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Zelkovamycin, a New Cyclic Peptide Antibiotic from *Streptomyces* sp. K96-0670

II. Structure Elucidation

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The structure of antibiotic zelkovamycin was elucidated as a cyclic peptide comprising glycyl, 2-aminobutanoyl, 2-amino-2-butenoyl, *N*-methyl glycyl, alanyl, 1,3-thiazoyl, 7-methoxytryptophanyl and 2-methyldehydrothreonyl residues. The sequence of the amino acids was established by spectroscopic studies including ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, ^{13}C-^{1}H HMQC, ^{13}C-^{1}H HMBC, ^{15}N-^{1}H HMQC and ^{15}N-^{1}H HMBC NMR experiments.

Zelkovamycin, an antibiotic, was isolated from the culture broth of *Streptomyces* sp. K96-0670. The fermentation, isolation and biological properties of zelkovamycin are described in the preceding paper[1]. We report herein the structure elucidation of zelkovamycin (Fig. 1).

Fig. 1. Structure of zelkovamycin.

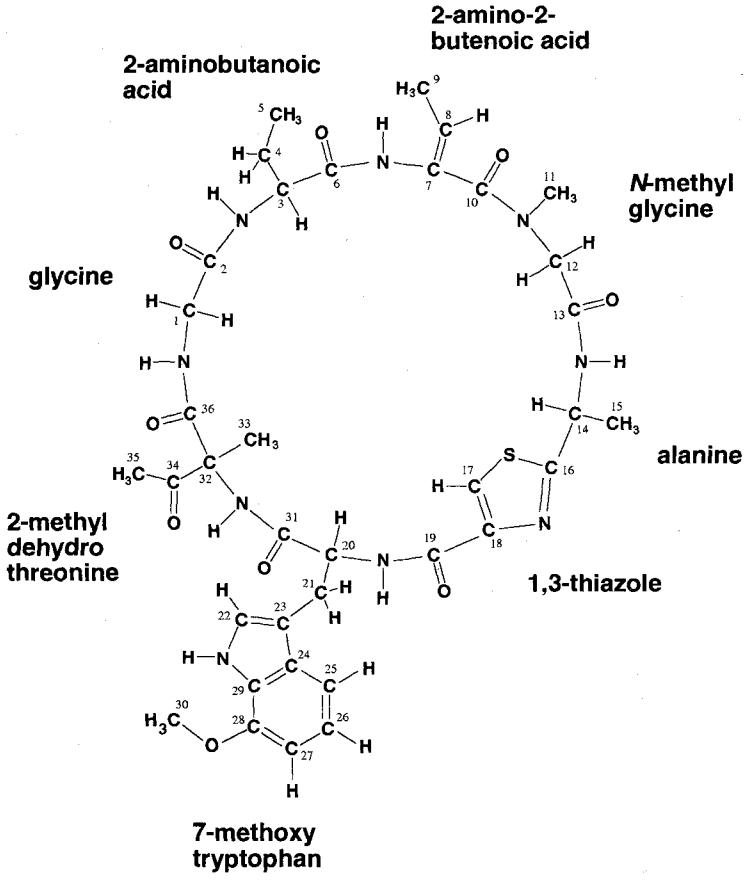


Table 1. Physico-chemical properties of zelkovamycin.

Appearance	White powder
Molecular formula	$C_{36}H_{45}N_9O_9S$
Molecular weight	779
FAB-MS (*m/z*) Positive	780 $[M+H]^+$
	802 $[M+Na]^+$
Negative	778 $[M-H]^-$
HRFAB-MS (*m/z*) (negative)	
MF-H	$C_{36}H_{44}N_9O_9S$
Calcd:	778.2982
Found:	778.2986
UV $\lambda_{max}^{CH_3OH}$ nm (ε)	205 (57,800)
	222 (59,300)
	244sh (24,000)
	284 (6,000)
	292 (5,300)
IR ν_{max}^{KBr} (cm^{-1})	3307, 1653, 1537,
	1495, 1406, 1362,
	1257, 1092
$[\alpha]_D^{28}$ (*c* 0.1, CH_3OH)	+ 84.3 °
Solubility	
Soluble:	CH_3OH, $CHCl_3$, CH_3CN, Acetone, C_2H_5OH, Ethyl acetate
Insoluble:	H_2O, *n*-Hexane
Color reaction	
Positive:	50% H_2SO_4
Negative:	Ninhydrin reagent

Methods

General Experimental Procedures

UV spectra were recorded on a Shimadzu UV-200S spectrophotometer. IR spectra were recorded on a Horiba FT-210 infrared spectrometer. Optical rotations were obtained with a JASCO DIP-370 digital polarimeter. EI-MS spectra were recorded on a JEOL JMS-D 100 mass spectrometer at 20 eV. FAB-MS spectra were recorded on a JMS-DX300 mass spectrometer. The various NMR spectra were obtained on a Varian XL-400 spectrometer.

Results

Physico-chemical Properties of Zelkovamycin

Physico-chemical properties of zelkovamycin are summarized in Table 1. The IR absorption at 1653 and 1537 cm^{-1} suggested the presence of carbonyl groups in the structure[2].

Structure of Zelkovamycin

The molecular formula of zelkovamycin was determined to be $C_{36}H_{45}N_9O_9S$ on the basis of HRFAB-MS measurement. The ^{13}C NMR spectrum ($CDCl_3$) showed 36 resolved peaks (Table 2), which were classified into seven methyl (one of which is an *O*-methyl), two *C*-methylene, two *N*-methylene, three *N*-methine, six sp^2 methine, one sp^3 quarternary, seven sp^2 quaternary and eight carbonyl carbons by analysis of the DEPT spectra. The ^1H NMR spectrum displayed 45 proton signals (Table 2, Fig. 2). The results support the molecular formula. The connectivity of proton and carbon atoms was established by the ^{13}C-^1H HMQC spectrum (Table 2). In addition to the molecular formula, seven proton signals (δ 10.86, 9.31, 8.89, 8.55, 7.98, 6.90 and 5.68) suggested the presence of NH protons. Furthermore, ^{15}N-^1H coupling of J_{NH} in the ^{15}N-^1H HMQC spectrum confirmed the seven NH residues (δ 139.5, 130.3, 128.8, 121.8, 119.6, 113.5 and 102.0). Analysis of the ^1H-^1H COSY spectrum revealed the eight partial structures I to VIII (Fig. 3). ^{13}C-^1H long range couplings of ^2J and ^3J observed in the ^{13}C-^1H HMBC experiment (Fig. 4) gave the following evidence:

1) The long range couplings from 1-NH (δ 5.68) to C-1 (δ 42.3) and C-36 (δ 166.9), from H-1 (δ 1.57) to C-2 (δ 170.7) and C-36, and from H-1 (δ 3.61) to C-2 and C-36 showed the presence of the glycine moiety containing the partial structure I.

2) The cross peaks from 3-NH (δ 6.90) to C-2, C-3 (δ 54.6) and C-4 (δ 21.0), from H-3 (δ 4.06) to C-2, C-4, C-5 (δ 10.2) and C-6 (δ 169.1), from H_2-4 (δ 2.00) to C-3, C-5 and C-6, and from H_3-5 (δ 0.91) to C-3 and C-4 indicated that the 2-aminobutanoic acid moiety containing the partial structure II is attached to the glycine moiety.

3) The long range couplings from 7-NH (δ 9.31) to C-6, C-7 (δ 130.4) and C-8 (δ 110.4), from H-8 (δ 5.17) to C-7, C-9 (δ 11.3) and C-10 (δ 169.0), and from H_3-9 (δ 1.87) to C-8 showed the presence of the 2-amino-2-butenoic acid moiety containing partial structure III and attachment to the 2-aminobutanoic acid moiety. Observation of NOE between H_3-9 (δ 1.87) and 7-NH (δ 9.31) elucidated that the stereochemistry of the C-7-C-8 olefin as Z.

4) The long range couplings from H_3-11 (δ 3.09) to C-10 and C-12 (δ 51.1), from H-12 (δ 4.99) to C-10, C-11 (δ 37.4) and C-13 (δ 167.0), and from H-12 (δ 3.40) to C-10, C-11 and C-13 showed the presence of the *N*-methyl glycine (sarcosine) moiety containing the partial structure

Table 2. NMR chemical shifts of zelkovamycin.

Carbon No.	^{13}C chemical shifts ppm[a]	^{1}H chemical shifts ppm[b]	^{15}N chemical shifts ppm[c]
1-NH		5.68 (1H, m)	102.0 (J_{NH}=94 Hz)
C-1	42.3	1.57 (1H, d, J=5.4 Hz)	
		3.61 (1H, d, m)	
C-2	170.7		
3-NH		6.90 (1H, d, J=6.5 Hz)	121.8 (J_{NH}=95 Hz)
C-3	54.6	4.06 (1H, m)	
C-4	21.0	2.00 (2H, m)	
C-5	10.2	0.91 (3H, d, J=7.3 Hz)	
C-6	169.1		
7-NH		9.31 (1H, s)	130.3 (J_{NH}=93 Hz)
C-7	130.4		
C-8	110.4	5.17 (1H, q, J=7.3 Hz)	
C-9	11.3	1.87 (3H, d, J=7.3 Hz)	
C-10	169.0		
12-N			108.9
C-11	37.4	3.09 (3H, s)	
C-12	51.1	3.40 (1H, d, J=16.7 Hz)	
		4.99 (1H, d, J=16.7 Hz)	
C-13	167.0		
14-NH		8.89 (1H, d, J=8.4 Hz)	128.8 (J_{NH}=94 Hz)
C-14	45.2	5.50 (1H, dq, J=8.4, 3.0 Hz)	
C-15	20.6	1.72 (3H, d, J=3.0 Hz)	
C-16	170.9		
C-17	122.8	8.09 (1H, s)	
18-N			307.9
C-18	150.4		
C-19	159.7		
20-NH		8.55 (1H, d, J=7.6 Hz)	113.5 (J_{NH}=95 Hz)
C-20	52.4	5.20 (1H, m)	
C-21	28.0	3.55 (1H, m)	
		4.18 (1H, dd, J=15.9, 3.8 Hz)	
22-NH		10.86 (1H, s)	139.5 (J_{NH}=101 Hz)
C-22	124.9	6.97 (1H, d, J=2.7 Hz)	
C-23	106.2		
C-24	116.0		
C-25	105.5	6.77 (1H, d, J=8.0 Hz)	
C-26	122.4	7.02 (1H, t, J=8.0 Hz)	
C-27	99.1	6.41 (1H, d, J=8.0 Hz)	
C-28	153.4		
C-29	136.6		
C-30	55.2	3.87 (3H, s)	
C-31	171.2		
32-NH		7.98 (1H, s)	119.6 (J_{NH}=94 Hz)
C-32	69.7		
C-33	19.8	1.70 (3H, s)	
C-34	200.5		
C-35	23.1	2.24 (3H, s)	
C-36	166.9		

[a] Each sample was dissolved in $CDCl_3$. Chemical shifts are shown with reference to $CDCl_3$ as 77.0 ppm.
[b] Chemical shifts are shown with reference to $CDCl_3$ as 7.26 ppm.
[c] Chemical shifts are shown with reference to formamide as 115 ppm.

IV and its attachment to the 2-amino-2-butenoic acid moiety.

5) The long range couplings from 14-NH (δ 8.89) to C-13, C-14 (δ 45.2) and C-15 (δ 20.6), from H-14 (δ 5.50) to C-13, C-15 and C-16 (δ 170.9), and from H_3-15 (δ 1.72) to C-14 and C-16 showed the presence of the alanine moiety containing the partial structure V and its attachment to the *N*-methyl glycine moiety.

6) The presence of 1,3-thiazole moiety was suggested by long range couplings from H-17 (δ 8.09) to C-16, C-18 (δ 150.4) and C-19 (δ 159.7) and the chemical shifts of C-16, C-17 (δ 122.8), C-18 and C-19 comparable to those

Fig. 2. ^1H NMR spectrum of zelkovamycin in $CDCl_3$.

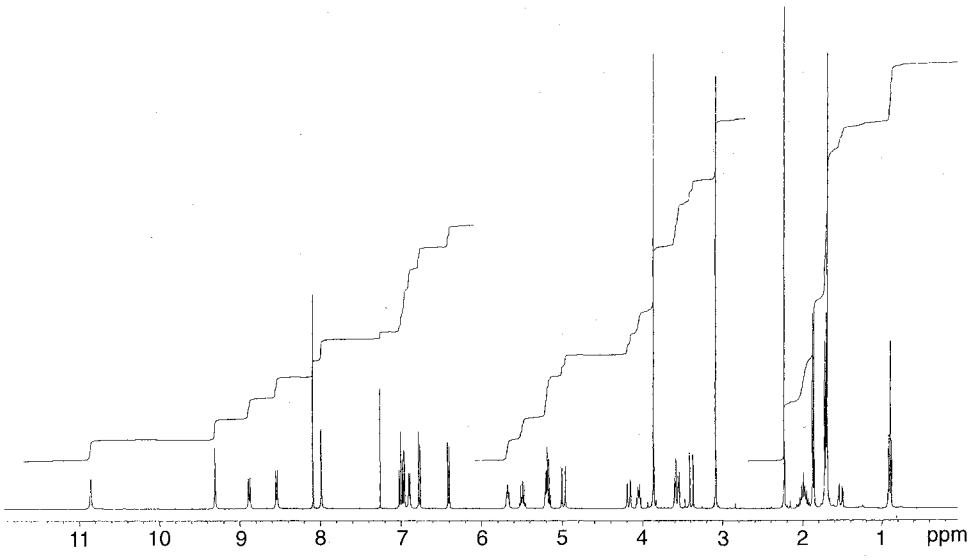
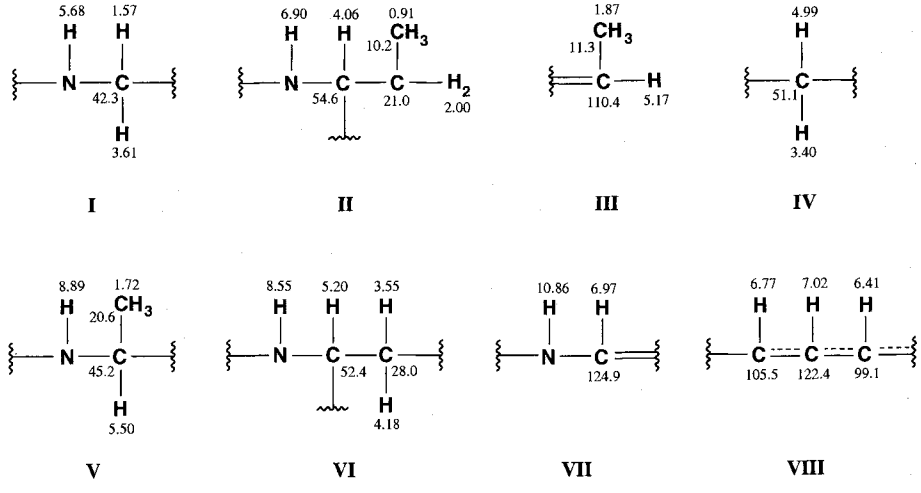


Fig. 3. Partial structures I to VIII of zelkovamycin.



of thiazole carbons[3~6]. These correlations show that the 1,3-thiazole moiety is attached to the alanine moiety.

7) In the ^{13}C-^1H HMBC experiments, the long range couplings were observed from 20-NH (δ 8.55) to C-19 and C-31 (δ 171.2), from H-20 (δ 5.20) to C-19 and C-31, from H_2-21 (δ 3.55, 4.18) to C-20 (δ 52.4), C-22 (δ 124.9), C-23 (δ 106.2), C-24 (δ 116.0) and C-31, from 22-NH (δ 10.86) to C-22 and C-29 (δ 136.6), from H-22 (δ 6.97) to C-21 (δ 28.0), C-23, C-24 and C-29, from H-25 (δ 6.77) to C-24, C-26 (δ 122.4), C-27 (δ 99.1) and C-29, from H-26 (δ 7.02) to C-24, C-25 (δ 105.5), C-27 and C-28 (δ 153.4), from H-27 (δ 6.41) to C-25, C-26, C-28 and C-29, and from H_3-30 (δ 3.87) to C-28. Therefore, the presence of the 7-methoxy tryptophan moiety containing the partial structures VI, VII and VIII was suggested and supported by a fragment ion peak (m/z 620) in the FAB-MS. These correlations show the attachment of the 7-methoxy tryptophan moiety to the 1,3-thiazole moiety.

Finally, 8) the cyclic structure was suggested by the long range couplings from 32-NH (δ 7.98) to C-31, C-33 (δ 19.8), C-34 (δ 200.5) and C-36, from H_3-33 (δ 1.70) to C-32 (δ 69.7), C-34 and C-36, and from H_3-35 (δ 2.24) to C-32 and C-34, indicating that the 2-methyl dehydro threonine moiety is attached to the glycine moiety. Furthermore, the cyclic peptide structure for zelk-

Fig. 4. ^{1}H-^{1}H COSY, ^{13}C-^{1}H HMQC and ^{13}C-^{1}H HMBC experiments of zelkovamycin.

^{1}H-^{1}H COSY: ▬, ^{13}C-^{1}H HMBC: H ⟶ C

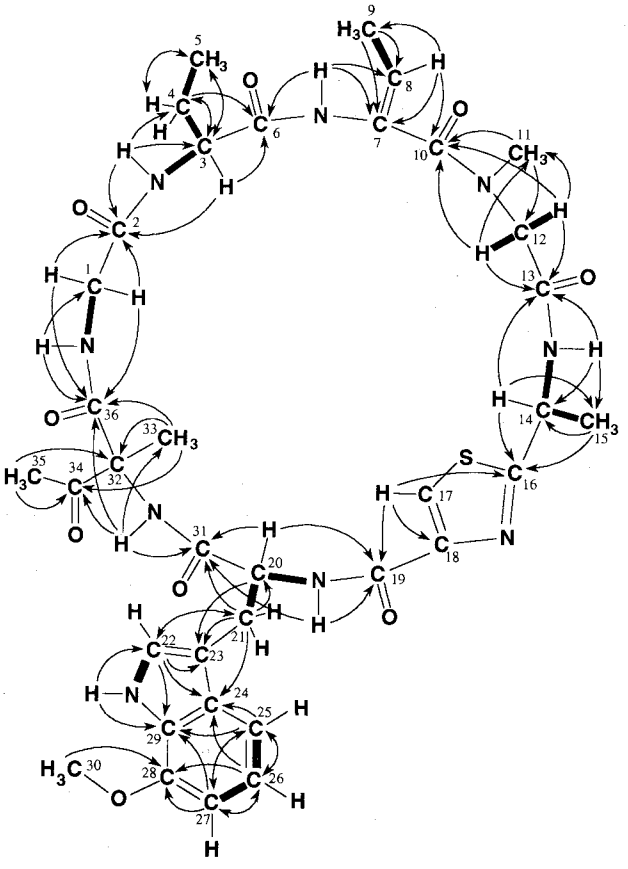
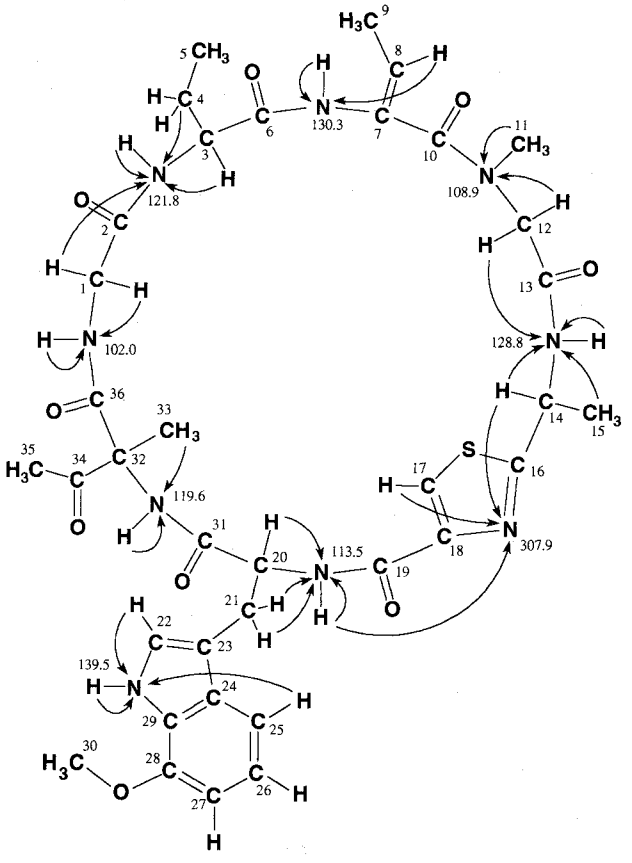


Fig. 5. ^{15}N-^{1}H HMQC and ^{15}N-^{1}H HMBC experiments of zelkovamycin.

^{15}N-^{1}H HMQC and HMBC: H ⟶ N



ovamycin was confirmed by ^{15}N-^{1}H heteronuclear NMR experiments. In fact, in the ^{15}N-^{1}H HMBC experiment (Fig. 5), ^{15}N-^{1}H long range couplings were observed from H-1 (δ 3.61) to 1-N (δ 102.0), from H-1 (δ 1.57), H-3 and H-4 to 3-N (δ 121.8), from H-8 to 7-N (δ 130.3), from H_3-11 and H-12 (δ 4.99) to 12-N (δ 108.9), from H-12 (δ 3.40, H-14 and H_3-15 to 14-N (δ 128.8), from H-14, H-17 and 20-NH to 18-N (δ 307.9), from H-20 and H_2-21 to 20-N (δ 113.5), from H-22 and H-25 to 22-NH (δ 139.5), and from H_3-33 to 32-N (δ 119.6).

Taken together, the structure of zelkovamycin was elucidated as cyclo[glycyl-(2-aminobutanoyl)-(2-amino-2-butenoyl)-(*N*-methylglycyl)-alanyl-1,3-thiazoyl-7-methoxytryptophanyl-(2-methyldehydrothreonyl)] (Fig. 1).

Discussion

The total structure of zelkovamycin was elucidated without chemical degradation by spectroscopic techniques including ^{15}N-^{1}H NMR. It was found to be a cyclic peptide composed of highly modified amino acids.

The cyclic peptide contains unique 7-methoxy tryptophan and 2-methyldehydrothreonine residues and a thiazole moiety derived from two amino acids. Cyclic peptide antibiotics A21459 A and B[3] and nosiheptide[4], produced by *Actinoplanes* and *Streptomyces* strains, respectively, also have a modified tryptophan residue in their structures. However, the modified positions are different among these compounds in that a methoxy group is attached at the 5-position in A21459 and a methylene group is attached at the 4-position in nosiheptide, whereas zelkovamycin has a tryptophan residue modified at the 7-position with a methoxy group. The 2-methyldehydrothreonine is unusual, and its biosynthesis has not been studied. Several cyclic peptides have been reported to contain thiazole residue(s)[3~6]. The thiazole ring is usually derived from cysteine and an amino acid such as glutamic acid[4,5], glycine[4,6], glu-

tamine[6], threonine[5], cysteine[4,5] or alanine[3]. As for zelkovamycin, the thiazole seems to originate from cysteine and alanine. Altogether, zelkovamycin shares several similarities with A21459.

Acknowledgment

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