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Cytotoxic Constituents of *Sorbaria sorbifolia* var. *stellipila*

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The acivity-guided fractionation upon the MeOH extract of the aerial parts of *Sorbaria sorbifolia* var. *stellipila* led to the isolation of two cucurbitacin-compounds, cucurbitacin D and cucurbitacin F, as active principles. Two compounds were shown to exhibit significant cytotoxicity against cultured human tumor cell lines, A-549, SK-OV-3, SK-MEL-2, XF-498, and HCT 15.

Key words : *Sorbaria sorbifolia* var. *stellipila*, Cucurbitacin D, Cucurbitacin F, Cytotoxicity

INTRODUCTION

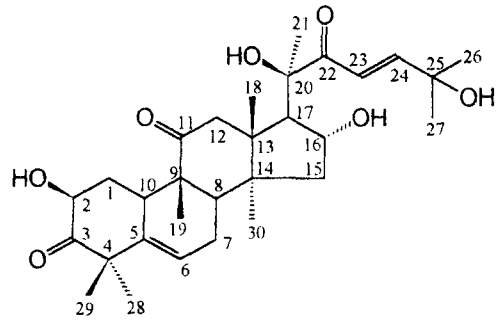
Earlier investigations on the chemical constituents of *S. sorbifolia* var. *stellipila* MAX. (Rosaceae) mainly dealt with the isolation of flavonoids such as quercetin-3-β-D-galactopyranoside (Zaitsev *et al.*, 1969), scutellarein-rhamnoside (Plouvier, 1969, Arisawa *et al.*, 1969), and sorbifolin (Munehisa *et al.*, 1970).

In a continuing search for plant-derived cytotoxic compounds, we found that the MeOH extract obtained from the aerial parts of *Sorbaria sorbifolia var. stellipila* exhibited significant cytotoxicity against A-549, SK-OV-3, SK-MEL-2, XF-498, and HCT-15. And the cytotoxicity was mainly concentrated in the CH_2Cl_2 soluble fraction. Activity-guided fractionation on the basis of the inhibitory activity upon the growth of tumor cells, *in vitro*, and repeated column chromatography afforded two cytotoxic compounds, which were characterized to be cucurbitacin D and F (Fig. 1). The compounds were isolated from this plant for the first time. In this paper, we report the isolation and structural elucidation of the compounds, and their cytotoxic activities against some cancer cell lines.

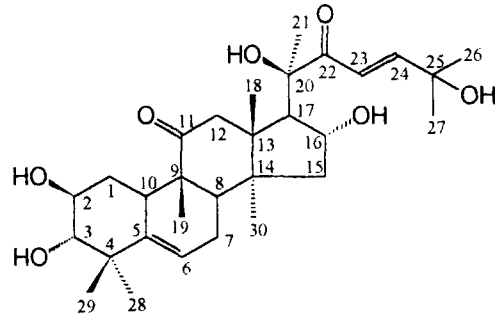
MATERIALS AND METHODS

Instruments and test for the cytotoxicity *in vitro*

Melting points were obtained on Gallenkamp melting pointing apparatus (uncorr.). ^{1}H-NMR spectra were run at 200 MHz and ^{13}C-NMR at 50 MHz and recorded by Brucker AC-200. The EI/MS (70 eV) were determined on a VG-VSEQ. The UV spectra were recorded on Shimadzu UV 240 UV-Visible recording spectrophotometer. And all experimental procedures, test for the cytotoxicity *in vitro*, were followed up the



Compound I (Cucurbitacin D)



Compound II (Cucurbitacin F)

Fig. 1. Compounds isolated from *Sorbaria sorbifolia* var. *stellipila*.

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Table I. ^{13}C-NMR chemical shifts of cucurbitacins

C	I*	II**	II***
1	36.0	34.8	33.6
2	71.6	71.7	69.4
3	212.3	81.9	79.9
4	50.2	43.3	41.9
5	140.4	142.7	141.8
6	120.3	121.2	120.1
7	23.8	24.7	23.5
8	42.3	44.3	42.3
9	48.3	49.7	47.4
10	33.7	34.9	32.9
11	213.0	216.0	213.0
12	48.6	49.9	48.5
13	48.2	49.4	47.7
14	50.7	51.9	50.1
15	45.5	46.6	45.6
16	71.4	71.6	69.3
17	57.2	59.4	58.8
18	20.0	20.6	19.8
19	19.2	19.8	18.4
20	78.0	79.9	78.5
21	24.0	25.4	24.9
22	202.4	204.9	204.0
23	118.9	120.0	117.5
24	155.9	155.3	153.6
25	71.1	71.5	69.2
26#	28.7	29.2	29.2
27#	29.5	29.4	29.4
28	21.2	22.3	21.9
29	29.3	25.4	24.9
30	20.0	20.7	20.0

*$CDCl_3$ solutions containing TMS as standard.
**CD_3OD solutions.
***DMSO-d_6 solutions containing TMS as standard.
#Signals corresponding to C-26 and C-27 of each compounds may be interchanged.

NCI's protocol based on the SRB method (Skehan *et al.*, 1990)

Extraction and Isolation

The aerial parts of *S. sorbifolia* var. *stellipila* (Rosaceae), which was collected May of 1995 at Odaesan, Kangwondo, Korea. A voucher speciman is deposited in the herbarium of college of Pharmacy, SungKyunKwan University. The crude material 10 Kg was extracted with MeOH for 5 hours at below 50°C (×3). The resultant MeOH extract was subjected to evaporation and suspended in water, followed by the successive solvent partition to give CH_2Cl_2 soluble fraction (63 g), EtOAc soluble fraction (10 g), *n*-BuOH soluble fraction (50 g) and of water soluble fraction (220 g) respectively. And each fraction was examined for the cytotoxicity *in vitro*, and it was found that the CH_2Cl_2 soluble fraction exhibited significant cytotoxic activity against some cell lines.

The CH_2Cl_2 soluble fraction was applied over silica gel column using gradient solvent system of *n*-hexane: EtOAc:MeOH (3:1:0 → 5:5:2) as eluents to give eight sub-fractions, whose fifth, sixth and seventh one was chromatographed with silica gel column eluting with chloroform:EtOAc (1:5) to afford two kinds of active compounde, 20 mg of compound I, 90 mg of compound II.

Table II. The cytotoxicity of compound I and II on some cancer cell lines

Compound	ED_{50}(μg/ml)*				
	A-549	SK-OV-3	SK-MEL-2	XF-498	HCT-15
I	6.6×10^{-2}	7.4×10^{-2}	3.1×10^{-2}	5.8×10^{-2}	5.8×10^{-2}
II	7.3×10^{-1}	6.6×10^{-1}	1.8×10^{-1}	6.5×10^{-1}	8.7×10^{-1}

*ED_{50} value of compounds against each cancer cell line, which was defined as a concentration (μg/ml) that caused 50% inhibition of cell growth *in vitro*.

Compound I (cucurbitacin D). colorless needle in MeOH, mp. 153-156°C, UV (λ_{max}):229 nm (MeOH), positive FABMS: *m/z* 539 $[M+Na]^+$, MS:m/z (rel. int.); 498 (M^+-H_2O, 12), 385 (13), 112 (19), 96 (100). ^{1}H-NMR ($CDCl_3$, δ):7.15 (1H, d, *J*=15.2 Hz, 24-H), 6.57 (1H, d, *J*=15.2 Hz, 23-H), 5.79 (1H, m, 6-H), 4.32 (1H, m, 2-H), 4.12 (1H, m, 16-H), 3.33 (1H, d, *J*=14.7 Hz, 12α-H), 2.78 (1H, m, 10-H), 2.62 (1H, d, *J*=14.7 Hz, 12β-H), 2.47 (1H, d, *J*=6.8 Hz, 17-H), 1.35 (12H, s, -$\underline{CH_3}$), 1.30, 1.31, 1.14, 0.98 (each 3H, s, -$\underline{CH_3}$), ^{13}C-NMR:Table I

Compound II (cucurbitacin F). colorless crystal in MeOH, mp. 247-249°C, UV (λ_{max}):230 nm (MeOH), positive FABMS: *m/z* 541 $[M+Na]^+$, MS:*m/z* (rel. int.) ; 500 (M^+-H_2O, 3), 387 (6), 112 (22), 96 (100). ^{1}H-NMR (CD_3OD, δ):6.97 (1H, d, *J*=15.4 Hz, 24-H), 6. 85 (1H, d, *J*=15.4 Hz, 23-H), 5.73 (1H, m, 6-H), 4.45 (1H, m, 2-H), 3.53 (1H, m, 16-H), 3.31 (1H, d, *J*=14.6 Hz, 12α-H), 2.84 (1H, d, *J*=9.3 Hz, 3-H), 2.58 (1H, m, 10-H), 2.47 (1H, d, *J*=14.7 Hz, 12β-H), 2.35 (1H, d, *J*=6.8 Hz, 17-H), 1.37, 1.31, 1.29, 1.19, 1.18, 1.10, 0.95, 0.94 (each 3H, s, -$\underline{CH_3}$), ^{13}C-NMR:Table II

RESULTS AND DISCUSSION

The MeOH extract of the aerial part of *S. sorbifolia* var. *stellipila* yielded two kinds of active compounds, which was traced according to the activity toward the growth of cultured human tumor cells. Two active principles comprised of common tetracyclic triterpene skeleton, called cucurbitacin, and each isolates was identified as cucurbitacin D (compound I) and cucurbitacin F (compound II), respectively (Fig. I), by the comparison of the physicochemical and spectral data of them with those of reported ones (Vincent *et al.*, 1983, Ryu *et al.*, 1994, Chun-tao, C., *et al.*, 1985)

Combined anaysis of FABMS and ^{1}H-, ^{13}C-NMR (DEPT) spectra of compound I indicated the molecular formular $C_{30}H_{44}O_7$, suggesting the double bond equivalents 9. ^{13}C-NMR spectrum showed the three

carbonyl" (δ 213.0, 212.3, 202,4 ppm) and four sp^2 carbon signals (δ 140.4, 120,3, 118.9, 155.9 ppm), hence the remaining 4 double bond equivalents should be tetracyclic. In the ^{1}H-NMR spectrum (200 MHz, $CDCl_3$), the signals due to three of olefinic (δ 7.15, 6.57, 5.79), eight methyls [1.35 (12H, s, -CH_3), 1.30, 1.31, 1.14, 0.98 (each 3H, s, -CH_3)] and two methines bearing oxygen (δ 4.32, 4.12) protons were observed. In the ^{13}C-NMR spectrum (50 MHz, $CDCl_3$), thirty carbon signals were observed, among which the presence of eight methyls (δ 29.5, 29.3, 28.7, 24.0, 21.2, 20.0, 20.0, 19.2) was comfirmed by their chemical shifts and DEPT experiment. The above results indicates that compound I was supposed to be a tetracyclic cucurbitacin-triterpenoid. The cucurbitacin structure was identified by comparison with those reported in the literatre (Vincent *et al.*, 1983, Ryu *et al.*, 1994, Chun-tao, C., *et al.*, 1985). And the detailed analysis of 2D-NMR (^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY) spectra provided the chemical structure of compound I as 19-norlanost-5, 23E-dien-3, 11, 22-trione-2β, 16α, 20β, 25-tetrahydroxy-9-methyl (cucurbitacin D) (Vincent *et al.*, 1983).

The FABMS of II gave a molecular ion at *m/z* 541 $[M+Na]^+$. The ^{1}H- and ^{13}C-NMR spectra of II was very similar to those of I, suggesting the same skeleton. The main difference was the absence of signal at 212.3 ppm of ^{13}C-NMR spectrum attributed in I to carbonyl group (C-3). The analysis of ^{1}H-, ^{13}C-NMR and MS spectra of II indicated that II possessed the same basic structure as I but carbonyl at C-3 of I was substituted with hydroxyl group. Therefore, the structure of II was established as 19-norlanost-5, 23E-dien-11, 22-dione-2β, 3α, 16α, 20β, 25-pentahydroxy-9-methyl (cucurbitacin F) (Chun-tao, C. *et al.*, 1985). This structure corresponds to ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY and DEPT experiment data.

It has been well known that a highly oxygenated tetracyclic triterpenoid group, like cucurbitacins, possessed a wide range of biological activities (Hylands *et al.*, 1986), and cytotoxic effect on cancer cell lines *in vitro* (Baek *et al.*, 1995, Ryu et al., 1994). Cytotoxic activity of I and II on A-549, SK-OV-3, SK-MEL-2, XF-498, and HCT 15 was tested by the procedure of NCI's SRB method (Skehan et al., 1990).

The cucurbitacins are mainly distributed in several species of Cucurbitceae, Cruciferae and Euphorbiaceae, etc. (Shrotria, 1976). Compound I and II were isolated for the first time from *S. sorbifolia* var. *stellipila* in Rosaceae.

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