

## Chemical Constituents of *Hoya buotii* Kloppenb.

Virgilio D. Ebajo Jr.<sup>1</sup>, Robert Brkljača<sup>2</sup>, Sylvia Urban<sup>2</sup>, Consolacion Y. Ragasa<sup>1,3\*</sup>

<sup>1</sup>Chemistry Department, De La Salle University, 2401 Taft Avenue, Manila 1004, Philippines. <sup>2</sup>School of Applied Sciences (Discipline of Chemistry), Health Innovations Research Institute (HIRi) RMIT University, GPO Box 2476V Melbourne, Victoria 3001, Australia. <sup>3</sup>Chemistry Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna 4024, Philippines.

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### ABSTRACT

Chemical investigation of the dichloromethane extracts of *Hoya buotii* Kloppenb. afforded taraxerone (**1**), taraxerol (**2**), a mixture of  $\beta$ -sitosterol (**3a**) and stigmasterol (**3b**) in about 2:1 ratio, and a mixture of  $\alpha$ -amyrin cinnamate (**4a**) and  $\beta$ -amyrin cinnamate (**4b**) in about 1:2 ratio from the stems; **1**, **2**, and **3a** from the roots; a mixture of **4a** and **4b** in about 3:2 ratio from the flowers; and **3a**, squalene (**5**) and saturated hydrocarbons from the leaves. The structures of **1-5** 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 *Hoyas* found in the Philippines, 88 of these are endemic to the country (Aurigie, 2013). *Hoya buotii* Kloppenb. is an endemic Philippine ornamental plant which was first collected from Mt. Banahaw, Quezon Province and was also found in the Sierra Madre Mountain Range of Luzon and Mt. Halcon in Mindoro (Aurigie, 2013).

The plant bears a cluster of creamy yellow flowers which resemble a starfish with reddish center (Aurigie, 2013). There are no reported chemical studies and biological activities on *H. buotii*. This study is part of our research on the chemical constituents of endemic Philippine *Hoyas*. We earlier reported the isolation of lupenone (**I**) and lupeol (**II**) from the roots; **II**, squalene (**III**) and  $\beta$ -sitosterol (**IV**) from the leaves; and betulin (**V**) from the stems of *H. mindorensis* Schlechter (Ebajo *et al.*,

2014). In another study, we reported the isolation of **II**,  $\alpha$ -amyrin (**VI**),  $\beta$ -amyrin (**VII**), lupeol acetate (**VIII**),  $\alpha$ -amyrin acetate (**IX**), and  $\beta$ -amyrin acetate (**X**) from the stems; and **VI**, bauerenol (**XI**), **III**, lutein (**XII**), **IV**, and stigmasterol (**XIII**) from the leaves of *H. multiflora* Blume (Ebajo *et al.*, 2015a). Recently, the isolation of  $\beta$ -amyrin cinnamate (**XIV**) and taraxerol (**XV**) from the stems; and **XV**, triglycerides (**XVI**), chlorophyll a (**VII**), and a mixture of **IV** and **XIII** from the leaves of *H. wayetii* Kloppenb has been reported (Ebajo *et al.*, 2015b). We report herein the isolation of taraxerone (**1**), taraxerol (**2**), a mixture of  $\beta$ -sitosterol (**3a**) and stigmasterol (**3b**) in about 2:1 ratio, and a mixture of  $\alpha$ -amyrin cinnamate (**4a**) and  $\beta$ -amyrin cinnamate (**4b**) in about 1:2 ratio from the stems; **1**, **2**, and **3a** from the roots; a mixture of **4a** and **4b** in about 3:2 ratio from the flowers; and **3a**, squalene (**5**), and saturated hydrocarbons from the leaves of *H. buotii*. To our knowledge this is the first report on the isolation of these compounds from *H. buotii*. Chemical structures of taraxerone (**1**), taraxerol (**2**),  $\beta$ -sitosterol (**3a**), stigmasterol (**3b**),  $\alpha$ -amyrin cinnamate (**4a**),  $\beta$ -amyrin cinnamate (**4b**), and squalene (**5**) from *H. buotii*.

\* Corresponding Author

Email: [consolacion.ragasa@dlsu.edu.ph](mailto:consolacion.ragasa@dlsu.edu.ph)

## MATERIALS AND METHODS

### General experimental procedure

$^1\text{H}$  (500 MHz) and  $^{13}\text{C}$  (125 MHz) NMR spectra were acquired in  $\text{CDCl}_3$  on a 500 MHz Agilent DD2 NMR spectrometer with referencing to solvent signals ( $\delta$  7.26 and 77.0 ppm). Column chromatography was performed with silica gel 60 (70-230 mesh). Thin layer chromatography was performed with plastic backed plates coated with silica gel  $\text{F}_{254}$  and the plates were visualized by spraying with vanillin/ $\text{H}_2\text{SO}_4$  solution followed by warming.

### Sample collection

The sample was collected from a garden in Calocan City, Philippines in March 2014. The sample was authenticated at the Philippine National Herbarium, Botany Division, National Museum of the Philippines. It was identified as *Hoya buotii* Kloppenb. by Danilo N. Tandang.

### General isolation procedure

A glass column 18 inches in height and 1.0 inch internal diameter was packed with silica gel. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in  $\text{CH}_2\text{Cl}_2$  (10% increment) as eluents. Fifty milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same  $R_f$  values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Two milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

### Isolation of the chemical constituents of the stems

The air-dried stems of *H. buotii* (71.5 g) were ground in a blender, soaked in  $\text{CH}_2\text{Cl}_2$  for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (2.3 g) which was chromatographed using increasing proportions of acetone in  $\text{CH}_2\text{Cl}_2$  at 10% increment. The 10% acetone in  $\text{CH}_2\text{Cl}_2$  fraction was rechromatographed (4  $\times$ ) using 5% EtOAc in petroleum ether to afford a mixture of **4a** and **4b** (3 mg) after washing with petroleum ether. The 20% acetone in  $\text{CH}_2\text{Cl}_2$  fraction was rechromatographed (3  $\times$ ) using 10% EtOAc in petroleum ether to afford **1** (6 mg) after washing with petroleum ether. The 30% acetone in  $\text{CH}_2\text{Cl}_2$  fraction was rechromatographed (3  $\times$ ) using 20% EtOAc in petroleum ether to afford **2** (12 mg) after washing with petroleum ether. The 40% acetone in  $\text{CH}_2\text{Cl}_2$  fraction was rechromatographed (2  $\times$ ) using  $\text{CH}_3\text{CN}:\text{Et}_2\text{O}:\text{CH}_2\text{Cl}_2$  (0.5:0.5:9, v/v) to yield a mixture of **3a** and **3b** (8 mg) after washing with petroleum ether.

### Isolation of the chemical constituents of the roots

The air-dried roots of *H. buotii* (7.2 g) were ground in a

blender, soaked in  $\text{CH}_2\text{Cl}_2$  for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (0.1 g) which was chromatographed using increasing proportions of acetone in  $\text{CH}_2\text{Cl}_2$  at 10% increment. The 20% acetone in  $\text{CH}_2\text{Cl}_2$  fraction was rechromatographed using 10% EtOAc in petroleum ether to afford **1** (4 mg) after washing with petroleum ether.

The 40% acetone in  $\text{CH}_2\text{Cl}_2$  fraction was rechromatographed using 15% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed using the same solvent (2  $\times$ ) to afford **2** (5 mg) after washing with petroleum ether. The more polar fractions were combined and rechromatographed using 20% EtOAc in petroleum ether to yield **3a** (3 mg) after washing with petroleum ether.

### Isolation of the chemical constituents of the flowers

The air-dried flowers of *H. buotii* (6.0 g) were ground in a blender, soaked in  $\text{CH}_2\text{Cl}_2$  for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (0.1 g) which was chromatographed using increasing proportions of acetone in  $\text{CH}_2\text{Cl}_2$  at 10% increment. The  $\text{CH}_2\text{Cl}_2$  fraction was rechromatographed (3  $\times$ ) using 5% EtOAc in petroleum ether to afford a mixture of **4a** and **4b** (5 mg) after washing with petroleum ether.

### Isolation of the chemical constituents of the leaves

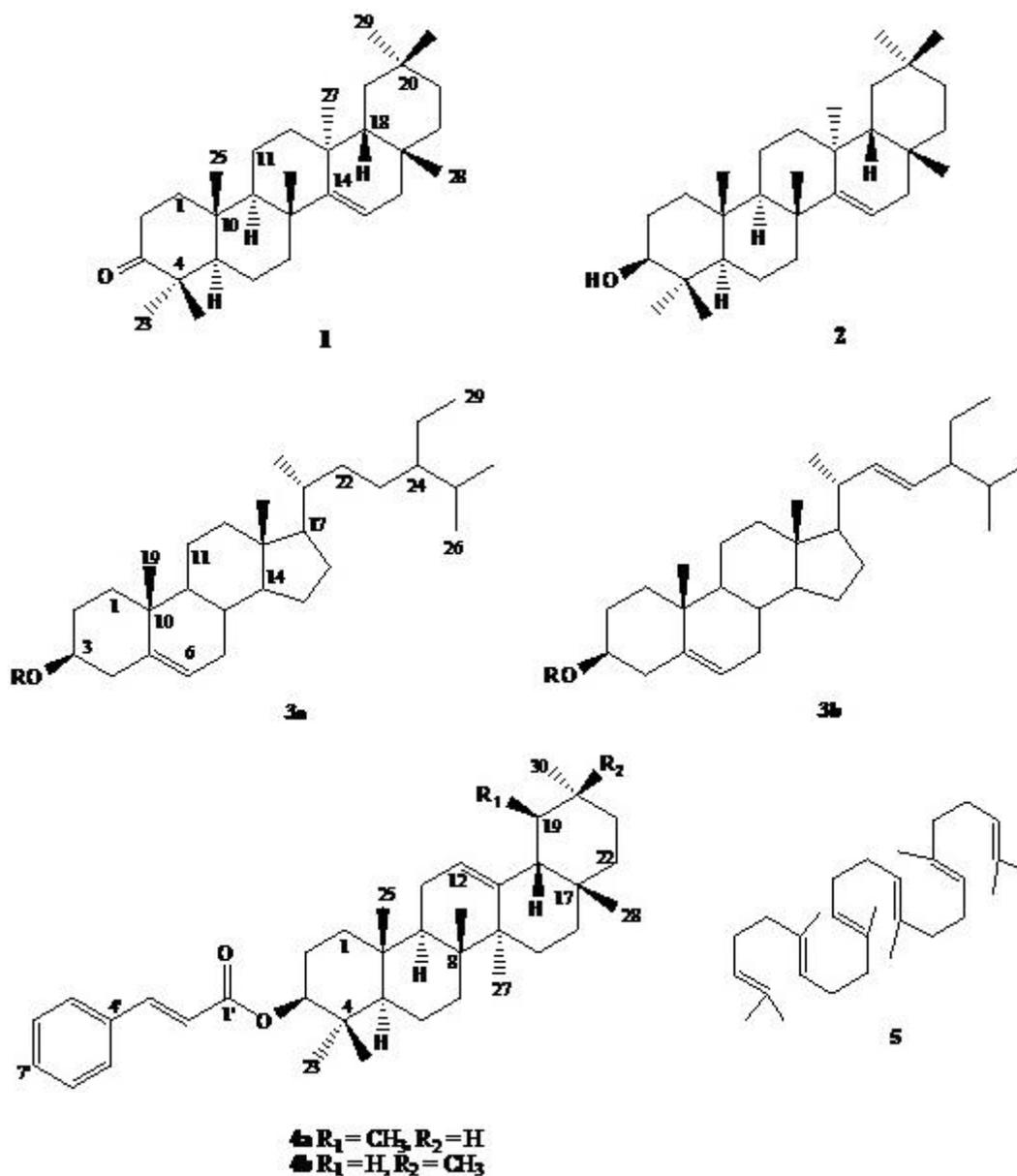
The air-dried leaves of *H. buotii* (71.5 g) were ground in a blender, soaked in  $\text{CH}_2\text{Cl}_2$  for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (6.5 g) which was chromatographed using increasing proportions of acetone in  $\text{CH}_2\text{Cl}_2$  at 10% increment.

The  $\text{CH}_2\text{Cl}_2$  was rechromatographed in petroleum ether. The less polar fractions were combined and rechromatographed in petroleum ether to yield saturated hydrocarbons (25 mg) after washing with petroleum ether.

The more polar fractions were combined and rechromatographed using 1% EtOAc in petroleum ether to afford **5** (9 mg). The 30% acetone in  $\text{CH}_2\text{Cl}_2$  fraction was rechromatographed (3  $\times$ ) using 20% EtOAc in petroleum ether to afford **3a** (6 mg).

### Taraxerone (**1**)

$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  5.54 (H-15), 1.07 (s, Me-23), 1.06 (s, Me-24), 1.08 (s, Me-25), 0.91 (s, Me-26), 1.13 (s, Me-27), 0.82 (s, Me-28), 0.95 (s, Me-29), 0.89 (s, Me-30);  $^{13}\text{C}$ -NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$ : 38.3 (C-1), 33.6 (C-2), 217.6 (C-3), 47.6 (C-4), 55.8 (C-5), 19.9 (C-6), 37.7 (C-7), 38.9 (C-8), 48.8 (C-9), 37.5 (C-10), 17.4 (C-11), 34.1 (C-12), 29.8 (C-13), 157.6 (C-14), 117.2 (C-15), 36.7 (C-16), 35.8 (C-17), 48.8 (C-18), 40.6 (C-19), 28.8 (C-20), 33.1 (C-21), 35.1 (C-22), 26.1 (C-23), 21.5 (C-24), 14.8 (C-25), 29.9 (C-26), 25.6 (C-27), 29.8 (C-28), 33.3 (C-29), 21.3 (C-30).

**Taraxerol (2)**

$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.20 (H-3), 5.52 (H-14), 0.92 (s, Me-23), 0.90 (s, Me-24), 0.97 (s, Me-25), 1.08 (s, Me-26), 0.82 (s, Me-27), 0.80 (s, Me-28), 0.94 (s, Me-29), 0.90 (s, Me-30);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  38.0 (C-1), 27.1 (C-2), 79.1 (C-3), 39.0 (C-4), 55.5 (C-5), 18.8 (C-6), 35.1 (C-7), 38.8 (C-8), 48.7 (C-9), 37.6 (C-10), 17.5 (C-11), 35.8 (C-12), 37.7 (C-13), 158.1 (C-14), 116.9 (C-15), 36.7 (C-16), 37.7 (C-17), 49.3 (C-18), 41.3 (C-19), 28.8 (C-20), 33.7 (C-21), 33.1 (C-22), 28.0 (C-23), 15.4 (C-24), 15.4 (C-25), 29.8 (C-26), 25.9 (C-27), 29.9 (C-28), 33.3 (C-29), 21.3 (C-30).

 **$\beta$ -Sitosterol (3a)**

$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.50 (m, H-3), 5.35 (d, 4.8, H-5), 0.66 (s, Me-18), 0.99 (s, Me-19), 0.93 (d, 6.6, Me-21),

0.84 (d,  $J = 6.6$ , Me-26), 0.83 (d,  $J = 6.0$ , Me-27), 0.86 (t,  $J = 6.0$ , Me-29).

**Stigmasterol (3b)**

$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.50 (m, H-3), 5.33 (d,  $J = 4.8$ , H-5), 0.68 (s, Me-18), 0.99 (s, Me-19), 1.01 (d,  $J = 6.6$ , Me-21), 5.13 (dd,  $J = 8.4, 15.6$  Hz, H-22), 5.00 (dd,  $J = 8.4, 15.0$  Hz, H-23), 0.84 (d,  $J = 6.6$  Hz, Me-26), 0.83 (d,  $J = 6.0$  Hz, Me-27), 0.80 (t,  $J = 6.0$  Hz, Me-29).

 **$\alpha$ -Amyrin cinnamate (4a)**

$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  4.65 (dd,  $J = 6.0, 10.1$  Hz, H-3), 5.13 (t,  $J = 3.8$  Hz, H-12), 0.92 (s, Me-23), 0.95 (s, Me-24), 1.01 (s, Me-25), 1.03 (s, Me-26), 1.08 (s, Me-27), 0.80 (s, Me-28), 0.80 (d,  $J = 5.9$  Hz, Me-29), 0.92 (d,  $J = 5.9$  Hz, Me-30), 7.67

(d,  $J = 16.0$  Hz, H-2'), 6.44 (d,  $J = 16.0$  Hz, H-3'), 7.53 (H-5', H-9'), 7.38 (H-6', H-8'), 7.38 (H-7');  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$ : 38.5 (C-1), 23.7 (C-2), 81.0 (C-3), 37.9 (C-4), 55.2 (C-5), 18.3 (C-6), 32.9 (C-7), 40.0 (C-8), 47.6 (C-9), 36.8 (C-10), 23.4 (C-11), 124.3 (C-12), 139.6 (C-13), 42.0 (C-14), 26.6 (C-15), 28.12 (C-16), 33.7 (C-17), 59.0 (C-18), 39.6 (C-19), 39.8 (C-20), 32.4 (C-21), 41.5 (C-22), 28.09 (C-23), 16.86, 16.90 (C-24, C-26), 15.6 (C-25), 23.2 (C-27), 28.7 (C-28), 17.5 (C-29), 21.4 (C-30), 166.8 (C-1'), 144.2 (C-2'), 118.8 (C-3'), 134.5 (C-4'), 128.0 (C-5', C-9'), 128.8 (C-6', C-8'), 130.1 (C-7').

#### ***$\beta$ -Amyrin cinnamate (4b)***

$^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  4.63 (t,  $J = 6.0$  Hz, H-3), 5.17 (t,  $J = 3.7$  Hz, H-12), 0.90 (s, Me-23), 0.93 (s, Me-24), 0.97 (s, Me-25), 0.96 (s, Me-26), 1.13 (s, Me-27), 0.82 (s, Me-28), 0.86 (s, Me-29), 0.85 (s, Me-30), 7.65 (d,  $J = 16.0$  Hz, H-2'), 6.43 (d,  $J = 16.0$  Hz, H-3'), 7.53 (H-5', H-9'), 7.37 (H-6', H-8'), 7.37 (H-7');  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$ : 38.3 (C-1), 23.7 (C-2), 81.0 (C-3), 37.9 (C-4), 55.2 (C-5), 18.3 (C-6), 32.6 (C-7), 39.8 (C-8), 47.5 (C-9), 38.5 (C-10), 23.5 (C-11), 124.3 (C-12), 139.6 (C-13), 41.7 (C-14), 26.1 (C-15), 26.9 (C-16), 32.5 (C-17), 59.0 (C-18), 46.8 (C-19), 31.1 (C-20), 34.7 (C-21), 37.1 (C-22), 28.1 (C-23), 16.9 (C-24), 15.7 (C-25), 16.8 (C-26), 26.0 (C-27), 28.4 (C-28), 33.3 (C-29), 23.7 (C-30), 166.8 (C-1'), 144.3 (C-2'), 118.8 (C-3'), 134.5 (C-4'), 128.0 (C-5', C-9'), 128.8 (C-6', C-8'), 130.1 (C-7').

#### ***Squalene (5)***

$^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  5.08-5.13 (6H, =CH), 1.58 (18H, allylic Me, *cis*), 1.66 (6H, allylic Me, *trans*), 1.94-2.07 (20H, allylic  $\text{CH}_2$ ).

#### **Hydrocarbons**

$^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.23 (br s), 0.85 (t, 6.5 Hz).

### **RESULTS AND DISCUSSION**

Silica gel chromatography of the dichloromethane extracts of *H. buotii* yielded taraxerone (**1**) (Ragasa and Cornelio, 2013a), taraxerol (**2**) (Tareq *et al.*, 2009; Ragasa *et al.*, 2014), and a mixture of  $\beta$ -sitosterol (**3a**) (Ragasa *et al.*, 2013b) and stigmasterol (**3b**) (Ragasa *et al.*, 2013b) from the roots and stems; another mixture of  $\alpha$ -amyrin cinnamate (**4a**) (Akihisa *et al.*, 2010) and  $\beta$ -amyrin cinnamate (**4b**) (Akihisa *et al.*, 2010) from the flowers; and **3a**, squalene (**5**) (Ragasa *et al.*, 2014b), and saturated hydrocarbons (Ragasa *et al.*, 2015) from the leaves. The structures of **1-5** and saturated hydrocarbons were identified by comparison of their NMR data with those reported in the literature.

The dichloromethane extracts of *H. buotii* yielded taraxerone (**1**), taraxerol (**2**), and a mixture of  $\beta$ -sitosterol (**3a**) and stigmasterol (**3b**) from the roots and stems; another mixture of  $\alpha$ -amyrin cinnamate (**4a**) and  $\beta$ -amyrin cinnamate (**4b**) from the flowers; and **3a**, squalene (**5**), and hydrocarbons from the leaves. These results indicate that *H. buotii* shares similar chemical

characteristics with other members of the genus *Hoya*: *H. wayetii* which yielded  $\beta$ -amyrin cinnamate (**4a**) and taraxerol (**2**) (Ebajo *et al.*, 2015b); *H. mindorensis* (Ebajo *et al.*, 2014), *H. multiflora* (Ebajo *et al.*, 2015a) and *H. wayetii* (Ebajo *et al.*, 2015b) which contained  $\beta$ -sitosterol and stigmasterol; and *H. mindorensis* (Ebajo *et al.*, 2014) and *H. multiflora* (Ebajo *et al.*, 2015a) which afforded squalene.

### **CONFLICTS OF INTEREST**

The authors declare no conflicts of interest.

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