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Steroids from the Aerial Parts of *Artemisia princeps* Pampanini

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ABSTRACT : Three stigmastane-type sterols and one ergostane-type sterol were isolated from the ethyl acetate soluble fraction of the aerial parts of *Artemisia princeps* Pampanini (Sajuarissuk). From the results of physico-chemical data including NMR, MS and IR, the chemical structures of the compounds were determined as stigmasta-5,22-dien-3β-ol (stigmasterol, 1), stigmast-5-en-3β-ol (β-sitosterol, 2), 5,8-epidioxy-5β,8β-ergosta-6,22-dien-3β-ol (ergosterol peroxide, 3), and β-sitosterol 3-O-β-D-glucopyranoside (daucosterol, 4).

Key words **:** *Artemisia princeps* Pampanini, Compositae, stigmasterol, β-sitosterol, ergosterol peroxide, daucosterol

INTRODUCTION

Artemisia species are distributed above 200 kinds in the world, especially about 38 kinds in Korea, but several species were unidentified (Tang *et al.*, 1992). Knowledge of these unidentified plants is very important because there is not only the potential to discover new alternatives for the treatments of illnesses, but also the conservation of plants. These species has been used traditionally as medicines, among them, the aerial parts of *Artemisia asiatica* (Compositae) have been used for stopping several kinds of bleedings, regulating menses and curing menstrual disorders as well as alleviating pain (Ryu *et al.*, 2004). The chemical constituents of genus *Artemisia* have been studied by a number of researchers. For example, terpenes, sesquiterpenes, lignans, phenylpropanoids have been isolated (Brown *et al*, 2003; Marco *et al*, 1997; Park *et al*, 1994). "Sajuarissuk" is a variety of *Artemisia princeps* Pampanini which is annual herb with 'Sajabalssuk' growing wildly in Ganghwa, Korea. The constituents of *A. princeps* Pampanini have been reported by Ryu, which are flavonoids such as eupatilin, jaceosidin (Ryu *et al*, 2004). In this paper the authors report the isolation and identification of four steroids from the aerial parts of *A. princeps* Pampanini.

MATERIALS AND METHODS

Plant material

The aerial parts of *Artemisia princeps* Pampanini (Sajuarissuk), which have been harvested at Ganghwa in 2002 and 2003, were offered from Ganghwa Agricultural R&D Center (Incheon). The *A. princeps* Pampanini stored for 2 and 3 years in the air was used in the experiments. A voucher specimen (KHU-NPCL-05228) was reserved at the Laboratory of Natural Products Chemistry, Kyung Hee University, Suwon, Korea.

Instrumentation

Uncorrected melting point was determined on a Fisher-John apparatus. Optical rotations were measured on a P-1010 digital polarimeter (JASCO, Japan). EIMS and FABMS were recorded on a JMS-700 (JEOL, Japan). IR spectra were run on a Spectrum One FT-IR spectrometer (Perkin Elmer, USA). ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were taken on a Varian Unity Inova AS 400 FT-NMR spectrometer (Varian, USA).

Isolation of steroids from the aerial parts of *Artemisia princeps* Pampanini

The dried aerial parts of *Artemisia princeps* Pampanini

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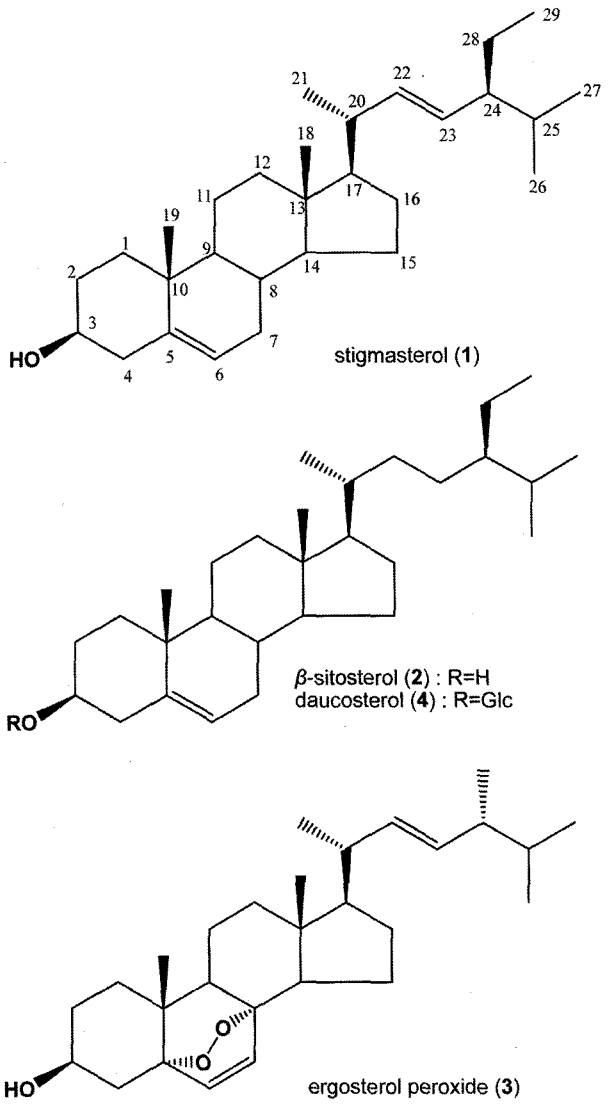

Fig. 1. Chemical structures of compounds **1-4** isolated from the aerial parts of *Artemisia princeps* Pampanini (Sajuarissuk).

(Sajuarissuk, 8.2 kg) were extracted at room temperature with 80% MeOH (40 L × 2). The extract was partitioned with water (3 L), EtOAc (3 L × 2) and *n*-BuOH (3 L × 2), successively. The EtOAc extract (SAE, 102 g) was applied to a silica gel (70-230 mesh) column (8 × 25 cm) chromatography (c.c.), and eluted with *n*-hexane - EtOAc (7 : 1 → 5 : 1 → 1 : 1, each 5 L) and $CHCl_3$ - MeOH (10 : 1 → 1 : 1, each 2 L) monitoring by thin layer chromatography (TLC) to produce 19 fractions (SAE1 to SAE19).

SAE6 (5.2 g) fraction was eluted with *n*-hexane - EtOAc (7 : 1, 2 L) from a silica gel c.c. (5 × 12 cm) to produce 10 fractions (SAE6-1 to SAE6-10). SAE6-7 (541 mg) fraction was subjected to a ODS c.c. (2 × 5 cm) eluted with acetone-acetonitrile (1 : 1, 1 L), sprayed with 10% H_2SO_4, and heated of the TLC to produce purified compound **1** (141 mg) showing the purple color, ultimately. SAE6-8 (232 mg) fraction was applied to a ODS c.c. (2 × 4 cm) and eluted with acetone-acetonitrile (1 : 1, 1L) to give 8 fractions (SAE6-8-1 to SAE6-8-8) and to yield compound **2** (42 mg). SAE8 (2.2 g) fraction was eluted with *n*-hexane-EtOAc (5 : 1, 3 L) from a silica gel c.c. (5 × 15 cm) to produce 11 fractions (SAE8-1 to SAE8-11). SAE8-4 (316 mg) fraction was applied to the silica gel c.c. (4 × 10 cm) eluted with *n*-hexane-EtOAc (7 : 1 → 5 : 1, each 1 L) to give compound **3** (21 mg). SAE14 (3.9 g) fraction was eluted with $CHCl_3$-MeOH (10 : 1, 2 L) from a silica gel c.c. (5×10 cm) to produce compound **4** (33 mg).

Stigmasterol (1) : white powder ($CHCl_3$); mp.163-165 ℃; $[\alpha]_D = -42.9^\circ$ ($c = 0.2$, $CHCl_3$); EIMS *m/z* : 412 $[M]^+$; IR (KBr, ν) 3460, 2930, 1650, 1550, 1330, 1060 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, δ) 5.32 (1H, br. d, J = 4.8 Hz, H-6), 5.15 (1H, dd, J = 15.6, 8.8 Hz, H-22), 4.99 (1H, dd, J = 15.6, 8.8 Hz, H-23), 3.47 (1H, m, H-3), 2.02 (1H, ddq, J = 8.8, 8.6, 6.8 Hz, H-20), 1.03 (3H, d, J = 6.8 Hz, H-21), 0.98 (3H, s, H-19), 0.83 (3H, d, J = 6.4 Hz, H-26), 0.80 (3H, t, J = 7.2 Hz, H-29), 0.77 (3H, d, J = 6.8 Hz, H-27), 0.65 (3H, s, H-18); ^{13}C-NMR (100 MHz, $CDCl_3$, δ) (Table 1).

β-Sitosterol (2) : white powder (*n*-hexane-$CHCl_3$); m.p.140-142 ℃; $[\alpha]_D = -29.2^\circ$ ($c = 0.2$, $CHCl_3$); EIMS *m/z*: 414 $[M]^+$; IR (KBr, ν) 3400, 1640, 1050, 802, 845, 830 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, δ) 5.32 (1H, br.d, J = 4.8 Hz, H-6), 3.51 (1H, m, H-3), 1.02 (3H, s, H-19), 0.88 (3H, d, J = 6.4 Hz, H-21), 0.81 (3H, t, J = 7.2 Hz, H-29), 0.80 (3H, d, J = 7.2 Hz, H-26), 0.77 (3H, d, J = 6.8 Hz, H-27), 0.64 (3H, s, H-18); ^{13}C-NMR (100 MHz, $CDCl_3$, δ) (Table 1).

Ergosterol peroxide (3) : white powder ($CHCl_3$); m.p. 180-182 ℃; $[\alpha]_D = +26.0^\circ$ ($c = 0.2$, $CHCl_3$); EIMS *m/z*: 428 $[M]^+$; IR (KBr, ν) 3360, 1460, 1380, 1040, 1030, 960, 940 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, δ) 6.48 (1H, d, J = 8.4, H-7), 6.22 (1H, d, J = 8.4 Hz, H-6), 5.18 (1H, dd, J = 7.6, 15.2 Hz, H-22), 5.09 (1H, dd, J = 7.6, 15.2 Hz, H-23), 3.91 (1H, m, H-3), 0.97 (3H, d, J = 6.4 Hz, H-21), 0.88 (3H, d, J = 7.2 Hz, H-28), 0.86 (3H, s, H-19), 0.81 (3H, d, J = 6.8 Hz, H-26), 0.78 (3H, s, H-18), 0.77 (3H, d, J = 6.4 Hz, H-27); ^{13}C-NMR (100 MHz, $CDCl_3$, δ) (Table 1).

Daucosterol (4) : colorless crystals (pyridine-MeOH-H_2O); m.p. 282-286 ℃; $[\alpha]_D$ -29.0° ($c = 0.06$, pyridine); pos. FABMS *m/z*: 577 $[M+1]^+$; IR (KBr, ν) 3320, 3030, 2935, 1645 cm^{-1}; ^{1}H-NMR (400 MHz, pyridine-d_5, δ) 5.34 (1H, d, J = 4.4 Hz, H-6), 5.06 (1H, d, J = 7.6 Hz, H-1'), 3.58 (1H, m, H-3), 1.02 (3H, d, J = 7.2 Hz, H-21), 0.94 (3H, s, H-19), 0.89 (3H, d, J = 6.4 Hz, H-26), 0.88 (3H, d, J = 8.4 Hz, H-27), 0.85 (3H, t, J = 6.8 Hz, H-29), 0.64 (3H, s, H-19); ^{13}C-NMR(100 MHz, pyridine-d_5, δ) (Table 1).

Table 1. ^{13}C-NMR chemical shifts (100 MHz) of steroids from the aerial parts of *Artemisia princeps* Pampanini (Sajuarissuk).

No. of Carbon	Compound 1†	Compound 2†	Compound 3†	Compound 4†
1	37.97	37.22	39.34	37.06
2	30.46	31.91	30.12	29.61
3	72.34	71.71	66.42	78.68
4	42.97	42.33	51.05	39.89
5	141.31	140.57	79.39	140.88
6	122.26	121.59	130.61	121.94
7	32.33	31.91	135.29	30.40
8	32.61	31.86	82.12	30.13
9	50.81	50.04	34.70	50.45
10	36.87	36.49	36.93	36.53
11	21.82	21.19	20.68	20.15
12	40.39	39.72	39.34	39.47
13	42.89	42.31	44.56	42.60
14	57.44	56.80	51.67	56.34
15	24.79	24.38	28.71	24.67
16	28.99	28.28	23.44	28.70
17	56.61	55.97	56.16	56.16
18	12.61	11.90	12.94	12.15
19	19.79	19.88	18.24	19.18
20	40.48	36.15	39.79	36.53
21	21.98	18.81	20.93	18.59
22	138.89	33.89	132.16	33.64
23	129.82	25.95	135.07	25.86
24	51.93	45.74	42.78	45.54
25	32.61	29.06	33.09	29.60
26	21.86	19.45	20.02	18.79
27	19.53	19.02	19.71	19.59
28	25.11	23.03	17.62	23.54
29	12.74	12.06		12.34
1'				102.61
2'				75.42
3'				78.13
4'				71.76
5'				78.56
6'				62.92

†**1**, **2**, **3**: in $CDCl_3$, **4**: in pyridine-d_5 (NMR solvent)

RESULTS AND DISCUSSION

When the EtOAc fraction of the 80% methanol extract of *A. princeps* Pampanini was developed on the silica gel TLC, the spots showed purple colorization by 10% H_2SO_4 solution and heating, which indicated the presence of steroids in the fraction. The repeated silica gel, ODS column chromatographies of EtOAc layer supplied four steroids, compounds **1**-**4**.

Compound **1**, white powder, showed absorbance bands due to hydroxyl (3460 cm^{-1}) and olefine (1650 cm^{-1}) groups in the IR spectrum and a molecular ion peak $[M]^+$ at *m/z* 412 in the EIMS spectrum. In the ^{1}H-NMR spectrum, three olefinic methine (δ_H 5.32, 5.15 and 4.99) and an oxygenated methine (δ_H 3.47) signals were observed. Also, in the high magnet field region, two singlet methyl (δ_H 0.98 and 0.65) including three doublet methyl (δ_H 1.03, 0.83 and 0.77) and a triplet methyl (δ_H 0.80) signals were observed. In the ^{13}C-NMR spectrum, 29 carbon signals consisting of an olefinic quaternary (δ_C 141.31), three olefinic methine (δ_C 138.89, 129.82 and 122.26), an oxygenated methine (δ_C 72.34) and six methyl (δ_C 21.98, 21.86, 19.79, 19.53, 12.74 and 12.61) signals were observed. These led to be a stigmastane-type steroid with two double bonds, an oxygenated carbon, two singlet methyl, three doublet methyl, and a triplet methyl signals. Compound **1** was eventually identified as stigmasta-5,22-dien-3β-ol (stigmasterol), through the comparison of several physical and spectroscopic data with those of the literatures (Forgo *et al*, 2004; Lim *et al*, 2005).

Compound **2**, white powder, showed absorbance bands resulting from the hydroxyl (3400 cm^{-1}) and olefine (1640 cm^{-1}) in the IR spectrum and a molecular ion peak $[M]^+$ at *m/z* 414 in the EIMS spectrum. In the ^{1}H-NMR spectrum, an olefinic methine (δ_H 5.32) and an oxygenated methine (δ_H 3.51) signals were observed. Also, a number of methylene and methine (δ_H 2.29-1.00) was observed. The two singlet methyl (δ_H 1.02 and 0.64), three doublet methyl (δ_H 0.88, 0.80 and 0.77) and a triplet methyl (δ_H 0.81) signals were observed. In the ^{13}C-NMR spectrum, 29 carbon signals consisting of a quaternary carbon (δ_C 140.57), an olefinic methine (δ_C 121.59), an oxygenated methine (δ_C 71.71) and six methyl (δ_C 19.88, 19.45, 19.02, 18.81, 12.06 and 11.90) signals were observed. Thus, we concluded that compound **2** was a sterol compound composed of one double bond and one hydroxyl group. Compound **2** was finally identified as stigmast-5-en-3β-ol (β-sitosterol) through the comparison of several physical and spectroscopic data with those of the literatures (Kim *et al*, 2004; Lim *et al*, 2005).

Compound **3**, white powder, showed absorbance bands due to hydroxyl (3360 cm^{-1}) and olefine (1460 cm^{-1}) groups in the IR spectrum and a molecular ion peak $[M]^+$ at *m/z* 428 in the EIMS spectrum. In the ^{1}H-NMR spectrum, two olefinic methine (δ_H 6.48 and 6.22) signals showing vicinal coupling with a coupling constant of 8.4 Hz, two other olefinic methine (δ_H 5.18 and 5.09) signals showing *trans* vicinal coupling with a coupling constant of 15.2 Hz, and an oxygenated methine (δ_H 3.91) signal were observed. Also, in the high magnet field region, four doublet (δ_H 0.97, 0.88, 0.81 and 0.77) and two singlet (δ_H 0.86 and 0.78) methyl signals were observed. In the ^{13}C-NMR, 28 carbon signals consisting of four olefinic

methine (δ_C 135.29, 135.07, 132.16 and 130.61), two oxygenated quaternary carbon (δ_C 82.12 and 79.39), an oxygenated methine (δ_C 66.4) and six methyl (δ_C 20.93, 20.02, 19.71, 18.24, 17.62 and 12.94) signals were observed. These led to conclude that compound **3** was as a ergostane-type steroid, with two double bonds, three oxygenated carbon signals and four doublet and two singlet methyl signals. Compound **3** was finally identified as 5,8-epidioxy-5α,8β-ergosta-6,22-dien-3β-ol (ergosterol peroxide) by comparison of several physical and spectroscopic data with those in the literatures (Takaishi *et al*, 1992; Bok *et al*, 1999; Nam *et al*, 2001; Kim *et al*, 2005a).

Compound **4**, colorless crystals, showed absorbance bands resulting from the hydroxyl (3320 cm^{-1}) and olefine (1645 cm^{-1}) in the IR spectrum and a molecular ion peak $[M+1]^+$ at *m/z* 577 in the FABMS spectrum. The NMR spectra of compound **4** were almost similar to those of compound **2**, exception with a sugar signals. In the ^{13}C-NMR spectrum, the sugar signals were identified as D-glucopyranose. Also, in the ^{1}H-NMR spectrum, the coupling constant of the anomeric proton (δ_H 5.06) signal was 7.6 Hz, the glucopyranose was confirmed to have β-glycosidic linkage. Compound **4** was eventually identified as β-sitosterol 3-O-β-D-glucopyranoside (daucosterol) through the comparison of other physical and spectroscopic data with those of the literatures (Kim *et al*, 2005b; Lendl *et al*, 2005). The sterols have been first isolated from this plant in this study.

For the investigation of constituents in the aerial parts of *Artemisia princeps* Pampanini, the compound **1-4** were isolated and identified. These compounds have the activities as follows. Stigmasterol was examined for reducing blood cholesterol level (Pollak *et al*, 1981). It was reported that β-sitosterol had uterotrophic effect as accelerating an acid phosphate activity, antivirus, antiinflammatory and antifebrile activities (Hyun *et al*, 1996). Ergosterol peroxide was examined for ACAT inhibitory activity with oleic acid anilide used as a positive control (Roth *et al*, 1992; Kim *et al*, 1994) and antiatherosclerosis activity (Kim *et al*, 2005a). Daucosterol was examined for cytotoxity on cancer cell (Hyun *et al*, 1996), FPTase inhibitory activity (Kim *et al*, 2004) and insecticidal and antifeedant effects (Carlos *et al*, 2005). Therefore, *Artemisia princeps* Pampanini, which was used as edibility in Ganghwa island, might be so useful for development of functional food and raw materials of medicine.

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