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Yaequinolones J1 and J2, Novel Insecticidal Antibiotics from *Penicillium* sp. FKI-2140

Ryuji Uchida,[†] Rie Imasato,[†] Kazuro Shiomi,[‡] Hiroshi Tomoda,*[,‡] and Satoshi Ōmura[†]

The Kitasato Institute and Kitasato Institute for Life Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan, School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

tomoda@lisci.kitasato-u.ac.jp

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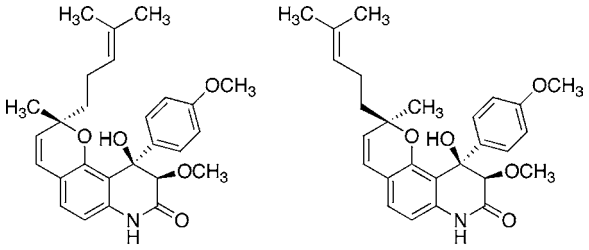
ABSTRACT



Yaequinolone J1(**1**) Yaequinolone J2 (**2**)

Two novel insecticidal antibiotics with a *p*-methoxyphenylquinolinone skeleton fused with a pyran ring, yaequinolones J1 (1) and J2 (2), have been isolated from *Penicillium* sp. FKI-2140, and structures were elucidated by spectroscopic studies including various NMR experiments. The relative stereochemistries were assigned by NOE experiments. Yaequinolones J1 and J2 showed toxicity against *Artemia salina* (brine shrimp) with the MIC value of 6.25 μg/mL.

In the course of our screening program for insecticidal antibiotics using *Artemia salina* (brine shrimp) as a test organism, we have reported several new bioactive metabolites of microbial origin.[1] Our continuous efforts rewarded us the discovery of two novel alkaloids having a *p*-methoxyphenylquinolinone skeleton fused with an isoprenyl pyran ring, yaequinolones J1 (**1**) and J2 (**2**). Microbial metabolites having such a ring system seem to be very unique and have not been previously reported. In this paper, the isolation and structure elucidation, including the stereochemistries of **1** and **2**, are described.

The production culture was initiated by transferring 1 mL of the seed culture of strain *Penicillium* sp. FKI-2140, isolated from a soil sample collected at Ishigakijima Island, Okinawa Prefecture, Japan, into 60 500 mL Erlenmeyer flasks containing 100 mL of the production medium, and the fermentation was carried out at 27 °C with rotation at 210 rpm. After 3 days, two flasks of the main culture were transferred into a 1 L Roux flask and incubated for 11 days at 27 °C. The culture broth (6 L) was treated with acetone, and the mixture was centrifuged to obtain the supernatant and concentrated under reduced pressure. Resulting aqueous layer was extracted with an equal volume of ethyl acetate twice. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give a dark brown oil. After treating with hexane/acetonitrile (1:1), acetonitrile

* To whom correspondence should be addressed. Tel: +81-3-5791-6241. Fax: +81-3-3444-6197.

[†] The Kitasato Institute and Kitasato Institute for Life Sciences, Kitasato University.

[‡] School of Pharmaceutical Sciences, Kitasato University.

(1) (a) Uchida, R.; Imasato, R.; Yamaguchi, Y.; Masuma, R.; Shiomi, K.; Tomoda, H.; Ōmura, S. *J. Antibiot.* **2005**, *58*, 397−404. (b) Shiomi, K.; Hatae, K.; Yamaguchi, Y.; Masuma, R.; Tomoda, H.; Kobayashi, S.; Ōmura, S. *J. Antibiot.* **2002**, *55*, 952−961. (c) Enomoto, Y.; Shiomi, K.; Matsumoto, A.; Iwai, Y.; Harder, A.; Kolbl, H.; Woodruff, H. B.; Omura, S. *J. Antibiot.* **2001**, *54*, 308−313.

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soluble fraction was applied to centrifugal partition chromatography (Sanki Engineering Ltd.) under the following conditions: solvent system, the upper and lower layers of a hexane/acetonitrile/chloroform (5:5:1) mixture were used as mobile and stationary phase, respectively; flow rate, 3 mL/min; rotation speed, 1200 rpm. The mobile phase of the solvent was introduced by ascending method.

After washing with the mobile phase (480 mL), the active fractions containing of **1** and **2** retained in the stationary phase were eluted with the lower layer and concentrated under reduced pressure to give crude **1** and **2**. They were subjected to preparative HPLC under the following conditions: column, Capcell Pak C18 (Shiseido, i.d. 20 × 250 mm); mobile phase, 50% acetonitrile; flow rate, 8 mL/min; detection, UV at 210 nm. Compounds **1** and **2** were eluted at retention times of 26 and 37 min, respectively. Each fraction was collected and concentrated to dryness to give pure **1** (3.0 mg) and **2** (2.0 mg) as pale yellow powders.

Yaequinolone J1 (**1**)[2] showed the molecular ion peak at *m*/*z* 449 $(M)^+$ in FABMS, and the molecular formula $C_{27}H_{31}NO_5$ was assigned on the basis of its HRFABMS [*m*/*z* 449.2230 $(M)^+$, Δ +2.8 mmu, indicating 13 degrees of unsaturation]. IR absorptions indicated the presence of hydroxyl (3496 cm^{-1}) and amide (1697 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **1** measured in chloroform-d_1 showed 31 protons and 27 carbon signals assigned on the basis of 2D NMR correlations. The multiplicity of the carbon signals was classified into 5 methyl, 2 methylene, 10 methine, and 10 quaternary carbons by analysis of the HMQC data. The connectivity of proton and carbon atoms was established by HMQC (Table 1). As shown by bold lines in Figure 1,

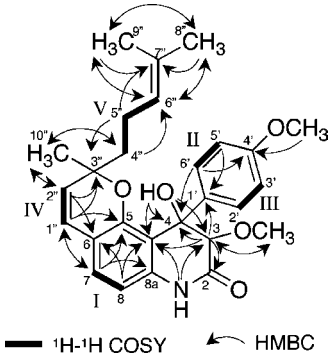

Figure 1. $^1H-^1H$ COSY and HMBC correlations for **1**.

five partial structures, I, II, III, IV (−CH═CH−), and V (−CH_2−CH_2−CH═), were deduced by the $^1H-^1H$ COSY. The $^1H-^{13}C$ long-range couplings of ^2J and ^3J in the HMBC are also shown in Figure 1, giving the following results. The

(2) Yaequinolone J1 (**1**): Pale yellow powder; $[\alpha]^{23}_D$ −65.6° (*c* 0.1, EtOH); IR (KBr) υ_{max} 3421, 2962, 1700, 1604, 1405, 1303, 1261, 1097, 1033, 806 cm^{-1}; UV (EtOH) λ_{max} 218 (ϵ 21700), 284 (ϵ 7900), 295 (ϵ 8600), 318 (ϵ 8000), 324 (ϵ 7200); ^1H and ^{13}C NMR data (Table 1); FABMS *m*/*z* 449 $(M)^+$; HRFABMS *m*/*z* 449.2230 (M^+; calcd for $C_{27}H_{31}NO_5$, 449.2202).

Table 1. ^1H (600 MHz) and ^{13}C NMR (150 MHz) Spectral Data of **1** and **2** in $CDCl_3$

	1		**2**	
position	δC[a]	δH (*J* in Hz)	δC[a]	δH (*J* in Hz)
1-NH		7.40, br s		7.49, br s
2	167.2		167.4	
3	85.1	3.80, d (1.1)	85.0	3.83, d (0.9)
4	78.1		78.0	
4a	114.3		114.6	
5	152.5		152.6	
6	118.0		118.5	
7	127.2	6.90, d (8.0)	127.1	6.93 d (8.0)
8	108.1	6.34, d (8.0)	108.2	6.36, d (8.0)
8a	136.2		136.2	
1′	133.7		134.1	
2′, 6′	127.5	7.19, d (8.4)	127.4	7.19, d (8.8)
3′, 5′	114.2	6.78, d (8.4)	113.9	6.79, d (8.8)
4′	159.8		159.8	
1″	122.2	6.28, d (9.6)	122.5	6.31, d (9.7)
2″	128.0	5.44, d (9.6)	128.4	5.50, d (9.7)
3″	80.8		80.5	
4″	41.1	1.40, m (2H)	41.3	1.67, m
				1.62, m
5″	22.2	1.69, m	23.0	2.09, m
		1.56, m		2.01, m
6″	123.7	4.71, t (7.1)	123.8	5.04, t (7.3)
7″	131.9		132.4	
8″	25.6	1.56, s	25.8	1.66, s
9″	17.4	1.31, s	17.7	1.57, s
10″	26.7	1.41, s	25.6	0.95, s
3-OCH_3	59.5	3.58, s	59.7	3.58, s
4′-OCH_3	55.3	3.74, s	55.4	3.77, s
4-OH		5.36, s		5.22, s

[a] ^{13}C NMR chemical shift values are assigned on the basis of 2D NMR correlations.

cross-peaks from H7 (δ 6.90) to C5 (δ 152.5) and C8a (δ 136.2), and from H8 (δ 6.34) to C4a (δ 114.3) and C6 (δ 118.0) suggested the presence of a 1,2,3,4-tetrasubstituted benzene ring containing the partial structure I. The coupling constant (8.0 Hz) observed between H7 and H8 (Table 1) supported that they are in the ortho position of the benzene ring. Further, the cross-peaks from amide proton NH (δ 7.40) to C3 (δ 85.1) and C4a, and the cross-peaks from oxymethine proton H3 (δ 3.80) to C2 (δ 167.2), C4 (δ 78.1), C4a, and 3-oxymethyl carbon (δ 59.5) and from OH4 (δ 5.36) to C3, C4, and C4a showed that a piperidinone ring is attached to the benzene ring, thus revealing the presence of a 3,4,5,6-tetrasubstituted quinolinone moiety. The cross-peaks from H2′ and H6′ (δ 7.19) to C4′ (δ 159.8) and from H3′ and H5′ (δ 6.78) to C1′ (δ 133.7) and C4′ suggested the presence of a 1,4-disubstituted benzene ring containing the partial structures II and III. Further, a cross-peak from the 4′-oxymethyl proton (δ 3.74) to C4′ showed the presence of a *p*-methoxyphenyl group. This benzene group proved to be connected to C4 by HMBC of H3 to C1′ and H2′ and H6′ to C4, thus revealing the presence of a *p*-methoxyphenylquinolinone skeleton. The cross-peaks from H1″ (δ 6.28) to C3″ (δ 80.8), from H2″ (δ 5.44) to C3″ and C10″ (δ

26.7), from $H_2$4″ (δ 1.40) to C6″ (δ 123.7) and C10″, from $H_2$5″ (δ 1.69, 1.56) to C3″ and C7″ (δ 131.9), from H6″ (δ 4.71) to C9″ (δ 17.4), from $H_3$8″ (δ 1.56) to C6″, C7″ (δ 131.9), and C9″ (δ 17.4), from $H_3$9″ (δ 1.31) to C6″, C7″, and C8″ (δ 25.6), and from $H_3$10″ (δ 1.41) to C2″(δ 128.0) and C4″ (δ 41.1) suggested the presence of 3,7-dimethylocta-1,6-diene presumably originated from a geranyl pyrophosphate. Connectivity of this moiety to C6 was shown by HMBC of H7 to C1″ (δ 122.2), H1″ to C5 and C7, as well as H2″ to C6. Furthermore, connection between C5 and C3″ through an oxygen atom was suggested by the molecular formula and the degrees of unsaturation. Summarizing all these data, the structure of **1** was elucidated as shown in Figure 1.

The relative stereochemistry of **1** was deduced from NOE experiments, and the computer-generated three-dimensional drawing is shown in Figure 2. The NOEs were observed

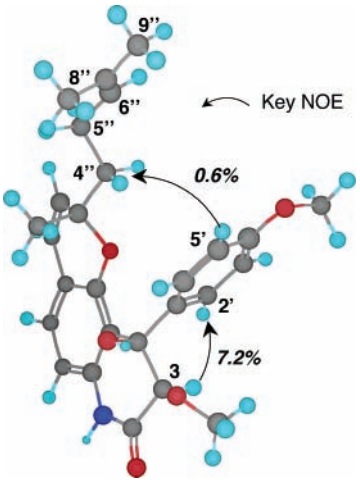

Figure 2. Key NOE correlations for **1**.

between H3 and phenyl proton H2′ (7.2%), but not observed between the 3-oxymethyl proton and H2′, suggesting that the methoxyl group at C3 and the *p*-methoxyphenyl group at C4 were oriented to the opposite side of the quinolinone plane. Furthermore, the NOEs were observed between H5′ and H4″ (0.6%) weakly, suggesting that the *p*-methoxyphenyl group and the dimethylallyl side chain were on the same side of the quinolinone plane. Thus, the relative stereochemistry of **1** is presumed to be 3*R**4*R**3″*S**. These conclusions were supported by the fact that the proton chemical shifts of the 3,7-dimethylocta-1,6-diene side chain at H″4, H″5, H″6, H″8, and H″9 were shifted to upfield due to the anisotropic effect of the *p*-methoxyphenyl group.

Yaequinolone J2 (**2**)[3] showed the molecular ion peak at *m*/*z* 449 $(M)^+$ in FABMS, and the molecular formula $C_{27}H_{31}NO_5$ was assigned to **2** on the basis of its HRFABMS [*m*/*z* 449.2210 $(M)^+$, Δ +0.8 mmu]. Similarity in physicochemical properties strongly suggested that **1** and **2** are structurally closely related. The ^{1}H and ^{13}C NMR spectra (Table 1) of **2** also resembled those of **1** except for the proton signals of $H_2$4″, $H_2$5″, H6″, $H_3$9″, and $H_3$10″. The structure of **2** was elucidated by 2D NMR (^{1}H−^{1}H COSY, HMQC, and HMBC) data and comparison with those of **1**. As shown by bold lines in Figure 3, the presence of five partial structures,

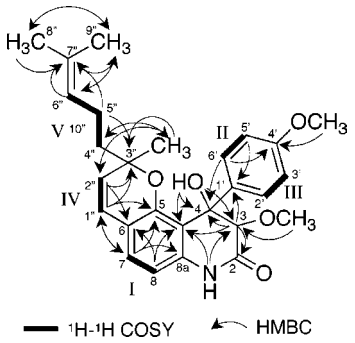

Figure 3. ^{1}H−^{1}H COSY and HMBC correlations for **2**.

I, II, III, IV (−CH═CH−), and V (−CH_2−CH_2−CH═), was indicated by the ^{1}H−^{1}H COSY. Their connectivity was proved by HMBC experiments, indicating that **2** has the same planar structure as **1**.

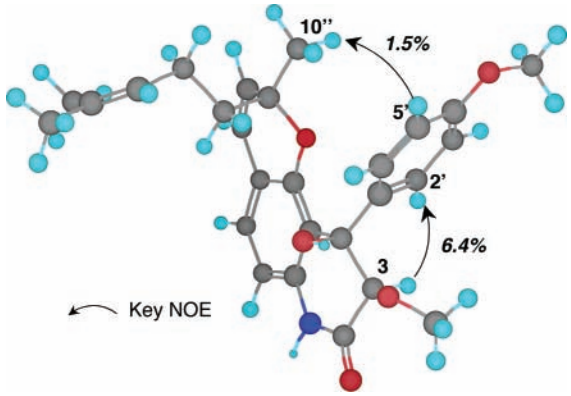

Figure 4. Key NOE correlations for **2**.

The relative stereochemistry of **2** was deduced according to NOE experiments (Figure 2). The NOEs were observed between H3 and phenyl proton H2′ (6.4%), but not observed between the 3-oxymethyl proton and H2′, suggesting that the methoxyl group at C3 and the *p*-methoxyphenyl group at C4 were oriented to the opposite side of the quinolinone plane. Furthermore, the NOEs observed between H5′ and methyl proton H10″ (1.5%) suggested that the *p*-methoxyphenyl group and the dimethylallyl side chain were on the

(3) Yaequinolone J2 (**2**): Pale yellow powder; $[\alpha]^{23}_D$ 181.7° (*c* 0.1, EtOH); IR (KBr) ν_{max} 3496, 2925, 1697, 1604, 1504, 1461, 1380, 1259, 1099, 1035, 806 cm^{-1}; UV (EtOH) λ_{max} 218 (ϵ 17900), 284 (ϵ 9300), 295 (ϵ 9800), 324 (ϵ 8700); ^{1}H and ^{13}C NMR data (Table 1); FABMS *m*/*z* 449 $(M)^+$; HRFABMS *m*/*z* 449.2210 (M^+; calcd for $C_{27}H_{31}NO_5$, 449.2202).

opposite side of the quinolinone plane. Thus, the relative stereochemistry of **2** is presumed to be 3*R**4*R**3′′*R**. These conclusions were supported by the upfield shift of the H10′′ signal due to anisotropic effect of the *p*-methoxyphenyl group.

Insecticidal activities of **1** and **2** were studied by a microplate assay using brine shrimp (*Artemia salina*). Compounds **1** and **2** showed the same potency with the same MIC values of 6.25 μg/mL.

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Supporting Information Available: Available ^{1}H NMR and two-dimensional NMR spectra of yaequinolones J1 (**1**) and J2 (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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