YM-47522, a Novel Antifungal Antibiotic Produced by *Bacillus* sp.

II. Structure and Relative Stereochemistry

TAKEO SUGAWARA, MITSUYOSHI SHIBAZAKI, HIDEAKI NAKAHARA† and KENICHI SUZUKI

Drug Serendipity Research Laboratories and †Molecular Chemistry Research Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd. 21 Miyukigaoka, Tsukuba, Ibaraki 305, Japan

(Received for publication October 18, 1995)

YM-47522 (**1**) was isolated from the fermentation broth of *Bacillus* sp. YL-03709B as an antifungal antibiotic. The structure of **1** was elucidated by spectroscopic analyses. YM-47522 (**1**) consisted of C_{13} carboxylic acid amide and cinnamate moieties. The relative stereochemistry was also proposed on the basis of chemical transformation into a 1,3-diol acetonide and its NMR data.

In the course of our screening program for novel antifungal antibiotics, the extract of the fermentation of *Bacillus* sp. YL-03709B showed marked antifungal activity against *Rhodotorula acuta* and *Pichia angusta* in the agar diffusion assay. As described in the preceding paper, YM-47522 (**1**) was isolated as an active principle, and was evaluated for biological properties. YM-47522 (**1**) contained an $\alpha,\beta,\gamma,\delta$-unsaturated carboxylic acid which was characterized by its C_{13} chain length. In this paper, we report the structure elucidation of **1**.

Results and Discussion

Structure of YM-47522 (1)

The physico-chemical properties of YM-47522 (**1**) are listed in Table 1. **1** had a molecular formula of $C_{24}H_{33}NO_4$, which was established by high-resolution FAB-MS and NMR data. The ^1H NMR (Fig. 1) and DEPT spectra indicated the presence of three methyls, three methylenes, two aliphatic methines, two oxygenated methines, six olefinic methines, two carbonyls, and a monosubstituted benzene ring, which accounted for nine degrees of unsaturation. The well-resolved ^1H and ^{13}C NMR signals allowed four partial structures, **a**~**d**, to be unambiguously assigned by COSY, HMQC, and HMBC spectra. Partial structures, **a**~**d**, were connected by interpretation of HMBC data, leading to the gross structure **1**. The ^1H and ^{13}C NMR chemical shifts are shown in Table 2.

The assignment of the C2-C9 portion in partial structure **a** was straightforward by analysis of the COSY spectrum, which revealed a diene system connecting a 1,3-diol unit through a methylene. The *Z* geometry of the C2, C3 double bond was derived from the coupling constant of 11.6 Hz between H2 and H3, whereas the geometry of the C4, C5 double bond was deduced to be *E* on the basis of the coupling constant of 15.3 Hz between H4 and H5. The contiguous nature of the C7-C8(C14)-C9 portion was easily assigned by tracing of cross peaks in the COSY spectrum. Further COSY analysis revealed that $H_2$6 correlated both with H5 and H7, completing the spin system in partial structure **a**. C7 and C9 were inferred to be oxygenated as judged by their

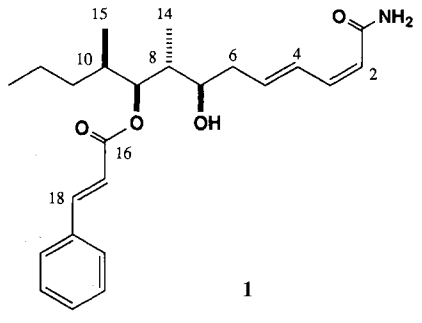

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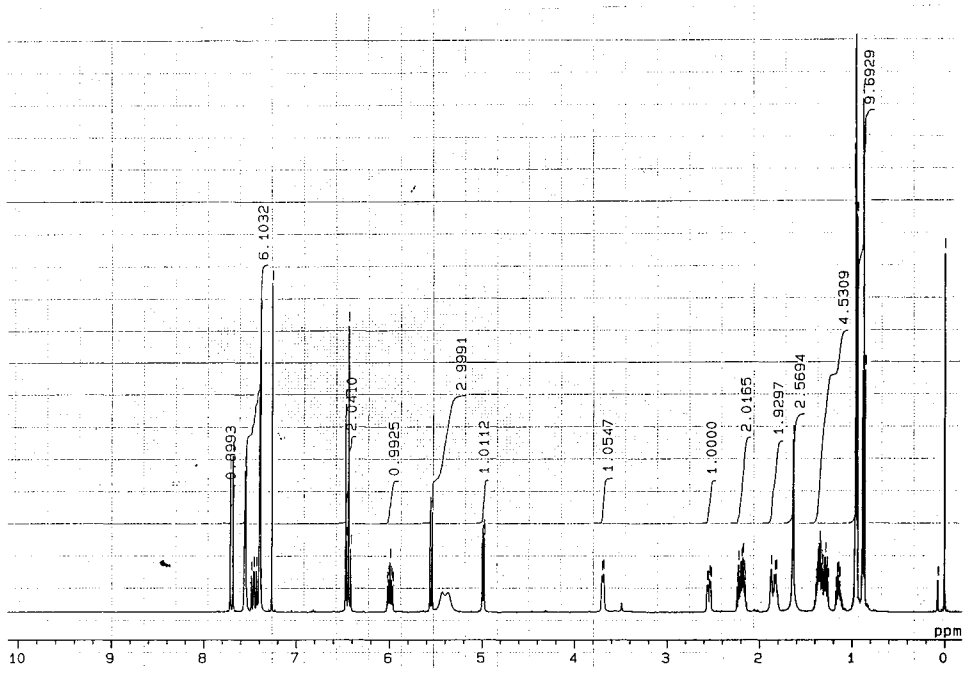
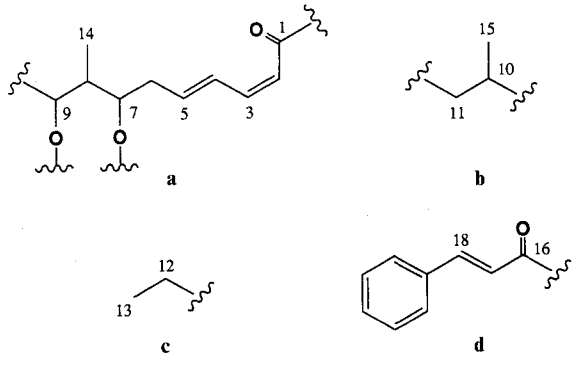
Table 1. Physico-chemical properties of YM-47522 (**1**).

Appearance	Colorless gum
Molecular weight	399
Molecular formula	$C_{24}H_{33}NO_4$
HRFABMS (*m/z*)	
Found:	400.2449 (MH^+)
Calcd:	400.2487
$[\alpha]^{25}_D$	-106.1° (*c* 0.33, MeOH)
UV (MeOH) λ_{max} nm (ε)	217 (19000), 222 (18000), 263 (31000)
IR ν_{max} (film) cm^{-1}	3340, 2960, 2940, 1700 1670, 1600, 1460

Fig. 1. ^{1}H NMR spectrum of YM-47522 (**1**) in $CDCl_3$ (500 MHz).





^{13}C chemical shifts at δ 71.6 and δ 77.7, respectively. An amide or ester carbonyl at δ 168.0, which showed HMBC correlations with H2 and H3, could be located at C1.

Partial structures **b** and **c** were established by interpretation of the COSY spectrum. In partial structure **b**, a doublet methyl protons at δ 0.96 coupled to H10, which in turn showed correlations with H11 and H11′. The C12-C13 unit in partial structure **c** was also straightforward by COSY data. Although overlapping signals of H11′ and $H_2$12 hampered the COSY analysis, C12 gave intense HMBC cross peaks with H11 and H11′, connecting partial structures **b** and **c**.

Simultaneous analyses of COSY and HMBC data suggested the presence of a monosubstituted benzene ring (C19-C24), a disubstituted double bond (C17, C18), and an amide or ester carbonyl (C16) in partial structure **d**. Furthermore, HMBC correlations (H17/C16, C19; H18/C16, C20, C24) allowed us to assign a cinnamic acid moiety for **d**. The coupling constant of 15.8 Hz between H17 and H18 suggested the *E* geometry of the C17, C18 double bond.

Connection of partial structures **a**~**d** was accomplished by interpretation of the HMBC spectrum.

Table 2. ^{1}H and ^{13}C NMR data of YM-47522 (**1**) in $CDCl_3$.

no.	^{13}C	^{1}H
1	168.0 (s)	
2	118.0 (d)	5.55 (d, 11.6)
3	142.1 (d)	6.44 (dd, 11.6, 11.6)
4	129.4 (d)	7.46 (dd, 15.3, 11.6)
5	140.6 (d)	5.99 (ddd, 15.3, 8.6, 6.7)
6	36.2 (t)	2.54 (brdd, 14.6, 6.7)
		2.21 (ddd, 14.6, 9.1, 8.6)
7	71.6 (d)	3.69 (brd, 9.1)
8	40.4 (d)	2.18 (m)
9	77.7 (d)	4.99 (dd, 9.8, 3.1)
10	34.2 (d)	1.83 (m)
11	36.2 (t)	1.28 (m)
		1.14 (m)
12	20.3 (t)	1.35 (m)
13	14.2 (q)	0.87 (t, 7.3)
14	11.4 (q)	0.95 (d, 5.5)
15	13.1 (q)	0.96 (d, 5.5)
16	167.0 (s)	
17	118.0 (d)	6.44 (d, 15.8)
18	145.2 (d)	7.69 (d, 15.8)
19	134.3 (s)	
20, 24	128.2 (d)	7.55 (dd, 6.1, 2.5)
21, 23	128.9 (d)	7.38 (m)
22	130.4 (d)	7.39 (m)

Though the HMBC cross peak between H9 and C10 was faint, H9 showed an intense correlation with C15. Therefore, connection between partial structures **a** and **b** was proved. Attachment of a cinnamate moiety (partial structure **d**) at C9 in partial structure **a** was apparent from an HMBC correlation between H9 and C16, which was supported by the low field chemical shift of H9 at δ 4.99. Considering the degree of unsaturation, an oxygen on C7 must be a hydroxyl group, and the remaining NH_2 group should form an amide terminus with C1 carbonyl. Thus, the gross structure of YM-47522 (**1**) was established.

Relative Stereochemistry of YM-47522 (**1**)

In order to determine the relative stereochemistry of YM-47522 (**1**), the 1,3-diol acetonide **3** was prepared. **1** was hydrolyzed with 0.2% KOH in MeOH, followed by treatment with 2,2-dimethoxypropane in the presence of *p*-TsOH to afford the acetonide **3**. (Scheme 1) Full ^1H and ^{13}C NMR assignments of **3** (Table 3) were straightforward by NMR data including COSY, HMQC, and HMBC spectra. In the ^1H NMR spectrum, both coupling constants for H7/H8 and H8/H9 were 10 Hz, indicating that a 1,3-diol acetonide unit existed in a chair conformation with H7, H8, and H9 in axial positions.[1,2] Furthermore, NOESY cross peaks were observed for $H_3$14/H7 and $H_3$14/H9, leading to the assignment of the relative stereochemistries of C7, C8, and C9 as 7*R**, 8*R**, and 9*S**. The proposed relative stereochemistries were supported by the RYCHNOVSKY's study,[3] in which *syn*-1,3-diol acetonides were expected to adopt a chair conformation, having ^{13}C chemical shifts of acetonide methyls approximately at 19 (axial) and 30 ppm (equatorial) and ketal carbons below 98.5 ppm, while *anti*-1,3-diol acetonides would exist in a twist-boat conformation, having methyl shifts between 23 and 26 ppm and ketal shifts above 100.5 ppm. The observed chemical shifts of acetonide methyls at δ 19.5 and 30.1 and a ketal carbon at δ 97.8 in **3** were in accordance with a *syn*-1,3-diol system, confirming 7*R** and 9*S** configurations. The relative stereochemistry of C10 was deduced on the basis of the vicinal coupling constant between H9 and H10 and NOESY data. The $^3J_{H9,H10}$ value of 2.1 Hz indicated that the dihedral angle between H9 and H10 was close to 90°.[4] Furthermore, in the NOESY spectrum of **3**, significant cross peaks were observed between H8 and $H_3$15, between H9 and H10, and between H10 and $H_3$14. These findings were only consistent with a 10*R** configuration. (Fig. 2) Thus, the relative stereochemistries of four chiral centers in **1** were defined as 7*R**, 8*R**, 9*S**, and 10*R**. The absolute stereochemistry for **1** remains to be determined.

YM-47522 (**1**) belongs to a new class of antifungal antibiotics,[5] and its mode of action is now under investigation.

Scheme 1.

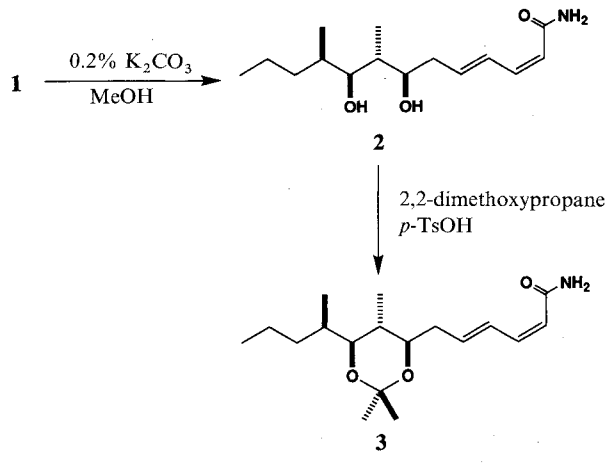
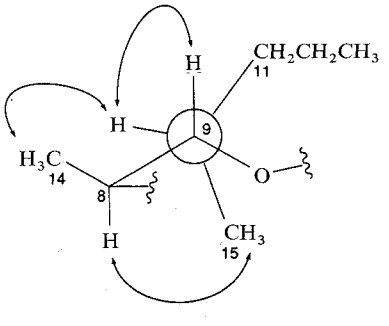


Table 3. ^1H and ^{13}C NMR data of **3** in $CDCl_3$.

no.	^{13}C	^1H
1	168.2 (s)	
2	117.1 (d)	5.54 (d, 11.0)
3	143.2 (d)	6.49 (dd, 11.0, 11.0)
4	128.1 (d)	7.45 (dd, 14.9, 11.0)
5	140.8 (d)	6.14 (ddd, 14.9, 7.3, 7.3)
6	36.7 (t)	2.53 (ddd, 14.6, 7.3, 3.1)
		2.32 (ddd, 14.6, 7.3, 7.3)
7	74.3 (d)	3.55 (ddd, 10.0, 7.3, 3.1)
8	35.1 (d)	1.45 (ddq, 10.0, 10.0, 6.7)
9	75.8 (d)	3.41 (dd, 10.0, 2.1)
10	32.8 (d)	1.65 (m)
11	36.3 (t)	1.28 (m)
12	20.5 (t)	1.31 (m)
13	14.3 (q)	0.89 (t, 7.0)
14	11.7 (q)	0.73 (d, 6.7)
15	12.5 (q)	0.81 (d, 6.7)
16	97.8 (s)	
$16Me_{ax}$	19.5 (q)	1.39 (s)
$16Me_{eq}$	30.1 (q)	1.34 (s)

Fig. 2. Extended Newman projection and NOESY correlations of **3**.



Experimental

General

^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-A500 NMR spectrometer. An ultraviolet spectrum was measured on a Shimadzu UV-2200 spectrophtometer. An infrared spectrum was recorded on a Hitachi 260-50 infrared spectrometer. An optical rotation was determined by a JASCO DIP-370 polarimeter. Mass spectra were measured on a VG ZAB-VSE mass spectrometer.

Preparation of Acetonide **3**

To 20 mg of YM-47522 (**1**) was added 1 ml of a 0.2% (w/v) solution of anhydrous K_2CO_3 in MeOH. The solution was stirred at room temperature for 62 hours, and then 40 μl of 0.1 N HCl was added. The reaction mixture was diluted with water, and extracted with EtOAc. The extract was subjected to preparative TLC ($CHCl_3$ - MeOH, 9 : 1) to give 6.9 mg of diol **2**. A solution of **2** (6.9 mg) in 400 μl of 2,2-dimethoxypropane was treated with 3 mg of *p*-toluenesulfonic acid monohydrate (*p*-TsOH), and stirred at room temperature for 1 hour. The reaction mixture was diluted with saturated aqueous $NaHCO_3$, and extracted with EtOAc. The extract was purified by preparative TLC ($CHCl_3$ - MeOH, 9 : 1) to afford 3.4 mg of acetonide **3**.

Acknowledgment

We thank Dr. HARUMITU IMAI, Clinical Pharmacology Research Laboratories of Yamanouchi Pharmaceutical Co., Ltd., for valuable discussions.

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