# NOVEL ANTIBIOTICS, AMYTHIAMICINS

## III. STRUCTURE ELUCIDATIONS OF AMYTHIAMICINS A, B AND C

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The structures of novel antimicrobial antibiotics, amythiamicins A, B and C, were elucidated by chemical degradations and NMR spectral analyses. The main frame from C-1 to C-41 of these antibiotics was the same as that of amythiamicin D. Amino acid autoanalyses of amythiamicins A, B and C showed that these have another one mole of serine and proline in comparison with amythiamicin D. Stereochemistries of both amino acids were determined to be L by chiral HPLC. These seryl-prolyl residues in amythiamicins A, B and C are attached at C-41 through an oxazoline ring, amide and ester bond, respectively.

Amythiamicins A, B, C and D, novel antimicrobial antibiotics, have been isolated from the fermentation broth of *Amycolatopsis* sp. MI481-42F4[1]. The structure elucidation of amythiamicin D was reported in the preceding paper[2]. This paper describes details of the structure elucidations of amythiamicins A, B and C.

### Structure of Amythiamicin A

The molecular formula of amythiamicin A was established to be $C_{50}H_{51}N_{15}O_8S_6$ by HRFAB-MS, $^{13}C$ NMR and elemental analysis. The acid hydrolysis (6 N HCl, 110°C, 16 hours) of amythiamicin A gave

Fig. 1. Structures of amythiamicins A, B, C and D.

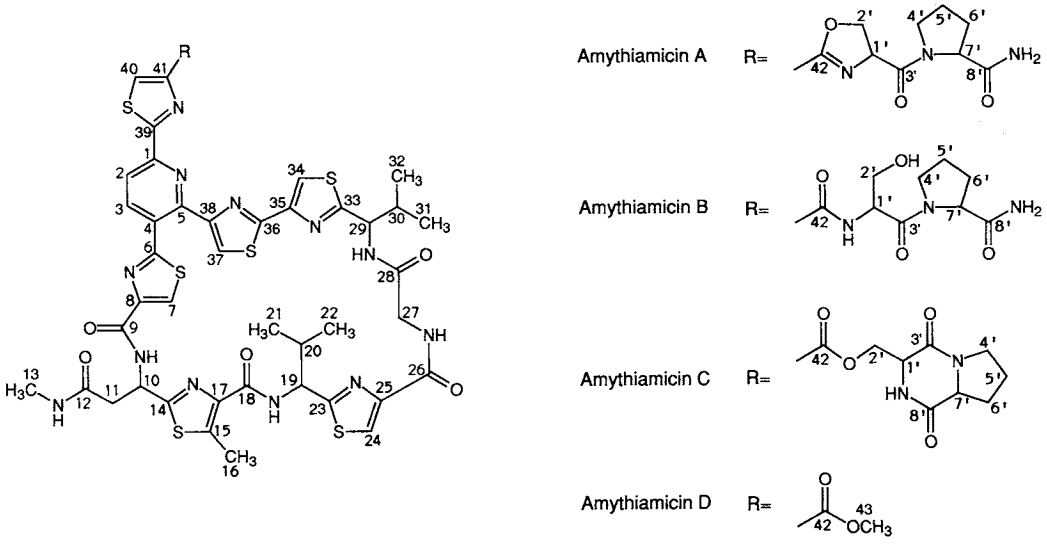


Table 1. Physico-chemical properties of amythiamicins A, B and C.

| Compound | A | B | C |
|---|---|---|---|
| Appearance | Colorless powder | Colorless powder | Colorless powder |
| FAB-MS (*m/z*) | 1,182 $(M+H)^+$, 1,181 $(M^-)$ | 1,200 $(M+H)^+$, 1,199 $(M^-)$ | 1,183 $(M+H)^+$, 1,182 $(M^-)$ |
| HRFAB-MS ($(M+H)^+$, *m/z*) | Calcd for $C_{50}H_{52}N_{15}O_8S_6$ | Calcd for $C_{50}H_{54}N_{15}O_9S_6$ | Calcd for $C_{50}H_{51}N_{14}O_9S_6$ |
| Calcd: | 1,182.2447 | 1,200.2553 | 1,183.2288 |
| Found: | 1,182.2463 | 1,200.2549 | 1,183.2285 |
| Molecular formula | $C_{50}H_{51}N_{15}O_8S_6$ | $C_{50}H_{53}N_{15}O_9S_6$ | $C_{50}H_{50}N_{14}O_9S_6$ |
| Elemental analysis calcd. for | $C_{50}H_{51}N_{15}O_8S_6 \cdot H_2O$ | | |
| Calcd: | C 50.03, H 4.45, N 17.50, O 11.99, S 16.03 | | |
| Found: | C 50.10, H 4.53, N 17.36, O 12.28, S 15.87 | | |
| Optical rotation | $[\alpha]_D^{28}$ +133° (*c* 0.745, DMSO) | $[\alpha]_D^{23}$ +155° (*c* 0.25, MeOH) | $[\alpha]_D^{24}$ +112° (*c* 0.25, MeOH) |
| UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$) | 203 (4.92), 221 (4.89), 250 (sh 4.73), 310 (4.55), 345 (sh 4.13) | 204 (4.78), 220 (4.75), 250 (sh 4.52), 308 (4.37), 345 (sh 3.97) | 203 (4.80), 221 (4.76), 250 (sh 4.52), 306 (4.40), 345 (sh 3.97) |
| $\lambda_{max}^{MeOH-HCl}$ | 203 (4.93), 223 (4.90), 250 (sh 4.72), 303 (4.62), 345 (sh 4.07) | 204 (4.77), 222 (4.75), 250 (sh 4.52), 308 (4.38), 345 (sh 3.97) | 203 (4.78), 221 (4.76), 250 (sh 4.53), 306 (4.41), 345 (sh 3.97) |
| $\lambda_{max}^{MeOH-NaOH}$ | 204 (5.46), 250 (sh 4.73), 309 (4.55), 345 (sh 4.93) | 202 (5.44), 250 (sh 4.52), 308 (4.37), 345 (sh 3.97) | 203 (5.44), 250 (sh 4.53), 306 (4.39), 345 (sh 3.97) |
| IR $\nu_{max}^{KBr}$ $cm^{-1}$ | 3300, 2980, 1660, 1540, 1500, 1250, 1100, 990, 760 | 3395, 2965, 1655, 1540, 1500, 1245, 1070, 755 | 3385, 3115, 2965, 1735, 1665, 1540, 1495, 1210, 755 |
| Solubility | | | |
| Soluble | MeOH, DMSO | MeOH, DMSO | MeOH, DMSO |
| Insoluble | *n*-Hexane, $H_2O$ | *n*-Hexane, $H_2O$ | *n*-Hexane, $H_2O$ |
| Color reaction | | | |
| Positive: | Phosphomolybdate-$H_2SO_4$, Rydon-Smith | Phosphomolybdate-$H_2SO_4$, Rydon-Smith | Phosphomolybdate-$H_2SO_4$, Rydon-Smith |
| Negative | Ninhydrin, Dragendorff | Ninhydrin, Dragendorff | Ninhydrin, Dragendorff |

one mole of proline, serine, glycine and three unusual amino acids described in the previous paper[2]. The structure elucidation of amythiamicin A was carried out spectroscopically by comparing the data with those of amythiamicin D.

Chemical shifts in the $^{1}H$ NMR and $^{13}C$ NMR of amythiamicin A are shown in Tables 2 and 3. Detailed NMR analyses of $^{1}H$ and $^{13}C$ NMR spectra, $^{1}H$-$^{1}H$ COSY and $^{1}H$-$^{13}C$ COSY spectra as well as heteronuclear multiple bond correlations (HMBC)[3] spectrum indicated that the main frame from C-1 to C-41 in amythiamicin A was the same as amythiamicin D. This identity was also confirmed by the fact that the acid hydrolysis of amythiamicin A with 1 N HCl at room temperature for 1 hour followed by methanolysis with 10% anhydrous HCl-MeOH at 80°C for 1 hour gave amythiamicin D (Fig. 3). Amythiamicin A differed from amythiamicin D in the part of the peptidic side chain consisting of one mole of serine and proline. The side chain at C-41 was determined as follows: The prolyl residue (C-4′ ~ C-8′) was assigned by the $^{1}H$-$^{1}H$ COSY and HMBC spectra as shown in Fig. 2. The $^{1}H$-$^{13}C$ long-range couplings from two amino protons (the 8′-$NH_2$ ($\delta_H$ 8.02, 8.40)) to C-7′ ($\delta_C$ 61.1) showed that *C*-terminal of the side chain was prolinamide. The seryl residue (C-1′ ~ C-3′) was also assigned by the $^{1}H$-$^{1}H$ COSY and HMBC spectra. The long-range cross peaks from the 1′-CH ($\delta_H$ 5.36), the 2′-$CH_2$ ($\delta_H$ 4.40, 5.32) (seryl residue) and the 4′-$CH_2$ ($\delta_H$ 3.98, 4.38) (prolyl residue) to an amide carbonyl (C-3′ ($\delta_C$ 168.5)) confirmed that the structure of the side chain was serylprolinamide. To satisfy the molecular formula of amythiamicin A, two moles of $H_2O$ should be lost between amythiamicin D free acid and the serylprolinamide. Indeed, an amide proton and a hydroxyl proton signals derived from seryl residue were not observed in the $^{1}H$ NMR spectrum. Therefore, it was deduced that the side chain was attached at C-41 through an oxazoline ring. On the basis of these results described above, the structure of amythiamicin A was determined as shown in Fig. 1.

## Structure of Amythiamicin B

The molecular formula of amythiamicin B was determined to be $C_{50}H_{53}N_{15}O_9S_6$ by HRFAB-MS and $^{13}C$ NMR analysis. The result of amino acid autoanalysis for amythiamicin B was the same as that of amythiamicin A.

As summarized in Tables 2 and 3, $^{1}H$ and $^{13}C$ chemical shifts of amythiamicin B were similar to those of amythiamicin A. However, one amino proton (the 1′-NH ($\delta_H$ 9.19)) and one hydroxyl proton (the 2′-OH ($\delta_H$ 7.36)) which were absent in amythiamicin A, were observed in the $^{1}H$ NMR spectrum of amythiamicin B. The $^{1}H$-$^{1}H$ COSY spectrum showed the partial structure (1′-NH–1′-H–2′-H–2′-OH). The molecular formula of amythiamicin B showed that one mole of $H_2O$ was added as compared with that of amythiamicin A. These results suggested that the oxazoline ring in amythiamicin A was opened by the hydrolysis of the carbon-oxygen bond. The HMBC data of amythiamicin B (Fig. 2) and the result of a mild acid hydrolysis for amythiamicin A (Fig. 3) also supported the fact that the seryl-prolyl residue was attached at C-41 through an amide bond. From the results described above, the structure of amythiamicin B was determined as shown in Fig. 1.

## Structure of Amythiamicin C

The molecular formula of amythiamicin C was established to be $C_{50}H_{50}N_{14}O_9S_6$ by HRFAB-MS and $^{13}C$ NMR analysis. The result of amino acid autoanalysis for amythiamicin C was also the same as that of amythiamicin A.

As summarized in Tables 2 and 3, $^{1}H$ and $^{13}C$ chemical shifts of amythiamicin C were similar to

Table 2. $^1$H NMR chemical shifts of amythiamicins A, B, C and D.

| Proton | Chemical shifts ($\delta_H$) in ppm | | | |
|---|---|---|---|---|
| | A* | B* | C* | D** |
| 2-H | 8.37 d (7.9) | 8.01 d (8.1) | 8.30 d (8.2) | 8.36 d (8.2) |
| 3-H | 8.15 d (7.9) | 8.15 d (8.1) | 8.17 d (8.2) | 8.13 d (8.2) |
| 7-H | 8.83 s | 8.84 s | 8.85 s | 8.39 s |
| 10-H | 5.73 dt (3.0, 9.1) | 5.73 dt (3.1, 8.9) | 5.73 dt (3.2, 9.1) | 5.44 dt (3.7, 9.1) |
| 11-$H_a$ | 1.70 br dd | 1.70 br dd | 1.70 m | 1.12 m |
| 11-$H_b$ | 3.44 dd (3.0, 16.5) | 3.45 dd (3.1, 16.5) | 3.45 dd (3.2, 16.1) | 2.76 dd (3.7, 16.5) |
| 13-$H_3$ | 2.66 d (4.3) | 2.62 d (4.6) | 2.66 d (4.6) | 2.66 d (4.9) |
| 16-$H_3$ | 2.61 s | 2.61 s | 2.61 s | 2.67 s |
| 19-H | 5.46 dd (4.6, 7.6) | 5.47 dd (4.7, 7.7) | 5.46 dd (4.6, 7.9) | 5.26 dd (4.6, 7.9) |
| 20-H | 2.30 m | 2.30 m | 2.30 m | 2.30 m |
| 21-$H_3$ | 0.94 d (6.7) | 0.94 d (6.7) | 0.94 d (6.7) | 0.90 d (6.9) |
| 22-$H_3$ | 0.94 d (6.7) | 0.95 d (6.7) | 0.94 d (6.7) | 1.00 d (6.9) |
| 24-H | 8.43 s | 8.43 s | 8.43 s | 8.13 s |
| 27-$H_a$ | 4.18 dd (3.7, 17.1) | 4.18 dd (3.7, 17.1) | 4.18 dd (3.7, 17.1) | 3.96 dd (3.4, 17.4) |
| 27-$H_b$ | 5.46 dd (8.8, 17.1) | 5.40 dd (9.3, 17.1) | 5.40 dd (9.0, 17.1) | 5.04 dd (9.4, 17.4) |
| 29-H | 5.30 dd (7.0, 7.8) | 5.33 dd (6.7, 7.8) | 5.33 dd (7.1, 7.8) | 5.01 dd (7.0, 7.8) |
| 30-H | 2.12 m | 2.11 m | 2.10 m | 2.09 m |
| 31-$H_3$ | 0.81 d (6.6) | 0.80 d (6.6) | 0.81 d (6.7) | 0.93 d (7.0) |
| 32-$H_3$ | 1.05 d (6.6) | 1.05 d (6.6) | 1.05 d (6.7) | 1.11 d (7.0) |
| 34-H | 7.70 s | 7.71 s | 7.71 s | 7.25 s |
| 37-H | 8.38 s | 8.38 s | 8.36 s | 8.24 s |
| 40-H | 8.42 s | 8.67 s | 8.54 s | 8.15 s |
| 43-$OCH_3$ | | | | 4.02 s |
| 1′-H | 5.36 dd (7.4, 9.5) | 5.64 ddd (6.7, 7.1, 7.7) | 4.86 br dd (3.3, 6.4) | |
| 2′-$H_a$ | 4.40 dd (9.5, 17.1) | 4.47 m | 5.15 dd (6.4, 11.6) | |
| 2′-$H_b$ | 5.32 dd (7.4, 17.1) | 4.55 m | 5.40 dd (3.3, 11.6) | |
| 2′-OH | | 7.36 t (5.2) | | |
| 4′-$H_a$ | 3.98 m | 3.85 m | 3.50 m | |
| 4′-$H_b$ | 4.38 m | 4.03 m | 3.63 m | |
| 5′-$H_a$ | 1.79 m | 1.76 m | 1.67 m | |
| 5′-$H_b$ | 2.12 m | 1.96 m | 2.30 m | |
| 6′-$H_a$ | 1.97 m | 2.14 m | 1.65 m | |
| 6′-$H_b$ | 2.25 m | 2.26 m | 2.23 m | |
| 7′-H | 4.93 dd (3.6, 8.4) | 5.10 dd (4.0, 7.5) | 4.29 t (8.2) | |
| 10-NH | 9.57 d (9.1) | 9.58 d (8.9) | 9.57 d (9.1) | 8.99 d (9.1) |
| 12-NH | 8.05 br q (4.3) | 8.07 br q (4.5) | 8.06 br q (4.6) | 6.90 q (4.9) |
| 19-NH | 9.14 d (7.6) | 9.14 d (77) | 9.14 d (7.7) | 8.81 d (7.9) |
| 27-NH | 9.08 dd (3.7, 8.8) | 9.08 dd (3.7, 9.3) | 9.08 dd (3.7, 9.0) | 7.94 dd (3.4, 9.4) |
| 29-NH | 10.16 d (7.0) | 10.18 d (6.7) | 10.18 d (7.1) | 7.22 d (7.0) |
| 1′-NH | | 9.19 d (7.7) | 9.82 br s | |
| 8′-$NH_a$ | 8.02 | 8.21 | | |
| 8′-$NH_b$ | 8.40 | 8.22 | | |

* Measured at 500 MHz in $C_5D_5N$; ppm from TMS.
** Measured at 500 MHz in $CDCl_3$; ppm from TMS.
The coupling constants (Hz) are in parentheses.

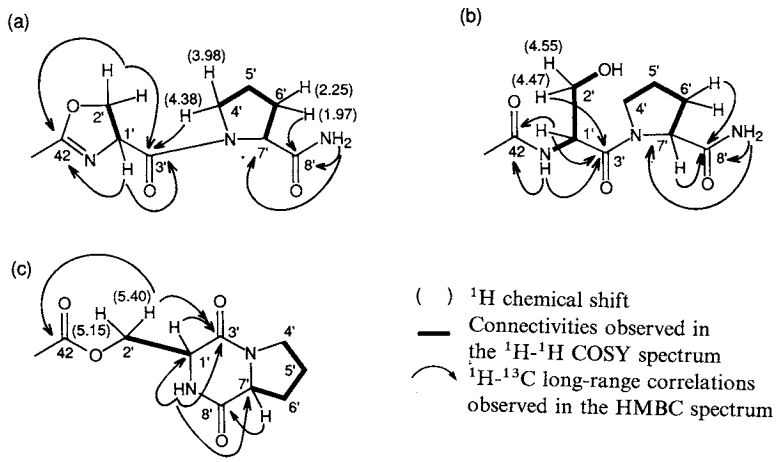
those of amythiamicin A, but one of the terminal amide protons in amythiamicin C was lost. In the HMBC spectrum, this amide proton (the 1′-NH ($\delta_H$ 9.82)) had long-range couplings with C-1′ ($\delta_C$ 55.3), C-3′ ($\delta_C$ 164.1) (seryl residue) and C-7′ ($\delta_C$ 59.6) (prolyl residue) as shown in Fig. 2. Amythiamicin C was also obtained from the acid hydrolysate (1 N HCl, 110°C, 1 hour) of amythiamicin A (Fig. 3). These results suggested that the oxazoline ring in amythiamicin A was opened by the hydrolysis of the carbon-nitrogen

Table 3. $^{13}C$ NMR chemical shifts of amythiamicins A, B, C and D.

| Position | Chemical shifts ($\delta_C$) in ppm | | | | Position | Chemical shifts ($\delta_C$) in ppm | | | |
|---|---|---|---|---|---|---|---|---|---|
| | A* | B* | C* | D** | | A* | B* | C* | D** |
| 1 | 151.1 s | 150.9 s | 151.0 s | 150.6 s | 27 | 41.9 t | 41.9 t | 41.9 t | 41.6 t |
| 2 | 118.6 d | 118.6 d | 118.6 d | 118.7 d | 28 | 171.3 s | 171.3 s | 171.3 s | 169.4 s |
| 3 | 141.1 d | 141.0 d | 141.1 d | 140.4 d | 29 | 60.0 d | 60.0 d | 60.1 d | 59.4 d |
| 4 | 127.9 s | 128.0 s | 128.0 s | 127.7 s | 30 | 33.8 d | 33.8 d | 33.8 d | 33.3 d |
| 5 | 150.6 s | 150.6 s | 150.6 s | 150.2 s | 31 | 19.3 q | 19.3 q | 19.3 q | 19.2 q |
| 6 | 165.6 s | 165.6 s | 165.6 s | 165.0 s | 32 | 19.7 q | 19.7 q | 19.7 q | 19.2 q |
| 7 | 126.3 d | 126.3 d | 126.4 d | 125.3 d | 33 | 175.4 s | 175.5 s | 175.5 s | 173.1 s |
| 8 | 151.0 s | 151.0 s | 150.9 s | 150.3 s | 34 | 115.5 d | 115.6 d | 115.6 d | 114.3 d |
| 9 | 161.5 s | 161.5 s | 161.5 s | 161.3 s | 35 | 149.0 s | 149.0 s | 149.0 s | 148.8 s |
| 10 | 49.0 d | 49.1 d | 49.0 d | 48.4 d | 36 | 160.8 s | 160.8 s | 160.8 s | 160.0 s |
| 11 | 38.9 t | 38.9 t | 38.9 t | 38.4 t | 37 | 124.2 d | 124.2 d | 124.3 d | 123.2 d |
| 12 | 170.4 s | 170.4 s | 170.5 s | 169.7 s | 38 | 154.8 s | 154.8 s | 154.7 s | 154.4 s |
| 13 | 26.2 q | 26.2 q | 26.2 q | 26.3 q | 39 | 169.1 s | 168.4 s | 168.9 s | 169.1 s |
| 14 | 169.5 s | 169.5 s | 169.5 s | 167.7 s | 40 | 128.2 d | 127.4 d | 132.2 d | 130.5 d |
| 15 | 140.2 s | 140.2 s | 140.2 s | 140.6 s | 41 | 146.0 s | 152.1 s | 148.3 s | 148.2 s |
| 16 | 12.1 q | 12.1 q | 12.1 q | 12.4 q | 42 | 160.4 s | 160.9 s | 161.4 s | 161.9 s |
| 17 | 143.1 s | 143.1 s | 143.1 s | 142.2 s | 43 | | | | 52.6 q |
| 18 | 162.0 s | 162.0 s | 162.0 s | 162.0 s | 1′ | 69.3 d | 53.9 d | 55.3 d | |
| 19 | 56.3 d | 56.2 d | 56.2 d | 56.3 d | 2′ | 69.5 t | 63.8 t | 64.4 t | |
| 20 | 35.3 d | 35.3 d | 35.3 d | 34.7 d | 3′ | 168.5 s | 170.4 s | 164.1 s | |
| 21 | 18.2 q | 18.2 q | 18.2 q | 18.1 q | 4′ | 47.7 t | 47.8 t | 45.6 t | |
| 22 | 18.5 q | 18.5 q | 18.5 q | 18.3 q | 5′ | 25.0 t | 25.0 t | 22.9 t | |
| 23 | 169.0 s | 169.0 s | 169.0 s | 168.7 s | 6′ | 29.9 t | 29.8 t | 28.7 t | |
| 24 | 124.6 d | 124.6 d | 124.7 d | 123.9 d | 7′ | 61.1 d | 61.1 d | 59.6 d | |
| 25 | 150*** s | 150*** s | 150*** s | 148.4 s | 8′ | 174.7 s | 174.9 s | 174.4 s | |
| 26 | 161.7 s | 161.7 s | 161.7 s | 161.5 s | | | | | |

*,** Measured at 125 MHz in (*) $C_5D_5N$ or (**) $CDCl_3$; ppm from TMS.
*** The value is obscure because of solvent interference.

Fig. 2. Partial structures of amythiamicins A (a), B (b) and C (c) elucidated by HMBC experiments.




bond and the formation of an ester bond, followed by the diketopiperazine ring formation. The presence of the ester bond was confirmed by IR spectrum (1735 $cm^{-1}$). The HMBC data suggested that the ester bond was between C-42 and the hydroxyl group of the seryl residue. From these results, the structure of

Fig. 3. Acid hydrolysis of amythiamicin A.

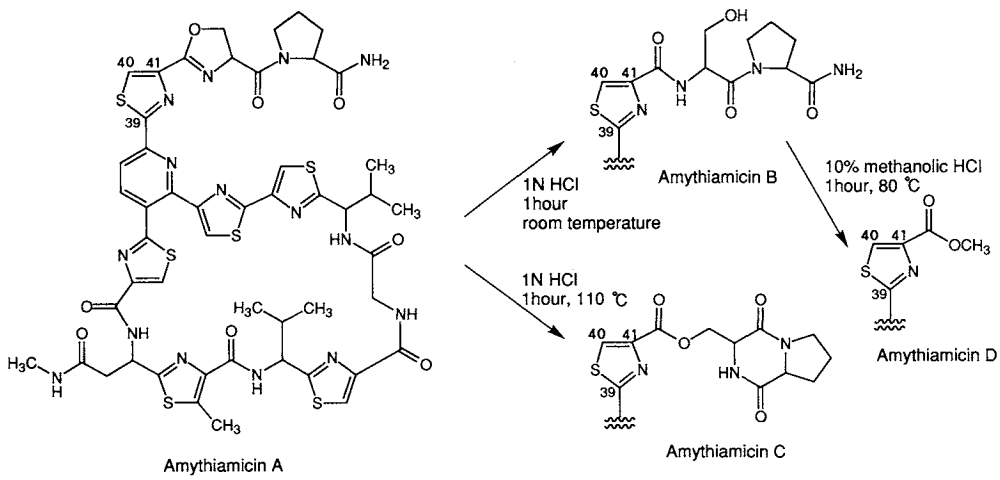



Table 4. HPLC data for amino acids in amythiamicins A, B and C on a CHIRALPAK WH column.

| Amino acids | Retention time (minute) | | | |
|---|---|---|---|---|
| | Authentic amino acids | Amino acids in amythiamicin A | Amino acids in amythiamicin B | Amino acids in amythiamicin C |
| L-Serine | 9.0 | 9.1 | 9.1 | 9.2 |
| D-Serine | 6.5 | — | — | — |
| L-Proline | 7.4 | 7.5 | 7.4 | 7.5 |
| D-Proline | 11.6 | — | — | — |

—: Not detected.

amythiamicin C was determined as shown in Fig. 1.

### Stereochemistries of Amino Acids of Amythiamicins A, B and C

Stereochemistries of the amino acids of amythiamicins A, B and C were elucidated by chiral HPLC. Their retention times in HPLC with a CHIRALPAK WH column of each amino acid obtained by the acid hydrolyses of amythiamicins A, B and C were compared with those of authentic amino acids (Table 4). The stereochemistries of serine and proline in amythiamicins A, B and C were all L. The three amino acids containing thiazole rings were all racemic mixtures[4]. Therefore, an X-ray crystallographic study of amythiamicin D is now in progress.

## Experimental

FAB-MS and HRFAB-MS were obtained on a JEOL JMS-SX 102 spectrometer. NMR spectra were recorded on a JEOL JNM-A500 spectrometer, IR spectra on a Hitachi I-5020 FT-IR spectrometer, and UV spectra on a Hitachi U-3210 spectrometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Amino acid autoanalyses were done with a Hitachi L-8500 amino acid autoanalyzer.

The stereochemistry of the amino acids of amythiamicins A, B and C were examined by HPLC with a chiral column. Analytical condition—Column: CHIRALPAK WH (i.d. 4.6×250 mm); mobile phase, 1 mM $CuSO_4$; column temperature, 50°C; flow rate, 1.5 ml/minute; detection, UV absorption at 220 nm.

### Acid Hydrolysis of Amythiamicin A

Amythiamicin A (32 mg) was hydrolyzed with 1 N HCl (3 ml) at room temperature for 1 hour in a

sealed tube. The reaction mixture was concentrated under reduced pressure. The residue was chromatographed on a Capcell Pak $C_{18}$ column with 45% $CH_3CN$, giving amythiamicin B (8 mg) as a colorless powder.

Amythiamicin A (20 mg) was also hydrolyzed with 1 N HCl (3 ml) at 110°C for 1 hour in a sealed tube. The reaction mixture was concentrated under reduced pressure. The residue was chromatographed on a Capcell Pak $C_{18}$ column with 48% $CH_3CN$, giving amythiamicin C (12 mg) as a colorless powder.

Amythiamicin B: FAB-MS *m/z* 1,200 $(M+H)^+$. $^1H$ and $^{13}C$ NMR data for amythiamicin B are shown in Tables 2 and 3.

Amythiamicin C: FAB-MS *m/z* 1,183 $(M+H)^+$. $^1H$ and $^{13}C$ NMR data for amythiamicin C are shown in Tables 2 and 3.

Methanolysis of Amythiamicin B

Amythiamicin B (10 mg) was treated with 10% anhydrous HCl-MeOH (2 ml) at 80°C for 1 hour in a sealed tube. The reaction mixture was concentrated under reduced pressure. The residue was chromatographed on a Capcell Pak $C_{18}$ column with 70% $CH_3CN$, giving amythiamicin D (6 mg) as a colorless powder.

Amythiamicin D: FAB-MS *m/z* 1,031 $(M+H)^+$. $^1H$ and $^{13}C$ NMR data for amythiamicin D are shown in Tables 2 and 3.

## References

1) SHIMANAKA, K.; N. KINOSHITA, H. IINUMA, M. HAMADA & T. TAKEUCHI: Novel antibiotics, the amythiamicins. I. Taxonomy, fermentation, isolation, physico-chemical properties, and antimicrobial activity. J. Antibiotics 47: 668~674, 1994

2) SHIMANAKA, K.; Y. TAKAHASHI, H. IINUMA, H. NAGANAWA & T. TAKEUCHI: Novel antibiotics, amythiamicins II. Structure eludiation of amythiamicin D. J. Antibiotics 47: 1145~1152, 1994

3) BAX, A. & M. F. SUMMERS: $^1H$ and $^{13}C$ assignments from sensitivity-enhanced detection of heteronuclear multiple-bond connectivity by 2D multiple quantum NMR. J. Am. Chem. Soc. 108: 2093~2094, 1986

4) SETO, Y.; K. TORII, K. BORI, K. INABATA, S. KUWATA & H. WATANABE: Studies of unusual amino acids and their peptides. V. The synthesis and the absolute configuration of β-(2-thiazolyl)-β-alanine present in bottromycin. Bull. Chem. Soc. Japan 47: 151~155, 1974