino, keto and phenyl functions. Thus, the only thing left for the structure determination is the connection between the two carbon chains described above.

Taking the bioactivity into account, the structure shown in Chart 1 was postulated for **I-a**. In order to prove the postulated structure by mass

Chart 1. Arphamenines and their derivatives.

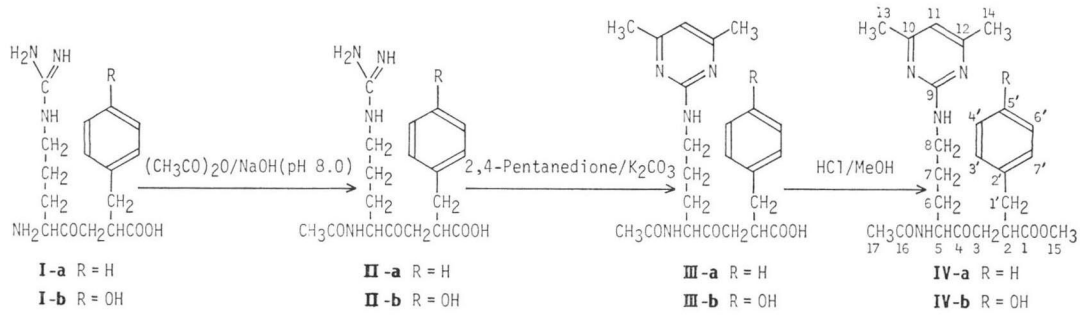


Fig. 1. Mass spectrum of **IV-a**.

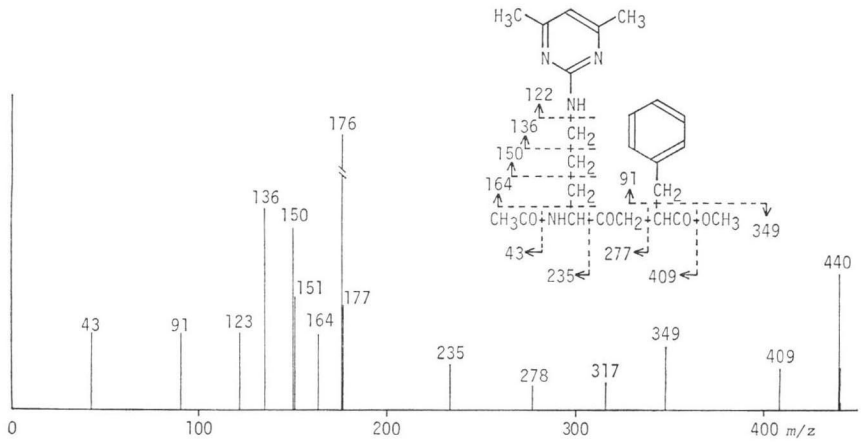


Table 1. ^{1}H NMR spectral data for arphamenines A, B and their derivatives.

Position[a]	Compounds[b]					
	I-a[c]	I-b[c]	III-a[d]	III-b[d]	IV-a[e]	IV-b[e]
2-CH	3.69~3.84 (m)	3.54~3.78 (m)	2.50~3.24 (m)	2.50~3.10 (m)	3.17 (m)	3.06 (m)
3-CH_2	3.34~3.69 (m)	3.14~3.54 (m)			2.49 (J=18.0, 4.0 Hz)	2.54 (J=11.0, 2.5 Hz)
					2.97 (J=18.0, 9.0 Hz)	3.11 (m)
5-CH	4.83 (m)	4.75 (m)	4.49 (m)	4.38 (m)	4.59 (m)	4.51 (m)
6-CH_2	2.35~2.72 (m)	2.30~2.62 (m)	1.42~1.84 (m)	1.60 (m)	1.63 (m)	1.08~1.50 (m)
7-CH_2	2.04~2.33 (m)	1.87~2.30 (m)				
8-CH_2	3.69~3.84 (m)	3.54~3.78 (m)	3.28~3.48 (m)	3.4 (m)	3.46 (m)	3.20~3.46 (m)
11-CH	—	—	6.30 (s)	6.36 (s)	6.34 (s)	6.36 (s)
13,14-CH_3	—	—	2.28 (s)	2.26 (s)	2.34 (s)	2.34 (s)
1′-CH_2	3.34~3.69 (m)	3.14~3.54 (m)	2.50~3.24 (m)	2.50~3.10 (m)	2.72 (J=13.5, 9.0 Hz)	2.52 (J=19.0, 3.5 Hz)
					3.30 (J=13.5, 6.0 Hz)	2.85 (J=19.0, 7.9 Hz)
Phenyl (5H)	7.82~8.00 (m)	—	7.08~7.36 (m)	—	7.1~7.3 (m)	—
Phenyl (4H)	—	7.30~7.67 (m)	—	6.83 (m)	—	6.89 (m)
15-CH_3	—	—	—	—	3.64 (s)	3.73 (s)
17-CH_3	—	—	1.96 (s)	1.94 (s)	1.99 (s)	1.98 (s)

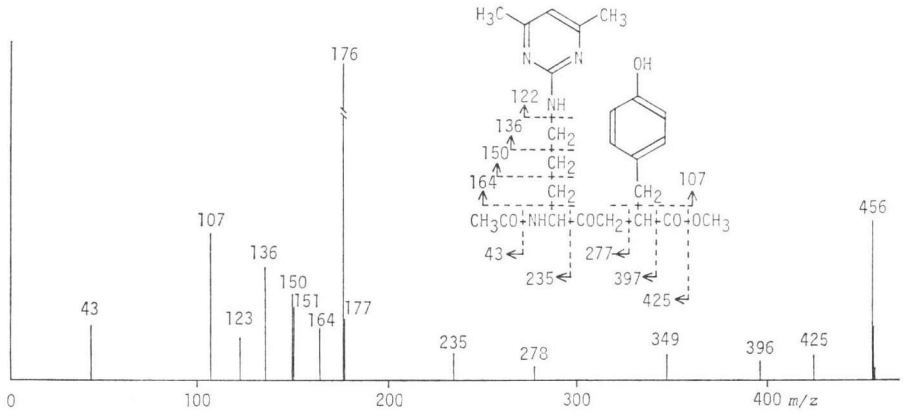
[a]) Numbering of carbon atoms are illustrated in Chart 1.
[b]) See Chart 1.
[c]) In D_2O (external TMS δ=0).
[d]) In CD_3OD - $CDCl_3$ (internal TMS δ=0).
[e]) In $CDCl_3$ (internal TMS δ=0).

Table 2. ^{13}C NMR spectral data for arphamenines A, B and their derivatives.

Position[a]	Compounds[b]			
	I-a[c]	I-b[c]	IV-a[d]	IV-b[d]
1	182.1 s	179.7 s	174.8 s	174.9 s
2	44.0 d	42.7 d	41.6 d	41.6 d
3	41.2 t	41.2 t	40.8 t	40.9* t
4	206.5 s	206.5 s	207.3 s	207.3 s
5	58.9 d	58.9 d	57.8 d	57.8 d
6	26.9 t	27.0 t	28.2 t	28.4 t
7	24.2 t	24.2 t	25.4 t	25.6 t
8	41.2 t	41.2 t	40.8 t	40.4* t
9	157.6 s	157.6 s	160.3 s	162.0 s
10, 12	—	—	167.3 s×2	167.6 s×2
11	—	—	109.5 d	109.9 d
13, 14	—	—	23.4 q×2	23.7 q×2
15	—	—	51.9 q	51.9 q
16	—	—	170.1 s	170.5 s
17	—	—	23.6 q	23.6 q
1′	38.2 t	36.8 t	37.7 t	36.5 t
2′	139.6 s	130.7 s	138.1 s	129.2 s
3′, 7′	129.5 d×2	131.3 d×2	128.6 d×2	130.1 d×2
4′, 6′	130.0 d×2	116.3 d×2	128.9 d×2	116.0 d×2
5′	127.6 d	155.1 s	126.8 d	155.6 s

a) Numbering of carbon atoms are illustrated in Chart 1.
b) See Chart 1.
c) In D_2O (internal dioxane $\delta=67.4$).
d) In $CDCl_3$ (internal TMS $\delta=0$).
* These signals may be exchanged.

Fig. 2. Mass spectrum of **IV-b**.



spectrometry, **I-a** was connected to a derivative in which the amino group was acetylated (**II-a**), the guanidino group was connected to dimethylpyrimidine[2] (**III-a**), and the carboxyl group was esterified (**IV-a**), as shown in Chart 1. The mass spectrum of **IV-a** thus obtained is shown in Fig. 1. The fragmentation pattern of the EI-mass spectrum of **IV-a** clearly supported the postulated structure. From the above-mentioned results, the structure of arphamenine A was determined to be 5-amino-8-guanidino-4-oxo-2-phenylmethyl octanoic acid.

Arphamenine B (**I-b**) was obtained as its monohydrochloride monohydrate, and its molecular formula was established as $C_{16}H_{24}N_4O_4 \cdot HCl \cdot H_2O$ by elemental analysis and field desorption mass spectrometry [m/z 337 (M+1)] as reported in a previous paper[1]. From the molecular formula, which has one more oxygen atom than **I-a**, UV [$\lambda_{max}^{H_2O}$ 275 nm (ε 1,040)] and ^{1}H NMR (Table 1) spectra, the structure of **I-b** was suggested to be *p*-hydroxy **I-a**. The potentiometric titration showed the presence of a phenolic function (pKa 9.8), in addition to carboxyl (3.8), amino (8.0) and guanidino (>12) groups. The ^{13}C NMR spectrum (Table 2) also supported the postulated structure.

In order to confirm the proposed structure, **IV-b** (see Chart 1) was derived from **I-b** in the same manner as for the preparation of **IV-a** from **I-a**, and analyzed by mass spectrometry (Fig. 2). The fragmentation pattern clearly supported the proposed structure; 5-amino-8-guanidino-2-(4-hydroxyphenylmethyl)-4-oxooctanoic acid.

The absolute configuration at C-2 and C-5 of arphamenine was determined by X-ray crystallographic analysis of **II-a** methyl ester hydrochloride. Crystals were grown from methanol-benzene (mp 215~216°C). A crystal of approximate dimensions 0.1×0.05×0.6 mm was chosen for X-ray study Diffraction data were collected on a Philips PW1100 diffractometer using CuKα radiation monochromated by a graphite plate. The crystal data are given in Table 3. Intensitites were measured by the $\theta-2\theta$ scan method with a scan speed of 0.0668°/second in θ. The scans were repeated twice when the total counts recorded in a single scan were less than 3000. A total of 2088 reflections were measured as above the 2σ (**I**) level out of 2304 within a 2θ range of 6°—150°. 566 h$\bar{k}l$ Friedel reflections were measured immediately after the measurement of corresponding hk$l$ reflections up to the 4th layer of l. The structure was solved by the heavy atom method coupled with the method of anomalous dispersion using f′ and f″ values of Cl for CuKα radiation. The result revealed directly the absolute configuration as shown in Fig. 3. It was further confirmed by the comparison of $|F_0(hkl)|^2 / |F_0(h\bar{k}l)|^2$ values with the calculated ones.

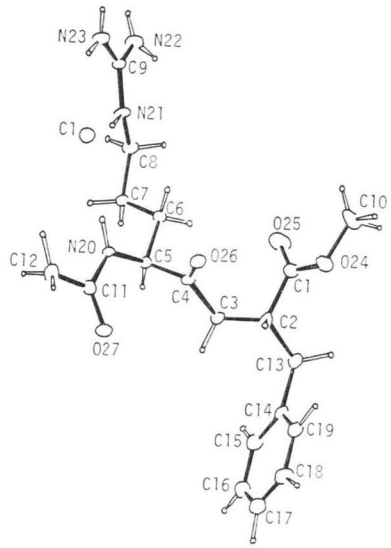
The refinement of the structural parameters was carried out with the method of block-diagonal-matrix least-squares including all the hydrogen atoms with isotropic temperature factors. The final R value was 0.038 allowing for the effect of anomalous dispersion for Cl*. The chloride ion forms hydrogen bonds to N20 [3.304(4)Å], N21 [3.163(3)Å] and N23 [3.391(4)Å] of the same molecule and to N22 [3.238(4)Å] of the neighbouring molecule (see Fig. 3).

Table 3. Crystal data.

N^5-Acetylarphamenine A methyl ester hydrochloride
$C_{19}H_{28}N_4O_4 \cdot HCl$, FW=412.9, Monoclinic $P2_1$,
a=10.831 (5), b=9.722 (5), c=11.771 (5)Å,
β=116.97 (6)°, U=1104.6 Å^3, Z=2,
D_{cal}=1.241 g/cm^3

Fig. 3. Crystal structure of N^5-acetylarphamenine A methyl ester hydrochloride.

The numbering of atoms are different from those in Chart 1 and Tables 1 and 2.



The X-ray crystallographic study confirmed the structure of arphamenine elucidated by the chemical study, and, further, indicated R and S configurations for the C2 and C5, respectively. Thus, the structure of arphamenine A was determined to be (2R,5S)-5-amino-8-guanidino-4-oxo-2-phenylmethyl octanoic acid, which is isosteric of L-arginyl-L-phenylalanine, a substrate of aminopeptidase B. It is interesting to note that arphamenines are a new class of aminopeptidase inhibitors, with a methylene ketone (–CO–

* A list of atomic parameters was sent to Cambridge Crystallographic Data Centre. The structure factor table mat can be obtained from one of the authors (Y. I.) upon request.

CH_2–) in the place of the scissile peptide bond (–CO–NH–) of the substrate.

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References

1) Umezawa, H.; T. Aoyagi, S. Ohuchi, A. Okuyama, H. Suda, T. Takita, M. Hamada & T. Takeuchi: Arphamenines A and B, new inhibitors of aminopeptidase B, produced by bacteria. J. Antibiotics 36: 1572~1575, 1983
2) Gilbert, III, H. F. & M. H. O'Leary: Modification of arginine and lysine in proteins with 2,4-pentanedione. Biochemistry 14: 5194, 1975