PRODUCTION, ISOLATION AND CHEMICAL CHARACTERIZATION OF MIMOSAMYCIN

HIROSHI FUKUMI*, FUMIO MARUYAMA, KAYOKO YOSHIDA and MAMORU ARAI
Central Research* and Fermentation Research Laboratories
Sankyo Co., Ltd., Shinagawa-ku, Tokyo, Japan

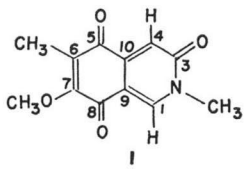
AKINORI KUBO and TADASHI ARAI
Division of Chemotherapy, Chiba Cancer Center Research Institute, Chiba, Japan

(Received for publication April 19, 1978)

A procedure is described for the large-scale production of mimosamycin, a satellite antibiotic found in the culture filtrate of *Streptomyces lavendulae* No. 314. The ^{1}H and ^{13}C NMR, and mass spectroscopic data, are explained in terms of the structure of mimosamycin.

From the culture filtrate of *Streptomyces lavendulae* No. 314, an antibiotic, which is responsible for antimycobacteria activity including streptomycin-resistant strains of human tubercle bacilli, was isolated and designated as mimosamycin.[1,2]

The structure of mimosamycin was determined as **I** by an X-ray crystallographic study and it became clear that mimosamycin belongs to a thus-far unprecedented structural class of antibiotics albeit a relatively simple ring system.[3]



I

This paper describes the large scale production of mimosamycin and analysis of the spectroscopic data of mimosamycin.

Production and Isolation

For the large-scale production of mimosamycin, *Streptomyces lavendulae* No. 314 was cultivated in a 6,000-liter fermentor containing 3,000 liters of a medium having the same ingredients as described in the previous paper.[1] Fermentation was carried out for 18.5 hours at 27°C under agitation at 120 rpm and aeration of 1,500 liters/min. Isolation procedures were improved somewhat as follows. The methylene chloride extract (700 liters) of the culture filtrate (2,800 liters) was concentrated to remove the solvent and 10% aqueous MeOH was then added to make up a volume of 1.9 liters. After washing with hexane the solution was diluted with water (1.5 liters) and extracted with $CHCl_3$ (3.7 liters) at pH 9.0. The concentrate of the extract was made up to a volume of 585 ml by addition of benzene. A 400-ml aliquot of the benzene solution was applied to a column of silica gel (Merck, Silica gel 60, 8 × 52 cm) and mimosamycin was eluted with benzene - AcOEt (4 : 1). Further purification of mimosamycin from the concentrate was performed by silica gel chromatography on a dry column (Woelm for dry column, 4.5 × 94 cm) developed with benzene - AcOEt (1 : 1). A yellow-colored band containing mimosamycin was extracted with $CHCl_3$ - MeOH (9 : 1) and the crude extract was chromatographed on silica gel (Merck, Silica gel 60, 3 × 23 cm) using $CHCl_3$ - MeOH (98 : 2) as an eluent. Recrystallization from MeOH gave 75 mg of mimosamycin, yellow prisms, melting at 219~221°C.

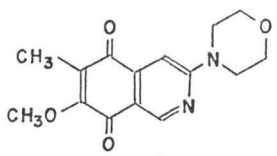
^{1}H- and ^{13}C-NMR Data*

The ^{1}H-NMR spectrum revealed that there are three methyl groups, showing singlets at 2.10, 3.69 and 4.20 ppm. The first one is from the C-methyl group and the rest must be due to the N-methyl group and O-methyl group. The signal at 8.28 ppm exhibited a nuclear OVERHAUSER effect (NOE) to signals at 3.69 and the extent of 17.6% area when the signal at 3.69 ppm was irradiated. This evidence indicated that the 8.28 ppm are due to the N-methyl and the proton at C_1, respectively. The chemical shift of the O-methyl group at a lower field (4.20 ppm) than the usual range (3.6~4.0 ppm) is explained in terms of the compression effect of the methyl group. The proton at C_4 resonated at 7.12 ppm and is coupled with the C_1 proton ($J_{H_1,H_4}=0.5$ Hz).

The signals of the ^{13}C-NMR spectrum were assigned as shown in Table 1. The assignment of the carbon chemical shifts was based on the comparison of the ^{13}C spectrum of a model compound and on the ^{1}H coupled ^{13}C spectra. The signals of the carbon coupled with the aromatic protons at C_1 and C_4 were easily differentiated from others. The signal at 133.6 ppm was assigned to the C_6 carbon for the reason that it was coupled with the signal of the C-methyl protons at 9.5 ppm. The assignment of the signals at 111.3 and 138.9 ppm to the quaternary carbons at C_9 and C_{10} was made from the comparison with the signals of 7-methoxy-6-methyl-3(4-morpholinyl)-5,8-isoquinolinedione (II)[4]. The spectrum contains three signals at 183.5, 177.3 and 162.8 ppm apparently due to the carbonyl carbons. The last was assigned to the lactam carbonyl carbon C_3 since it was coupled with the proton at C_1 ($J=2.6$ Hz). The first and second signals are ascribed to the carbonyl carbon at C_5 and C_8, respectively, on the basis that the C_8 carbon resonance would shift upfield under the influence of the methoxy group attached to the vicinal carbon.[5] The remaining signal at 159.5 ppm was assigned to the quaternary carbon at the C_7 position. $^{1}J_{CH}$ coupling constants of the two compounds are shown in Table 2.

Table 1. Carbon chemical shifts of **I** and **II** in $CDCl_3$ solution (δ)

Compound	C_1	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	CH_3	OCH_3
I	142.1	162.8	116.7	183.5	133.6	159.5	177.3	111.3	138.9	9.6	61.3
II	149.3	161.2	100.8	185.3	130.5	158.8	179.1	115.8	139.1	9.2	61.2



II

Table 2. $^{1}J_{CH}$ Coupling constants in Hz.

Compound	J_{C-1}	J_{C-4}	J_{CH_3}	J_{OCH_3}
I	176	173	130	147
II	181	166	129	142

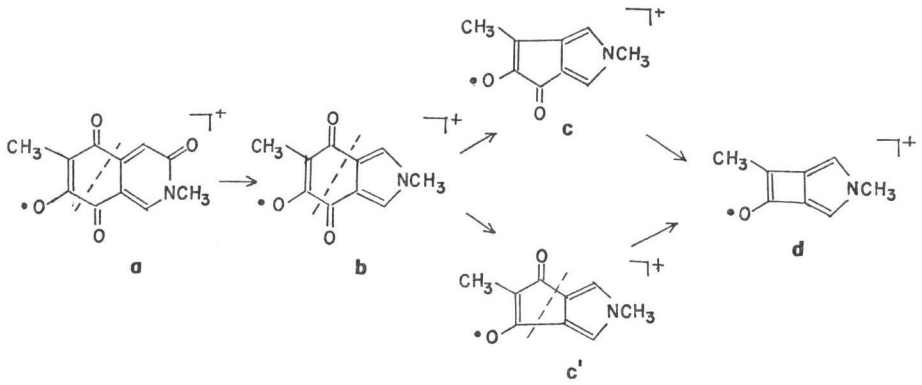
Mass Spectral Data**

The mass spectrum reveals the parent peak (M^+, $C_{12}H_{11}NO_4$, *m/e* 233), which is the base peak. The spectrum contains prominent peaks of a series resulting from the loss of a methyl radical (M^+-CH_3, $C_{11}H_8NO_4$, *m/e* 218) and the subsequent loss of three carbonyls (M^+-CH_3-CO, $C_{10}H_8NO_3$, *m/e* 190;

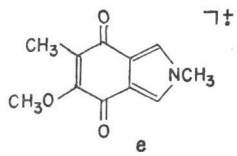
* ^{1}H-NMR spectra were recorded on a Varian HA-100 spectrometer operating at 100 MHz at 35°C. ^{13}C-NMR spectra were obtained on a Varian XL-100A spectrometer operating at 25.2 MHz at 30°C.

** Mass spectra were recorded on a JEOL JMS-OISG at 75 eV using a direct inlet system.

$M^+-CH_3-2\,CO$, $C_9H_8NO_2$, *m/e* 162; $M^+-CH_3-3\,CO$, C_8H_8NO, *m/e* 134). The loss of a methyl radical is more pronounced from either the M^+-nCO (*m/e* 205, 177, 149) or the ion of M^+-CH_3O (*m/e* 203). Plausible structures can be assigned as **a**, **b**, **c**, **c'**, **d** (in either order) to the abundant fragment ions based on a fragment sequence, which is apparent in the spectrum of methyl ether of phthiocol and related naphthoquinones.[6]



The other sequence of fragmentation is the successive loss of three carbonyls (M^+-CO, $C_{11}H_{11}NO_3$, *m/e* 205; $M^+-2\,CO$, $C_{10}H_{11}NO_2$, *m/e* 177; M^+-3CO, $C_9H_{11}NO$, *m/e* 149) from the molecular ion without the loss of the methyl (or methoxy) radical. This decomposition sequence may occur initially with the formation of **e** as evidenced by the spectrum of 2-methyl-3-isoquinolone.[7]



An additional noteworthy feature of the spectrum is the presence of a *m/e* 83 ion, which corresponds to $C_4H_3O_2$ ($O{=}C{=}\underset{\displaystyle CH_3}{\underset{|}{C}}{-}C{\equiv}O^+$) resulting from the fission at the dotted line of **a**, **b** (or **c'**).

References

1) Arai, T.; K. Yazawa, Y. Mikami, A. Kubo & K. Takahashi: Isolation and characterization of satellite antibiotics, mimosamycin and chlorocarcins from *Streptomyces lavendulae*, streptothricin source. J. Antibiotics 29: 398~407, 1976
2) Mikami, Y.; K. Yokoyama, A. Omi & T. Arai: Identification of producer and biological activities of new antibiotics, mimosamycin and chlorocarcins. J. Antibiotics 29: 408~414, 1976
3) Fukumi, H.; H. Kurihara, T. Hata, C. Tamura, H. Mishima, A. Kubo & T. Arai: Mimosamycin, a novel antibiotic produced by *Streptomyces lavendulae* No. 314: structure and synthesis. Tetrahed. Lett. 1977: 3825~3828, 1977
4) Fukumi, H.; H. Kurihara & H. Mishima: Total synthesis of mimosamycin. Chem. Pharm. Bull. 26: 2175~2180, 1978
5) Höfle, G.: ^{13}C-NMR-spektroskopie chinoider Verbindungen-I: Substituierte *p*-Benzochinone und 2-Cyclohexenone. Tetrahedron 32: 1431~1436, 1976
6) Bowie, J. H.; D. W. Cameron & D. W. Williams: Studies in mass spectrometry. I. Mass spectra of substituted naphthoquinones. J. Am. Chem. Soc. 87: 5094~5099, 1965
7) Evans, D. A.; G. F. Smith & M. A. Wahid: The tautomerism of 3-hydroxyisoquinolines. J. Chem. Soc. 1967(B): 590~595, 1967