Debilosides A–C: Three New Megastigmane Glucosides from *Equisetum debile*

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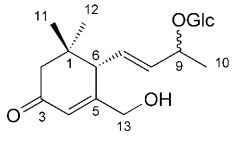
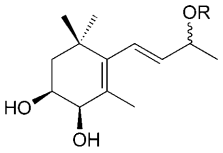
Debilosides A–C (**1**–**3**), three new megastigmane glucosides, were isolated from the whole plant of *Equisetum debile*, together with the four known constituents blumenol A, corchoinoside C, sammangaoside A, and (3*S*,5*R*,6*R*,7*E*,9*S*)-9-[(*β*-D-glucopyranosyl)oxy]megastigm-7-ene-3,5,6-triol (**4**). Their structures were elucidated on the basis of in-depth spectroscopic analyses including 2D-NMR techniques.

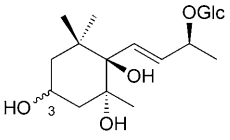
Introduction. – *Equisetum debile* ROXB. (Equisetaceae), distributed widely in Southeast China, has a long history as a Chinese folk medicine for the treatment of acute hepatitis, urethritis, and conjunctivitis, as well as against diarrhea [1]. To our knowledge, no previous study on the chemical constituents of this plant was reported. In the course of our phytochemical investigations of *E. debile*, we now report the isolation and characterization of three new megastigmane glucosides, debilosides A–C (**1**–**3**), and of four known norisoprenoids, blumenol A [2], corchoinoside C [3], sammangaoside A [4], and (3*S*,5*R*,6*R*,7*E*,9*S*)-9-[(*β*-D-glucopyranosyl)oxy]megastigm-7-ene-3,5,6-triol (**4**) [5].



1 (9*S*)
1a (9*R*)



2 (9*S*) R = Glc
2a (9*R*) R = Glc-(2→1)-Api



3 (3*R*)
4 (3*S*)

Glc = *β*-D-Glucopyranosyl, Api = apiosyl

Results and Discussion. – Debiloside A (**1**) was obtained as a white, amorphous powder. Its ESI mass spectrum displayed the $[M+H]^+$ ion peak at *m/z* 387, in accord with the molecular formula $C_{19}H_{30}O_8$. The ^{1}H- and ^{13}C-NMR spectra of **1** (*Table*) indicated a *β*-glucopyranosyl (Glc) unit (*δ*(C) 101.8, 78.8, 78.6, 75.5, 72.2, 63.3; *δ*(H) 4.27 (*d*, *J* = 8.0 Hz, 1 H)). The aglycone part of the NMR spectra indicated three Me groups (*δ*(H) 1.01 (*s*); 1.03 (*s*); 1.28 (*d*, *J* = 6.6 Hz)), a tertiary CH_2OH group (*δ*(H) 4.18,

Table. *^{1}H-NMR Data of* **1**–**3**. At 400 MHz in CD_3OD; δ in ppm, J in Hz. Arbitrary atom numbering.

	1	**2**	**3**
CH_2(2)	2.10 (*d*, *J* = 16.8), 2.55 (*d*, *J* = 16.8)	1.43 (*dd*, *J* = 12.2, 3.4), 1.76 (*t*, *J* = 12.2)	1.56 (*dd*, *J* = 13.8, 5.0), 1.79 (*dd*, *J* = 13.8, 3.6)
H–C(3)	–	3.75 (*dt*, *J* = 12.0, 3.9)	4.08 (*tt*, *J* = 5.0, 3.6)
H–C(4) or CH_2(4)	6.15 (br. *s*)	3.81 (br. *d*, *J* = 3.9)	1.72 (*dd*, *J* = 13.9, 5.0), 2.04 (*dd*, *J* = 13.9, 3.6)
H–C(6)	2.71 (*d*, *J* = 9.3)	–	–
H–C(7)	5.78 (*dd*, *J* = 15.6, 9.3)	6.14 (*d*, *J* = 15.8)	6.22 (*d*, *J* = 15.8)
H–C(8)	5.57 (*dd*, *J* = 15.6, 7.5)	5.41 (*dd*, *J* = 15.8, 8.0)	5.59 (*dd*, *J* = 15.8, 8.4)
H–C(9)	4.46 (*dq*, *J* = 7.5, 6.6)	4.52 (*dq*, *J* = 8.0, 6.4)	4.52 (*dq*, *J* = 8.4, 6.5)
Me(10)	1.28 (*d*, *J* = 6.6)	1.31 (*d*, *J* = 6.4)	1.30 (*d*, *J* = 6.5)
Me(11)	1.01 (*s*)	1.07 (*s*)	1.15 (*s*)
Me(12)	1.03 (*s*)	1.05 (*s*)	0.90 (*s*)
CH_2(13) or Me(13)	4.18 (*dd*, *J* = 17.2, 1.5), 4.10 (*dd*, *J* = 17.2, 1.5)	1.85 (*s*)	1.18 (*s*)
H–C(1′)	4.27 (*d*, *J* = 8.0)	4.38 (*d*, *J* = 7.7)	4.39 (*d*, *J* = 8.0)
H–C(2′,3′,4′,5′)	3.1–3.3 (*m*, 4 H)	3.1–3.3 (*m*, 4 H)	3.0–3.3 (*m*, 4 H)
CH_2(6′)	3.84 (*dd*, *J* = 11.8, 2.2), 3.63 (*dd*, *J* = 11.8, 5.5)	3.84 (*dd*, *J* = 11.8, 2.2), 3.63 (*dd*, *J* = 11.8, 5.5)	3.86 (*dd*, *J* = 11.8, 2.2), 3.65 (*dd*, *J* = 11.8, 5.5)

4.10 (2*dd*, *J* = 17.2, 1.5 Hz each)), three olefinic H-atoms (δ(H) 6.15 (br. *s*); 5.78 (*dd*, *J* = 15.6, 9.3 Hz); 5.57 (*dd*, *J* = 15.6, 7.5 Hz)), one *AB*-type CH_2 resonance (δ(H) 2.55, 2.10 (2*d*, *J* = 16.8 Hz each)), and two CH (δ(H) 2.71 (*d*, *J* = 9.3 Hz); 4.46 (*dq*, *J* = 7.5, 6.6 Hz)). The ^{13}C-NMR spectrum of **1** showed 13 C-atom signals: three Me, two CH_2, and five CH, as well as three quaternary C-atoms for the aglycone part. These data indicated a megastigma-4,7-dien-3-one [6].

The ^{13}C-NMR data of **1** were almost superimposable with those of inamoside (**1a**) [6], except for the obvious differences at C(7) ($\Delta\delta = -1.4$)), C(8) (+2.0), and C(9) (−2.5) for **1** relative to **1a**. This indicated that compound **1** was the 9-epimer of **1a**. According to *Calis et al.* [7], ^{13}C-NMR is of particular diagnostic value in assigning the absolute configuration at C(9) of 9-hydroxy-4,7-megastigmadien-3-one 9-*O*-glycosides, with δ(C) values of 74 *vs.* 77 ppm for the corresponding (9*S*) and (9*R*) forms, respectively [7]. Hence, we assigned the (9*S*) configuration to **1**, based on an observed chemical shift of δ(C) 75.3. The (*S*)-configuration at C(6) was deduced by circular dichroism (CD). The CD spectrum of **1** showed $\Delta\varepsilon$ values of +83.0 and −5.9 mdeg at 247 and 320 nm wavelength, respectively, identical with those of **1a**[1]). Therefore, the structure of debiloside A (**1**) was identified as (6*S*,7*E*,9*S*)-9-[(*β*-D-glucopyranosyl)-oxy]megastigma-4,7-dien-3-one.

Compound **2** had the molecular formula $C_{19}H_{32}O_8$, as determined by HR-ESI-MS for the $[M+Na]^+$ signal observed at *m/z* 411.2017 (calc. 411.1995). Similar to **1**, the ^{13}C-NMR spectrum of **2** displayed 19 C-atom signals, comprising one Glc and one C_{13} norisoprenoid moiety, the latter including four Me, one CH_2, and five CH groups, as well as three quaternary C-atoms.

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[1]) The absolute configuration of inamoside (**1a**) was determined by the authors to be (6*S*,9*R*), but was falsely drawn as (6*R*,9*R*) in [6].

In the ^{1}H-NMR spectrum of **2**, the following moieties were identified: four Me groups at δ(H) 1.05 (*s*), 1.07 (*s*), 1.85 (*s*), and 1.31 (*d*, $J=6.4$ Hz); two (*E*)-conjugated olefinic H-atoms at δ(H) 6.14 and 5.41 ($^3J=15.8$ Hz); three oxygenated CH [δ(H) 4.52 (*dq*, $J=8.0$, 6.4); 3.75 (*dt*, $J=12.0$, 3.9); 3.81 (br. *d*, $J=3.9$ Hz)]; and one CH_2 (δ(H) 1.43, 1.76 ($^2J=12.2$ Hz)). These data were in accord with a trihydroxymegastigma-5,7-diene such as ebracteatoside A (**2a**) [8].

The aglycone of **2** gave rise to nearly the same ^{13}C-NMR signals as that of **2a**. Differences were observed for the side-chain atoms C(7) to C(10), with $\Delta\delta$ values of +2.2, −1.2, −2.2, and +1.2, respectively for the signals of **2** *vs.* **2a**, suggesting that the former was the (9*S*)-isomer of **2a** regarding the aglycone portion. For the analogue platanionoside B (=(3*R*,9*R*,7*E*)-megastigma-5,7-dien-3,9-diol 3,9-bis-*O*-β-D-glucopyranoside) [9], a δ(C) value of 78.3 was reported for C(9), which further confirmed the proposed (9*S*)-configuration of **2** [7]. Therefore, the structure of debiloside B was identified as (3*S*,4*R*,9*S*)-9-[(β-D-glucopyranosyl)oxy]megastigma-5,7-diene-3,4-diol.

Debiloside C (**3**) was obtained as a white, amorphous powder. HR-ESI-MS indicated the empirical formula $C_{19}H_{34}O_9$, based on the $[M+Na]^+$ signal at *m/z* 429.2092. The ^{1}H- and ^{13}C-NMR spectra of **3** (*Table*) exhibited the characteristics of a hydroxylated megastigm-7-ene β-D-glucopyranoside.

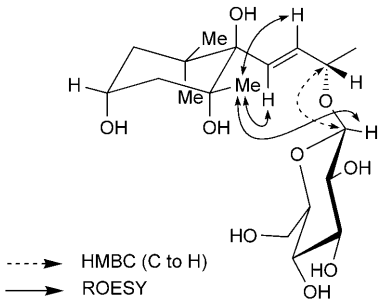

Figure. *Key HMBC and ROESY correlations for* **3**

The ^{1}H,^{1}H-COSY and HMBC spectra revealed that **3** had the same planar structure as **4** (*Figure*). The ^{1}H-NMR signals at δ(H) 4.08 (*tt*, $J=5.0$, 3.6 Hz, H−C(3)) indicated that H−C(3) was equatorial. NOE Cross-peaks between Me−C(5) and H−C(7), H−C(8), as well as H−C(1′) were observed, indicating that both the side chain and the 5-Me group were in equatorial positions on the cyclohexane ring. The configuration at C(9) of **3** was determined to be (*S*), as derived by comparison of the corresponding ^{13}C-NMR data with those of **4** [7]. From these data, the structure of compound **3** was unequivocally deduced as (3*R*,5*R*,6*R*,7*E*,9*S*)-9-[(β-D-glucopyranosyl)oxy]megastigm-7-ene-3,5,6-triol.

Experimental Part

General. Column chromatography (CC): silica gel (200–300 or 400 mesh; *Qingdao Haiyang, Co.*, China), *Sephadex LH-20* (*Pharmacia Biotech*, Sweden), *RP-18* (*Greenherbs Sci & Tech Development*

Co., Ltd., China). UV Spectra: *Cary 300Bio* UV/VIS spectrophotometer; λ_{max} in nm. Optical rotations: *Perkin-Elmer-341* polarimeter; λ in nm ($\Delta\varepsilon$ in mdeg). IR Spectra: *Nicolet Magna-750 FTIR* spectrometer, with KBr pellets; in cm^{-1}. NMR Spectra: *Bruker DRX-400* apparatus, at 400 (^1H) or 100 MHz (^{13}C), in CD_3OD soln.; δ in ppm rel. to Me_4Si, J in Hz. ESI- and HR-ESI-MS: *LCQ-Deca* and *Q-Tof-Ultima* mass spectrometers, resp.; in *m/z* (rel. %).

Plant Material. Equisetum debile ROXB. was collected from Xiamen, Fujian Province, China, in October 2001, and identified by Prof. *Shan-Hao Jiang*. A voucher specimen (No. 01-092) was deposited at the Herbarium of the Shanghai Institute of Materia Medica.

Extraction and Isolation. The dried whole plant of *E. debile* (10 kg) was consecutively extracted with $CHCl_3$ and 70% aq. EtOH. After solvent evaporation, the EtOH extract was suspended in H_2O (4 l) and extracted with AcOEt (3×4 l), and then with BuOH (3×4 l). The AcOEt fraction (20 g) was subjected to column chromatography (CC) (SiO_2; $CHCl_3$/MeOH 100:1 → 5:1): fractions *Fr. A1–A16*. *Fr. A5* (1.36 g) yielded solids (0.43 g), which were purified by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 2:1) to afford blumenol A (260 mg). *Fr. A8* (1.26 g) was purified by repeated CC (1. SiO_2, $CHCl_3$/MeOH 10:1; 2. *Sephadex LH-20*, MeOH; 3. *RP-18*, MeOH/H_2O 3:2) to provide corchoinoside C (27 mg). The above BuOH-soluble extract (40 g) was separated into *Fr. B1–B20* through CC (SiO_2; MeOH/$CHCl_3$ 0:100 → 50:50). *Fr. B8* (1.03 g) was repeatedly chromatographed (1. SiO_2, $CHCl_3$/ MeOH 10:1; 2. *Sephadex LH-20*, $CHCl_3$/MeOH 1:1; 3. *RP-18*, MeOH/H_2O 3:1) to afford sammangaoside A (36 mg) and **1** (7 mg). *Fr. B10* (2.32 g) gave an amorphous solid, which was subjected to CC (1. *Sephadex LH-20*, MeOH; 2. *RP-18*, MeOH/H_2O 2:1) to provide **2** (90 mg), and **3** (18 mg). *Fr. B14* (3.24 g) was purified by repeated CC (1. *Sephadex LH-20*, MeOH; 2. SiO_2; $CHCl_3$/MeOH 8:1; 3. *RP-18*, MeOH/H_2O 4:1) to provide **4** (11 mg).

Debiloside A (=*(6S,7E,9S)-9-[(β-*D*-Glucopyranosyl)oxy]megastigma-4,7-dien-3-one*; **1**). White, amorphous powder. UV (MeOH): 235.5. $[\alpha]_D^{16} = +91.0$ ($c = 0.235$, MeOH). CD ($c = 1.2$ g l^{-1}; MeOH): 204 (−19.2), 212 (0), 247 (+83), 284 (0), 320 (−5.9), 368 (0). IR (KBr): 3423, 2929, 1652, 1369, 1039. ^1H-NMR: see the *Table.* ^{13}C-NMR (100 MHz, CD_3OD): 22.7 (*q*, C(10)); 27.9 (*q*, C(12)); 28.3 (*q*, C(11)); 37.7 (*s*, C(1)); 49.7 (*t*, C(2)); 52.9 (*d*, C(6)); 63.3 (*t*, C(6′); 64.7 (*t*, C(13)); 72.2 (*d*, C(4′); 75.3 (*d*, C(9)); 75.5 (*d*, C(2′)); 78.6 (*d*, C(5′)); 78.8 (*d*, C(3′)); 101.8 (*d*, C(1′)); 123.0 (*d*, C(4)); 131.7 (*d*, C(8)); 137.5 (*d*, C(7)); 168.4 (*s*, C(5)); 202.5 (*s*, C(3)). ESI-MS (pos./neg.): 409 ($[M+Na]^+$), 387 ($[M+H]^+$), 385 ($[M-H]^-$). HR-ESI-MS: 409.1850 ($[M+Na]^+$, $C_{19}H_{30}NaO_8^+$; calc. 409.1838).

Debiloside B (=*(3S,4R,9S)-9-[(β-*D*-Glucopyranosyl)oxy]megastigma-5,7-diene-3,4-diol*; **2**). Amorphous powder. UV (MeOH): 227.5. $[\alpha]_D^{24} = -127.8$ ($c = 0.917$, MeOH). IR (KBr): 3396, 2927, 1637, 1363, 1060, 1039. ^1H-NMR: see the *Table.* ^{13}C-NMR (100 MHz, CD_3OD): 20.0 (*q*, C(13)); 22.2 (*q*, C(10)); 27.5 (*q*, C(11)); 30.4 (*q*, C(12)); 37.7 (*s*, C(1)); 41.4 (*t*, C(2)); 62.6 (*t*, C(6′)); 67.7 (*d,* C(3)); 71.5 (*d*, C(4′)); 72.5 (*d*, C(4)); 74.8 (*d*, C(2′)); 75.2 (*d*, C(9)); 77.9 (*d*, C(5′)); 78.1 (*d*, C(3′)); 100.7 (*d*, C(1′)); 129.1 (*s*, C(5)); 130.8 (*d*, C(7)); 137.2 (*d*, C(8)); 141.9 (*s*, C(6)). ESI-MS: 411 ($[M+Na]^+$), 389 ($[M+H]^+$). HR-ESI-MS: 411.2017 ($[M+Na]^+$, $C_{19}H_{32}NaO_8^+$; calc. 411.1995).

Debiloside C (=*(3R,5R,6R,7E,9S)-9-[(β-*D*-Glucopyranosyl)oxy]megastigm-7-ene-3,5,6-triol*; **3**). Amorphous powder. $[\alpha]_D^{20} = -34.9$ ($c = 0.930$, MeOH). IR (KBr): 3415, 2969, 2927, 1646, 1369, 1078, 1039. ^1H-NMR: see the *Table.* ^{13}C NMR (100 MHz, CD_3OD): 22.9 (*q*, C(10)); 27.1 (*q*, C(13)); 28.1 (*q*, C(12)); 29.4 (*q*, C(11)); 39.1 (*s*, C(1)); 42.4 (*t*, C(4)); 44.7 (*t*, C(2)); 63.2 (*t*, C(6′)); 69.5 (*d*, C(3)); 72.2 (*d*, C(4′)); 75.5 (*d*, C(2′)); 76.1 (*d*, C(9)); 78.0 (*d*, C(5)); 78.4 (*d*, C(5′)); 78.8 (*d*, C(3′)); 80.8 (*s*, C(6)); 100.9 (*d*, C(1′)); 132.9 (*d*, C(8)); 136.9 (*d*, C(7)). ESI-MS: 429 ($[M+Na]^+$). HR-ESI-MS: 429.2092 ($[M+Na]^+$, $C_{19}H_{34}NaO_9^+$; calc. 429.2101).

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