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New Polyaromatic Metabolites from a Marine-Derived Fungus *Penicillium* sp.

Elin Julianti,[†] Jung-Ho Lee,[†] Lijuan Liao,[†] Wanki Park,[‡] Sunghyouk Park,[†] Dong-Chan Oh,[†] Ki-Bong Oh,*[,‡] and Jongheon Shin*[,†]

Natural Products Research Institute, College of Pharmacy, Seoul National University, San 56-1, Sillim, Gwanak, Seoul 151-742, Korea, and Department of Agricultural Biotechnology, College of Agriculture & Life Science, Seoul National University, San 56-1, Sillim, Gwanak, Seoul 151-921, Korea

shinj@snu.ac.kr; ohkibong@snu.ac.kr

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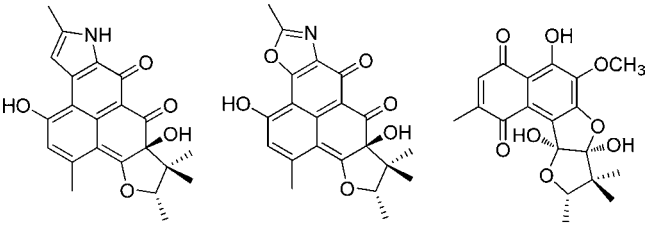
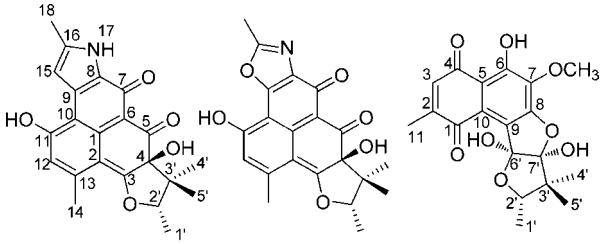
ABSTRACT



1 2 3

Herqueiazole (1), herqueioxazole (2), and herqueidiketal (3), polyaromatic metabolites with a novel skeletal class, were isolated from the marine-derived fungus *Penicillium* sp. Based on the combined spectroscopic analyses, the structures of 1 and 2 were determined to be the first examples of pyrrole- and oxazole-containing phenalenone compounds, respectively, whereas 3 possessed a novel skeleton with a highly oxidized naphthoquinone moiety. Compound 3 exhibited moderate cytotoxicity and significant inhibitory activity against sortase A.

Marine microorganisms are widely recognized as new frontiers in natural products research. In particular, the actinomycete bacteria and higher fungi from diverse marine environments are very prolific sources of structurally unique and biologically active metabolites.[1] Since the 1990s, more than a few hundred novel compounds have been annually isolated from these organisms, which significantly contributes to both the chemical and biomedical aspects of natural products research.[2] The bioactivities of these marine-derived microbial metabolites are highly diverse but with a focus on cytotoxic and antimicrobial activities. Among the antimicrobial-related bioactivities, the sortase enzymes are regarded as being a promising target for therapy because Gram-positive bacteria proteins are displayed on the cell surface with these enzymes.[3]



1 2 3

During the course of our search for bioactive compounds from marine fungi obtained from Korea, we collected a strain of the fungus *Penicillium* sp. (strain number F011) from a marine sediment whose crude extract exhibited moderate cytotoxicity toward the A549 cell-line (LC_{50} 28.7 μg/mL). In addition, the ESI-MS profile indicated the presence of secondary metabolites. The strain was subsequently cultivated on a large scale in liquid

[†] Natural Products Research Institute, College of Pharmacy.

[‡] Department of Agricultural Biotechnology, College of Agriculture & Life Science.

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media, and the bioactive constituents were isolated using diverse chromatographic methods. Herein, we report the structures and bioactivity of herqueiazole (**1**), herqueioxazole (**2**), and herqueidiketal (**3**), polyaromatic compounds of a new skeletal class.

Table 1. NMR Assignments for **1** and **2** (in DMSO-d_6, J in Hz)[a]

	1		**2**	
no.	δ_H	δ_C	δ_H	δ_C
1		140.0		138.6
2		116.3		116.4
3		167.1		172.3
4		77.1		77.8
5		194.1		193.1
6		95.2		93.0
7		182.7		180.6
8		134.4		130.1
9		117.3		154.2
10		111.8		112.9
11		166.4		166.0
12	6.65, s	116.4	6.87, s	117.1
13		148.4		149.7
14	2.58, s	23.3	2.69, s	23.3
15	6.33, s	101.9		
16		134.3		163.1
18	2.37, s	13.3	2.70, s	13.9
1′	1.43, d (6.5)	13.0	1.48, d (6.5)	12.8
2′	4.87, q (6.5)	88.9	5.04, q (6.5)	90.5
3′		46.2		46.1
4′	1.29, s	16.6	1.37, s	16.3
5′	0.78, s	16.4	0.88, s	16.1
4-OH	7.01, s		7.41, s	
11-OH	15.35, s		14.44, s	
17-NH	10.98, s			

[a] Data were measured at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR.

The molecular formula of compound **1** was deduced to be $C_{22}H_{21}NO_5$ based on HRFABMS analysis. Most signals in the ^{13}C NMR spectrum were located in the aromatic (δ 170−95) region, which, combined with the 13 degrees of unsaturation inherent in the molecular formula, revealed the polyaromatic nature of this compound (Table 1). The presence of several absorption maxima in the UV spectrum (209, 262, 276, and 436 nm) supported this interpretation. In addition, the IR absorption bands at 3382 (br) and 1728 cm^{-1} indicated the presence of hydroxy and carbonyl functionalities, respectively, whose corresponding carbon signals were observed at δ 77.1 and 194.1, respectively, in the ^{13}C NMR spectra. However, the ^{13}C NMR data showed additional signals in the region of δ 165−185, which suggested the potential for related functionalities, such as phenolic and polyunsaturated carbonyl groups, in this compound.

Based on this information, the planar structure of **1** was determined by a combination of diverse 2-D NMR experiments. In particular, the long-range carbon−proton correlations in the HMBC data, which was further extended by D-HMBC data, were crucial for the structural elucidation of this proton-deficient compound.[4] First, an allylic (J_{vs}) ^{1}H COSY coupling between the aromatic proton at δ 6.65 and the benzylic methyl proton at δ 2.58 revealed that these substituents were in an *ortho*-orientation. The HMBC correlations of these substituents and a hydroxy proton at δ 15.35 with neighboring carbons revealed the presence of a 1-hydroxy-3-methylbenzene-type moiety (Figure 1a). Because of the lack of an adjacent proton, the carbon on the opposite site (C-1) of the benzene and the other substituents (C-2 and C-10) remain undetermined. A ^{1}H COSY correlation between the aromatic proton at δ 6.33 and the methyl proton at δ 2.37 suggested the presence of another allylic- or benzylic-type relationship. The HMBC correlations of these substituents and an exchangeable proton at δ 10.98 with neighboring carbons revealed the presence of a five-carbon moiety. The chemical shifts of the four aromatic carbons (C-8 and C-16 $\approx \delta$ 135, C-9 and C-15 $< \delta$ 120) and the downfield shift of the exchangeable proton indicated that this moiety is indeed a 2-methylpyrrole. The attachment of this group at C-10 of the 1-hydroxy-3-methylbenzene was confirmed based on the long-range correlation between H-15 and C-10. Finally, the HMBC correlations of the three upfield methyl protons at δ 1.43, 1.29, and 0.78, a methine proton at δ 4.87, and a hydroxy proton at δ 7.01 with neighboring carbons revealed a partial structure that consisted of eight carbons (i.e., $CH_3CH(O)C(CH_3)_2C$-(OH)(C=O)C=). Although it was not directly determined from the HMBC data, consideration of the molecular formula revealed that the oxygen-bearing carbons at δ 167.1 and 88.9 were connected by an ether bridge. Therefore, the partial structure is a hydrofuran-type ring (C-3, C-4, C-2′, and C-3′) substituted by hydroxy and methyl groups (Figure 1a). The attachment of a carbonyl carbon (C-5) at the hydroxy-bearing C-4 was also determined via a three-bond correlation between the hydroxy proton and a carbon at δ 194.1.

The connectivity of these three partial structures as well as the construction of a whole planar structure accommodating the remaining three quaternary carbons at δ 182.7, 140.0, and 95.2 was accomplished by D-HMBC analysis performed with NMR parameters optimized with a 1 Hz carbon−proton coupling constant. First, the connection of the hydroxymethylbenzene with the pyrrole was determined by the HMBC correlation of H-15/C-10 and was further supported by the four-bond correlations at 11-OH/C-9 and H-15/C-11 in the D-HMBC data. The connection between the hydroxymethylbenzene and hydrofuran moiety was confirmed by the correlations at H-12/C-3 and 4-OH/C-2. The three quaternary carbons at δ 182.7, 140.0, and 95.2 were placed at C-7, C-1, and C-6, respectively, by the correlations at H-12/C-1, H-14/C-1, 11-OH/C-1, 4-OH/C-6, and H-15/C-7 (Figure 1c). Therefore, the planar structure of **1** was defined to be a pentacyclic compound in a new skeletal class. A literature study revealed that compound **1** is structurally similar to herqueinones

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Table 2. NMR Assignments for **3** (in $CDCl_3$, J in Hz)[a]

no.	δ_H	δ_C
1		188.0
2		149.4
3	6.75, q (1.5)	136.4
4		189.2
5		111.5
6		157.2
7		138.2
8		153.6
9		125.6
10		121.8
11	2.15, d (1.5)	16.6
1′	1.14, d (6.3)	14.1
2′	3.55, q (6.3)	80.8
3′		45.9
4′	1.09, s	16.2
5′	1.13, s	18.4
6′		117.2
7′		104.6
6-OH	12.90, s	
7-OMe	4.16, s	61.1
6′-OH	4.59, s	
7′-OH	8.17, s	

[a] Data were measured at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR.

from the fungus *Penicillium herquei* and *P. atrovenetum*.[5−8] Although these and structurally related phenalenone metabolites have been obtained from diverse fungal strains,[9−12,15−17] to the best of our knowledge, the presence of an additional three-carbon unit (C-15, C-16, and C-18) and a nitrogenous substituent culminating in the formation of a methylpyrrole is unprecedented.

Compound **1** possessed asymmetric carbon centers at C-4 and C-2′. The NOESY data showed cross-peaks at 4-OH/H-2′, 4-OH/H-5′, H-1′/H-4′, and H-2′/H-5′, which suggests the opposite orientation between the 4-OH and the C-1′ methyl group. Based on X-ray analysis from the stereochemical assignment of isoherqueinone,[8,13,14] which is a phenalenone metabolite that possesses the same

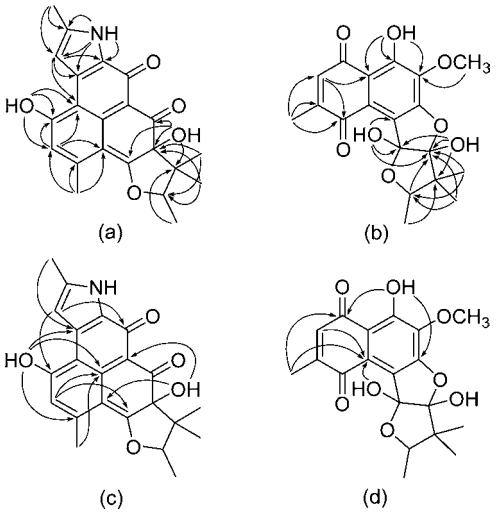

Figure 1. Selected HMBC correlations (J_{CH} = 8 Hz, a and b) and D-HMBC correlations (J_{CH} = 1 Hz, c and d) for **1** and **3**.

hydroxyfuran moiety, our NOESY data are consistent with 4*S** and 2′*S** relative configurations. Therefore, the structure of herqueiazole (**1**) was determined to be a pyrrole-containing phenalenone derivative.

Herqueioxazole (**2**) was isolated as a brown solid and was determined to be $C_{21}H_{20}NO_6$ based on HRFABMS analysis. The spectroscopic data of this compound were similar to those of **1**, which suggested that it possessed the same polyaromatic nature. However, a detailed examination of the NMR data revealed significant changes in the carbon and proton signals at the pyrrole and in the vicinity of **1**, with the disappearance of the C-15 methine, which was the most noticeable difference (Table 1). In conjunction with the appearance of an additional oxygen in the molecular formula, these changes were accommodated by the replacement of the pyrrole with an oxazole unit. This interpretation was confirmed through a combination of 2-D NMR analyses in which a crucial long-range correlation was found at H-18/C-8 in the HMBC data. The relative configurations of **2** were assigned as 4*S** and 2′*S**, identical to **1**, via NOESY analysis. Thus, the structure of herqueioxazole (**2**) was determined to be a polyaromatic oxazole, which is the first example of an oxazole-containing phenalenone compound related to the herqueinones.

The molecular formula of herqueidiketal (**3**) was deduced to be $C_{19}H_{20}O_8$ based on HRFABMS analysis. Although the spectroscopic data for this compound were reminiscent of those obtained for other phenalenone metabolites, the highly distinctive ^{13}C and ^{1}H NMR data indicated a requirement for an independent structural elucidation. The 12 carbon signals in the aromatic and carbonyl regions of the ^{13}C NMR spectrum (Table 2) coupled with the 10 degrees of unsaturation from the molecular formula indicated the presence of a naphthoquinone-type moiety (plus a double bond or ketal/acetal-type functionality), which was supported by the UV absorption maxima at 222, 280, and 438 nm and by IR

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absorption bands at 1729 and 1604 cm^{-1}. The similar chemical shifts of the carbonyl carbons at δ 189.2 and 188.0 suggested that the naphthoquinone is indeed a *para*-diketone. The presence of characteristic proton signals of the three upfield methyls at δ 1.14, 1.13, and 1.09 and a methine at δ 3.55 in the ^{1}H NMR spectrum indicated that **3** possessed a trimethylhydrofuran moiety similar to those of **1** and **2**.

The structure of **3** was determined by combined 2-D NMR analyses, which included HMBC and D-HMBC experiments (Figure 1b and 1d). The ^{1}H COSY data showed an allylic coupling (J = 1.5 Hz) between the olefinic proton at δ 6.75 and the methyl proton at δ 2.15. The long-range couplings of the latter proton with the neighboring carbons observed first in the HMBC and then expanded by the D-HMBC data revealed the presence of a benzoquinone moiety. The long-range couplings of the phenolic and methoxy protons at δ 12.90 and 4.16, respectively, with aromatic carbons allowed the placement of these substituents at an *ortho*-orientation on the benzene ring. The connection between the benzoquinone and benzene, as well as the presence of a naphthoquinone moiety, was determined by key long-range correlations at H-3/C-5, H-3/C-10, H-11/C-10, 6-OH/C-4, 6-OH/C-5, and 6-OH/C-8. Although the carbon at δ 125.6 failed to show any correlation with the protons of the naphthoquinone, an analysis of the partial structure and chemical shift confirmed its placement at C-9.

The aliphatic moiety of compound **3** was first investigated by the comparison of its ^{1}H and ^{13}C NMR data with those of **1** and **2**, which revealed the presence of a trimethylhydroxyfuran moiety similar to that of the congeners. However, the downfield shifts of the two quaternary carbons at δ 117.2 and 104.6, combined with two hydroxy protons at δ 8.17 and 4.59 in the NMR spectra, suggested the presence of an additional functionality. Several carbon−proton correlations in the HMBC and D-HMBC data suggested the substitution of the two hydroxyl groups at C-6′ and C-7′ of the trimethylhydrofuran moiety. The attachment of this moiety at C-9 of the naphthoquinone was determined based on the long-range correlations at 6′-OH/C-9 and 6′-OH/C-10 (Figure 1b and 1d). Although no direct evidence of the other linkage was observed in the NMR data, the downfield chemical shift of the C-8 carbon at δ 153.6 and the remaining 1 degree of unsaturation from the molecular formula placed an ether linkage between C-8 and C-7′. The presence of the two hemiketal groups at C-6′ and C-7′ was also firmly established.

Compound **3** possessed asymmetric centers at C-2′, C-6′, and C-7′, whose configurations were assigned by the NOESY analysis in which a series of cross-peaks at H-1′/6′-OH, H-1′/7′-OH, H-2′/H-5′, H-4′/6′-OH, H-4′/7′-OH, and H-5′/7-OMe assigned the 1′*S**, 6′*S**, and 7′*S** configurations. The NOESY data obtained in MeCN-d_3 also provided the identical result (Supporting Information). Therefore, the structure of herqueidiketal (**3**) was determined to be a highly oxidized naphthoquinone metabolite containing additional rings, which represents a novel carbon skeleton.

Phenalenone compounds exhibit diverse bioactivities, such as antimicrobial and anti-HIV activities, as well as the inhibition of DNA polymerase and human leukocyte elastase.[12,15−17] In our bioactivity measurements, **3** was moderately active against the lung carcinoma A549 cell line (LC_{50} 17.0 μM), whereas the others were only marginally active (67.3 and 176.7 μM for **1** and **2**, respectively; 3.3 μM for doxorubicin as a positive control). Compound **3** significantly inhibited (IC_{50} 23.6 μM, 116.2 μM for *para*-hydroxymercuribenzoic acid (pHMB) as a positive control) the action of *Staphylococcus aureus* sortase A (SrtA). Therefore, this compound is the first example of a naphthoquinone that exhibits this enzyme−inhibitory activity.

In summary, three novel natural products were isolated from a marine-derived fungus. The structures of **1** and **2** were determined to be the first examples of pyrrole- and oxazole-containing phenalenones, respectively. In addition, **3** possessed a novel skeleton with a highly oxidized naphthoquinone moiety. The bioactivity studies indicated that **3** exhibited moderate cytotoxicity and potent inhibitory activity against *S. aureus* SrtA. Because the sortase family of enzymes are not found in mammals, this compound has the potential to be a starting candidate for the design of new therapeutic agents.

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Supporting Information Available. Full experimental procedures, spectroscopic and analytical data, with ^{1}H, ^{13}C, and 2D NMR spectra of **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.