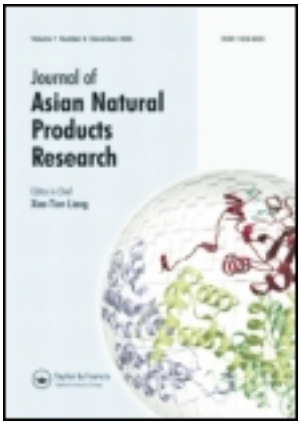

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Chemical constituents of *Equisetum debile*

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Three new compounds, debilitriol (**1**), debilignanoside (**2**), and equisetumine (**3**), along with nine known compounds, were isolated from the whole plant of *Equisetum debile*. Their structures were elucidated by spectral and chemical methods.

Keywords: *Equisetum debile*; debilitriol; debilignanoside; equisetumine

1. Introduction

Equisetum debile Roxb. (Equisetaceae) is a fern distributed widely in the south of China, Southeast Asia, and India, which has a long history as a Chinese folk medicine for the treatment of acute hepatitis, urethritis, conjunctivitis, and diarrhea [1]. Pharmacological studies disclosed that the alcohol extraction of *E. debile* could decrease the level of triglyceride and total cholesterol in rats and triglyceride in rabbits [2]. A few studies on *E. debile* [3–5] reported the existence of megastigmane glucosides, phenol glycosides, lignan glycosides, and flavonoids and their glycosides. As a continuous interest in Chinese folk medicinal plants, we investigated systematically the chemical constituents of the title plant, and obtained 12 compounds, including a new phenylhexane debilitriol (**1**), a new 8-*O*-4′ neolignan glucoside debilignanoside (**2**), and a new alkaloid equisetumine (**3**) (Figure 1). The structures of these new compounds were determined to be (rel-2*R*,4*S*)-6-(3-hydroxy-4-methoxyphenyl)hexane-1,2,4-triol (**1**), (7*R*,8*S*)-guaiaglycerol-β-coniferyl ether 9-*O*-β-D-glucopyranoside (**2**), and 2-pentyl-1,5,9-triazacyclotridecan-4-one (**3**), respectively, on the basis of spectral evidence.

2. Results and discussion

Debilitriol (**1**) was obtained as viscous syrup. Its negative and positive ESI-MS gave pseudomolecular ion peaks at *m/z* 255 $[M - H]^-$ and 279 $[M + Na]^+$, respectively, in agreement with the molecular formula $C_{13}H_{20}O_5$, which was also confirmed by the HR-ESI-MS at *m/z* 279.1205 $[M + H]^+$.

The ^1H, ^{13}C NMR, and HSQC spectra of **1** showed ^1H and ^{13}C signals for a 1,3,4-trisubstituted aromatic ring at δ_H 6.79 (1H, d, $J = 8.2$ Hz), 6.67 (1H, d, $J = 2.0$ Hz), and 6.63 (1H, dd, $J = 8.2$, 2.0 Hz), as well as δ_C 147.9 (s), 147.7 (s), 137.3 (s), 121.0 (d), 117.1 (d), and 113.5 (d), a methoxyl at δ_H 3.81 (3H, s) and δ_C 57.1, four methylenes at δ_C 68.4, 42.4, 41.8, and

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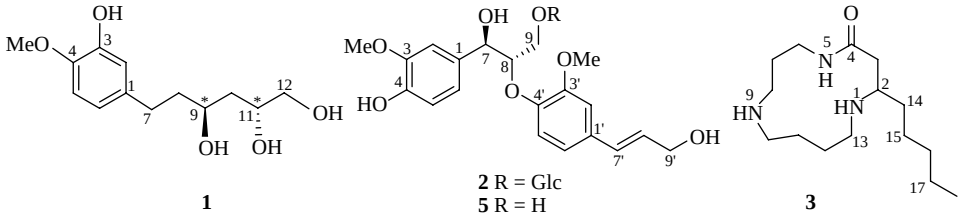


Figure 1. Structures of compounds **1**–**4**.

32.9, and two oxygen-bearing methines at δ_C 69.1 and 70.9, indicating a phenylhexane derivative. The aliphatic C_6 moiety was elucidated to be $-CH_2CH_2CH(OH)CH_2CH(OH)CH_2OH$ based on the $^1H-^1H$ COSY cross-peaks of H_2-12/H-11, H-11/H_2-10, H_2-10/H-9, H-9/H_2-8, and H_2-8/H_2-7 (Figure 2). The MeO was assigned on C-4 of the aromatic ring by HMBC cross-peaks of MeO/C-4, H-2/C-4, and H-6/C-4 (Figure 2). The relative stereochemistry of **1** was determined to be 9,11-*trans* by the ^1H signals of 10-CH_2 and appeared as triplet (δ 1.52, t, $J = 6.2$ Hz) rather than two sets of multiplets [6]. The structure of **1** was, therefore, established to be (rel-2*R*,4*S*)-6-(3-hydroxy-4-methoxyphenyl)hexane-1,2,4-triol.

Debilignanoside (**2**) was obtained as a white amorphous powder. It had the molecular formula of $C_{26}H_{34}O_{12}$ as indicated by the HR-ESI-MS. The acid hydrolysis and GC analysis, together with NMR signals at δ_H 4.27 (d, $J = 7.7$ Hz) and δ_C 105.2 (d), 78.4 (2C, d), 75.7 (d), 72.0 (d), and 63.1(t), revealed a β-D-glucopyranosyl unit in the molecule of **2**.

In the ^1H NMR spectrum of **2**, the aglycone moiety was observed to contain two 1,3,4-trisubstituted aromatic rings [δ 7.06 (d, $J = 1.8$ Hz), 6.88 (dd, $J = 8.1$, 1.8 Hz), and 6.74 (d, $J = 8.1$ Hz); 7.02 (d, $J = 1.7$ Hz), 6.98 (d, $J = 8.3$ Hz), and 6.90 (dd, $J = 8.3$, 1.7 Hz)], a pair of *E*-double bonds (δ 6.51 and 6.24, $J_{gemical} = 15.7$ Hz), and two methoxyls (δ 3.85 and 3.81, each 3H, s). The ^{13}C NMR spectrum of **2** showed 20 carbon signals (2 × OCH_3, 2 × OCH_2, 2 × OCH, 8 × =CH, and 6 × =C) for the aglycone, which were almost superposed with those of guaiacylglycerol-β-coniferyl ether (**4**) [7] except for a downfield shift of C-9 ($\Delta\delta_{2-4} = 7.0$) due to the glycosidation effect. The above evidence indicated the structure of **2** to be a 9-*O*-β-D-glucopyranoside of **4**, which was further substantiated by the HMBC experiment (Figure 2). The absolute configuration of **2** was determined to be 7*R*,8*S* on the basis of positive CD curve in the range of 210–250 nm [8] and the coupling constant of 5.4 Hz between H-7 and H-8 [9].

Equisetumine (**3**) was obtained as yellowish oil with positive Dragendorff's reaction. Its molecular formula was

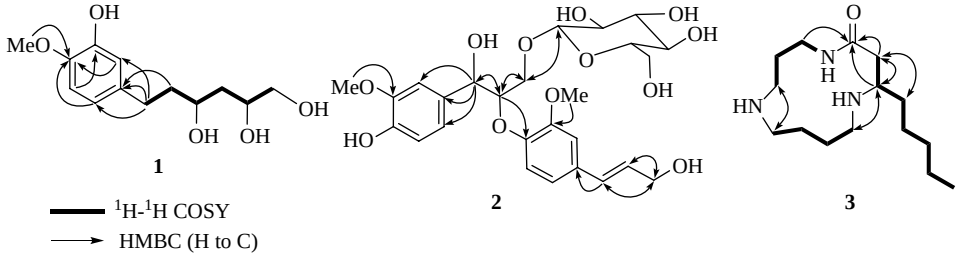


Figure 2. Selected $^1H-^1H$ COSY and HMBC correlations of **1**–**3**.

determined to be $C_{15}H_{31}N_3O$ by the HR-EI-MS. The IR bands exhibited absorptions of NH (3408 and 3261 cm^{-1}) and CONH (1651 cm^{-1}). The ^{13}C NMR spectrum displayed 15 carbon signals ($1 \times CH_3$, $12 \times CH_2$, $1 \times CH$, and $1 \times CON$), indicating a monocyclic alkaloid. The HSQC and $^1H-^1H$ COSY spectra of **3** (Figure 2) revealed three isolated spin systems: (i) $-^5NH^6CH_2^7CH_2^8CH_2-$, (ii) $-^{10}CH_2^{11}CH_2^{12}CH_2^{13}CH_2-$, and (iii) $-^3CH_2^2CH(NH)(^{14}CH_2^{15}CH_2^{16}CH_2^{17}CH_2^{18}CH_3)-$. In the HMBC spectrum, marked correlations were observed at H_2-6/C-4, H_2-3/C-4, H-2/C-13, and H_2-8/C-10 (Figure 2), indicating the three fragments to be connected through two *N*-atoms and an amide group to form a monocyclic structure.

Equisetumine is a rare macrocyclic polyamine alkaloid, biogenetically, which might be derived from spermidine (**5**) and γ-hydroxycaprylic acid (**6**) via a condensation reaction (Figure 3). Spermidine alkaloids were also reported from *Equisetum palustre* L. [10], *Clerodendrum myricoides* [11], *Meehania fargesii* [12], *Dracocephalum tanguticum* [13], etc.

These known compounds were identified to be coumaric acid, *p*-hydroxybenzoic acid, ferulic acid, 5-hydroxymethyl-2-furfuraldehyde [14], equisetumoside B [15], guaiacylglycerol-β-coniferyl ether (**4**) [7], (+)-lariciresinol 9-*O*-β-D-glucopyranoside [16], (+)-isolariciresinol 3a-*O*-β-D-glucopyranoside [17], and thymidine [18] by comparison with published physical and spectroscopic data and/or with authentic samples.

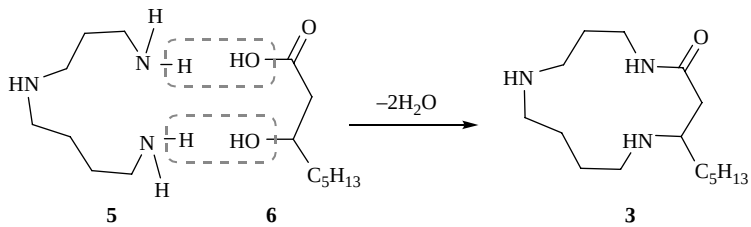
3. Experimental

3.1 General experimental procedures

Optical rotations were determined on a PerkinElmer 341 polarimeter. The IR spectra were recorded on a Nicolet-Magna-750-FT-IR spectrometer. CD analysis was carried out on a Jasco J-810 spectropolarimeter. The NMR spectra were recorded on a Bruker AV-400 spectrometer with TMS as internal standard. ESI-MS and HR-ESI-MS were obtained on an Esquire 3000plus and a Q-TOF-Ultima mass spectrometers, respectively. EI-MS and HR-EI-MS spectra were obtained on a MAT-95 mass spectrometer. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd, Qingdao, China), D-1400 macroporous resin (Yangzhou Pharmaceutical Factory, Yangzhou, China), RP-18 silica gel (150–200 mesh, Fuji Silysia Chemical Ltd, Aichi, Japan), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) were used for column chromatography (CC). Silica gel $HSGF_{254}$ (Yantai Jiangyou Guijiao Kaifa, Co., Yantai, China) was used for TLC. GC analysis was carried out on a PerkinElmer Sigma-115 gas chromatograph.

3.2 Plant material

The whole plants of *E. debile* were collected from Anji County, Zhejiang Province, China, in October 2009. The



5 **6** **3**

Figure 3. Possible biogenetic pathway of **3**.

plant was identified by Dr X.Q. Tan of PLA 98th Hospital. A voucher sample (No. 20091013) is deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

3.3 Extraction and isolation

The dried whole plant of *E. debile* (5 kg) was powdered and extracted with 25 liters 95% EtOH for three times. The concentrated extract was suspended in water and partitioned orderly with petroleum ether (PE), EtOAc, and BuOH. The EtOAc-soluble fraction (20 g) was subjected to CC of silica gel with gradient $CHCl_3$–MeOH (50:1, 25:1, 10:1, and 5:1) as eluents to give subfractions E1–E4. Fraction E1 offered **3** (40 mg) by repeated silica gel CC (PE–acetone, 5:1). E2 furnished 5-hydroxymethyl-2-furfuraldehyde (90 mg) after purification of repeated silica gel CC ($CHCl_3$–MeOH, 20:1). Fraction E3 was separated into subfractions E3.1–E3.5 through a silica gel column with gradient $CHCl_3$–MeOH (15:1 → 5:1) as eluents. E3.1 and E3.2 yielded solids, which were recrystallized to afford coumaric acid (18 mg) and *p*-hydroxybenzoic acid (13 mg), respectively. Compounds **4** (15 mg), **1** (5 mg), and ferulic acid (7 mg) were obtained from E3.3 after repeated CC of silica gel ($CHCl_3$–MeOH, 10:1).

BuOH fraction (37 g) was subjected to macroporous resin CC with gradient EtOH–H_2O (0, 20, 40, 60, and 95%) as eluents to gain B1–B5. Fraction B3 was separated into subfractions B3.1–3.5 by CC of RP-18 (60% MeOH). Fr.B3.2 yielded (+)-lariciresinol 9-*O*-β-D-glucopyranoside (25 mg) as solids. Fr.B3.3 offered **2** (18 mg) and equisetumoside B (11 mg), and Fr.B3.4 afforded (+)-isolariciresinol 3a-*O*-β-D-glucopyranoside (8 mg) after purification of Sephadex LH-20 CC (75% MeOH), respectively. Fraction B4 yielded thymidine (42 mg) as needles.

3.3.1 Debilitriol (*1*)

Colorless viscous syrup. $[\alpha]_D^{24}$ −5.3 ($c = 0.417$, MeOH). IR (KBr) ν_{max}: 3419, 1604, 1452, and 1512 cm^{-1}; ^1H NMR spectral data (400 MHz, CD_3OD): δ 6.79 (d, $J = 8.2$ Hz, H-5), 6.67 (d, $J = 2.0$ Hz, H-2), 6.63 (dd, $J = 8.2$, 2.0 Hz, H-6), 3.84 (m, H-11), 3.80 (m, H-9), 3.81 (s, 4-OMe), 3.47 (dd, $J = 9.6$, 5.0 Hz, H_a-12), 3.43 (dd, $J = 9.6$, 6.5 Hz, H_b-12), 2.66 (dt, $J = 9.7$, 7.7 Hz, H_a-7), 2.52 (dt, $J = 9.7$, 7.7 Hz, H_b-7), 1.70 (td, $J = 7.7$, 6.3 Hz, H_2-8), and 1.52 (t, $J = 6.2$ Hz, H_2-10). ^{13}C NMR spectral data (100 MHz, CD_3OD): δ 147.9 (C-3, s), 147.7 (C-4, s), 137.3 (C-1, s), 121.0 (C-6, d), 117.1 (C-2, d), 113.5 (C-5, d), 70.9 (C-11, d), 69.1 (C-9, d), 68.4 (C-12, t), 57.1 (OMe, q), 42.4 (C-10, t), 41.8 (C-8, t), and 32.9 (C-7, t). ESI-MS: *m/z* 279 $[M + Na]^+$, 255 $[M - H]^-$. HR-ESI-MS: *m/z* 279.1205 $[M + Na]^+$ (calcd for $C_{13}H_{20}O_5Na$, 279.1208).

3.3.2 Debilignanoside (*2*)

White amorphous powder. $[\alpha]_D^{20}$ +5.5 ($c = 0.583$, MeOH). CD (MeOH): $\Delta\varepsilon_{215\,nm}$ + 2.5, $\Delta\varepsilon_{225\,nm}$ + 4.7, $\Delta\varepsilon_{240\,nm}$ + 3.5, $\Delta\varepsilon_{248\,nm}$ + 4.7. IR (KBr) ν_{max}: 3419 (OH), 1605, 1452, and 1512 (Ar) cm^{-1}. ^1H NMR spectral data (400 MHz, CD_3OD): δ 7.06 (d, $J = 1.8$ Hz, H-2), 7.02 (d, $J = 1.7$ Hz, H-2′), 6.98 (d, $J = 8.3$ Hz, H-5′), 6.90 (dd, $J = 8.3$, 1.7 Hz, H-6′), 6.88 (dd, $J = 8.1$, 1.8 Hz, H-6), 6.74 (d, $J = 8.1$ Hz, H-5), 6.51 (br d, $J = 15.7$ Hz, H-7′), 6.24 (dt, $J = 15.7$, 5.6 Hz, H-8′), 4.97 (d, $J = 5.4$ Hz, H-7), 4.49 (td, $J = 6.4$, 5.4 Hz, H-8), 4.27 (d, 7.7 Hz, H-1″), 4.19 (2H, dd, $J = 5.6$, 1.1 Hz, H_2-9′), 3.85 (3H, s, MeO-3′), 3.83 (2H, d, $J = 6.4$, H_2-9), 3.81 (3H, s, MeO-3), 3.78 (dd, $J = 11.6$, 1.9 Hz, H_a-6″), 3.64 (dd, $J = 11.6$, 5.5 Hz, H_b-6″), and 3.40–3.15 (4H, m, H-3″, H-4″, H-2″, and H-5″). ^{13}C NMR spectral data (CD_3OD, 100 MHz): δ 152.1 (C-3′, s), 149.3 (C-4′, s), 149.2 (C-3, s), 147.5 (C-4, s), 134.2 (C-1, s), 133.5 (C-1′, s),

131.9 (C-7′, d), 129.0 (C-8′, d), 121.2 (C-6, d), 121.1 (C-6′, d), 118.9 (C-5′, d), 116.3 (C-5, d), 112.4 (C-2, d), 111.7 (C-2′, d), 105.2 (C-1″, d), 85.2 (C-8, d), 78.4 (C-3″ and C-5″, each d), 75.7 (C-2″, d), 74.1 (C-7, d), 72.0 (C-4″, d), 69.5 (C-9, t), 64.2 (C-9′, t), 63.1 (C-6″, t), 57.0 (MeO-3′, q), and 56.9 (MeO-3, q). ESI-MS: *m/z* 561 $[M + Na]^+$, 537 $[M - H]^-$. HR-ESI-MS: *m/z* 561.1949 $[M + Na]^+$ (calcd for $C_{26}H_{34}O_{12}Na$, 561.1948).

3.3.3 *Equisetumine (**3**)*

Yellowish oil. $[\alpha]_D^{20}$ +18 ($c = 0.7$, $CHCl_3$). IR (KBr) ν_{max}: 3408, 3261, 3076, 2929, 2658, 1651, 1556, 1441, 1367, 1178, 1066, and 629 cm^{-1}. ^1H NMR spectral data ($CDCl_3$, 400 MHz): δ 8.39 (1H, t, $J = 5.7$ Hz, NH-5), 3.73 (1H, m, H_a-6), 3.20–3.10 (4H, m, H-2, H_b-6, and H_2-8), 2.96 (1H, m, H_a-13), 2.92 (1H, m, H_a-10), 2.82 (1H, m, H_b-10), 2.62 (1H, m, H_b-13), 2.42 (1H, m, H_a-3), 2.38 (1H, m, H_b-3), 2.20–1.90 (3H, m, H_2-7 and H_a-11), 1.90–1.75 (4H, m, H_b-11, H_2-12, and H_a-14), 1.50–1.20 (7H, H_b-14, H_2-17, H_2-16, and H_2-15), and 0.87 (3H, t, $J = 6.8$ Hz, Me-18). ^{13}C NMR spectral data ($CDCl_3$, 100 MHz): δ 173.0 (s, C-4), 55.3 (d, C-2), 49.3 (t, C-10), 48.9 (t, C-8), 44.2 (t, C-13), 41.6 (t, C-3), 38.4 (t, C-6), 31.94 (t, C-16), 31.90 (t, C-14), 26.7 (t, C-7), 26.1 (t, C-11), 26.0 (t, C-12), 24.7 (t, C-15), 22.6 (t, C-17), and 14.0 (q, C-18). EI-MS: *m/z* 269 (M^+, 11), 252 (19), 226 (28), 198 (100), 155 (43), 140 (37), 126 (37), 100 (44), and 84 (70). HR-EI-MS: *m/z* 269.2455 $[M]^+$ (calcd for $C_{15}H_{31}N_3O$, 269.2467).

3.4 *Acid hydrolysis of 2*

Compound **2** (1 mg) was refluxed in 2 N HCl–dioxane (1:1 v/v, 2 ml) for 2 h. On cooling, the mixture was neutralized with $NaHCO_3$. After extraction with EtOAc, the aqueous layer was concentrated by blowing with N_2. The residue was purified by CC of Sephadex LH-20 (MeOH–H_2O 1:1, v/v) to give the sugar moiety. The purified sugar and standard D-glucose (Sigma-Aldrich, St Louis, MO, USA) were treated with L-cysteine methyl ester hydrochloride (2 mg) in pyridine (1 ml) at 60°C for 1 h. Then, the solution was treated with *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (0.02 ml) at 60°C for 1 h. The supernatant was subjected to GC analysis to identify the sugars. Conditions for GC were capillary column, DB5-MS (30 m × 0.25 mm × 0.25 μm), oven temperature program, 180–300°C at 6°C/min; injection temperature 350°C; carrier gas, He at 1 ml/min. D-Glucose was detected by comparing its retention time with that of the authentic sample ($t_R = 12.30$ min).

Acknowledgements

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