Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(2); 353-355

ISSN: 0975-4873

Research Article

An Isoflavone from *Wrightia pubescens*

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Available Online: 15th March, 2015

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[3] and the latex is used against dysentery[4]. Chinese medicine preparations containing *W. pubescens* for acute upper respiratory infection of children[5], 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, polyprenols 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, oleanolic acid, friedelin, β-sitosterol, and stigmasterol from the twigs, while the leaves afforded β-sitosterol, stigmasterol**,** squalene, chlorophyll a, and polyprenol[12].

In an earlier study on *Wrightia pubescens* collected from the DLSU-STC riparian forest and reforested area, the isolation and identification of ursolic acid, oleanolic acid, squalene, β-sitosterol and chlorophyll a from the leaves; and ursolic acid, oleanolic 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 VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^1H 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_2SO_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

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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 were 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 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. 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 CH_2Cl_2 for 3 days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (3.8g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9 by volume ratio) to afford **1** (12 mg) after trituration with petroleum ether.

Wrightiadione (**1**): ^{1}H NMR (600 MHz, $CDCl_3$): 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'); ^{13}C NMR (150 MHz, $CDCl_3$): 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).

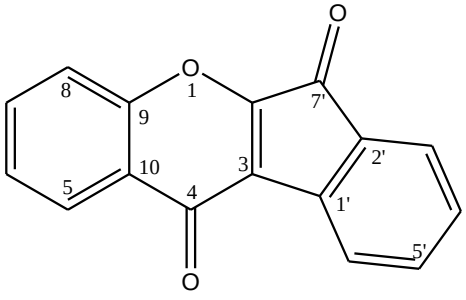
RESULTS AND DISCUSSION

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 ^{1}H 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 ^{1}H NMR spectrum, indicating that the aromatic rings do not contain hydroxyl.

The ^{13}C 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 δ182.56. 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 $C_{16}H_8O_3$. 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 ^{13}C 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 carbonyls (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



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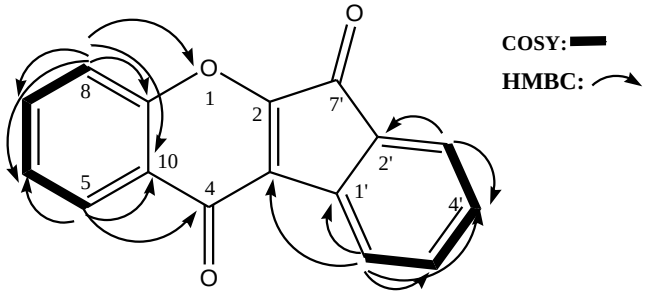

Fig. 1. ^{1}H-^{1}H COSY and ^{1}H-^{13}C long-range correlations of **1**

benzopyran part of **1**, **l**ong-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)[14].

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