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Megastigmanes from the aerial part of *Euphorbia heterophylla*

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ABSTRACT

Three unprecedented megastigmanes together with twelve known compounds were isolated from the aerial part of *Euphorbia heterophylla*. The structural elucidation was based on extensive uses of spectroscopic data. The absolute configuration assignment of **1** was based on NOESY correlations and comparison of the experimental and calculated ECD spectra. Selected isolates obtained in sufficient quantity were evaluated for their cytotoxic activity.

1. Introduction

Euphorbia heterophylla (Euphorbiaceae), wild poinsettia or painted spurge known in Thailand as "ya yang" (Smitinand, 2014), is an herbaceous plant that can grow up to 50 cm in height. The leaf extract was reported to possess laxative, antigonorrhoeal, insecticidal and piscicidal properties and was used as a cure for convulsants, coughs, migraines and warts. It was also documented to have wound healing properties (James and Friday, 2010).

No phytochemical study of *E. heterophylla* has been reported. Preliminary cytotoxic activity evaluation of the CH_2Cl_2 and MeOH extracts of the aerial part of this plant showed IC_{50} values of 57.3 and 4.2 μg/mL against KB cells, respectively, thus prompting us to investigate its biologically active chemical constituents. This study reports the isolation of three new megastigmanes (**1**-**3**) (Fig. 1) together with 12 known constituents that were elucidated as (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol, $[\alpha]_D^{31}$ −26.8 (*c* 0.19, MeOH) {lit. $[\alpha]_D^{26}$ −25.7 (*c* 1.52, MeOH) (Takeda et al., 2000); $[\alpha]_D^{22}$ −21.1 (*c* 0.38, MeOH) (Otsuka et al., 2003); $[\alpha]_D^{26}$ −25.1 (*c* 0.99, MeOH) (Yu et al., 2002)}, (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol-3-*O*-*β*-D-glucopyranoside, $[\alpha]_D^{31}$ −14.2 (*c* 1.24, MeOH) {lit. $[\alpha]_D^{24}$ −38.0 (*c* 1.00, MeOH) (Otsuka et al., 2003)}, blumenol A (vomifoliol), $[\alpha]_D^{23}$ +117.7 (*c* 1.07, $CHCl_3$) {lit. $[\alpha]_D^{22}$ +41 (*c* 0.01, $CHCl_3$) (Hammami et al., 2004), (Cutillo et al., 2005; Galbraith and Horn, 1973)}, (6*S*)-dehydrovomifoliol, $[\alpha]_D^{25}$ +164.9 (*c* 0.39, MeOH) {lit. $[\alpha]_D^{28}$ +139.0 (*c* 0.42, MeOH) (Kai et al., 2007); $[\alpha]_D^{20}$ +134.4 (*c* 0.11, MeOH) (Kim et al., 2004); $[\alpha]_D^{20}$ +219 (*c* 0.10, $CHCl_3$) (Park et al., 2011)}, (3*S*,5*R*,6*R*,7*E*)-3,5,6-trihydroxy-7-ene-9-oxo-megastigmane, $[\alpha]_D^{25}$ −20.0 (*c* 0.17, MeOH) {lit, Tan et al., 1989; Zhang et al., 2010}, *ent*-kaurane-3*β*,16*β*,17-triol, $[\alpha]_D^{31}$ −14.8 (*c* 0.09, MeOH) {lit. $[\alpha]_D^{28}$ −38.9 (*c* 0.36, pyridine) (Li et al., 1990), (Wu et al., 1995)}, 7,11,15-trimethyl-3-methylene-hexadecan-1,2-diol (phytene-1,2-diol) (Brown et al., 2003; Urones et al., 1987), glut-5-en-3*β*-ol (El-Seedi, 2005), β-sitosterol, sitosterol-*β*-D-glucopyranoside, stigmast-4-en-3*α*,6*β*-diol (Shi et al., 2008) and lupeol acetate (Bhattacharyya and Barros, 1986; Ng et al., 2015). The structural identification was based on their spectroscopic data, and for (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol and blumenol A (vomifoliol), not only a comparison of their optical rotation values with literature data but also a comparison of their calculated and experimental ECD spectra were used for absolute configuration assignment (Figs. S1 and S2). Based on these calculation results, the optimized calculation parameters were therefore applied to acquire the calculated ECD spectrum of **1** (Figs. 4 and S3).

2. Results and discussion

2.1. Characterization of compounds 1-3

Compound **1** was isolated as a colourless liquid with a molecular formula of $C_{13}H_{20}O_4$ based on its HRESIMS corresponding to *m/z* 263.1257 $[M + Na]^+$ (calcd for $C_{13}H_{20}O_4Na$, 263.1254). The ^{13}C NMR spectrum revealed 13 carbon resonances comprising three methyls, three methylenes, three methines, one quaternary, two oxygenated

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tertiary carbons and one keto carbon. The ^{1}H-^{1}H COSY spectrum showed correlations of H-7/H-8/H-9/H-10. The 3-oxo-megastigman-7-ene moiety was established from the HMBC spectrum which exhibited cross-peaks between H-2/C-1, C-3, C-4, C-6, C-11, C-12; H-4/C-2, C-3, C-5, C-6, C-13; H-7/C-1, C-5, C-6, C-8, C-9; and H-8/C-6, C-7, C-9, and C-10 (Fig. 2). The presence of a tetrahydrofuran moiety with the ether linkage between C-5 and C-11 was revealed from the HMBC cross-peaks between H-11/C-1, C-2, C-5, C-6, and C-12. The coupling constant $J_{H\text{-}7/H\text{-}8}$ with a value of 15.9 Hz indicated a *trans*-double bond between C-7/C-8. Based on the above data, compound **1** was proposed to have a planar structure similar to that of drummondol (Powell and Smith, 1981). The absolute configuration, particularly at C-9, of drummondol first obtained from *Sesbania drummondii*, $[\alpha]^{23}_D$ −21 (*c* 1.03, MeOH), was not established (Powell et al., 1986; Powell and Smith, 1981). Later, drummondol obtained from *Cynanchum liukiuense*, having $[\alpha]^{25}_D$ +2.7 (*c* 0.30, MeOH), was reported as 1*R*,5*R*,6*S*,7*E*,9*R*-drummondol (Abe et al., 2000), while the β-D-glucopyranoside of a related compound (spionoside B, (9*S*)-drummondol-9-*O*-β-D-glucopyranoside) isolated from *Capparis spinosa* fruits, having $[\alpha]^{20}_D$ −51.2 (*c* 2.0, MeOH), gave drummondol that was reported to have a 1*R*,5*R*,6*S*,7*E*,9*S* configuration after hydrolysis (Çalış et al., 2002). Assignments of the configuration at C-9 of drummondol from both reports were based on a modified Mosher's ester method. In the present study, the NOESY spectrum of compound **1** revealed NOE interactions between H-7/H_a-11, H_3-12, H_3-13; H_β-2/H_b-11, H_3-12 and H_2-4/H_3-13, as shown in Fig. 3. On the basis of the optical rotation of **1** with $[\alpha]^{23}_D$ +15.4 (*c* 0.24, MeOH) and a comparison of the experimental and calculated ECD spectra (Fig. 4), compound **1** was thus proposed as (1*S*,5*S*,6*S*,7*E*,9*S*)-drummondol and named heterophyllol.

Compound **2** was obtained as a sticky liquid with molecular formula $C_{19}H_{30}O_9$ based on its HRESIMS. The ^{13}C NMR spectrum showed 19 carbon resonances. The ^{1}H and ^{13}C NMR spectra of **2** showed similar patterns of resonances as those found in **1**, with additional resonances of a β–glucopyranosyl group showing a dioxygenated methine group at δ_H 4.34 (d, *J* =7.8 Hz) and δ_C 102.8 and oxymethylene resonances at δ_H 3.87, 3.63, and δ_C 62.9. The HMBC spectrum showed cross-peaks between H-9/C-1′ as well as H-1′/C-9, indicating connectivity of C-9-*O* to C-1′ of the glucosyl group (Fig. 2). To specify whether the glucose is D- or L-, acid hydrolysis of the glucoside is needed. Although direct acid hydrolysis of **2** could not be carried out due to the scarcity of **2**, D-glucose $[\alpha]^{25}_D$ +16.2 (*c* 0.40, H_2O); lit. $[\alpha]^{23}_D$ +53.2 (*c* 0.1, H_2O) (Perrone et al., 2012) was obtained after (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol-3-*O*-β-D-glucopyranoside was hydrolysed with 5% HCl. It was thus assumed that all glucose found in this study is D-glucose. Based on the optical rotation of **2**, $[\alpha]^{23}_D$ +4.1 (*c* 0.91, MeOH), as compared to spionoside B, $[\alpha]^{20}_D$ −51.2, and the co-occurrence of **1** in this plant, compound **2** was thus elucidated as (1*S*,5*S*,6*S*,7*E*,9*S*)-drummondol-9-*O*-β-D-glucopyranoside and given the name heterophylloside A.

Compound **3** was obtained as an amorphous solid. Its HRESIMS indicated a molecular formula of $C_{19}H_{34}O_9$ based on $[M + Na]^+$ at *m/z* 429.2123 (calcd for $C_{19}H_{34}O_9Na$, 429.2095) with $[\alpha]^{23}_D$ -14.2 (c 0.22, MeOH). The successive ^{1}H-^{1}H connectivities from H-2, H-3 and H-4 and H-7, H-8, H-9 and H-10 were revealed from its ^{1}H,^{1}H COSY spectrum. The *trans*-double bond between C-7/C-8 was proposed from the $J_{H\text{-}7/H\text{-}8}$ value of 15.9 Hz. The presence of a β-glucopyranosyl moiety was evident in particular from the ^{1}H and ^{13}C NMR chemical shifts of an anomeric group at δ_H 4.41 (d, *J* =8.0 Hz) and δ_C 101.8. Most of the ^{1}H and ^{13}C NMR resonances were very similar to those of (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol-3-*O*-β-D-glucopyranoside, which was also isolated in this study. However, H-3 was found to resonate at δ_H 4.17 as quintet-like (*J* =3.5 Hz) instead of at ca. δ_H 4.19 as triplet of triplets (*J* = 11.7 and 4.3 Hz) as previously reported (Otsuka et al., 2003) and found for (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol-3-*O*-β-D-glucopyranoside. The NOESY spectrum showed similar NOE correlations as those found in (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol-3-*O*-β-D-glucopyranoside except that the NOE cross-peak between H-3/H_3-12 was missing (Fig. 3). The above evidence indicated that these two compounds possess different configurations at C-3. Direct acid hydrolysis of **3** was not performed due to the scarcity of **3**. However, on the basis of the result obtained from acid hydrolysis of (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol-3-*O*-β-D-glucopyranoside, which provided D-glucose, compound **3**, i.e., heterophylloside B, was thus concluded to be (3*R*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol-3-*O*-β-D-glucopyranoside.

Among the nine compounds, (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol,

blumenol A (vomifoliol), (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol-3-*O*-β-D-glucopyranoside, (3*S*,5*R*,6*R*,7*E*)-3,5,6-trihydroxy-7-ene-9-oxo-megastigmane, (6*S*)-dehydrovomifoliol, *ent*-kaurane-3β,16β,17-triol, 7,11,15-trimethyl-3-methylene-hexadecan-1,2-diol (phytene-1,2-diol), stigmast-4-en-3α,6β-diol and glut-5-en-3β-ol obtained in sufficient quantity, only glut-5-en-3β-ol showed IC_{50} values of 9.72 ± 0.58 and 9.86 ± 1.07 μM, while the positive control compound (doxorubicin) exhibited IC_{50} values of 0.43 ± 0.01 and 0.16 ± 0.01 μM against HelaS3 and KB cells, respectively.

3. Experimental section

3.1. General experimental procedures

Melting points were measured using an Electrothermal melting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO DIP 1020 polarimeter, and ECD spectra were recorded on a JASCO J-810 apparatus. The IR spectra were obtained on a Perkin-Elmer 1760x FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded with Bruker AVANCE 400 MHz and Bruker AVANCE III HD 400 MHz NMR spectrometers. Chemical shifts are referenced to the residual solvent signals (MeOH-d_4: δ_H 3.30 and δ_C 49.0 ppm). HRESIMS was recorded on a Bruker DaltonicsmicroTOF mass spectrometer. Column chromatographic separations were performed using silica gel 60 (less than 0.063 mm and 0.063−0.200 mm, Merck) and silica gel 60 RP-18 (40−63 μm, Merck), Dianion HP-20 (Supelco Analytical, Bellefonte,

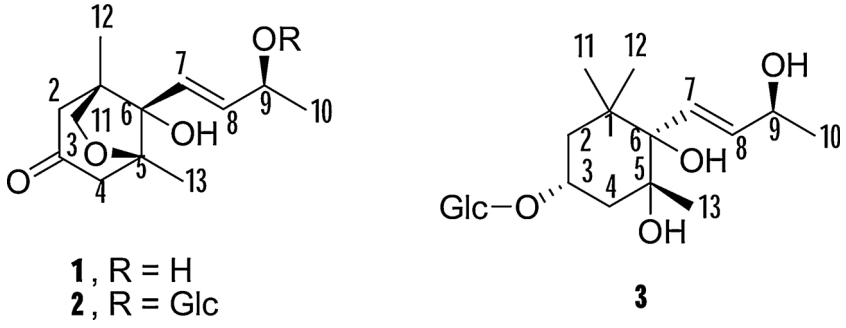

Fig. 1. Structures of compounds **1**-**3**.

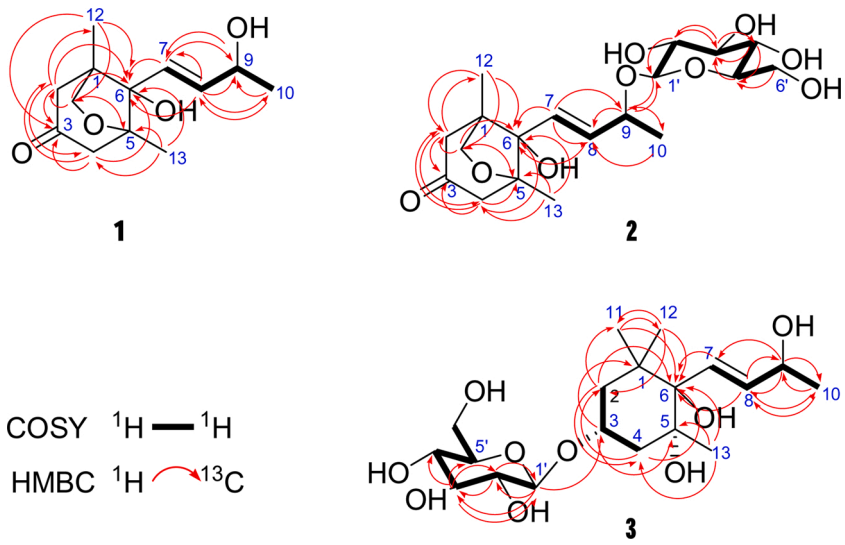

Fig. 2. COSY and HMBC correlations of **1**, **2** and **3**.

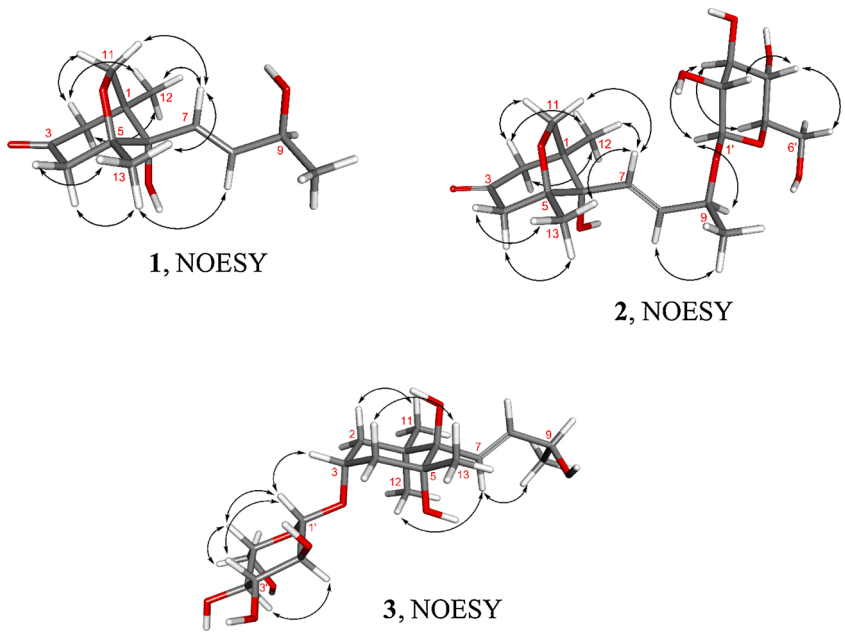

Fig. 3. The NOESY correlations of **1**-**3**.

PA) and Sephadex LH-20 (bead size of 25−100 μm, Sigma, Pharmacia Inc.). Thin-layer chromatography was performed using precoated silica gel 60 F_{254} and silica gel 60 RP-18 F_{254}s aluminium sheets (Merck).

3.2. Plant material

The aerial part of *Euphorbia heterophylla* (Euphorbiaceae) was obtained from Thepraksa subdistrict, Sangka district, Surin province [GPS of collection site: 14°29′55.5″N 103°49′35.9″E (14.498750, 103.826629)] in May 2015. A voucher specimen (SSEH-1/2015) was maintained at the Department of Chemistry, Ramkhamhaeng University.

3.3. Extraction and isolation

The aerial part (12.4 kg) was ground and soaked at ambient temperature successively in hexanes, CH_2Cl_2 and MeOH for seven days, and the filtrate was then concentrated. The extraction was repeated twice for each solvent to obtain hexanes (130.2 g), CH_2Cl_2 (60.0 g) and MeOH (492.5 g) extracts. The CH_2Cl_2 extract (60.0 g) was fractionated by column chromatography (CC, silica gel, hexanes−CH_2Cl_2 90:10 to CH_2Cl_2-MeOH 30:70) to obtain eight fractions. Fraction 3 (2.25 g) was purified by CC (silica gel, hexanes−CH_2Cl_2 50:50 to CH_2Cl_2-MeOH 90:10) to give five subfractions (3.1–3.5). Subfraction 3.3 (528.6 mg) was further purified by CC (silica gel, hexanes−CH_2Cl_2 86:14 to 70:30) to give three subfractions (3.3.1–3.3.3), and subfraction 3.3.2 (436.8 mg) after CC (silica gel, hexanes−CH_2Cl_2 80:20) gave three subfractions (3.3.2.1–3.3.2.3). Subfraction 3.3.2.2 (293.8 mg) was further purified by CC (silica gel, hexanes-EtOAc 99.5:0.5) to give lupeol acetate (5.9 mg). Subfraction 3.4 (64.7 mg) was purified by CC (silica gel, hexanes-EtOAc 98:2 to 95:5) to obtain glut-5-en-3β-ol (6.4 mg). Fraction 5 (13.4 g) was fractionated (silica gel, CH_2Cl_2-MeOH 100:0 to 30:70) to give four subfractions (5.1–5.4). Subfraction 5.3 (8.4 g) was purified by CC (silica gel, CH_2Cl_2-MeOH 98:2 to 90:10) to give three subfractions (5.3.1−5.3.3), and subfraction 5.3.2 (2.8 g) was fractionated (Sephadex LH-20, MeOH) to give three subfractions. Subfraction 5.3.2.2 (1.1 g) was

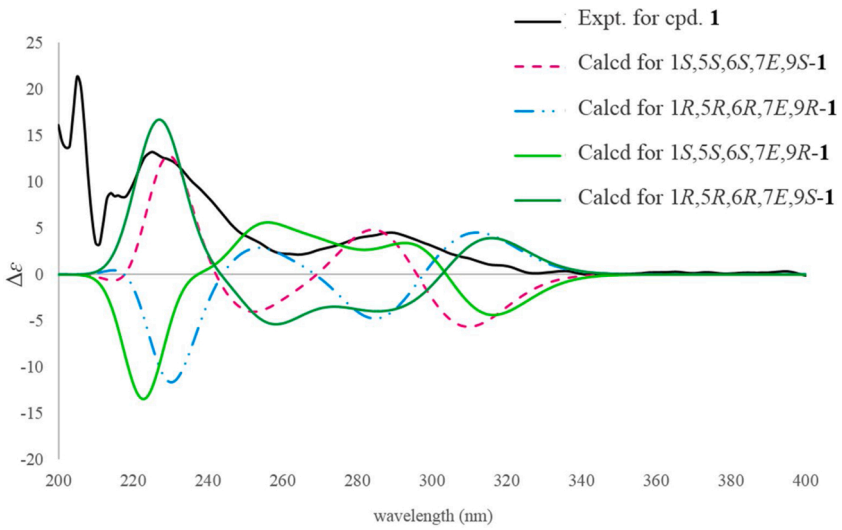

Fig. 4. The experimental and calculated ECD spectra for **1**.

further purified by CC (silica gel, hexanes-EtOAc 70:30 to 30:70) to give six subfractions (5.3.2.2.1−5.3.2.2.6), and subfraction 5.3.2.2.3 (36.9 mg) provided (3*S*,5*R*,6*R*,7*E*)-3,5,6-trihydroxy-7-ene-9-oxo-megastigmane (1.7 mg) after CC (silica gel, hexanes-EtOAc 75:25 to 65:35). Subfraction 5.3.2.2.4 (14.6 mg) after further purification (silica gel, CH_2Cl_2-MeOH 98:2) provided (6*S*)-dehydrovomifoliol (3.9 mg). Subfraction 5.3.2.2.5 (10.7 mg) provided blumenol A (vomifoliol). Fraction 6 (3.3 g) was fractionated (silica gel, hexanes-EtOAc 70:30 to 20:80) to give five subfractions (6.1–6.5), and subfraction 6.2 (210.3 mg) was purified by CC (silica gel, hexanes-EtOAc 60:40) to give four subfractions (6.2.1−6.2.4). Subfraction 6.2.2 (25.3 mg) gave *ent*-kaurane-3*β*,16*β*,17-triol (12.2 mg) after recrystallization. Fraction 7 (1.3 g) was fractionated (silica gel, hexanes-EtOAc 70:30 to 20:80 and then Sephadex LH-20, MeOH) to give four subfractions (7.1–7.4). Subfraction 7.3 (124.3 mg) after successive CC (silica gel, hexanes-EtOAc 65:35 to 55:45 and then silica gel, CH_2Cl_2-MeOH 98:2 to 97:3) provided compound **1** (29.0 mg). Fraction 8 (1.64 g) was fractionated (Sephadex LH-20, CH_2Cl_2-MeOH 30:70 and then RP-18, MeOH-H_2O 65:35 to 100:0) to give three subfractions (8.1–8.3). Subfraction 8.2 (176.8 mg) after fractionation (Sephadex LH-20, MeOH) gave three subfractions (8.2.1−8.2.3), and subfraction 8.2.2 (58.9 mg) provided (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol (2.2 mg) after further CC (silica gel, CH_2Cl_2-MeOH 94:6).

The MeOH extract (492.5 g) was fractionated using Dianion HP-20 (H_2O-MeOH 100:0 to 0:100) to give seven fractions. Fraction 2 (16.6 g) was purified by CC (Sephadex LH-20, MeOH and then RP-18, MeOH-H_2O 10:90 to 100:0) to give four subfractions (2.1–2.4). Subfraction 2.2 (3.8 g) after CC (Sephadex LH-20, MeOH) gave three subfractions, and subfraction 2.2.2 (1.23 g) after further CC (silica gel, CH_2Cl_2-MeOH 92:8 to 90:10) provided compound **2** (9.1 mg) and compound **3** (2.2 mg). Subfraction 2.3 (1.3 g) was purified by CC (silica gel, CH_2Cl_2-MeOH 96:4 to 80:20) to give three subfractions (2.3.1−2.3.3), and subfraction 2.3.1 provided (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol-3-*O*-*β*-D-glucopyranoside (21.4 mg). Fraction 5 (15.23 g) after purification (silica gel, CH_2Cl_2-MeOH 100:0 to 80:20) gave three subfractions (5.1–5.3). Subfraction 5.2 (222.6 mg) was purified by CC (silica gel, hexanes−CH_2Cl_2 30:70 to 0:100 and then silica gel, hexanes-EtOAc 85:15 to 70:30) to obtain 7,11,15-trimethyl-3-methylene-hexadecan-1,2-diol (phytene-1,2-diol) (6.8 mg). Subfraction 5.3 (6.1 mg) gave sitosterol-*β*-D-glucopyranoside. Fraction 6 (14.2 g) was fractionated by CC (silica gel, CH_2Cl_2-MeOH 100:0 to 88:12) to give four subfractions (6.1–6.4), and subfraction 6.2 (41.7 mg) provided β-sitosterol after recrystallization. Subfraction 6.3 (379.8 mg) was purified by CC (silica gel, CH_2Cl_2-MeOH 99:1 to 96:4) to give four subfractions, and subfraction 6.3.3 (29.7 mg) gave stigmast-4-en-3*α*,6*β*-diol (2.5 mg) after recrystallization from CH_2Cl_2-MeOH 60:40.

3.4. (1S,5S,6S,7E,9S)-drummondol, heterophyllol (1)

Colourless oil; $[\alpha]_D^{23}$ +15.4 (*c* 0.24, MeOH); FTIR (ATR) ν_{max} 3382, 2969, 2928, 2877, 1701, 1659, 1454, 1380, 1299, 1245, 1174, 1144, 1041, 1008, 980, 943, 887, 846, 801, 765 cm^{-1}; ^1H NMR (MeOH-d_4, 400 MHz) and ^{13}C NMR (MeOH-d_4, 100 MHz) see Table 1; HRESIMS *m/z* 263.1257 $[M + Na]^+$ (calcd for $C_{13}H_{20}O_4Na$, 263.1254).

3.5. (1S,5S,6S,7E,9S)-drummondol-9-O-β-D-glucopyranoside, heterophylloside A (2)

Sticky liquid; $[\alpha]_D^{23}$ +4.1 (*c* 0.91, MeOH); FTIR (ATR) ν_{max} 3352, 2972, 2929, 2879, 1699, 1649, 1452, 1376, 1306, 1247, 1153, 1071, 1028, 1010, 927, 890, 847 cm^{-1}; ^1H NMR (MeOH-d_4, 400 MHz) and ^{13}C NMR (MeOH-d_4, 100 MHz) see Table 1; HRESIMS *m/z* 425.1822 $[M + Na]^+$ (calcd for $C_{19}H_{30}O_9Na$, 425.1782).

3.6. (3R,5R,6R,7E,9S)-megastigman-7-ene-3,5,6,9-tetrol-3-O-β-D-glucopyranoside, heterophylloside B (3)

Sticky liquid; $[\alpha]_D^{23}$ −14.2 (*c* 0.22, MeOH); FTIR (ATR) ν_{max} 3326, 2963, 2922, 2878, 1646, 1452, 1418, 1369, 1319, 1285, 1103, 1072, 1026, 1016, 985, 935, 884 cm^{-1}; ^1H NMR (MeOH-d_4, 400 MHz) and ^{13}C NMR (MeOH-d_4, 100 MHz) see Table 1; HRESIMS *m/z* 429.2123 $[M + Na]^+$ (calcd for $C_{19}H_{34}O_9Na$, 429.2095).

3.7. Acid hydrolysis of (3S,5R,6R,7E,9S)-megastigman-7-ene-3,5,6,9-tetrol-3-O-β-D-glucopyranoside

(3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol-3-*O*-*β*-D-glucopyranoside (8.5 mg) and 5% HCl (8 mL) were heated at 70 °C for 5 h and then left to cool to room temperature. The reaction mixture was then partitioned with EtOAc (3 × 10 mL) to give an EtOAc extract, from which the aglycone, (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol (3.2 mg) with R_f 0.3 [silica gel TLC, thickness 0.2 nm, CH_2Cl_2-MeOH (94:6)], was obtained after concentration. The aqueous phase was neutralized with saturated $NaHCO_3$ and concentrated to give an aqueous extract (5.2 mg). Column chromatography (Sephadex LH-20, MeOH) gave D-glucose (4.0 mg) with R_f 0.2 (CH_2Cl_2-MeOH, 90:10). The optical rotation value was measured after 24 h of dissolution in H_2O: D-glucose, $[\alpha]^{25}_D$ +16.2 (*c* 0.40, H_2O).

Table 1
^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data of **1-3** in MeOH-d_4.

	1		**2**		**3**	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	–	45.7, C	–	47.0, C	–	38.6, C
2*α*	2.75 [a] (16.9)	50.9, CH_2	2.77 d (17.1)	51.6, CH_2	1.90 dd[c] (14.4, 3.5)	42.1, CH_2
2*β*	2.37 d (16.9)		2.19 d (17.1)		1.66 dd (14.4, 3.5)	
3	–	209.7, C	–	213.1, C	4.17 quint-like (3.5)	75.5, CH
4	2.75 [a] (17.1) 2.21 d (15.9)	50.6, CH_2	2.74 d (17.3) 2.25 d (17.3)	52.1, CH_2	1.94 brs [c]	37.7, CH_2
5	–	83.7, C	–	85.4, C	–	76.4, C
6	–	80.5, C	–	81.3, C	–	80.1, C
7	5.88 d (15.9)	128.7, CH	5.89 d (15.7)	130.7, CH	6.16 d (15.9)	131.2, CH
8	5.94 dd (15.9, 5.0)	134.9, CH	6.05 dd (15.7, 6.4)	135.2, CH	5.78 d (15.9, 6.3)	135.7, CH
9	4.46 dq (6.3, 5.0) quint (6.3)	68.3, CH	4.48 quint (6.4)	78.0, CH	4.34 quint (6.3)	69.5, CH
10	1.32 d (6.4)	23.9, CH_3	1.34 d (6.4)	21.6, CH_3	1.26 d (6.3)	24.1, CH_3
11*a*	3.73 dd (8.9 2.6)	76.4, CH_2	3.80 dd (8.9, 2.6)	77.6, CH_2	1.27	28.1, CH_3
11*b*	3.78 d (8.9)		3.73 d (8.9)			
12	0.90 s	17.9, CH_3	0.96 s	18.3, CH_3	0.88 s	28.0, CH_3
13	1.07 s	21.4, CH_3	1.06 s	21.9, CH_3	1.10 s	26.8, CH_3
1′			4.34 d (7.8)	102.8, CH	4.41 d (8.0)	101.8, CH
2′			3.18 t (7.8)	75.3, CH	3.16 t (8.0, 8.9)	75.3, CH
3′			3.23 [b]	78.1, CH	3.37 t (8.9)	78.3, CH
4′			3.23 [b]	71.7, CH	3.30 [d]	71.7, CH
5′			3.33 [e]	78.2, CH	3.28 [d]	78.2, CH
6′			3.87 d (11.7) 3.63 dd (11.7, 3.0)	62.9, CH_2	3.86 d (11.7) 3.66 dd (11.7, 4.7)	62.8, CH_2

[a–d]Overlapped signals; [e]obscured by the solvent signal.

3.8. ECD calculation

The absolute configurations of compound **1**, (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol and blumenol A (vomifoliol) were obtained using DataWarrior software (Sander et al., 2015) to generate random low-energy conformers with the MMFF94s force field. The conformational search results were selected for further geometry optimization and electronic excitation energy calculations. Geometrical optimizations of the structures were made using density functional theory calculations at the hybrid density functional B3LYP/6−31G(d) level. The electronic excitation energy calculations for generating ECD spectra were computed with the time-dependent density functional theory (TDDFT) method at the CAM-B3LYP/6−31G(2d,p) level and C-PCM solvation model (MeOH as the solvent). All calculations were performed with Gaussian09 software (Frisch et al., 2013). The ECD spectra were simulated with overlapping Gaussian functions, and the excited states were analysed using GaussSum software (O'Boyle et al., 2008).

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2021.05.006.

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