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MEGASTIGMANE *O*-GLUCOPYRANOSIDES FROM *Litsea glutinosa*

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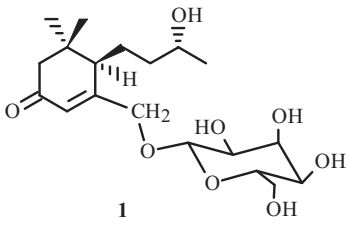
UDC 547.972

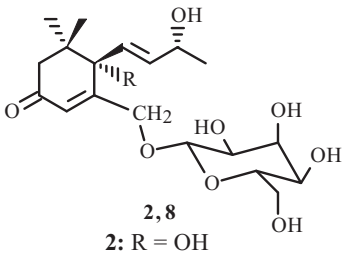
Litsea glutinosa (Lour.) C. B. Rob., (Lauraceae family) [1] is an evergreen medium-sized tree, which is locally known as "Chan Gao Shu." Our previous investigation on *L. glutinosa* resulted in the isolation of new aporphine alkaloids and flavone glycoside [2, 3]. In this study, we report the isolation and identification of ten known megastigmane *O*-glucopyranosides **1–10** for the first time from the *n*-BuOH-soluble fraction of an EtOH extract of *L. glutinosa*.

The leaves and twigs of *L. glutinosa* were collected from Xishuangbanna County of Yunnan Province, P. R. China, in October 2002 and identified by Prof. Yu Chen of Kunming Institute of Botany. Powdered materials of *L. glutinosa* (12.0 kg) were repeatedly extracted with EtOH at room temperature. The extract was then concentrated under reduced pressure to give a brown syrup, which was partitioned in H_2O and extracted with solvents into a petroleum ether-fraction (80 g), an EtOAc-fraction (54 g), and an *n*-BuOH-fraction (108 g). The *n*-BuOH-soluble fraction was subjected to silica gel column chromatography eluted with $CHCl_3$–MeOH (9:1–1:1) and MeOH to afford eight fractions (I–VIII). The resulting fractions were resubmitted to silica gel column chromatography, Sephadex LH-20, and RP-18 to yield compound **1–10**.

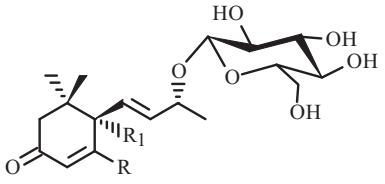
The structures of megastigmane *O*-glucopyranosides **1–10** were elucidated on the basis of their MS, ^{1}H NMR, and ^{13}C NMR spectra. All experimental data were in agreement with their IR, UV, 1D, and 2D NMR data in the literature and some were compared with corresponding authentic samples. These megastigmane glycosides are reported for the first time from the genus *Litsea*.



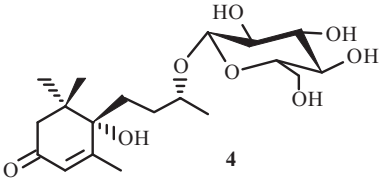
1



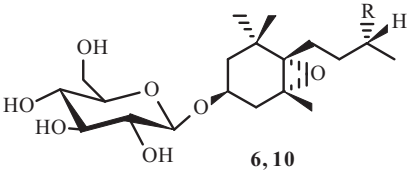
2, 8
2: R = OH
8: R = H



3, 5, 7, 9
3: R = CH_3, R_1 = OH
5: R = CH_3, R_1 = H
7: R = CH_2OH, R_1 = OH
9: R = CH_2OH, R_1 = H



4



6, 10
6: R = OH
10: R,H = O

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 0009-3130/12/4802-0346

Euodionoside G (1). $C_{19}H_{32}O_8$, amorphous powder. FAB-MS (Gly, *m/z*, %): 387 ([M – 1]$^-$, 100), 325 (30), 265 (8), 225 (35). IR (KBr, ν, cm^{-1}): 3430, 2924, 1655, 1620, 1370, 1075. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 2.55, 2.01 (each 1H, d, J = 17.5, H-2a, 2b), 6.18 (1H, s, H-4), 2.03 (1H, m, H-7), 1.55 (2H, m, H-8), 3.68 (1H, m, H-9), 1.17 (3H, d, J = 6.0, H-10), 1.03 (3H, s, CH_3-11), 1.11 (3H, s, CH_3-12), 4.53, 4.38 (each 1H, dd, J = 1.8, 16.5, CH_3-13), 4.33 (1H, d, J = 7.9, H-1′), 3.26 (1H, m, H-2′), 3.35 (1H, m, H-3′), 3.28 (1H, m, H-4′), 3.28 (1H, m, H-5′), 3.89, 3.67 (each 1H, dd, J = 2.0, 11.8, H-6′). ^{13}C NMR (125 MHz, CD_3OD, δ): 37.3 (C-1), 48.8 (C-2), 202.2 (C-3), 123.2 (C-4), 167.9 (C-5), 47.8 (C-6), 27.9 (C-7), 39.7 (C-8), 68.9 (C-9), 23.6 (C-10), 28.9 (C-11), 27.7 (C-12), 70.8 (C-13), 103.5 (C-1′), 75.0 (C-2′), 78.0 (C-3′), 71.6 (C-4′), 78.1 (C-5′), 62.8 (C-6′) [4]. The compound was directly compared with authentic samples.

Apocynoside II (2). $C_{19}H_{30}O_9$, amorphous powder. FAB-MS (Gly, *m/z,* %): 401 ([M – 1]$^-$, 100), 325 (4), 265 (2), 221 (12). ^{1}H NMR (500 MHz, C_5D_5N, δ, ppm, J/Hz): 2.68, 2.39 (each 1H, d, J = 16.7, CH_2-2), 6.95 (1H, s, H-4), 6.28 (1H, d, J = 15.5, H-7), 6.40 (1H, dd, J = 5.4, 15.5, H-8), 4.65 (1H, m, H-9), 1.40 (3H, d, J = 6.4, H-10), 1.08 (3H, s, CH_3-11), 1.21 (3H, s, CH_3-12), 5.25, 4.87 (each 1H, dd, J = 1.9, 17.8, CH_2-13), 4.85 (1H, d, J = 7.7, H-1′), 3.85 (1H, m, H-2′), 4.08 (1H, m, H-3′), 4.25 (1H, m, H-4′), 4.18 (1H, m, H-5′), 4.44, 4.36 (each 1H, dd, J = 2.2, 11.9, H-6′). ^{13}C NMR (125 MHz, C_5D_5N, δ): 37.3 (C-1), 48.8 (C-2), 202.2 (C-3), 123.2 (C-4), 167.9 (C-5), 47.8 (C-6), 27.9 (C-7), 39.7 (C-8), 68.9 (C-9), 23.6 (C-10), 28.9 (C-11), 27.7 (C-12), 70.8 (C-13), 103.5 (C-1′), 75.0 (C-2′), 78.0 (C-3′), 71.6 (C-4′), 78.1 (C-5′), 62.8 (C-6′) [5]. The compound was directly compared with authentic samples.

Roseoside (3). $C_{19}H_{30}O_8$, amorphous powder. FAB-MS (Gly, *m/z*, %): 387 ([M + 1]$^+$, 70), 225 ([M + H – 163]$^+$), 207 (100), 115. ^{1}H NMR (MeOD, ppm, δ, J/Hz): 5.87 (1H, s, H-5), 5.86, 5.85 (each 1H, overlap, H-7, H-8), 4.41 (1H, m, H-9), 4.33 (1H, d, J = 7.8, Glc-1′), 3.84, 3.63 (each 1H, m, Glc-6′), 2.50, 2.16 (each 1H, d, J = 17.0, H-2), 3.23–3.33 (4H, m, Glc-H), 1.92 (3H, s, CH_3-13), 1.29 (3H, d, J = 6.6, CH_3-10), 1.02, 1.01 (each 3H, s, CH_3-11, 12). ^{13}C NMR (MeOD, ppm, δ): 42.4 (C-1), 50.7 (C-2), 201.2 (C-3), 167.3 (C-4), 127.2 (C-5), 80.0 (C-6), 131.5 (C-7), 135.3 (C-8), 77.3 (C-9), 21.2 (C-10), 23.4, 24.7 (C-11, 12), 19.6 (C-13), 102.8 d, 78.1 d, 78.2 d, 75.3 d, 71.7 d, 62.9 (Glc) [6]. The compound was directly compared with authentic samples.

Blumenol C Glucoside (4). $C_{19}H_{32}O_7$, amorphous powder. FAB-MS (Gly), *m/z* 373 ([M + 1]$^+$). ^{1}H NMR (C_5D_5N, ppm, δ): 5.89 (1H, s, H-5), 2.51, 2.15 (each 1H, d, J = 17.0, H-2), 1.90 (3H, s, CH_3-13), 1.40 (3H, d, J = 6.0, CH_3-10), 0.96, 1.01 (each 3H, s, CH_3-11, 12), 4.93 (1H, d, J = 7.5, H-1′). ^{13}C NMR (C_5D_5N, ppm, δ): 36.8 (C-1), 47.7 (C-2), 199.2 (C-3), 125.7 (C-4), 165.2 (C-5), 51.6 (C-6), 25.7 (C-7), 36.5 (C-8), 76.3 (C-9), 21.9 (C-10), 27.4 (C-11), 28.7 (C-12), 24.6 (C-13), 104.3 d, 78.1 d, 78.6 d, 75.3 d, 71.6 d, 62.9 (Glc) [7].

(6*R*,7*E*,9*R*)-9-Hydroxy-megastigma-4,7-dien-3-one 9-*O*-*β*-D-glucopyranoside (5). $C_{19}H_{30}O_7$, amorphous powder. FAB-MS *m/z* 369 [M – H]$^-$. ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 2.45, 2.05 (each 1H, d, J = 16.4, H-2a, 2b), 5.88 (1H, s, H-4), 2.68 (1H, d, J = 9.0, H-6), 5.67 (1H, dd, J = 9.0, 15.4, H-7), 5.79 (1H, dd, J = 6.4, 15.4, H-8), 4.40 (1H, m, H-9), 1.30 (3H, d, J = 6.4, H-10), 0.99 (3H, s, CH_3-11), 1.05 (3H, s, CH_3-12), 1.93 (3H, CH_3-13), 4.35 (1H, d, J = 7.9, H-1′). ^{13}C NMR (100 MHz, CD_3OD, δ): 37.2 (C-1), 48.5 (C-2), 202.3 (C-3), 126.5 (C-4), 166.0 (C-5), 56.9 (C-6), 128.9 (C-7), 138.3 (C-8), 77.2 (C-9), 21.3 (C-10), 27.8 (4C-11), 28.2 (C-12), 24.0 (C-13), 102.5 (C-1′), 75.3 (C-2′), 78.2 (C-3′), 71.6 (C-4′), 77.9 (C-5′), 62.8 (C-6′) [8].

Alangionoside E (6). $C_{19}H_{31}O_8$, amorphous powder. FAB-MS (MNBA) *m/z* 387 ([M – H]$^-$). ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 1.35 (1H, dd, J = 10, 13, H-2_{ax}), 1.75 (1H, ddd, J = 2, 3, 13, H-2_{eq}), 2.40 (1H, ddd, J = 2, 5, 14, H-4_{eq}), 1.75 (1H, dd, J = 7, 14, H-4_{ax}), 4.30 (1H, m, H-9), 3.90 (1H, m, H-3), 5.65 (1H, dd, J = 6, 16, H-8), 5.93 (1H, dd, J = 1, 16, H-7), 1.24 (3H, d, J = 6.0, H-10), 0.97 (3H, s, CH_3-11), 1.15 (3H, s, CH_3-12), 1.21 (3H, CH_3-13), 4.35 (1H, d, J = 7.8, H-1′), 3.13 (1H, m, H-2′), 3.64 (each 1H, dd, J = 5, 12, H-6′a), 3.85 (1H, dd, J = 2, 12, H-6′b). ^{13}C NMR (125 MHz, CD_3OD, δ): 35.7 (C-1), 45.8 (C-2), 73.5 (C-3), 38.3 (C-4), 67.9 (C-5), 71.5 (C-6), 125.9 (C-7), 139.3 (C-8), 68.9 (C-9), 23.6 (C-10), 25.4 (C-11), 29.7 (C-12), 20.4 (C-13), 103.5 (C-1′), 75.0 (C-2′), 77.9 (C-3′), 71.6 (C-4′), 77.9 (C-5′), 62.8 (C-6′) [9].

Spinoside A (7). $C_{19}H_{30}O_9$, amorphous powder. ESI-MS *m/z* 425 ([M + Na]$^+$). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.50, 2.10 (each 1H, d, J = 16.4, H-2), 5.97 (1H, s, H-4), 5.93 (1H, d, J = 15.6, H-7), 5.56 (1H, dd, J = 6.2, 15.6, H-8), 4.41 (1H, m, H-9), 1.15 (3H, d, J = 6.4, H-10), 0.90 (3H, s, CH_3-11), 0.89 (3H, s, CH_3-12), 4.25, 4.01 (each 1H, d, J = 18.6, CH_3-13), 4.09 (1H, d, J = 7.7, H-1′), 2.97 (1H, m, H-2′), 3.07–3.00 (3H, m, H-3′, 4′, 5′), 3.64 (1H, d, J = 12, H-6′a), 3.43 (1H, dd, J = 4.5, 12, H-6′b). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 41.4 (C-1), 49.5 (C-2), 197.5 (C-3), 121.3 (C-4), 167.2 (C-5), 77.3 (C-6), 131.8 (C-7), 131.3 (C-8), 72.1 (C-9), 22.3 (C-10), 23.6 (C-11), 23.1 (C-12), 59.2 (C-13), 100.1 (C-1′), 73.2 (C-2′), 77.0 (C-3′), 70.1 (C-4′), 77.3 (C-5′), 61.2 (C-6′) [10]. The compound was directly compared with authentic samples.

Apocynoside I (8). $C_{19}H_{30}O_8$, amorphous powder. FAB-MS (Gly) *m/z* 386 ([M – 1]$^-$). ^{1}H NMR (300 MHz, C_5D_5N, δ, ppm, J/Hz): 2.53, 2.16 (each 1H, d, J = 16.5, CH_2-2), 6.72 (1H, s, H-4), 5.82 (1H, dd, J = 8.9, 15.3, H-7), 5.94 (1H, dd, J = 5.2, 15.4, H-8), 4.55 (1H, m, H-9), 1.40 (3H, d, J = 6.4, H-10), 0.95 (3H, s, CH_3-11), 0.89 (3H, s, CH_3-13), 4.68 (2H, J = 14.9, CH_2-13), 4.85 (1H, d, J = 7.6, H-1′), 3.89 (1H, m, H-2′), 4.13 (1H, m, H-3′), 4.22 (1H, m, H-4′), 4.11 (1H, m, H-5′), 4.44 (2H, m, H-6′). ^{13}C NMR (75 MHz, C_5D_5N, δ): 36.3 (C-1), 48.8 (C-2), 198.4 (C-3), 123.6 (C-4), 161.2 (C-5), 51.1 (C-6), 141.3 (C-7), 125.6 (C-8), 62.5 (C-9), 24.6 (C-10), 27.7 (C-11), 27.2 (C-12), 69.2 (C-13), 103.5 (C-1′), 75.1 (C-2′), 78.4 (C-3′), 71.6 (C-4′), 78.2 (C-5′), 62.8 (C-6′) [5].

Euodionoside F (9). $C_{19}H_{32}O_8$, amorphous powder. FAB-MS (Gly), *m/z* 388 ([M – 1]$^-$). ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 6.62 (1H, s, H-4), 4.31, 4.17 (each 1H, dd, J = 1, 18, H-13a, b), 3.81 (1H, m, H-9), 2.57, 2.05 (1H, d, J = 17, H-2a, b), 1.92 (1H, m, H-6), 1.81–1.53 (4H, m, CH_2-7, 8), 1.23 (3H, d, J = 6.0, CH_3-10), 1.01 (3H, s, CH_3-11), 1.12 (3H, s, CH_3-12), 4.33 (1H, d, J = 8, H-1′), 3.84 (1H, m, H-6′a), 3.66 (1H, dd, J = 6, 11, H-6′b), 3.12 (1H, m, H-2′), 3.37–3.28 (3H, m, H-3′, 4′, 5′). ^{13}C NMR (125 MHz, CD_3OD, δ): 37.9 (C-1), 48.9 (C-2), 202.4 (C-3), 121.5 (C-4), 172.3 (C-5), 48.5 (C-6), 27.2 (C-7), 37.4 (C-8), 77.8 (C-9), 21.8 (C-10), 27.8 (C-11), 29.0 (C-12), 65.3 (C-13), 104.1 (C-1′), 75.7 (C-2′), 78.2 (C-3′), 71.7 (C-4′), 77.7 (C-5′), 63.0 (C-6′) [4].

Euodionoside A (10). $C_{19}H_{30}O_8$, amorphous powder. FAB-MS (Gly) *m/z* 385 ([M – 1]$^-$). ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.05 (1H, d, J = 16, H-7), 6.21 (1H, d, J = 16, H-8), 2.37 (1H, dd, J = 7, 15, H-4_{eq}), 2.00 (1H, dd, J = 10, 15, H-4_{ax}), 4.00 (1H, m, H-3), 2.30 (3H, s, CH_3-10), 1.25 (3H, s, CH_3-11), 1.18 (3H, s, CH_3-12), 4.31 (1H, d, J = 8, H-1′), 3.13 (1H, dd, J = 8, 9, H-2′), 3.45–3.25 (3H, m, H-3′, 4′, 5′), 3.88 (1H, dd, J = 2, 12, H-6′a), 3.66 (1H, dd, J = 6, 12, H-6′b). ^{13}C NMR (125 MHz, CD_3OD, δ): 35.9 (C-1), 41.3 (C-2), 72.4 (C-3), 38.5 (C-4), 67.3 (C-5), 72.1 (C-6), 143.5 (C-7), 134.4 (C-8), 200.3 (C-9), 27.4 (C-10), 25.1 (C-11), 27.6 (C-12), 21.1 (C-13), 103.1 (C-1′), 75.3 (C-2′), 78.2 (C-3′), 71.8 (C-4′), 78.1 (C-5′), 63.1 (C-6′) [4].

Megastigmane and its glycosides are a currently expanding class of compounds. Even with only 13 carbon atoms in the basic skeleton of megastigmane, several oxidation steps and glycosylation afforded many kinds of megastigmane derivatives and their glycosidic forms. Recently, a variety of megastigmane (ionol) glycosides have been isolated from various plant families, including Vitaceae, Apocynaceae, Beridaceae, Staphyleaceae, Rutaceae, Euphorbiaceae, Moraceae, and Cucurbitaceae, and the number of families with this skeleton is increasing [4, 11–14]. Moreover, it seems that the ability to oxidize the megastigmane and its glycosides at C-9 is a common factor, and di-, tri- and tetra-oxygenated megastigmane and its glycosides are often widely distributed in nature. In this study, through a combination of silica gel, RP C-18, and Sephadex LH-20 column chromatography, ten known megastigmane *O*-glucopyranosides **1–10** were isolated for the first time from the traditional Chinese medicine *L. glutinosa* (Lour.) C. B. Rob. All isolated compounds were oxygenated at C-3 and -9 positions. Megastigmane derivatives have, to date, never been reported from the genus *Litsea*. The result of the present study suggests that *L. glutinosa* was a source of megastigmane glycosides. The presence of this class of naturally occurring compounds in the genus *Litsea* could stimulate future phytochemical genomics studies.

ACKNOWLEDGMENT

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