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A NEW INDENE DERIVATIVE FROM THE MARINE FUNGUS *Phomopsis* SP. (No. GX7-4A)

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*A new indene derivative named phomoindene A (**1**) and four known compounds were isolated from the marine fungus* Phomopsis *sp. (No. GX7-4A). Their structures were determined by spectroscopic methods, mainly 1D and 2D NMR. Preliminary pharmacological tests revealed that phomoindene A exhibited little cytotoxic activity towards KB, KBv 200, and MCF-7 cell lines.*

Keywords: natural product, indene derivative, marine fungus, cytotoxic activity, prenyl, NMR assignment.

Fungi of *Phomopsis* sp. have been widely investigated for natural products and proven to produce a variety of chemically unique and biologically interesting low molecular weight metabolites [1, 2]. In our continuing search for new metabolites from marine fungi from the South China Sea [3, 4], we discovered a new indene derivative named phomoindene A (**1**) together with four known compounds: terramide A (**2**), terretonin (**3**), monochlorosulochrin (**4**), and butyrolactone II (**5**). Their structures were determined by spectroscopic methods, mainly 1D and 2D NMR. Preliminary pharmacological test revealed that its IC_{50} values of cytotoxic activity againt KB, KBv 200, and MCF-7 cell lines were above 50 μmoL/mL.

Compound **1** was isolated as a pale wax. HR-EI-MS (380.1619) in conjunction with the NMR data revealed the molecular formular as $C_{23}H_{24}O_5$, which indicated twelve degrees of unsaturation. FT-IR spectrum showed bands for the carbonyl group (1738 cm^{-1}) and hydroxyl group (3363 cm^{-1}). ^{13}C NMR and DEPT spectra revealed a prenyl group [δ_C 17.8 (CH_3), 25.8 (CH_3), 134.1 (C), 121.4 (CH), 28.9 (CH_2)], a 1,4-substituted benzene ring [δ_C 121.9 (C), 129.5 (2CH), 115.9 (2CH), 156.7 (C)], a 1,2,3,4-substituted benzene ring [δ_C 129.0 (CH), 131.1 (CH), 126.6 (C), 152.9 (C), 137.1 (C), 124.4 (C)], an ethylene group [δ_C 128.3 (C), 114.9 (CH)], a methylene carbon (δ_C 38.6), an oxygenated tertiary carbon (δ_C 86.1), a carbonyl group (δ_C 169.4), and a methoxyl group (δ_C 53.7). For the 9 degrees of unsaturation corresponding to the double bonds, compound **1** should possess three rings. The whole structure was assembled by an HMBC experiment. Correlation from 8-OME to C-8 formed the methyl formate group. Correlations from H-3 to C-1, 2, 3a, 4, 5 formed the indene framework. Correlations from H-1″ to C-5, C-6, C-7 located the prenyl group on C-6. Correlation from H-3′ to C-1′ connected the benzene moiety to C-1′. Correlations from H-1′ to C-2, C-3 connected C-1′ to C-2. In the NOE experiment, the weak coupling signal between H-1′ and H-3 established the C-1′, C-2 double bond to be in the *Z* configuration. The coupling signal between H-4″ and H-2″ confirmed the assignment of C-4. So, the structure of compound **1** was established to be 1,7-dihydroxy-2(*Z*)-(4-hydroxybenzylidene)-6-(3-methylbut-2-enyl)-indan-1-carboxylic acid methyl ester.

Phomoindene A (**1**) possesses a new skeleton. To our knowledge, indene derivatives are rare in the field of nature products. Its biosynthetic route is still unclear and worth further investigation. There is a literature report on a similar structure synthesized by Murray et al. [5]. The structures of other compounds were established by comparing their spectroscopy data with those in the literature [6–9].

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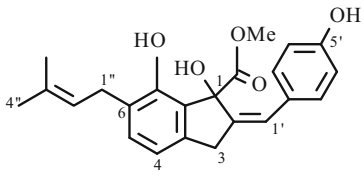
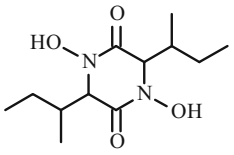
TABLE 1. NMR Data of Phomoindene A (**1**) (500 MHz, $CDCl_3$, δ, ppm, J/Hz)

C atom	δ_C (DEPT)	δ_H	HMBC	NOESY
1	86.1 (C)			
2	128.4 (C)			
3	38.6 (CH_2)	3.55 (d, J = 14.8) 3.49 (d, J = 14.8)	C-1, 2, 3a, 4, 5	H-1′
3a	124.4 (C)			
4	129.0 (CH)	6.59 (d, J = 8.0)	C-3, 5, 7	
5	131.1 (CH)	6.53 (d, J = 8.0)	C-3, 4, 6, 7	
6	126.6 (C)			
7	152.9 (C)			
7a	137.1 (C)			
8	169.4 (C)			
1′	114.9 (CH)	6.51 (s)	C-2, 3	H-3
2′	121.9 (C)			
3′/7′	129.5 (CH)	7.62 (d, J = 8.8)	C-2, 1′, 4′/6′, 5′	H-4′/6′
4′/6′	115.9 (CH)	6.92 (d, J = 8.8)	C-2′, 3′/7′, 5′	H-3′/7′
5′	156.7 (C)			
1″	28.9 (CH_2)	3.14 (d, J = 7.2)	C-5, 6, 7, 2″, 3″	H-2″, 5″
2″	121.4 (CH)	5.09 (t, J = 7.2)	C-1″, 4″, 5″	H-1″, 4″
3″	134.1 (C)			
4″	25.8 (CH_3)	1.68 (s)	C-2″, 3″, 5″	H-2″, 5″
5″	17.8 (CH_3)	1.63 (s)	C-2″, 3″, 4″	H-1″, 4″
8-OME	53.7 (CH_3)	3.78 (s)	C-8	



1



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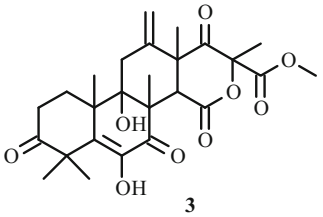

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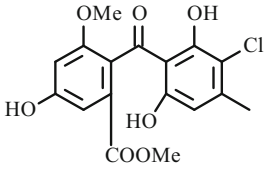

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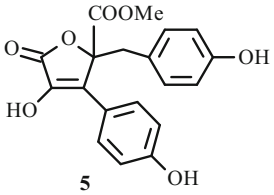

5

EXPERIMENTAL

Melting points were determined on an X-4 micro-melting point apparatus and were uncorrected. Optical rotations were measured on a SCHMIDT + HAENSCH POLARTRONIC HH W5 polarimeter and were uncorrected. IR spectra were measured on a Bruker VECTOR 22 spectrophotometer. ^1H and ^{13}C NMR data were recorded on a Varian Inova 500 MB NMR spectrometer operating at 500 and 125 MHz for ^1H and ^{13}C, respectively (TMS as internal standard). HR-EI-MS were measured on a Thermo MAT95XP high-resolution mass spectrometer and EI-MS on a Thermo DSQ EI-mass spectrometer. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd.) was used for column chromatography (CC).

Fungus Material and Culture Conditions. *Phomopsis* sp. (No. GX7-4A) was isolated from the mangrove sediment of BeiHai, GuangXi, China and deposited in the Department of Applied Chemistry, Zhongshan University, Guangzhou, GuangDong, China. Starter cultures were maintained on cornmeal seawater agar. Plugs of agar supporting mycelial growth were cut and transferred aseptically to a 500 mL Erlenmeyer flask containing 200 mL of liquid medium (glucose 10 g/L, peptone 2g/L, yeast extract 1 g/L, NaCl 30 g/L, pH 6.0). The flask was incubated at 28°C. After shaking on a rotary shaker for 3–5 days, the mycelium was aseptically transferred to a 1000 mL Erlenmeyer flask containing the culture liquid (400 mL). The flask was then incubated at 20° for 21 days. The cultures (200 L) were filtered through cheesecloth. The filtrate was concentrated

to 5 L below 50°C and extracted three times with an equal volume of ethyl acetate. The combined organic extracts were subjected to silica gel column chromatography, eluting with a gradient of petroleum ether to ethyl acetate to yield **1** (565 mg), **2** (41 mg), **3** (18 mg), **4** (3 mg), and **5** (156 mg).

1,7-Dihydroxy-2(*Z*)-(4-hydroxybenzylidene)-6-(3-methylbut-2-enyl)-indan-1-carboxylic Acid Methyl Ester (1). Pale wax; $[\alpha]_D^{20}$ –25.8° (*c* 0.31, MeOH); FT-IR (KBr, ν_{max}, cm^{-1}): 3363 (st), 2959 (m), 1738 (st), 1670 (st), 1610 (st), 1517 (st), 1438 (st), 1387 (st), 1261 (st), 1180 (st), 1065 (m), 1035 (m), 838 (m); for ^1H NMR, ^{13}C NMR and 2D NMR data ($CDCl_3$), see Table 1.

EI-MS *m/z* (rel. int.): 380 (23.7), 364 (2.8), 348 (34.5), 320 (4.4), 293 (13.9), 291 (18.3), 265 (12.2), 237 (13.7), 223 (12.0), 205 (21.5), 190 (13.9), 177 (32.8), 175 (100.0), 133 (29.0), 131 (59.4), 119 (25.3), 91 (40.6); HR-EI-MS $[M^+]$ *m/z*: 380.1619 (calcd for $C_{23}H_{24}O_5$, 380.1618).

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