Arch. Pharm. Res. Vol. 17, No. 1, pp. 45-47, 1994

A Cytotoxic Component from Angelicae Koreanae Radix against L1210 and HL-60 Cells

KiHwan Bae, JongMyung Ji, JongSeong Kang and ByungZun Ahn

College of Pharmacy, Chungnam National University, Taejon 305-764, Korea

(Received November 6, 1993)

A cytotoxic sesquiterpene against L1210 and HL-60 cells was isolated from Angelicae Koreanae Radix (buk-kang-hwal). The component was identified as bisabolangelone by means of chemical and physical methods. The ED_{50} values of it were 1.20 μg/ml against L1210 cells and 2.30 μg/ml against HL-60 cells. Bisabolangelone was found in buk-kang-hwal but not in kang-hwal.

Key words: Cytotoxic activity, L1210 cell, HL-60 cell, Angelicae Koreanae Radix, Kang-hwal, Buk-kang-hwal, Bisabolangelone

INTRODUCTION

Since the early 1950s, a strong effort has been put into the search for novel anticancer agents from natural products. The first clinically useful compounds isolated from natural products were the *Catharanthus alkaloids*. The antineoplastic activity of Catharanthus roseus (Apocynaceae) was discovered independently by Canadian and American research teams (Neuss *et al.*, 1964). Their combined efforts lead to the isolation and structure elucidation of the active bis-indole alkaloids, vinblastine, vincristine, leurosine and leurosidine in the late 1950s and early 1960s.

The novel diterpenoid taxol has become one of the most important lead compounds to emerge from the screening of natural products in recent years. It was first isolated in 1971 by Wall and his collaborators, and it showed significant activity against various leukemias, the Walker 256 carcinosarcoma, Sarcoma 180, and the Lewis lung tumor (Hamburger *et al.*, 1991).

Our approach to the study of cytotoxic components from crude drugs has been driven by the concept that the developed antitumor agents such as vinblastine, vincristine (Neuss *et al.*, 1964) and taxol (Hamburger et al., 1991). As a part of our continuing studies on cytotoxic natural products, the authors have undertaken the screening test on the cytotoxic activity of the medicinal plants against L1210 cell and HL-60 cell (Lee *et al.*, 1986; Bae *et al.*, 1992).

Correspondence to: KiHwan Bae, College of Pharmacy, Chungnam National University, Taejon 305-764, Korea

Angelicae Koreanae Radix has been used in Korea, China and Japan for the treatment of headache and rhumatalgia. Angelicae Koreanae Radix is commercially available in the herb markets under the name of kang-hwal or buk-kang-hwal. The genus Angelica is a large and circumboreal one, probably to be excluded from the southern hemisphere, and is by far the largest genus of Umbelliferae in the Korean flora. Approximately 20 species of the genus Angelica are classified in Korea (Lee, 1989; Woo *et al.*, 1980).

In this paper, we report about the isolation and identification of a cytotoxic component from Angelicae Koreanae Radix.

MATERIALS AND METHODS

Crude Drug

Angelicae Koreanae Radix was purchased from crude drug stores (Jungdo, Jeil and Dongil) in Taejon, Aug. 15-20, 1991.

Reagents and Instruments

Penicillin, streptomycin, dimethylsulfoxide (DMSO) and sodium bicarbonate were purchased from Sigma, Kiesel gel 60 (70-230mesh) and precoated TLC plate (silica gel GF254) were from Merk. UV/VIS spectrophotometer PU 8800 (Pye Unicam), Infrared spectrophotometer model 783 (Perkin-Elmer), NMR spectrometer (Varian EM-360), and Thiele mp apparatus were used.

Cultivation and Maintenance of Cells

L1210 and HL-60 cells were grown in screw cap tubes (10×160 mm) at 37℃ and transferred twice a week.

Determination of ED_{50}

The value of ED_{50} which is the concentration of a test compound to inhibit the growth of tumor cells by 50% to control was determined with the protocol of National Cancer Institute (Thayer *et al.*, 1971).

Sample Preparation for Screening (Table I)

Sample 50 g was extracted with MeOH 200 ml for 3hrs. After filtration, the filter cake was extracted once again. The combined filtrates were concentrated to dried mass. The mass was suspended into water, and extracted with hexane, ether, and EtOAc in sequence. Each fraction was dried and weighed. Each fraction 10 mg was dissolved in EtOH 1 ml or DMSO 1 ml to make a stock solution for the measurement of the cytotoxicity.

Table I. Cytotoxicity of each fraction[a]

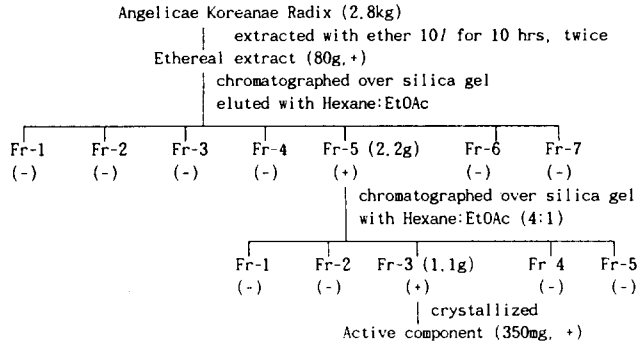
Fractions	ED_{50} (μg/ml)[b]	
	L1210[c]	HL-60[d]
Hexane	15.0	14.5
Ether	4.2	4.5
Ethyl acetate	>20.0	>20.0
Water	>20.0	>20.0

[a]Cytotoxicity of these fractions were measured with known methods (Thayer *et al.*, 1971). [b]Mean ED_{50} values were obtained from the tests repeated three times. [c]Fisher's medium supplemented with horse serum in 10% was used. [d]RPMI medium enriched with fetal bovine serums in 10% was used.

Isolation of a Cytotoxic Component (Scheme I)

The powdered root 2.8 kg was refluxed with ether 10 l for 24 hrs on water bath. The ethereal solution was filtred, dried and weighed (80 g). The ethereal extract was chromatographed over silica gel and eluted with hexane-EtOAc increasing EtOAc ratio. Among fractions 1-7, fr.5 showed the activity, therefore, fr.5 was re-chromatographed over silica gel. Cytotoxic activity was found in subfraction 3, which was almost one compound. Repeated crystallization gives pale yellowish platelets (350 mg).

Component I (bisabolangelone, Fig. 1): colorless or pale yellow platelets, mp. 156-7°, 2,4-dinitrophenylhydrazine test solution gives orange-red colored precipitation in EtOH. UVmax 246 nm, IR (KBr, cm^{-1}): 3,300 (OH), 1640 (α,β-unsaturated ketone with hydroxyl). ^{1}H-



Scheme I. Extraction and isolation of a cytotoxic component from Angelicae Koreanae Radix.
(+) indicates the ED_{50} value is below 5 μg/ml, (−) above 20 μg/ml.

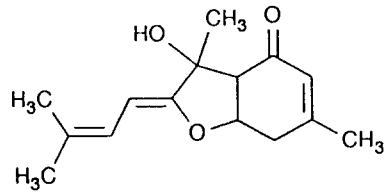


Fig. 1. Cytotoxic component from Angelicae Koreanae Radix.

NMR ($CDCl_3$, ppm): 1.63 (3H, s, CO-CH_3), 1.73 and 1.79 (3Hx2, s, -CH=C(CH_3)$_2$), 2.03 (3H, s, -CH=C-CH_3), 2.67 (1H, d, J=6.7 Hz, CH_3(OH)C-CH-CO), 2.72 (2H, d, exhibiting slight splits, J=6.7Hz, CH=C-CH_2-CH), 3.32 (1H, s, disappears with D_2O, OH), 4.85 (1H, q, J=6.7 Hz, -O-CH(CH)-CH_2-), 5.36 (1H, m, =CH-CH =C(CH_3)$_2$).

RESULTS AND DISCUSSION

Isolation of a Cytotoxic Component

The cytotoxic activities of the solvent fractions against L1210 and HL-60 cells were shown in Table I. Among four solvent fractions, the ethereal fraction showed strongest activity (ED_{50} values were 4.2 μg/ml and 4.5 μg/ml against L1210 and HL-60 cells, respectively), and hexane fraction is moderately active (ED_{50} values were 15.0 μg/ml and 14.5 μg/ml against L1210 and HL60 cells, respectively). The EtOAc and water fractions were inactive. According to the solubility, the cytotoxic components must be lipophilic. The ethereal extract was repeatedly chromatographed over silica gel column gradiently with hexane-EtOAc, whereby a TLC-pure, cytotoxic fraction was obtained. Recrystallization of the fraction gave a colorless platelets, mp. 156-7°. A hydrazone reaction of the component with 2,4-dinitrophenylhydrazine test solution gave an orange-red colored precipitation, implying the presence of a con-

jugated carbonyl group. The presence of the carbonyl group could be proven by IR and UV absorptions at 1640 cm^{-1} and 246 nm, respectiveley. The lower field absorptions of the conjugated enone at 1640 cm^{-1} and hydroxyl group at 3300 cm^{-1} suggest that a strong intramolecular hydrogen bond exists between both groups. There are four methyls in PMR; at 1.63, 1.73, 1.79 and 2.03 ppm. The methyl group at 2.03 ppm must be located in C-3 of enone group. This verifies the presence of enone group. Two methyls at 1.73 and 1.79 ppm correspond to typical isoprene methyl groups. The component containing all of isobutene, 3-methylenone and hydroxyl groups, isolated from *Angelica* species, is bisabolagelone. All the spectroscopic data of both substances were identical. Bisabolangelone, a well known sesquiterpene, is isolated from the seeds of *Angelica silvestris* (Novotny *et al.*, 1966) and buk-kang-hwal, the roots of *Angelica* spp. (Hata *et al.*, 1971).

Stability and Cytotoxicity of Bisabolangelone

Bisabolangelone transforms gradually into yellow resinous substance during storage. This transformation occurs more quickly under exposure to sunlight. The transformed substance showed no cytotoxic activity both in L1210 and HL-60 cells.

Differences in Cytotoxicity of *Angelica* species

The original species of Angelicae Koreanae Radix has been known as *Angelica koreana* Max. But as indicated by Hata *et al.*, the one of buk-kang-hwal is different from kang-hwal. According to the results of our chemotaxonomic and cytotoxic studies, bisabolangelone was only found in buk-kang-hwal but not in kang-hwal. These findings could be used for the identification key of kang-hwal and buk-kang-hwal. The botanical origin of buk-kang-hwal should be clarified furthermore.

CONCLUSIONS

The cytotoxic component of Angelicae Koreane Radix was identified to be bisabolangelone, a well known sesquiterpene isolated from the seeds of *Angelica silvestris* and buk-kang-hwal (the root of *Angelica* spp.). Its ED_{50} values against L1210 and HL-60 cells were 1.20 μg/ml and 2.30 μg/ml, respectively. Bisabolangelone was transformed photochemically into yellow resinous substance during storage, which showed no cytotoxic activity both in L1210 and HL-60 cells.

Among collected Angelicae Koreanae Radix, buk-kang-hwal (originated from *Angelica* spp. but its species was not identified yet) showed not only the cytotoxic activity but also the existence of bisabolangelone. On the other hand, kang-hwal showed no cytotoxic activity and no existence of bisabolangelone.

ACKNOWLEDGEMENT

This study was supported partly by Ohwoon grant (Kolon Co. Ltd.).

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