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Triterpenes and sterols from *Hoya diversifolia* Blume

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ABSTRACT

Chemical investigation of the dichloromethane extracts of *Hoya diversifolia* Blume led to the isolation of *β*-amyrin cinnamate (**1**), squalene (**2**), *β*-sitosterol (**3**), a mixture of *β*-amyrin (**4a**), *α*-amyrin (**4b**) and lupeol (**4c**) in a 4:2:1 ratio and saturated hydrocarbons from the leaves; and **2**, taraxerol (**5**), lupeol cinnamate (**6**), and a mixture of **3** and stigmasterol (**7**) in a 2:1 ratio from the stems. The structures of **1**-**7** were identified by comparison of their NMR data with those reported in the literature.

INTRODUCTION

Hoya plants are also called wax plants due to the waxy appearance of their leaves or flowers. There are at least 109 species of *Hoya* found in the Philippines, 88 of these are endemic to the country. *Hoya diversifolia* Blume is an indigenous Philippine ornamental plant found in Palawan and Luzon, specifically Quezon Province (Aurigue, 2013). It is distributed in Myanmar, Indo-China, peninsular Thailand, Peninsular Malaysia, Singapore, Sumatra and Java (Mansur, 2003). An earlier study reported that *H. diversifolia* afforded apigenin-*O*-glycoside, apigenin-di-*C*-glycoside, vitexin and isovitexin. The extract of this plant exhibited strong antinematodal activity against the pine wood nematode *Bursaphelenchus xylophilus*. In Malaysia and Vietnam, a decoction of the leaves of *H. diversifolia* is used as a bath to treat rheumatism (Mansur, 2003). Another study reported that *H. diversifolia* contains 11.1% free terpenoids and 81.7% triterpene cinnamates (Warnaar, 1984). This study is part of our research on the chemical constituents of Philippine native hoyas. We earlier reported the isolation of lupenone and lupeol from the roots; lupeol, squalene and *β*-sitosterol from the leaves; and betulin from the stems of *H. mindorensis* Schlechter (Ebajo *et al.*, 2014). In another study, we reported the isolation of lupeol, *α*-amyrin, *β*-amyrin, lupeol acetate, *α*-amyrin acetate, and *β*-amyrin acetate from the stems; and *α*-amyrin, bauerenol, squalene, lutein, *β*-sitosterol, and stigmasterol from the leaves of *H. multiflora* Blume (Ebajo *et al.*, 2015a). Recently, the isolation of *β*-amyrin cinnamate and taraxerol from the stems; and taraxerol, triglycerides, chlorophyll a, and a mixture of *β*-sitosterol and stigmasterol from the leaves of *H. wayetii* Kloppenb has been reported (Ebajo *et al.*, 2015b).

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Furthermore, the isolation of taraxerol, taraxerone, a mixture of *β*-sitosterol and stigmasterol in about 2:1 ratio, and a mixture of *α*-amyrin cinnamate and *β*-amyrin cinnamate in about 1:2 ratio from the stems; taraxerol, taraxerone, and *β*-sitosterol from the roots; a mixture of *α*-amyrin cinnamate and *β*-amyrin cinnamate in about 3:2 ratio from the flowers; and squalene, *β*-sitosterol, and saturated hydrocarbons from the leaves of *H. buotii* has been reported (Ebajo *et al.*, 2015c). We report herein the isolation of *β*-amyrin cinnamate (**1**), squalene (**2**), β-sitosterol (**3**), and a mixture of *β*-amyrin (**4a**), *α*-amyrin (**4b**) and lupeol (**4c**) in a 4:2:1 ratio and saturated hydrocarbons from the leaves; and **2**, taraxerol (**5**), lupeol cinnamate (**6**), and a mixture of **3** and stigmasterol (**7**) in a 2:1 ratio from the stems of *H. diversifolia.* The chemical structures of **1-7** are presented in Fig. 1.

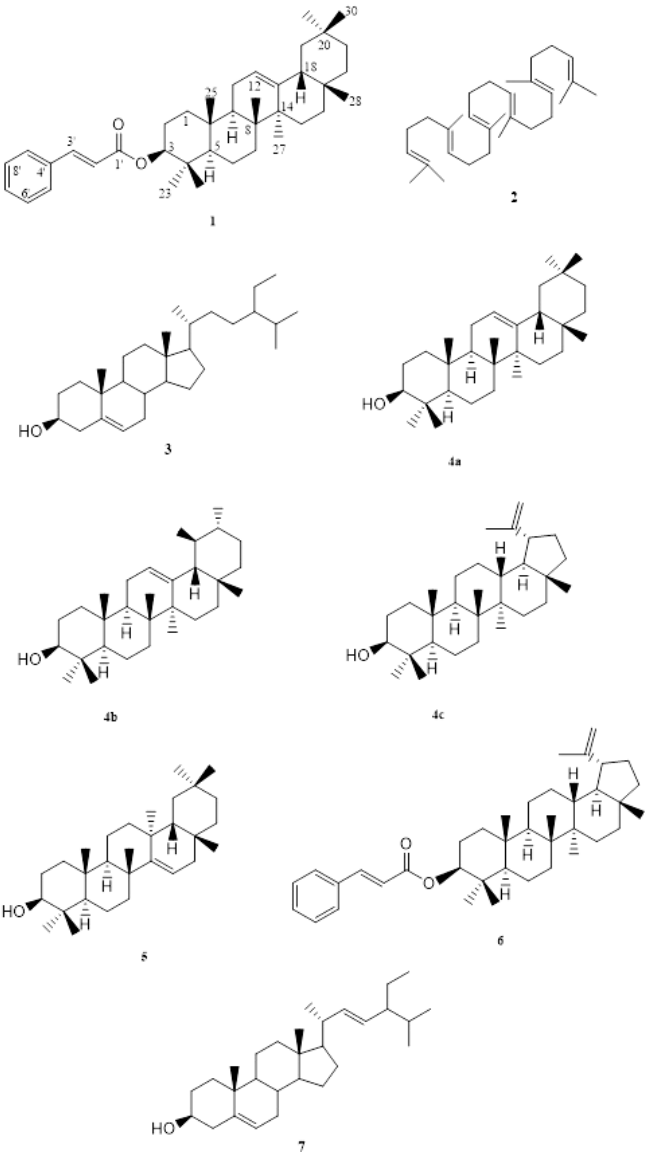

Fig 1. Chemical structures of *β*-amyrin cinnamate (**1**), squalene (**2**), β-sitosterol (**3**), *β*-amyrin (**4a**), *α*-amyrin (**4b**), lupeol (**4c**), taraxerol (**5**), lupeol cinnamate (**6**), and stigmasterol (**7**) from *Hoya diversifolia.*

MATERIALS AND METHODS

General Experimental Procedure

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR spectra. Column chromatography was performed, with silica gel 60 (70-230 mesh). Thin layer chromatography, was performed with plastic backed plates coated with silica gel F_{254} and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming.

Sample Collection

Healthy vines of *Hoya diversifolia* Blume were collected from the Philippine Nuclear Research Institute *Hoya* Germplasm Collection with Accession Number H.034 under MTA No. 2015-002 dated January 20, 2015. The plants were propagated by stem cuttings from an established material originally obtained from Lucban, Quezon Province, Luzon Island, Philippines. The species is authenticated by one of the authors (FBA).

General Isolation Procedure

The air-dried leaves (157.2 g), and stems (383.9 g) of *H. diversifolia* were ground in a blender, soaked in CH_2Cl_2 for three days and then filtered. The filtrates were concentrated under vacuum to afford crude extracts of leaves (9.1 g), and stems (11.75 g) which were each chromatographed by gradient elution with CH_2Cl_2, followed by increasing amounts of acetone at 10% increment by volume as eluents. A glass column 18 inches in height and 1 inch internal diameter was used for the fractionation of the crude extracts. Eleven 20 mL fractions were collected. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography of fractions from the crude extracts. 2 mL fractions were collected. Fractions with spots of the same R_f values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. Rechromatography and final purifications were conducted using Pasteur pipettes as columns. 1 mL fractions were collected.

Isolation of Chemical Constituents of the Leaves

The 10% acetone in CH_2Cl_2 fraction from the chromatography of the crude extract was rechromatographed using 1% EtOAc in petroleum ether. 1 mL fractions were collected. Fractions 7-12 yielded **2** (5 mg), while fractions 1-5 afforded saturated hydrocarbons (6 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed using 2.5% EtOAc in petroleum ether and portions of 2 mL fractions were collected. Fractions 17-21 were combined to yield **1** (3 mg) after washing with petroleum ether. The 40% acetone in CH_2Cl_2 fraction was rechromatographed using 10% EtOAc in petroleum ether, followed by 15% EtOAc in petroleum ether. 1 mL fractions were collected. Fractions 5-6 eluted with 10% EtOAc in petroleum ether afforded a mixture of

4a, **4b** and **4c** (4 mg) after washing with petroleum ether. The 10-13 fractions eluted with 15% EtOAc in petroleum ether afforded **3** (5 mg) after washing with petroleum ether.

Isolation of Chemical Constituents of the Stems

The 10% acetone in CH_2Cl_2 fraction from the chromatography of the crude extract was rechromatographed using petroleum ether and 1 mL fractions were collected. Fraction 16 afforded **2** (5 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed using 2.5% EtOAc in petroleum ether and 1-mL fractions were collected. Fractions 11-19 from this column were combined and rechromatographed using 1% EtOAc in petroleum ether. 1 mL fractions were collected. The 12th fraction afforded **6** (2 mg) after washing with petroleum ether. The 30% acetone in CH_2Cl_2 fraction was rechromatographed using 15% EtOAc in petroleum ether and 1 mL fractions were collected. Fractions 15-20 were combined and rechromatographed using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) and 1 mL fractions were collected. Fractions 10-12 yielded **5** (3 mg) after washing with petroleum ether. The 40% acetone in CH_2Cl_2 fraction was rechromatographed using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) and 1 mL fractions were collected. Fractions 5-10 were combined and rechromatographed using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) and 1 mL fractions were collected. Fractions 3-5 were combined to yield a mixture of **3** and **7** (2 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of *H. diversifolia* yielded **1**–**7** and saturated hydrocarbons. The NMR spectra of **1** are in accordance with data reported in the literature for β-amyrin cinnamate (Akihisa *et al.*, 2010; Ebajo *et al.*, 2015c); **2** for squalene (Ragasa *et al.*, 2014b; 2014c); **3** for *β*-sitosterol (Ragasa *et al.*, 2013b); **4a** for *β*-amyrin (Ragasa *et al.*, 2013b); **4b** for *α*-amyrin (Ragasa *et al.*, 2013b); **4c** for lupeol (Ragasa *et al.*, 2013b); **5** for taraxerol (Tareq *et al.*, 2009; Ragasa *et al.*, 2014a; Ebajo *et al.*, 2015b, 2015c); **6** for lupeol cinnamate (Akihisa *et al.*, 2010; Lakshmi *et al.*, 2014); **7** for stigmasterol (Ragasa *et al.*, 2013b); and saturated hydrocarbons (Ebajo *et al.*, 2015c).

The 4:2:1 ratio of the mixture of *β*-amyrin (**4a**), *α*-amyrin (**4b**) and lupeol (**4c**) was deduced from the intensities and integrations of the ^{1}H NMR resonances for the olefinic protons of **4a** at δ 5.16 (t, *J* = 3.6 Hz) (Ragasa *et al.*, 2013b), **4b** at δ 5.11 (t, *J* = 3.6 Hz) (Ragasa *et al.*, 2013b), and **4c** at δ 4.67 (d, *J* = 2.4 Hz) and 4.55. (d, *J* = 2.4 Hz) (Ragasa *et al.*, 2013b). The 2:1 ratio of *β*-sitosterol (**3**) and stigmasterol (**7**) was deduced from the intensities and integrations of the ^{1}H NMR resonances for olefinic protons at δ 5.35 (d, *J* = 4.8 Hz, H-5) and methyl protons at δ 0.66 (s) for **3** and olefinic protons at δ 5.35 (d, *J* = 4.8 Hz, H-5), 5.13 (dd, J = 8.4, 15.6 Hz) and 5.00 (dd, J = 8.4, 15.0 Hz) and the methyl protons at δ 0.68 (s) for **7** (Ragasa *et al.*, 2013b). These results indicate that *H. diversifolia* shares similar chemical characteristics with other members of the genus *Hoya*: *H. wayetii* (Ebajo *et al.*, 2015b) and *H. buotii* (Ebajo *et al.*, 2015c) which yielded *β*-amyrin cinnamate (**1**) and taraxerol (**5**); *H. mindorensis* (Ebajo *et al.*, 2014), *H. multiflora* (Ebajo *et al.*, 2015a), *H. wayetii* (Ebajo *et al.*, 2015b) and *H. buotii* (Ebajo *et al.*, 2015c) which contained *β*-sitosterol (**3**) and stigmasterol (**7**); *H. mindorensis* (Ebajo *et al.*, 2014), *H. multiflora* (Ebajo *et al.*, 2015a) and *H. buotii* (Ebajo *et al.*, 2015c) which afforded squalene (**2**); and *H. multiflora* (Ebajo *et al.*, 2015a) which yielded *β*-amyrin (**4a**) and *α*-amyrin (**4b**).

CONCLUSION

Hoya diversifolia Blume is an ornamental plant native to the Philippines and other Asian countries with chemical constituents from dichloromethane extracts identified as follows: β-amyrin cinnamate, squalene, β-sitosterol, a mixture of β-amyrin, α-amyrin and lupeol in a 4:2:1 ratio, and saturated hydrocarbons from the leaves; and taraxerol, lupeol cinnamate, and a mixture of β-sitosterol and stigmasterol in a 2:1 ratio from the stems.

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