STUDIES ON BOTTROMYCINS

I. ^{1}H AND ^{13}C NMR ASSIGNMENTS OF BOTTROMYCIN A2, THE MAIN COMPONENT OF THE COMPLEX

MIYUKI KANEDA

Institute of Pharmaceutical Sciences,
Hiroshima University School of Medicine,
1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan

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Bottromycins were first isolated from *Streptomyces bottropensis* by WAISVISZ *et al.*[1] and are especially active against Gram-positive bacteria and *Mycoplasma*[1~3]. The complete structure of bottromycin A2 (**1**), the main active component of the complex, was first proposed by NAKAMURA *et al.* in 1966 as a linear peptide[4,5], but the structure was revised in 1976 by TAKAHASHI *et al.*[6] to a partly cyclic structure by means of CI and EI mass and ^{1}H and ^{13}C NMR spectroscopy and chemical degradation studies. In 1983, SCHIPPER[7] reinvestigated the structure of **1** using ^{1}H and ^{15}N NMR spectroscopy and re-revised it to the structure in which the chain moiety links to the cyclic moiety through the imino nitrogen of the amidine group as shown in Fig. 1.

From the bottromycin complex, four other components were isolated[3,5,8] and to them, NAKAMURA *et al.* gave linear peptide structures analogous to **1**. The linear structures of bottromycins B1 and B2 were synthesized by YAMADA *et al.*[9], but they differed from the natural antibiotics. These facts prompted me to attempt unambiguous structure elucidation of the components other than **1** of the complex. For that purpose, it could be essential to establish the unequivocal NMR assignments of the all protons and carbons of bottromycin A2, which is the main component and of which considerable sample is available. Although some ^{1}H and ^{13}C NMR data of **1** have so far been described in the literature[4,6,7], those assignments are only partial and not useful for complete structure assignment. The present paper describes the complete ^{1}H and ^{13}C NMR assignments of **1**, which include several revisions of the assignments reported heretofore. Furthermore, the re-revised structure of **1** proposed by SCHIPPER[7] has been confirmed by this work.

The ^{1}H and ^{13}C NMR spectra of **1** were measured in $CDCl_3$ on a Jeol JNM-FX400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The ^{13}C multiplicity data were obtained from DEPT experiments. The complete assignments of proton and carbon signals of **1** were established as shown in Table 1 based on 2D NMR experiments such as ^{1}H-^{1}H correlation spectroscopy (COSY) (Fig. 2), ^{1}H-^{13}C COSY (Fig. 3) and correlation *via* long range coupling (COLOC) experiments (the *J* values, 5 Hz, 7 Hz and 10 Hz) (Fig. 4). The basis for these assignments is described in the following. The carbon, nitrogen and sulfur atoms constituting the skeleton of **1** are tentatively numbered as shown in Fig. 1.

First of all, in the methyl signal region of the ^{1}H NMR spectrum of **1**, there are four doublet methyl signals. Two of them in the highest field at δ_H 0.68 and δ_H 0.80, which correspond to δ_C 19.6 and δ_C 20.1, respectively, in the ^{13}C NMR spectrum, both correlate with the identical methine proton at δ_H 2.78 in the ^{1}H-^{1}H COSY spectrum. Obviously these

Fig. 1. Structure of bottromycin A2 (**1**).

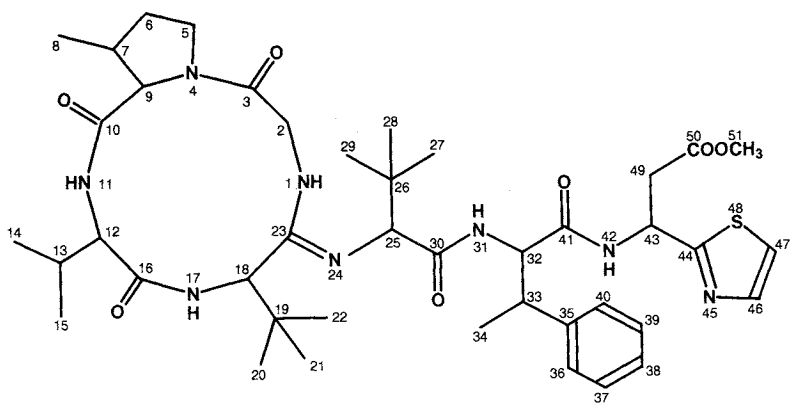


Fig. 2. ^{1}H-^{1}H COSY spectrum of bottromycin A2 in $CDCl_3$.

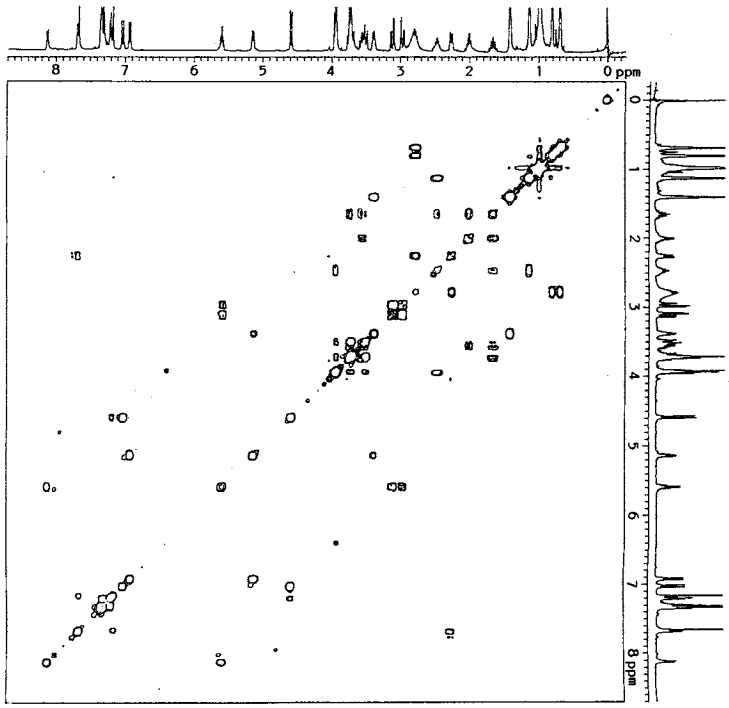


Fig. 3. ^{1}H-^{13}C COSY spectrum of bottromycin A2 in $CDCl_3$.

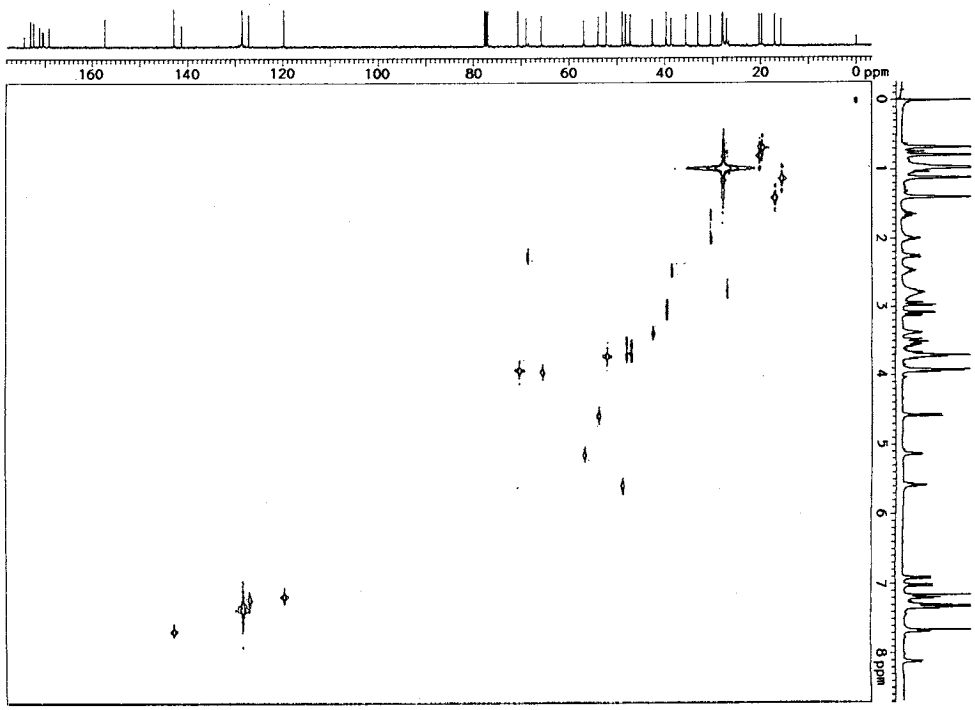
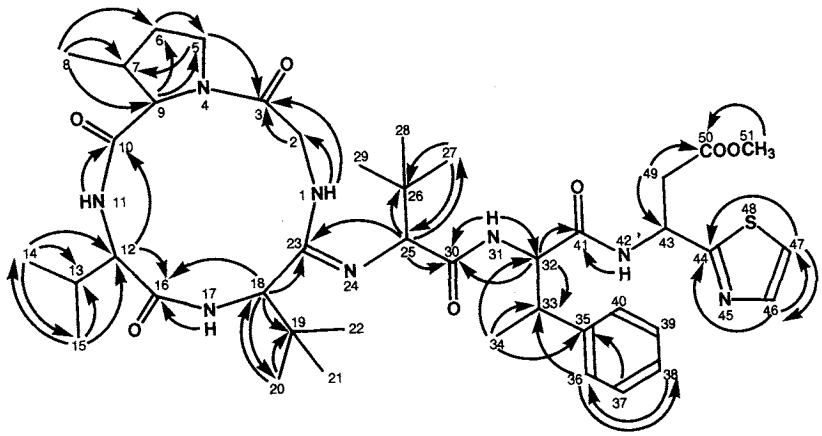


Fig. 4. Summary of long range ^{1}H-^{13}C couplings observed by COLOC experiments on bottromycin A2.
⟶ $^{1}H \rightarrow {}^{13}C$ long range coupling.



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

two methyl signals are due to the methyls of the valine moiety and this methine is 13-H. The methine signal at δ_H 2.26 which couples with this 13-H can be ascribed to 12-H and the NH doublet at δ_H 7.68 which couples with 12-H can be assigned to 11-NH. The methyl doublet at δ_H 1.40 (δ_C 16.8) couples with a methine signal at δ_H 3.38, which in turn couples with a methine signal at δ_H 5.13. An exchangeable doublet signal at δ_H 6.92 couples with this methine signal at δ_H 5.13. Judging from this CH_3–CH–CH–NH– system, these protons can be assigned to 34-H_3, 33-H, 32-H and 31-NH, respectively, of the 3-methyl-3-phenyl-L-alanine moiety. The above doublet signal at δ_H 6.92 was originally assigned by NAKAMURA and UMEZAWA[4], and NAKAMURA *et al.*[5] to the olefinic proton of a Δ^1-isocaproic acid moiety in the proposed linear structure and is here revised. The remaining doublet methyl signal at δ_H 1.12, which corresponds to the carbon signal at δ_C 15.5, couples with a methine signal at δ_H 2.46, which in turn couples with a methine signal at δ_H 3.94 and one proton at δ_H 1.65 of a methylene group. This proton at δ_H 1.65 correlates with two protons at δ_H 3.54 and δ_H 3.73 which both assemble to the carbon signal at δ_C 47.0. These are due to 8-H_3, 7-H, 9-H, 6-H_2 and 5-H_2, in this order, of the *cis*-3-methyl-L-proline moiety. Six methyl signals of the two tertiary butyl groups of two 2-amino-3,3-dimethylbutyric acid moieties concentrate at δ_H 0.98 as a broad singlet and they appear at δ_C 27.7 (27.69) and δ_C 27.8 (27.83) with almost the same intensity in the ^{13}C NMR spectrum.

The former carbon signal shows, in the COLOC spectra, long range ^{13}C-^{1}H coupling with 18-H which appears as a doublet at much lower field at δ_H 4.58 than 25-H (δ_H 3.91, s) and couples with an exchangeable proton at δ_H 7.02 (d, J=10.5 Hz). The latter carbon signal also shows long range ^{13}C-^{1}H coupling with 25-H. Therefore, C-20, 21, 22 appear at δ_C 27.7 and C-27, 28, 29 appear at δ_C 27.8. Two quaternary charbons C-19 and C-26 in each 2-amino-3,3-dimethylbutyric acid moiety can be located at δ_C 33.0 and δ_C 35.4, respectively, by the COLOC experiments in which C-19 and C-26 have connectivities with 18-H and 25-H, respectively. The quaternary sp^2 carbon C-23 in the amidine group can be located at δ_C 157.2 also by the COLOC experiments because C-23 shows long range ^{13}C-^{1}H couplings with 18-H and 25-H. Another quaternary sp^2 carbon C-44 in the thiazole ring, appears at δ_C 170.2 which shows long range ^{13}C-^{1}H couplings with the characteristic sp^2 protons of the thiazole ring, 46-H (δ_H 7.65, d, J=3.1 Hz) and 47-H (δ_H 7.15, d, J=3.3 Hz). TAKAHASHI *et al.*[6] reported the assignment of C-44 to δ 157.2 with no evidence, but it is here revised. Proton signals due to 42-NH (δ_H 8.12, d, J=7.7 Hz), 43-H (δ_H 5.58) and 49-H_2(δ_H 2.96 and δ_H 3.10) of the 3-(2-thiazolyl)-β-alanine moiety couple to one another in this order in the ^{1}H-^{1}H COSY spectrum. Among five NH protons, 1-NH appears at the highest field at δ_H 3.93 as is expected from its amidine structure instead of amide structure. 2-H_2 (δ_H 3.49 and 3.71) in the glycine moiety couple with this 1-NH in the ^{1}H-^{1}H COSY

Table 1. ^{1}H and ^{13}C NMR chemical shift data of bottromycin A2 in $CDCl_3$.

Position	δ_C (ppm)	δ_H (ppm) (J, Hz)	Assignment	Amino acid
N-1		3.93 br s	NH	Glycine
C-2	48.0 t	3.49 m, 3.71 m	CH_2	
C-3	169.1 s		C=O	
C-5	47.0 t	3.54 m, 3.73 m	CH_2	*cis*-3-Methyl-L-proline
C-6	30.3 t	1.65 m, 2.00 m	CH_2	
C-7	38.5 d	2.46 m	CH	
C-8	15.5 q	1.12 d, J=6.8	CH_3	
C-9	65.5 d	3.94 d, J=8.3	CH	
C-10	174.3 s		C=O	
N-11		7.68 d, J=5.7	NH	L-Valine
C-12	68.8 d	2.26 dd, J=5.7, 11.8	CH	
C-13	26.9 d	2.78 m	CH	
C-14	19.6 q	0.68 d, J=6.8	CH_3	
C-15	20.1 q	0.80 d, J=6.4	CH_3	
C-16	171.2 s		C=O	
N-17		7.02 d, J=10.5	NH	2-Amino-3,3-dimethylbutyric acid
C-18	53.7 d	4.58 d, J=10.7	CH	
C-19	33.0 s		=C	
C-20	27.7 q	0.98 br s	CH_3	
C-21	27.7 q	0.98 br s	CH_3	
C-22	27.7 q	0.98 br s	CH_3	
C-23	157.2 s		C=N	
C-25	70.5 d	3.91 s	CH	2-Amino-3,3-dimethylbutyric acid
C-26	35.4 s		=C	
C-27	27.8 q	0.98 br s	CH_3	
C-28	27.8 q	0.98 br s	CH_3	
C-29	27.8 q	0.98 br s	CH_3	
C-30	173.0 s		C=O	
N-31		6.92 d, J=9.4	NH	3-Methyl-3-phenyl-L-alanine
C-32	56.7 d	5.13 dd, J=4.2, 9.2	CH	
C-33	42.4 d	3.38 m	CH	
C-34	16.8 q	1.40 d, J=7.2	CH_3	
C-35	141.1 s		=C	
C-36	128.4 d	7.34 br d	=CH	
C-37	128.3 d	7.30 br t	=CH	
C-38	127.0 d	7.19 br t	=CH	
C-39	128.3 d	7.30 br t	=CH	
C-40	128.4 d	7.34 br d	=CH	
C-41	172.4 s		C=O	
N-42		8.12 d, J=7.7	NH	3-(2-Thiazolyl)-β-alanine
C-43	48.7 d	5.58 ddd, J=5.7, 7.5, 7.7	CH	
C-44	170.2 s		=C	
C-46	142.7 d	7.65 d, J=3.1	=CH	
C-47	119.6 d	7.15 d, J=3.3	=CH	
C-49	39.5 t	2.96 dd, J=5.7, 16.9 3.10 dd, J=7.5, 16.9	CH_2	
C-50	170.5 s		C=O	
C-51	52.0 q	3.71 s	OCH_3	

spectrum. Six carbonyl carbons, namely five amide carbonyl groups and one methoxycarbonyl group, can all be located based on an assembly of three COLOC experiments, where they correlate with adjacent NH protons and α- and/or nearby protons of amino acids as seen in Fig. 4. 3-CO, for example,

has connectivities with 2-H_2, 1-NH and 5-H_2. Thus, 3-CO is located at δ 169.1, 10-CO at δ 174.3, 16-CO at δ 171.2, 30-CO at δ 173.0, 41-CO at δ 172.4 and 50-CO at δ 170.5. Table 1 presents the total ^1H and ^{13}C NMR assignments of bottromycin A2 here established.

Separation and purification of the other components[3,5,8] of the bottromycin complex are now in progress. Careful investigation of their ^1H and ^{13}C NMR data on the basis of the NMR assignments of bottromycin A2 here obtained should afford their structures.

As for bottromycin A2, the absolute configurations of C-18 and C-25 are still ambiguous[4,10~12], and are also under study.

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