Two New Monoterpene Peroxide Glycosides from *Aster scaber*

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The aerial part of *Aster scaber* THUNB. (Asteraceae) yielded two new monoterpene peroxide glycosides, (3*S*)-3-*O*-(3′,4′-diangeloyl-β-D-glucopyranosyloxy)-7-hydroperoxy-3,7-dimethylocta-1,5-diene (1) and (3*S*)-3-*O*-(3′,4′-diangeloyl-β-D-glucopyranosyloxy)-6-hydroperoxy-3,7-dimethylocta-1,7-diene (2), and five known compounds, α-spinasterol (3), germacra-4(15),5,10(14)-triene-1β-ol (4), 7-methoxy-4(15)-oppositen-1β-ol (5), 6α-methoxy-4(15)-eudesmane-1β-ol (6) and α-spinasterol 3-*O*-β-D-glucopyranoside (7). The structures were established by chemical and spectroscopic methods.

Key words *Aster scaber* THUNB; Asteraceae; monoterpene peroxide glycoside; (3*S*)-3-*O*-(3′,4′-diangeloyl-β-D-glucopyranosyloxy)-7-hydroperoxy-3,7-dimethylocta-1,5-diene; (3*S*)-3-*O*-(3′,4′-diangeloyl-β-D-glucopyranosyloxy)-6-hydroperoxy-3,7-dimethylocta-1,7-diene

Aster scaber THUNB. (Asteraceae) is widespread and cultivated as culinary vegetables in Korea.[1] *Aster* species have been used in traditional Chinese medicine to treat bruises, snakebite, headache and dizziness.[2] Recently, triterpene glycosides[3,4] and volatile compounds[5] have been reported from *Aster scaber*. In our previous study on this plant, we reported four quinic acid derivatives and their inhibitory activities against human immunodeficiency virus-1 (HIV-1) integrase.[6] In our continuing study of this plant, we have isolated two new monoterpene peroxide glycosides (**1**, **2**), together with three known sesquiterpenes (**4**—**6**) and two known sterols (**3**, **7**). The present paper describes the isolation and structural characterization of these two new monoterpene peroxide glycosides (**1**, **2**).

Five known compounds, α-spinasterol (**3**),[7] germacra-4(15),5,10(14)-triene-1β-ol (**4**),[8] 7-methoxy-4(15)-oppositen-1β-ol (**5**),[9] 6α-methoxy-4(15)-eudesmane-1β-ol (**6**)[9] and α-spinasterol 3-*O*-β-D-glucopyranoside (**7**)[7] were characterized by comparing their physical and spectroscopic data with those reported in the literatures.

Compound **1** was obtained as a colorless oil and positive peroxide test.[10] Its molecular formula was determined as $C_{26}H_{40}O_{10}$ by HR-ESI-MS spectrum. Its IR spectrum displayed absorption bands at 3450 and 1721 cm^{-1}, indicating the presence of hydroperoxy and ester groups. The ^{1}H- and ^{13}C-NMR spectra exhibited the presence of two angeloyl groups[11] and a hexose sugar. ^{1}H-NMR coupling constants and ^{13}C-NMR chemical shifts data suggested that the sugar was glucose, and its $J_{1'2'}$ (8.0 Hz, diaxial) and $J_{3'4'}$ (9.6 Hz, diaxial) values in the ^{1}H-NMR spectrum suggested that the sugar is β-D-glucopyranose.[12,13] In addition, the ^{1}H-NMR spectrum showed signals by three tertiary methyl groups at δ 1.30, 1.32 and 1.39, an aliphatic methylene at δ 2.35 (1H, dd, J=14.6, 7.3 Hz) and 2.40 (1H, dd, J=14.6, 7.3 Hz), five olefinic protons at δ 5.25 (1H, d, J=17.0 Hz), 5.26 (1H, d, J=10.8 Hz), 5.59 (1H, d, J=15.8 Hz), 5.70 (1H, dt, J=15.8, 7.3 Hz) and 5.88 (1H, dd, J=17.0, 10.8 Hz), and hydroperoxy proton at δ 8.07 (1H, br s). The ^{13}C-NMR spectrum showed ten carbon resonances in addition to the signals of two angeloyl and a glucosyl groups. These ^{1}H- and ^{13}C-NMR data led to the monoterpene structure 3-hydroxy-7-hydroperoxy-3,7-dimethylocta-1,5-diene. The relationship of the two angeloyl groups, the glucose, and the monoterpene moiety was established by the analysis of the ^{1}H–^{1}H COSY, HMQC and HMBC spectra. In the HMBC spectrum, long-range connectivities were observed between the H-3′ (δ 5.29) and the angeloyl ester carbons C-1″ (δ 168.5), the H-4′ protons (δ 5.09) and C-1‴ (δ 167.6), and the anomeric H-1′ proton (δ 4.56) and the C-3 carbon (δ 80.8), respectively. Thus, the gross structure of **1** was suggested to be 3-*O*-(3′,4′-diangeloyl-β-D-glucopyranosyloxy)-7-hydroperoxy-3,7-dimethylocta-1,5-diene.

The reductive cleavage of **1** with $LiAlH_4$ afforded 3-*O*-β-D-glucopyranosyloxy-7-hydroxy-3,7-dimethylocta-1,5-diene (**1a**), which was identified by its ^{1}H-NMR spectrum.[14] Enzymatic hydrolysis of **1a** afforded (3*S*)-3,7-dihydroxy-3,7-dimethylocta-1,5-diene (**1b**) and D-glucose, which were identified by its ^{1}H-NMR spectrum[13,15] for **1b** and by co-TLC for glucose with authentic D-glucose, respectively. Based on the above evidence, the structure of **1** was determined as (3*S*)-3-*O*-(3′,4′-diangeloyl-β-D-glucopyranosyloxy)-7-hydroperoxy-3,7-dimethylocta-1,5-diene.

Compound **2** was obtained as a colorless oil and positive peroxide test.[10] Its molecular formula was determined as $C_{26}H_{40}O_{10}$ by HR-ESI-MS spectrum. The IR, ^{1}H- and ^{13}C-NMR spectra of **2** were very similar with those of **1**. The only differences in the ^{1}H-NMR spectra were observed in the signals of the monoterpene moiety. Comparing the ^{1}H-NMR spectrum of **1** with that of **2**, the absence of the signal at δ 5.73 (1H, dt, J=15.9, 7.4 Hz) and 5.59 (1H, d, J=15.9 Hz), and the appearance of new signals at δ 4.99 (1H, s) and 5.00 (1H, s) in the ^{1}H-NMR spectrum of **2** indicated the shift of the hydroperoxide group from C-7 to C-6 in the monoterpene moiety. This was also supported by the appearance of a new proton signal at δ 4.32 (1H, br t, J=5.4 Hz). These observations of the monoterpene moiety led to the structure of 3-hydroxy-6-hydroperoxy-3,7-dimethylocta-1,7-diene. The relationship of the two angeloyl groups, the glucose, and the monoterpene moiety was established by the analysis of the ^{1}H–^{1}H COSY, HMQC and HMBC spectra. The reductive

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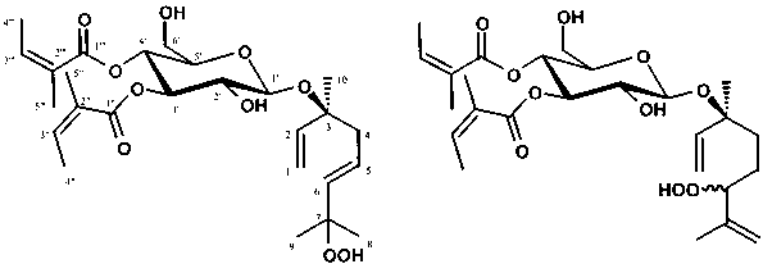

Fig. 1. Structures of Compounds **1** and **2**

cleavage of **2** with $LiAlH_4$ afforded 3-*O*-*β*-D-glucopyranosyloxy-6-hydroxy-3,7-dimethylocta-1,7-diene (**2a**), which was identified by its ^{1}H-NMR spectrum.[14] Enzyme hydrolysis of **2** afforded (3*S*)-3,6-dihydroxy-3,7-dimethylocta-1,7-diene (**2b**) and D-glucose, which were identified by its ^{1}H-NMR spectrum[13] for **2b** and by co-TLC for glucose with authentic D-glucose, respectively. Based on the above evidence, the structure of **2** was determined as (3*S*)-3-*O*-(3′,4′-diangeloyl-*β*-D-glucopyranosyloxy)-6-hydroperoxy-3,7-dimethylocta-1,7-diene. Although we have tried to determine the configuration of the OOH group by some chemical methods, we could not obtain a conclusive result.

Experimental

General Mps: Gallenkamp melting point apparatus (uncorr.) NMR: Bruker AMX 500 and Varian UNITY INOVA 500. IR: Nicolet model 205 FT-IR spectrophotometer. MS: VG70-VSEQ mass spectrometer. HPLC: JAI LC-908 model with refractive index detector, UV detector and Econosil C18 10u column (250 mm×22 mm). Column chromatography: silica gel (Merck, 70—230 mesh and 230—400 mesh). TLC: Merck precoated Si gel F_{254} plates and RP-18 F_{254s} plates. LPLC: Merck Lichroprep Lobar®-A Si 60 (240×10 mm).

Plant Materials Cultivated *Aster scaber* plants were purchased in ChukRyung Mt., Kyungi-Do, Korea in September 1997. A voucher specimen (SKK-98-001) is deposited in the College of Pharmacy at Sung Kyun Kwan University.

Extraction and Isolation The dried and chopped aerial parts of *Aster scaber* (2.1 kg) were extracted with MeOH two times at room temp. and once at 50 °C for 5 h. The resultant MeOH extract (200 g) was subjected to successive solvent partitioning to give CH_2Cl_2 (28 g), EtOAc (17 g) and *n*-BuOH (33 g) soluble fractions. The CH_2Cl_2 soluble fraction (28 g) was chromatographed on a silica gel column using a gradient solvent system of hexane–EtOAc (5 : 1—0 : 1) to give eight subfractions (F1—F8). The subfraction F5 was chromatographed over silica gel eluting with hexane–EtOAc (2 : 1) to give two subfractions (F51, F52). The first subfraction (F51) was subjected to Sep-Pak (RP-18, 40% acetonitrile) and purified by HPLC (40% acetonitrile, RP-18) to yield **1** (25 mg) and **2** (13 mg). The second subfraction (F52) was purified by recrystallization from the same solvent to afford **3** (240 mg). Supernant of F52 was chromatographed over silica gel Lobar®-A column (hexane–CH_2Cl_2–EtOAc, 9 : 1 : 1) to afford **4** (8 mg), **5** (4 mg) and **6** (10 mg). Subfraction F7 was chromatographed on silica gel (hexane–EtOAc–CH_2Cl_2–MeOH, 2 : 1 : 1 : 0.5) and recrystallized from the same solvent to afford **7** (20 mg).

(3*S*)-3-*O*-(3′,4′-Diangeloyl-*β*-D-glucopyranosyloxy)-7-hydroperoxy-3,7-dimethylocta-1,5-diene (**1**): Colorless oil. $[\alpha]_D^{20}$: −13.1° (*c*=0.48, $CHCl_3$). UV $\lambda_{max}^{(MeOH)}$ nm (log *ε*): 216 (4.29). IR $\nu_{max}^{(CHCl_3)}$ cm^{-1}: 3450, 3021, 2983, 1721, 1650. HR-ESI-MS *m*/*z*: 535.2526 ([M+Na]$^+$, Calcd for $C_{26}H_{40}O_{10}Na$ 535.2519); ^{1}H-NMR (500 MHz, $CDCl_3$) *δ*: 1.31 (3H, s, H-8),1.33 (3H, s, H-9), 1.40 (3H, s, H-10), 1.80 (3H, m, H-5‴), 1.83 (3H, m, H-5″), 1.91/1.92 (each 3H, d, *J*=7.1 Hz, H-4″, H-4‴), 2.35 (1H, dd, *J*=13.6, 7.4 Hz, H-4a), 2.41 (1H, dd , *J*=13.6, 7.4 Hz, H-4b), 2.54 (1H, br s, 6′-OH), 2.82 (1H, br s, 2′-OH), 3.50 (1H, ddd, *J*=9.9, 5.1, 2.6 Hz, H-5′), 3.60 (2H, br m, H-2′, H-6′a), 3.67 (1H, br m, H-6′b), 4.56 (1H, d, *J*=8.0 Hz, H-1′), 5.09 (1H, t, *J*=9.6 Hz, H-4′), 5.24 (1H, dd, *J*=17.6, 0.9 Hz, H-1$_{trans}$), 5.26 (1H, dd, *J*=10.8, 0.9 Hz, H-1$_{cis}$), 5.29 (1H, t, *J*=9.6 Hz, H-3′), 5.59 (1H, d, *J*=15.9 Hz, H-6), 5.73 (1H, dt, *J*=15.9, 7.4 Hz, H-5), 5.88 (1H, dd, *J*=17.6, 10.8 Hz, H-2), 6.08 (2H, m, H-3″, H-3‴), 8.07 (1H, br s, OOH). ^{13}C-NMR (500 MHz, $CDCl_3$) *δ*: 16.5/16.4 (q, C-4″, C-4‴), 21.1/21.0 (q, C-5″, C-5‴), 24.4 (q, C-10), 24.7 (q, C-8), 25.1 (q, C-9), 44.5 (t, C-4), 62.2 (t, C-6′), 68.9 (d, C-4′), 73.5 (d, C-2′), 74.9 (d, C-3′), 75.0 (d, C-5′), 80.8 (s, C-3), 82.5 (s, C-7), 98.7 (d, C-1′), 116.8 (t, C-1), 126.9 (d, C-5), 127.8/127.3 (s, C-2″, C-2‴), 138.1 (d, C-6), 140.9/139.8 (d, C-3″, C-3‴), 142.5 (d, C-2), 167.6 (s, C-1‴), 168.5 (s, C-1″).

(3*S*)-3-*O*-(3′,4′-Diangeloyl-*β*-D-glucopyranosyloxy)-6-hydroperoxy-3,7-dimethylocta-1,7-diene (**2**): Colorless oil. $[\alpha]_D^{20}$: −5.1° ($CHCl_3$; *c*=0.15); UV $\lambda_{max}^{(MeOH)}$ nm (log *ε*): 218 (4.24); IR $\nu_{max}^{(CHCl_3)}$ cm^{-1}: 3450, 2924, 1719, 1650; HR-ESI-MS *m*/*z*: 535.2494 ([M+Na]$^+$, Calcd for $C_{26}H_{40}O_{10}Na$ 535.2519); ^{1}H-NMR (500 MHz, $CDCl_3$) *δ*: 1.38 (3H, s, H-10), 1.54—1.68 (2H, m, H-4, H-5), 1.72 (3H, s, H-9), 1.81 (3H, br s, H-5‴), 1.84 (3H, br s, H-5″), 1.92 (6H, d, *J*=7.0 Hz, H-4″, H-4‴), 2.52 (1H, br s, 6′-OH), 2.83 (1H, br s, 2′-OH), 3.50 (1H, ddd, *J*=9.6, 4.8, 2.0 Hz, H-5′), 3.60 (1H, br m, H-2′, H-6′a), 3.67 (1H, br m, H-6′b), 4.32 (1H, br t, *J*=5.4 Hz), 4.58 (1H, d, *J*=7.9 Hz, H-1′), 4.99/5.00 (each 1H, s, H-5), 5.07 (1H, t, *J*=9.6 Hz, H-4′), 5.23 (1H, d, *J*=17.3 Hz, H-1$_{trans}$), 5.24 (1H, d, *J*=11.1 Hz, H-1$_{cis}$), 5.33 (1H, t, *J*=9.6 Hz, H-3′), 5.82 (1H, dd, *J*=17.3, 11.1 Hz, H-2), 6.08 (2H, m, H-3″, H-3‴), 8.20 (1H, br s, OOH). ^{13}C-NMR (500 MHz, $CDCl_3$) *δ*: 16.5/16.4 (q, C-4″, C-4‴), 18.2 (q, C-9), 21.0/21.0 (q, C-5″, C-5‴), 24.1 (q, C-10), 25.7 (t, C-4), 37.4 (t, C-5), 62.3 (d, C-6′), 69.0 (d, C-4′), 73.5 (d, C-2′), 74.8 (d, C-3′), 75.0 (d, C-5′), 81.0 (s, C-3), 89.8 (d, C-6), 98.7 (d, C-1′), 114.6 (t, C-8), 116.6 (t, C-1), 128.0/127.4 (s, C-2″, C-2‴(×2)), 140.9/139.4 (d, C-3″, C-3‴), 142.6 (d, C-2), 144.1 (s, C-7), 167.8 (s, C-1‴), 168.4 (s, C-1″).

Reductive Cleavage[16] of 1 and 2 $LiAlH_4$ (10 mg) was added to a solution of **1** (5 mg) in 1 ml of THF. After 12 h reflux, 0.1 ml H_2O, 0.1 ml 25%NaOH and 0.3 ml H_2O were added to reaction mixture, successively. The mixture was filtered and the organic layer concentrated to afford **1a** (2 mg). The reduction of **2** (5 mg) was achieved in the same way to afford **2a** (2 mg).

(3*S*)-3-*O*-*β*-D-Glucopyranosyloxy-7-hydroxy-3,7-dimethylocta-1,5-diene (**1a**): $[\alpha]_D^{20}$: −17.2° (MeOH; *c*=0.1); ^{1}H-NMR (500 MHz, CD_3OD) *δ*: 1.26 (6H, s, H-8, H-9), 1.34 (3H, s, H-10), 2.34 (2H, d, *J*=6.7 Hz, H-4), 3.14—3.29 (4H, m, H-2′, 3′, 4′ , 5′), 3.62 (1H, dd, *J*=11.9, 5.8 Hz, H-6′a), 3.80 (1H, dd, *J*=11.9, 2.4 Hz, H-6′b), 4.36 (1H, d, *J*=7.9 Hz, H-1′), 5.20 (1H, dd, *J*=11.0, 1.2 Hz, H-1a), 5.21 (1H, dd, *J*=17.4, 1.2 Hz, H-1b), 5.60 (1H, d, *J*=15.6 Hz, H-6), 5.66 (1H, dt, *J*=15.6, 6.7 Hz, H-5), 5.93 (1H, dd, *J*=17.4, 11.0 Hz, H-2).

(3*S*)-3-*O*-*β*-D-Glucopyranosyloxy-6-hydroxy-3,7-dimethylocta-1,7-diene (**2a**): $[\alpha]_D^{20}$: −6.2° (MeOH; *c*=0.4); ^{1}H-NMR (500 MHz, CD_3OD): *δ* 1.37 (3H, s, H-10), 1.50—1.66 (4H, m, H-4, 5), 1.69 (3H, s, H-9), 3.14—3.29 (4H, m, H-2′, 3′, 4′, 5′), 3.62 (1H, dd, *J*=11.9, 5.8 Hz, H-6′a), 3.80 (1H, dd, *J*=11.9, 2.4 Hz, H-6′b), 3.96 (1H, t, *J*=6.1 Hz, H-6), 4.80 (1H, br s, H-8a), 4.90 (1H, br s, H-8b), 5.19 (1H, dd, *J*=11.0, 1.5 Hz, H-1a), 5.23 (1H, dd, *J*=17.7, 1.5 Hz, H-1b), 5.91 (1H, dd, *J*=17.7, 11.0 Hz, H-2).

Enzymatic Hydrolysis[13] A solution of **1a** (2 mg) and 10 mg cellulase (Merck) in 1 ml H_2O was stirred at 37 °C for 24 h, followed by solvent partitioning with chloroform and water to afford **1b** (0.8 mg) and D-glucose, respectively. Enzymatic hydrolysis of **2** was achieved in the same way to afford **2b** (0.8 mg) and D-glucose.

(3*S*)-3,7-Dihydroxy-3,7-dimethylocta-1,5-diene (**1b**): $[\alpha]_D^{20}$: +3.6° (MeOH; *c*=0.016) ^{1}H-NMR (500 MHz, $CDCl_3$) *δ*: 1.22 (3H, s, H-10), 1.26 (6H, s, H-8, 9), 2.24 (1H, dd, *J*=14.4, 7.6 Hz, H-4), 2.31 (1H, dd, *J*=13.7, 6.7 Hz, H-4), 5.07 (1H, dd, *J*=10.7, 1.2 Hz, H-1a), 5.21 (1H, dd, *J*=17.4, 1.2 Hz, H-1b), 5.63 (1H, dt, *J*=15.8, 6.7 Hz, H-5), 5.73 (1H, d, *J*=15.8 Hz, H-6), 5.93 (1H, dd, *J*=17.4, 10.7 Hz, H-2).

(3*S*)-3,6-Dihydroxy-3,7-dimethylocta-1,7-diene (**2b**): ^{1}H-NMR (500 MHz, $CDCl_3$) *δ*: 1.30 (3H, s, H-10), 1.72 (3H, s, H-9), 4.08 (1H, t, *J*=5.0 Hz, H-6), 4.86 (1H, br s, H-8a), 4.96 (1H, br s, H-8b), 5.08 (1H, dd, *J*=10.7,

1.2 Hz, H-1a), 5.23 (1H, dd, J=17.4, 1.2 Hz, H-1b), 5.91 (1H, dd, J=17.4, 10.7 Hz, H-2).

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