Arch. Pharm. Res. Vol.19, No.6, pp. 559-561, 1996

Isolation of a Cytotoxic Agent from Asiasari Radix

Jong Dae Park*, Nam In Baek, You Hui Lee and Shin Il Kim

Korea Ginseng and Tobacco Research Institute, Taejeon 305-345, Korea

(Received July 18, 1996)

A minor cytotoxic compound was isolated by bioassay-guided fractionation from Asiasari Radix and identified as aristolactam III (**1**) on the basis of spectral data and chemical evidence. This is the first report on the isolation of compound **1** from Asiasarum genus. Compound 1 exhibited a significant cytotoxic activity against the three kinds of human cancer cell lines (A-549, SK-MEL-2 and SK-OV-3).

Key words : Asiasari Radix, Aristolochiaceae, Aristolactam III, Cytotoxicity, Antitumor agent

INTRODUCTION

In the course of searching for the cytotoxic principles from natural medicinal plants, we have found that the ethyl acetate soluble fraction of Asiasari Radix, which has traditionally been used as an analgesic, an antitussive and a anodyne (Shougakukan, 1985) , showed potent cytotoxicities against several human cancer cell lines. Asiasari Radix was generally prepared from *Asiasarum sieboldi* F. Maekawa or *A. heterotropoides* F. Maekawa var. *mandshuricum* F. Maekawa (Aristolochiaceae). A number of investigators isolated methyl eugenol , elemicin, γ-asarone, (-)-asarinin, (-)-sesamin (Nagasawa, 1961), amides (Yasuda *et al.*, 1981), asarinols A, B (Hashimoto *et al.*, 1990), lignan glycosides (Yahara *et al.*, 1990), flavonoid glycosides (Hashimoto *et al.*, 1992) from Asiasari Radix. 3',4'-Dimethoxycinnamaldehyde and xanthoxylol have recently been reported as anti-allergic compounds in the root of *Asiasarum sieboldi* (Hashimoto *et al.*, 1994) and also N-isobutyldodecatetraeneamide, as the antitussive principle from Asiasari Radix (Kosuge *et al.*, 1978). However, no paper has been so far published on the antitumoral principles of Asiasari Radix. This paper will describe the isolation and identification of a cytotoxic principle from Asiasari Radix.

MATERIALS AND METHODS

General procedure

Melting points were determined on a Fisher-John apparatus and uncorrected. The EI-MS spectrum was determined on a Varian MAT 212MS equipped with a Varian 3700GLC. UV spectra was measured with a Pye Unicom PU8000. IR spectrum was taken with a Perkin-Elmer Model 599B. ^{1}H-NMR spectrum was measured with a Bruker AMX 400 spectrometer operating at 400 MHz and TMS was used as an internal standard and chemical shifts were expressed in δ(ppm). Silica gel 60 (Merck, 70-230 mesh) was used for column chromatography.

Plant material

Asiasari Radix was commercially purchased from crude drug market in Taejeon , Korea. The plant material was deposited in our laboratory and identified by Prof. Yong Pyo Lim, Dept. of Horticulture, College of Agriculture, Chungnam National University.

Isolation of active compound

Asiasari Radix (600 g) were extracted with methanol to obtain a methanol extract (48 g). The extract was partitioned with EtOAc/ Water. The EtOAc soluble fraction (12 g), which showed strong cytotoxic activity, was chromatographed by silica gel column chromatography with hexane/EtOAc (10 : 1→1 : 1) to get the active fraction, fr. AS-EA-6 (380 mg). Fraction AS-EA-6 was repeatedly separated by silica gel column chromatography, and the active fraction , AS-EA-6-5 (74 mg) was obtained from $CHCl_3$/MeOH (30 : 1) eluent. Further separation of fr. AS-EA-6-5 by Rp-18 reverse column chromatography with MeOH/H_2O (8 : 1) gave the active compound **1** (4.2 mg), which was purified by HPLC (column: μ-Bondapak C_{18}, MeOH/H_2O=10 : 1). Compound **1**: yellow powder, m.p: 294-296°, UV λ_{max} nm (logε) EtOH : 235 (4.42),

Correspondence to: Jong Dae Park, Korea Ginseng and Tobacco Research Institute, 302 Shinsung-dong, Yousung-ku Taejeon 305-345, Korea

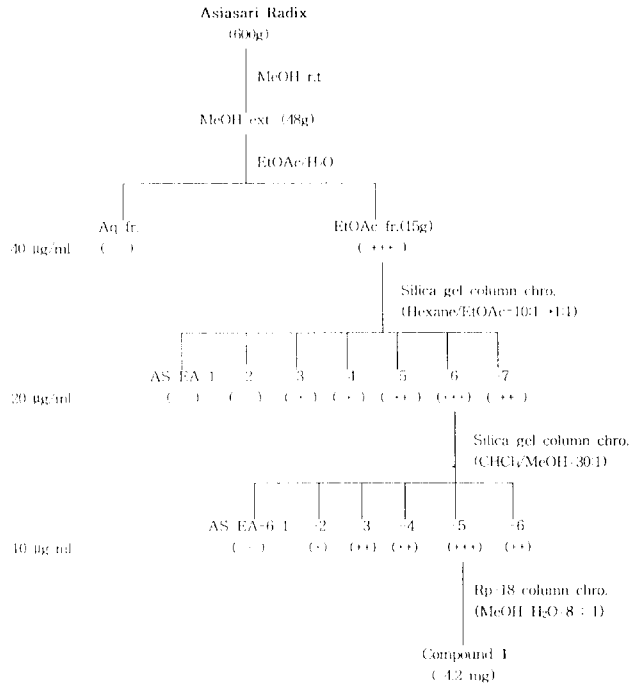


Fig. 1. Isolation of active compound from Asiasari Radix.

252 (4.30), 267 (4.28), 278 (4.36), 296 (4.24), 330 (4.00), 344 (4.02), 398 (4.12), IRv KBr (max) cm^{-1} : 3190 (NH), 1690 (C=O), 1370, 1045, 860, EI-MS:m/z(rel. int, %) : 293 (M^+, 95), 278 (M^+-CH_3, 100), 262 (M^+-OCH_3, 8), 250 (M^+-CO -NH, 36), 232 (4), 207 (10), 164 (19)., ^{1}H-NMR (DMSO-d_6, δ ppm) : 10.84 (1H, brs, NH), 7.92 (1H, d, J=1.0Hz, H-5), 7.49 (1H, s, H-2), 7.25 (1H, dd, J=7.2, 1.0Hz, H-7), 7.84 (1H, d, J=7.2Hz, H-8), 7.06 (1H, s, H-9), 6.42 (2H, s, -CH_2O_2), 3.94 (3H, s, -OCH_3).

Cytotoxic assay

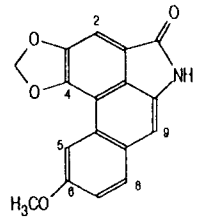
A549 (human lung carcinoma), SK-OV-3 (human ovary adenocarcinoma), and SK-MEL-2 (human malignant melanoma) were grown in RPMI 1640 medium with 5% fetal bovine serum. The initial concentration of each cells in medium was adjusted to 5×10^4 cells/ml, and incubated for 48 h. at 37°C under the 5% CO_2. For cytotoxicity assay against A549, SK-MEL-2 and SK-OV-3 cell lines, 1×10^5 cells in 1 ml of the medium were seeded into each well of 24 well plates, and preincubated for 24 h. at 37°C under the 5% CO_2, followed by incubation with varying concentration of the compounds for 48 h. The experiment was carried out according to the sulforhodamin B method of the NCI protocol (Skehan *et al.*, 1990). Cytotoxicity of the substances at various concentrations against each cell line was calculated as the net growth inhibition (%), Y, of cells as compared with that of control. 5-Fluorouracil was used as a positive control. The value of ED_{50} (μg/ml) which is the concentration of a test substance to inhibit the growth of cell line was determined graphically plotting the concentration of the test substance vs. Y. The effectiveness of bioassay-guided fractionation was evaluated as follows : inhibition ratio (%) ; over 70% (+++), 70<Y≤40% (++), 40<Y≤10% (+), below 10% (-).

RESULTS AND DISCUSSION

The EtOAc soluble fraction of Asiasari Radix has been found to exhibit the cytotoxic activity against several human cancer cell lines. Bioassay-directed fractionation by column chromatography of the active fraction led to an isolation of aristolactam type alkaloid (**1**). Compound **1**, yellow powder, mp 294-296°, showed one Dragendorff positive spot on TLC and a molecular ion at m/z 293 in the EI-MS. The IR absorption of **1** showed the presence of amine group (3190 cm^{-1}) and carbonyl group (1690 cm^{-1}). The UV absorption of **1** (235, 252, 267, 278, 296, 330, 344 and 398 nm) suggested the typical absorption pattern of aristolactam skeleton, which is commonly found in Aristolochia species (Houghton *et al.*, 1991). It was also supported by its mass fragmentation pattern showing the prominent ion peaks at m/z 262 and 250 corresponding to the elimination of one methoxyl and one lactam group, respectively. Its ^{1}H-NMR spectrum showed, in the sp^2 region, the signals of five protons consisting of a pair of ABX type signals at δ 7.92 (1H, d, J=1.0 Hz), 7.25 (1H, dd, J=7.2, 1.0 Hz), 7.84 (1H, d, J=7.2 Hz) and two proton signals at δ 7.06 (1H, s) and 7.49 (1H, s). Besides the above signals, one methoxyl signal at δ 3.94 (3H, s) and one lactam amine signal at 10.84 (1H, brs) were observed. From the above results, compound **1** was assumed to be an aristolactam with one methoxyl substituent at C-6. The direct comparison of the physical and spectral data of **1** with those of aristolactam III revealed both compounds to be identical (Priestap, 1985). Therefore, compound **1** was identified as aristolactam III, which had already been isolated from the rhizome of *Aristolochia argentina*. To our best knowledge, this is the first report of isolation of **1** from Asiasarum genus.

As shown in Table 1, the cytotoxicity of compound **1** was evaluated against several cancer cell lines, which had originated from various human carcinomas. **1** was shown to exhibit significant cytotoxicities against A549, SK-MEL-2 and SK-OV-3 cell lines with ED50 values of 10.2, 8.6 and 9.8 g/ml, respectively. Whereas 5-Fluorouracil, the reference drug, showed cytotoxic activity about two times as strong as compound **1**, suggesting that compound **1** has potential as a source of anticancer drug with possible cytotoxicity. From the above result, it is very difficult to give any definite conclusion on the an-

Table I. Cytotoxic activities of an active compound isolated from Asiasari Radix against human cancer cell lines



1

Compound[b]	ED_{50} (μg/ml)[a]		
	A549	SK-MEL-2	SK-OV-3
1	10.2[c]	8.6	9.6
5-Fluorouracil	1.8	4.7	4.2

[a]ED_{50} value represents the concentration of a compound required for 50% inhibition of cell growth.
[b]Each compound was examined with five concentrations in duplicate.
[c]The values were the range from at least four experiments.

ticancer agent from Asiasari Radix, but it may indicate that **1** plays partially an important role in the development of anticancer drug. For the evaluation of this compound as an anticancer drug, further detailed *in vivo* tests would be necessary.

REFERENCES CITED

Hashimoto, K., Katsuhara, T., Itoh, H., Ikeya, Y., Okada, M. and Mitsuhashi, H., Monoterpenes from Asiasari Radix from *Asiasarum* Sp. *Phytochemistry*, 29, 3571-3574 (1990).

Hashimoto, K., Katsuhara, T., Niitsu, K., Ikeya, Y., Okada, M. and Mitsuhashi, H., Two glycosides from roots of *Asiasarum sieboldi*. *Phytochemistry*, 31, 2477-2480 (1992)

Hashimoto, K., Yanagisawa, T., Okui, Y., Maruno, M. and Fujita, T., Studies on anti-allergic components in the roots of *Asiasarum sieboldi*. *Planta Medica*, 60, 124-127 (1994).

Houghton, P. and Ogutveren, M., Aristolochic acids and aristolactams from *Aristolochia auricularia*. *Phytochemistry*, 30, 253-254 (1991).

Kosuge, T., Yokota, M., Nukuya, H., Goto and Nagasawa, M., Studies on antitussive principles of Asiasari Radix. *Chem. Pharm. Bull.*, 26, 2284-2285 (1978)

Nagasawa, M., Analysis of crude drugs by infrared spectrophotometry. II. Analysis of Asiasari Radix by infrared spectrophotometry. *Yakugaku Zasshi*, 81, 129-138(1961).

Priestap, H., Seven aristolactams from *Aristolochia argentina*. *Phytochemistry*, 24, 849-852 (1985).

Shougakukan, *The dictionary of Chinese drug*. Vol II., Shanghai Science and Technology Publisher and Shougakukan, Tokyo, PP. 1805, 1985.

Skehan, P., Storeng, R., Scudiero, D., Monks, D., 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).

Yahara, S., Kato, K. and Nohara, T., Studies on the water soluble portion in Asiasari Radix. *Shoyakugaku Zasshi*, 44, 331-334(1990).

Yasuda, I., Takeya, K. and Itokawa, H., Structures of amides from *Asiasarum hetereotropoides* Maek. var. *mandshuricum* Maek. *Chem. Pharm. Bull.*, 29, 564-566 (1981).