

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-3 β ,4 β -diol (**compound 3**) and two known compounds, stigmasta-5-en-3 β -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). ¹H-NMR (400 MHz), and ¹³C-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 \times 2). The MeOH extracts were partitioned with water (2 L), EtOAc (2 L \times 2), and *n*-butanol (2 L \times 2), successively. The EtOAc extract (ESE, 44 g) was subjected to silica gel (SiO₂, 200 g) column chromatography (c.c.) (6 \times 12 cm) and eluted with *n*-hexane-EtOAc (10:1 \rightarrow 7:1 \rightarrow 5:1 \rightarrow 3:1 \rightarrow 1:1, v/v, 2000 mL) and

chloroform (CHCl₃)-MeOH (10:1 \rightarrow 7:1 \rightarrow 5:1 \rightarrow 3:1 \rightarrow 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 SiO₂ (100 g) c.c. (3 \times 12 cm) to yield five fractions (ESE5-1~ESE5-5). ESE5-5 (285 mg, Ve/Vt 0.23-0.34) was subjected to SiO₂ (100 g) c.c. (3 \times 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 SiO₂ (130 g) c.c. (3 \times 16 cm) and eluted with *n*-hexane-EtOAc (5:1 \rightarrow 3:1, v/v, 1800 mL) to yield compound **1** (6.9 mg, Ve/Vt 0.35-0.43; SiO₂ TLC R_f 0.5, CHCl₃-MeOH=15:1). ESE-17 (584 mg, Ve/Vt 0.63-0.68) was eluted with CHCl₃-MeOH (7:1, v/v, 2400 mL) through SiO₂ (90 g) c.c. (3 \times 10 cm) to produce three fractions (ESE17-1~ESE17-3). ESE-17-2 (180 mg, Ve/Vt 0.32-0.75) was separated by SiO₂ (100 g) c.c. (4 \times 20 cm) and eluted with CHCl₃-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 SiO₂ (ODS, 50 g) c.c. (4 \times 20 cm) and eluted with acetone-acetonitrile (CH₃CN) (1:1) to yield compound **2** (15 mg, Ve/Vt 0.45-0.53; SiO₂ TLC R_f 0.3, CHCl₃-MeOH=7:1). ESE5-4 (299 mg, Ve/Vt 0.19-0.23) was subjected to SiO₂ (150 g) c.c. (4 \times 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 SiO₂ (150 g) c.c. (4 \times 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 \times 10 cm) and eluted with MeOH-H₂O (1:1) to produce compound **3** (59 mg, Ve/Vt 0.80-0.95; ODS TLC R_f 0.25, MeOH-H₂O=10:1).

Stigmasta-5-en-3 β -ol (β -sitosterol, compound 1): White powder (CHCl₃); mp 140-142 °C; [α]_D -29.2° (c 0.2, CHCl₃); IR (KBr window in CHCl₃) 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 (CHCl₃); mp 298-299 °C; [α]_D -48.2° (c 1.0, C₃H₅N); IR (KBr window in CHCl₃) 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-3 β ,4 β -diol (compound 3): White powder (CHCl₃); mp 183–185 °C; $[\alpha]_D^{25}$ –48.2° (c 1.0, C₅H₅N); IR (KBr window in CHCl₃) 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 C₂₉H₄₈O₂); ¹H-NMR (400 MHz, C₅D₅N, 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); ¹³C-NMR (100 MHz, C₅D₅N, 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 H₂O. From the EtOAc fraction, three steroids were isolated through repeated SiO₂ and ODS column chromatography. Two known compounds, **1** and **2**, were identified as stigmasta-5-en-3 β -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 C₂₉H₄₈O₂ was determined by HREIMS ($[M]^+$, m/z 428.3640). In the ¹H-NMR spectrum (400 MHz, C₅D₅N), 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 ¹³C NMR spectrum (100 MHz, C₅D₅N) indicated the presence of 29 carbon signals including two double bonds, consisting of an olefin quaternary carbon

signal at δ_C 139.0 (C-8), and three olefin methine signals [δ_C 138.7 (C-22), δ_C 129.6 (C-23), and δ_C 118.8 (C-7)], two oxygenated methine carbon signals [δ_C 73.7 (C-4) and δ_C 73.0 (C-3)], and six methyl carbon signals [δ_C 21.8 (C-21), δ_C 21.4 (C-26), δ_C 19.3 (C-27), δ_C 16.0 (C-19), δ_C 12.7 (C-29), and δ_C 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 [δ_C 51.1 (C-9), δ_C 45.2 (C-5), and δ_C 55.5 (C-14)] by J_3 correlation and an olefin quaternary carbon signal at δ_C 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 sp³ methylene proton signals (δ_H 2.81 and δ_H 1.86, H-6); between the H-6 signal and a sp³ 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

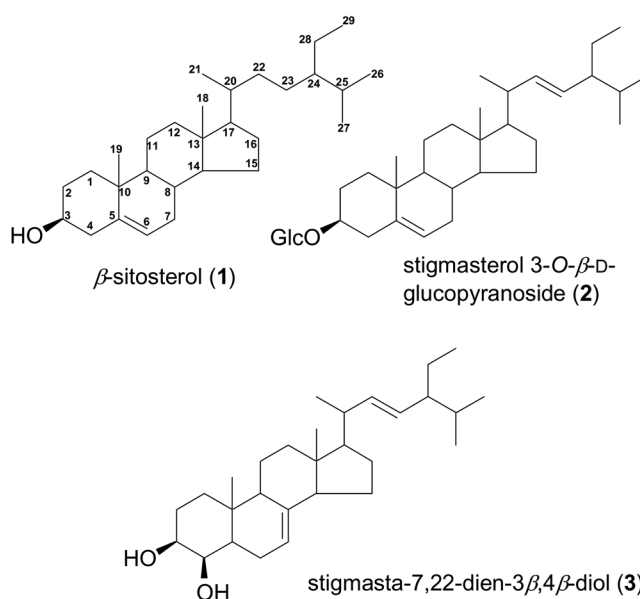


Figure 1. Chemical structures of sterols isolated from whole plants of *Eriocaulon sieboldianum*.

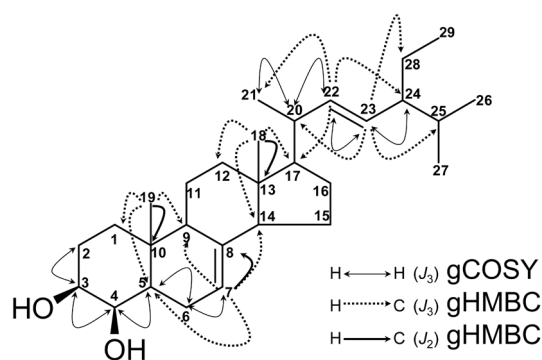


Figure 2. ^1H - ^1H coupling (J_2 and J_3) and ^1H - ^{13}C long-range correlations (J_2 and J_3) observed in the gCOSY and gHMBC spectra of compound **3**. The two-way arrows indicate the coupling (J_3) between proton and proton signals in the gCOSY spectrum, and dotted-line and solid-line arrows indicate 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 [δ_{C} 45.2 (C-5) and δ_{C} 51.1 (C-9)], a methylene carbon signal at δ_{C} 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 [δ_{C} 55.5 (C-14) and δ_{C} 56.1 (C-17)], a methylene carbon signal at δ_{C} 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-3 β ,4 β -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-2 α ,3 β -diol,¹⁰ stigmasta-5,22-dien-3 β ,7 α -diol,^{9,11} stigmasta-5,22-dien-3 β ,7 β -diol,^{9,11} 24-methylene-27-methylcholesta-5-en-3 β ,7 α -diol,¹²

24-methylene-27-methylcholesta-5-en-3 β ,7 β -diol,¹² recursterol [stigmasta-7,9(11)-diene-3 β ,6 α -diol],¹³ stigmasta-5,22-diene-3 β ,25-diol,¹⁴ and stigmast-5-en-3 α ,26-diol¹⁵ 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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