Steroids from the Whole Plants of *Leucaena Leucocephala*

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Received January 10, 2009; revised February 21, 2009; accepted February 23, 2009

Abstract

Four steroids, 5α,8α-epidioxy-(24ξ)-ergosta-6,22-dien-3β-ol (1), β-sitosterol (2), β-sitostenone (3), and stigmastenone (4), along with 10 known compounds were isolated from the whole plants of *Leucaena leucocephala* (Leguminosae). Lupeol (5), 1,3-dipalmitoyl-2-oleoylglycerol (6), methylparabene (8) and isovanillic acid (9) were found for the first time from the species. The structure of these compounds were characterized and identified by spectra analyses.

Keywords: Steroids, *Leucaena Leucocephala*, Leguminosae

1. Introduction

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

Investigation on the MeOH extract of the plants has led to the isolation of 14 compounds, four steroids: 5α,8α-epidioxy-(24ξ)-ergosta-6,22-dien-3β-ol (1) ([Figure 1](#fig1){ref#}) [3], β-sitosterol (2) ([Figure 2](#fig2){ref#}) [4], β-sitostenone (3) ([Figure 3](#fig3){ref#}) and stigmastenone (4) ([Figure 4](#fig4){ref#}) [5]; one terpenoid: lupeol (5) [6]; one glyceride: 1,3-dipalmitoyl-2-oleoylglycerol (6) [7]; one alkanoïd: linoleic acid (7) [8]; two benzzenoids: methylparabene (8) [9] and isovanillic acid (9) [10]; and five chlorophylls: pheophytin-a (10) [11], pheophorbide a methyl ester (11) [12], methyl-13β-hydroxy-(13β-S)-pheophorbide-b (12) [13], 13β-hydroxy-(13′-S)-pheophytin-a (13) [14] and aristophyll-C (14) [15]. These compounds were obtained and characterized by the comparison of their physical and spectral data (UV, IR, NMR and MS) with values obtained in the literature. Among them, 1, 5, 6, 8 and 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 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* (5.0 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),

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Chemical structure of 5α,8α-epidioxy-(24ξ)-ergosta-6,22-dien-3-ol (1).
enriched with acetone to furnish 10 fractions (1-1 to 1-10). Fraction 1-1 (1.72 g) was re-subjected to Si gel chromatography, 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 \( \beta \)-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 to 4-7). Fraction 4-5 (0.62 g) was further purified by another silica gel column using n-hexane/acetone to obtain methylparabene (8) (8 mg, 0.0049%) and isovanillic acid (9) (12 mg, 0.0074%). The air-dried brown beans of \( L. \) leucocephala (5.0 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 \( \beta \)-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 to 2-8). Fraction 2-3 (2.33 g) was re-subjected to Si gel chromatography, 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-24ζ ergosta-6,22-dien-3β-ol (1) (43 mg, 0.0251%).

The air-dried leaves of \( L. \) leucocephala (5.0 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 to 3-12). Fractions 3-7 (2.97 g) was re-subjected to Si gel chromatography, eluting with n-hexane/acetone (40:1) to obtain 13β-hydroxy-(13S)-pheophytin-a (13) (12 mg, 0.0075%) and methyl-13β-hydroxy-(13S)-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 (5.0 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%).

2. Acknowledgements

This investigation was supported by a grant from the
National Science Council of Republic of China (NSC 97-2320-B-242-002-MY3).

3. References


