Tetrahedron Letters,Vol.28,No.47,pp 5861-5864,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

STRUCTURE OF PAMAMYCIN-607, AN AERIAL MYCELIUM-INDUCING SUBSTANCE OF *STREPTOMYCES ALBONIGER*

Satoru Kondo[a], Kazuhisa Yasui[a], Masato Katayama[a], Shingo Marumo*[a], Tadao Kondo[b] and Hiroyuki Hattori[c]

a) Department of Agricultural Chemistry, Nagoya University, Nagoya 464, Japan
b) Chemical Instrument Center, Nagoya University, Nagoya 464, Japan
c) National Institute for Basic Biology, Okazaki 444, Japan

Summary: Pamamycin-607 with aerial mycelium-inducing activity has been isolated from *Streptomyces alboniger*, and its structure with the relative stereochemistry has been determined as a novel ionophore compound (1) on the basis of spectral analysis mainly by 2D ^{1}H-^{13}C and ^{1}H-^{1}H correlation NMR and NOE difference spectroscopy.

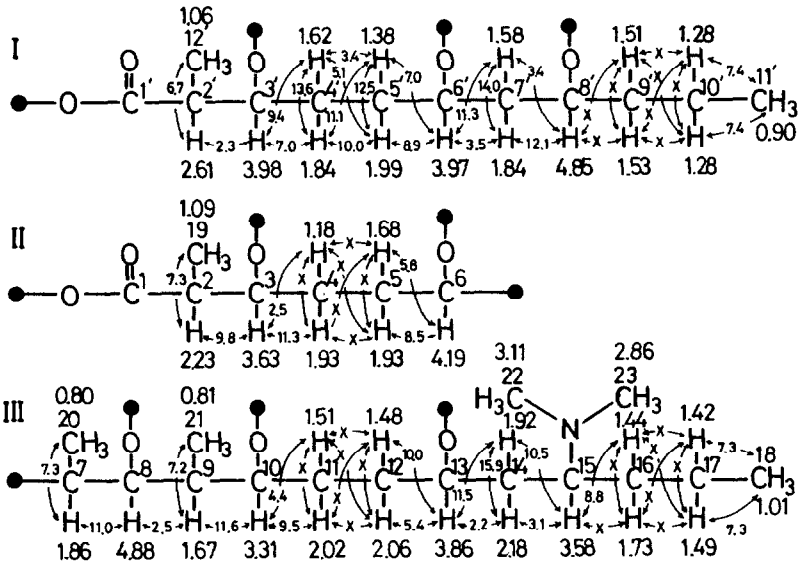
Actinomycetes are an important group of microorganisms that produce a number of antibiotics. The productivity of some useful antibiotics and enzymes in actinomycetes has recently been shown to be closely correlated with cell differentiation of these actinomycetes,[1] such as aerial mycelium formation from substrate mycelium and spore formation. A-factor that was isolated from *Streptomyces griseus* was thus identified as an autoregulating substance of both streptomycin biosynthesis and spore formation.[2] Several other bioregulators in actinomycetes were reviewed by Khokhlov.[3] A-factor-related metabolites were recently isolated from *Streptomyces virginiae*.[4] McCann and Pogell in 1979 discovered a new antibiotic, pamamycin, isolated from *Streptomyces alboniger*.[5] The antibiotic also stimulated aerial mycelium formation in the producing strain. They obtained a mixture of four pamamycins of MW 621, 635, 649 and 663, and postulated the molecular formula, $C_{36}H_{63}NO_7$, for the major component (MW 621) of pamamycins, however, their structures could not be elucidated.

In our continuing research on aerial mycelium-inducing substances in actinomycetes,[6] we have isolated a new pamamycin of MW 607 in a pure form from *Streptomyces alboniger* IFO 12738.[7] The isolated compound, named pamamycin-607, at 0.1 μg/paper disk induced aerial mycelium formation in an aerial mycelium-negative mutant of *Streptomyces alboniger*, and also exhibited antibiotic activity against gram positive bacteria and pathogenic fungi. We now describe the structure elucidation of pamamycin-607 as 1, which has been shown to be a unique ionophore compound of an eighteen membered macrodiolide ring with a dimethylamino group-bearing side chain.

The physicochemical properties of pamamycin-607 are colorless oil, $[\alpha]_D^{33}$ +22.8°(c 0.26, MeOH), $C_{35}H_{61}NO_7$(HRMS observed m/z 607.44621 error 1.4),[8] UV λ_{max}^{MeOH} 215 nm(ε 470), IR $\nu_{max}^{CHCl_3}$ 2960, 2890, 1740, 1460, 1385, 1270, 1190, 1130, 1110, 1075 and 1055 cm^{-1}.

The ^{13}C-(50[9] and 125 MHz) and ^{1}H-NMR(500 MHz) spectra of pamamycin-607 in $CDCl_3$(or acetone-d_6), the spectra of which were sharpened upon addition of CF_3COOD, were extensively

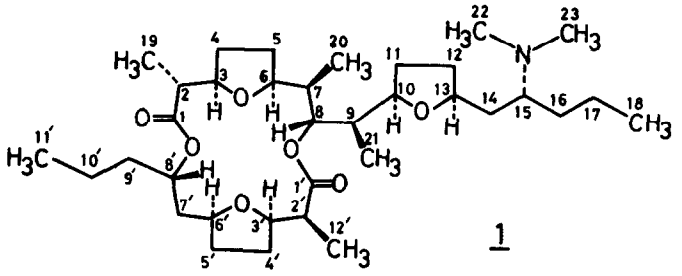
Fig. 1. Assignments of ^{1}H-NMR(500 MHz) Signals in $CDCl_3$ and the Three Partial Structures of Pamamycin-607(x : J value is unclear)



analysed by ^{1}H-^{1}H COSY and ^{1}H 2D-J and ^{1}H-^{13}C COSY with the aid of proton spin decoupling, thus all of the carbons and protons in the molecule could be assigned and the three partial structures I, II and III were deduced, as shown in Fig. 1. The presence of dimethylamino group was recognized by two singlet signals of two methyls in CF_3COOD-$CDCl_3$ that were changed into two doublets by replacing CF_3COOD to CF_3COOH. Seven oxygens in the molecule were assigned to four of two ester groups(^{13}C-NMR: δ 173.9(s), 71.0(d), 173.2(s), 74.4(d); ν_{max} 1740 cm^{-1}), and three ethereal oxygens, because of no OH absorption in IR spectrum and no other carbonyl signal in ^{13}C-NMR spectrum. Two ester groups were also ascertained by reduction of pamamycin-607 with $LiAlH_4$ in diethyl ether to afford two diol products A and B.[10]

Connecting of these three partial structures to build up the plain structure of pamamycin-607 was carried out as follows. The C_6 of II could be connected to the C_7 of III, by observing that the coupling constant between H_6 and H_7 that was never observed in the ^{1}H-NMR spectrum of pamamycin-607 itself newly appeared (J=1 Hz) in that of the bis(p-bromobenzoyl) derivative of the diol B. The C_6-C_7 bond thus formed was further confirmed by NOE enhancement between H_6 and H_7. NOE experiments showed the spacial proximity between two protons in three pairs, i.e., H_3-H_6, H_{10}-H_{13} and $H_{3'}$-$H_{6'}$, in every pair the two protons were connected through five covalent bonds, thus the presence of three tetrahydrofuran rings were suggested. This suggestion was confirmed by large $^{1}J_{C-H}$ values of C_4-H_4(131.8 Hz), C_5-H_5(133.1 Hz), C_{11}-H_{11} (132.2 Hz), C_{12}-H_{12}(131.8 Hz), $C_{4'}$-$H_{4'}$(133.1 Hz), and $C_{5'}$-$H_{5'}$(133.1 Hz), all of which were the characteristic J values of tetrahydrofuran ring.[11] Since the molecule consisted of two parts, I and II+III, which were connected by two ester bonds, the C_1-carbonyl of II should bind with the $C_{8'}$-O- of I, and the $C_{1'}$-carbonyl of I with the C_8-O- of III, respectively, thus an eighteen membered macrodiolide ring was formed, and the plain structure of pamamycin-607 was built up, as shown in Fig. 2. In order to confirm the proposed structure, the reduction products, diols A and B, were extensively analyzed by mass fragmentation[10] and their bis(p-bromobenzoyl)

Fig. 2. The Structure with Relative Stereochemistry of Pamamycin-607

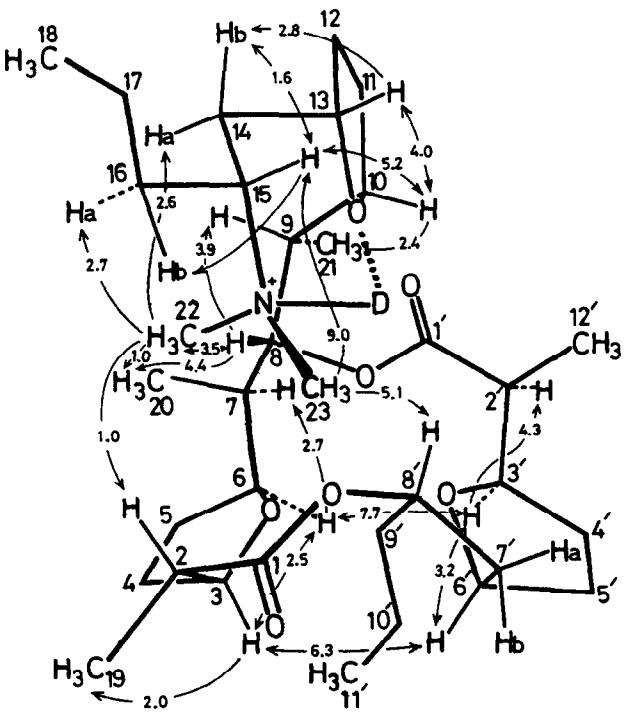


1

derivatives by ^{1}H-^{1}H COSY.

The relative stereochemistry of pamamycin-607 was established by NOE difference spectroscopy and J_{H-H} values of its CF_3COOD salt in acetone-d_6(or $CDCl_3$)(Fig. 3). Namely, an irradiation of 22-CH_3 enhanced the signals of H_8, H_2, H_{14a}, H_{16a} and 20-CH_3, and that of 23-CH_3 enhanced those of H_8, and H_{15}, indicating that 22-CH_3 should hung over H_8, H_2 and 20-CH_3, and 23-CH_3 over $H_{8'}$, respectively, therefore, all of H_8, H_2, 20-CH_3 and $H_{8'}$ were oriented on the upper side of the macrodiolide ring. The NOE enhancements of respective signal group of 23-CH_3, H_{16b}, H_{14b} and H_{13} upon irradiation of H_{15}, and of H_{15}, H_{14b} and H_{10} upon irradiation of H_{13}, indicate that there is a sequential proton group, H_{10}-H_{13}-H_{14b}-H_{15}(-23-CH_3)-H_{16b}, and protons in the group are spacially close to each neighboring proton, and an examination of the spacial interrelation of these protons with the aid of a Dreiding model, suggests the formation of six-membered ring conformation by hydrogen-bonding between $\underline{D}$-N^+-23-CH_3 and the oxygen of the tetrahydrofuran ring in the side chain. This suggestion was confirmed by further experiments that 1) an irradiation of $\underline{H}$-N^+(δ 8.649) that prepared by replacing CF_3COOD to CF_3COOH, enhanced the signals of H_8, H_{13} and H_{15}, 2) the large J values of H_{13}-H_{14a}(11.5 Hz) and of H_{14a}-H_{15}(10.5 Hz), and the small values of H_{13}-H_{14b}(2.2 Hz) and of H_{14b}-H_{15} (3.1 Hz), indicate that H_{13}, H_{14a} and H_{15} are axial and H_{14b} is equatorial on the six-membered chair conformation.

Fig. 3. NOE Enhancements (%) between Protons of Pamamycin-607·CF_3COOD in Acetone-d_6



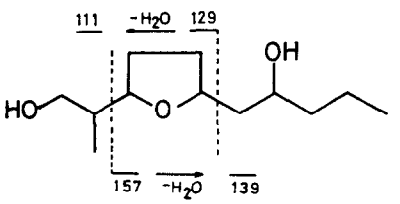
The *cis*-substituents at α,α'-positions of three tetrahydrofuran rings were shown by NOE enhancements between H_3-H_6, H_{10}-H_{13} and $H_{3'}$-$H_{6'}$, respectively. The NOE enhancements were also observed between H_3-$H_{6'}$ and H_6-$H_{3'}$, but no observation between any of these four protons and 22- and 23-methyls, indicating that the H_3, H_6, $H_{3'}$, and $H_{6'}$ should be disposed to the down side of the

macrodiolide ring. The stereochemistries at C_7, C_8, C_9 and $C_{2'}$ were determined by respective NOE enhancement and J value (Hz), i.e., C_7: NOE (20-CH_3 - 22-CH_3) and $J_{6-7}\simeq 0$, C_8: NOE (H_8 - 22-CH_3) and J_{7-8}=11.0, C_9: NOE (H_{10} - 21-CH_3) and J_{9-10}=11.6, $C_{2'}$: NOE ($H_{2'}$ - $H_{3'}$) and $J_{2'-3'}$= 2.3.

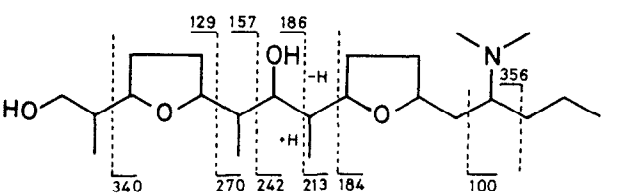
The structure of pamamycin-607 with the relative stereochemistry thus has been elucidated as shown in Fig. 2. The J values of H_2-H_3, H_8-H_9, $H_{6'}$-$H_{7'a}$, $H_{6'}$-$H_{7'b}$, $H_{7'a}$-H_8, and $H_{7'b}$-H_8, were in good agreement with the proposed relative stereochemistry. The absolute stereochemistry of pamamycin-607 and the structures of other pamamycin analogues are now under investigation.

References and Notes

1) M. Okanishi, T. Ohta and H. Umezawa, J. Antibiotics, 23, 45(1970).
2) A. S. Khokhlov, L. N. Anisova, I. I. Tovarova, E. M. Klerner, I. V. Kovalenko, O. I. Krasilnikova, E. Ya Kornitskaya and S. A. Pliner, Z. Allg. Mikrobiol., 13, 647(1973).
3) A. S. Khokhlov, in "Overproduction of Microbial Products ", ed. by V. Krumphanzl, B. Sikyta and Z. Vanek, Academic Press, 1982, pp.97-110
4) Y. Yamada, K. Sugamura, K. Kondo, M. Yanagimoto and H. Okada, J. Antibiotics, 40, 496 (1987).
5) P. A. McCann and B. M. Pogell, J. Antibiotics, 32, 673(1979).
6) Aerial mycelium-inducing substances of *Streptoverticillium sp.* and *Streptomyces ambofaciens* have been investigated by S. Kondo, K. Yasui and S. Marumo: The preliminary announcements at Annual Meeting of the Agricultural Chemical Society of Japan were summarized in The Abstracts of Papers, p.288(1984) and p.357(1986).
7) The isolation and biological activity of pamamycin-607 are to be published in Agric. Biol. Chem., 51(1987).
8) Mass spectrum of pamamycin-607: m/z 607(M^+, 3.4%), 592(1.1), 578(4.5), 564(24), 550(1.0), 494(1.8), 353(1.0), 296(1.4), 263(1.9), 254(5.7), 213(2.9), 186(3.7), 157(7.6), 143(4.8), 111(7.0) and 100(100).
9) ^{13}C-NMR(50 MHz) data of pamamycin-607 in acetone-d_6(ppm, TMS)(multiplicity, $^1J_{C-H}$, position); 173.9(s, C_1), 47.2(d, 143.3, C_2), 82.5(d, 144.0, C_3), 30.6(t, 131.8, C_4), 17.8(t, 133.1, C_5), 76.4(d, 146.5, C_6), 37.4(d, 107.4, C_7), 74.4(d, 146.5, C_8), 41.0(d, 123.3, C_9), 80.3(d, 146.5, C_{10}), 29.6(t, 132.2, C_{11}), 31.1(t, 131.8, C_{12}), 78.4(d, 142.8, C_{13}), 34.1(t, 126.1, C_{14}), 67.5(d, 145.3, C_{15}), 29.3(t, 133.9, C_{16}), 19.8(t, 123.9, C_{17}), 14.1 (q, 127.0, C_{18}), 14.0(q, 127.0, C_{19}), 9.8(q, 127.3, C_{20}), 10.4(q, 127.0, C_{21}), 36.6(q, 142.8, C_{22}), 43.1(q, 141.6, C_{23}), 173.2(s, $C_{1'}$), 41.6(d, 128.2, $C_{2'}$), 78.4(d, 146.5, $C_{3'}$), 27.5(t, 133.1, $C_{4'}$), 31.7(t, 133.1, $C_{5'}$), 74.5(d, 146.5, $C_{6'}$), 38.9(t, 132.4, $C_{7'}$), 71.0(d, 151.4, $C_{8'}$), 37.1(t, 130.6, $C_{9'}$), 18.3(t, 127.6, $C_{10'}$), 14.2(q, 127.0, $C_{11'}$) and 8.7(q, 129.0, $C_{12'}$).
10) Mass spectrum of the diol A: m/z 186(M^+-30, 17%), 157(100), 155(30), 139(33), 129(49), 121(27), 111(32), 95(16), 85(65) and 71(96). Mass spectrum of the diol B: m/z 399(M^+, 0.8%), 357(2.7), 356(12), 340(1.5), 270(2.3), 262(1.7), 254(1.5), 242(5.0), 213(2.3), 207 (2.7), 198(2.3), 186(2.3), 170(7.5), 157(3.1), 142(2.6), 129(8.7), 111(5.4) and 100(100)



diol A



diol B

11) J. B. Stothers, Carbon-13 NMR Spectroscopy, ed. by A. T. Blomquist and H. Wasserman, Academic Press, New York, 1972, p.342.

(Received in Japan 30 June 1987)