New Members of the Trichothecene Family

TAKEO SUGAWARA*, AKIHIRO TANAKA, KOJI NAGAI†, KENICHI SUZUKI† and GEN OKADA††

Medicinal Chemistry Research III, Chemistry Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 21, Miyukigaoka, Tsukuba-shi, Ibaraki 305, Japan
†Microbiology Research, Drug Serendipity Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 1-1-8 Azusawa, Itabashi-ku, Tokyo 174, Japan
††Japan Collection of Microorganisms, Institute of Physical and Chemical Research (RIKEN) 2-1 Hirosawa, Wako-shi, Saitama 351-01, Japan

(Received for publication April 11, 1997)

We have identified two new members of the trichothecene family YM-47524 (**1**) and YM-47525 (**2**) as fungicidal metabolites, which were produced by an unidentified fungal strain YL-03713F. These compounds were isolated by bioactivity-guided fractionation using agar diffusion assay for antifungal antibiotics. In this report, we describe the fermentation, isolation, structure elucidation, and biological properties of **1** and **2**.

Strain YL-03713F was isolated from a deadwood sample of *Acer* sp. collected in Ibaraki, Japan. Colonies grew slowly on malt extract agar reaching 25 mm in diameter after two weeks at 24°C. The surface of colonies was white to pale yellow, and the reverse was colored pale yellowish brown. The strain produced no reproductive structures even on sterilized natural substrates. The absence of morphological characteristics implied that this fungus was treated as a deuteromycete in the Agonomycetes[1].

Vegetative mycelia of the producing strain YL-03713F growing on potato-dextrose agar were used to prepare fermentation inoculum in a 500-ml Erlenmeyer flask containing 100 ml of a medium consisting of glucose 1%, potato starch 2%, yeast extract 0.5%, Polypepton (Nihon Pharmaceutical Co., Ltd.) 0.5% and $CaCO_3$ 0.4%. After incubation at 24°C for three days on a rotary shaker at 220 rpm, the seed culture was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of a medium consisting of glucose 3%, corn steep liquor 1%, dried yeast 0.5%, Pharmamedia (Traders Protein Co., Ltd.) 0.5%, soybean meal 0.5% and $CaCO_3$ 0.2% The pH of the medium was adjusted to 6.5. The fermentation was carried out at 24°C for five days on a rotary shaker at 220 rpm.

A total of 2.5 liters of the fermentation broth was filtered, and the filtrate was extracted with EtOAc at pH 3. The extract was subjected to silica gel flash chromatography with increasing amounts of MeOH in $CHCl_3$. The active fractions, eluted with $CHCl_3$ and $CHCl_3$/MeOH (98 : 2), were combined, and then were recrystallized from hexane twice to give a mixture of **1** and **2**. The mixture was well separated by reversed phase HPLC on *L*-column (Chemicals Inspection and Testing Inst., Japan) using CH_3CN/H_2O (1 : 1) to afford YM-47524 (**1**, 6.5 mg) and YM-47525 (**2**, 10.6 mg).

The physico-chemical properties of **1** and **2** are listed in Table 1. YM-47524 (**1**) had a molecular formula of $C_{33}H_{44}O_{11}$, which was established by high-resolution EI-MS and NMR spectra. The ^1H and ^{13}C NMR data (Table 2) were characteristic of macrocyclic trichothecenes, which were divided mainly into two groups: roridin series of macrocyclic diesters and verrucarin series of triesters[2]. Inspection of the NMR data suggested that **1** was closely related to roridin A (**3**)[3]. However, additional signals, assignable to a *trans*-crotonyl ester, were observed in **1**, which was found to be attached to the C-8 position of the central trichothecene part on the basis of HMBC correlations from C-1″ to H-8 and H-3″, and the chemical shifts of the C-8 methine (δ_C 68.0 and δ_H 5.25). The gross structure of **1** was straightforward by interpretation of 2D NMR spectra including COSY, CH-COSY, HMBC, and ROESY (rotating-frame Overhauser enhancement spectroscopy). The assignment of the *cis*, *trans*-dienic function in the macrocyclic portion was made firm by the $^3J_{HH}$ coupling constants of 11.3 Hz for the C-9′, C-10′ double bond and 15.2 Hz for the C-7′, C-8′ double bond. The stereochemistry of the central ring was proposed to be the same as commonly known trichothecenes both by observation of the significant ROESY cross peaks (H11/H3b, 15a, 15b; H4/H15a) and by comparison of spectral data with those in the literature[4]. The α configuration of a crotonyl moiety at C-8 was assigned by the ^{13}C chemical shifts of C-15 (δ

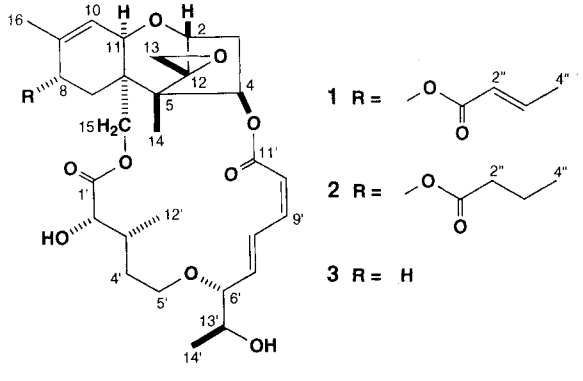

Table 1. Physico-chemical properties of YM-47524 (**1**) and YM-47525 (**2**).

	1	**2**
Appearance	Colorless crystalline solid	Colorless crystalline solid
Molecular weight	616	618
Molecular formula	$C_{33}H_{44}O_{11}$	$C_{33}H_{46}O_{11}$
HREIMS (*m/z*)		
Found:	616.2857 (M^+)	618.3038 (M^+)
Calcd:	616.2884	618.3040
$[\alpha]_D^{25}$	+100.5° (*c* 0.58, MeOH)	+152.4° (*c* 0.84, MeOH)
UV (MeOH) λ_{max} nm (ε)	207 (17200), 262 (17300)	262 (18100)
IR ν_{max} (film) cm^{-1}	3500, 2980, 1710, 1640, 1600, 1440	3450, 2970, 1720, 1640, 1600, 1420

Table 2. ^1H and ^{13}C NMR data of YM-47524 (**1**) and YM-47525 (**2**) in $CDCl_3$.

No.	**1**		**2**	
	^{13}C	^1H	^{13}C	^1H
2	78.8 (d)	3.84 (d, 4.9)	78.8 (d)	3.84 (d, 4.8)
3	34.8 (t)	2.47 (dd, 15.8, 7.9)	34.8 (t)	2.47 (dd, 15.5, 8.2)
		2.23 (ddd, 15.8, 4.9, 4.9)		2.22 (ddd, 15.5, 8.2, 4.8)
4	73.7 (d)	5.78 (dd, 7.9, 4.9)	73.7 (d)	5.78 (dd, 8.2, 8.2)
5	49.3 (s)		49.3 (s)	
6	42.4 (s)		42.3 (s)	
7	26.0 (t)	2.19 (brs)	26.2 (t)	2.21 (dd, 14.0, 5.5)
				2.11 (br d, 14.0)
8	68.0 (d)	5.25 (br s)	67.8 (d)	5.28 (br d, 5.5)
9	136.8 (s)		136.7 (s)	
10	123.5 (d)	5.70 (br d, 5.5)	123.5 (d)	5.67 (br d, 5.5)
11	66.6 (d)	3.71 (d, 5.5)	66.6 (d)	3.69 (d, 5.5)
12	65.0 (s)		65.1 (s)	
13	47.6 (t)	3.09 (d, 3.9)	47.6 (t)	3.09 (d, 3.6)
		2.82 (d, 3.9)		2.81 (d, 3.6)
14	7.5 (q)	0.79 (s)	7.4 (q)	0.80 (s)
15	65.6 (t)	a 4.40 (d, 12.2)	65.5 (t)	a 4.36 (d, 12.2)
		b 4.57 (d, 12.2)		b 4.58 (d, 12.2)
16	20.5 (q)	1.76 (br s)	20.4 (q)	1.74 (br s)
1′	174.6 (s)		174.3 (s)	
2′	75.7 (d)	3.91 (dd, 7.3, 2.4)	75.5 (d)	4.02 (dd, 7.6, 2.7)
2′-OH		2.76 (d, 7.3)		2.80 (d, 7.6)
3′	37.6 (d)	1.91 (m)	37.4 (d)	1.99 (br qd, 6.8, 2.7)
4′	33.2 (t)	1.72 (m)	33.5 (t)	1.74 (br t, 5.8)
		1.67 (m)		
5′	70.3 (t)	3.52 (dd, 9.1, 4.9)	70.2 (t)	3.54 (t, 5.8)
6′	84.0 (d)	3.63 (br d, 3.0)	84.0 (d)	3.64 (br d, 3.4)
7′	139.3 (d)	5.98 (dd, 15.2, 3.0)	139.4 (d)	5.98 (dd, 15.9, 3.4)
8′	125.9 (d)	7.62 (dd, 15.2, 11.6)	126.0 (d)	7.63 (dd, 15.9, 11.6)
9′	144.0 (d)	6.64 (dd, 11.6, 11.3)	144.1 (d)	6.64 (dd, 11.6, 11.6)
10′	117.4 (d)	5.77 (d, 11.3)	117.3 (d)	5.77 (d, 11.6)
11′	166.3 (s)		166.3 (s)	
12′	14.6 (q)	1.08 (d, 7.3)	14.5 (q)	1.10 (d, 6.8)
13′	70.8 (d)	3.59 (br q, 6.1)	70.8 (d)	3.60 (br q, 6.1)
13′-OH		2.72 (br s)		2.73 (br s)
14′	18.2 (q)	1.19 (d, 6.1)	18.2 (q)	1.19 (d, 6.1)
1″	166.1 (s)		173.5 (s)	
2″	121.8 (d)	5.67 (dd, 15.3, 1.3)	36.0 (t)	2.14 (t, 7.6)
3″	146.7 (d)	6.94 (dq, 15.3, 6.7)	18.5 (t)	1.60 (qt, 7.6, 7.6)
4″	18.1 (q)	1.88 (dd, 6.7, 1.3)	13.6 (q)	0.93 (t, 7.6)

65.5) and C-16 (δ 20.5), as supported by the fact that introduction of an α-acyl substituent at C-8 caused a downfield shift of C-15 (*ca.* 2 ppm) and an upfield shift of C-16 (*ca.* 2~3 ppm), whereas a β-substituent caused an upfield shift of C-16 (4~5 ppm), having little effect on C-15[5]. In contrast to **1**, baccharinol, a closely related macrocyclic trichothecene having a β-hydroxyl group at C-8, was reported to show the chemical shifts of δ 64.4 for C-15 and δ 18.3 for C-16[6]. With regard to the macrocyclic region in **1**, careful comparison of NMR data with those for roridin A and its only known stereoisomer isororidin A (C-13′ epimer) indicated that **1** had the same configuration for four chiral centers of C-2′, C-3′, C-6′ and C-13′ as roridin A rather than isororidin A. Especially notable was that C-6′, adjacent to C-13′, resonated at δ 84.0 in **1**, which was close to that for roridin A at δ 83.7, whereas this carbon shifted to δ 82.6 in isororidin A. In addition, the C-2′ methine proton at δ 3.91 exhibited a doublet of doublets pattern in **1** due to coupling to H-3′ and the hydroxyl proton with $^3J_{HH}$ values of 2.4 and 7.3 Hz, respectively, which was in good accordance both with coupling constants of 3 and 6 Hz for H-2′ in roridin A and with its X-ray crystallography[7]. These findings strongly suggested that the relative configurations for **1** and roridin A were identical. Recent study on trichothecene antibiotics indicated that most of roridins retained an *R* configuration at C-6′ as a result of ring closure at this point from corresponding nonmacrocyclic intermediates, trichoverrins, whose absolute structures had been established[5]. Therefore, the stereochemistry for C-2′, C-3′, C-6′, and C-13′ was tentatively assigned as *S*, *R*, *R*, and *R*, respectively, by relating **1** to roridin A. Since it was reported that baccharinoids possessing a hydroxyl group at C-8 position were derived from roridins *via* biosynthetic routes of a plant *Baccharis megapotamica*[8], roridin A was most likely to serve as a precursor for **1** also in this fungus.

YM-47525 (**2**) had a molecular formula of $C_{33}H_{46}O_{11}$, which corresponded to that given for **1** with two additional hydrogens. The ^1H and ^{13}C NMR data were almost identical to those of **1**, except that a butylyl group was present in **2** instead of a crotonyl group in **1**. Thus, **2** was a reduced derivative of **1**.

Macrocyclic trichothecenes possessing an ester function at C-8 were first reported by B. B. JARVIS as roridin K acetate and verrucarin L acetate[5], although a number of nonmacrocyclic trichothecenes have such substituents[2].

YM-47524 (**1**) and YM-47525 (**2**) inhibited the growth of *Candida albicans* at concentrations up to 6.25 μg/ml, whereas *Candida tropicalis*, *Cryptococcus neoformans*, *Aspergillus niger*, *Mucor hiemalis* and *Trichophyton interdigitale* were not affected by both compounds at 50 μg/ml. Both **1** and **2** were also inactive against *Staphylococcus aureus* and *Escherichia coli* at 50 μg/ml.

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