SHORT REPORT

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A New Dibenzofuran from the Barks of *Sorbus commixta*

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Abstract: A new dibenzofuran derivative, 1,2,4-trimethoxydibenzofuran-3,9-diol **(7)**, was isolated from the ethyl acetate fraction of *Sorbus commixta* barks, along with six known compounds, lupeol **(1)**, betulin **(2)**, betulinic acid **(3)**, ursolic acid **(4)**, *β*-sitosterol **(5)** and *β*-pyrufuran **(6)**. Their structures were determined by NMR spectroscopic analysis, including of ^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC and HMBC spectra data. Cytotoxic activities of seven compounds were evaluated in five cancer cell lines, HEp-2, A549, MCF-7, PC-3 and SKOV-3 at the concentrations ranging from 10 to 100 μM.

Keywords: *Sorbus commixta;* triterpenes; dibenzofuran; 1,2,4-trimethoxydibenzofuran-3,9-diol; cytotoxicity.

1. Plant Source

The barks of *S. commixta* was collected from Gamjeong-ri, Dong-Myeon, Chuncheon, Kangwon Province, Korea, in August 2015. It was identified by Prof. Young Soo Bae, College of Forest and Environmental Sciences, Kangwon National University, and has been stored in the Herbarium of College of Pharmacy, Kangwon National University (KNUP-SC-1).

2. Previous Studies

Sorbus commixta Hedl (Rosaceae) is mainly distributed in Korea and China. It has used for the treatment of neuralgia, cough and expectorant in folk remedies [1-3]. Until now, the study on the *S. commixta* has been mainly focused on the berries of *S. commixta*, and their biological activities, anti-inflammatory and antioxidant effect, have been studied [4-6]. The anticancer activity of the bars of *S. commixta* has been previously reported, [7] but whether single compounds have the anti-proliferation activity against cancer cells have not reported yet. Previous research on the plant had reported the isolation of sorbic acid, chalcone glucoside, prunasin, amygdalin and triterpenoids such as lupenone

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and lupeol[4,8-9]. Recently, the methanol extract from the barks of *S. commixta* was shown to have antioxidant, anti-inflammatory and the potent radical scavenging activity [4,5].

3. Present Study

In the present study, the ethyl acetate fraction of the barks of *S. commixta* showed the cytotoxic activity against the cancer cells. Further, we isolated seven compounds (**1**-**7**) from the barks of *S. commixta* and evaluated their cytotoxic activities against five cancer cell lines, HEp-2, A549, MCF-7, PC-3 and SKOV-3.

Extraction and isolation: The dried barks of *S. commixta* (2.3 kg) were extracted three times with 80% methanol for 3h. The extract was concentrated under reduced pressure to obtained total extract (183.5g), which was then suspended in water and sequentially partitioned with *n*-hexane, EtOAc and *n*-butanol to give four fractions. The EtOAc fraction (46.6 g) was subjected to silica gel C.C eluting with gradient of *n*-hexane/EtOAc and $CHCl_3$/MeOH to afford ten fractions (Fr. 1 - 10). Among these, Fr. 4, 5 and 6 were used to obtain compounds **1-7**.

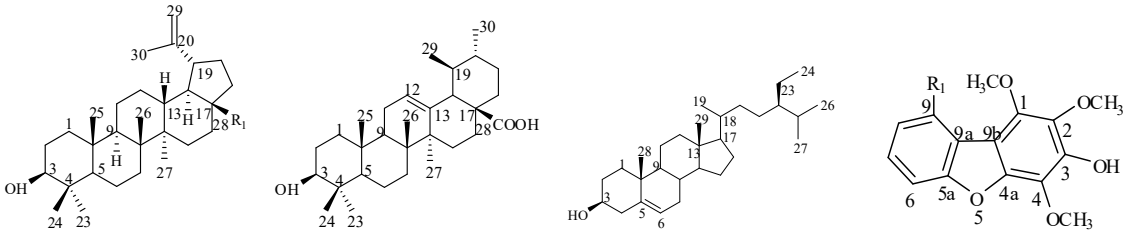
Fr. 4 and 5 were subjected to normal phase silica gel C.C eluting with *n*-hexane/EtOAc (5:1 to 0:100) to give four and six sub-fraction, respectively (Fr. 4.1 - 4.4 and Fr 5.1 – 5.6). The sub-fraction Fr. 4.3 was re-crystallized with MeOH to yield compound **1** (5.8 mg). The sub-fraction Fr.5.6 was purified by C_{18} HPLC (MeOH/H_2O, 3:2) to yield compound **5** (4.4 mg). Fr. 6 was subjected to reverse phase silica gel C.C eluting with MeOH/H_2O (9.5:0.5, isocratic) to give four sub-fractions (Fr. 6.1 - 6.4). The sub-fraction Fr. 6.1 was purified by C_{18} HPLC (MeOH/H_2O, 3.75:1.25) to yield compound **6** (3.1 mg) and compound **7** (5.4 mg). Compound **3** (4.0mg) was isolated from the sub-fraction Fr. 6.2 by recrystallization in MeOH. The residual solution of the sub-fraction Fr. 6.2 was purified by C_{18} HPLC (MeOH/H_2O, 3:2) to yield compound **2** (1.9 mg). Compound **4** (1.6 mg) was isolated the sub-fraction Fr. 6.4 by C_{18} HPLC (MeOH/H_2O, 3.5:1.5).

Seven compounds were isolated from the EtOAc fraction using various chromatographic techniques. Compounds were identified as lupeol **(1)**[10], betulin **(2)**[11], betulinic acid **(3)**[11], ursolic acid **(4)** [12], β-sitosterol **(5)**[10], β-pyrufuran **(6)**[13] and 1,2,4-trimethoxydibenzofuran-3,9-diol **(7)**, respectively. The compound **7** isolated for the first time from nature. Their structures were determined by NMR spectroscopic analysis, including ^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC, and HMBC spectra data, which were acquired in the Central Laboratory of Kangwon National University (Figure 1).

1,2,4-trimethoxydibenzofuran-3,9-diol **(7)** : yellowish syrup; UV (MeOH): λ_{max} (log ε), 301 (3.97), 280 (3.88), 285 (3.83); HRESIMS *m/z* 289.0708 $[M-H]^-$ (calcd. for *m/z* 289.0712 for $C_{15}H_{14}O_6$); ^{1}H and ^{13}C NMR data see Table 1 and supporting information.

Cytotoxicity: HEp-2 cells were cultured in Dulbecco's modified Eagle's media (DMEM) and MCF-7, A549, PC-3 and SKOV3 cells were cultured in Roswell Park Memorial Institute medium (RPMI) supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin and 100 μg/mL streptomycin respectively. The cells were maintained at 37°C in a humidified atmosphere at air (95%) and CO_2 (5%). Cell cytotoxicity was measured by MTT assay.

The ^{1}H NMR spectrum of **7** exhibited three methoxyl signals at δ 4.18 (3H, *s*, OCH_3-1), 4.16 (3H, *s*, OCH_3-4) and 3.97 (3H, *s*, OCH_3-2), and two hydroxyl signals at δ 8.61 (1H, *s*, OH-9) and 5.91 (1H, *s*, OH-3). The ^{13}C NMR spectrum displayed twelve down-fielded carbons at around δ 109.4 – 150.9 deduced the presence of two aromatic groups, and three up-fielded carbons at δ 62.4 (OCH_3-4), 61.4 (OCH_3-2) and 61.2 (OCH_3-1). The HSQC spectrum confirmed that three aromatic proton existed through the correlation between δ^H 7.24 (1H, *d*, *J* = 8.1 Hz, H-7) and δ^C 127.8 (C-7), δ^H 7.00 (1H, *d*, *J* = 8.1 Hz, H-6) and δ^C 109.3 (C-8), and δ^H 6.79 (1H, *d*, *J* = 8.1 Hz, H-8) and δ^C 102.9 (C-6). (Table 1).



1 $R_1 = CH_3$
2 $R_1 = CH_2OH$
3 $R_1 = COOH$

4

5

6 $R_1 = H$
7 $R_1 = OH$

Figure 1. Structures of compounds **1-7** isolated from the barks of *S. commixta*

Table 1. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra data of compounds **6** and **7** in $CDCl_3$ (δ in ppm, J in Hz).

Compound	**6**		**7**	
Position	δ_H	δ_C	δ_H	δ_C
1		128.8		129.4
2		136.6		135.8
3		141.1		141.3
4		142.7		139.3
4a		143.0		142.6
5a		155.9		156.9
6	7.50 (1H, m)	111.2	7.00 (1H, *d, J*=8.2)	102.9
7	7.35 (1H, m)	125.7	7.24 (1H, *m*)	127.8
8	7.29 (1H, m)	123.0	6.79 (1H, *d, J*=8.1)	109.3
9		121.9		150.9
9a		123.5		111.7
9b		114.6		109.4
OCH_3-1	4.05 (3H, s)	60.9	4.18 (3H, *s*)	61.2
OCH_3-2	4.18 (3H, s)	61.2	3.97 (3H, *s*)	61.4
OCH_3-4	3.99 (3H, s)	61.5	4.16 (3H, *s*)	62.4

The ^1H and ^{13}C NMR spectroscopic data of compound **7** showed a quite similar pattern with **6**, β-pyrufuran, except for the signal corresponding to a hydroxyl group. In the HMBC spectra, δ 8.61 (1H, *s*, OH-9) showing the strong hydrogen signal of the hydroxyl moiety was strongly correlated with δ 111.7 (C-9a), which described that the hydroxyl moiety was located at δ 150.9 (C-9), not 102.9 (C-6) (Figure 2 and See Supporting file). Also, the correlation signals of δ 8.61 (1H, *s*, OH-9) with δ 150.9 (C-9) and 109.3 (C-8) proved the presence of the hydroxyl moiety of δ 150.9 (C-9).

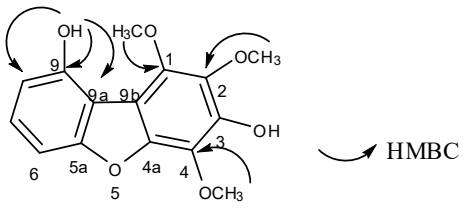


Figure 2. Chemical structure and HMBC correlations of compound **7**

Three concentrations (1, 50 and 100μM) of seven compounds and the positive control, docetaxel, were measured to confirm cytotoxicity in five cancer cell lines (HEp-2, MCF-7, A549, PC-3 and SKOV-3). Compounds **2**, **3** and **4** showed significant cytotoxicity in HEp-2 and A549 at a concentration of 100μM, compared to the positive control, docetaxel (Table 2). But, compounds **1**, **5**, **6** and **7** showed no or weak activities against five cancer cell (data not shown).

Table 2. The cytotoxic effects of compounds **2-4** against five cancer cell lines.

	HEp-2	A549	MCF-7	PC-3	SKOV-3
2	43.5±2.32***	38.0±3.26***	-12.4±4.74	19.2±7.68	6.1±4.92
3	37.2±3.08***	41.0±4.75***	23.3±22.76	20.4±5.91	15.8±16.86
4	84.8±0.23***	80.8±0.31***	80.8±0.22***	76.6±3.89***	77.1±0.36***
docetaxel	83.6±1.69***	64.8±3.52***	-	88.4±3.73***	77.6±0.77***

Analysis of cell cytotoxicity of 100μM Compounds 2-4 using the MTT assay in cancer cell liens at 4 hours post-UV at 590 nm irradiation. *Columns*, mean of three experiments; SD. *, p<0.05; **, p<0.01; ***, p<0.001 for control (untreated) versus treated.

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Supporting Information

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