New Polyoxygenated Farnesylcyclohexenones, Deacetoxyyanuthone A and Its Hydro Derivative from the Marine-Derived Fungus *Penicillium* sp.

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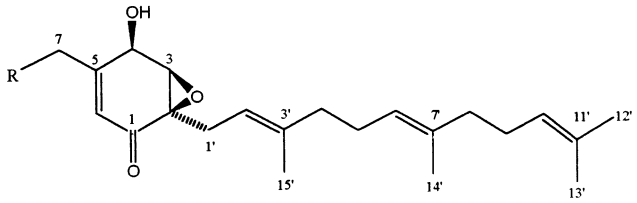
New polyoxygenated farnesylcyclohexenones, 7-deacetoxyyanuthone A (**1**) and its 2,3-hydro derivative (**2**), were isolated together with the known farnesylquinones (**3**, **4**) from a marine isolate of the genus *Penicillium*. The structures of the new deacetoxyyanuthone A (**1**) and its 2,3-hydro derivative (**2**) were assigned by spectroscopic methods, including 2D NMR and CD for the Cotton effect of α-epoxyketone experiments. Compounds **1** and **3** showed moderate in vitro cytotoxicity in a panel of five human tumor cell lines, and **1** also exhibited mild in vitro antibacterial activity against methicillin-resistant and multidrug-resistant *Staphylococcus aureus* (MIC, 50 μg/mL).

During a search for bioactive constituents from marine microorganisms,[1] we previously isolated farnesylquinones (**3**, **4**) from the marine-derived fungus *Penicillium* sp.[2] In a continuing study on the more polar fractions of the same fungus, we have isolated two new polyoxygenated farnesylcyclohexenones (**1**, **2**), which are proposed to be oxidized derivatives of farnesylquinone. We report here on the isolation and structural elucidation of these new compounds.

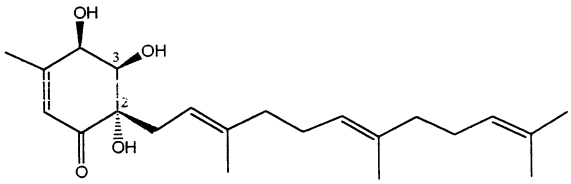
Deacetoxyyanuthone A (**1**) was isolated as a yellow oil. A molecular formula of $C_{22}H_{32}O_3$, which gave seven degrees of unsaturation, was established by HRFABMS and ^{13}C NMR methods. The IR absorption spectrum of **1** showed bands that are characteristic of hydroxyl (3431 cm^{-1}) and enone (1680, 1029 cm^{-1}) functionalities. The ^1H and ^{13}C NMR spectra, including DEPT, showed five olefinic methyl singlets, two oxygenated methines, one oxygenated quaternary carbon, four olefinic methines, four olefinic quaternary carbons, one carbonyl carbon, one diastereotopic methylene, and four other methylenes. The overall NMR data, which were similar to the farnesylquinones **3** and **4**[2] as well as yanuthone A (**1a**),[3] indicated the presence of a farnesyl moiety, a trisubstituted enone, and a trisubstituted epoxy group. The presence of an enone chromophore and an epoxy group was further supported by UV spectral data [203 nm (log ϵ 4.6), 238 nm (log ϵ 4.2)] and by oxygenated carbon signals [δ61.6 (s), C-2; 59.2 (d), C-3] located considerably upfield, respectively.

The connection of functional groups in **1** was achieved on the basis of 2D NMR (COSY, HMQC, HMBC) correlations, which allowed all carbons and their respective protons to be assigned. Diagnostic HMBC correlations from H_2-1′ to C-1, C-2, and C-3 and from H-2′ to C-2 showed the connection of C-2 and C-1′ in **1**. On the basis of all of the foregoing evidence, the structure of **1** was proposed as the 7-deacetoxy analogue of yanuthone A (**1a**).[3]

The stereochemistry illustrated for **1** is based on NOE experiments, which had established that **1** and **1a** possessed the identical relative stereochemistry at all centers from the same CD spectral data. Key NOE correlations from H-3 to H-4 and from H-3 to H-2′ were critical in establishing the relative structure of 7-deacetoxyyanuthone A (**1**) as shown. A *cis* relationship between the epoxide and the hydroxyl at C-4 was further supported by comparing the coupling constant of **1** (J_{H3-H4} = 3.0 Hz) with values reported for similar epoxycyclohexenones, yanuthone A (**1a**) (J = 2.5 Hz),[3] macrophorins (J = 2.5 Hz),[4] and (+)-isopanepoxydon (J = 3.0 Hz).[5]



7-deacetoxyyanuthone A (**1**): R=H
yanuthone A (**1a**): R=OAc



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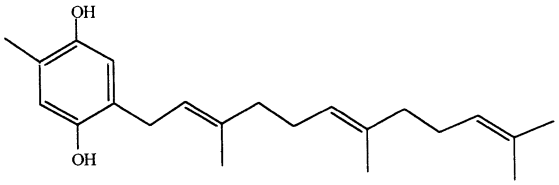

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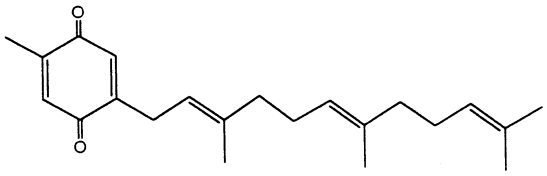

4

The absolute stereochemistry of the 7-deacetoxyyanuthone A (**1**) was investigated using CD. The CD spectrum

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Table 1. ^{1}H (δ, mult, J) and ^{13}C (δ, mult) NMR Data for 7-Deacetoxyyanuthone A (**1**) and Its 2,3-Hydro Derivative (**2**)[a]

carbon no.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		193.3 (s)		190.5 (s)
2		61.6 (s)		68.4 (s)
3	3.69 (d, 3.0)	59.2 (d)	4.28 (d, 3.5)	74.5 (d)
4	4.41 (br, s)	67.6 (d)	4.73 (br, s)	68.8 (d)
5		155.8 (s)		158.2 (s)
6	5.77 (s)	123.6 (d)	5.95 (s)	123.9 (d)
7	2.00 (s)	20.1 (q)	2.07 (s)	20.2 (q)
1′	2.49 (dd, 15.5, 7.0)	26.0 (t)	2.85 (br, d, 8.0)	32.3 (t)
	2.80 (dd, 15.5, 7.0)			
2′	5.01 (t, 7.0)	116.1 (d)	5.33 (dd, 7.0, 3.0)	117.1 (d)
3′		139.8 (s)		140.5 (s)
4′	1.97 (m)[b]	39.6 (t)	2.13 (m)[c]	39.9 (t)
5′	2.07 (m)[b]	26.4 (t)	2.17 (m)[c]	26.3 (t)
6′	5.07 (t, 7.0)	123.8 (d)	5.08 (m)[d]	123.7 (d)
7′		135.2 (s)		135.5 (s)
8′	1.99 (m)[b]	39.7 (t)	1.98 (m)[c]	39.7 (t)
9′	2.03 (m)[b]	26.7 (t)	2.11 (m)[c]	26.7 (t)
10′	5.09 (t, 7.0)	124.3 (d)	5.10 (m)[d]	124.3 (d)
11′		131.3 (s)		131.5 (s)
12′	1.68 (s)	25.7 (q)	1.69 (s)	25.7 (q)
13′	1.60 (s)	17.7 (q)	1.61 (s)	17.7 (q)
14′	1.59 (s)	16.0 (q)	1.61 (s)	16.1 (q)
15′	1.63 (s)	16.3 (q)	1.70 (s)	16.5 (q)

[a] Recorded in $CDCl_3$ at 400 MHz (^{1}H) and 100 MHz (^{13}C). [b−d] Overlapped.

of **1** showed a positive first Cotton effect at 340 nm ($\Delta\epsilon$, +2.8), which was identical to those of macrophorins of *R*-stereochemistry.[4] Thus, the absolute configuration of asymmetric centers for **1** was determined to be 2*R*, 3*R*, and 4*R*, which was further supported by the octant rule of α-epoxyketone.[6]

The 2,3-hydro derivative (**2**) was obtained as a yellowish oil, and HRFABMS and ^{13}C NMR methods established the molecular formula to be $C_{22}H_{34}O_4$. The general features of its UV, IR, and NMR spectra (Table 1) closely resembled those of 7-deacetoxyyanuthone A (**1**), except that the NMR signals assigned to the epoxy group had shifted downfield from δ_H 3.69 (H-3), δ_C 59.2 (C-3), and δ_C 61.6 (C-2) for compound **1** to δ_H 4.28 (H-3), δ_C 74.5 (C-3), and δ_C 68.4 (C-2) for compound **2** (Table 1).

Detailed analyses of the ^{1}H and ^{13}C NMR spectra of **2**, including the results from DEPT, COSY, HMQC, HMBC, and NOESY experiments, suggested the metabolite (**2**) is the 2,3-hydro derivative derived from opening the epoxide ring of compound **1**.

The relative stereochemistry of the metabolite **2** was determined by the coupling constant and the NOESY data. The coupling constant of H-3 and H-4 was observed to be 3.5 Hz, clearly suggesting a *cis* relationship with the *trans* orientation of $J_{H3-H4} = 8.0-9.0$ Hz.[7,8] An NOE correlation was observed between H-3 and H-4, but it was not observed in H_2-1′ or H-2′, indicating therefore that 3-OH and 4-OH should be positioned on the same side, but 2-OH and 3-OH were inferred to be in a *trans* relationship.

The absolute stereochemistry of the asymmetric carbon centers in **2** remains to be assigned.

Compounds **1**, **3**, and **4** were evaluated for cytotoxicity against a small panel of five human tumor cell lines and for antibacterial activity against methicillin-resistant and multidrug-resistant *Staphylococcus aureus*. Compounds **1** and **3** showed moderate in vitro cytotoxicity in a small panel of five human tumor cell lines (Table 2), and **1** also exhibited mild in vitro antibacterial activity against methicillin-resistant and multidrug-resistant *S. aureus* (MIC = 50 μg/mL), respectively.

Table 2. Cytotoxicities (ED_{50}, μg/mL) of Compounds **1**, **3**, and **4** against Human Solid Tumor Cells[a]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	7.74	6.35	3.86	10.04	10.07
3	4.73	5.31	4.80	5.94	6.11
4	25.44	37.29	18.41	38.07	42.56
doxorubicin	0.02	0.09	0.04	0.02	0.79

[a] A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT15, human colon cancer.

Experimental Section

General Experimental Procedures. The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.[1]

Fungal Isolation and Culture. The fungal strain (culture #MFA 577) was isolated from the surface of the polymeric cord collected in Bijin Island, Gyeongnam Province, in 2000 and identified as a *Penicillium* sp. based on fatty acid methyl ester analysis (Korean Culture Center of Microorganisms, Seoul, Korea), similarity index 0.862. The fungus was cultured (20 L) for 30 days (static) at 29 °C in SWS medium: soytone (0.1%), soluble starch (1.0%), and seawater (100%).

Extraction and Isolation. The mycelium and broth were separated by filtration. The mycelial mat was freeze-dried and then exhaustively extracted with CH_2Cl_2−MeOH (1:1). The combined extract (4.8 g) was subjected to reversed-phase flash column chromatography on YMC ODS-A gel. Elution was performed with H_2O−MeOH (stepwise, 0−100% MeOH) to yield four fractions. Further purification of fraction 3 (410 mg) over silica gel column chromatography using *n*-hexane−EtOAc (15:1−5:1), followed by HPLC (YMC ODS-A, 10 × 250 mm) (MeOH), yielded the farnesylhydroquinone (**3**, 61 mg) and sesquiterpene quinone (**4**, 12.0 mg). Fraction 4 (84 mg) containing compounds **1** and **2** was further separated by using a column of Si gel [*n*-hexane−EtOAc (5:1−1:1)] as the eluent and purified by HPLC (ODS-A, MeOH) to furnish **1** (18.0 mg) and **2** (3.0 mg).

7-Deacetoxyyanuthone A (1): yellow oil; $[\alpha]_D$ +3.1° (*c* 0.5, $CHCl_3$); IR (neat) ν_{max} 3431, 1680, 1440, 1381, 1274, 1108, 1029, 999, 871 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 203 (4.6), 238 (4.2) nm; CD (MeOH) ($\Delta\epsilon$) 340 (+2.8), 256 (−1.2); see Table 1 for NMR spectral data; LRFABMS *m*/*z* 367 $[M + Na]^+$ (5), 345 $[M + H]^+$ (15); HRFABMS *m*/*z* 345.2432 (calcd for $C_{22}H_{33}O_3$, 345.2430).

Compound 2: unstable yellow oil; $[\alpha]_D$ −2.1° (*c* 0.3, $CHCl_3$); IR (neat) ν_{max} 3436, 1670, 1439, 1379, 1229, 1098, 1039, 998, 882, 832 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 203 (4.2), 238 (4.0) nm; CD (MeOH) ($\Delta\epsilon$) 352 (+0.2), 257 (−2.2); see Table 1 for NMR spectral data; LRFABMS *m*/*z* 385 $[M + Na]^+$, 363 $[M + H]^+$; HRFABMS *m*/*z* 363.2538 (calcd for $C_{22}H_{35}O_4$, 363.2535).

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