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NOTE

Cytotoxic Ophiobolins Produced by *Bipolaris* sp.

AHN, JONG-WOONG*, MI-KYOUNG LEE, SANG-UN CHOI, CHONG-OCK LEE, AND BYUNG-SUP KIM[1]

Korea Research Institute of Chemical Technology, P.O. Box 107, Yusong, Taejon 305-600, Korea
[1]Department of Horticulture, Kangnung National University, Kangnung 201-702, Korea

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Abstract 6-Epiophiobolin A, 3-anhydro-6-epiophiobolin A, and ophiobolin I were isolated from culture broth of *Bipolaris* sp. as cytotoxic agents against human tumor cells. Both 6-epiophiobolin A and 3-anhydro derivatives demonstrated significant cytotoxicity, having IC_{50} values in the range of 1 μg/ml, which indicates that they have comparable cytotoxic potential with that of etoposide. The activity of ophiobolin I was, however, very weak compared with those of 6-epiophiobolin A and etoposide.

Key words: *Bipolaris* sp., cytotoxic, human tumor cells, ophiobolins

As part of our continuing search for novel antitumor agents from fungal metabolites, a strain NDGP-E classified as *Bipolaris* sp. was selected for study since the culture broth of the strain was found to exhibit significant cytotoxicity against various human tumor cell lines.

Cytotoxicity-guided chromatographic fractionation led to the isolation of three active principles, 6-epiophiobolin A(**1**), 3-anhydro-6-epiophiobolin A(**2**), and ophiobolin I(**3**) (Fig. 2). While the compounds **1**, **2**, and **3** had been isolated as phytotoxins from the same genus of fungus [3, 12], we rediscovered these compounds in the course of our antitumor screening. Here, we report that the three compounds (**1-3**) were isolated as antitumor substances showing cytotoxicity against human tumor cell lines *in vitro*.

The producing strain NDPG-E was isolated from the Poaceae weed, *Leersia japonica*, and identified as a *Bipolaris* sp. based on its cultural and morphological properties [1, 2]. The strain was cultivated in a Difco potato dextrose broth. Batch cultures of 100 ml in 500-ml Erlenmeyer flasks were incubated at 26°C on a rotary shaker at 150 rpm for 7 days.

*Corresponding author
Phone: 82-42-860-7164; Fax: 82-42-860-7160;
E-mail: jwahn@pado.krict.re.kr

The isolation of **1**, **2**, and **3** was carried out using a bioassay guided isolation scheme, monitoring the cytotoxic activity against human lung cancer cells (A-549). The culture supernatant obtained from the culture broth (10 liters) was stirred for several hours with the adsorber resin Amberlite XAD-16. The active principles could be eluted from the resin with methanol and were

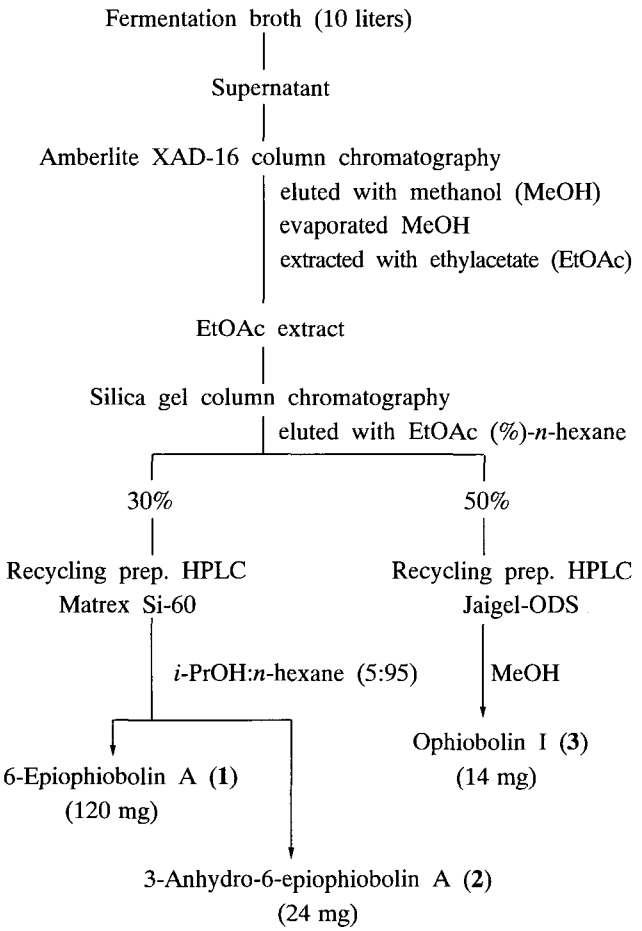
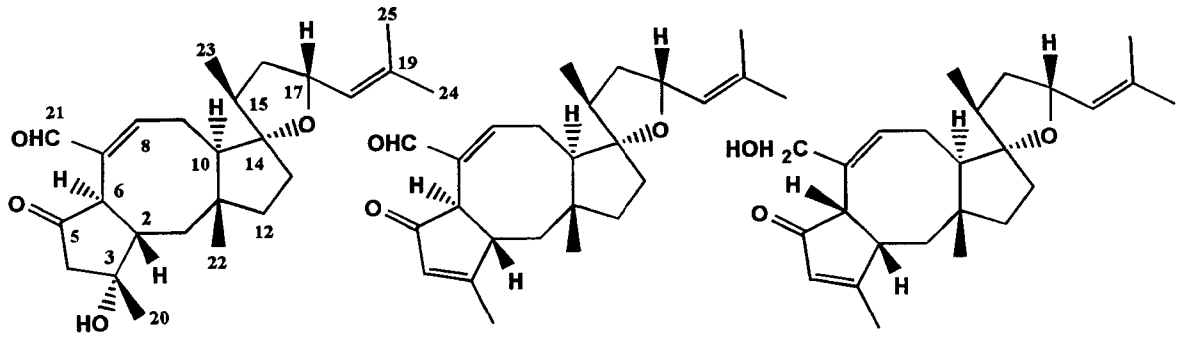


Fig. 1. Isolation and purification procedure for the ophiobolins.



1 **2** **3**

Fig. 2. Chemical structures of active compounds **1–3**.

purified by column chromatography on silica gel. Final purification using recycling preparative HPLC gave pure **1** (120 mg), **2** (24 mg), and **3** (14 mg). The isolation scheme for these compounds is shown in Fig. 1.

The compounds **1-3** were isolated as colorless oil. The molecular formulas of the compounds **1**, **2**, and **3** were established to be $C_{25}H_{36}O_4$, $C_{25}H_{34}O_3$, and $C_{25}H_{36}O_3$, respectively, by HREI-MS and ^{13}C NMR. These compounds are soluble in methanol, acetone, ethyl acetate, and chloroform, while insoluble in n-hexane and water. The R_f values of the compounds **1-3** on silica gel TLC developed with benzene-acetone (4:1) were 0.46, 0.51 and 0.31, respectively. Comparison of the three compounds by UV, IR, NMR, and MS showed that they were structurally closely related. All three compounds turned out to be known ones by comparison of their spectra with literature data [3, 12]. A set of mass fragmentation ions characteristic of the ophiobolin A ring nucleus, namely, m/z 273, 176, and 165 were observed in the MS spectra of **1** and **2**. As summarized in Table 1, the ^1H NMR spectrum of **3** showed a doublet at δ 3.48 ($J = 2.9$ Hz) assigned for H-6 and a double doublet at δ 2.59 ($J = 2.9$ and 14.5 Hz) for H-2 and these were consistent with *cis* ring fusion.

The activity of the three compounds and etoposide (reference) against five human tumor cell lines is listed in Table 2. Except for **3**, they exhibited significant cytotoxic activity, having IC_{50} values in the range of 1 μg/ml, which indicates that compounds **1** and **2** have similar cytotoxic potential to that of the reference etoposide. Since there is no significant difference in the activity of **1** and its dehydration product (**2**), 3-OH of **1**

Table 1. ^1H NMR spectral data for compounds **1**, **2**, and **3**[a].

Position	1[b]	2[b]	3[c]
2	2.09 ddd (4.6, 10.5, 12.1)	2.60 m	2.59 dd (2.9, 14.5)
4a	2.39 brd (16.6)	6.02 s	5.74 s
4b	3.03 d (16.6)		
6	3.33 d (10.9)	3.39 d (4)	3.48 d (2.9)
8	6.89 dd (2, 7.1)	6.80 ss (2.3, 6.5)	5.61 d (4.3)
9a	2.33 ddd (7.1, 13.5, 19.2)	2.31 ddd (6.6, 14.1, 19.3)	
9b	2.76 ddd (2, 4.2, 19.2)	2.81 ddd (2.3, 3.8, 19.3)	1.70 m
10	2.58 dd (4.2, 13.5)	2.63 dd (3.8, 14.1)	1.26 m
15	2.19 dd (6.9, 13.7)	2.19 dd (6.9, 13.7)	1.98 dd (6.9, 14.4)
17	4.59 dd (7.3, 15.5)	4.57 dd (7.1, 15.6)	4.65 dd (7.2, 15)
18	5.11 d (8.7)	5.10 d (8.6)	5.37 d (8.7)
20-CH_3	1.39 s	2.03 s	1.40 s
21a	9.19 s	9.29 s	4.45 brt (10)
21b			3.98 d (13.7)
22-CH_3	0.83 s	0.85 s	0.81 s
23-CH_3	1.02 d (7.1)	1.01 s (6.9)	0.81 d (6.9)
24-CH_3	1.67 s	1.63 s	1.55 d (1.2)*
25-CH_3	1.72 s	1.68 s	1.60 d (1.2)*

[a] ^1H chemical shift values (δppm) followed by multiplicity and then the coupling constant (*J*/Hz) in parentheses.
[b] In $CDCl_3$.
[c] In C_6D_6.
*These values may be interchanged.

Table 2. Cytotoxicities of compounds **1–3** and etoposide (reference).

Compounds	IC_{50} (μg/ml)				
	A-549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	1.6	1.6	0.9	1.6	1.6
2	1.9	1.8	1.6	1.8	1.7
3	>10	>10	>10	>10	>10
Etoposide	2.1	1.1	1.0	0.7	2.5

A-549: Human lung adenocarcinoma. SK-OV-3: Human ovarian adenocarcinama. SK-MEL-2: Human malignant melanoma. XF498: Human CNS carcinoma. HCT15: Human colon adenocarcinoma. The cytotoxic activities against the above human tumour cell lines were determined colorimetrically at 520 nm after staining viable cells with 0.4% SRB (sulforhodamine B) solution [9, 11].

seemed to have little effect on the cytotoxicity. **3** was less active.

Although many kinds of ophiobolin derivatives are known as phytotoxins [3, 4, 12], β-glucan formation inhibitors [6], nematocides [10, 13], calmodulin antagonists [5, 8], and antifungal antimicrobial agents [7], the antitumor activities of these compounds have never been reported so far. It is, therefore, interesting to investigate the cytotoxic activities against human tumor cells of ophiobolin derivatives.

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