# Communications to the Editor

[Chem. Pharm. Bull. 33(7)3053—3056(1985)]

## STRUCTURE OF LAVENDOMYCIN, A NEW PEPTIDE ANTIBIOTIC

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On the basis of chemical and spectroscopic evidence, the antibiotic lavendomycin ($C_{29}H_{50}N_{10}O_8$) has been shown to be a hexapeptide 1 containing a new amino acid, 3-methylarginine, in the molecule.

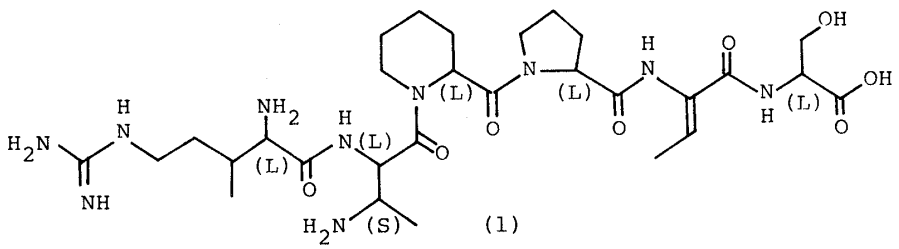
KEYWORDS——peptide antibiotic; Streptomyces lavendulae; lavendomycin; structure elucidation

Lavendomycin (1), recently isolated from Streptomyces lavendulae subsp. sp., is a novel hexapeptide with potent antibacterial activity.[1] Here we describe the structure of this antibiotic based on chemical and spectroscopic evidence.

Lavendomycin was isolated as colorless needles from MeOH: $C_{29}H_{50}N_{10}O_8$ 2HCl (FABMS and FDMS, m/z 667 ($MH^+$) and elemental analysis[1]); mp > 210°C (dec); $[\alpha]_D^{21}$ -47.4° (c 0.40, $H_2O$); IR (Nujol) 3400, 3200, 3000-2400, 1680(sh), 1635 $cm^{-1}$; UV end absorption; pKa 2.9, 6.2, 7.8, ca.13; positive ninhydrin and Sakaguchi reactions.

Acid hydrolysis (6N HCl, 110°C, 24 h) of 1 gave five ninhydrin positive products, which were all isolated by a Dowex 50 x 8 column chromatography as follows. Elution with 0.2 M pyridine-AcOH buffer (pH 3.1) gave the three neutral amino acids and elution with the buffer (pH 4.8) gave the two basic amino acids. The former three amino acids were identified as L-proline (Pro), L-pipecolinic acid (Pip), and L-serine (Ser), respectively, by comparison of their $^1$H-NMR and CD spectra with those of authentic samples. One of the latter two amino acids was determined to be a diastereomeric mixture (ca. 2:1)[2] of 2,3-diaminobutyric acid (Dab) by the $^1$H- and $^{13}$C-NMR and FD mass [m/z 119 ($MH^+$)] spectra. Crystallization from 80% EtOH led to the isolation of the major isomer, which was identified as erythro-L-Dab by mp (197-200°C; lit. 202-204°C[3]) and ORD spectrometry [molar rotation, +2000° (225 nm, 1N HCl); lit. +2200° (225 nm, 1.5N HCl)[4]].

The remaining amino acid 2 [FDMS, m/z 189 ($MH^+$)] was shown to be L-3-methyl-



(1)

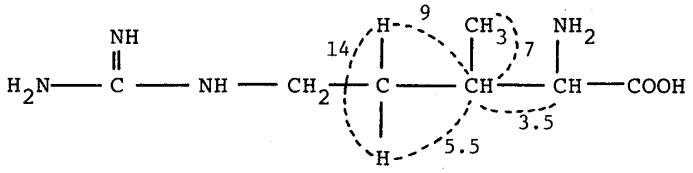


Fig. 1. Structure of 2 with $^{1}H$-$^{1}H$ Relationships in Hz

arginine (3-MeArg) on the following grounds. The amino acid 2 showed a positive Sakaguchi reaction, indicating the presence of a guanidyl group in 2. With the aid of spin decoupling studies (Fig. 1), all the proton signals in the $^{1}H$-NMR spectrum (270 MHz, $D_2O$) were assigned: 1.04 (3H, d, J=7.0Hz, 3-$CH_3$), 1.63(1H, m, 4-H), 1.82 (1H, m, 4-H), 2.29(1H, m, 3H), 3.32(2H, m, 5-$H_2$), 3.79(1H, d, J=3.5Hz, 2-H), which were consistent with the $^{13}C$-NMR data (67.5MHz, $D_2O$): 14.1(q, 3-$CH_3$), 31.8(t, C-4), 32.0(d, C-3), 39.4(t, C-5), 59.5(d, C-2), 157.4(s, C=NH), 173.9 ppm (s, COOH). The absolute configuration at C-2 was deduced to be L from the ORD data: molar rotation, +3000° (225 nm, 1N HCl), +3100° (207 nm, $H_2O$).[5]

The equimolar ratio of the above five amino acid residues in 1 was shown by $^{13}C$- and $^{1}H$-NMR spectroscopies[6] of 1. In addition, the spectra clearly showed the presence of a methylvinyl group: two signals at 128.1(s) and 136.2(d) ppm in the $^{13}C$-NMR spectrum and two proton signals at δ6.84(1H, q, J=7.0Hz) and 1.75(3H, d, J=7.0Hz) in the $^{1}H$-NMR spectrum. This methylvinyl group was identified as dehydro-2-aminobutyric acid as follows. After catalytic hydrogenation (10% Pd-C, MeOH, 3 atm) of 1, the product was subjected to acid hydrolysis (6N HCl, 110°C, 24 h) to give, along with the other five amino acids described above, α-aminobutyric acid which was identified by amino acid analysis using a standard sample. It was concluded, therefore, that dehydro-2-aminobutyric acid (ΔAbu) exists in 1. The relatively high chemical shift (δ1.62) of the methyl protons in the $^{1}H$-NMR spectrum of 1 (DMSO-$d_6$) led to the conclusion that the stereochemistry of the ΔAbu residue is Z.[7]

For determination of the amino acid sequence in 1, the Edman degradation method[8] was applied to 1 in combination with mass spectral analysis.[9] The PTH amino acids obtained in the first and second steps were determined to be PTH-3-MeArg (3) and PTH-$N^{\beta}$-PTC-Dab (5), respectively, by their EI mass spectral data:

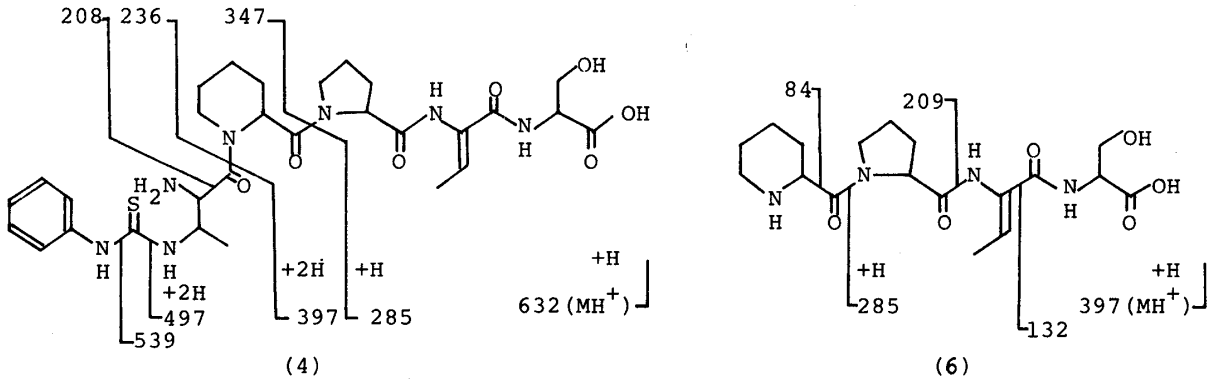



Fig. 2. Fragmentations in the SI Mass Spectra of 4 and 6

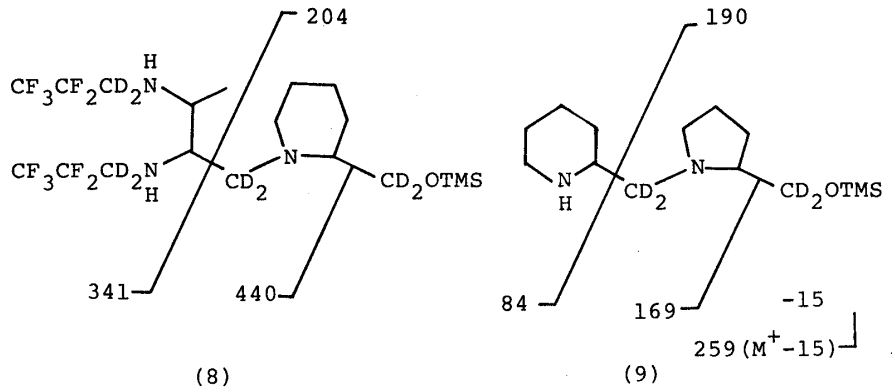
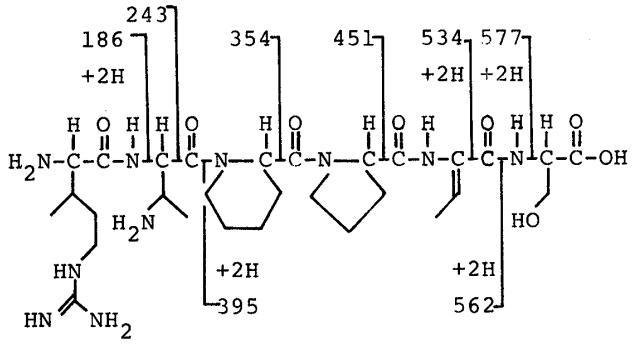



Fig. 3. Fragmentations in the EI Mass Spectra of 8 and 9

3, m/z 305($M^+$); 5, m/z 370($M^+$). On the other hand, the residual peptide 4 in the first step was deduced to be the phenylthiocarbamyl derivative of the pentapeptide lacking 3-MeArg from the lavendomycin molecule (FABMS and SIMS, m/z 632($MH^+$)). These data, together with the mass spectra (FDMS and SIMS, m/z 397($MH^+$)) of the residual peptide 6 in the second step, led to the conclusion that Dab is located at the second position from the N-terminus and the β-amino group of Dab is free in 1. Although the PTH amino acid and the residual peptide 7 in the third step could not be characterized, the residual peptide was shown to contain Pro and Ser by acid hydrolysis of 7 followed by amino acid analysis of the hydrolysate. Ser was determined to be at the C-terminus in 1 by hydrazinolysis: treatment of 1 with hydrazine (100°C, 6 h) gave Ser as the sole amino acid detectable on amino acid analysis. Together with these chemical data, analysis of the SIMS fragmentation ions[10] of 4 and 6 also gave information on the peptide sequence of 1 (see Fig. 2).



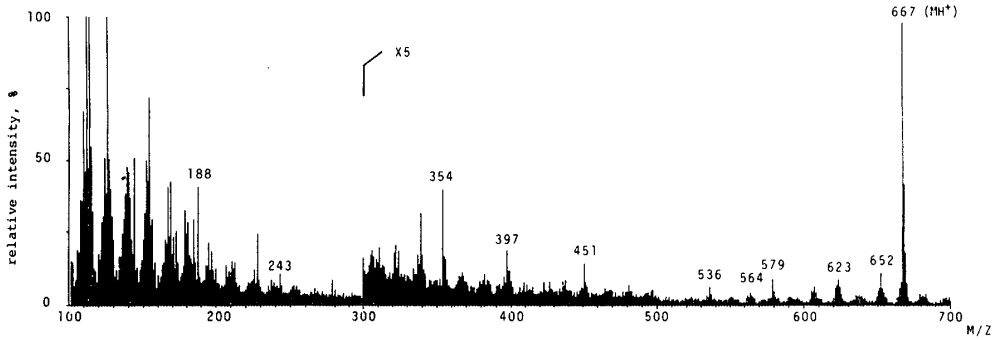




Fig. 4. FAB Mass Spectrum of Lavendomycin (1)

Further supports of the peptide sequence in 1 was obtained by using the GC-MS method developed by Biemann et al.[11] The perfluoro-deuteroalkylated peptide derivatives were prepared by the following treatments[12] of 1: 1) 1N NaOH, 37°C, 30h, 2) 5% HCl-MeOH, 3) $(CF_3CF_2CO)_2O$, 4) $LiAlD_4$, 5) $Et_2NSiMe_3$, and submitted to GC-MS spectrometry, in which two fragments 8 and 9 were characterized as shown in Fig. 3, thus supporting the presence of the sequence Dab-Pip-Pro in 1.

The above chemical and spectral evidence unequivocally determined the peptide sequence of 1 as 3-MeArg-Dab-Pip-Pro-ΔAbu-Ser. Moreover, the FAB mass spectral data of lavendomycin itself was quite consistent with this peptide sequence as shown in Fig. 4. In conclusion, the structure of lavendomycin including its absolute configuration was established to be as shown in 1 except for the configuration at C-3 of the 3-methylarginine residue.

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6) The $^1H$- and $^{13}C$-NMR data of lavendomycin are as follows. $^1H$-NMR ($D_2O$, 270MHz, ext. DSS as $\delta_0$): 1.02(3H, d, J=6.9Hz), 1.27(3H, d, J=6.9Hz), 1.75(3H, d, J=7.0Hz), 1.40 to 2.35(12H, m), 2.44(1H, m), 3.17 to 3.45(3H, m), 3.55 to 3.90(6H, m), 4.10(1H, d, J=5.0Hz), 4.38(1H, dd, J=4.3, 5.3Hz), 4.55(1H, t, J=7.6Hz), 5.01(1H, t, J= 5.2Hz), 5.38(1H, d, J=4.3Hz), 6.84(1H, q, J=7.0Hz); $^{13}C$-NMR ($D_2O$, 67.5Hz, ext Benzene as δ128.7): 13.4(q), 13.7(q), 14.0(q), 19.0(t), 24.0(t), 25.0(t), 25.7(t), 29.9(t), 31.6(t), 32.9(d), 39.3(t), 44.2(t), 48.6(d), 48.6(t), 52.0(d), 54.2(d), 57.7(d), 58.2(d), 61.4(d), 62.9(t), 128.1(s), 136.2(d), 157.3(s), 166.2(s), 169.3(s), 170.2(s), 172.6(s), 174.7(s), 176.4(s).

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(Received May 7, 1985)