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Anti-Complementary Activity of Protostane-Type Triterpenes from *Alismatis Rhizoma*

Sang Myung Lee[1], Jung Hee Kim[1], Ying Zhang[2], Ren Bo An[1,2], Byung Sun Min[1], Hyouk Joung[1], and Hyeong Kyu Lee[1]

[1]Laboratory of Immunomodulator, Korea Research Institute of Bioscience, Biotechnology, P. O. Box 115, Yusong, Daejeon 305-333, Korea and [2]Yan Bian Institute for Drug Control, Yanji 133000, China

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Four protostane-type triterpenes, alisol B 23-acetate (**1a**), alisol C 23-acetate (**2a**), alisol B (**3a**), and alisol A 24-acetate (**4a**), were isolated from the rhizome of *Alismatis plantago-aquatica* L. var. *orientale* Samuelson (Alismataceae) and eleven protostane derivatives (compounds **1**-**11**) were obtained by selective modification from alisol B 23-acetate (**1a**). These compounds were investigated for their anti-complement activity against the classical pathway of the complement system. Alisol B (**3a**) and alisol A 24-acetate (**4a**) exhibited anti-complement activity with IC_{50} values of 150 and 130 μM. Among the synthetic derivatives, the tetrahydroxylated protostane triterpene (**9**) showed moderate inhibitory activity with IC_{50} value of 97.1 μM. Introduction of an aldehyde group at C-23 (**10**; IC_{50} value, 47.7 μM) showed the most potent inhibitory effect on the complement system *in vitro*.

Key words: Alismatis rhizoma, Protostane-type triterpene, Anti-complementary activity

INTRODUCTION

The complement system is a major effector of the humoral immunity and is activated by a cascade mechanism through an antigen-antibody mediated process (classical pathway, CP), antibody independent process (alternative pathway, AP), or mannan binding lectin/MBL-associated serine protease (MBL/MASP) (Park *et al.*, 1999). The proteolytic cascade allows for a tremendous amplification, next step activated enzyme later in the cascade, which in turn cleaves non-enzyme such as C3, C4, and C5. The pathway converge the C3 convertase step leading to C5 convertase and self-assembly of the membrane attack complex (MAC). In complement activation, the complement components induce the release of mediators from the mast cells and lymphocyte, which cause a variety of disease (i.e., rheumatoid arthritis, osteoarthritis, atopic dermatitis, lung fluid inflammationand, and atherosclerotic lesion) as well as can be fated if occurring after organ transplantation (Lichtman and Pober, 1997). It is, therefore, effect normally beneficial for the host, whereas they can also cause reverse effects depending on the site as extent and duration of complement activation. The modulation of complement activity can be important to treatment of inflammations.

During the screening of plant extracts, the complement inhibiting property of the EtOAc-soluble fraction of Alismatis Rhizoma (rhizome of *Alisma plantago-aquatica* L. var. *orientale* Samuelsson, Alismataceae) was investigated. This plant has been used in Korea and China as a diuretic agent (Namba, 1993), and the components were characterized to possess anti-complement activity *in vivo* (Matsuda *et al.*, 1998) and cytotoxicity against L1210, K562, A549, SK-OV3, B16-F10, and HT1080 tumor cell lines (Lee *et al.*, 2001). This paper deals with the anti-complementary activity of four natural protostane-type triterpenes isolated from Alismatis Rhizoma and eleven synthetic derivatives from alisol B 23-acetate.

MATERIALS AND METHODS

General procedures

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

Correspondence to: Byung Sun Min, Laboratory of Immunomodulator, Korea Research Institute of Bioscience and Biotechnology, P. O. Box 115, Yusong, Daejeon 305-333, Korea
Tel: 82-42-860-4359, Fax: 82-42-860-4309
E-mail: bsmin@kribb.re.kr

Varian STAR 3400CX spectrometer. Optical rotations were measured on Perkin-Elmer 241 polarimeter. IR spectra were measured on Jasco Infrared spectrophotometer IR Report-100. NMR spectra were measured on Bruker AC 300 instrument equipped with a 5 mm ^{1}H and ^{13}C probe operating at 300 and 75 MHz, respectively.

Materials

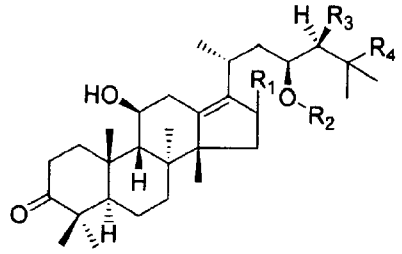
Silica gel (230-400 mesh; Merck) was used for column chromatography and pre-coated TLC was performed on silica gel $60F_{254}$. Chemical reagents were purchased from Aldrich Chemical Company. Solvents used were extra pure grade and obtained from local suppliers. Tetrahydrofuran (THF) was distilled under argon from sodium/benzophenone ketyl immediately prior to use. Methylene chloride was distilled under argon from calcium hydride.

Extraction and isolation

The dried rhizome of *A. plantago-aquatica* L. var. *orientale* (4 kg) was extracted with MeOH (7 day at room temperature twice) and the MeOH solution was then evaporated to dryness. The residue (500 g) was diluted in H_2O and partitioned with hexane and EtOAc, successively. The EtOAc-soluble fraction (60 g) was subsequently fractionated on silica gel (8×80 cm) column chromatography and eluted using a gradient of hexane and acetone (9:1 → 1:1, v/v), to give five fractions (fr. 1, 3 g; fr. 2, 15 g; fr. 3, 30 g; fr. 4, 9 g; fr. 5, 2 g). Fractions 2 and 3 were further chromatographed on silica gel and eluted with hexane/acetone (4:1, v/v), and then recrystallization (in hexane/EtOAc) yielded four compounds **1a** (3.1 g), **2a** (0.6 g), **3a** (0.2 g), and **4a** (0.2 g) (Lee *et al.*, 2001).

Preparation of alisol B 23-acetate derivatives

The eleven protostane derivatives (**1-11**) were prepared by published methods (Lee *et al.*, 2002).



	R_1	R_2	R_3	R_4
1a	H_2	Ac	—O—	
2a	O	Ac	—O—	
3a	H_2	H	—O—	
4a	H_2	H	OAc	OH

Scheme 1. Compounds from EtOAc-soluble fraction of *Alismatis Rhizoma*

Determination of anti-complement activity through the classical pathway

Anti-complement activity was determined by modified method of Mayer (Kabat and Mayer, 1961) as described previously (Oh *et al.*, 1998). For the classical pathway assay, a diluted solution of normal human serum (80 μL) collected from a healthy volunteer (Man) was mixed with gelatin veronal buffer (80 μL) with or without sample. The mixture was preincubated at 37 °C for 30 min, and then sensitized sheep red blood cells (40 μL) were added (Klerx *et al.*, 1983). After incubation under the same conditions, the mixture was centrifuged and the optical density of supernatant (100 μL) was measured at 405 nm. Anti-complementary activity was determined as a mean of triplicates. The purities of compounds used for the assay were above 95% checked by HPLC.

RESULTS AND DISCUSSION

Repeated column chromatography on silica gel and then solvent recrystallization of the EtOAc-soluble fraction from the MeOH extract of the rhizome of *A. plantago-aquatica* L. var. *orientale* led to the isolation of four protostane-type triterpenes, such as alisol B 23-acetate (**1a**), alisol C 23-acetate (**2a**), alisol B (**3a**) and alisol A 24-acetate (**4a**), by comparison with the spectral data reported previously (Murata *et al.*, 1970). Among four compounds, **3a** and **4a** exhibited inhibitory effects on complementary system with IC_{50} values of 150 μM and 130 μM. Meanwhile, **1a** and **2a** were completely incapable of inhibiting complement activity. These observations indicate that a hydroxyl group at C-23 in protostane-type triterpene is essential for significant anti-complement activity, whereas an acetoxyl group at C-23 or 24 is less important. The importance of the hydroxyl group in the aliphatic chain of triterpene was also emphasized by ganoderiols isolated from *Ganoderma lucidum* to maximize anti-complement activity (Min *et al.*, 2001).

Table I. Anti-complementary activity of protostane-type derivatives against complement system *in vitro*.

Compound	IC_{50} values (μM)[a]	Compound	IC_{50} values (μM)
1a	> 200	**5**	> 200
2a	> 200	**6**	> 200
3a	150.4 ± 0.1	**7**	> 200
4a	130.5 ± 0.3	**8**	> 200
1	> 200	**9**	97.1 ± 0.2
2	> 200	**10**	47.7 ± 0.5
3	> 200	**11**	> 200
4	> 200	Tiliroside[b]	102.2

[a]The values represent the mean ± S.D of three expriments.
[b]It was used as positive control (Jung *et al.*, 1998).

For the research of structure-activity relationship about protostane-type triterpenoids, eleven synthetic derivatives were obtained by modification from alisol B 23-acetate (**1a**) (Lee *et al.*, 2002) and were tested for their anti-complement activity. Among them, compound **10** contained an aldehyde group at C-23 in the aliphatic chain and showed most potent anticomplementary activity with IC_{50} value of 47.7 μM. Compound **8** existed an aldehyde moiety at C-24 and an acetoxyl group at C-23 in the skeleton, but was inactive. In addition, compounds **9**, which was existed three hydroxyl groups in the aliphatic chain of protostane, exhibited inhibitory activity with IC_{50} value of 97.1 μM. However, compound **1** contained two hydroxyl groups at C-24 and -25, and an acetyl group at C-23 exhibited inactive in this assay system. As results, the presence of a hydroxyl group or an aldehyde group instead of an acetyl group in the side chain of protostane-type triterpene was essential for the anti-complementary activity. This biological activity of the hydroxyl or aldehyde group in protostane-type triterpene was also supported by their cytotoxic activity against tumor cell lines (Lee *et al.*, 2002).

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