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Antitumor activity of *Trichosanthes kirilowii*

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The activity-directed fractionation upon the MeOH extract of the root of *Trichosanthes kirilowii* led to the isolation of eight cucurbitane triterpenes namely cucurbitacin B (**I**), isocucurbitacin B (**II**), cucurbitacin D (**III**), isocucurbitacin D (**IV**), 3-*epi*-isocucurbitacin B (**V**), dihydrocucurbitacin B (**VI**), dihydroisocucurbitacin B (**VII**) and dihydrocucurbitacin E (**VIII**), as active principles. All isolates were shown to exhibit significant cytotoxicity against cultured human tumor cells, including A-549, SK-OV-3, SK-MEL-2, XF-498 and HCT 15, with an exceptionally high potency.

Key words: *Trichosanthes kirilowii*, Cucurbitacin B, Cucurbitacin D, Isocucurbitacin B, Isocucurbitacin D, 3-*epi*-isocucurbitacin B, Dihydrocucurbitacin B, Dihydroisocucurbitacin B, Dihydrocucurbitacin E, Antitumor

INTRODUCTION

The plant *Trichosanthes kirilowii* (Cucurbitaceae), called "Kwalu" in Korean, is one of the important herb drugs in northeast Asian Countries since ancient times. It has been widely used as a remedy for the regulation of the water balance and for the pyretolysis or as an anti-inflammatory agent. Earlier investigation upon the chemical constituents of *Trichosanthes kirilowii* was mainly dealt with the isolation of fatty acid or its methyl ester, amino acids such as citrulline and arginine, some sterols including the campesterol, sitosterol and stigmasterol, *etc.* (Kanaoka *et al.*, 1982), glycans named trichosan A, B, C, D and E (Hikino *et al.*, 1989), and a plant protein called trichosanthin which was regarded as a promising abortifacient or antitumor agent (Feng *et al.*, 1986).

In the course of continuing search for the tumour inhibitors of plant origin, the methanolic extract of the root or the seed of *Trichosanthes kirilowii* was found to exhibit a significant inhibitory activity with an exceptionally high potency upon the growth of some human tumor cells carried out in cell culture. And it was also found that such a cytotoxic activity of *Trichosanthes kirilowii* was mainly concentrated in the CH_2Cl_2 soluble fraction, whereas the water soluble fraction, presumably believed to contain the trichosanthin which was reported to be a prominent antitumor agent, was shown to exhibit negligibly poor activity. These results suggested that this plant could contain another active compounds, totally different from the plant protein trichosanthin and this suggestion prompted us to reinvestigate the active constituents of *Trichosanthes kirilowii* guided by the bioassay. Present paper dealt with the isolation of active principles **I-VIII**, from the root of *Trichosanthes kirilowii* by the way of the activity-directed fractionation on the basis of the inhibitory activity upon the growth of tumor cells, *in vitro*.

MATERIALS AND METHODS

^{1}H-NMR spectra were run at 300 MHz and ^{13}C-NMR at 75 MHz and recorded by Bruker AM-300. Low resolution MS (70 eV) were taken with a direct inlet and recorded by JMS-DX303 mass spectrometer (JEOL). Human tumor cells used in the experiment, *i.e.*, A549 (non small cell lung), SK-OV-3 (ovary), SK-MEL-2 (skin), XF498 (central nerve system) and HCT-15 (colon) were obtained from the National Cancer Institute(NCI) in the USA, which were currently used in the NCI's *in vitro* anti-cancer drug screening. Stock cell cultures were grown in T-25 (falcon) flasks containing 10 ml of RPMI-1640 medium with glutamine, sodium bicarbonate and 5% fetal calf serum, which were dissociated with 0.25% trypsin and 30 mM 1,2-cyclohexane-

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diaminetetraacetic acid (CDTA) in PBS in case of transfer or dispense before experiment.

Test for the cytotoxicity *in vitro*

All experimental procedures were followed up the NCI's *protocol* based on the SRB method (Skehan *et al.*, 1990). Detailed experimental procedures were described on the previous paper (Ryu *et al.*, 1992).

Extraction and Isolation

The root of *Trichosanthes kirilowii*, which was commercially available, was purchased at market and 6 kg of the crude material was extracted with MeOH by reflux for 4 hours. The resultant MeOH extract was subjected to evaporation and suspended in water, followed by the successive solvent partition with CH_2Cl_2 and EtOAc, and finally gave 38 g of CH_2Cl_2 soluble fraction, 13 g of EtOAc soluble fraction and 110 g of water soluble fraction. The CH_2Cl_2 soluble fraction was adsorbed in 1 kg of neutral alumina (Al_2O_3, activity 1, Merck.), and was eluted with 5 L of CH_2Cl_2 and then washed with 5 L of MeOH. The eluate and wash was pooled up and evaporated to dryness to give 7 g of Fr.N. The alumina gel was further eluted with 2.5% NH_3/MeOH 5 L and the eluate was collected and evaporated to give 25 g of Fr.A. Each fraction, *i.e.*, Fr.N, Fr.A, Fr.EtOAc and Fr.H_2O was examined for the cytotoxicity in vitro, and it was found that the total activity of the crude MeOH extract was almost completely recovered in the Fr.N. Therefore, the Fr.N was subjected to the SiO_2 column chromatography and the repeated preparative TLC using various solvent system, such as CH_2Cl_2/MeOH gradient system, 3% CH_2Cl_2 in MeOH, heaxane : EtOAc (3 : 2, 2 : 1 or 1 : 1) and 25% EtOAc in CH_2Cl_2, which finally yielded eight kinds of active principles, *i.e.*, 8 mg of **VIII**, 10 mg of **VII**, 80 mg of **III**, 90 mg of **VI**, 15 mg of **V**, 300 mg of **I**, 15 mg of **IV** and 20 mg of **II** by the order of Rf value.

Compound **I** (*cucurbitacin* B). yield 0.005%, colorless needle in *dil.* MeOH, mp. 180-183℃, $[\alpha]_D$ +82 (c, 0.2; MeOH), UV(λ_{max}); 229 nm (MeOH), MS: m/z (rel. int.); 498 (M^+-AcOH, 19), 480(6), 455(12), 385(12), 370 (12), 111(40), 96(100). ^{1}H-NMR ($CDCl_3$, δ): 7.02 (1H, d, J=15.6 Hz, 24-H), 6.45 (1H, d, J=15.6 Hz, 23-H), 5.74 (1H, m, 6-H), 4.38 (1H, dd, J=12.8, 5.9 Hz, 2-H), 4.32 (1H, m, 16-H), 3.20 (1H, d, J=14.5 Hz, 12α-H), 2.74 (1H, m, 10-H), 2.65 (1H, d, J=14.5 Hz, 12β-H), 2.48 (1H, d, J=7.0 Hz, 17-H), 1.98 (3H, s, -OAc), 1.54, 1.52, 1.40, 1.32, 1.30, 1.25, 1.04 and 0.95 (each 3H, s, -CH_3), ^{13}C-NMR: Table I.

Compound **II** (*isocucurbitacin* B). yield 0.0013%, colorless needle in *dil.* MeOH, mp. 220-222℃, $[\alpha]_D$ +35 (c, 0.2; $CHCl_3$), UV(λ_{max}); 229 nm (MeOH), MS: m/z (rel. int.); 498(M^+-AcOH, 15), 455(12), 385(12), 370(12), 111(34), 96(100). ^{1}H-NMR ($CDCl_3$, δ): 7.03 (1H, d, J=15.6 Hz, 24-H), 6.44 (1H, d, J=15.6 Hz, 23-H), 5.91 (1H, m, 6-H), 4.32 (1H, m, 16-H), 4.25 (1H, brs, 3-H), 3.08 (1H, d, J=14.5 Hz, 12α-H), 2.70 (1H, m, 10-H), 2.58 (1H, d, J=14.5 Hz, 12β-H), 2.42 (1H, d, J=7.0 Hz, 17-H), 1.98 (3H, s, -OAc), 1.52, 1.50, 1.42, 1.32, 1.25, 1.15, 0.98 and 0.82. (each 3H, s, -CH_3), ^{13}C-NMR: Table I.

Compound **III** (*cucurbitacin* D). yield 0.0003%, colorless needle in *dil.* MeOH, mp. 150-155℃, $[\alpha]_D$ +50 (c, 0.1; MeOH), UV(λ_{max}); 229 nm (MeOH), MS: m/z (rel. int.); 498(M^+-H_2O, 25), 480(6), 385(12), 370(12), 111(40), 96(100). ^{1}H-NMR ($CDCl_3$, δ): 7.07 (1H, d, J=15.2 Hz, 24-H), 6.58 (1H, d, J=15.2Hz, 23-H), 5.72 (1H, m, 6-H), 4.35 (1H, dd, J=12.9, 5.9 Hz, 2-H), 4.28 (1H, m, 16-H), 3.24 (1H, d, J=14.5 Hz, 12α-H), 2.68 (1H, m, 10-H), 2.64 (1H, d, J=14.5 Hz, 12β-H), 2.48 (1H, d, J=7.0 Hz, 17-H), 1.30 and 1.28 (each 6H, s, -CH_3), 1.33, 1.23, 1.01, 0.92 (each 3H, s, -CH_3), ^{13}C-NMR: Table I.

Compound **IV** (*isocucurbitacin* D). yield 0.0003%, colorless needle in *dil.* MeOH, mp. 185-190℃, $[\alpha]_D$ +35 (c, 0.1; $CHCl_3$), UV(λ_{max}); 229 nm (MeOH), MS: m/z (rel. int.); 498 (M^+-H_2O, 20), 480(8), 385(12), 370 (12), 111(50), 96(100). ^{1}H-NMR ($CDCl_3$, δ): 7.08 (1H, d, J=15.2 Hz, 24-H), 6.59 (1H, d, J=15.2 Hz, 23-H), 5.91 (1H, m, 6-H), 4.32 (1H, m, 16-H), 4.27 (1H, brs, 3-H), 3.12 (1H, d, J=14.5 Hz, 12α-H), 2.70 (1H, m, 10-H), 2.62 (1H, d, J=14.5 Hz, 12β-H), 2.48 (1H, d, J=7.0 Hz, 17-H), 1.33 (6H, s, -CH_3), 1.35, 1.29, 1.22, 1.17, 0.94 and 0.87 (each 3H, s, -CH_3), ^{13}C-NMR: Table I.

Compound **V** (3-*epi-isocucurbitacin* B). yield 0.0003 %, white amorphous powder, $[\alpha]_D$ −25 (c, 0.2; $CHCl_3$), UV(λ_{max}); 229 nm (MeOH), MS: m/z (rel. int.); 498 (M^+-AcOH, 10), 480(5), 455(12), 385(15), 369(12), 111 (15), 96(100). ^{1}H-NMR ($CDCl_3$, δ): 7.02 (1H, d, J=15.6 Hz, 24-H), 6.43 (1H, d, J=15.6 Hz, 23-H), 5.81 (1H, m, 6-H), 4.32 (1H, m, 16-H), 4.09 (1H, brs, 3-H), 3.08 (1H, d, J=14.5 Hz, 12α-H), 2.94 (1H, m, 10-H), 2.62 (1H, d, J=14.5 Hz, 12β-H), 2.45(1H, d, J=7.0 Hz, 17-H), 1.98(3H, s, -OAc), 1.53, 1.51, 1.41, 1.36, 1.32, 1.05, 0.94 and 0.83(each 3H, s, -CH_3), ^{13}C-NMR: Table I.

Compound **VI** (23,24-*dihydrocucurbitacin* B). yield 0.0015%, white amorphous powder, $[\alpha]_D$ +50 (c, 0.2; $CHCl_3$), UV(λ_{max}); end absorption (MeOH), MS: m/z (rel. int.); 500 (M^+-AcOH, 38), 482(20), 402(75), 385 (75), 368(45), 113(98), 96(52), 43(100). ^{1}H-NMR ($CDCl_3$, δ): 5.74 (1H, m, 6-H), 4.38 (1H, dd, J=12.8, 5.9 Hz, 2-H), 4.32 (1H, m, 16-H), 3.20 (1H, d, J=14.5 Hz, 12α-H), 2.72 (1H, m, 10-H), 2.65 (1H, d, J=14.5 Hz, 12β-H), 2.48 (1H, d, J=7.0 Hz, 17-H), 1.90 (3H, s, -OAc), 1.40, 1.38, 1.36, 1.33, 1.31, 1.24, 1.02 and 0.91 (each 3H, s, -CH_3), ^{13}C-NMR: Table I.

Table I. ^{13}C-NMR Chemical Shifts* of Cucurbitacins

C	I	II	III	IV	V	VI	VII	VIII
1	36.0	38.8	36.0	38.8	32.2	35.9	38.8	114.8
2	71.6	210.6	71.6	210.6	211.0	71.1	210.6	144.5
3	212.2	80.2	212.2	80.2	79.4	212.2	80.2	198.6
4	50.2	46.7	50.3	46.7	40.9	50.2	46.7	47.5
5	140.4	138.1	140.5	138.2	139.9	140.3	138.2	136.8
6	120.3	121.9	120.3	121.9	122.0	120.3	121.8	120.7
7	23.9	23.9	23.9	23.9	23.9	23.8	23.8	23.6
8	42.4	42.7	42.4	42.7	42.4	42.2	42.6	41.5
9	48.4	48.5	48.4	48.3	48.2	47.9	48.3	48.4
10	33.7	36.2	33.7	36.2	36.4	33.6	36.3	34.7
11	213.0	211.8	213.0	211.9	212.4	212.9	211.8	212.8
12	48.6	48.6	48.6	48.5	48.7	48.1	48.6	48.8
13	48.1	47.9	48.2	48.1	47.9	48.3	48.2	48.9
14	50.6	50.6	50.8	50.7	50.6	50.5	50.6	50.0
15	45.3	45.3	45.5	45.4	45.5	45.4	45.4	45.7
16	71.2	71.2	71.4	71.3	71.3	70.9	71.0	71.0
17	58.2	58.0	57.3	57.4	58.1	57.7	57.7	57.8
18	20.0	19.8	20.1	20.0	19.0	19.8	20.0	19.8
19	18.9	18.8	19.2	19.0	18.5	18.7	18.8	18.2
20	78.2	78.1	78.1	78.1	78.2	78.9	78.9	78.9
21	23.8	23.8	23.8	23.8	23.7	24.4	24.4	24.5
22	202.5	202.3	202.6	202.8	202.4	213.8	213.9	213.9
23	120.4	120.3	119.0	119.0	120.3	30.6	30.6	30.6
24	151.9	152.0	155.8	155.7	151.9	34.6	34.8	34.7
25	79.3	79.3	71.1	71.1	79.3	81.2	81.2	81.2
26#	26.4	26.4	29.0	29.0	26.4	26.1	26.1	26.1
27#	26.0	25.9	29.5	29.5	25.9	25.7	25.8	25.8
28	21.9	21.9	21.2	20.9	24.4	21.2	21.0	20.2
29	29.3	24.1	29.3	24.1	27.6	29.2	24.1	27.9
30	20.1	20.0	20.1	20.1	19.9	19.9	19.8	20.1
-OAc	170.2	170.2			170.2	170.3	170.3	170.3
	21.9	21.9			21.9	22.3	22.4	22.4

*In ppm downfield from TMS; $CDCl_3$ solutions containing TMS as standard.
#Signals corresponding to C-26 and C-27 of each compounds may be interchanged.

Compound **VII** (23,24-*dihydroisocucurbitacin* B). yield 0.00017%, white amorphous powder, UV(λ_{max}); end absorption, MS: m/z (rel. int.); 500 (M^+-AcOH, 15), 443(12), 402(85), 385(80), 368(65), 113(85), 96(95), 43(100). ^{1}H-NMR ($CDCl_3$, δ): 5.88 (1H, m, 6-H), 4.20 (1H, m, 16-H), 3.84 (1H, brs, 3-H), 3.08 (1H, d, J=14.5 Hz, 12α-H), 2.70 (1H, m, 10-H), 2.58 (1H, d, J=14.5 Hz, 12β-H), 2.42 (1H, d, J=7.0 Hz, 17-H), 1.92 (3H, s, -OAc), 1.43, 1.40, 1.37, 1.30, 1.24, 1.16, 0.94 and 0.82 (each 3H, s, -C<u>H</u>$_3$), ^{13}C-NMR: Table I.

Compound **VIII** (23,24-*dihydrocucurbitacin* E). yield 0.00013%, white amorphous powder, UV(λ_{max}); 267 nm (MeOH), MS: m/z(rel. int.); 498 (M^+-AcOH, 15), 403 (15), 402(13), 369(12), 248(35), 113(80), 96(55), 43(100). ^{1}H-NMR ($CDCl_3$, δ): 5.92 (1H, d, J=2.7 Hz, 1-H), 5.75 (1H, m, 6-H), 4.29 (1H, m, 16-H), 3.50 (1H, brs, 10-H), 3.22 (1H, d, J=14.5 Hz, 12α-H), 2.72 (1H, d, J=14.5 Hz, 12β-H), 2.52 (1H, d, J=7.0 Hz, 17-H), 1.92 (3H, s, -OAc), 1.43, 1.40, 1.39, 1.37, 1.33, 1.22, 1.00 and 0.96 (each 3H, s, -C<u>H</u>$_3$), ^{13}C-NMR: Table I.

Compound **VIIIa** (23,24-*dihydrocucurbitacin* E *diacetate*). Four mg of **VIII** was treated with Ac_2O and pyridine by the conventional manner to give 4 mg of **VIIIa**, white amorphous powder, UV(λ_{max}); 230 nm (MeOH), MS: m/z (rel. int.); 582 (M^+-AcOH, 15), 564 (5), 540(14), 525(20), 485(100), 442(25), 425(60), 383 (90), 177(50), 164(70), 113(75), 43(80). ^{1}H-NMR ($CDCl_3$, δ): 6.32 (1H, d, J=2.5 Hz, 1-H), 5.78 (1H, m, 6-H), 5.14 (1H, m, 16-H), 3.50 (1H, brs, 10-H), 3.22 (1H, d, J=14.5 Hz, 12α-H), 2.74 (1H, d, J=14.5 Hz, 12β-H), 2.68 (1H, d, J=7.0 Hz, 17-H), 2.19 (3H, s, 1-OAc), 1.96 (3H, s, 16-OAc), 1.92 (3H, s, 25-OAc), 1.54 and 1.23 (each 6H, s, -C<u>H</u>$_3$) 1.42, 1.28, 1.27 and 1.07 (each 3H, s, -C<u>H</u>$_3$).

RESULTS AND DISCUSSION

The methanolic extract of the root of *Trichosanthes*

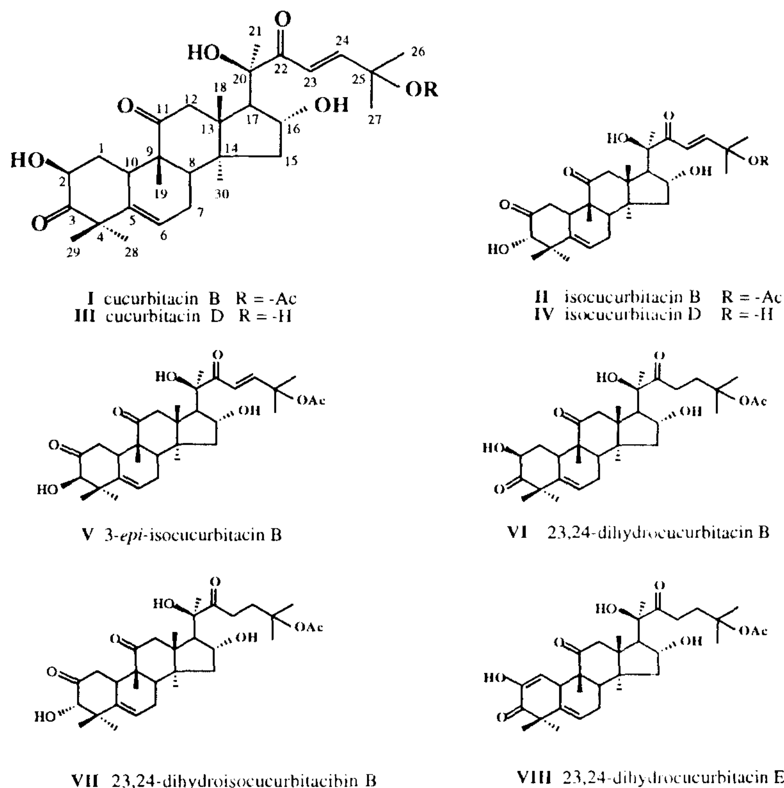

Fig. 1. Cucurbitacins isolated from *Trichosanthes kirilowii.*

kirilowii yielded eight kinds of active principles, **I-VIII**, which was traced according to the activity-oriented fractionation monitoring the inhibitory activity toward the growth of cultured human tumor cells. All of them comprised of common tetracyclic triterpene skeleton, called cucurbitane, and each isolates was identified as *cucurbitancin* B **I**, *isocucurbitacin* B **II**, *cucurbitacin* D **III**, *isocucurbitacin* D **IV**, *3-epi-isocucurbitacin* B **V**, *23,24-dihydrocucurbitacin* B **VI**, *23,24-dihydroisocucurbitacin* B **VII** and *23,24-dihydrocucurbitacin* E **VIII**, respctively (Fig.1), by the comparison of the physicochemical and spectral data of them with those of reported ones (Kupchan *et al.*, 1973 and 1977, Arisawa *et al.*, 1984). Especially, the carbon chemical shifts of each compounds(Table I) showed good accordance with those found in literature (Yamada *et al.*, 1978. and Velde *et al.*, 1983). In case of no reference was found, *i.e.*, **II**, **IV**, **V** and **VII**, the ^{13}C chemical shifts were assigned by the comparison with those of related compounds, **I** or **II**. The compound **II**, **IV** and **V** could be regarded as an artifact of **I** and **III**, produced during the SiO_2 column chromatography (Kupchan *et al.*, 1978). The inhibitory activity of the isolates upon the growth of each human tumor cells, *in vitro* was examined, and the ED_{50} value of each compounds against tumor cells was calculated and tabulated on Table II. All isolates, especially **I**, **III** and **V**, were exhibited a marked activity against each tumor cells with an exceptionally high potency compared with those of any other compounds which was reported as an antitumor agent, even higher than that of adriamycin or taxol (Lien *et al.*, 1984).

The cytotoxic activity of cucurbitacins isolated from *Trichosanthes kirilowii* was presumed to be predominantly due to the enone moiety of side chain from C-20 to C-27, attached to C-17, because the marked diminution of cytotoxicity was observed in the case of **VI**, **VII** and **VIII**, which lacked the enone structure. The detailed structure activity relationships of cucurbitacins will be reported later (Ryu *et al.*, 1994).

The cucurbitacins, to which these isolates belong, are a group of highly oxygenated tetracyclic triterpenes having a cucurbitane skeleton characterized by a 19 (10→9β) *abeo*-10α-lanostane. During last two decades, this group of compounds has been investigated extensively on the isolations from natural resources (Hylands *et al.*, 1986 and Laurie *et al.*, 1985, *etc.*), the structure analysis by X-ray crystallography (Kupchan *et al.*, 1971) and the partial synthesis (Kupchan *et al.*, 1967, 1972 and 1973), because a number of compounds in this group, for example, cucurbitacin B **I**, D **II**, or E has been reputed for their cytotoxic properties (Konopa *et al.*, 1974), antifertility activity (Shohat *et al.*, 1972), antiinflammatory activity (Yesilada *et al.*, 1988) and as

Table II. The inhibititory activity of cucurbitacins from *Trichosanthes kirilowii* toward the growth of tumour cells *in vitro*

Compound	ED_{50} (μg/ml)*				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
I	4.1×10^{-5}	6.2×10^{-5}	1.0×10^{-5}	7.1×10^{-5}	8.2×10^{-5}
II	0.05	0.10	0.06	0.08	0.20
III	4.6×10^{-3}	3.5×10^{-3}	0.1×10^{-3}	2.0×10^{-3}	5.3×10^{-3}
IV	0.2	1.2	0.06	0.6	0.8
V	7.7×10^{-3}	40×10^{-3}	1.2×10^{-3}	86×10^{-3}	52×10^{-3}
VI	0.7	3.7	0.2	2.2	1.8
VII	3.5	9.8	0.7	7.8	6.3
VIII	0.8	2.6	0.4	1.2	1.0
adriamycin	0.1	0.2	0.1	0.2	2.4

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

plant growth regulators(Shrotria *et al.*, 1976). Especially, the cytotoxic activity of cucurbitacins against various tumor cell lines with an unusual potency *in vitro* still rendered them as a promising antitumor agents. Actually, they are still on the consideration or under investigations to be developed as an antitumor agent, even though they were once regarded just as a simple toxic compound by the reason of their low LD_{50} value (<1.0 mg/kg, b.w. mouse. Konopa *et al.*, 1974).

These cucurbitacins were known to be widely distributed in the Cucurbitaceae family, even though in low content (Shrotria *et al.*, 1976), and recently, Kitajima *et al.* (1986) reported that **I** and **III** was isolated from *Trichosanthes kirilowii* var. *japonicum* with a poor yield as 0.0008% of **I** and 0.0006% of **III**.

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REFERENCES CITED

Arisawa, M., Pezzuto, J. M., Kinghorn, A. D., Cordell, G. A. and Farnsworth, N. R., Plant anticancer agents XXX., Cucurbitacins from *Ipomopsis aggregata* (Polemoniaceae). *J. Pharm. Sci.*, 73(3), 411-413 (1984).

Feng, Z., Ng, T. B., Li, W. W. and Yeung, H. W., Improved Isolation and further characterization of beta-trichosanthin, a ribosome-inactivating and abortifacient protein from tubers of *Trichosanthes cucumeroides* (Cucurbitaceae). *Int. J. Biochem.*, 23, 561-567 (1991).

Hikino, H., Yoshizawa, M., Yutaka, S., Oshima, Y. and Konno, C. Isolation and hypoglycemic activity of trichosans A, B, C, D and E: glycans of *Tricosanthes kirilowii* roots. *Planta Med.*, 55(4), 349-350 (1989).

Hylands, P. J. and Magd, M. S., Cucurbitacins from *Acanthosicyos horridus*. *Phytochemistry*, 25(7), 1681-1684 (1986).

Kanaoka, M., Yoshizaki, M. and Fujino, H., Studies on the constituents of *Trichosanthes* species. I. *Chem. Pharm. Bull.*, 30(7), 2570-2574 (1982).

Kitajima, J. and Tanaka, Y., a) Studies on the constituents of *Trichosanthes* root. I. *Yakugaku Zasshi*, 109(4), 250-255 (1989); b) Studies of the constituents of *Trichosanthes* root. IV. *ibid.*, 109(9), 677-679 (1989).

Konopa, J., Matuszkiewicsz, A., Hrabowska, M. and Onoszka, K., Cucurbitacines, cytotoxic and antitumor substances from *Bryonia alba* L. *Arzneim. Forsch.*, 24 (11), 1741-1743 (1974).

Kupchan, S. M., Gray, A. H. and Grove, M. D., Tumor inhibitors. XXIII., The cytotoxic principles of Marah oreganus H. *J. Med. Chem.*, 10, 337-340 (1967).

Kupchan, S. M. and Tsou, G., The structure and partial synthesis of Fabacein. *J. Org. Chem.*, 38, 1055-1056 (1972).

Kupchan, S. M., Sigel, C. W., Guttman, L. J., Restivo, R. J. and Bryan, R. F., Datiscoside, a novel antileukemic cucurbitacin glycoside from *Datisca glomerata*. *J. Am. Chem. Soc.*, 94(4), 1353-1354 (1972).

Kupchan, S. M., Tsou, G. and Sigel, C. W., Datiscacin, a novel cytotoxic cucurbitacin 20-acetate from *Datisca glomerata*. *J. Org. Chem.*, 38(7), 1420-1421 (1973).

Kupchan, S. M., Meshulam, H. and Sneden, T., New cucurbitacins from *Phormium tenax* and *Marah oreganus*. *Phytochemistry*, 17, 767-769 (1978).

Laurie, W. A., McHale, D. and Sheridan, J. B., A cucurbitacin glycoside from *Picrorhiza kurrooa*. *Phytochemistry*, 24(11), 2659-2661 (1985).

Lien, E. J. and Li, W. Y., Structure-activity relationship analysis of chinese anticancer drugs and related plants. pressed by *Oriental Healing Arts Institute*. 47-49 (1984).

Ryu, S. Y., Choi, S. U., Lee, C. O. and Zee, O. P., Antitumor activity of *Psoralea corylifolia*. *Arch. Pharm.*

Res., 15(4), 356-359 (1992).
Ryu, S. Y., Choi, S. U., Lee, S. H., Lee, C. O., No, Z. and Ahn, J. W., Cytotoxicity of cucurbitacin *in vitro*. *Arch. Pharm. Res.*, 17, in press (1994).
Shohat, B., Beemer, A. M., Gitter, S. and Lavie, D., Antifertility activity of dihydroelatericin A in the female mouse. *Experientia.*, 28, 1203-1205 (1972).
Shrotria, A., Cucurbitacins-A new class of growth sustances. *Botanica*, 26, 28-31 (1976).
Skehan, P., Streng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S. and Boyd, M. R., New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer. Inst.*, 82, 1107-1112 (1990).
Velde, V. V. and Lavie, D., ^{13}C NMR spectroscopy of cucurbitacins. *Tetrahedron*, 19(2), 317-321 (1983).
Yamada, Y., Hagiwara, K., Iguchi, K., Takahashi, Y. and Suzuki, S., Carbon-13 NMR spectral assignments of cucurbitacin aglycones. *Chem. Lett.*, 319-322 (1978).
Yesilada, E., Tanaka, S., Sezik, E. and Tabata, M., Isolation of an anti-inflammatory principle from the fruit of *Ecballium elaterium*. *J. Nat. Prods.*, 51(3), 504-508 (1988).