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# Structure elucidation of β-stigmasterol and β-sitosterol from *Sesbania grandiflora* (Linn.) Pers. and β-carotene from *Heliotropium indicum* Linn. by NMR spectroscopy

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The flowers of *Sesbania grandiflora* (Linn.) Pers. afforded a 3:2 mixture of β-stigmasterol **(1a)** and β-sitosterol **(1b)**, and oleanolic acid **(2)**, while the leaves of *Heliotropium indicum* Linn. yielded β-carotene **(3)**, lutein **(4)**, a mixture of polyprenols **(5)**, and a mixture of b-stigmasterol and b-sitosterol. The structures of **1a**, **1b**, and **3** were elucidated by extensive 1D and 2D NMR analyses. Antimicrobial activity tests on **3** indicated that it has high activity against *Pseudomonas aeruginosa* and *Aspergillus niger*, slight activity against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Trichophyton mentagrophytes*, and inactive against *Bacillus subtilis*.

***Keywords:*** *Sesbania grandiflora* (Linn.) Pers.; Leguminosae; *Heliotropium indicum* Linn.; Boraginaceae; β-stigmasterol, β-sitosterol; oleanolic acid; lutein; β-carotene; polyprenols; antimicrobial activity

## INTRODUCTION

*Sesbania grandiflora* (Linn.) Pers., commonly known as katuray is a tree planted for its edible flowers and pods. The juice and flowers of the plant are used as a remedy for nasal catarrh and headache. The flowers are also used as emollient and laxative [1]. Earlier studies on the plant reported the isolation of flavonoids triterpenes, and sterols [2–10].

*Helitropium indicum* Linn., commonly known as higad-higaran is a common weed found in open waste places throughout the Philippines. It is used in folk medicine to treat and prevent various skin diseases and allergic skin rashes like boils, gum-boils, and pimples [1]. Furthermore, recent studies have shown that the extracts of the plant possess good wound healing activity in rats [11]. Previous studies on the plant reported the isolation of alkaloids [12–23], sterols and terpenoids [19–24].

This study reports the isolation and structure elucidation of an approximately 3:2 mixture of β-stigmasterol **(1a)** and β-sitosterol **(1b)** from the flowers of *Sesbania grandiflora* (Linn.) Pers. and β-carotene **(3)** from the leaves of *Heliotropium indicum* Linn. Although **1a**, **1b** and **3** have been commonly isolated from plants, this is the first report on their full structure elucidation by extensive 1D and 2D NMR spectroscopy. Oleanolic acid **(2)** was also isolated from *S. grandiflora* (Linn.) Pers., while *H. indicum* Linn. also afforded lutein **(4)**, a mixture of polyprenols **(5)**, and a mixture of β-stigmasterol and β-sitosterol. To the best of our knowledge this is the first report on the isolation of **3-5** from *H. indicum* Linn.

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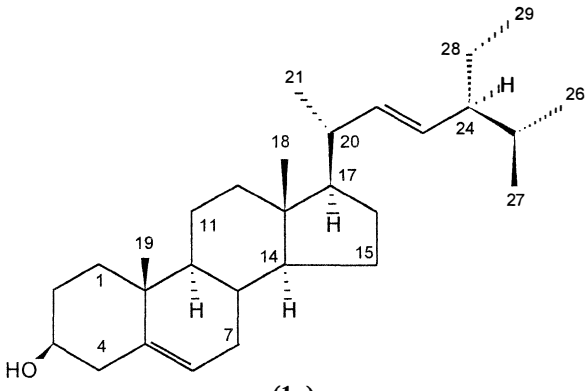
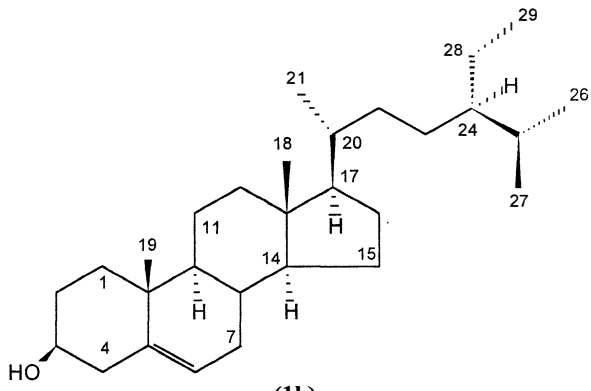


(1a)



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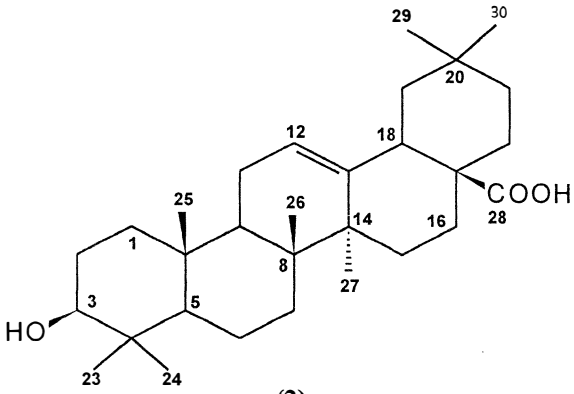

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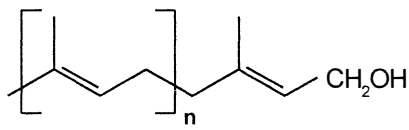
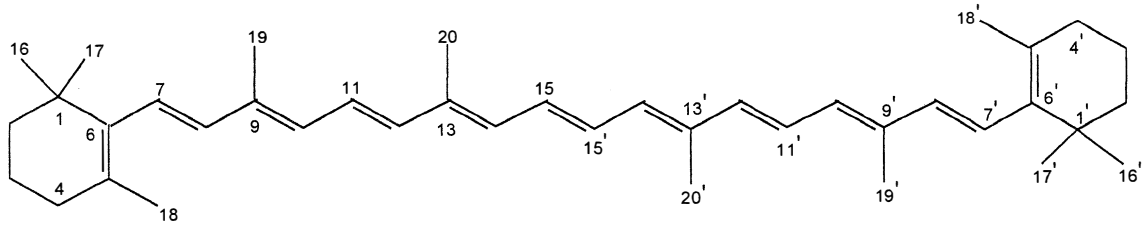


where n=8 to 12

(5)



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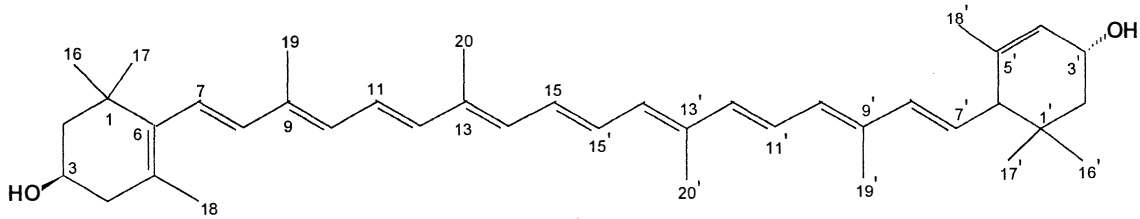

(4)

## RESULTS AND DISCUSSION

The dichloromethane extract of the air-dried flowers of *Sesbania grandiflora* (Linn.) Pers. yielded an approximately 3:2 mixture of β-stigmasterol **(1a)** and β-sitosterol **(1b)**, and oleanolic acid **(2)**. The structures of **1a** and **1b** were elucidated by extensive 1D and 2D NMR spectral analysis as follows.

The $^1$H-NMR spectrum of **1** indicated resonances for a mixture of two compounds based on integrals and disparity in single hydrogen peaks. Resonances with large intensities and integrals were attributed to the major compound, while small resonance intensities and integrals were assigned to the minor compound. The $^1$H-NMR spectrum of the major compound **1a** shown in Table 1 indicated resonances for three olefinic methine protons at δ 5.02 (dd, J = 8.4, 15.1 Hz), 5.15 (dd, J = 8.4, 15.1 Hz), and 5.35 (d, J = 4.7 Hz); a carbinyl proton at δ 3.53 (m); and six methyl protons at δ 0.70 (s), 0.80 (t, J = 6.0 Hz), 0.83 (d, J = 6.1 Hz), 0.84 (d, J = 6.4 Hz), 1.01 (s) and 1.02 (d, J = 6.8 Hz). The J-modulated $^{13}$C-NMR spectral data (Table 1) indicated resonances for twenty-nine carbon atoms with the following functionalities: four olefinic carbons (δδ 121.7, 129.2, 138.3, 140.7); a carbinyl

**Table 1. 300 MHz $^1$H-NMR, 100 MHz $^{13}$C-NMR, HMBC, and NOESY correlations of 1a**

| Position | $\delta_C$ | $\delta_H$ mult. (J Hz) | HMBC Correlations | NOESY Correlations |
|---|---|---|---|---|
| 1 | 37.2 | 1.08 | C-2, C-3, C-5, C-9, C-10, C-19 | H-1b, H-2b, H-3, H-9 |
| | | 1.83 | C-2, C-3, C-5, C-10 | H-1a, H-2a, H-11a, H-19 |
| 2 | 31.6 | 1.49 | C-1, C-3, C-13 | H-1b, H-2b, H-4b, H-19 |
| | | 1.82 | C-3, C-4, C-10 | H-1a, H-2a, H-3 |
| 3 | 71.8 | 3.53 | – | H-1a, H-2b, H-4a |
| 4 | 42.27 | 2.24 | C-2, C-3, C-5, C-6,C-10 | H-3, H-6, H-7a |
| | | 2.28 | C-2, C-3, C-5, C-6,C-10 | H-2a, H-6, H-19 |
| 5 | 140.7 | – | – | – |
| 6 | 121.7 | 5.35 (1H, d, J=4.7 Hz) | C-7, C-10, C-13 | H-4a, H-4b, H-7a, H-7b |
| 7 | 31.9 | 1.53 | C-5, C-6, C-8, C-9, C-14 | H-4a, H-6, H-7b, H-9, H-14 |
| | | 1.98 | C-5, C-6, C-8, C-9, C-14 | H-6, H-7a, H-8, H-15a, H-19 |
| 8 | 31.9 | 1.46 | C-7, C-9, C-14 | H-7b, H-15a, H-18, H-19 |
| 9 | 50.1 | 0.94 | C-1, C-7, C-8, C-10, C-11, C-19 | H-1a, H-7a,H-11b,H-12b, H-14 |
| 10 | 36.5 | – | – | – |
| 11 | 21.06 | 1.45 | C-8, C-9, C-12 | H-1b, H-12a, H-18, H-19 |
| | | 1.48 | C-4, C-8, C-9, C-12 | H-9, H-12b |
| 12 | 39.7 | 1.15 | C-4, C-11, C-18 | H-11a, H-12b, H-21 |
| | | 1.97 | C-9, C-14 | H-9, H-11b, H-12a, H-14, H-17 |
| 13 | 42.19 | – | – | – |
| 14 | 56.8 | 1.00 | C-7, C-8, C-9, C-15, C-16, C-17, C-18 | H-7a, H-9, H-12b, H-15b, H-17 |
| 15 | 24.4 | 1.06 | C-8, C-14, C-17 | H-7b, H-8, H-15a, H-15b, H-16a |
| | | 1.55 | C-4, C-8, C-14, C-16 | H-14, H-15a, H-17 |
| 16 | 28.9 | 1.27 | C-15, C-17, C-20 | H-15a, H-16b, H-18, H-20 |
| | | 1.71 | C-15 | H-16a, H-17 |
| 17 | 55.9 | 1.13 | C-4, C-12, C-14,C-16, C-18, C-20, C-22 | H-12b, H-14, H-15b, H-16b, H-22 |
| 18 | 12.04 | 0.70 s (Me) | C-4, C-12, C-14, C-17 | H-8, H-11a, H-15a, H-16a, H-19,H-20, H-21 |
| 19 | 19.4 | 1.01 s (Me) | C-1, C-5, C-9, C-10 | H-1b, H-2a, H-4b, H-7b,H-8, H-11a, H-18 |
| 20 | 40.5* | 2.04 | C-17, C-22, C-23 | H-16a, H-18, H-21, H-23 |
| 21 | 21.09 | 1.02 d (6.8 Hz, Me) | C-17, C-20, C-22 | H-12a, H-18, H-21 |
| 22 | 138.3* | 5.15 dd (8.4, 15.1 Hz) | C-17, C-20, C-21, C-23, C-24 | H-13, H-24 |
| 23 | 129.2* | 5.02 dd (8.4, 15.1 Hz) | C-20, C-22, C-24, C-25, C-28 | H-20, H-28, H-29 |
| 24 | 51.2* | 1.53 | C-23, C-26, C-27 | H-22, H-26, H-28 |
| 25 | 31.9 | 1.44 | C-23, C-24 | H-26, H-27, H-28 |
| 26 | 21.2 | 0.84 d (6.4 Hz, Me) | C-24, C-25, C-27 | H-24, H-25 |
| 27 | 18.97 | 0.83 d (6.1 Hz, Me) | C-24, C-25, C-26 | H-25, H-28 |
| 28 | 25.4 | 1.15 | C-23, C-24, C-29 | H-23, H-24, H-25, H-27, H-29 |
| 29 | 12.3 | 0.80 t (6.0 Hz, Me) | C-24, C-28 | H-23, H-28 |

* Main differences between the two compounds

carbon (δδ 71.8); seven methine carbons (δδ 31.9 (2×), 40.5, 50.1, 51.2, 55.9, 56.8); two quaternary carbons (δδ 36.5, 42.19); nine methylene carbons (δδ 21.06, 24.4, 25.4, 28.9, 31.6, 31.9, 37.2, 39.7, 42.27); and six methyl carbons (δδ 12.04, 12.3, 18.97, 19.4, 21.09, 21.2). These are characteristic resonances of a sterol with two olefins and an alcohol.

The COSY 2D NMR spectrum of **1a** showed correlations for five spin systems: (1) $H_2$-1/$H_2$-2/H-3/$H_2$-4; (2) H-6/$H_2$-7/H-8/H-9/$H_2$-11/$H_2$-12/H-14/$H_2$-15/$H_2$-16; (3) H-20/$H_3$-21; (4) H-22/H-23/H-24/H-25/$H_3$-27; (5) $H_2$-28/$H_3$-29 (Fig. 1).

The $^1$H and $^{13}$C assignments of **1a** (Table 1) were verified by 2D Heteronuclear Single Quantum Coherence (HSQC) experiments, while connectivities were verified by Heteronuclear Multi Bond Coherence (HMBC) (Table 1 and Fig. 1). The hydroxyl group

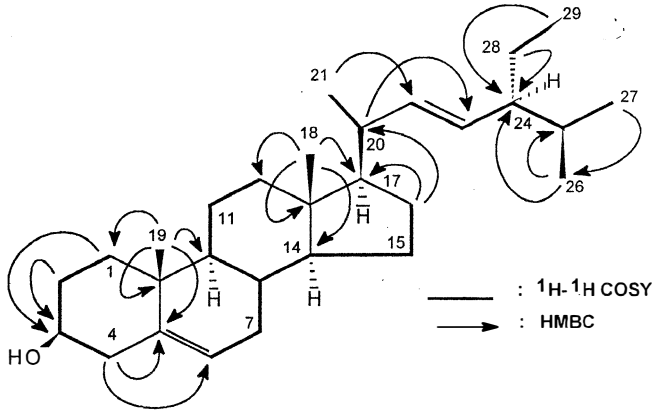


*Fig. 1. $^1$H-$^1$H COSY correlations and Key $^1$H-$^{13}$C long-range correlations for* ***1a.***