Arch Pharm Res Vol 25, No 5, 608-612, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

# Chemical Modification of Alisol B 23-acetate and Their Cytotoxic Activity

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*(Received May 20, 2002)*

The twelve-protostane analogues were synthesized from alisol B 23-acetate and assessed for their *in vitro* antitumor activity against six different human and murine tumor cell lines. Of the compounds synthesized, 23*S*-acetoxy-24*R*(25)-epoxy-11$\beta$,23*S*-dihydroxyprotost-13(17)-en-3-hy-droxyimine (**12**) exhibited significant cytotoxic activities against A549, SK-OV3, B16-F10, and HT1080 tumor cells with $ED_{50}$ values of 10.0, 8.7, 5.2, and 3.1 μg/ml, respectively. Furthermore, 23*S*-acetoxy-13(17),24*R*(25)-diepoxy-11$\beta$-hydroxyprotost-3-one (**5**), 13(17),24*R*(25)-diepoxy-11$\beta$, 23*S*-dihydroxyprotostan-3-one (**6**), 24*R*,25-epoxy-11$\beta$,23*S*-dihydroxyprotost-13(17)-en-3-one (**7**), and 11β, 23*S*,24*R*,25-tetrahydroxyprotost-13(17)-en-3-one (**9**) showed moderate cytotoxic activities against B16-F10 and HT1080 tumor cells. These results mean that a hydroxyimino group at C-3 position in the protostane-type terpene enhances cytotoxic activity.

**Key words:** Alismatis Rhizoma, Protostane, Cytotoxic activity

## INTRODUCTION

In the previous paper, we reported the isolation and cytotoxic activity of four protostane-type triterpenes, alisol B 23-acetate, alisol C 23-acetate, alisol B, and alisol A 24-acetate, from Alismatis Rhizoma (Lee *et al.*, 2001). It had been reported that alisol B 23-acetate and alisol A 24-acetate showed moderate cytotoxic activity against several tumor cells with $ED_{50}$ values of 10~20 μg/ml, whereas alisol B exhibited significant cytotoxic activity against SK-OV-3, B16-F10, and HT1080 with $ED_{50}$ values of 7.5, 7.5, and 4.5 μg/ml, respectively (Lee *et al.*, 2001). The protostane-type triterpenes showed apoptotic activity against vascular smooth muscle cell line (A7r5) and human acute lymphoblastic leukemia cell line (CEM cells). Since the triterpenoids have a common steroid-like structure, they are expected to have steroid-like pharmacological activities (Chen *et al.*, 2001).

In the present study, we synthesized a series of protostane derivatives from alisol B 23-acetate isolated from Alismatis Rhizoma in order to enhance the cytotoxicity, as well as to determine *in vitro* cytotoxic activity against several tumor cell lines.

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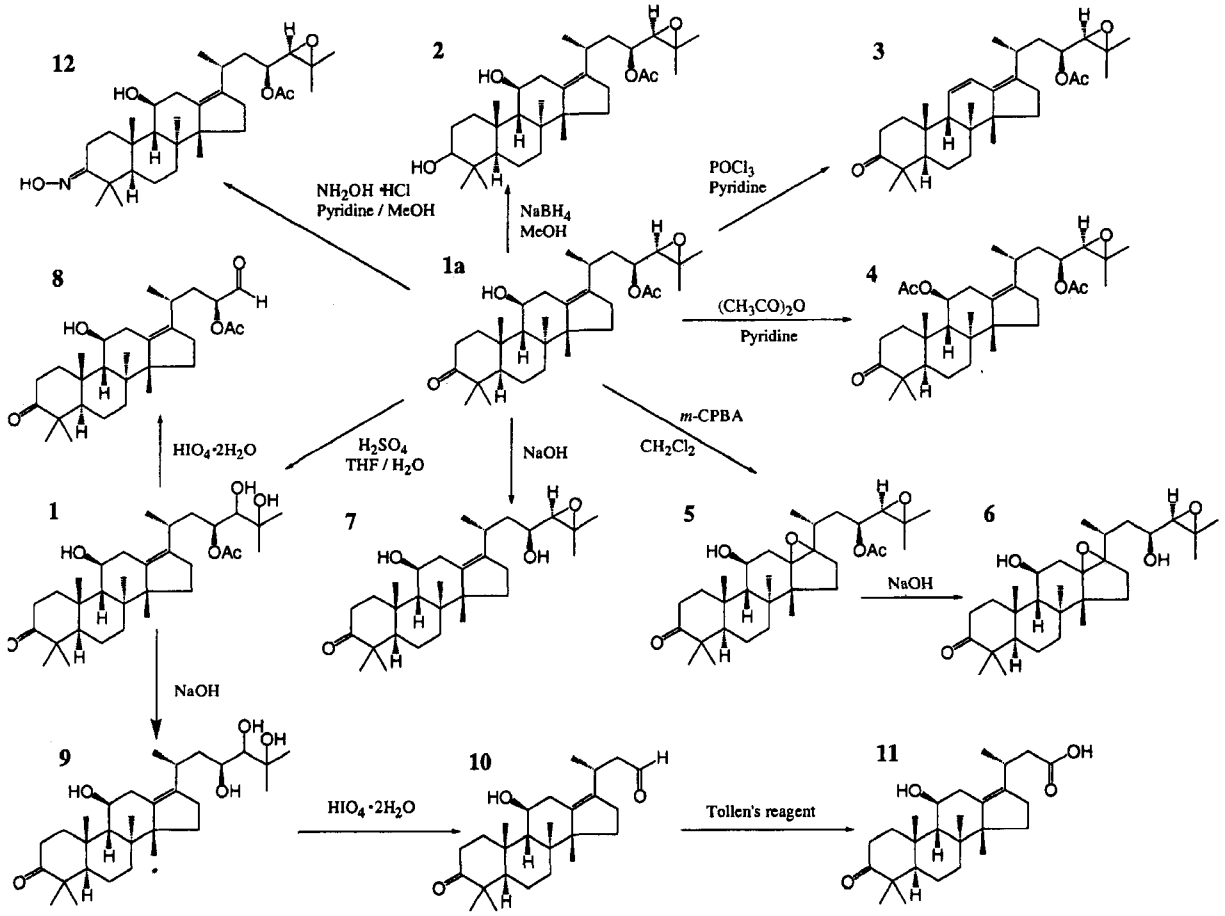
## RESULTS AND DISCUSSION

The twelve-protostane analogues were prepared by published method as shown in Scheme (Murata *et al.*, 1970). Hydration of epoxy ring in alisol B 23-acetate (**1a**) with sulfuric acid afforded alisol A 23-acetate (**1**). Reduction of **1a** with sodium borohydride gave dihydroalisol B 23-acetate (**2**), while dehydration of **1a** with phosphor oxychloride in pyridine yielded the 11(12),13(17)-diene derivative (**3**). Acetylation of **1a** with acetic anhydride in pyridine afforded alisol B 11, 23-diacetate (**4**). Epoxydation of alisol B 23-acetate with *m*-chloroperoxybenzoic acid (*m*-CPBA) in methylene chloride to give 13(17), 23(24) diepoxy derivative (**5**). Treatment of **1** with periodic acid and sodium hydroxide yielded the respective derivatives, **8** and **9**. Tetranoraldehyde **10** was obtained from **9** with periodic acid. Compound **10** was treated with Tollens reagent to afford **11**. Compound **12,** containing a hydroxyimine functional group at position 3 in protostane skeleton, was prepared from **1a** with hydroxylamine hydrochloride in methanolic pyridine.

In an attempt to examine the cytotoxic activity of compounds synthesized, *in vitro* experiments were performed on six tumor cell lines, such as L1210, K562, A549, SK-OV3, B16-F10, and HT1080. **1a** and other compounds isolated from Alismae Rhizoma were found to be slightly active (Lee *et al.*, 2001). To develop more active




**Scheme 1.** Chemical modification

compound, a number of protostane-type triterpenoid analogues were synthesized from **1a,** a major component of Alismatis Rhizoma (Matsuda *et al.*, 1999). As result, twelve analogues could be obtained, which were tested *in vitro* above mentioned cell line system and the results are shown in Table II. From these results, it is apparent that introduction of hydroxyl at C-24, 25 position, acetoxy at C-23 (**9**), and epoxy ring at C-11, 13 (**5**, **6**) exhibited inferior activity. Most of compounds tested exhibit moderately cytotoxic activity ($ED_{50}$ value, some 20 μg/ml). However, compound **12** showed a significant activity against A549, SK-OV-3, B16-F10 and HT1080 with $ED_{50}$ values of 10, 8.7, 5.2, and 3.1 μg/ml, respectively. These results mean that a hydroxyimino group at C-3, hydroxy moiety at C-24, 25 and epoxy ring at C-11, 13 positions in the protostane-type terpen enhances cytotoxic activity.

## 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. IR spectra were measured by a Jasco Infrared spectrophotometer IR Report-100. NMR spectra were measured on a Bruker AC 300 instrument equipped with a 5 mm $^1H$ and $^{13}C$ probe operating at 300, and 75 MHz, respectively. All $^1H$ chemical shifts were referenced to TMS used as an internal standard. Silica gel (70-230 mesh; Merck) was used for column chromatography and TLC was performed on silica gel $60F_{254}$.

### Materials

Chemical reagents were purchased from Aldrich Chemical Company. Solvents 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.

### *In Vitro* Cytotoxity

*In vitro* cytotoxic activity was measured with murine and human tumor cells. Six different tumor cells, L1210 (mouse

**Table I.** $^{13}$C-NMR Spectral Data of Compounds 1-12 ($CDCl_3$, 75 MHz)

| C No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 1a |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 31.0 | 32.8 | 32.3 | 31.1 | 30.9 | 30.6 | 31.6 | 31.0 | 31.7 | 31.0 | 31.0 | | 30.9 |
| 2 | 33.8 | 36.8 | 33.6 | 33.4 | 33.7 | 33.6 | 33.7 | 33.7 | 32.5 | 33.7 | 33.7 | | 36.9 |
| 3 | 220.0 | 79.2 | 220.0 | 220.0 | 220.0 | 220.0 | 220.0 | 220.0 | 222.2 | 220.0 | 220.0 | 168.6 | 220.1 |
| 4 | 46.9 | 52.8 | 46.5 | 46.9 | 40.3 | 40.3 | 46.9 | 46.9 | 48.9 | 46.9 | 46.9 | | 47.1 |
| 5 | 48.6 | 39.4 | 47.1 | 48.3 | 48.8 | 48.8 | 48.5 | 48.5 | 50.5 | 48.5 | 48.5 | | 48.7 |
| 6 | 20.0 | 18.4 | 19.4 | 19.8 | 20.1 | 20.1 | 20.5 | 20.0 | 22.0 | 20.0 | 20 | | 20.3 |
| 7 | 34.3 | 34.5 | 35.5 | 34.2 | 35.0 | 35.0 | 34.4 | 34.2 | 33.0 | 34.2 | 34.3 | | 4.4 |
| 8 | 40.4 | 40.4 | 40.0 | 40.7 | 40.3 | 40.3 | 40.6 | 40.7 | 42.5 | 40.6 | 40.5 | | 40.9 |
| 9 | 49.9 | 48.4 | 46.9 | | 49.2 | 49.2 | 49.7 | 49.8 | 51.7 | 49.7 | 49.7 | | 50.1 |
| 10 | 36.9 | 37.9 | 37.0 | 36.9 | 36.9 | 36.9 | 36.9 | 37.0 | 38.9 | 36.9 | 36.9 | | 37.1 |
| 11 | 70.1 | 70.4 | 121.0 | 71.4 | 68.7 | 68.7 | 69.9 | 70.1 | 71.9 | 70.1 | 70.1 | | 70.3 |
| 12 | 34.4 | 34.6 | 128.0 | | 36.9 | 36.9 | 34.4 | 34.4 | 33.5 | 34.5 | 34.4 | | 34.7 |
| 13 | 138.0 | 139 | 140.0 | 137.0 | 73.3 | 73.3 | 138.0 | 139.2 | 139.5 | 137.2 | 137 | 136.8 | 138.2 |
| 14 | 57.0 | 57.1 | 55.2 | 56.9 | 49.9 | 49.9 | 57.0 | 57.1 | 59.0 | 57.0 | 56.9 | | 57.1 |
| 15 | 30.6 | 30.6 | 31.5 | 30.5 | 31.0 | 31.0 | 30.6 | 34.2 | 31.5 | 30.5 | 30.5 | | 30.9 |
| 16 | 29.1 | | 29.1 | 29.1 | 26.3 | 26.3 | 29.1 | 29.1 | 29.5 | 30.0 | 29.4 | | 29.4 |
| 17 | 135.0 | 134.0 | 137.0 | 135.0 | 77.8 | 77.8 | 135.0 | 133.7 | 137.6 | 134.6 | 134.5 | 134.2 | 134.2 |
| 18 | 23.1 | 22.8 | 22.9 | 22.7 | 19.2 | 19.2 | 22.6 | 23.9 | 24.6 | 24.0 | 24.0 | | 23.4 |
| 19 | 25.5 | 25.0 | 25.7 | 25.3 | 25.7 | 25.7 | 25.5 | 25.7 | 26.0 | 25.6 | 25.6 | | 25.9 |
| 20 | 29.0 | 27.8 | 27.7 | 27.7 | 31.8 | 31.8 | 27.7 | 28.1 | 28.2 | 27.2 | 29.3 | | 28.1 |
| 21 | 20.7 | 19.4 | 20.4 | 20.1 | 16.7 | 16.7 | 20.2 | 20.0 | 21.3 | 19.9 | 20.0 | | 20.4 |
| 22 | 33.7 | 33.8 | 53.6 | 33.4 | 35.9 | 35.9 | 38.8 | | 42.1 | 49.0 | 57.7 | | 37.0 |
| 23 | 73.8 | 71.5 | 71.6 | 73.1 | 72.1 | 72.1 | 69.1 | 170.4 | 71.4 | 202.2 | 177.7 | | 71.7 |
| 24 | 78.7 | 65.1 | 65.1 | 65.1 | 64.9 | 64.9 | 67.8 | 198.3 | 76.1 | | | | 65.2 |
| 25 | 71.8 | 8.4 | 58.4 | 58.3 | 59.1 | 59.1 | 59.3 | | 74.1 | | | | 58.6 |
| 26 | 24.1 | 16.6 | 19.6 | 19.4 | 20.0 | 20.0 | 19.1 | | 27.5 | | | | 19.6 |
| 27 | 27.5 | 24.7 | 24.9 | 24.6 | 24.6 | 24.6 | 24.8 | | 26.6 | | | | 24.9 |
| 28 | 29.5 | 26.6 | 29.3 | 26.5 | 29.5 | 29.5 | 29.5 | 29.5 | 30.3 | 29.2 | 29.5 | | 29.8 |
| 29 | 20.0 | 20.1 | 19.9 | 19.9 | 19.8 | 19.8 | 10.1 | 19.7 | 22.0 | 19.1 | 19.4 | | 20.3 |
| 30 | 23.1 | 24.1 | 24.7 | 24.0 | 24.6 | 24.6 | 24.0 | 22.6 | 25.0 | 22.9 | 22.9 | | 23.4 |
| $OCO\underline{C}H_3$ | 21.0 | 21.2 | 21.1 | 21.7 | 21.1 | 21.1 | | | | | | | 20.6 |
| $O\underline{C}OCH_3$ | 170.7 | 170.0 | 170.0 | 170.0 | 170.0 | 170.0 | | | | | | 164.6 | 170.0 |
| $OCO\underline{C}H_3$ | | | | 21.0 | | | | | | | | | |
| $O\underline{C}OCH_3$ | | | | 170.0 | | | | | | | | | |

leukemia), K562 (chronic myelogenous leukemia), A549 (human lung carcinoma), SK-OV-3 (ovary adenocarcinoma), B16-F10 (mouse melanoma), and HT1080 (acetabulum fibrosarcoma) were purchased from National Cancer Institute (NCI) in U.S.A. The cytotoxic activity was carried out previously before (Kwon et al., 1998) using sulforhodamine B (SRB) method (Skehan et al., 1990). $ED_{50}$ value was determined graphically by plotting the viability versus the concentration of the test sample.

**23*S*-acetoxy-11β,24*R*,25-trihydroxyprotost-13(17)-en-3-one (1)**- A solution of **1a** (1 g) in a mixture of $H_2O$ (5 ml) and THF (5 ml) was reduced with 0.2 N $H_2SO_4$ (in $H_2O$) 2 ml at 50 °C for 12 h. The reaction mixture was treated with $H_2O$ (50 ml) and extracted with chloroform (50 ml). The organic layer was dried over sodium sulfate anhydrous (10 g), and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluting with hexane : acetone, 5:1) to give **1** (yield: 86%). Appearance: white needless (in methanol), mp: 187-188°C, $[\alpha]_D^{25}$: +120° ($CHCl_3$;c 0.96), IR $\nu_{max}^{KBr}$ ($cm^{-1}$): 3400, 1730, 1680. $^1$H-NMR ($CDCl_3$) δ: 097, 0.99, 1.00, 1.05, 1.13, 1.15, 1.29 (3H each, s), 1.07 (3H, d, $J$ = 6.2 Hz), 1.75 (1H,d, $J$ = 11.0 Hz, H-9), 2.18 (3H, s), 3.88 (2H, overlapped, H-11, 23), 4.59 (1H, d, $J$ = 1.0 Hz, H-24), $^{13}$C-NMR (see Table I).

**23*S*-acetoxy-24*R*,25-epoxy-3a,11β-dihydroxyprotost-13(17)-ene (2)**- To a stirred solution of **1a** (200 mg) in MeOH (2 ml), $NaBH_4$ (39.0 mg) was added in small portion during 30 min at room temperature, and then stirring was

continued for further 2 h. Following the procedures described above and after flash chromatography (hexane:acetone, 20:1), yield: 97%. Appearance: white powder (in methanol), mp: 135-136°C, $[\alpha]_D^{25}$ : +46.8° ($CHCl_3$; c 0.96), IR $\nu_{max}^{KBr}$ ($cm^{-1}$): 3400, 1720, 1650. $^1$H-NMR ($CDCl_3$) δ: 0.80, 0.92, 0.99, 1.12, 1.19, 1.30, 1.32 (3H, each s), 1.01 (3H, d, *J* = 6.2 Hz), 1.84 (3H, s, -O-COC<u>H</u>$_3$), 2.72 (1H, d, *J* = 8.5 Hz, H-24), 3.25 (1H, dd, *J* = 8.26 Hz, H-3), 3.75 (1H, m, H-11), 4.60 (1H, m, H-23), $^{13}$C-NMR (see Table I).

**23*S*-acetoxy-24*R*,25-epoxyprotost-11(12),13(17)-dien-3-one (3)**- To solution of **1a** (120 mg) in pyridine (1 ml), $POCl_3$ (0.14 ml in pyridine 1 ml) was drop wise during 30 min with ice-cooling and the mixture was allowed to stand at room temperature for 2 h. Following the procedure described above and after flash chromatography (hexane : acetone, 10:1), yield: 83%. Appearance: colorless plates (in methanol), mp: 154-155°C, $[\alpha]_D^{25}$: +53°($CHCl_3$ ; c 0.96), IR $\nu_{max}^{KBr}$ ($cm^{-1}$): 1740, 1680. $^1$H-NMR ($CDCl_3$) δ: 0.88, 0.90, 0.96, 1.04, 1.31, 1.35 (3H, each s), 1.05 (3H, d, *J* = 6.2 Hz), 2.06 (3H, s, -O-COC<u>H</u>$_3$), 2.73 (1H, d, *J* = 8.5 Hz), 4.62 (1H, m, H-23), 5.54 (1H, d, *J* = 10 Hz), 6.11 (2H, dd, *J* = 7.1, 1.2 Hz, 1,12-H), $^{13}$C-NMR (see Table I).

**11β,23*S*-diacetoxy-24*R*,25-epoxyprotost-13(17)-en-3-one (4)** To solution of **1a** (200 mg) in pyridine (1 ml), acetic anhydride (1 ml) was added and stirred at 70°C for 6 h. Following the procedure described above and after flash chromatography (hexane : acetone, 5:1), yield: 89%. Appearance: colorless plates (in methanol), mp: 147-148 °C, $[\alpha]_D^{25}$ : +117.5($CHCl_3$ ; c 0.96), IR $\nu_{max}^{KBr}$ ($cm^{-1}$): 1730, 1695. $^1$H-NMR ($CDCl_3$) δ: 0.98, 1.01, 1.05, 1.06, 1.15, 1.29, 1.32 (3H each s), 1.04 (3H, d *J* = 6.2 Hz), 2.01 (6H, s, -O-COC<u>H</u>$_3$ 2), 2.69 (1H, d, *J* = 8.5 Hz, H-24), 4.54 (1H, m, H-23), 4.89 (1H, m, H-11). $^{13}$C-NMR (see Table I).

**23*S*-acetoxy-13(17),24*R*(25)-diepoxy-11β-hydroxyprotost-3-one (5)**- To solution of *m*-CPBA (77 mg) in $CH_2Cl_2$, **1a** (200 mg) was added and stirred at room temperature for 12 h. Following the procedure described above and after flash chromatography (hexane : acetone, 10:1), yield: 94%. Appearance: colorless plates (in methanol), mp: 195-196°C, $[\alpha]_D^{25}$ : +139.4° ($CHCl_3$ ; c 0.96), IR $\nu_{max}^{KBr}$ ($cm^{-1}$): 3470, 1720, 1680. $^1$H-NMR ($CDCl_3$) δ: 1.04, 1.06, 1.07, 1.09, 1.10, 1.32, 1.37 (3H each, s), 1.05 (3H, d, *J* = 7.7 Hz), 2.07 (3H, s), 2.77 (1H, d, *J* = 8.8 Hz, H-24), 4.02 (1H, m, H-11), 4.86 (1H, m, H-23), $^{13}$C-NMR (see Table I).

**13(17),24*R*(25)-diepoxy-11,23*S*-dihydroxyprotostan-3-one (6)** To solution of **5** (200 mg) in MeOH (3 ml), NaOH (0.2 N, in MeOH, 3 ml) was added and stirred at room temperature for 6 h. Following the procedure described above and after flash chromatography (Hexane : acetone, 5:1), yield: 46%. Appearance: colorless needles (in ethyl acetate), mp: 105-106°C, $[\alpha]_D^{25}$ :+110.2°($CHCl_3$ ; c 0.96), IR $\nu_{max}^{KBr}$ ($cm^{-1}$): 3400, 1680 $cm^{-1}$, $^1$H-NMR ($CDCl_3$) δ: 1.06, 1.07, 1.08, 1.10, 1.18, 1.32, 1.33,(3H each, s), 1.06 (3H, d, *J* = 7.7 Hz), 2.07 (1H, d, *J* = 8.1 Hz, H-24), 3.69 (1H, m, H-23), 4.05 (1H, m, H-11), $^{13}$C-NMR (see Table I).

**24*R*,25-epoxy-11β,23*S*-dihydroxyprotost-13(17)-en-3-one (7)**- Synthetic method of **7** (yield; 93.7%) was same as **6.** Appearance : colorless plates (in ethyl acetate), mp: 165-168°C, IR $\nu_{max}^{KBr}$ ($cm^{-1}$): 3400, 1695, $^1$H-NMR ($CDCl_3$) δ: 0.99, 1.05, 1.07, 1.12, 1.24, 1.31 (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, Ha-12), 2.91 (2H, m, Hb-22, 20), 3.20 (1H, m, H-23), 3.87 (1H, m, H-11), $^{13}$C-NMR (see Table I).

**Oxidation of 1 with $HIO_4$ (8)** To solution of **1** (200 mg) in dioxane (6 ml), $HIO_4$ $2H_2O$ (200 mg) in $H_2O$ (2 ml) was dropwised and stirred at 50°C for 2 h. Following the procedures described above and after flash chromatography (Hexane : acetone, 5:1), yield: 88%. Appearance: colorless plates (in ethyl acetate), mp: 115-116°C, IR $\nu_{max}^{KBr}$ ($cm^{-1}$): 3450, 1720 $cm^{-1}$, $^1$H-NMR ($CDCl_3$) δ: 0.98-1.43 (3H × 6 overlapped), 2.19 (3H, s, OCOC<u>H</u>$_3$), 2.60 (1H, dd, *J* = 5.5, 13.2 Hz, Ha-12), 3.83 (1H, ddd, *J* = 5.5, 10.6, 11.0 Hz, H-11), 4.69 (1H, m, H-23), 9.50 (1H, s, -C<u>H</u>O), $^{13}$C-NMR (see Table I).

**11β,23S,24R, 25-tetrahydroxyprotost-13(17)-en-3-one (9)** A solution of **1** (300 mg) in NaOH (0.2 N, 3 ml) was stirred at room temperature for 3 h. Following the procedures described above and after flash chromatography (Hexane : acetone, 5:1), yield: 88%. Appearance: white powder (in methanol), mp : 101-102°C, IR $\nu_{max}^{KBr}$ ($cm^{-1}$): 3400, 1680 $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ: 1.00 (3H, s), 1.06 (6H, s), 1.07, 1.14, 1.22, 1.27 (3H each, s), 1.02 (3H, d, *J* = 6.9 Hz), 1.66 (1H, m, Ha-22), 1.75 (1H, d, *J* = 10.6 Hz, H-9), 2.79 (1H, dd, *J* = 5.8, 13.2 Hz, Ha-12), 3.00 (1H, br s, H-24), 3.77 (1H, dd, *J* = 3.5, 9.3 Hz, H-23), 3.88 (1H, m, H-11), $^{13}$C-NMR (see Table I).

**Oxidation of 9 with $HIO_4$ (10)** - To solution of **9** (400 mg) in dioxane (12 ml), $HIO_4$ $2H_2O$ (400 mg) in $H_2O$ (4 ml) was dropwised and stirred at 50°C for 2 h. Following the procedures described above and after flash chromatography (Hexane : acetone, 20:1), yield: 88%. Appearance: colorless plates (in ethyl acetate), mp: 179-178°C, IR $\nu_{max}^{KBr}$ ($cm^{-1}$): 3425, 1700, 1680 $cm^{-1}$, $^1$H-NMR ($CDCl_3$) : 0.94-1.11 (3H × 5 overlap), 2.75 (1H, dd, *J* = 5.5, 13.2 Hz, Ha-12), 3.87 (1H, ddd, *J* = 5.5, 10.6, 11.0 Hz, H-11), $^{13}$C-NMR (see Table I).

**Table II.** *In vitro* anti-tumor activities.

| Compound | $ED_{50}$ (μg/ml)[a] | | | | | |
|---|---|---|---|---|---|---|
| | L1210[c] | K562[d] | A549[d] | SK-OV-3[d] | B16-F10[d] | HT1080[d] |
| 1 | >20 | >20 | >20 | >20 | >20 | >20 |
| 2 | >20 | >20 | >20 | >20 | >20 | >20 |
| 3 | >20 | >20 | >20 | >20 | >20 | >20 |
| 4 | >20 | >20 | >20 | 15 | >20 | >20 |
| 5 | >20 | >20 | >20 | >20 | 17 | 18 |
| 6 | >20 | >20 | >20 | >20 | 17 | 18 |
| 7 | >20 | >20 | >20 | >20 | 7.5 | 5.1 |
| 8 | >20 | >20 | >20 | >20 | >20 | >20 |
| 9 | >20 | >20 | 14 | >20 | 6.5 | 8.9 |
| 10 | >20 | >20 | >20 | >20 | 18 | >20 |
| 11 | >20 | >20 | >20 | >20 | >20 | >20 |
| 12 | 19 | >20 | 10 | 8.7 | 5.2 | 3.1 |
| **1a**[d] | 16 | 18 | 20 | 14 | >20 | >20 |
| Etoposide[e] | 0.3 | 1.0 | 0.3 | 3.1 | 0.3 | 0.6 |

[a]Cytotoxicity of these compounds were assessed by the procedure of Thayer *et al.* and SRB method.
[b]cultured RPMI 1640 medium with 5% FBS and results are mean of triplicate.
[c]L1210 cell was cultured Fisher's medium with 10% horse serum.
[d]Alisol B 23-acetate as starting materal from alismatis Rhizoma (Lee *et al.*,2001).
[e]positive control.

**Oxidation of S-9 with Tollen's reagent (11)** To solution of **10** (100 mg) in MeOH (0.5 ml), Tollen's reagent was dropwised during 30 min. The reaction mixture was heated for 3 h at 50°C and naturalization with dilute HCl solution, and then added ethyl acetate (10 ml) with vigorous stirring. After 1 h stirred, the acquired ethyl acetate fraction was dehydrated over sodium sulfate anhydrous (10 g) and the residue was purified by column chromatography on silica gel (eluting with hexane: acetone, 5:2) to give compound **11**, yield:68%. Appearance: colorless needles (in ethyl acetate), mp : 112-113°C, IR $\nu_{max}^{KBr}$ ($cm^{-1}$): 3400, 1700, 1680 $cm^{-1}$. $^1$H-NMR (CDCl3) δ:0.97-1.25 (3H × 5 overlapped), 2.72 (1H, dd, *J* = 5.5, 13.2 Hz, Ha-12), 3.88 (1H, m, H-11), $^{13}$C-NMR (see Table I).

**23*S*-acetoxy-24*R*(25)-epoxy-11β,23*S*-dihydroxyprotost-13(17)-en-3-hydroxyimine (12)** A mixture of $NH_2OHHCl$ in pyridine (0.1 ml) was added to a solution of **1a** (100 mg), in MeOH (8 ml). After refluxed for 10 h, the reaction mixture added ice water (10 ml) and extracted with $CH_2Cl_2$. The $CH_2Cl_2$ extract was concentrated in vacuum and chromatographed on silica gel (hexane /acetone, 4:1) to give **12**, yield: 93%. Appearance: colorless needles (in ethyl acetate), mp: 112.7-113.5 °C, IR $\nu_{max}^{KBr}$ ($cm^{-1}$): 3400, 1738, 1720 $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ:0.88-1.56 (3H overlapped), 2.02 (3H, s, -O-COC<u>H</u>$_3$), 2.05 (1H, dd, *J* = 5.5, 13.2 Hz, Ha-12), 2.15 (1H, d, *J* = 8.4 Hz, H-24), 3.74 (1H, m, H-11), 5.09 (1H, m, H-23), 9.81 (1H, br. S, =N-O<u>H</u>), $^{13}$C-NMR (see Table I).

## ACKNOWLEDGEMENTS

This work was supported by Korea Research Foundation Grant (KRF-1999-042-F6104).

## REFERENCES

Chen H. W., Hsu M. J., Chien C. T., Huang H. C., Effect of alisol B acetate, a plant triterpene, on apoptosis in vascular smooth muscle cell and lymphocytes. *Eur. J. Pharmacol.*, 419, 127-138 (2001).

Kwon B. M., Lee S. H., Choi S. U., Park S. H., Lee C. O., Cho Y. K., Sing N. D., Bok S. H., Synthesis and *in vitro* cytotoxicity of cinnamaldehydes to human solid tumor cells. *Arch. Pharm. Res.*, 21, 147-152 (1988).

Lee S. M., Kho Y. H., Min B. S., Kim J. H., Na M. K., Kang S. J., Maeng H. Y., Bae K. H., Cytotoxic triterpenoides from Alismatis Rhizoma. *Arch. Pharm. Res.*, 24, 524-526 (2001).

Matsuda H., Kageura T., Toguchida I., Murakami T., Kishi A., Yoshikawa M., Effect of sesquiterpenes and triterpenes from the Rhizome of Alisma oriental on nitric oxid production in lipopolysaccharide-activated macrophages. *Bioorg. Med. Chem. Lett.*, 9, 3081-3086 (1999).

Murata T., Shinohara M., Miyamoto M., Biological-active triterpenes of Alismatis Rhizoma. *Chem. Pharm . Bull.*, 18, 1369-1384 (1970).

Skehan P., Storeng S., Studiero D., Monke A., McMahon J., Vistica D., Waren J. T., Bodesh H., Kenny, S., Boyd M. R., New colorimetric cytotoxity assay for anticancer-drug screening. *Natl. Cancer Inst.*, 82, 1107-1112 (1990).