Studies on Bottromycins

II. Structure Elucidation of Bottromycins B2 and C2

MIYUKI KANEDA

Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan

(Received for publication June 5, 2002)

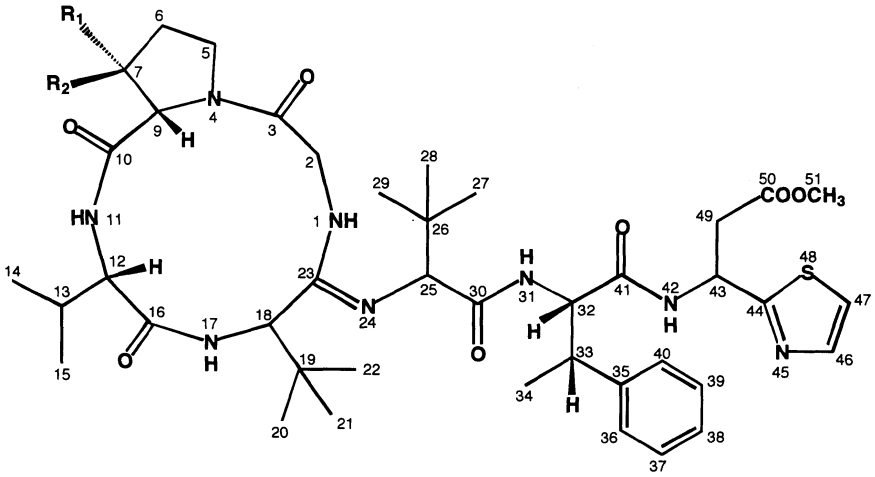
Bottromycins were first isolated from *Streptomyces bottropensis* by WAISVISZ *et al.*[1] and were found especially active against Gram-positive bacteria and Mycoplasma[1~3]. The structure of bottromycin A2 (**1**), the main active component of the complex, was first presented by NAKAMURA *et al.* in 1966 as a linear peptide[4,5] composed of seven constituent amino acid residues including four unusual amino acid residues. However, the structure was revised in 1976 by TAKAHASHI *et al.*[6] as a partly cyclic structure. In 1983, SCHIPPER[7] finally established the structure of **1**, using ^{1}H and ^{15}N NMR spectroscopy, in which the chain moiety links to the cyclic moiety through the imino nitrogen of the amidine group as shown in Fig. 1.

The re-revised structure of bottromycin A2 led the two other components of the bottromycin complex, bottromycins B2 and C2[3,5,8], to be reinvestigated. For this purpose, I have carried out the unequivocal NMR assignments of all the protons and carbons of bottromycin A2 (**1**), which is the main component and whose sample is abundantly available. The established NMR assignments were reported in the preceding paper[9] and confirmed the re-revised structure of **1** proposed by SCHIPPER[7]. In this paper, I report the structure elucidation of bottromycins B2 (**2**) and C2 (**3**), which has been made by the analyses of their one and two-dimensional NMR spectra (^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H COSY, HSQC, HMBC and ROESY) and MS spectral data. During the examination of their NMR data, comparison with the published NMR assignments[9] of **1** was found extremely useful. The carbon, nitrogen and sulfur atoms constituting the skeleton of **2** and **3** are numbered as shown in Fig. 1 according to the tentative numbering of **1**[9].

Bottromycin B2 (2)

A pure sample of bottromycin B2 (**2**) has been obtained as white powder from the crude mixture of the bottromycin complex by the chromatographic methods reported[3]. Low and high-resolution FAB-MS spectra were obtained on a JEOL JMS-SX102 spectrometer. From the FAB-MS data of **2**, the molecular weight of 808 was obtained and the

Fig. 1. Structures of bottromycins A2 (**1**), B2 (**2**) and C2 (**3**).

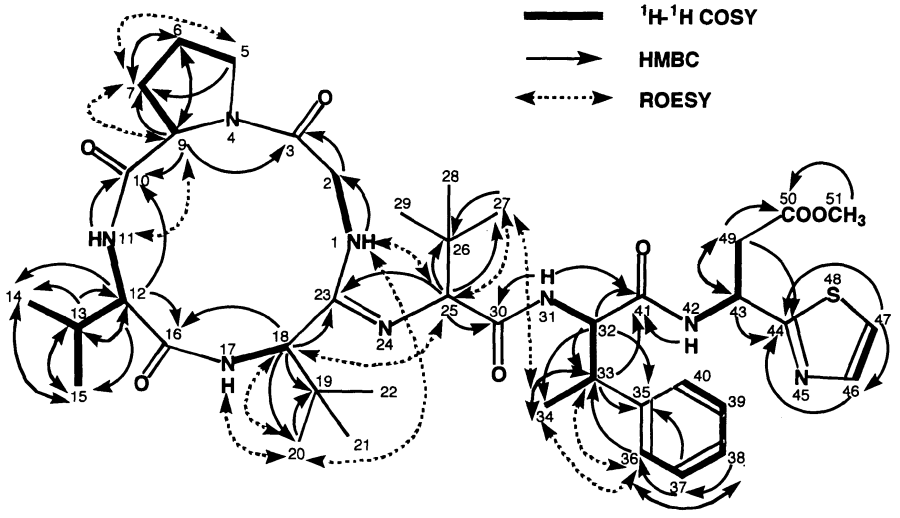


Bottromycin A2 (1): R_1 = CH_3 (C-8), R_2 = H
Bottromycin B2 (2): R_1 = R_2 = H
Bottromycin C2 (3): R_1 = CH_3 (C-8), R_2 = CH_3 (C-8')

* Corresponding author: mikan@hiroshima-u.ac.jp

molecular formula was determined to be $C_{41}H_{60}N_8O_7S$ from HRFAB-MS (found $(M+H)^+$, *m/z* 809.4364; calcd for $C_{41}H_{61}N_8O_7S$, *m/z* 809.4384) and ^{13}C NMR data. This molecular formula is smaller than that of **1** by one CH_2 unit, which indicates that one methyl group of **1** is displaced by one H in **2**. The ^1H and ^{13}C NMR spectra were measured in $CDCl_3$ on a JEOL JNM ALPHA-400 spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C. The ^{13}C multiplicity data were obtained from DEPT experiments. NAKAMURA *et al.* reported[3] that on acid hydrolysis **2** gave L-proline instead of the *cis*-3-methyl-L-proline residue obtained from **1**, but all the other amino acids liberated were the same as **1**. As a matter of fact, the ^1H and ^{13}C NMR spectra of **2** were very similar to those of **1** except for the signals of each proline derivative moiety particularly its carbon signals. In Table 1, the ^{13}C NMR chemical shifts of proline, methylproline and 3,3-dimethylproline residue in **2**, **1** and **3** respectively, are compared. As seen in the table, the methyl signal at δ_C 15.5 in **1** was absent in **2**, and the methine carbon signal at δ_C

Fig. 2. Selected ^1H-^1H COSY, HMBC and ROESY correlations for bottromycin B2.



Correlations concerning 21-CH_3, 22-CH_3 and 28-CH_3, 29-CH_3 are the same as 20-CH_3 and 27-CH_3, respectively, and those concerning 39-CH and 40-CH are the same as 37-CH and 36-CH, respectively.

Fig. 3. Selected ^1H-^1H COSY, HMBC and ROESY correlations for bottromycin C2.

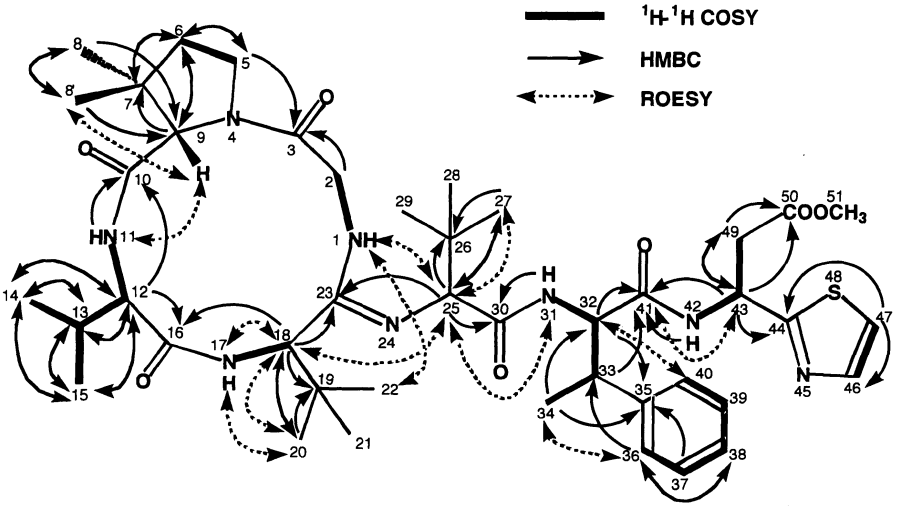


Table 1. Comparison of ^{13}C NMR chemical shifts of each proline moiety of bottromycins B2 (**2**), A2 (**1**) and C2 (**3**).

Position	B2 (**2**) (L-proline)	A2 (**1**) (*cis*-3-methyl-L-proline)	C2 (**3**) (3,3-dimethyl-L-proline)
C-5	47.5t	47.0t	45.9t
C-6	22.8t	30.3t	36.1t
C-7	32.9t	38.5d	43.4s
C-8		15.5q	24.9q
C-8′			28.3q
C-9	61.0d	65.5d	71.6d
C-10	176.7s	174.3s	174.9s

38.5 (C-7) in **1** changed to a methylene signal at δ_C 32.9 in **2**, confirming the existence of proline residue in **2** instead of methylproline in **1**.

The complete assignments of proton and carbon signals of each amino acid residue in **2** were established as shown in Table 2 based on 2D NMR experiments such as ^1H-^1H COSY, HSQC, HMBC and ROESY and by comparison with the NMR assignment data of **1**[9]. The amino acid sequence of **2** was confirmed by long range H–C correlations observed in the HMBC experiments and NOE correlations in the ROESY experiments as shown in Fig. 2. The structure of **2** has thus been established as shown in Fig. 1, which preserves the formation of a cyclic moiety linked with a linear chain moiety similar to that of **1**.

Bottromycin C2 (3)

A pure sample of bottromycin C2 (**3**) has also been obtained from the crude mixture of the bottromycin complex by the chromatographic means reported[3]. The FAB-MS spectrum of **3** gave the molecular weight of 836 and the molecular formula was determined to be $C_{43}H_{64}N_8O_7S$ from HRFAB-MS (found $(M+H)^+$, *m/z* 837.4642; calcd for $C_{43}H_{65}N_8O_7S$, *m/z* 837.4697) and ^{13}C NMR data. This is larger than that of **1** by one CH_2 unit. NAKAMURA *et al.* reported[3] that on acid hydrolysis of **3**, an unidentified proline derivative ($C_7H_{13}NO_2$) was obtained instead of *cis*-3-methyl-L-proline from **1** or proline from **2**, but all the other amino acids liberated were the same as **1**. NAKAMURA *et al.* suggested that **3** would contain either a dimethylproline or an ethylproline residue in the molecule. The ^1H and ^{13}C NMR spectra of **3** were quite similar to those of **1**, but the methyl carbon signal at δ_C 15.5 in **1** was absent in **3**. Instead, two methyl signals were observed at δ_C 24.9 and δ_C 28.3 as shown in Table 1. Furthermore, the methine carbon signal at δ_C 38.5 in **1** was absent in **3**, but instead, one quaternary carbon signal was observed at δ_C 43.4 in **3**. These data could be fully explained by exchanging the 3-methylproline residue of **1** for a 3,3-dimethylproline residue in **3**. In Table 1, the ^{13}C NMR chemical shifts of proline, 3-methylproline and 3,3-dimethylproline residue in **2**, **1** and **3** respectively, are compared. Interestingly, successive substitution of a methyl group at C-7 of the proline residue in **2** that yields **1** and then **3** has produced large and significant influences on all of the carbon chemical shifts of each proline derivative residue of **1** and **3**.

The complete assignments of proton and carbon signals of each amino acid residues of **3** were established as shown in Table 2 in the same manner as **2** based on 2D NMR experiments such as ^1H-^1H COSY, HSQC, HMBC and ROESY and by comparison with the NMR assignment data of **1**[9]. The amino acid sequence of **3** was confirmed by HMBC and ROESY spectral data of **3** as shown in Fig. 3. Thus the structure of **3** has been elucidated as shown in Fig. 1.

Table 2. ^{1}H and ^{13}C NMR assignments for bottromycins B2 (**2**) and C2 (**3**) in $CDCl_3$.

Position	Bottromycin B2 (**2**)		Bottromycin C2 (**3**)		Amino acid residue
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	
N-1		4.04 t-like		3.95 t-like	Glycine
2	48.1	3.56 dd(12.2, 2.7), 3.77 dd(12.2, 4.1)	47.9	3.69*, 3.84 dd(12.1, 4.2)	
3	169.1		169.4		
N-4					L-Proline for **2** or 3,3-Dimethyl-L-proline for **3**
5	47.5	3.67 m	45.9	3.70*	
6	22.8	1.94 m	36.1	1.66 ddd(12.4, 4.0, 4.0), 1.84 ddd(12.4, 7.9, 7.9)	
7	32.9	1.97 m, 2.28 m	43.4		
8			24.9	1.12 s	
8'			28.3	1.16 s	
9	61.0	4.08 dd(9.3, 2.1)	71.6	3.92 s	
10	176.7		174.9		
N-11		7.66*		7.36*	L-Valine
12	68.3	2.32 m	69.1	2.47 dd(11.8, 5.5)	
13	26.9	2.76 m	26.9	2.82 m	
14	19.5	0.70 d(6.6)	19.7	0.75 d(6.7)	
15	19.5	0.75 d(6.4)	20.2	0.81 d(6.5)	
16	171.1		171.0		
N-17		7.15 d(10.5)		6.99 d(10.5)	2-Amino-3,3-dimethylbutyric acid
18	53.9	4.59 d(10.8)	54.0	4.62 d(10.5)	
19	33.0		32.9		
20	27.7	0.98 br s	27.7	1.00 br s	
21	27.7	0.98 br s	27.7	1.00 br s	
22	27.7	0.98 br s	27.7	1.00 br s	
23	157.3		157.3		
N-24					2-Amino-3,3-dimethylbutyric acid
25	70.4	3.92 s	70.4	3.93 s	
26	35.4		35.4		
27	27.8	0.96 br s	27.8	0.97 br s	
28	27.8	0.96 br s	27.8	0.97 br s	
29	27.8	0.96 br s	27.8	0.97 br s	
30	172.8		172.6		
N-31		6.91 d(8.8)		7.00 d(8.0)	3-Methyl-3-phenyl-L-alanine
32	57.1	5.01 dd(8.6, 4.1)	57.5	4.97 dd(8.2, 4.4)	
33	41.9	3.39 m	41.9	3.40 m	
34	16.1	1.37 d(7.1)	15.1	1.34 d(7.1)	
35	141.2		141.3		
36	128.5	7.34 br d	128.8	7.37 br d	
37	128.3	7.31 br t	128.0	7.33 br t	
38	127.0	7.21*	127.2	7.23 br t	
39	128.3	7.31 br t	128.0	7.33 br t	
40	128.5	7.34 br d	128.8	7.37 br d	
41	172.0		172.0		
N-42		7.47 d(6.4)		6.89 br d(7.1)	3-(2-Thiazolyl)-β-alanine
43	48.4	5.62 ddd(7.4, 6.8, 5.6)	48.3	5.55 m	
44	170.1		169.7		
N-45					
46	142.6	7.66 d(3.3)	142.9	7.66 d(3.2)	
47	119.8	7.20 d(3.3)	119.7	7.21 d(3.2)	
S-48					
49	39.3	2.93 dd(16.8, 5.5), 3.06 dd(16.8, 6.8)	39.3	2.88 dd(16.8, 5.6), 3.03 dd(16.8, 6.2)	
50	170.6		170.4		
51	52.1	3.70 s	52.1	3.69 s	

* Signal pattern was unclear due to overlapping.

Similarly to bottromycin A2 (**1**)[10~13], the absolute configurations of C-18, C-25 and C-43 of bottromycins B2 (**2**) and C2 (**3**) are still ambiguous and remain to be established.

Acknowledgment

The author is very grateful to Dr. SHOSHIRO NAKAMURA, Professor Emeritus of Hiroshima University, for kindly providing bottromycin complex mixture and for valuable discussions.

References

1) WAISVISZ, J. M.; M. G. VAN DER HOEVEN, J. VAN PEPPEN & W. C. M. ZWENNIS: Bottromycin. I. A new sulfur-containing antibiotic. J. Am. Chem. Soc. 79: 4520~4521, 1957
2) NAKAMURA, S.; T. CHIKAIKE, K. KARASAWA, N. TANAKA, H. YONEHARA & H. UMEZAWA: Isolation and characterization of bottromycins A and B. J. Antibiotics, Ser. A 18: 47~52, 1965
3) NAKAMURA, S.; T. YAJIMA, YOUNG-CHI LIN & H. UMEZAWA: Isolation and characterization of bottromycin A2, B2, C2. J. Antibiotics, Ser. A 20: 1~5, 1967
4) NAKAMURA, S. & H. UMEZAWA: The structure of bottromycin A2, a new component of bottromycins. Chem. Pharm. Bull. 14: 981~986, 1966
5) NAKAMURA, S.; N. TANAKA & H. UMEZAWA: Bottromycin A1, A2 and their structures. J. Antibiotics, Ser. A 19: 10~12, 1966
6) TAKAHASHI, Y.; H. NAGANAWA, T. TAKITA, H. UMEZAWA & S. NAKAMURA: The revised structure of bottromycin A2. J. Antibiotics 29: 1120~1123, 1976
7) SCHIPPER, D.: The revised structure of bottromycin A2. J. Antibiotics 36: 1076~1077, 1983
8) MILLER, W. J.; L. CHAIET, G. RASMUSSEN, B. CHRISTENSEN, J. HANNAH, A. KATHRINE MILLER & F. J. WOLF: Bottromycin. Separation of biologically active compounds and preparation and testing of amide derivatives. J. Med. Chem. 11: 746~749, 1968
9) KANEDA, M.: Studies on bottromycins. I. ^{1}H and ^{13}C NMR assignments of bottromycin A2, the main component of the complex. J. Antibiotics 45: 792~796, 1992
10) NAKAMURA, S.; T. CHIKAIKE, H. YONEHARA & H. UMEZAWA: Isolation, characterization and structural elucidation of new amino acids from bottromycin A. Chem. Pharm. Bull. 13: 599~602, 1965
11) AROLD, H.; M. EULE & S. REISSMANN: Synthesis and configuration of α-amino-β-phenylbutyric acid. Z. Chem. 9: 447~448, 1969
12) SETO, Y.; K. TORII, K. BORI, K. INABATA, S. KUWATA & H. WATANABE: Unusual amino acids and their peptides. Synthesis and the absolute configuration of β-(2-thiazolyl)-β-alanine present in bottromycin. Bull. Chem. Soc. Jpn. 47: 151~155, 1974
13) TSUCHIHASHI, G.; S. MITAMURA & K. OGURA: Synthesis of (*S*,*S*)-2-amino-3-phenylbutyric acid. Bull. Chem. Soc. Jpn. 52: 2167~2168, 1979