

Cytotoxic Triterpenoides from *Alismatis Rhizoma*

SangMyung Lee¹, YungHee Kho¹, ByungSun Min¹, JinHee Kim¹, MinKyun Na², ShinJyung Kang³, HackYoung Maeng³, and KiHwan Bae²

¹Korea Research Institute of Bioscience and Biotechnology, Taejon 305-600, and ²College of Pharmacy, Chungnam National University, Taejon 305-764, ³Department of Life Science, Joongbu University, Keumsan Chungnam 312-702, Korea

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Four prostane-type triterpenes were isolated from a methanol extract of *Alismatis Rhizoma* by bioassay-guided isolation using *in vitro* cytotoxic assay. The compounds were identified as alisol B 23-acetate (1), alisol C 23-acetate (2), alisol B (3), alisol A 24-acetate (4) by spectroscopic methods. Amongst the compounds, alisol B (3) showed significant cytotoxicity against SK-OV3, B16-F10, and HT1080 cancer cell lines with ED₅₀ values of 7.5, 7.5, 4.9 µg/ml, respectively.

Key words: *Alismatis Rhizoma*, Alisol B, Cytotoxicity

INTRODUCTION

Alismatis Rhizoma is originated from the rhizome of *Alisma plantago-aquatica* L. var. *orientale* Samuelson or *A. canaliculatum* A. Br. et Bouche (Alismataceae). Prostane-type triterpenes (Murata *et al.*, 1970, Fukuyama *et al.*, 1998), guaiane type sesquiterpenes (Yoshikawa *et al.*, 1992) and kaurane type diterpene (Yamaguchi *et al.*, 1994) have been reported as the main constituents from these plants. Pharmacological studies have been revealed on repairing action to cholinergic acetyltransferase and antihepatic disease (prostane-type), antihypertensive (alisol) and diuretic (orientalol A-C) activities (Yamahara *et al.*, 1989, Yamada *et al.*, 1995, Yamaguchi *et al.*, 1994, Yoshikawa *et al.*, 1992, 1994).

In the course of searching for cytotoxic compounds from natural sources, we have isolated four prostane-type triterpenes from *Alismatis Rhizoma*. We herein report the isolation, structural determination, and the cytotoxicity against several cancer cell lines of these compounds.

MATERIALS AND METHODS

General procedures

Melting points were determined on an Electrothermal Series IA9100 apparatus. EI-mass spectra were obtained

on Varian STAR 3400CX spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at room temperature. IR spectra were measured by a Jasco Infrared spectrophotometer IR Report-100. Some NMR spectra were measured on a Bruker AC 300 instrument equipped with a 5 mm ¹H and ¹³C probe operating at 300 and 75 MHz, respectively. All ¹H chemical shifts were referenced to TMS. Silica gel (70-230 mesh; Merck) was used for column chromatography while TLC was performed on silica gel 60F₂₅₄.

Plant material

Alismatis Rhizoma was purchased from oriental medicine market in Taejon Korea. It was identified by one of the authors (K. Bae). A voucher specimen (CNU2292) is deposited at the Herbarium of College of Pharmacy, Chungnam National University.

Extraction and isolation

The dried rhizoma (4 kg) was extracted with MeOH (3 L × 2) at room temperature for 7 days. After removal of the solvent *in vacuo*, the residue (500 g) was suspended in H₂O and extracted with hexane (2 L × 2), ethyl acetate (2 L × 2) and butanol (2 L × 2), successively. The ethyl acetate extract (60 g) was fractionated by silica gel column chromatography (70-230 mesh, 1.5 kg), eluting with hexane and increasing proportion acetone (9:1 → 1:1). Fractions were combined by monitoring with TLC (hexane/acetone/acetic acid-12:3:0.1, pre-coated silica gel 60) and puri-

Correspondence to: KiHwan Bae, College of Pharmacy, Chungnam National University, Taejon 305-764, Korea
E-mail: baekh@cnu.ac.kr

fied by chromatography on silica gel eluted with hexane-ethyl acetate (5:1), and recrystallization in ethyl acetate/hexane gave four triterpenoids, compounds **1** (3.1 g), **2** (0.6 g), **3** (0.2 g) and **4** (0.2 g).

Alisol B 23-acetate (1): colorless prism (hexane/ethyl acetate); mp. 170-172°C; $[\alpha]_D^{25}$: +121° (CHCl₃; c 0.92); IR ν_{\max}^{kBr} cm⁻¹: 3460, 1740, 1700; EI-MS (*m/z*): 514 (M⁺, C₃₂H₅₀O₅), ¹H NMR (CDCl₃) δ : 0.97, 1.01, 1.03, 1.04, 1.14, 1.31, 1.33 (3H each, s), 1.06 (3H, d, *J* = 6.6 Hz), 1.72 (1H, d, *J* = 11.0 Hz, H-9), 2.06 (3H, s, OAc), 2.55 (1H, dd, *J* = 5.5, 13.2 Hz, Ha-12), 2.72 (1H, d, *J* = 8.5 Hz, H-24), 3.80 (1H, m, H-11), 4.61 (1H, m, H-23).

Alisol C 23-acetate (2): colorless prism (hexane/ethyl acetate); mp. 233-234°C; $[\alpha]_D^{25}$: +139.4° (CHCl₃; c 0.96); IR ν_{\max}^{kBr} cm⁻¹: 3550, 1715, 1700; EI-MS (*m/z*): 528 (M⁺, C₃₂H₄₈O₆); ¹H NMR (CDCl₃) δ : 0.89, 1.07, 1.08, 1.10, 1.23, 1.29, 1.33 (3H, each, s), 1.20 (3H, d, *J* = 7.0 Hz), 2.07 (3H, s), 1.76 (1H, m, H α -22), 1.84 (1H, d, *J* = 18.1 Hz, Ha-15), 2.60 (1H, m, H-20), 2.69 (1H, m, H-2), 2.72 (1H, d, *J* = 8.4 Hz, H-24), 4.00 (1H, m, H-11), 4.52 (1H, m, H-23).

Alisol B (3): white needles (methanol); mp. 165-168°C; $[\alpha]_D^{25}$: +130° (CHCl₃; c 0.96); IR ν_{\max}^{kBr} cm⁻¹: 3430, 1700; EI-MS (*m/z*): 472 (M⁺, C₃₀H₄₈O₄); ¹H NMR (CDCl₃) δ : 0.99, 1.05, 1.07, 1.12, 1.24 (3H each, s), 1.02 (3H, d, *J* = 8.8 Hz), 1.55 (1H, m, Ha-22), 1.73 (1H, d, *J* = 10.6 Hz, H-9), 2.69 (1H, d, *J* = 8.1 Hz, H-24), 2.79 (1H, dd, *J* = 5.9, 13.3 Hz, H α -12), 2.91 (2H, m, H β -22,20), 3.20 (1H, m, H-23), 3.87 (1H, m, H-11).

Alisol A 24-acetate (4): white needles (methanol); mp. 194-196°C; $[\alpha]_D^{25}$: +130° (CHCl₃; c 0.96); IR ν_{\max}^{kBr} cm⁻¹: 3450, 1740, 1700; EI-MS (*m/z*): 514 ([M-H₂O]⁺, C₃₂H₅₂O₆); ¹H NMR (CDCl₃) δ : 0.98, 0.99, 1.00, 1.06, 1.14, 1.16, 1.30 (3H each, s), 1.08 (3H, d, *J* = 6.2 Hz), 1.75 (1

H, d, *J* = 11.0 Hz, H-9), 2.19 (3H, s), 3.89 (2H, overlapped, H-11, 23), 4.60 (1H, d, *J* = 1.0 Hz, H-24).

In vitro cytotoxicity

In vitro cytotoxicity was measured with murine (L1210, leukemia. B16-F10, melanoma) and three different human cancer cell lines, A549 (lung adenocarcinoma), SK-OV3 (ovarian) and K562 (leukemia) were purchased from National Cancer Institute (NCI) in U. S. A. The cytotoxic activity was carried out using sulforhodamine B (SRB) method (Skehan *et al.*, 1990). ED₅₀ value was determined graphically by plotting the viability versus the concentration of the test sample.

RESULTS AND DISCUSSION

The methanol extract of *Alismatis Rhizoma* was fractionated with hexane, ethyl acetate, and butanol, successively. Of the solvent fractions, the ethyl acetate-soluble fraction showed cytotoxic activity against L1210 cells with ED₅₀ value of 19 μ g/ml, while other fractions displayed no cytotoxic activity (ED₅₀ value, >20 μ g/ml). Bioassay-guided isolation of ethyl acetate fraction afforded four prostane-type triterpenes (**1-4**).

Compound **1**, colorless crystals, was isolated as a major compound from this plant. The IR spectrum was consistent with the presence of hydroxyl (3460 cm⁻¹) and carbonyl groups (1740, 1700 cm⁻¹), the latter was further supported by the ¹³C NMR spectrum (δ 170.0, 220.1). ¹H NMR spectrum showed characteristic peaks of prostane-type at δ 0.97, 1.01, 1.03, 1.04, 1.14, 1.31, 1.33 (3H, each s), 2.72 (1H, d, *J* = 8.5 Hz, H-24), and 4.52 (1H, m, H-23). The ¹³C NMR spectrum indicated the presence of an olefin group (C-13, 17) signals at δ 138.2, 134.2. Consequently, compound **1** was determined as alisol B 23-acetate (Murata *et al.*, 1970).

Compound **2** was isolated as colorless prisms. The ¹³C NMR spectrum of **2** was quite similar to that of **1**, except the presence of carbonyl signal arising from C-16 at δ 207.2. This was further identified a prominent peak at 1670 cm⁻¹ in IR spectrum. From the spectral data, the structure of **2** was confirmed as alisol C-23 acetate (Murata *et al.*, 1970).

The ¹³C and ¹H NMR spectra of compound **3** were very similar to those of **1**. Comparison of the NMR data, however, **3** showed a hydroxyl at δ 69.1 instead of an acetoxy group connected to C-23 (δ 71.1) of **1**. This compound was confirmed as alisol B (Murata *et al.*, 1970). Compound **4**, colorless needles, was also similar to spectral data of **1**, except two oxygen-bearing carbons at δ 73.9 (C-25) and 78.7 (C-24) instead of epoxy carbons of **1**. The signals (δ_C 69.0, δ_H 3.89) indicated the presence of a hydroxyl at C-23. From these results, **4** was confirmed as alisol A 24-acetate (Murata *et al.*, 1970).

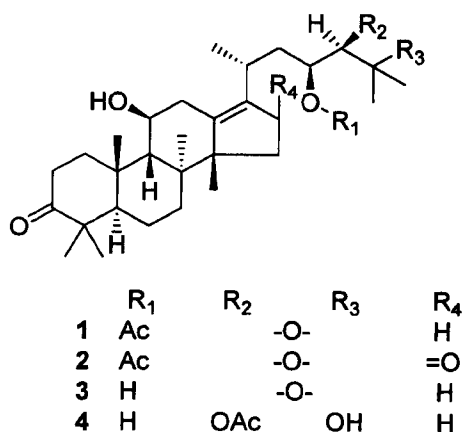


Fig. 1. Structures of Compounds **1-4**

Table I. ^{13}C NMR Spectral Data of Compounds 1-4 (CDCl_3 , 75 MHz)

Carbon No.	1	2	3	4
1	30.9	31.1	31.6	31.0
2	36.9	33.7	33.7	34.4
3	220.1	218.6	220.3	220.2
4	47.1	47.1	46.9	46.9
5	48.7	48.6	48.5	48.5
6	20.3	20.2	20.5	20.0
7	4.4	35.0	34.4	34.9
8	40.9	40.3	40.6	40.4
9	50.1	49.0	49.7	49.6
10	37.13	37.1	36.9	36.9
11	70.3	69.8	69.9	70.0
12	34.7	35.8	34.4	34.5
13	138.2,	176.3	138.0	137.9
14	57.1	49.8	57.0	57.0
15	30.9	45.9	30.6	30.7
16	29.4	207.2	29.1	29.1
17	134.2	138.2	135.0	135.2
18	23.4	23.2	22.6	23.2
19	25.9	25.7	25.5	25.5
20	28.1	26.9	27.7	27.4
21	20.4	20.3	20.2	20.0
22	37.0	35.3	38.8	40.5
23	71.7	71.9	69.1	69.0
24	65.2	64.9	67.8	78.7
25	58.6	58.7	59.3	73.9
26	19.6	20.0	19.1	27.9
27	24.9	24.7	24.8	26.5
28	29.8	29.7	29.5	29.5
29	20.3	19.5	10.1	20.7
30	23.4	23.2	24.0	24.1
-O-COCH ₃	20.6	21.5		20.0
-O-COMe	170.0	169.5		170.7

Table II. Cytotoxicity of Compounds 1-4

Compounds	ED ₅₀ (μg/ml)*					
	L1210	K562	A549	SK-OV3	B16-F10	HT1080
1	16	18	>20	14	>20	>20
2	14	>20	>20	18	18	>20
3	18	>20	>20	7.5	7.5	4.9
4	18	15	14	10	19	15

*Results are mean of triplicate.

All compounds were tested for their cytotoxicity against L1210, K562, A549, SK-OV3, B16-F10 and HT1080 tumor cell lines by SRB method. The results (ED₅₀ values) are summarized in Table II. Compound 1, 2, and 4 showed weak inhibitory activity with ED₅₀ values of 10~20 μg/ml against all tested cancer cells, but alisol B (3) exhibited significant cytotoxic activity against SK-OV3, B16-F10, and HT1080 with ED₅₀ values of 7.5, 7.5, and 4.5 μg/ml, respectively. These results suggested that the important cytotoxic compound was to be alisol B (3) in ethyl acetate fraction of *Alismatis Rhizoma*.

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