Pergamon

Tetrahedron Letters, Vol. 38, No. 43, pp. 7573-7576, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01785-1

Structural Elucidation of YM-75518, A Novel Antifungal Antibiotic Isolated from *Pseudomonas* sp. Q38009

Ken-ichi Suzumura*, Isao Takahashi †, Hisao Matsumoto, Koji Nagai†, Boenjamin Setiawan‡, Ratna Murni Rantiatmodjo‡, Ken-ichi Suzuki† and Noriaki Nagano

Molecular Chemistry Research Lab., Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba-shi, Ibaraki 305, Japan,

†Drug Serendipity Research Lab., Yamanouchi Pharmaceutical Co., Ltd., 1-1-8 Azusawa, Itabashi-ku, Tokyo 174, Japan

‡PT. Kalbe Farma, JL. Jend. A. Yani (Pulo Mas), Jakarta 13210, Indonesia

Abstract : An antifungal antibiotic, YM-75518, was isolated from the fermentation broth of *Pseudomonas* sp. Q38009. Structural elucidation of YM-75518 was accomplished through extensive 2D NMR spectroscopy including ^{15}N-^{1}H HMQC and ^{15}N-^{1}H HMBC at natural abundance. YM-75518 consisted of a unique 15-membered macrolactone ring and a methoxy imino structure. © 1997 Elsevier Science Ltd.

In the course of our screening program for new antifungal substances, we isolated YM-75518(**1**) from the fermentation broth of *Pseudomonas* sp. Q38009. The extensive spectroscopic analysis, including the recently developed Pulsed Field Gradient ^{15}N-^{1}H HMQC[1] and ^{15}N-^{1}H HMBC[2,3,4] NMR techniques at natural abundance, revealed the unique skeleton of **1**. The structure of **1** comprised a 15-membered ring and a methoxy imino structure. We report the chemical structure of **1** in this paper.

Q38009 was isolated from a soil sample collected in Indonesia. The fermentation broth (3 liters) was extracted with acetone-H_2O (70:30) and filtered. The filtrate was concentrated to an aqueous solution, adjusted to pH 7.0 and extracted with EtOAc. The extract was subjected to silicagel column chromatography developed with a solvent system of benzene-acetone (90:10). The active fraction was purified by HPLC (STR-PREP-ODS_M) with a solvent of CH_3CN-THF-MeOH-H_2O (40:30:15:15) to yield an active compound YM-75518 (**1**, 4mg), $[\alpha]_D^{25}$=-13.9° (c 3.70, in MeOH), as an amorphous powder[5]. The molecular formula of **1** was determined to be $C_{27}H_{32}N_2O_8$ on the basis of positive-ion high resolution FAB-MS (obsd $[M+H]^+$ m/z 513.2240, Δ 0.3mmu for $C_{27}H_{33}N_2O_8$). The IR spectral data[5] had two strong carbonyl absorptions at 1745 and 1730 cm^{-1} and an amide carbonyl absorption at 1647 cm^{-1}.

The ^{1}H NMR spectrum in DMSO-d_6 displayed well-resolved signals. The bond connectivities from the C11 methine proton to the C15 methine proton were revealed by the COSY and HOHAHA experiments. The C12 methyl protons (δ_H 1.28) showed a correlation to the C11(δ_H 5.16) methine proton which was coupled to the vinyl proton H13 (δ_H 5.40) in the COSY spectrum. The olefinic protons H13 and H14 (δ_H 5.53) showed a relatively large coupling (15.3Hz) consistent with an (*E*)-olefin. The COSY spectrum showed the signal H14 to be coupled to an oxygenated methine proton H15 (δ_H 4.69) in turn coupled to an exchangeable proton (δ_H 4.99). Furthermore, C11 was assumed to be oxygenated as judged by its ^{13}C chemical shift at δ_C 71.8 and the C11 oxygenated methine proton (δ_H 5.16) showed the correlation to the C10 (δ_C 169.6) carbonyl carbon in the HMBC spectrum. Thus, C11 was considered to be attached to an ester bond. The C19 methylene protons (δ_H 2.69, 3.14) coupled to the H18 olefinic proton (δ_H 5.01) which showed allylic coupling to the H17 methyl group (δ_H 1.70). The connection between C15 and C16 was derived from the HMBC correlations (H17 to C15, H14

and H15 to C16). (*Z*)-Geometry for the C16-C18 olefin was deduced from the NOE correlations (H17-H18, H15-H19). Analyses of COSY and HMBC data suggested the presence of a benzene ring (C20-C25). The HMBC correlations (H19 to C21, C25) showed the connection between C19 and C20. The downfield shift at C24 (δ_C 154.1) indicated that C24 was substituted directly to an electron with drawing group and the exchangeable proton (δ_H 9.84) showed the HMBC correlation to C23, C24 and C25, suggesting the presence of a hydroxyl group at C24 position.

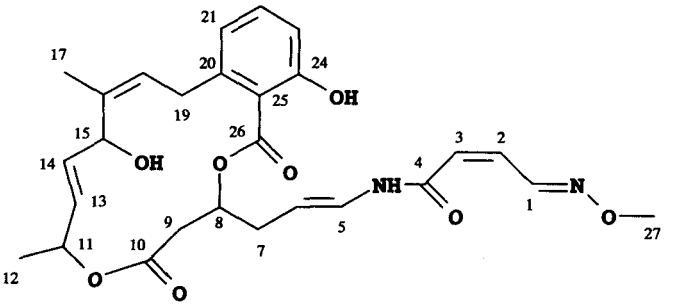

Figure 1. Structure of YM-75518(**1**)

Furthermore, HMBC showed a long-range correlation from H23 (δ_H 6.71) and H8 (δ_H 5.45) to the ester carbonyl C26 (δ_C 166.4). In the COSY spectrum the C9 methylene protons (δ_H 2.45, 2.72) were correlated to an oxygenated C8 methine (δ_H 5.45) which was coupled to a methylene at C7 (δ_H 2.36). These methylene protons were correlated to an olefinic proton H6 (δ_H 5.25) which coupled to H5 (δ_H 6.76). In the ^{1}H NMR spectrum, H5 showed a relatively large coupling (14.0 Hz) to H6, consistent with an (*E*)-olefin and H5 was also coupled to the doublet D_2O exchangeable proton (δ_H 10.24, J=9.8 Hz). In the HMBC experiment, the H8 proton showed correlation to the C10 (δ_H 169.6) ester carbonyl carbon and the chemical shift of C9 (δ_C 37.4) suggested that C9 was attached to the C10 carbonyl carbon. Thus, the 15-membered ring structure was established. The D_2O exchangeable proton (δ_H 10.24, J=9.8 Hz), coupled to H5, showed the correlation to the nitrogen signal at δ_N 141 in the PFG ^{15}N-^{1}H HMQC spectrum[6] at natural abundance. Therefore, it was revealed that the D_2O exchangeable proton (δ_H 10.24) was a NH proton. Furthermore, the HMBC correlation H5, H3 and H2 to the carbonyl carbon C4 (δ_C 161.6) suggested that the C4 carbonyl carbon was an amide carbon. The ^{15}N chemical shift was consistent with amide nitrogen chemical shift[7]. In the COSY spectrum, the H3 (δ_H 6.12) olefinic proton showed a coupling to H2 (δ_H 6.52) which in turn coupled to the H1 (δ_H 8.98) proton. The coupling constant between H3 and H2 was 10.4Hz, implying (*Z*)-olefin. The carbon at C27 (δ_C 62.7) was assumed to be an oxygenated methyl group as judged by its ^{13}C chemical shift. Because the molecular formula was derived to be $C_{26}H_{29}NO_5$ from HR FABMS analysis, the leaving substructures are CH_3O- (C27, δ_C 62.7) and one nitrogen. It is deduced that the methoxy imino structure (CH_3O-N=) was linked to the C1 position, which was supported by the NOE enhancement observed between H1 and H27 and the downfield ^{13}C shift δ 147.4 (C1). Furthermore, we obtained the direct information on the C1 carbon bonded to the imino nitrogen. In the ^{15}N-^{1}H

HMBC spectrum[6] (Fig. 2), the cross peaks from H1 (δ_H, 8.98, d, J=10.4 Hz), H2 (δ_H 6.52, t, J=10.4 Hz) and H27 (δ_H 3.86, s) to δ_N 408 ^{15}N signal were observed and δ_N 408 was consistent with a imino nitrogen chemical shift[7].

Table 1. ^1H NMR and ^{13}C NMR Chemical Shifts[a] and HMBC[b] and COSY Correlations of YM-75518 in DMSO-d_6

No	^{13}C	^1H NMR δ (mult, J(Hz), int)	HMBC correlations	COSY correlations
1	147.4	8.98 (d, 10.4, 1H)	C-2	H-2
2	133.2	6.52 (t, 10.4, 1H)	C-4	H-3
3	126.2	6.12 (d, 10.4, 1H)	C-1, C-4	H-2
4	161.6			
5	125.9	6.76 (dd, 14.0, 9.8, 1H)	C-4, C-7	H-6, NH(δ_H 10.24)
6	107.3	5.25 (dt, 14.0, 7.9, 1H)	C-5	H-5, H-7
7	33.9	2.36 (m, 2H)	C-5, C-6, C-8, C-9	H-6, H-8
8	70.9	5.45 (m, 1H)	C-10, C-26	H-7, H-9
9a	37.4	2.45 (dd, 17.1, 11.2,1H)	C-7, C-8	H-8
9b		2.72 (d, 17.1, 1H)		
10	169.6			
11	71.8	5.16 (dq, 6.7, 8.5, 1H)	C-10, C-14	H-12, H-13
12	19.8	1.28 (d, 6.7, 3H)	C-11, C-13	H-11
13	130.3	5.40 (dd, 15.3, 8.5, 1H)	C-12, C-14, C-15	H-11, H-14
14	133.8	5.53 (dd, 15.3, 10.2, 1H)	C-11, C-13, C-16	H-13, H-15
15	70.8	4.69 (dd, 10.2, 3.7, 1H)	C-16	H-14, OH(δ_H 4.99)
16	138.6			
17	19.2	1.70 (s, 3H)	C-15, C-16, C-18	
18	123.2	5.01 (d, 10.4, 1H)	C-15, C-17	H-19
19a	31.2	3.14 (dd, 17.7, 10.4, 1H)	C-16, C-18, C-20, C-21	H-18
19b		2.69 (δ, 17.7, 1H)	C-25	
20	138.8			
21	119.0	6.54 (δ, 7.9, 1H)	C-19, C-23, C-25	H-22
22	129.9	7.12 (t, 7.9, 1H)	C-20, C-24	H-21, H-23
23	112.9	6.71 (d, 7.9, 1H)	C-21, C-25, C-26	H-22
24	154.1			
25	122.1			
26	166.4			
27	62.4	3.86 (s, 3H)		
NH(C4)		10.24 (d, 9.8, 1H)	C-4	H-5
OH(C15)		4.99 (d, 3.7, 1H)	C-14, C-15, C-16	H-15
OH(C24)		9.84 (s, 1H)	C-23, C-24, C-25	

[a] Recorded at 500MHz (^1H) and 125MHz (^{13}C) [b] The long-range coupling constants in HMBC experiment were optimized for 8Hz

The compounds possessing a 15-membered macrolactone ring have never been reported except aplidite A[8]. Aplidite A (**2**) was isolated from an Australian marine tunicate *Aplidium* sp., which has the same molecular formula. The structural difference between **1** and **2** is that **2** possesses an amino group and an orthonitrite structure, while **1** is an amide group and a methoxy imino structure. In this structural elucidation, we were able to reveal the presence of amide and the methoxy imino structure using ^{15}N nitrogen chemical shifts, because we can obtain the direct evidence for the functional group containing a nitrogen atom by ^{15}N-^1H HMQC and ^{15}N-^1H HMBC methods.

In conclusion, the structure of YM-75518 was determined by ^{15}N-^1H HMQC and ^{15}N-^1H HMBC at natural abundance. The structure of YM-75518 consisted of a unique 15-membered macrolactone ring and a

methoxy imino structure.

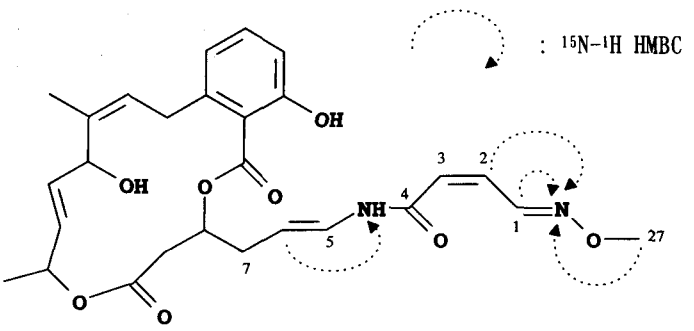

Figure 2. ^{15}N-^{1}H HMBC correlations for **1** in DMSO-d_6

YM-75518(**1**) showed weak antifungal activity against *Rhodotorula acuta* alone among the tested microorganisms. Further biological evaluation of **1** is in progress, and the stereochemistry will be reported in due course.

References and Notes

1. Hurd, E. R.; John, K. B. *J. Magn. Reson.* **1991**, 91, 648-653.
2. Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *Tetrahedron Lett.* **1996**, 37, 1447-1448.
3. Martin, E. G.; Crouch, C. R.; Andrews, W. C. *J. Heterocyclic Chem.* **1995**, 32, 1759-1766.
4. Willker, W.; Leibfritz, D.; Kerssebaum, R.; Bermel, W. *Magn. Reson. Chem.* **1993**, 31,287-292.
5. Physico-chemical properties of YM-75518 (**1**) ; Appearance; white powder, m.p. 126-128°C, $[\alpha]_D^{25}$=-13.9 (c 3.70, in MeOH), IR (KBr) 3440, 2920, 1750, 1730, 1650, 1530, 1450, 1390, 1260 cm^{-1}, UV (EtOH) λ_{max} 282 (ε 9000) 211 (ε 11000), ^{13}C NMR (125MHz, CD_3OD) δ 148.7 (C1), 135.6 (C2), 126.2 (C3), 164.3 (C4), 126.9 (C5), 109.9 (C6), 35.7 (C7), 73.1 (C8), 39.0 (C9), 171.9 (C10), 73.8 (C11), 20.2 (C12), 132.7 (C13), 135.1 (C14),73.4 (C15), 139.4 (C16). 19.7 (C17), 125.7 (C18), 33.2 (C19), 141.4 (C20), 121.0 (C21), 132.0 (C22), 114.5 (C23), 156.8 (C24), 122.2 (C25) and 170.1 (C26)
6. The ^{15}N-^{1}H HMQC and ^{15}N-^{1}H HMBC data were obtained on a JNM ALPHA 500 spectrometer, fitted with a 5mm inverse gradients probe. Data were obtained from nondegassed solution of 4 mg in 0.55 mL DMSO-d_6. 512 increments with 64 scans per increment were acquired. The gradient strength value was G1:G2=4.94:1.0 G cm^{-1}. Data sets consisted of 512 FIDs (*t1*) and 1024 data points in *t2*. A relaxation delay interval of 2.0s was set for each pulse sequence. The spectrum was zero-filled to 1024 points in t1 prior to Fourier transformation, ^{15}N chemical shifts were referenced to 0 ppm for NH_3
7. Levy, C. G.; Lichter, L. R. *Nitrogen-15 Nuclerar Magnetic Resonance Spectroscopy*, A Wiley-Interscience Publication, **1979**.
8. Murray, L.; Lim, K. T.; Currie, G.; Capon, *Aust. J. Chem.* **1995**, 48, 1253-1266.

(*Received in Japan* 25 *July* 1997; *accepted* 29 *August* 1997)