Tetrahedron Letters,Vol.29,No.12,pp 1401-1404,1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Press plc

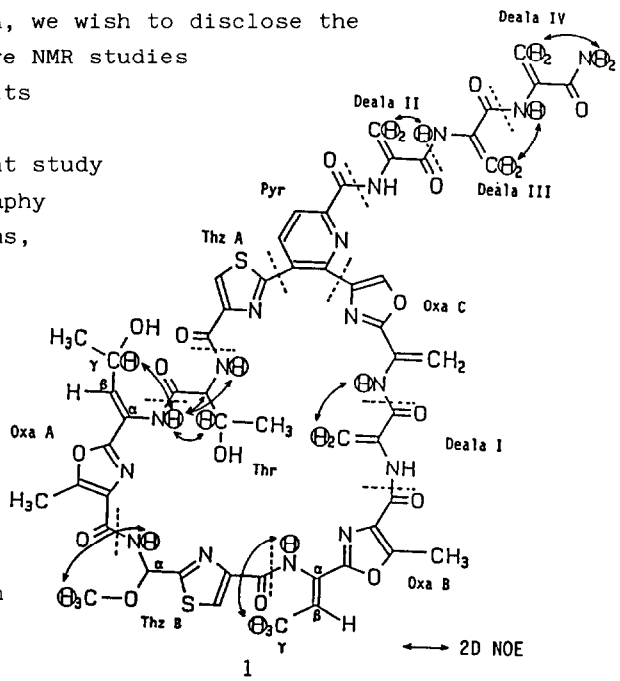
THE STRUCTURES OF SULFOMYCIN I AND BERNINAMYCIN A

Hiroshi Abe,*[a] Katsuhiko Kushida,[b] Yoshinori Shiobara,[c] and Mitsuaki Kodama[c]
[a]Organic Chemistry Research Laboratory, Tanabe Seiyaku Co., Ltd., Toda, Saitama 335, Japan. [b]Varian Instruments Ltd., Shinjuku-ku, Tokyo 160, Japan. [c]Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan.

Abstract: The structure of sulfomycin I, a modified peptide antibiotic, was determined on the basis of chemical and sepctroscopic evidence. The revised structure of structurally related antibiotic, berninamycin A, was also proposed.

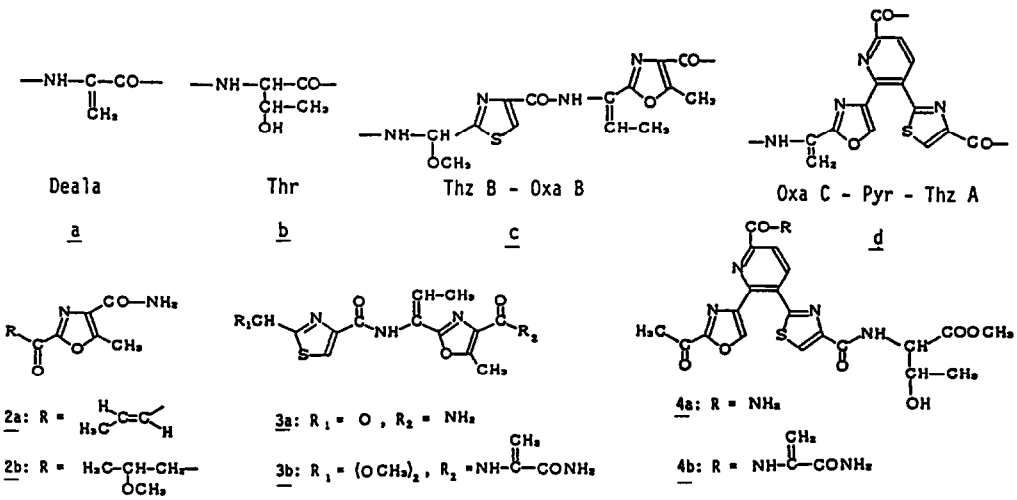
Sulfomycin I (1) is a novel sulfur-containing peptide antibiotic produced by Streptomyces viridochromogenes subsp. sulfomycini ATCC 29776 and exhibits strong inhibitory activity against gram-positive bacteria.[1] Its antibacterial spectra and cross resistance studies suggested that it belongs to a family of thiostrepton antibiotics.[1] In the earlier reports,[2,3] we revealed the presence of the four structural units a-d in the antibiotic. However, because of the complex nature of 1, further structural studies using degradation methods were difficult to continue. Recent developments in NMR techniques stimulated our interests in the re-examination of the novel antibiotic. In the present communication, we wish to disclose the structure of 1 based on the intensive NMR studies of 1 and new fragments obtained by its mild hydrolysis.

Sulfomycin I used in the present study was obtained by repeated chromatography followed by recrystallization (prisms, m.p. > 280 °C). The molecular formula was determined as $C_{54}H_{52}N_{16}O_{16}S_2$ (MW 1244) from analytical and mass spectral data (FAB-MS, m/z 1245 (MH^+)). In order to obtain larger degradation fragments, 1 was treated with 6N HCl at room temperature in MeOH-dioxane, giving five new products 2a,b, 3a, and 4a,b[4] after chromatographic separation. Although the NMR spectrum of 1 does not show signals corresponding to the side chains of 2a or 2b, these products



1

must be derived from the oxazole A (Oxa A) residue through dehydration and hydrolysis, since the signals assignable to the allyl alcohol moiety are observed in the spectrum of 1 (δ 1.20 (δ-CH_3),4.46 (γ-CH), and 6.27 (β-CH)). Long-range C-H couplings were observed between the carbonyl carbon of Oxa A (δ 161.10) and NH (δ 8.36) and α-CH (δ 6.46) protons of thiazole B (Thz B) residue in the LSPD spectrum of 1. Therefore, these two residues can be connected. Threonine (Thr) residue, the existence of which has been reported previously,[1a] was connected to the Oxa A, since NOEs were observed between NH proton of Oxa A (δ 9.38) and the protons of Thr (NH (δ 8.19), α-CH (δ 4.34) and β-CH (δ 4.14)). Consequently, the sequence Thr-Oxa A-Thz B-Oxa B was ascertained. The configuration of the ethylenic linkage in Oxa A and B was determined by the NOEs between NH (δ 10.04 (Oxa B)) and γ-CH (δ 1.75 (Oxa B)) in both cases (Figure 1). The formation of the hydrolysis products 4a and 4b was very informative and, first of all, Thr residue in the tetra peptide sequence assigned above was found to be attached to Thz A of sub-unit d. The NMR spectra of 1 disclosed the presence of four exomethylene groups (δ 5.78 and 6.40 (Deala I), 5.95 and 6.58 (Deala II), 5.67 and 5.72 (Deala III), 5.65 and 6.12 (Deala IV)), which are present in the form of dehydroalanine (Deala) residue in 1. The above results revealed that one of the Deala is attached to the pyridine ring of sub-unit d. The presence of three consecutive Deala side chain with C-terminal primary carboxamide was evidenced by 2D NOE (Figure 1), in which cross peaks were observed between the following pair of protons; NH_2-cis CH (Deala IV), NH (Deala IV)-cis CH (Deala III) and NH (Deala III)-cis CH (Deala II). The remaining problem is the location of the fourth Deala (Deala I). Careful re-examination of the previous methanolysis products[3] of 1 led to the isolation of a new sulfomycinate 3b[4] having Deala residue, allowing the attachment of Deala I to Oxa B residue and, subsequently, the C-terminal of Deala I to Oxa C residue. The latter connection leading to macrocyclic structure was confirmed by the NOE between NH proton of Oxa C (δ 9.99) and a olefinic proton of Deala I (δ 5.78) (Figure 1).



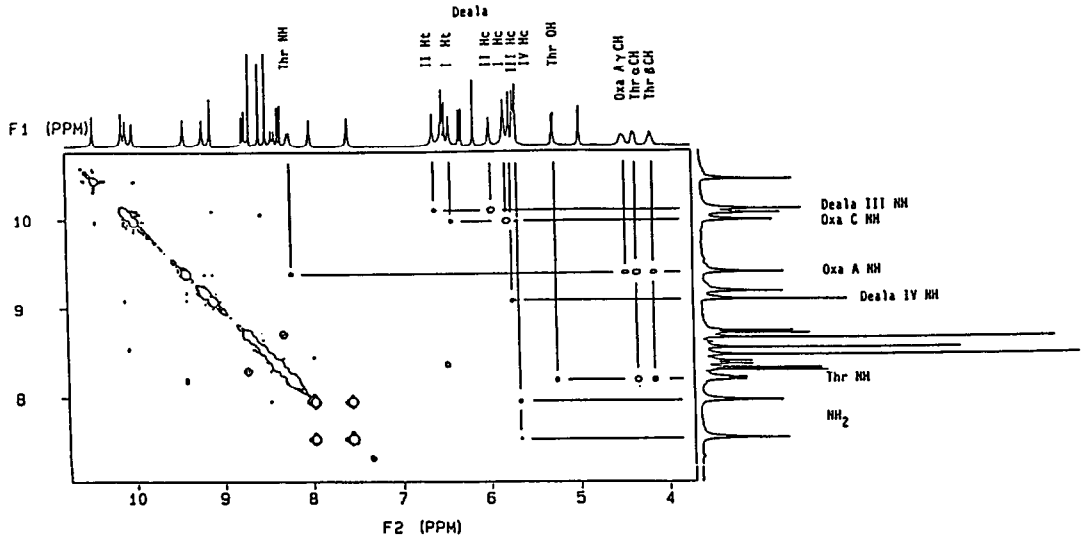
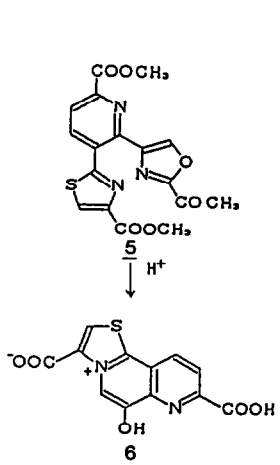
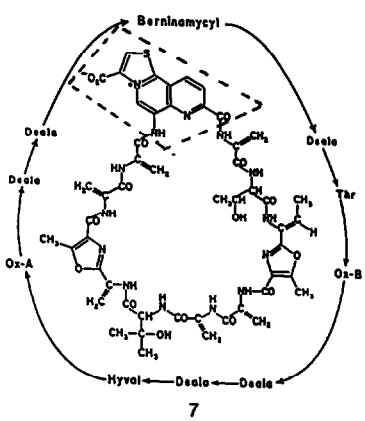


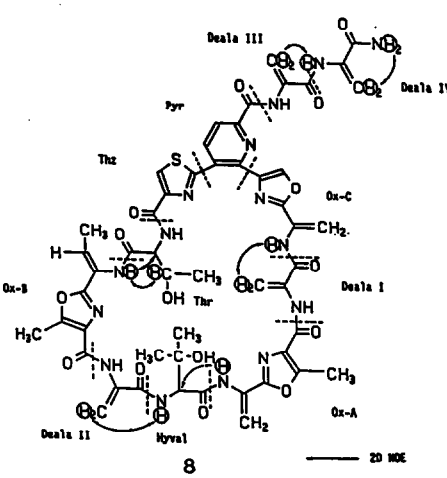
Figure 1. Phase-sensitive two dimentional NOE spectrum of sulfomycin I (300 MHz, in DMSO-d_6 at room temperature).

The extensive NMR analysis of 1 using ^1H-^1H and ^1H-^{13}C correlation 2D spectra as well as COLOC pules sequence[5] enabled us to assign all of 52 protons including 13 exchangeable protons and all 54 carbons. The results were fully compatible with the structure 1. The structure thus obtained was confirmed by X-ray analysis of 1.[6]

The structure 7 containing berninamycyl unit (indicated by dotted line) was proposed by Rinehalt,Jr. for berninamycin A,[7] a modifide peptide antibiotic structurally related to 1. The presence of the berninamycyl unit rests on the isolation of berninamycinic acid (6) on acid hydrolysis of the antibiotic. However, as 6 was obtained by the acid treatment of 1 as well as dimethyl sulfomycinate (5),[3] the antibiotic have an alternative possibility to contain the sub-unit d instead of berninamycyl unit. Careful analysis of COSY and 2D NOE spectra of berninamycin A verified the presence of C-terminal







primary carboxamide (δ 7.50 and 7.93) attached to two Deala residues. This result is difficult to explain based on the Rinehart s structure. At the same time, the sequences Thr-Oxa B, Deala II-Hyval-Oxa A, and Deala I-Oxa C were confirmed by NOEs indicated in the structure 8. Based on these evidences coupled with the result of FAB-MS (m/z 1146 (MH^+)), we wish to propose a new structure 8 for berninamycin A.

1) a) T. Egawa, K. Umino, Y. Tamura, M. Shimizu, K. Kaneko, M. Sakurazawa, S. Awataguchi, and T. Okuda, J. Antibiotics, 12, 12(1969); b) N. Nakanishi, T. Oshida, S. Yano, K. Takeda, T. Yamaguchi, and Y. Ito, Prasmid, 15, 217(1986).

2) H. Abe, M. Ikeda, T. Takaishi, Y. Ito, and T. Okuda, Tetrahedron Lett., 735(1977); H. Abe, T. Takaishi, Y. Ito, and T. Okuda, Heterocycles, 8, 461(1977).

3) H. Abe, T. Takaishi, T. Okuda, K. Aoe, and T. Date, Tetrahedron Lett., 2791(1978).

4) 2a: mp 161-5°C; UV(MeOH) 289nm (15,000); IR(Nujol) 3420, 1680, 1620, 1530cm^{-1}; ^1H NMR (100MHz, $CDCl_3$)δ 2.04 (3H, dd, J=6.4, 1.0), 2.76 (3H, s), 6.04 (1H, bs), 6.86 (1H, bs) ppm; ^{13}C NMR (25MHz, $CDCl_3$)δ 12.20 (q), 18.75 (q), 126.31 (d), 130.58 (s), 147.84 (d), 155.18 (s), 157.28 (s), 163.11 (s) ppm. 2b: mp 119.5-120°C; UV(MeOH) 273nm (11,000); IR(Nujol) 3480, 1700, 1690, 1610, 1530cm^{-1}; ^1H NMR δ 1.27 (3H, d, J=6.1), 2.75 (3H, s), 2.93 (1H, dd, J=15.9, 5.4), 3.33 (3H, s), 3.44 (1H, dd, J=15.9, 7.6), 4.01 (1H, m), 6.38 (1H, bs), 6.92 (1H, bs) ppm; ^{13}C NMR (25 MHz, $CDCl_3$)δ 12.17 (q), 19.34 (q), 45.66 (t), 15.40 (q), 73.22 (d), 130.64 (s), 154.62 (s), 157.31 (s), 163.16 (s), 186.01 (s) ppm. 3a: mp 237-240°C (dec.); UV(MeOH) 247nm (21,400); IR(Nujol) 3460, 3380, 1685, 1625, 1520cm^{-1}; NMR (100MHz, DMSO-d_6 1.78 (3H, d, J=7.1), 2.54 (3H, s), 6.60 (1H, q, J=7.1), 7.36 (2H, bs), 8.88 (1H, d, J=1.2), 10.02 (1H,d,J=1.2), 10.08 (1H, bs) ppm. 3b: mp 179-180°C, IR(Nujol) 3360, 3180, 1670, 1620, 1500cm^{-1}; ^1H NMR (100MHz; $CDCl_3$)δ 1.92 (3H, d, J=7.0), 2.66 (3H, s), 3.48 (6H, s), 5.34 (1H, t, J=1.0), 5.68 (1H, s), 5.94 (2H, bs), 6.60 (1H, d, J=1.0), 6.70 (1H, q, J=7), 8.24 (1H, s), 8.78 (1H, bs), 9.40 (1H, bs) ppm. 4a: amorphous; ^1H NMR (400MHz; $CDCl_3$)δ 1.29 (3H, d, J=6.4), 2.47 (3H, s), 3.37 (1H, bs), 3.79 (3H, s), 4.47 (1H, m), 4.80 (1H, dd, J=9.2, 2.8), 6.47 (1H, d, J=3.5), 7.87 (1H, d, J=3.5), 8.13 (1H, d, J=9.2), 8.14 (1H, d, J=8.1), 8.23 (1H, d, J=8.1), 8.26 (1H, s), 8.31 (1H, s) ppm; ^{13}C NMR (65MHz, DMSO-d_6)δ 20.24 (q), 26.10 (q), 52.03 (q), 57.55 (d), 66.07 (d), 121.08 (d), 126.95 (d), 128.59 (s), 140.52 (s), 140.90 (d), 144.11 (d), 146.12 (s), 148.94 (s), 150.67 (s), 156.47 (s), 160.41 (s), 163.87 (s), 164.85 (s), 170.74 (s), 184.97 (s) ppm.

5) H. Kessler, C. Griesinger, J. Zarbock, and H. R. Loosli, J. Magn. Reson, 57, 331(1984).

6) The results of X-ray analysis will be reported elsewhere.

7) J. M. Liesch and K. L. Rinehart, Jr., J. Am. Chem. Soc., 99, 1645(1977).

(Received in Japan 4 December 1987)