A New Sterol from Whole Plants of *Eriocaulon sieboldianum*

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Eriocaulon sieboldianum (Eriocaulaceae) is an aquatic annual herb that grows in shallow ponds or paddy fields of Korea, Japan, China, and Africa. *E. sieboldianum* has been used as a traditional medical treatment for headaches and toothaches.[1] Despite its medical potential, no study has reported any chemical components of *E. sieboldianum*. In this paper, we report the isolation of three steroids from *E. sieboldianum*. Three stigmastane-skeleton sterols were isolated from the ethyl acetate (EtOAc) soluble fraction of whole plants of *E. sieboldianum*. From the results of spectroscopic data including NMR, MS, and IR, the chemical structures of the isolates were identified as one new compound, stigmasta-7,22-dien-3β,4β-diol (**compound 3**) and two known compounds, stigmasta-5-en-3β-ol (β-sitosterol, **compound 1**) and stigmasterol 3-*O*-β-D-glucopyranoside (**compound 2**).

Experimental Section

Plant Materials. Whole plants of *E. sieboldianum* were purchased at the Kyeongdong market in Seoul, Korea and identified by Prof. Dae-Keun Kim, Woosuk University, Jeonju, Korea. A voucher specimen (KHU041027) was reserved at the laboratory of natural products chemistry, Kyung Hee University, Yongin, Korea.

Instruments. Melting points were determined on a Fisher-John's Apparatus (Fisher Scientific, Chicago, USA) and uncorrected. Optical rotations were measured on a JASCO P-1010 digital polarimeter (Jasco, Tokyo, Japan). IR spectra were run on a Perkin Elmer Spectrum One FT-IR spectrometer (Perkin Elmer, Norwalk, USA). EIMS and FABMS were recorded on a JEOL JMS 700 (JEOL, Tokyo, Japan). ^{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, California, USA).[2]

Isolation of Sterols. Whole plants of *E. sieboldianum* (4.0 kg) were extracted 3 times for 24 h at room temperature with 80% aqueous methanol (MeOH, 18 L × 2). The MeOH extracts were partitioned with water (2 L), EtOAc (2 L × 2), and *n*-butanol (2 L × 2), successively. The EtOAc extract (ESE, 44 g) was subjected to silica gel (SiO_2, 200 g) column chromatography (c.c.) (6 × 12 cm) and eluted with *n*-hexane-EtOAc (10:1 → 7:1 → 5:1 → 3:1 → 1:1, v/v, 2000 mL) and chloroform ($CHCl_3$)-MeOH (10:1 → 7:1 → 5:1 → 3:1 → 1:1, v/v, 2000 mL), and monitored by thin layer chromatography (TLC) to produce twelve four fractions (ESE1~ESE24). ESE5 [500 mg, Ve/Vt (elution volume/total volume) 0.21-0.25] was eluted with *n*-hexane-EtOAc (2:1, v/v, 2100 mL) through SiO_2 (100 g) c.c. (3 × 12 cm) to yield five fractions (ESE5-1~ESE5-5). ESE5-5 (285 mg, Ve/Vt 0.23-0.34) was subjected to SiO_2 (100 g) c.c. (3 × 10 cm) and eluted with *n*-hexane-EtOAc (7:1, v/v, 1800 mL) to produce six fractions (ESE5-5-1~ESE5-5-6). ESE5-5-5 (63 mg, Ve/Vt 0.77-0.85) was purified by SiO_2 (130 g) c.c. (3 × 16 cm) and eluted with *n*-hexane-EtOAc (5:1 → 3:1, v/v, 1800 mL) to yield compound **1** (6.9 mg, Ve/Vt 0.35-0.43; SiO_2 TLC R_f 0.5, $CHCl_3$-MeOH=15:1). ESE-17 (584 mg, Ve/Vt 0.63-0.68) was eluted with $CHCl_3$-MeOH (7:1, v/v, 2400 mL) through SiO_2 (90 g) c.c. (3 × 10 cm) to produce three fractions (ESE17-1~ESE17-3). ESE-17-2 (180 mg, Ve/Vt 0.32-0.75) was separated by SiO_2 (100 g) c.c. (4 × 20 cm) and eluted with $CHCl_3$-MeOH (12:1, v/v, 1300 mL) to give four fractions (ESE17-2-1~ESE17-2-4). ESE-17-2-2 (68 mg, Ve/Vt 0.20-0.40) was subjected to octadecyl SiO_2 (ODS, 50 g) c.c. (4 × 20 cm) and eluted with acetone-acetonitrile (CH_3CN) (1:1) to yield compound **2** (15 mg, Ve/Vt 0.45-0.53; SiO_2 TLC R_f 0.3, $CHCl_3$-MeOH=7:1). ESE5-4 (299 mg, Ve/Vt 0.19-0.23) was subjected to SiO_2 (150 g) c.c. (4 × 10 cm) and eluted with *n*-hexane-EtOAc (2:1, v/v, 2100 mL) to produce 15 fractions (ESE5-4-1~ESE5-4-15). ESE5-4-3 (200 mg, Ve/Vt 0.30-0.45) was separated by SiO_2 (150 g) c.c. (4 × 10 cm, *n*-hexane-EtOAc = 5:1, v/v, 2200 mL) to produce nine fractions (ESE5-4-3-1~ESE5-4-3-9). ESE5-4-3-2 (66 mg, Ve/Vt 0.33-0.65) was purified using ODS (50 g) c.c. (3 × 10 cm) and eluted with MeOH-H_2O (1:1) to produce compound **3** (59 mg, Ve/Vt 0.80-0.95; ODS TLC R_f 0.25, MeOH-H_2O=10:1).

Stigmasta-5-en-3β-ol (β-sitosterol, compound 1): White powder ($CHCl_3$); mp 140-142 °C; $[\alpha]_D$ −29.2° (*c* 0.2, $CHCl_3$); IR (KBr window in $CHCl_3$) ν_{max} 3400, 1640, 1050, 802, 845, 830 cm^{-1}; EIMS *m/z* 414[M]$^+$ (77), 396 (20), 382 (14), 367 (8), 329 (14), 315 (11).[3-5]

Stigmasterol 3-*O*-β-D-glucopyranoside (compound 2): White powder ($CHCl_3$); mp 298-299 °C; $[\alpha]_D$ −48.2° (*c* 1.0, C_5H_5N); IR (KBr window in $CHCl_3$) ν_{max} 3476, 2944, 1646, 1556, 1370, 1340, 1214, 1168, 1114, 1062, 1026 cm^{-1};

positive FABMS *m/z* 597 $[M+Na]^+$.[5-7]

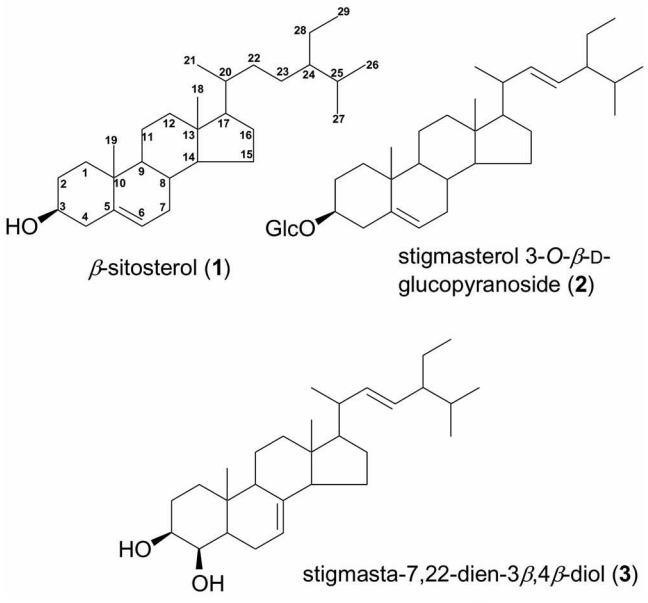
Stigmasta-7,22-dien-3β,4β-diol (compound 3): White powder ($CHCl_3$): mp 183-185 °C; $[\alpha]_D$ −48.2° (*c* 1.0, C_5H_5N) : IR (KBr window in $CHCl_3$) ν_{max} 3420, 1660, 1250, 862, 845, 830 cm^{-1}; EIMS *m/z* 428 $[M]^+$ (64), 417 (100), 410 (52), 377 (23), 351 (12), 316 (10), 287 (82), 271 (38), 253 (42); HREIMS *m/z*: 428.3640 (calcd. 428.3654 for $C_{29}H_{48}O_2$); ^{1}H-NMR (400 MHz, C_5D_5N, δ) 5.35 (1H, br. d, *J* = 3.2 Hz, H-7), 5.20 (1H, dd, *J* = 15.2, 8.8 Hz, H-22), 5.07 (1H, dd, *J* = 15.2, 8.4 Hz, H-23), 4.14 (1H, br. s, H-4), 3.84 (1H, br. d, *J* = 11.2 Hz, H-3), 2.81 (1H, br. dd, *J* = 10.0, 7.6, 3.2 Hz, H-6ax), 2.21 (1H, br. ddd, *J* = 13.2, 11.2, 7.6 Hz, H-2ax), 2.04 (1H, qdd, *J* = 6.4, 8.8, 9.6 Hz, H-20), 2.00 (1H, br. td, *J* = 13.2 Hz, H-12), 1.87 (1H, m, H-14), 1.86 (1H, br. td, *J* = 10.0 Hz, H-6eq), 1.81 (1H, m, H-2eq), 1.69 (1H, m, H-9), 1.58 (1H, m, H-24), 1.40 (1H, br. d, *J* = 7.6 Hz, H-5), 1.39 (3H, s, H-19), 1.26 (1H, m, H-17), 1.08 (3H, d, *J* = 6.4 Hz, H-21), 0.90 (3H, d, *J* = 6.8 Hz, H-26), 0.89 (3H, t, *J* = 7.6 Hz, H-29), 0.85 (3H, d, *J* = 6.4 Hz, H-27), 0.62 (3H, s, H-18); ^{13}C-NMR (100 MHz, C_5D_5N, δ) 139.0 (C-8), 138.7 (C-22), 129.6 (C-23), 118.8 (C-7), 73.7 (C-4), 73.0 (C-3), 56.1 (C-17), 55.5 (C-14), 51.5 (C-24), 51.1 (C-9), 45.2 (C-5), 43.6 (C-13), 41.3 (C-20), 39.8 (C-12), 38.3 (C-1), 34.9 (C-10), 32.3 (C-25), 29.1 (C-16), 27.1 (C-6), 26.7 (C-2), 25.8 (C-28), 23.6 (C-15), 21.8 (C-21), 21.5 (C-11), 21.4 (C-26), 19.3 (C-27), 16.0 (C-19), 12.7 (C-29), 12.4 (C-18).

Results and Discussion

Whole plants of *E. sieboldianum* were extracted with 80% aqueous MeOH, and the concentrated extract was partitioned with EtOAc, *n*-BuOH, and H_2O. From the EtOAc fraction, three steroids were isolated through repeated SiO_2 and ODS column chromatography. Two known compounds, **1** and **2**, were identified as stigmasta-5-en-3β-ol (β-sitosterol, yield: 1.7×10^{-7} %, **1**) and stigmasterol 3-*O*-β-D-glucopyranoside (yield: 3.7×10^{-6} %, **2**), respectively, through the comparison of spectroscopic data with the literature.[3-7] Although stigmasterol, an aglycon of compound **2**, frequently occurs in plants, the glycoside has very rarely been reported from natural sources.

Compound **3** (yield: 1.5×10^{-5} %), a white powder, showed absorbance bands due to hydroxyl (3420 cm^{-1}) and olefin (1660 cm^{-1}) in the IR spectrum. The molecular ion peak $[M]^+$ was detected at *m/z* 428 in the EIMS spectrum and a molecular formula of $C_{29}H_{48}O_2$ was determined by HREIMS ($[M]^+$, *m/z* 428.3640). In the ^{1}H-NMR spectrum (400 MHz, C_5D_5N), three olefin methine signals [δ_H 5.35 (H-7), δ_H 5.20 (H-22), and δ_H 5.07 (H-23)], two oxygenated methine signals [δ_H 4.14 (H-4) and δ_H 3.84 (H-3)], and six methyl signals composed of two singlet [δ_H 1.39 (H-19) and δ_H 0.62 (H-18)], three doublet [δ_H 1.08 (H-21), δ_H 0.90 (H-26), and δ_H 0.85 (H-27)] and a triplet [δ_H 0.89 (H-29)] methyl group were observed. This evidence suggested that this compound was a steroid. The ^{13}C NMR spectrum (100 MHz, C_5D_5N) indicated the presence of 29 carbon signals including two double bonds, consisting of an olefin quaternary carbon signal at δc 139.0 (C-8), and three olefin methine signals [δc 138.7 (C-22), δc 129.6 (C-23), and δc 118.8 (C-7)], two oxygenated methine carbon signals [δc 73.7 (C-4) and δ_C 73.0 (C-3)], and six methyl carbon signals [δc 21.8 (C-21), δc 21.4 (C-26), δc 19.3 (C-27), δc 16.0 (C-19), δc 12.7 (C-29), and δc 12.4 (C-18)]. This information led us to conclude that compound **3** was a stigmastane-type steroid with two hydroxyl groups and two double bonds.

One double bond was demonstrably located between C-22 and C-23 because of their specific coupling patterns (J_3 = 15.2 Hz, *trans*-conformation between H-22 and H-23).[3-5,9] Determination of the final structure of **3**, including the location of the functional group, was accomplished by 2D NMR experiments, including gradient correlated spectroscopy (gCOSY), gradient heteronuclear single quantum correlation (gHSQC), and gradient heteronuclear multiple bonding connectivity (gHMBC). In the gHMBC spectrum, the olefin methine proton signal at δ_H 5.35 (H-7) showed cross peaks with 3 methine carbon signals [δc 51.1 (C-9), δc 45.2 (C-5), and δc 55.5 (C-14)] by J_3 correlation and an olefin quaternary carbon signal at δc 139.0 (C-8) by J_2 correlation. Therefore, the two double bonds were determined to be located between C-7 and C-8, and between C-22 and C-23. Some cross peaks observed in the gCOSY spectrum of compound **3** such as between an olefin proton signal (δ_H 5.35, H-7) and sp^3 methylene proton signals (δ_H 2.81 and δ_H 1.86, H-6); between the H-6 signal and a sp^3 methine proton signal (δ_H 1.40, H-5); between the H-5 signal and an oxygenated methine proton signal (δ_H 4.14, H-4); between the H-4 signal and another oxygenated methine proton signal (δ_H 3.84, H-3); and between the H-3 signal and sp3 methylene signals (δ_H 2.21 and δ_H 1.81, H-2) led to the conclusion that the two hydroxyl groups were at C-3 and C-4. The results indicated that two hydroxyl groups were



β-sitosterol (**1**)

stigmasterol 3-*O*-β-D-glucopyranoside (**2**)

stigmasta-7,22-dien-3β,4β-diol (**3**)

Figure 1. Chemical structures of sterols isolated from whole plants of *Eriocaulon siboldianum*.

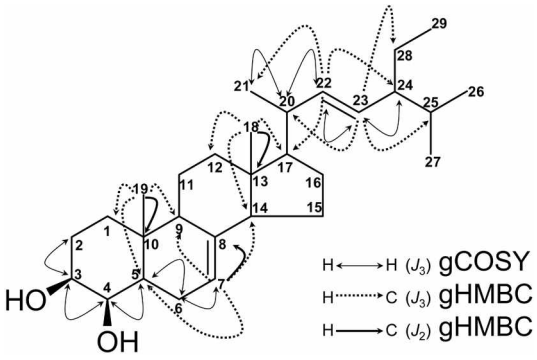


Figure 2. ^{1}H-^{1}H coupling (J_3) and ^{1}H-^{13}C long-range correlations (J_2 and J_3) observed in the gCOSY and gHMBC spectra of compound **3**. The two-way arrows indicate the coupling (J_3) between proton and proton signals in the gCOSY spectrum, and dotted-line and solid-line arrows indicate the long-range correlations of J_2 and J_3, respectively, between proton and carbon signals in the gHMBC spectrum.

located at C-3 and C-4 of the A ring of stigmastane-sterol (Figure 2). Both of the hydroxyl groups were revealed to have β-conformation from the coupling patterns of br. s between H-4 (equatorial, α-conformation) and H-5 (axial, α-conformation), br. s between H-3 (axial, α-conformation) and H-4 (equatorial, α-conformation), and doublet (J = 11.2 Hz) between H-2ax (β-conformation) and H-3 (axial, α-conformation).[8] The singlet methyl signal at δ_H 1.39 (H-19) showed cross peaks with two methine carbon signals [δc 45.2 (C-5) and δc 51.1 (C-9)], a methylene carbon signal at δc 38.3 (C-1) by J_3 correlation, and a quaternary carbon signal at 34.9 (C-10) by J_2 correlation in the gHMBC spectrum. Another singlet methyl signal at δ_H 0.62 (H-18) exhibited cross peaks with two methine carbon signals [δc 55.5 (C-14) and δc 56.1 (C-17)], a methylene carbon signal at δc 39.8 (C-12) by J_3 correlation, and a quaternary carbon signal at 43.6 (C-13) by J_2 correlation in the gHMBC spectrum. The new compound was identified as stigmasta-7,22-dien-3β,4β-diol. The comparison of spectroscopic and physicochemical data of compound **3** with those of several known stigmastane sterols having two hydroxyl and two olefin groups such as stigmasta-7,25-dien-2α,3β-diol,[10] stigmasta-5,22-dien-3β,7α-diol,[9,11] stigmasta-5,22-dien-3β,7β-diol,[9,11] 24-methylene-27-methylcholesta-5-en-3β,7α-diol,[12] 24-methylene-27-methylcholesta-5-en-3β,7β-diol,[12] recursterol [stigmasta-7,9(11)-diene-3β,6α-diol],[13] stigmasta-5,22-diene-3β,25-diol,[14] and stigmast-5-en-3α,26-diol[15] led to confirmation of the structure. Moreover, the identified sterols **1** and **2** were also isolated for the first time from *E. sieboldianum*.

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