YM-24074, a New Peptide Antibiotic

II. Structural Elucidation

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YM-24074 (formerly called YL-01869P) is a new antibiotic and type I collagenase inhibitor. The structure of this compound was elucidated by spectroscopic analysis and confirmed by acid degradation to be *N*-(1″-acetyl-3″-methylbutyl)-2-[2′-[(*N*-hydroxycarbamoyl)methyl]heptanoyl]-hexahydropyridazine-3-carboxamide. The stereochemistry of two of three chiral centers was determined by degradation products to be 3*S*, 2′*R*.

In our on-going screening program for new antibiotics, we found and isolated YM-24074 as a new MRSA active antibiotic. YM-24074 also exhibited type I collagenase inhibition. In another paper[1] we will describe the taxonomy, fermentation conditions of *Streptomyces* sp. YL-01869P, isolation procedure, physico-chemical properties and biological activity of YM-24074. In the present paper we describe the structural elucidation by spectroscopic analysis, especially NMR spectra, and acid degradation and determination of the absolute configuration of two chiral centers by comparison with authentic samples in HPLC or optical rotation.

Structures of YM-24074 and its Methyl Ether

IR (1660 and 1540 cm^{-1}) and ^{13}C NMR (177.7, 171.9 and 169.8 ppm in $CDCl_3$) spectra suggested that YM-24074 was a peptide antibiotic. Its molecular weight, 426, and molecular formula, $C_{21}H_{38}N_4O_5$, were determined by the FAB-MS and HR-MS spectra. As the methyl ether of YM-24074, produced by treatment with diazomethane, showed better NMR spectra than YM-24074 itself, YM-24074 methyl ether was used in NMR analysis. NMR spectra were measured in $CDCl_3$ and $CDCl_3$ - CD_3OD (10 : 1) as solvents. The INEPT and ^{13}C-^{1}H COSY spectra revealed the existence of five methyl groups, nine methylene groups, four methine groups and four carbonyl groups. ^{1}H-^{1}H COSY and HMBC NMR spectra suggested three partial structures (I ~ III) (Fig. 2). The carbonyl chemical shift (169.8 ppm) in the ^{13}C NMR spectrum and positive color reaction with $FeCl_3$ revealed the existence of a hydroxamic acid unit (IV). The remaining amide group (V) was suggested by the molecular formula. The nitrogen of partial structure (V) must be the same as either N-1 or N-2 in partial structure (I). One ring should be formed to account for the degree of unsaturation. The partial structures could not be connected in $CDCl_3$ or $CDCl_3$ - CD_3OD (10 : 1) solvents. We therefore measured the HMBC spectrum in DMSO-d_6. ^{13}C-^{1}H couplings were recognized between 1-H in partial structure (I) and the C-1′ in partial structure (V), and between 2′-H in partial structure (II) and C-1′. As the possibility of a substituted urea N(1)-C(1′)-N(2) was eliminated by the chemical shift (177.7 ppm) of the carbonyl group in partial structure (V)[2], the

Fig. 1. Structures of YM-24074 and its methyl ether.

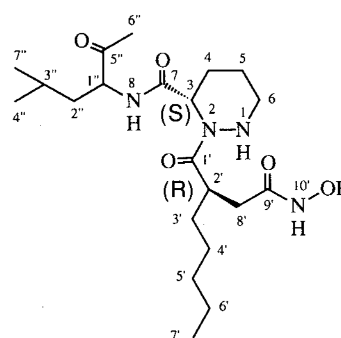
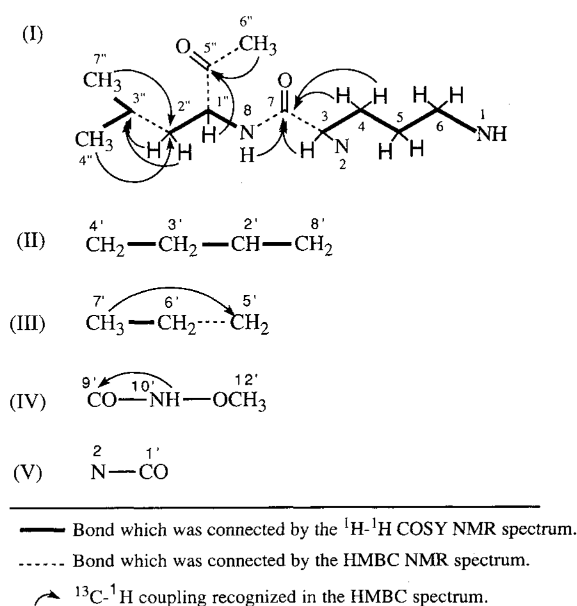


Table 1. NMR spectral data for YM-24074 methyl ether.

^{13}C Shift	^{1}H Shift	^{1}H-^{1}H connection	Assignment
207.9	—	—	5″
177.7	—	—	1′
171.9	—	—	7
169.8	—	—	9′
63.8	3.70	—	12′
57.1	4.53	8, 2″	1″
50.3	5.13	4	3
46.8	3.00, 2.89	1, 5	6
39.2	1.58, 1.45	1″	2″
36.6	3.95	3′, 8′	2′
34.9	2.35, 2.10	2′	8′
32.5	1.57, 1.46	2′, 4′	3′
31.6	1.25	—	5′
26.8	2.21	—	6″
26.2	1.25	3′	4′
26.0	2.07, 1.89	3, 5	4
24.8	1.68	4″, 7″	3″
22.9	0.93	3″	4″ or 7″
22.2	1.25	7′	6′
21.2	0.93	3″	7″ or 4″
20.7	1.74, 1.58	4, 6	5
13.7	0.86	6′	7′
	9.50*	—	10′
	7.69*	1″	8
	4.87*	6	1

ppm in $CDCl_3$ - MeOD (10 : 1).
* ppm in $CDCl_3$.

Fig. 2. Partial structures of YM-24074 methyl ether.



hexahydropyridazine structure was suggested with N-1 and N-2 connected. As for the carbonyl groups in partial structures (IV) and (V), ^{13}C-^{1}H couplings were recognized between 8′-H_2 in partial structure (II) and C-9′ and between 2′-H and C-1′. There were two ways to connect C-9′ with partial structure (II), by 1) C-8′ or 2) C-2′. The sequence resulting in the first case would be C(1′)-C(2′)-C(8′)-C(9′)-N(10′)-OCH_3, and C(1′)-C(8′)-C(2′)-C(9′)-N(10′)-OCH_3 in the second case. We then measured NOE and decoupled spectra. In the NOE experiment NOE was recognized between 10′-H and OCH_3 but not between 10′-H and 2′-H nor 10′-H and 8′-H_2. When 2′-H was decoupled, height change on the 10′-H peak was not observed by comparison with the 8-H peak as a standard. In contrast, enhancement on the 10′-H peak was observed (3.9 and 6.3%, respectively) on decoupling of each 8′-H_2 (1.97 ppm, 2.16 ppm, in DMSO-d_6). These enhancements could be deduced to reflect long range coupling between 10′-H and 8′-H_2 and therefore suggested case 1), involving the connection of C-9′ and C-8′. Remaining component (III) could be consequently connected with C-4′ in partial structure (II), though direct evidence could not be obtained due to close chemical shifts of methylene groups in both patial structures. The resulting structures of YM-24074 methyl ether and YM-24074 are shown in Fig. 1.

Acid Degradation

Acid degradation of YM-24074 was done to confirm the structure shown in Fig. 1. The degradation was carried out under two conditions. The first, with 6 N HCl - MeOH (2 : 1) at 110°C for 18 hours, produced compounds **A**～**D**. The second was with 6 N HCl at 110°C for 20 hours and produced compounds **E** and **F**, together with hexahydropyridazic acid (**B**) and ornithine (**C**). In the first case, the reaction mixture was extracted twice with EtOAc. The aqueous layer was dried *in vacuo* to evaporate HCl. The residue was dissolved with H_2O and absorbed on Dowex 50W (H^+) and eluted with 1 N HCl. The eluates were dried *in vacuo* and purified by silica gel column chromatography. Five ninhydrin-positive spots were detected on silica gel TLC with BuOH - AcOH - H_2O (4 : 1 : 2) as solvent. The three main components (compounds **A**～**C**) showed the Rf values of 0.44, 0.28 and 0.071, respectively. Pure samples were obtained by preparative silica gel TLC. In the second case, the reaction mixture was twice extracted with ethyl ether. The aqueous layer was dried *in vacuo* to evaporate HCl, and the residue was dissolved with H_2O, absorbed on Dowex 50W (H^+) and eluted with a gradient from H_2O to 1 N NH_4OH. Compound **E** was obtained together with hexahydropyridazic acid and ornithine. The ether layer was dried to give compound **F**. The structures of compounds **A**, **D**, **E** and **F** were elucidated by the MS

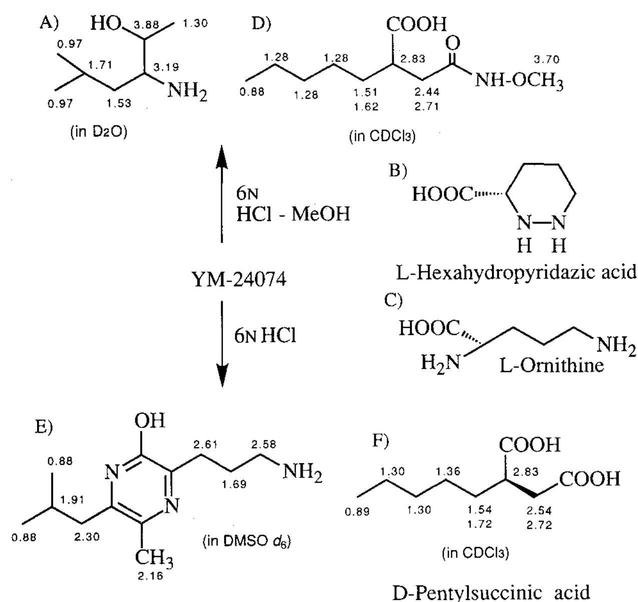


Fig. 3. Acid degradation products of YM-24074 and their ^{1}H NMR chemical shifts (ppm).

and NMR spectra, and are shown in Fig. 3 with chemical shifts in the ^{1}H NMR spectra. The structure of compounds **B** and **C** were clarified by comparing authentic samples of hexahydropyridazic acid synthesized in our laboratory and purchased ornithine (Wako, Japan) on silica gel TLC and HPLC. The structure of YM-24074 was confirmed by these degradation products, especially compounds **A**, **B** and **D**. Though compound **A** is a reduced structure compared with YM-24074, it might have been reduced by the reducing activity of hexahydropyridazic acid[3].

Stereochemistry of YM-24074

Compounds **B** and **C** were compared with authentic samples on chiral HPLC with Crown Pack CR (Daicel Chemical, Japan). Analytical conditions for compound **B** were as follows: solvent, aqueous $HClO_4$, pH 1.5; flow rate, 0.4 ml/minute; temperature, 0°C; detection, 200 nm UV. Compound **B** was detected at 20.1 minutes and determined to be L-hexahydropyridazic acid. Conditions for compound **C** were as follows: solvent, aqueous $HClO_4$, pH 0.6; flow rate, 0.4 ml/minute; temperature, 25°C; detection, 200 nm UV. Compound **C** was detected at 11.5 minutes and determined to be L-ornithine. Compound **F** was determined to be (+)-D-pentylsuccinic acid by optical rotation, $[\alpha]_D^{25}$ +17.3 (in EtOH, *c* 0.3)[4]. From the experimental results for compounds **B**, **C** and **F**, the chiral centers of 3 and 2′ were determined to be *S* and *R*, respectively.

Discussion

YM-24074 is a peptide antibiotic with hydroxamic acid as its active center. The inhibition mechanism of hydroxamate against metalloprotease is known to involve chelation with the enzyme's essential metal atom. Several hydroxamates are known to inhibit metalloproteases, including enkephalinase[5,6], aminopeptidase[7] and thermolysin[8]. Type IV collagenase is also a metalloendopeptidase and a key enzyme for tumor proliferation. Recently hydroxamate inhibitors produced by microorganisms have been reported, such as IC101[9], BE16627B[10] and matlystatins[11]. Matlystatin was chemically synthesized[12] and the chiral centers were determined. The same group also synthesized YM-24074[13] and speculated on the stereochemistry of the compound. Our results accord with their speculations. The stereochemistry of the third chiral center 1″ is under study. On acid degradation we obtained the unexpected products **A** and **E**. The former was mentioned above and the latter may be formed by condensation of the aminoketone corresponding to compound **A** with ornithine **C**, and subsequent oxdation. This reaction might have been caused by alkaline conditions in condensed solution.

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