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Epoxyphomalin A and B, Prenylated Polyketides with Potent Cytotoxicity from the Marine-Derived Fungus *Phoma* sp.

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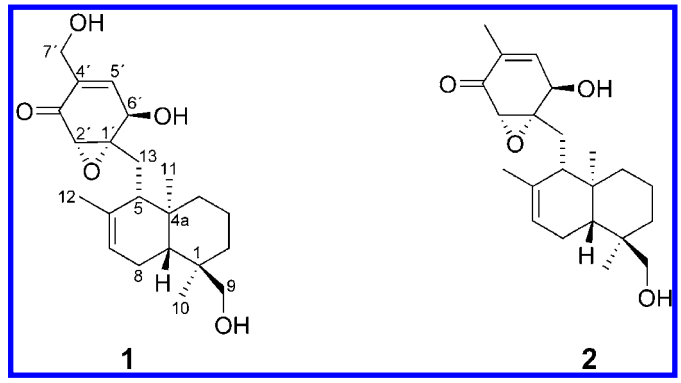
ABSTRACT



Chemical investigation of a strain of the marine-derived fungus *Phoma* sp. has led to the discovery of epoxyphomalin A (1) and B (2), two new prenylated polyketides with unusual structural features. Epoxyphomalin A (1) showed superior cytotoxicity at nanomolar concentrations toward 12 of a panel of 36 human tumor cell lines. In COMPARE analyses, the observed cytotoxic selectivity pattern of 1 did not correlate with those of reference anticancer agents with known mechanisms of action.

Cancer is a leading cause of death worldwide.[1] Natural products from plants and bacteria play an invaluable role in the treatment of tumors, and two-thirds of all anticancer drugs in clinical use are derived from these sources.[2] Secondary metabolites of fungal origin become increasingly recognized as an auspicious resource for new antitumoral agents.[3] Intriguingly, endophytic filamentous fungi were shown to produce the well-established anticancer drug taxol[4] and the drug lead compounds camptothecin[5] and podophyllotoxin,[6] whose biosynthesis was formerly solely ascribed to the respective host plants. The potential of fungal metabolites to become lead structures in the anticancer drug discovery process is apparent from the investigation and development

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Table 1. ^{1}H and ^{13}C NMR Spectral Data for Compounds **1** and **2** in d_6-Acetone (δ in ppm, J in Hz)

		1		**2**	
position	DEPT	δ_C	δ_H	δ_C	δ_H
1	qC	38.2		38.2	
2	CH_2	36.3	eq 1.24, m	36.4	eq 1.25, m
			ax 1.54, m		ax 1.53, m
3	CH_2	18.8	1.51, m	18.8	1.54, m
4	CH_2	39.6	ax 0.93, m	39.6	ax 0.93, m
			eq 1.79, m		eq 1.79, m
4a	qC	36.5		36.5	
5	CH	47.3	1.60, m	47.4	1.60, m
6	qC	135.4		135.4	
7	CH	122.9	5.38, br s	123.0	5.38, s
8	CH_2	24.1	1.88, m	24.2	1.91, m
8a	CH	43.6	1.63, m	43.7	1.64, m
9	CH_2	71.4	3.00, d (11.0)	71.4	3.00, dd (5.6, 10.7)
			3.31, d (11.0)		3.31, dd (5.6, 10.7)
10	CH_3	18.1	0.80, s	18.2	0.80, s
11	CH_3	14.7	0.83, s	14.7	0.83, s
12	CH_3	22.1	1.67, s	22.1	1.67, s
13	CH_2	26.2	2.10, dd (8.1, 16.1)	26.2	2.10, dd (8.3, 16.1)
			2.31, d (16.1)		2.30, d (16.1)
1′	qC	65.9		65.8	
2′	CH	57.5	3.25, d (1.1)	57.4	3.26, d (1.0)
3′	qC	195.6		196.1	
4′	qC	136.3		132.6	
5′	CH	140.2	6.73, dd (1.8, 5.1)	142.0	6.53, dd (1.8, 5.1)
6′	CH	67.1	4.58, br d (5.1)	67.3	4.47, dd (5.1, 8.5)
7′	CH_2/CH_3	59.0	4.14, d (15.0)	15.4	1.73, s
			4.26, d (15.0)		
6′-OH	OH		4.77, brs		4.66, d (8.5)
7′-OH	OH		not observed		
9-OH	OH		3.62, brs		3.55, t (5.6)

of derivatives of illudin S,[7] halimide,[8] and fumagillin[9] which advanced already into phase I and II clinical studies.

As part of our continued search for cytotoxic anticancer lead structures from marine-derived filamentous fungi, the facultative marine fungus *Phoma* sp. was obtained from the marine sponge *Ectyplasia perox*, collected from the Caribbean Sea, Dominica. The crude lipid extract of this fungal isolate was found to have strong cytotoxic properties toward six cancer cell lines. Bioassay-guided fractionation led to the isolation of two new and structurally most unusual compounds, named epoxyphomalin A (**1**) and B (**2**). This paper reports on the isolation, structure elucidation, and biological evaluation of the two new sesquiterpenoid compounds, of which compound **1** exhibited a remarkable high level of cytotoxic activity.

The fungus was cultivated on a solid biomalt medium with added artificial sea salt. Successive fractionation of the crude EtOAc extract of the fungal mycelium and medium by chromatography on silica and Sephadex LH-20 material, followed by reversed-phase HPLC, yielded compounds **1** and **2**.

The molecular formula of compound **1** was determined to be $C_{22}H_{32}O_5$ on the basis of HRESIMS measurements (*m*/*z* $[M + Na]^+$ 399.2138). The molecular formula implies seven degrees of unsaturation. The IR spectrum showed the presence of hydroxyl and carbonyl groups (3364, 1676 cm^{-1}), whereas the ^{13}C NMR spectrum (Table 1) disclosed 22 signals due to three methyl groups, seven sp^3 methylene units of which two are hydroxlated, two sp^2 methines, four sp^3 methines, and three sp^2 and three sp^3 quaternary carbons. The ^{1}H and ^{13}C NMR spectra (Table 1) indicated the presence of a carbonyl function (δ_C 195.6), two carbon−carbon double bonds (δ_C 136.3 (C), 140.2 (CH), 135.4 (C), 122.9 (CH)), three hydroxylated carbon atoms (δ_C 67.1, 71.4, 59.0), and the presence of an epoxide based on resonance signals at δ_C 65.9 (C) and 57.5 (CH), δ_H 3.25 (1H, d, $J = 1.1$). The two double bonds, the carbonyl group, and the epoxide moiety as deduced from NMR and IR spectra accounted for four degrees of unsaturation, and the remaining three degrees required the presence of three rings. Interpretation of the $^{1}H-^{1}H$ COSY, HMBC and HSQC spectra allowed the deduction of the planar structure of compound **1**. The $^{1}H-^{1}H$

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COSY spectrum suggested the presence of partial fragments A−D (Figure 1), which were extended and connected to the

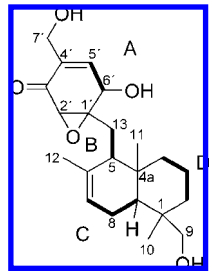

Figure 1. Fragments A−D deduced from ^{1}H−^{1}H COSY correlations for compound **1**.

skeletal framework using HMBC correlations. Long-range correlations from H-8a to C-9 and C-10, from H_3-10 to C-2 as well as C-9, and from H_2-9 to C-1 and C-2 established the connection between fragments C and D via C-1. HMBC cross peaks of H-5 to C-8a and C-6, from H_3-11 to C-4, C-4a, C-5, C-13, from H_3-12 to C-7, and from H_2-13 to C-6 evidenced the connection of substructure B to C and D via C-6 and C-4a, respectively. Accordingly, a decalin ring system was deduced as a partial structure of **1**. Several further significant HMBC correlations, e.g. of H-2′ to C-1′ and C-3′, H_2-7′ to C-3′, C-5′, and of H-6′ to C-1′, C-2′, C-4′, and C-5′, proved that fragment A and the carbons C-1′−C-4′ were part of a cyclohexenone ring, and the hydroxylated methylene C-7′ and the epoxide moiety were attached to this ring. Finally, an HMBC cross peak of H_2-13 to C-1′ showed the cyclohexenone ring to be connected to the decalin skeleton through C-13.

Diagnostic NOE correlations between the resonances of H_3-10 to H-2eq, H-4eq, and H_3-11 and NOEs from H-4ax to H-2ax, H-5, and H-8a were indicative of the relative configuration of the decalin portion of **1** as 1*R**, 4a*R**, 5*S**, 8a*R**.

The absolute configuration of the epoxydon moiety was established employing a combination of CD measurements and ^{1}H−^{1}H coupling constant analysis. Application of the empirical inverse octant rule[10] for cyclohexene oxides to the observed negative Cotton effect at λ 340 nm ($\Delta\varepsilon$ −5.31) determined the absolute configuration of the epoxide as 1′*S* and 2′*S*.[11] Knowing the absolute configuration at the epoxide ring, the stereochemistry at C-6′ was defined by comparison of ^{1}H−^{1}H coupling constants with partially reduced model epoxyquinones. Considering that epoxydons exist preferably in a boat conformation, in which the epoxide and the keto group possess an opposite relative orientation,[10,11] diagnostic short- and long-range couplings can be employed to determine whether a *cis*-oid or *trans*-oid relationship is given regarding the epoxide group and the C-6′-OH group. For *trans*-isomers ^{1}H−^{1}H short-range coupling constants $^3J_{5',6'}$ of 5 Hz and long-range W-coupling constants $^4J_{2',6'}$ of 1 Hz are observed, while in *cis*-isomers typically 2.5 and 0 Hz were measured, respectively.[12] Since the obtained coupling constants of **1** (Table 1) for $^3J_{5',6'}$ (5.1 Hz) and $^4J_{2',6'}$ (1.1 Hz) are consistent with a *trans*-oid relationship, the 6′*R* configuration is suggested for this compound, for which we propose the trivial name epoxyphomalin A.

From accurate mass measurement, compound **2** was found to have a molecular formula of $C_{22}H_{32}O_4$ (*m*/*z* [M + Na]$^+$ 383.2189). The spectroscopic data of **2** were very similar to those of **1** suggesting a related planar structure (Table 1). The major difference between the two sets concerned C-7′. The aforementioned difference can be explained by the presence of a methyl (δ_C 15.4) instead of the hydroxylated methylene group as found in compound **1**. Accordingly, the loss of 16 mass units supported the absence of one oxygen atom. ^{1}H−^{1}H COSY correlation of H-5′ (δ_H 6.53) to H_3-7′ (δ_H 1.73) further supported the above deduction.^{1}H−^{1}H COSY, HSQC, and HMBC experiments allowed the complete assignment for structure **2**.

The absolute configuration of the cyclohexenone part of compound **2** is similar to that in **1** since the obtained coupling constants, $^3J_{5',6'}$ showed values of 5.1 Hz and long-range constants $^4J_{2',6'}$ of 1 Hz, and since the CD curves of both compounds were identical (see Supporting Information). The relative configuration for the decalin part was also determined from the 2D NOESY correlations. NOEs between the resonances of H_3-10 and H_3-11 to H-4eq and from H-4ax to H-5 and H-8a were indicative of the relative configuration of the decalin portion of **2** to be the same as in **1**. For compound **2**, the trivial name epoxyphomalin B is proposed.

The cytotoxic effects of compounds **1** and **2** were investigated using a monolayer cell survival and proliferation assay in a panel of 36 human tumor cell lines, comprising 14 different solid tumor types. Epoxyphomalin A (**1**) and B (**2**) were found to be active exhibiting mean IC_{50} values of 0.11 and 1.25 μg/mL, respectively. Epoxyphomalin A (**1**) showed significant in vitro tumor cell selectivity toward 12 of the 36 tested tumor cell lines, which indicates 33% of selectivity (using an individual IC_{50} value <1/3 of the mean IC_{50} value as selectivity threshold; see Tables 2 and S2 in Supporting Information). IC_{50} values in these above average sensitive cell lines ranged from 0.010 μg/mL (breast cancer MAXF 401NL) to 0.038 μg/mL (adeno lung cancer LXFA 629 L) (Table 2). The observed cytotoxic selectivity pattern of epoxyphomalin A (**1**) did not correlate with those of any of the standard cytotoxic compounds with known mechanisms of action as deduced by COMPARE analyses, possibly indicating a multiple or an unknown mode of action. Epoxyphomalin B (**2**) exhibited 22% of selectivity, and IC_{50} values in the eight above average sensitive cell lines ranged from 0.251 μg/mL (pleuramesothelioma PXF 1752 L) to 0.402 μg/mL (bladder cancer BXF T24) (Table 2). In the COMPARE analysis, the cytotoxic selectivity pattern of epoxyphomalin B (**2**) correlates with the proteasome inhibitor tyropeptin A.

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Table 2. In Vitro Cytotoxic Activity of Epoxyphomalin A (**1**) and B (**2**) in Human Tumor Cell Lines

tumor type	cell line	(**1**) IC_{50} [μg/mL]	(**2**) IC_{50} [μg/mL]
bladder	BXF 1218 L	0.017	0.606
	BXF T24	0.374	0.402
glioblastoma	CNXF 498NL	0.022	0.904
	CNXF SF268	0.354	0.338
colon	CXF HCT116	0.329	2.245
	CXF HT29	0.198	0.593
stomach	GXF 251 L	0.034	11.420
head and neck	HNXF 536 L	0.247	0.326
lung	LXF 1121 L	0.381	1.584
	LXF 289 L	0.426	3.604
	LXF 526 L	0.430	10.000
	LXF 529 L	0.079	9.501
	LXF 629 L	0.038	1.920
	LXF H460	0.307	10.513
breast	MAXF 401NL	0.010	0.501
	MAXF MCF7	0.116	0.398
melanoma	MEXF 276 L	0.047	3.764
	MEXF 394NL	0.278	0.338
	MEXF 462NL	0.058	2.443
	MEXF 514 L	0.383	1.425
	MEXF 520 L	0.316	1.920
ovary	OVXF 1619 L	0.258	0.278
	OVXF 899 L	0.080	2.623
	OVXF OVCAR3	0.017	0.910
pancreas	PAXF 1657 L	0.027	1.481
	PAXF PANC1	0.330	0.523
prostate	PRXF 22RV1	0.034	0.589
	PRXF DU145	0.745	1.813
	PRXF LNCAP	0.937	0.774
	PRXF PC3M	0.017	0.546
mesothelioma	PXF 1752 L	0.033	0.251
kidney	RXF 1781 L	0.469	2.656
	RXF 393NL	0.080	1.098
	RXF 486 L	0.034	3.657
	RXF 944 L	0.316	0.380
uterus	UXF 1138 L	0.031	1.811
mean		0.114	1.249

Epoxyphomalin A and B are structurally related to a small family of sesquiterpene cyclohexenones formed by diverse analogues of macrophorin,[13] tauranin,[14] peyssonol A,[15] and hyatellaquinone.[15,16] Only the epoxyphomalins and macrophorins, however, are composed of a decalin ring system linked to an epoxydon moiety, and as such are unique chemical entities.

Epoxydon and its congeners are well-studied compounds, typically isolated from fungi of the genera *Penicillium*,[12] *Phoma*,[11a] *Panus*,[11b] *Apiospora*,[17] and *Phyllosticta*.[18] Their biosynthesis is of polyketidic nature and was established by feeding experiments.[17] The decalin system in **1** and **2** derives from isoprenoid precursors. In the case of the epoxyphomalins, it can be envisioned that the substructures are first connected to each other to give a farnesylated epoxycyclohexenone. A similar hypothetic intermediate was detected in the fungus *Aspergillus niger* and termed 22-deacetyl-yanuthone A.[19] Subsequent cyclization and hydroxylation of the isoprenoid moiety would yield epoxyphomalin A (**1**).

Intriguingly, the biosynthetically related compounds of the above-mentioned group of cyclohexenone sesquiterpenoids were obtained from phylogenetically diverse fungi[13,14] or occurred in unrelated genera of marine macroorganisms such as the red alga *Peyssonnelia* sp. and the marine sponge *Hyatella intestinalis*.[15] It is thus of interest to note that the fungus of this study was isolated from a marine sponge. Some of these compounds show interesting biological activity; e.g., 4′-oxomacrophorin A has been isolated as an immunosuppressant,[13d] while peyssonol A and B have been obtained from an extract active toward HIV reverse transcriptase.[15] Tauranin[20] and macrophorin A[13a] have been found to show cytotoxic activities.

While further experiments are in preparation to study the mode of action, our initial data suggest a potential for therapeutic development of an epoxyphomalin-derived anticancer drug. It is noteworthy that the structurally closely related compound **2** exhibits much weaker cytotoxic activity and that the compound (+)-epiepoxydon that we obtained in a former study had only weak cytotoxicity.[16] Moreover, (+)-epoxydon monoacetate, obtained during the same study,[16] was not cytotoxic. From these data, it can be concluded that the cancer cell toxicity is not due to the overall reactivity of the epoxide moiety but strongly dependent on the presence of the decalin ring system and on the appropriate stereochemistry of the epoxydon structural part.

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Supporting Information Available: Experimental procedures, NMR and CD spectra, and cytotoxicity data for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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