An Isoflavone from *Wrightia pubescens*

Consolacion Y. Ragasa\(^1,2\)*, Vincent Antonio S. Ng\(^2\), Mariquit M. De Los Reyes\(^3,4\), Emelina H. Mandia\(^5\), and Chien-Chang Shen\(^5\)

\(^1\)Chemistry Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna 4024, Philippines
\(^2\)Chemistry Department, De La Salle University, 2401 Taft Avenue, Manila 1004, Philippines
\(^3\)Biology Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna 4024, Philippines
\(^4\)Biology Department, De La Salle University, 2401 Taft Avenue, Manila 1004, Philippines
\(^5\)National Research Institute of Chinese Medicine, 155-1, Li-Nong St., Sec. 2, Taipei 112, Taiwan

ABSTRACT

Chemical investigation of the dichloromethane extract of the twigs of *Wrightia pubescens* (R.Br.) led to the isolation of an isoflavone, wrightiadione (1). The structure of 1 was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by mass spectrometry.

Keywords: *Wrightia pubescens* (R.Br.), Apocynaceae, isoflavone, wrightiadione

INTRODUCTION

*Wrightia pubescens* (R.Br.) of the family Apocynaceae is one of the eight known species of *Wrightia* in Malesia\(^1\). Locally known as “lanete” in the Philippines, *Wrightia pubescens*, which can grow up to 35 m tall in deciduous lowland thickets and forests, is also found in mainland China, India and Australia\(^2,3\). In traditional medicine, the root and bark extracts from the tree are used to treat scrofula and rheumatic arthralgia\(^4\) and the latex is used against dysentery\(^5\). Chinese medicine preparations containing *W. pubescens* for acute upper respiratory infection of children\(^6\), intractable hiccups\(^6\), and osteoarthritis\(^7,8\) have been reported previously. The plant’s latex has been shown to have inhibitory activities on prostaglandin E2 (PGE\(_2\)) production and cyclooxygenase 2 (COX-2) protein expression in RAW 264.7 mouse macrophages and these were associated to the anti-inflammatory and antinociceptive properties of the plant\(^9\).

This study is part of our research on the chemical constituents of trees found at the De La Salle University – Science and Technology Complex (DLSU–STC) riparian forest and reforested area. The trees studied included *Dysoxylum gaudichaudianum* (A. Juss.) Miq., *Kibatalia gitingensis* (Elm.) Woodson, *Pipturus arborescens* (Link) C.B. Rob., and *Wrightia pubescens* (R.Br.). The isolation of squalene, β-sitosterol, polypropenols and triglycerides from the leaves of *Dysoxylum gaudichaudianum* (A. Juss.) Miq. has been reported\(^10\). Furthermore, the dichloromethane extract of the leaves of *D. gaudichaudianum* exhibited IC\(_{50}\) values of 7.35 and 13.19 μg/mL against breast cancer (MCF-7) and colon cancer (HT-29) cells, respectively\(^10\). *Kibatalia gitingensis* (Elm.) Woodson afforded isoscopoletin from the twigs and ursolic acid, squalene, α-amyrin acetate and lupeol acetate from both leaves and twigs\(^11\). *Pipturus arborescens* (Link) C.B. Rob. yielded ursolic acid, oleic acid, friedelien, β-sitosterol, and stigmasterol from the twigs, while the leaves afforded β-sitosterol, stigmasterol, squalene, chlorophyll a, and polypropenol\(^12\).

In an earlier study on *Wrightia pubescens* collected from the DLSU-STS riparian forest and reforested area, the isolation and identification of ursolic acid, oleic acid, squalene, β-sitosterol and chlorophyll a from the leaves; and ursolic acid, oleic acid and α-amyrin acetate from the twigs were reported\(^13\). This study reports on the isolation of an isoflavone, wrightiadione (1) from the twigs of *W. pubescens*. To the best of our knowledge, this is the first report on the isolation of 1 from *W. pubescens*.

MATERIALS AND METHODS

General Experimental Procedure

NMR spectra were recorded on a Varian VNMR spectrometer in CDCl\(_3\) at 600 MHz for \(^1\)H NMR and 150 MHz for \(^13\)C NMR spectra. EIMS was obtained on a Thermo Focus GC & DSQII spectrometer. 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\(_2\)SO\(_4\) solution followed by warming.

Sample Collection

Samples of leaves and twigs of *Wrightia pubescens* (R.Br.) were collected from the De La Salle University – Science and Technology Complex (DLSU–STC) riparian forest in

*Author for Correspondence*
February 2014. The samples were authenticated by one of the authors (EHM) and deposited at the De La Salle University Herbarium with voucher specimen #915.

**General Isolation Procedure**

A glass column 20 inches in height and 2.0 inches internal diameter was packed with silica gel. The crude extract from the leaves was fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10% increment) as eluents. One hundred milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same Rf 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. Five milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

**Isolation**

The twigs of *W. pubescens* were air-dried for about one week. The air-dried twigs (391.4 g) were ground in a blender, soaked in CH₂Cl₂ for 3 days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (3.8 g) which was chromatographed using increasing proportions of acetone in CH₂Cl₂ at 10% increment. The 40% acetone in CH₂Cl₂ fraction was rechromatographed (3x) using CH₃CN:Et₂O:CH₂Cl₂ (0.5:0.5:9 by volume ratio) to afford I (12 mg) after trituration with petroleum ether.

**Wrightiadione (1):** ¹H NMR (600 MHz, CDCl₃): δ 7.89 (dd, J = 0.6, 6.6 Hz, H-5), 7.41 (dt, J = 1.2, 7.8 Hz, H-6), 7.77 (dt, J = 1.8, 7.8 Hz, H-7), 8.60 (d, J = 8.4 Hz, H-8), 8.01 (dd, J = 0.6, 8.4 Hz, H-3'), 7.83 (dt, J = 1.2, 7.8 Hz, H-4'), 7.65 (dt, J = 1.2, 7.8 Hz, H-5'), 8.41 (dd, J = 1.2, 7.8 Hz, H-6'); ¹³C NMR (150 MHz, CDCl₃): δ 144.32 (C-2), 158.09 (C-3), 182.56 (C-4), 125.40 (C-5), 127.20 (C-6), 138.28 (C-7), 118.00 (C-8), 146.32 (C-9), 121.91 (C-10), 123.72 (C-1'), 146.61 (C-2'), 130.72 (C-3'), 135.13 (C-4'), 130.24 (C-5'), 127.54 (C-6'), C-7' not observed; EIMS m/z (rel. int.) 248.03 [M⁺] (100), 220.04 (42), 192.05 (36), 164.03 (13), 143.99 (9), 124.08 (12), 116.04 (7), 101.98 (28), 90.03 (15), 76.05 (35), 75.05 (26), 63.05 (15).

**RESULTS AND DISCUSSION**

Silica gel chromatography of the dichloromethane extract of the twigs of *Wrightia pubescens* led to the isolation of an isoflavone, wrightiadione (1). The structure of 1 was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by mass spectrometry as follows. The ¹H NMR spectrum of 1 gave resonances for three aromatic proton doublet of doublet at δ 7.89 (J = 0.6, 6.6 Hz), 8.01 (J = 0.6, 8.4 Hz) and 8.41 (J = 1.2, 7.8 Hz); an aromatic proton doublet at δ 8.60 (J = 8.4 Hz); and four aromatic proton doublet of triplet at δ 7.41 (J = 1.2, 7.8 Hz), 7.77 (J = 1.8, 7.8 Hz), 7.83 (J = 1.2, 7.8 Hz), and 7.65 (J = 1.2, 7.8 Hz). The large and small coupling constants indicated ortho and meta coupling, respectively. These resonances suggested two aromatic rings with four adjacent protons in each ring. The coupled protons were verified from the COSY spectrum which indicated two isolated spin systems: H-5/H-6/H-7/H-8 and H-3'/H-4'/H-5'/H-6' (Fig. 1). No proton singlet was detected in the ¹H NMR spectrum, indicating that the aromatic rings do not contain hydroxyl.

The ¹³C NMR spectrum gave resonances for fifteen carbons with the following functionalities: four deshielded non-protonated aromatic carbons at δ 158.09, 144.32, 146.32 and 146.61; two relatively shielded non-protonated aromatic carbons at δ 121.29 and 123.72; eight protonated aromatic carbons at δ 138.28, 135.13, 130.72, 130.24, 127.54, 125.40, 127.20 and 118.00; and a conjugated carbonyl carbon at δ 182.56. These are characteristic resonances for an isoflavone.

The EIMS of 1 gave a stable molecular ion of m/z 248.03 [M⁺] (100%), which corresponded to a molecular formula of C₁₆H₁₀O₃. The molecular formula indicated an index of hydrogen deficiency (IHD) of thirteen. The two aromatic rings, one carbonyl and an additional double bond accounted for ten IHD. The ¹³C NMR spectrum indicated only fifteen carbon resonances, while the EIMS gave sixteen carbons. Since there is no aliphatic carbon, then the missing carbon should be a carbonyl and 1 should have two additional rings to account for the three remaining IHD. The presence of two carbonyls in 1 was supported by the fragmentation pattern in the EIMS which gave two peaks at 220.04 (42%) and 192.05 (36%) resulting from the loss of two carbons (C=O) from the molecular ion, m/z 248.03 [M⁺] (100%). The protons attached to carbon atoms were assigned from HSQC 2D NMR data (see experimental), and the structure of 1 was elucidated by analysis of the HMBC 2D NMR data. Key HMBC correlations are shown in Fig. 1. Thus, the carbonyl was assigned to C-4 based on long-range correlation between H-5 and this carbon. From the

![Fig. 1. ¹H-¹H COSY and ¹H-¹³C long-range correlations of 1](image-url)
benzopyran part of 1, long-range correlations were observed between H-5 and C-6, C-7, C-9 and C-10. Furthermore, correlations were also observed between H-8 and C-6, C-7, C-9, and C-10. From the other part of 1, H-6' was long-range correlated to C-2, C-1', C-4' and C-5'. Correlations were also observed between H-3' and C-2', C-1', C-4' and C-5'. All long-range correlations observed are consistent with the structure of 1.

Literature search revealed that 1 is an isoflavone, wrightiadione. The second carbonyl was also not detected in the $^{13}$C NMR spectrum of wrightiadione which was isolated from *Wrightia tomentosa*. The structure of this isoflavone was confirmed by x-ray analysis$^{14}$. This compound exhibited cytotoxic activity against the murine P388 lymphocytic leukemia cell line (ED$_{50}$, 1.1μg/ml)$^{19}$.

REFERENCES