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Prenylterphenyllin and Its Dehydroxyl Analogs, New Cytotoxic Substances from a Marine-derived Fungus *Aspergillus candidus* IF10

Hong Wei, Hirohiko Inada, Asami Hayashi, Kenichi Higashimoto, Patamaporn Pruksakorn, Sayo Kamada, Masayoshi Arai, Shunsuke Ishida, Motomasa Kobayashi

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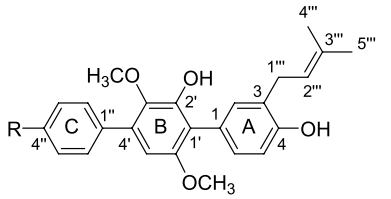
Abstract Three novel cytotoxic substances named prenylterphenyllin (**1**), 4″-deoxyprenylterphenyllin (**2**), and 4″-deoxyisoterprenin (**3**) were isolated from a cultured marine-derived fungus of *Aspergillus candidus* IF10 together with 4″-deoxyterprenin (**4**). Their chemical structures were elucidated on the basis of 2D NMR analysis. These compounds **1**∼**4** showed cytotoxic activity against human epidermoid carcinoma KB cells (KB3-1) with IC_{50} of 8.5, 3.0, 2.5, and 4.5 μg/ml, respectively.

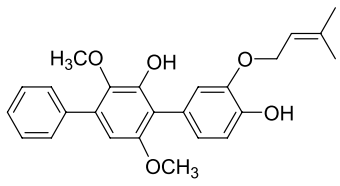
Keywords prenylterphenyllin, cytotoxic, marine-derived fungus, *Aspergillus candidus*

In recent years, marine microorganisms have been paid much attention as a significant source for new drug leads [1, 2]. During course of our search for anti-cancer agent from marine-derived microbe, three novel cytotoxic substances named prenylterphenyllin (**1**), 4″-deoxyprenylterphenyllin (**2**), and 4″-deoxyisoterprenin (**3**) were isolated from a cultured marine-derived fungus of *Aspergillus candidus* IF10 together with 4″-deoxyterprenin (**4**) (Fig. 1). In this paper, the fermentation, isolation, and structure elucidation of these compounds are presented.

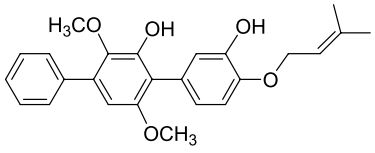
The fungal strain IF10 was isolated from the marine sediment collected from a depth of 50 m off Gokasyo Gulf, Mie Prefecture, Japan, and identified as *A. candidus* by morphological analysis [3] (Fig. 2) and BLAST search of 28S rDNA sequence using DNA Data Bank of Japan (DDBJ). 28S rDNA sequence of IF10 strain (575 nucleotides) showed 100% of homology with those of



Prenylterphenyllin (**1**): R = OH
4''-Deoxyprenylterphenyllin (**2**): R = H



4''-Deoxyisoterprenin (**3**)



4''-Deoxyterprenin (**4**)

Fig. 1 Structures of prenylterphenyllins and terprenins.

M. Kobayashi (Corresponding author), **H. Wei, H. Inada, A. Hayashi, K. Higashimoto, P. Pruksakorn, S. Kamada, M. Arai, S. Ishida:** Graduate School of Pharmaceutical Sciences, Osaka University, Yamada-oka 1-6, Suita, Osaka 565-0871, Japan,
E-mail: kobayasi@phs.osaka-u.ac.jp

A. candidus NRRL303 (Accession No. AF433067) and *A. candidus* NRRL4809 (Accession No. U28765). The cytotoxic activity of these compounds was evaluated by MTT assay method using human epidermoid carcinoma KB cells (KB3-1). The concentration (IC_{50}) of test compound causing 50% inhibition of the growth of KB3-1 cells was defined as an index of cytotoxicity.

The slant culture of the strain *A. candidus* IF10 was inoculated into a 500-ml Erlenmeyer flask containing 200 ml of MG medium [consisting of 2.0% malt extract (Difco, NJ, USA), 2.0% glucose, 0.1% bact peptone (Difco, NJ, USA) in artificial seawater (Yashima Pure Chemicals, Osaka, Japan)] at 30°C for 3 days. Then, 40 ml aliquots of the culture were transferred into 5-liter Erlenmeyer flasks containing 1.0 liter of MG medium and cultured under static conditions at 30°C for 2 weeks. A typical time course of the fermentation is shown in Fig. 3. **1** was detected in the culture broth at day 10 after inoculation, and its concentration reached level of 4.5 mg/liter at day 14. The 2 weeks old culture was extracted with 2-butanone, and the 2-butanone-soluble portion was further partitioned into an *n*-hexane - 90% aq MeOH mixture to furnish a MeOH extract (3.8 g). The MeOH extract (2.1 g) was fractionated by SiO_2 gel column chromatography (*n*-hexane - EtOAc) to give eight fractions (A~H) on the guidance of bioassay. Fraction C (73 mg) was then subjected to SiO_2 gel column chromatography (*n*-hexane - $CHCl_3$ - EtOAc=4 : 1 : 1) to afford **1** (14 mg). Fraction A (50 mg) was separated by SiO_2 gel column chromatography (*n*-hexane - $CHCl_3$ - EtOAc=10 : 10 : 1) to give four fractions (A-1 to A-4). Fraction A-4 (14 mg) was purified by HPLC (Cosmosil 5SL-II, *n*-hexane - $CHCl_3$ - EtOAc=4 : 4 : 1) to furnish **2** (2.5 mg). Then, fraction A-3 (20 mg) was separated by HPLC (Cosmosil 5SL-II, *n*-hexane - $CHCl_3$ - EtOAc= 5 : 5 : 1) to obtain an active fraction A-3-1 (8.0 mg), which was further purified by reversed phase HPLC (Cosmosil $5C_{18}$-MS-II, CH_3CN - H_2O=55 : 45) to afford **3** (1.0 mg) and **4** (4.5 mg).

Fig. 2 *Aspergillus candidus* IF10 under microscope.

The ESI TOF-MS of **1** [White powder. IR ν_{max} (KBr) cm^{-1}: 3420, 2936, 1610, 1522. UV λ_{max}^{MeOH} nm (ε): 205 (40200), 277 (20000). ESI TOF-MS: *m/z* 429 $(M+Na)^+$. HR-ESI TOF-MS: found *m/z* 429.1660 $(M+Na)^+$. Calcd for $C_{25}H_{26}O_5Na$: 429.1678.] showed a quasi-molecular ion peak at *m/z* 429 $(M+Na)^+$, and the molecular formula was determined as $C_{25}H_{26}O_5$ by HR-ESI TOF-MS in conjunction with NMR analysis. The ^1H- and ^{13}C-NMR analyses indicated that **1** possesses an isoprenyl group [δ_H 5.39 (1H, m); 3.39 (2H, d, *J*=7.1 Hz); 1.78 (3H, s); 1.76 (3H, s); δ_C 134.6, 121.8, 29.8, 25.8, 17.9], two methoxyl groups [δ_H 3.74 (3H, s); 3.45 (3H, s); δ_C 60.5, 56.0], three phenolic hydroxyl groups [δ_H 5.90 (s); 5.32 (s); 5.29 (s)], and eight aromatic protons [δ_H 7.52 (2H, d, *J*=8.2 Hz); 7.21 (2H, m); 6.91 (2H, d, *J*=8.2 Hz); 6.88 (1H, d, *J*=8.9 Hz); 6.45 (1H, s)]. The detailed analysis of the

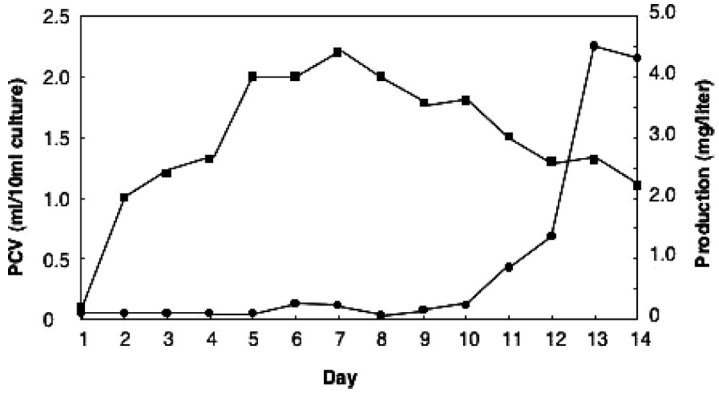


Fig. 3 Time course of prenylterphenyllin (**1**) production from *Aspergillus candidus* IF10.

●: Production of **1**, ■: PCV, packed cell volume (ml) from 10 ml of the whole culture broth.

COSY, HMQC, and HMBC spectra of **1** clarified the presence of three phenyl rings and an isopentenyl moiety as shown in Fig. 4. The connection of the ring structures was also defined on the basis of the NOESY correlations of **1** (Fig. 5). Thus, the strong NOE correlations [from H-2″ and H-6″ (δ_H 7.52, 2H, d, J=8.2 Hz) to H-5′ (δ_H 6.45, s) and 3′-OCH_3 (δ_H 3.45, s); from 6′-OCH_3 (δ_H 3.74, s) to H-5′ and H-2 and H-6 (δ_H 7.21, overlap); from 2′-OH (δ_H 5.29, s) to 3′-OCH_3] were observed.

The ^{1}H- and ^{13}C-NMR spectra of **2** [White powder. IR ν_{max} (KBr) cm^{-1}: 3485, 2930, 1604, 1564. UV λ_{max}^{MeOH} nm (ε): 204 (43200), 275 (17500). ESI TOF-MS: m/z 413 $(M+Na)^+$. HR-ESI TOF-MS: found m/z 413.1756 $(M+Na)^+$. Calcd for $C_{25}H_{26}O_4Na$: 413.1729.] (Tables 1 and 2) were closely similar to those of **1**, except for the signals assignable to the C ring. The structure of the C ring was deduced as phenyl group by 2D NMR analysis. Then, **2** was defined to be 4″-dehydoxyl analog of **1**. **4** was identified with 4″-deoxyterprenin [4], which has been isolated from the culture of a fungus *A. candidus* by Kamigauchi *et al.*

The molecular formula of **3** [White powder. IR ν_{max} (KBr) cm^{-1}: 3493, 2935, 1585, 1518. UV λ_{max}^{MeOH} nm (ε): 203 (45000), 277 (15400). ESI TOF-MS: m/z 429 $(M+Na)^+$. HR-ESI TOF-MS: found m/z 429.1683 $(M+Na)^+$. Calcd for $C_{25}H_{26}O_5Na$: 429.1678.] was determined as $C_{25}H_{26}O_5$ on the basis of NMR and HR-ESI

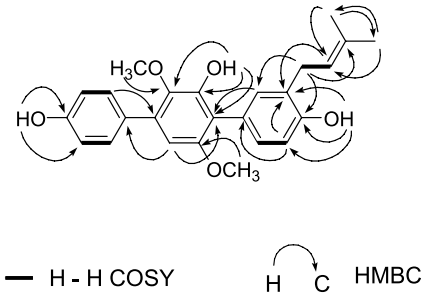


Fig. 4 COSY and key HMBC correlations of **1**.

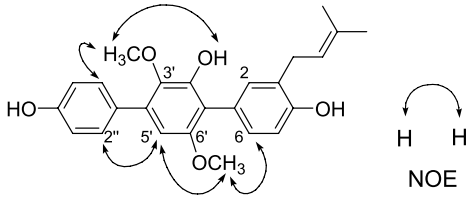


Fig. 5 Key NOE correlations of **1**.

Table 1 ^{1}H-NMR data for prenylterphenyllin (**1**), 4″-deoxyprenylterphenyllin (**2**), 4″-deoxyisoterprenin (**3**), and 4″-deoxyterprenin (**4**) (600 MHz in $CDCl_3$, δ (mult., J (Hz)))

Position	δ (ppm, J in Hz)			
	1	**2**	**3**	**4**
2	7.21 (m)	7.23 (s)	6.99 (s)	7.06 (s)
5	6.88 (d, 8.9)	6.88 (d, 8.8)	7.00 (d, 7.1)	6.96 (s)
6	7.21 (m)	7.22 (d, 8.8)	6.97 (d, 7.1)	6.96 (s)
5′	6.45 (s)	6.48 (s)	6.47 (s)	6.48 (s)
2″	7.52 (d, 8.2)	7.63 (d, 7.4)	7.62 (d, 7.4)	7.64 (d, 7.4)
3″	6.91 (d, 8.2)	7.45 (t, 7.4)	7.44 (dd, 7.4, 7.6)	7.45 (t, 7.4)
4″		7.37 (t, 7.4)	7.38 (t, 7.6)	7.37 (t, 7.4)
5″	6.91 (d, 8.2)	7.45 (t, 7.4)	7.44 (dd, 7.4, 7.6)	7.45 (t, 7.4)
6″	7.52 (d, 8.2)	7.63 (d, 7.4)	7.62 (d, 7.4)	7.64 (d, 7.4)
1‴	3.39 (d, 7.1)	3.40 (d, 7.2)	4.57 (d, 6.8)	4.61 (d, 6.7)
2‴	5.39 (m)	5.40 (m)	5.50 (m)	5.24 (m)
4‴	1.78 (s)	1.79 (s)	1.71 (s)	1.75 (s)
5‴	1.76 (s)	1.77 (s)	1.79 (s)	1.81 (s)
3-OH				5.70 (s)
4-OH	5.29 (s)	5.19 (s)	5.71 (s)	
2′-OH	5.90 (s)	5.90 (s)	5.89 (s)	5.92 (s)
3′-OCH_3	3.45 (s)	3.44 (s)	3.44 (s)	3.44 (s)
6′-OCH_3	3.74 (s)	3.74 (s)	3.74 (s)	3.75 (s)
4″-OH	5.32 (s)			

Table 2 ^{13}C-NMR data for **1**, **2**, **3**, and **4** (150 MHz in $CDCl_3$)

Position	δ_C (ppm)			
	1	**2**	**3**	**4**
1	125.1	125.1	124.5	125.9
2	132.3	132.3	114.6	117.0
3	126.5	126.4	145.5	145.5
4	153.6	153.7	145.2	145.2
5	115.6	115.6	114.2	111.5
6	129.8	129.9	123.7	122.2
1′	116.4	116.8	116.9	116.6
2′	147.2	147.2	147.3	147.2
3′	138.7	138.8	138.8	138.8
4′	132.3	132.7	132.8	132.8
5′	103.7	103.9	104.0	103.8
6′	153.4	153.5	153.5	153.4
1″	130.4	138.1	138.1	138.1
2″	130.0	128.8	128.8	128.7
3″	115.3	128.5	128.5	128.4
4″	155.3	127.5	127.5	127.4
5″	115.3	128.5	128.5	128.4
6″	120.0	128.8	128.8	128.8
1‴	29.8	30.0	65.7	65.6
2‴	121.8	121.8	119.3	119.4
3‴	134.6	134.7	138.7	138.7
4‴	17.9	17.9	18.2	17.9
5‴	25.8	25.8	25.8	25.8
3′-OCH_3	60.5	60.7	60.9	60.7
6′-OCH_3	56.0	56.0	56.0	56.0

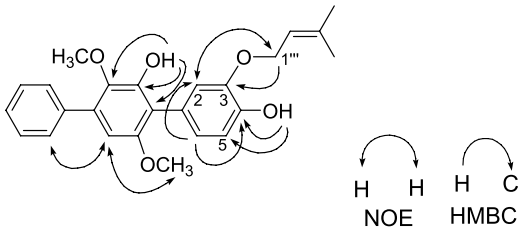


Fig. 6 Key NOE and HMBC correlations of **3**.

TOF-MS analysis. The ^{1}H- and ^{13}C-NMR spectra of **3** were almost superimposable to those of **4** (Tables 1 and 2), and **3** was presumed to be an isomer of **4** concerning with position of the *O*-isoprenyl group. As shown in Fig. 6, the position of the *O*-isoprenyl group in **3** was defined based on the HMBC correlations [from H-1‴ (δ_H 4.57, 2H, d, J=6.8 Hz) to C-3 (δ_C 145.5); from 4-OH (δ_H 5.71, s) to C-4 (δ_C 145.2) and C-5 (δ_C 114.2); from H-6 (δ_H 6.97, d, J=7.1 Hz) to C-2 (δ_C 114.6) and C-4] and the NOE correlation between H-1‴ and H-2 (δ_H 6.99, s).

1, **2**, **3**, and **4** exhibited cytotoxic activity against KB3-1 with IC_{50} of 8.5, 3.0, 2.5, and 4.5 μg/ml, respectively. So far, many related compounds have been reported as anti-proliferative agents [5~7]. Recently, Kamigauchi *et al.* [4] reported that terprenins including **4** exhibited immunosuppressive activity. Further biological study for these terphenyllins and terprenins are under way.

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