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STRUCTURE OF OXAZOLOMYCIN, A NOVEL β-LACTONE ANTIBIOTIC

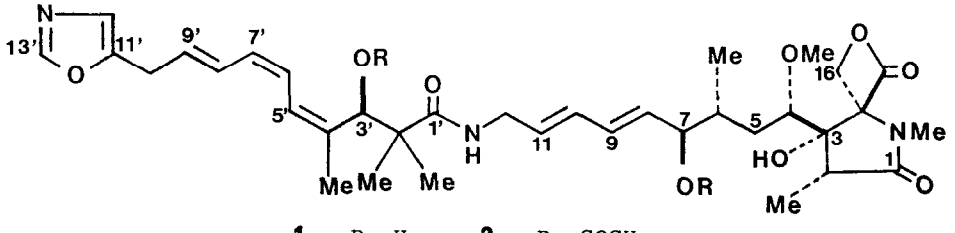
Tatsuya MORI, Kanji TAKAHASHI, Masato KASHIWABARA, and Daisuke UEMURA*
Faculty of Liberal Arts, Shizuoka University, Ohya, Shizuoka 422, Japan
Chuji KATAYAMA
Department of Chemistry, Faculty of Science, Nagoya University, Chikusa, Nagoya 464, Japan
Shuichi IWADARE, Yoshikazu SHIZURI, Ryuji MITOMO
Fumio NAKANO, and Akinori MATSUZAKI
Banyu Pharmaceutical Co. Ltd., Nihonbashi Honcho, Chuo-ku, Tokyo 103, Japan

Summary: The structure of oxazolomycin exhibiting antitumor activity has been elucidated.

Oxazolomycin was discovered in the course of a search for antibiotics with inhibitory activity against Ehrlich ascites tumor. This antibiotic was produced by Streptomyces sp.,[1] but the physico-chemical properties resembled those of resistaphylin reported by Kaken group in 1971.[2] Although direct comparison of oxazolomycin with resistaphylin has not made, it seems that they are identical. Despite of a novel molecule, however, the structure has not been determined. Therefore, we wish to report herein the structure of oxazolomycin.

The fermentation broth of Streptomyces sp. was adjusted to pH 7.0 with phosphate buffer, and then extracted with two portions of n-butyl acetate. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to give an oily material. The crude extracts were separated by column chromatography with silicic acid. Each elution was carefully tested by activity against Ehrlich ascites tumor in vivo. Further purification of active constituents was successfully performed by HPLC using the LiChrosorb RP-8 Hibar column. Two antibiotics, oxazolomycin[3] and neooxazolomycin[4] were obtained.

Oxazolomycin (**1**) also exhibits activity against P-388 leukemia and gram-positive bacteria. The molecular formula, $C_{35}H_{49}N_3O_9$, was suggested by com-



1 R= H **2** R= $COCH_3$

bination of the molecular weight determining [FAB/positive: m/Z 656 $(M+H)^+$][5] and elementary analysis of amorphous oxazolomycin. The characteristic properties of oxazolomycin in spectral analysis are as follows: it belongs to triene antibiotics, judging from the UV-absorption[6] at 265 (ε, 28000), 275 (ε, 34000) and 285 nm (ε, 27000), and the IR spectrum reveals the presence of β-lactone attributed to absorption at 1825 cm^{-1}.[7] Oxazolomycin (**1**) was easily acetylated with Ac_2O and pyridine, furnished a diacetate **2**, the ^{1}H-NMR spectrum of which was shown in Table 1. Compound **2** was degraded by ozonolysis (i. O_3/MeOH, -40°C, ii. $NaBH_4$, ambient, iii. Ac_2O/py), giving a methyl ester **3**[8] and a mixture of triacetates which was treated with aq. NH_3 in MeOH. The resulting triols were separated by preparative TLC (SiO_2, 15% MeOH in $CHCl_3$) and then independently acetylated under ordinary condition to afford threo acetate **4a**[9] and erythro acetate **4b**,[9] respectively. Compound **3** was converted to the p-bromobenzoate **5** by partial hydrolysis with aq. NH_3 in MeOH, followed by treatment with p-bromobenzoyl chloride in pyridine. Recrystallization of **5** from acetone and n-hexane afforded well-formed, orthorhombic crystals; m.p. 160-161°C. Its structure was unambiguously determined by X-ray crystallographic analysis. The space group is $P2_12_12_1$ and lattice constants are a= 0.105 nm, b= 0.302 nm and c= 0.097 nm. The structure was solved by using of MULTAN and refined to R= 8.2% (its antipode: 8.7%) by block-digonal least-square method. A view of the molecule of **5** is shown in Figure 1.

On the other hand, the structure of **4a** was completely determined by chemical transformation from L-(+)-pantolactone,[10] containing one asymmetric center assigned to be S. Treatment of this starting material with benzyl trichloroacetimidate and BF_3 gave a benzyl ether **6**[11]; $[\alpha]_D$= -114° (c 1.9, $CHCl_3$). Compound **6** was converted to **7**[11]; 34% yield, $[\alpha]_D$= -20° (c 0.49, $CHCl_3$), and to its erythro isomer; 19% yield, $[\alpha]_D$= +4.4° (c 4.5, $CHCl_3$), by treatment with MeMgI in THF and then with LAH in situ. Oxidation of **7** with PDC in CH_2Cl_2 was carried out to obtain **8**. Stereochemistry of **8** was confirmed by observation of NOE between H3' and H4' in the ^{1}H-NMR spectrum. The lactone **8** was converted to synthetic threo **4a** by treatment with ethanolamine, by hydrogenolysis with Pd-C and H_2, and then by acetylation with Ac_2O/py. Thus obtained **4a** was consistent with natural threo **4a** in the spectroscopic data and optical rotation; synthetic **4a**: $[\alpha]_D$= +8.7° (c 0.99, $CHCl_3$), natural **4a**: $[\alpha]_D$= +9.2° (c 0.09, $CHCl_3$).

Oxazolomycin contains the conjugated triene as described before. Furthermore, the UV-absorption at 230 nm (ε, 32000) also indicated the conjugated diene chromophore in **1**. Complete analysis of the ^{1}H-NMR spectrum (Table 1) of **2** clearly suggested the existence of two partial structures; $-CH_2CH\underset{E}{=}CHCH\underset{Z}{=}CHCH=CCH_3-$ and $-CONHCH_2CH\underset{E}{=}CHCH\underset{E}{=}CHCHOCOCH_3-$. Therefore, the residual molecular formula is necessarily C_3H_2NO, which corresponds to the oxazole ring or to the isooxazole ring. By the following experiment, the presence of 5-substituted oxazole was indicated. Under hydrogenation by using Pd-C as catalyst in AcOEt, oxazolomycin yielded decahydrooxazolomycin[FDMS, m/Z 688 $(M+Na)^+$; ^{1}H-NMR (360 MHz, $CDCl_3$) 6.73 (1H, br.s, H12'), 7.75 (1H, s, H13');

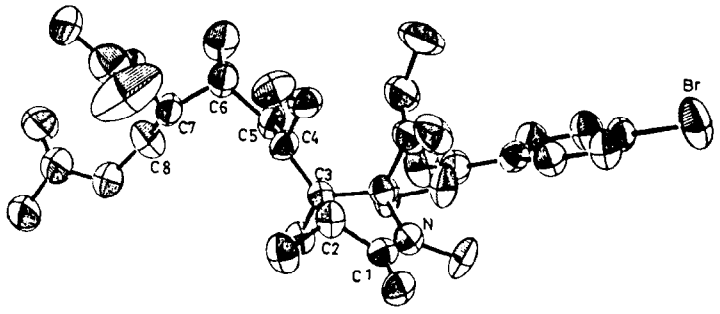
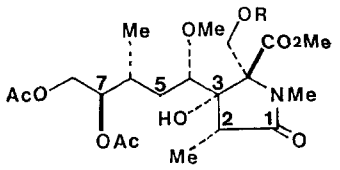
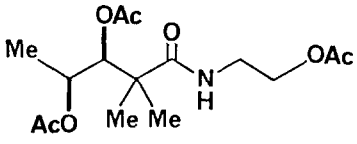


Figure 1. ORTEP drawing of the structure of **5**.

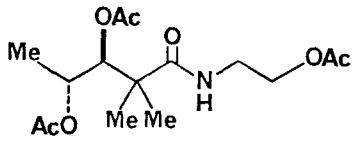


3 R= $COCH_3$

5 R= COC_6H_4Br



4a

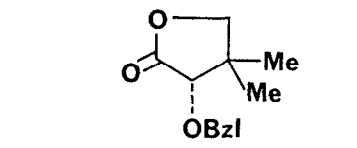


4b

Table 1. ^{1}H-NMR Signals and Decoupling for Diacetate **2**[a]

Assignment	δ, multi	J, Hz	Change on Irradn.
6-Me	1.00, d (3)	6.8	H6 s
H5	1.18, m (1)		H4 collapsed
2'-Me	1.23, s (6)		
2-Me	1.25, d (3)	7.4	H2 s
4'-Me	1.77, br.s (3)		
H5, H6	1.94, m (2)		H7 collapsed
			H4 collapsed
$-COCH_3$	2.07, s (6)		
H2	2.40, q (1)	7.4	
NMe	2.93, s (3)		
OMe	3.37, s (3)		
H4	3.50, m (1)		
H10'	3.52, d (2)	7.2	
H12	3.90, br.dd (2)	5.4, 6.3	NH collapsed
H16	4.40, d (1)	6.2[b]	
H16	4.75, d (1)	6.2[b]	
H7	5.23,[c] dd (1)	5.9, 7.7	H6 d, 7.7
			H8 d, 5.9
H8	5.56 , dd (1)	7.7, 14.9	H7 d, 14.9
H11	5.71, dt (1)	6.3, 14.4	H10 t, 6.3
H9'	5.59, dt (1)	7.2, 15.3	H10' d, 15.3
H3'	5.85,[d] s (1)		
H7'	6.00, dd (1)	11.3, 11.3	H6' d, 11,3
			H8' d, 11.3
NH	6.06[e], br.t (1)	5.4	H12 br.s
H10	6.15, dd (1)	10.0, 14.4	
H9	6.25, dd (1)	10.0, 14.9	H8 d, 10.0
H6'	6.35, dd (1)	11.3, 11.3	
H5'	6.48, br.d (1)	11.3	H6' br.s
			4'-Me d, 11.3
H8'	6.62, br.dd (1)	11.3, 15.3	H10' dd, 11.3, 15.3
H12'	6.80, br.s (1)		H10' s
H13'	7.80, s (1)		

a. 360 MHz, $CDCl_3$, b. AB pattern, c.δ3.94 (1H, dd, J= 6.2, 6.2 Hz) in **1**, d. δ4.63 (1H, s) in **1**, e. exchangeable with D_2O at 50°C.



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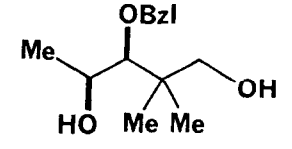

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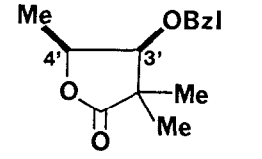

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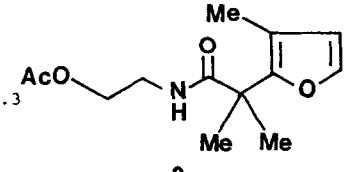

9

^{13}C-NMR (25.1 MHz, $CDCl_3$) 121.6 (d, C12'), 150.1 (d, C13'), 153.4 (s, C11')].[12] Finally, the Z configuration of trisubstituted double bond was confirmed by observation of 5.9% NOE between C4'-Me and H5'. Therefore, ozonolysis of oxazolomycin itself but not its acetate **2** (i. O_3/MeOH, ii. $NaBH_4$, iii. Ac_2O/py) gave compound **9**. This result is also consistent with configuration on C4'-double bond.

Thus, oxazolomycin has been refined as structure **1**, characterized by a unique β-lactone,[13] an oxazole and a triene. Now, studies on the structure-activity relationship are currently under way.

Acknowledgments: We are grateful to Professors Y. Hirata (Meijo University) and Y. Kishi (Harvard University) for the NMR and MS measurements.

REFERENCES AND NOTES

1) The characteristics of the strain will be published separately.
2) Resistaphylin was isolated from Streptomyces antibioticus No. K-869 as a new antibacterial antibiotic. S. Aizawa, M. Shibuya and S. Shirato, J. Antibiotics 1971, 24, 393.
3) The LD_{50} dose for **1** by the intraperitoneal injection in mice was 10.6 mg/kg.
4) The structure of neooxazolomycin will be reported in this issue.
5) The negative ion peak was also observed at m/Z 654 $(M-H)^-$. The molecular formula was previously proposed to be $C_{24}H_{34}N_2O_7$.[2]
6) A.I. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products", Pergamon Press, 1964, p.53.
7) K. Yamada, S. Takada, S. Nakamura and Y. Hirata, Tetrahedron 1968, 24, 199. Methanolysis of oxazolomycin gave a methyl ester in which the $-CH_2OH$ grouping was observed.
8) **3**: $C_{22}H_{35}NO_{11}$; IR($CHCl_3$) 3350, 1720, 1685 cm^{-1}; ^{1}H-NMR (90MHz, $CDCl_3$) 1.00 (3H, d, J=6.0Hz), 1.22(3H, d, J=7.4Hz), 2.05(9H, s), 2.70(1H, q, J=7.4Hz), 2.81(3H, s), 3.00(1H, br.s, exchangeable with D_2O), 3.40(1H, m), 3.40(3H, s), 3.70(3H, s), 3.97(1H, dd, J=7.5, 12Hz), 4.36(1H, dd, J=3.0, 12Hz), 4.43(1H, d, J=13Hz), 4.89(1H, d, J=13Hz), 5.00(1H, m).
9) The J value between H3' and H4' revealed the stereochemistry of **4**a and **4**b; **4**a: $J_{3',4'}$= 5.8 Hz, **4**b: $J_{3',4'}$ =3.3 Hz, see A.A. Bothner-By and C. Naar-Collin, J. Am. Chem. Soc. 1962, 84, 743.
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13) J.S. Wells, J.C. Hunter, G.L. Astle, J.C. Sherwood, C.M. Ricca, W.H. Trejo, D.P. Bonner and R.B. Sykes, J. Antibiotics 1982, 35, 814.

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