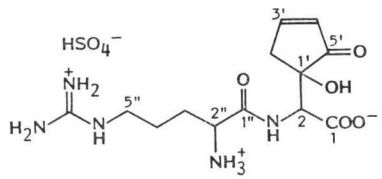
LL-BM726: A NOVEL DIPEPTIDE ANTIBIOTIC

Sir:

A number of years ago during the course of a program designed to uncover Gram-negative antibiotics, a weakly active material was produced by fermentations of an unidentified Streptomycete (Lederle culture BM726). This strongly basic (Sakaguchi positive), water-soluble antibiotic $[\alpha]_D^{25}$ $+28\pm2°$ (*c* 0.7, H_2O), was isolated from mash filtrate using a dextran weak cation exchanger followed by elution with a salt gradient. The active material was then recovered from the salt solutions by use of granular carbon. A recent re-examination of the spectral data along with some new mass spectral results (fast atom bombardment mass spectrometry-FABMS) have enabled us to unravel the structure as that of **I**, a dipeptide consisting of L-arginine and a novel cyclopentenonyl glycine.



I

Elemental analyses of **I** indicated the formula $C_{13}H_{21}O_5N_5 \cdot 2H_2SO_4 \cdot 2H_2O$. Confirmation was obtained by high-resolution FABMS which showed a strong $(M+H)^+$ ion at *m/z* 328.1619. Esterification with methanol under Fisher conditions gave the methyl ester **II** (M+H=342, FABMS) and catalytic reduction of **I** over palladium-charcoal gave a dihydro derivative **III** (M+H=330).

The 400 MHz ^1H NMR spectrum of **I** in D_2O showed arginyl resonances which match exactly in chemical shift and splitting pattern those recorded in the literature[1] at 400 MHz for an *N*-terminal arginyl pentapeptide. Chemical confirmation of arginine was obtained by TLC comparison with an authentic sample following 6 N hydrochloric acid hydrolysis and by isolation (in low yield) of the crystalline phenylthiohydantoin of L-arginine after treatment of **I** with phenylisothiocyanate under Edman conditions. Attempts to isolate the cyclopentenonyl glycine from a second stage Edman were not successful due to decomposition into several products that we were unable to isolate.

The vinyl and methylene ^1H NMR signals of the cyclopentenonyl moiety comprise a 4-proton system that uniquely accounts for the splitting patterns observed (Table 1). The H-2′, geminal protons resonate at δ 2.60 and 3.10, each as two

Table 1. NMR chemical shifts (D_2O, pH 6.5) in ppm and δ values.

Carbon position	I		III	
	^{13}C (20 MHz)	^1H (400 MHz)	^{13}C (75 MHz)	^1H (300 MHz)
1	174.0 (s)*		174.2	
2	60.7 (d)	4.60 (s)**	59.1	4.62 (s)
1′	77.7 (s)		79.9	
2′	41.2 (t)	2.60, 3.10 (each dt, 20, 1.2, 2.5 Hz)	36.9	1.6~2.0 (m)
3′	166.8 (d)	7.80 (dt, 6.1, 2.5 Hz)	33.5	1.6~2.0 (m)
4′	131.9 (d)	6.20 (dt, 6.1, 1.2 Hz)	39.3	2.45 (m)
5′	211.0 (s)		221.1	
1″	170.6 (s)		170.4	
2″	53.7 (d)	4.12 (t)	53.8	4.10 (t)
3″	24.2 (t)	1.95 (m)	24.2	1.95 (m)
4″	28.8 (t)	1.65 (m)	28.8	1.70 (m)
5″	41.2 (t)	3.20 (t)	41.2	3.23 (t)
6″	157.5 (s)		157.6	

* Off-resonance multiplicity.

** Multiplicity.

doublets of triplets due to a large 20 Hz geminal coupling and two other very small couplings of 2.5 and 1.2 Hz with the H-3′ and H-4′ vinyl protons at δ 7.80 and 6.20 respectively. These latter two signals each appear as partially superimposed doublets of triplets with a $J_{3',4'}$ of 6.1 Hz. In the ^{13}C NMR spectrum of **I** (Table 1) the ketone carbonyl signal resonates at 211 ppm which shifts downfield to 224 ppm in the dihydro derivative, **III**. These chemical shifts agree well with the corresponding signals in the spectra of cyclopentenone (208 ppm) and cyclopentanone (218 ppm)[2,3]. Also, the vinyl carbon resonances at 166.8 and 131.9 ppm agree nicely with those in the spectrum of cyclopentenone at 165.1 and 133.8 ppm[2].

The ^{13}C signals at 60.7 and 77.2 ppm are assigned to C-2 and C-1′ respectively of the cyclopentenonyl glycine subunit. The methine signal at 60.7 ppm is very broad relative to the others in the spectrum except for the carboxyl signal at 174 ppm which is also broad and of low intensity. In contrast, these two signals are quite sharp in the spectrum of the methyl ester **II** and with normal intensities.

The UV spectra in H_2O of **I** and **II** are characteristic of cyclopentenone with $\lambda_{max}^{H_2O}$ at 218 nm (ε 4,000). Carbonyl IR bands (KBr) at 1710 and 1675 cm^{-1} for **I**, 1740, 1720 and 1675 cm^{-1} for the ester **II** and 1740 and 1675 cm^{-1} for the dihydro derivative **III** are consistent with the carbonyl functionality present in these three compounds.

Mass spectral data, particularly from collision activation (CA) spectra of **I** and **III**, show fragment ions which confirm the above spectral and chemical deductions. The relevant fragments

Fig. 1.

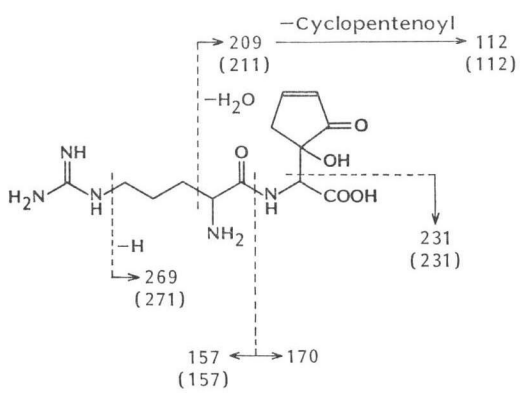
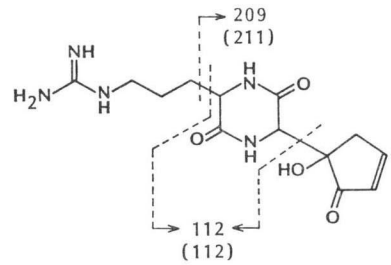


Fig. 2.



are shown in Fig. 1 for **I** with those values pertaining to the dihydro derivative, **III**, in parentheses.

Strong fragment ions are observed at 209 and 112 mass units which we postulate as originating from the loss of water to form a diketopiperazine, followed by the losses of the arginyl and cyclopentenonyl side chain moieties as shown in Fig. 2. These ions are even more prominent in the spectrum of the methyl ester as might be anticipated.

Except for the *S*-configuration of the arginine moiety, the stereochemistry of the other two chiral centers is unknown. A CD curve of **I** shows a positive Cotton effect at 302 nm ($\Delta\varepsilon$ +0.72) for the ketone n-π^* transition and based on SNATZKE's studies suggests the *R*-configuration at 1′ [4]. However, the lack of a close model makes this assignment tenuous.

It is noteworthy that synthetic (1′*R*,2*S*)-(2′-cyclopentene)glycine is a potent inhibitor of growth of *Escherichia coli*[5,6]. More recently both (1′*R*,2*S*)- and (1′*S*,2*S*)-(2′-cyclopentene)-glycine were isolated from seeds of *Hydrocarpus anthelminthica*[7].

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References

1) KRISHNA, N. R.; G. A. HEAVNER & J. B. VAUGHN, Jr.: Proton nuclear magnetic resonance investigation of the active site fragment of splenin, an immunoregulatory polypeptide. J. Am. Chem. Soc. 105: 6930~6934, 1983
2) LEVY, G. C.; R. L. LICHTER & G. L. NELSON: Carbon-13 Nuclear Resonance Spectroscopy. 2nd Ed., A Wiley-Interscience Publication, John Wiley and Sons, New York, 1980
3) JOHN, L. F. & W. C. JANKOWSKI: Carbon-13 NMR Spectra. A Wiley-Interscience Publication, John Wiley and Sons, New York, 1972
4) SNATZKE, G.: Optical Rotary Dispersion and Circular Dichroism. p. 218, Heijden and Sons, London, 1967
5) DENNIS, R. L.; W. J. PLANT, C. G. SKINNER, G. L. SUTHERLAND & W. SHIVE: Synthesis of 2-cyclopentene-1-glycine, an inhibitory amino acid analog. J. Am. Chem. Soc. 77: 2362~2364, 1955
6) SANTOSO, S.; T. KEMMER & W. TROWITZSCH: Separation and determination of configuration of the stereoisomers of *N*-acetyl-2-(2′-cyclopentenyl)glycine. Liebigs Ann. Chem. 1981: 658~667, 1981
7) CRAMER, U.; A. G. REHFELDT & F. SPENER: Isolation and biosynthesis of cyclopentenylglycine, a novel nonproteinogenic amino acid in Flacourtiaceae. Biochemistry 19: 3074~3080, 1980