STUDIES ON BOTTROMYCINS

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

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(Received for publication October 14, 1991)

Bottromycins were first isolated from Streptomyces bottropensis by WAISVISZ et al.¹⁾ and are especially active against Gram-positive bacteria and $My coplasma^{1 \sim 3}$. 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 ¹H and ¹³C NMR spectroscopy and chemical degradation studies. In 1983, SCHIPPER⁷⁾ reinvestigated the structure of 1 using ¹H and ¹⁵N 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 ¹H and ¹³C 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 ¹H and ¹³C NMR assignments of 1, which include several revisions of the assignments reported heretofore. Furthermore, the re-revised structure of 1 proposed by SCHIPPER⁷⁾ has been confirmed by this work.

The ¹H and ¹³C NMR spectra of 1 were measured in CDCl₃ on a Jeol JNM-FX400 spectrometer at 400 MHz for ¹H and 100 MHz for ¹³C. The ¹³C 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 ¹H-¹H correlation spectroscopy (COSY) (Fig. 2), ¹H-¹³C COSY (Fig. 3) and correlation *via* long range coupling (COLOC) experiments (the *J* values, 5 Hz, ⁷Hz 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 ¹H NMR spectrum of 1, there are four doublet methyl signals. Two of them in the highest field at $\delta_{\rm H}$ 0.68 and $\delta_{\rm H}$ 0.80, which correspond to $\delta_{\rm C}$ 19.6 and $\delta_{\rm C}$ 20.1, respectively, in the ¹³C NMR spectrum, both correlate with the identical methine proton at $\delta_{\rm H}$ 2.78 in the ¹H-¹H COSY spectrum. Obviously these

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





Fig. 2. ¹H-¹H COSY spectrum of bottromycin A2 in CDCl₃.

Fig. 3. ¹H-¹³C COSY spectrum of bottromycin A2 in CDCl₃.



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



Connectivities to 21-CH₃, 22-CH₃ and 28-CH₃, 29-CH₃ are the same as to 20-CH₃ and 27-CH₃, 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 $\delta_{\rm H}$ 2.26 which couples with this 13-H can be ascribed to 12-H and the NH doublet at $\delta_{\rm H}$ 7.68 which couples with 12-H can be assigned to 11-NH. The methyl doublet at $\delta_{\rm H}$ 1.40 ($\delta_{\rm C}$ 16.8) couples with a methine signal at $\delta_{\rm H}$ 3.38, which in turn couples with a methine signal at $\delta_{\rm H}$ 5.13. An exchangeable doublet signal at $\delta_{\rm H}$ 6.92 couples with this methine signal at $\delta_{\rm H}$ 5.13. Judging from this CH₃-CH-CH-NH- system, these protons can be assigned to 34-H₃, 33-H, 32-H and 31-NH, respectively, of the 3-methyl-3-phenyl-L-alanine moiety. The above doublet signal at $\delta_{\rm H}$ 6.92 was originally assigned by NAKAMURA and UMEZAWA⁴⁾, 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 $\delta_{\rm H}$ 1.12, which corresponds to the carbon signal at $\delta_{\rm C}$ 15.5, couples with a methine signal at $\delta_{\rm H}$ 2.46, which in turn couples with a methine signal at $\delta_{\rm H}$ 3.94 and one proton at $\delta_{\rm H}$ 1.65 of a methylene group. This proton at $\delta_{\rm H}$ 1.65 correlates with two protons at $\delta_{\rm H}$ 3.54 and $\delta_{\rm H}$ 3.73 which both assemble to the carbon signal at $\delta_{\rm C}$ 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 $\delta_{\rm H}$ 0.98 as a broad singlet and they appear at $\delta_{\rm C}$ 27.7 (27.69) and $\delta_{\rm C}$ 27.8 (27.83) with almost the same intensity in the ¹³C NMR spectrum.

The former carbon signal shows, in the COLOC spectra, long range ¹³C-¹H coupling with 18-H which appears as a doublet at much lower field at $\delta_{\rm H}$ 4.58 than 25-H ($\delta_{\rm H}$ 3.91, s) and couples with an exchangeable proton at $\delta_{\rm H}$ 7.02 (d, J = 10.5 Hz). The latter carbon signal also shows long range ¹³C-¹H coupling with 25-H. Therefore, C-20, 21, 22 appear at $\delta_{\rm C}$ 27.7 and C-27, 28, 29 appear at $\delta_{\rm C}$ 27.8. Two quaternary charbons C-19 and C-26 in each 2-amino-3,3-dimethylbutyric acid moiety can be located at $\delta_{\rm C}$ 33.0 and $\delta_{\rm C}$ 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 $\delta_{\rm C}$ 157.2 also by the COLOC experiments because C-23 shows long range ¹³C-¹H couplings with 18-H and 25-H. Another quaternary sp^2 carbon C-44 in the thiazole ring, appears at $\delta_{\rm C}$ 170.2 which shows long range ¹³C-¹H couplings with the characteristic sp^2 protons of the thiazole ring, 46-H ($\delta_{\rm H}$ 7.65, d, J = 3.1 Hz) and 47-H ($\delta_{\rm H}$ 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 ($\delta_{\rm H}$ 8.12, d, J = 7.7 Hz), 43-H (δ_{H} 5.58) and 49-H₂(δ_{H} 2.96 and $\delta_{\rm H}$ 3.10) of the 3-(2-thiazolyl)- β -alanine moiety couple to one another in this order in the ¹H-¹H COSY spectrum. Among five NH protons, 1-NH appears at the highest field at $\delta_{\rm H}$ 3.93 as is expected from its amidine structure instead of amide structure. 2-H₂ ($\delta_{\rm H}$ 3.49 and 3.71) in the glycine moiety couple with this 1-NH in the ¹H-¹H COSY

N-1 3.93 br s NH Glycine C-2 48.0 1 3.49 m, 3.71 m CH2 C-3 169.1 s C-0 C-5 47.0 1 3.54 m, 3.73 m CH2 C-6 30.3 1 1.65 m, 2.00 m CH2 C-7 38.5 d 2.46 m CH C-8 15.5 q 1.12 d, J=6.8 CH3 C-10 174.3 s C=0 C=0 N-11 7.66 d, J=5.7 NH L-Valine C-12 68.8 d 2.26 dd, J=5.7 NH L-Valine C-14 19.6 q 0.68 d, J=6.8 CH3 C=0 N-11 7.62 d, J=0.5 NH 2-Amino-3,3-dimethylbutyric acid C-18 53.7 d 4.58 d, J=10.7 CH C+0 C-19 30.0 s -C C C C-20 27.7 q 0.98 br s CH3 C-21 C7.7 q 0.98 br s C-22 7.7 q 0.98 br s CH3 C-22 C-23 <	Position	$\delta_{ m c}~({ m ppm})$	$\delta_{\rm H}$ (ppm) (J, Hz)	Assignment	Amino acid
$ \begin{array}{ccccc} C-2 & 48.0 t & 3.49 m, 3.71 m & CH_2 & C-0 & C-5 & 64.0 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 & cis-3-Methyl-L-proline & C-6 & 30.3 t & 1.65 m, 2.00 m & CH_2 & cis-3-Methyl-L-proline & C-6 & C-7 & 38.5 d & 2.46 m & CH & CH_2 & cis-3-Methyl-L-proline & C-7 & 38.5 d & 2.46 m & CH_2 & C-9 & cis-3 & 3.44 d, J=8.3 & CH & C-10 & 17.43 s & C-0 & C-0 & C-0 & C-0 & C-10 & C-10 & 17.43 s & C-10 & C-10 & C-10 & C-10 & 0.68 d, J=5.7 & NH & L-Valine & C-112 & 68.8 d & 2.26 dd, J=5.7 & NH & L-Valine & C-12 & cis-3-Methyl-L-proline & C-10 & 2.7.7 q & 0.98 br s & CH_3 & C-10 & C-10 & C-10 & 2.7.7 q & 0.98 br s & CH_3 & C-22 & 27.7 q & 0.98 br s & CH_3 & C-22 & 27.7 q & 0.98 br s & CH_3 & C-20 & C-20 & C-25 & C-N & C-25 & C-N & C-25 & C-25 & C-25 & C-N & C-25 & C-$	N-1	• •	3.93 br s	NH	Glycine
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$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	C-3	169.1 s		C=O	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	C-5	47.0 t	3.54 m, 3.73 m	CH ₂	cis-3-Methyl-L-proline
$ \begin{array}{cccccc} C-7 & 38.5 \ d & 2.46 \ m & CH \\ C-8 & 15.5 \ q & 1.12 \ d, J=6.8 & CH \\ C-9 & 65.5 \ d & 3.94 \ d, J=8.3 & C+0 \\ \hline \\ C-10 & 174.3 \ s & C-0 \\ \hline \\ $	C-6	30.3 t	1.65 m, 2.00 m	CH_2	
C-8 15.5 q 1.12 d, $J=6.8$ CH C-9 65.5 d 3.94 d, $J=8.3$ C-O N-11 7.68 d, $J=5.7$ NH L-Valine C-12 68.8 d 2.26 dd, $J=5.7$ NH L-Valine C-13 20.9 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-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-24 27.7 q 0.98 br s CH ₃ C-25 70.5 d 3.91 s C-C C-26 35.4 s -C-O C-27 27.8 q 0.98 br s CH ₃ C-28 27.8 q 0.98 br s CH ₃ <	C-7	38.5 d	2.46 m	\mathbf{CH}	
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$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C-13	26.9 d	2.78 m	CH	
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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-23 157.2 s C-N 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 C-O N-31 6.92 d, $J = 9.4$ NH 3-Methyl-3-phenyl-t-alanine C-32 56.7 d 5.13 dd, $J = 42.9.2$ CH C-34 16.8 q 1.40 d, $J = 7.2$ CH ₃ C-35 141.1 s -C C C-36 128.4 d 7.34 br d -CH C-38 127.0 d 7.19 br t -CH C-38 127.0 d 7.19 br t -CH C-40 128.4 d<	C-15	20.1 q	0.80 d, $J = 6.4$	CH_3	
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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 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-30 173.0 s C-O CH N-31 6.92 d, J=9.4 NH 3-Methyl-3-phenyl-t-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 14.1 s -C C C-36 128.4 d 7.34 br d -CH C-38 127.0 d 7.19 br t -CH C-39 128.3 d 7.30 br t -CH C-39 128.3 d 7.30 br t	N-17		7.02 d, $J = 10.5$	NH	2-Amino-3,3-dimethylbutyric acid
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	C-18	53.7 d	4.58 d, $J = 10.7$	CH	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	C-19	33.0 s		- = C	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C-20	27.7 q	0.98 br s	CH ₃	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C-21	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 2-Amino-3,3-dimethylbutyric acid C-27 27.8 q 0.98 br s CH ₃ 2-Amino-3,3-dimethylbutyric acid C-28 27.8 q 0.98 br s CH ₃ -C C-30 173.0 s C=O -C N-31 6.92 d, J=9.4 NH 3-Methyl-3-phenyl-t-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 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-40 128.4 d 7.34 br d -CH C-41 172.4 s -CO -C 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	C-22	27.7 q	0.98 br s	CH3	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C-23	157.2 s		C=N	
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 $6.92 \text{ d}, J=9.4$ NH 3 -Methyl-3-phenyl-L-alanine C-32 56.7 d $5.13 \text{ dd}, J=4.2, 9.2$ CH C-33 42.4 d 3.38 m CH C-34 16.8 q $1.40 \text{ d}, J=7.2$ CH_3 C-36 128.4 d 7.34 br d =CH C-36 128.3 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 C=O N+42 $8.12 \text{ d}, J=7.7$ NH $3-(2-\text{Thiazolyl})-\beta-alanine$ C-43 48.7 d $5.58 \text{ ddd}, J=5.7, 7.5, 7.7$ CH C-44 <	C-25	70.5 d	3.91 s	СН	2-Amino-3,3-dimethylbutyric acid
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C-26	35.4 s		=C	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	C-27	27.8 q	0.98 br s	CH_3	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C-28	27.8 q	0.98 br s	CH3	
C-30173.0 sC=ON-316.92 d, $J=9.4$ NH3-Methyl-3-phenyl-L-alanineC-3256.7 d5.13 dd, $J=4.2$, 9.2CHC-3342.4 d3.38 mCHC-3416.8 q1.40 d, $J=7.2$ CH ₃ C-35141.1 s=CC-36128.4 d7.34 br d=CHC-37128.3 d7.30 br t=CHC-38127.0 d7.19 br t=CHC-39128.3 d7.30 br t=CHC-40128.4 d7.34 br d=CHC-41172.4 sC=ON-428.12 d, $J=7.7$ NH3-(2-Thiazolyl)- β -alanineC-4348.7 d5.58 ddd, $J=5.7, 7.5, 7.7$ CHC-44170.2 s=CC-46142.7 d7.65 d, $J=3.1$ =CHC-47119.6 d7.15 d, $J=3.3$ =CHC-4939.5 t2.96 dd, $J=5.7, 16.9$ CH ₂ 3.10 dd, $J=7.5, 16.9$ C=O $C=O$ C-50170.5 sC=OC-5152.0 q3.71 sOCH ₃	C-29	27.8 q	0.98 br s	CH_3	
N-316.92 d, $J=9.4$ NH3-Methyl-3-phenyl-L-alanineC-3256.7 d5.13 dd, $J=4.2$, 9.2CHC-3342.4 d3.38 mCHC-3416.8 q1.40 d, $J=7.2$ CHC-35141.1 s=CC-36128.4 d7.34 br d=CHC-37128.3 d7.30 br t=CHC-38127.0 d7.19 br t=CHC-39128.3 d7.30 br t=CHC-40128.4 d7.34 br d=CHC-41172.4 sC=ON-428.12 d, $J=7.7$ NH3-(2-Thiazolyl)- β -alanineC-44170.2 s=CC-46142.7 d7.65 d, $J=3.1$ C-47119.6 d7.15 d, $J=3.3$ C-4939.5 t2.96 dd, $J=5.7, 16.9$ C-4939.5 t2.96 dd, $J=7.5, 16.9$ C-50170.5 sC=OC-50170.5 sC=OC-5152.0 q3.71 sCC+O	C-30	173.0 s		C=O	
C-32 56.7 d 5.13 dd, $J=4.2, 9.2$ CH C-33 42.4 d 3.38 m 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-40 128.4 d 7.34 br d = CH C-40 128.4 d 7.34 br d = CH C-41 172.4 s C=O = CH C-41 170.2 s = C = C C-44 170.2 s = C = C C-45 142.7 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 CH ₂ 3.10 dd, J=7.5, 16.9 CH 2.90 C=O	N-31		6.92 d, J=9.4	NH	3-Methyl-3-phenyl-L-alanine
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C-32	56.7 d	5.13 dd, $J = 4.2, 9.2$	CH	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	C-33	42.4 d	3.38 m	CH	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	C-34	16.8 q	1.40 d, $J = 7.2$	CH ₃	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	C-35	141.1 s		=C	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	C-36	128.4 d	7.34 br d	=CH	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	C-37	128.3 d	7.30 br t	=CH	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	C-38	127.0 d	7.19 br t	=CH	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	C-39	128.3 d	7.30 br t	=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, $J=5.7, 16.9$ CH2 3.10 dd, $J=7.5, 16.9$ C=O C-50 170.5 s C=O C-51 52.0 q 3.71 s OCH3	C-40	128.4 d	7.34 br d	=CH	
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, $J=5.7, 16.9$ CH ₂ 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	C-41	172.4 s		C=0	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	N-42		8.12 d, J=7.7	NH	3-(2-Thiazolyl)- β -alanine
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C-43	48.7 d	5.58 ddd, $J = 5.7, 7.5, 7.7$	CH	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C-44	170.2 s		=C	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C-46	142.7 d	7.65 d, $J = 3.1$	=CH	
C-49 39.5 t $2.96 \text{ dd}, J=5.7, 16.9$ CH_2 $3.10 \text{ dd}, J=7.5, 16.9$ $C=O$ C-50 170.5 s $C=O$ C-51 52.0 q 3.71 s OCH_3	C-47	119.6 d	7.15 d, $J = 3.3$	=CH	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C-49	39.5 t	2.96 dd, $J = 5.7$, 16.9	CH ₂	
C-50 170.5 s C=O C-51 52.0 q 3.71 s OCH ₃			3.10 dd, J = 7.5, 16.9		
C-51 52.0 q 3.71 s OCH ₃	C-50	170.5 s		C=O	
- 2	C-51	52.0 q	3.71 s	OCH ₃	

Table 1. ¹H and ¹³C NMR chemical shift data of bottromycin A2 in CDCl₃.

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-H₂, 1-NH and 5-H₂. 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 ¹³C 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 ¹³C 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^{$4,10 \sim 12$}, and are also under study.

Acknowledgment

The author is grateful to Professor S. NAKAMURA of this institute for his kind gift of bottromycin A2, his encouragement and helpful discussions.

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