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SECONDARY METABOLITES FROM *Leucaena leucocephala*

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Leucaena leucocephala (Leguminosea) is a small, leguminous plant native to tropical America, now widely distributed in southern Asia and neighboring islands [1]. Previous studies have show that gallocatechin, epigallocatechin, catechin, and epicatechin extracted from the roots of *L. leucocephala* exhibit nitrification inhibition against the bacterium *Nitrosomonas europaea* [2]. *L. leucocephala* was chosen for further phytochemical investigation. The MeOH extract of its plants was subjected to solvent partitioning and chromatographic separation to afford 14 pure substances. The chemical constituents in the plants of *L. leucocephala* were separated by column chromatography.

Investigation on the MeOH extract of the plants has led to the isolation of 14 compounds. These are four steroids: 5α,8α-epidioxy-(24ξ)-ergosta-6,22-dien-3β-ol (**1**) [3], β-sitosterol (**2**) [4], β-sitostenone (**3**), and stigmastenone (**4**) [5]; one triterpenoid: lupeol (**5**) [6]; one glyceride: 1,3-dipalmitoyl-2-oleoylglycerol (**6**) [7]; one alkaloid: linoleic acid (**7**) [8]; two benzenoids: methylparaben (**8**) [9] and isovanillic acid (**9**) [10]; and five chlorophylls: pheophytin-a (**10**) [11], pheophorbide a methyl ester (**11**) [12], methyl-13^2-hydroxy-(13^2-*S*)-pheophorbide-b (**12**) [13], 13^2-hydroxy-(13^2-*S*)-pheophytin-a (**13**) [14], and aristophyll-C (**14**) [15]. These compounds were obtained and characterized by comparison of their physical and spectral data (UV, IR, NMR, and MS) with values obtained in the literature. Among them, **1–9** were found for the first time from the species.

The specimen of *L. leucocephala* was collected from Pingtung County, Taiwan in December, 2008. A voucher specimen was characterized by Dr. Jin-Cherng Huang of the Department of Forest Products Science and Furniture Engineering, National Chiayi University, Chiayi, Taiwan and deposited in the School of Medical and Health Sciences, Fooyin University, Kaohsiung County, Taiwan.

The air-dried seeds of *L. leucocephala* (2.1 kg) were extracted with MeOH (80 L × 6) at room temperature, and the MeOH extract (162.5 g) was obtained upon concentration under reduced pressure. The MeOH extract was chromatographed over silica gel (800 g, 70~230 mesh) using *n*-hexane–acetone as eluent to produce 5 fractions. Part of fraction 1 (6.11 g) was subjected to Si gel chromatography by eluting with *n*-hexane–acetone (40:1), enriched with acetone to furnish 10 fractions (1-1–1-10). Fraction 1-1 (1.72 g) was resubjected to Si gel chromatography by eluting with *n*-hexane–acetone (80:1) to obtain linoleic acid (**7**) (58 mg, 0.0357%). Part of fraction 3 (6.94 g) was subjected to Si gel chromatography by eluting with *n*-hexane–acetone (50:1) to obtain β-sitosterol (**2**) (15 mg, 0.0092%). Part of fraction 4 (6.77 g) was subjected to Si gel chromatography by eluting with *n*-hexane–acetone (8:1), then enriched with acetone to furnish 7 fractions (4-1–4-7). Fraction 4-5 (0.62 g) was further purified by another silica gel column using *n*-hexane–acetone to obtain methylparaben (**8**) (8 mg, 0.0049%) and isovanillic acid (**9**) (12 mg, 0.0074%).

The air-dried brown beans of *L. leucocephala* (3.2 kg) were extracted with MeOH (80 L × 6) at room temperature, and the MeOH extract (171.5 g) was obtained upon concentration under reduced pressure. The MeOH extract was chromatographed over silica gel using *n*-hexane–acetone as eluent to produce 6 fractions. Part of fraction 1 (7.02 g) was subjected to Si gel chromatography by eluting with *n*-hexane–acetone (40:1) to obtain β-sitostenone (**3**) (6 mg, 0.0035%) and stigmastenone (**4**) (4 mg, 0.0023%). Part of fraction 2 (8.49 g) was subjected to Si gel chromatography by eluting with *n*-hexane–acetone (10:1), then enriched with acetone to furnish 8 fractions (2-1–2-8). Fraction 2-3 (2.33 g) was resubjected to Si gel chromatography by eluting with *n*-hexane–acetone (40:1) to obtain lupeol (**5**) (74 mg, 0.0431%). Part of fraction 3 (7.76 g) was subjected to Si gel chromatography by eluting with *n*-hexane–acetone (40:1) to obtain 1,3-dipalmitoyl-2-oleoylglycerol (**6**) (9 mg, 0.0052%). Part of fraction 6 (13.89 g) was subjected to Si gel chromatography by eluting with *n*-hexane–acetone (4:1), then enriched with acetone to obtain 5α,8α-epidioxy-(24ξ)-ergosta-6,22-dien-3β-ol (**1**) (43 mg, 0.0251%).

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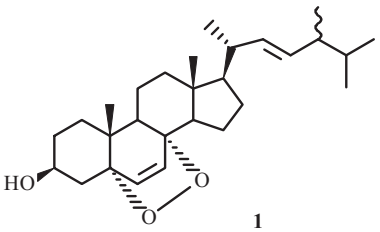

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The air-dried leaves of *L. leucocephala* (3.1 kg) were extracted with MeOH (80 L × 6) at room temperature, and the MeOH extract (159.5 g) was obtained upon concentration under reduced pressure. The MeOH extract was chromatographed over silica gel using *n*-hexane–acetone as eluent to produce 7 fractions. Part of fraction 1 (6.62 g) was subjected to Si gel chromatography by eluting with *n*-hexane–acetone (50:1) to obtain pheophytin-a (**10**) (6 mg, 0.0038%). Part of fraction 3 (7.56 g) was subjected to Si gel chromatography by eluting with *n*-hexane–acetone (8:1), then enriched with acetone to furnish 12 fractions (3-1–3-12). Fraction 3-7 (2.97 g) was resubjected to Si gel chromatography by eluting with *n*-hexane–acetone (40:1) to obtain 13^2-hydroxy-(13^2-*S*)-pheophytin-a (**13**) (12 mg, 0.0075%) and methyl-13^2-hydroxy-(13^2-*S*)-pheophorbide-b (**12**) (9 mg, 0.0056%). Part of fraction 6 (17.15 g) was subjected to Si gel chromatography by eluting with *n*-hexane–acetone (4:1) to obtain aristophyll-C (14) (4 mg, 0.0025%).

The air-dried green beans of *L. leucocephala* (1.3 kg) were extracted with MeOH (80 L × 6) at room temperature, and the MeOH extract (146.5 g) was obtained upon concentration under reduced pressure. The MeOH extract was chromatographed over silica gel using *n*-hexane–acetone as eluent to produce 8 fractions. Part of fraction 4 (7.19 g) was subjected to Si gel chromatography by eluting with *n*-hexane–acetone (8:1) to obtain pheophorbide a methyl ester (**11**) (8 mg, 0.0055%).

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