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Chemical constituents from ethyl acetate extract of the leaves of *Rourea harmandiana* Pierre

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Abstract:

***Rourea harmandiana* Pierre is a species which belongs to the family of Connaraceae. This plant is found abundantly in central of Vietnam (Thua Thien - Hue, Da Nang). Chemical study on the ethyl acetate extract of *Rourea harmandiana* leaves has led to the isolation of four compounds including vomifoliol (1), boscialin (2), abscisic acid (3) and *p*-coumaric acid (4). The structures of these compounds have been identified by NMR, MS spectroscopic data and comparison with the reported literature. These compounds were isolated from *Rourea harmandiana* species as well as from the genus *Rourea* for the first time.**

Keywords: **abscisic acid, boscialin, *p*-coumaric acid, *Rourea harmandiana*, vomifoliol.**

Classification number: **2.2**

Introduction

Rourea harmandiana Pierre. is a climbing plant of the *Rourea* genus, that belongs to the Connaraceae family. This plant, which is one of 7 Rourea species found in Vietnam, is distributed in Hai Van mountain areas [1]. *Rourea* species have been used in traditional medicine to treat rheumatism, diabetes, dysentery and to promote wound healing [2]. Several classes of natural compounds such as flavonoids, triterpenoids, coumarins, quinones have been isolated from *Rourea* plants. The isolated compounds and *Rourea* plant extracts have been tested for several biological properties such as antibacterial, anti-inflammatory, antioxidant and anti-diabetic activities, and have shown positive results [3, 4].

In the biological screening program of Vietnamese plants, the MeOH extracts of *Rourea oligophlebia* and *R. harmandianan* showed good antibacterial activity against several microbial strains. We have reported the chemical study of *Rourea oligophlebia* species collected in Ben En national park, Thanh Hoa province [5]. In continuation of our search for bioactive compounds from *Rourea* species in Vietnam, four compounds including vomifoliol (**1**), boscialin (**2**), abscisic acid (**3**) and *p*-coumaric acid (**4**) were isolated from the ethyl acetate extract of the leaves of *R. harmadiana*. Their chemical structures were elucidated by different spectroscopic methods. This is the first study of the *R. harmandiana*. These compounds were firstly isolated from this species as well as from the genus *Rourea*.

Experimental

Plant materials

The leaves of *R. harmandiana* were collected at Phu Loc district, Thua Thien - Hue province, Vietnam in October

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2010. The plant sample was identified by Dr. Nguyen The Cuong, Institute of Ecological and Biological Resources, VAST. A voucher specimen (VN-2149) was provided to the Institute of Ecological and Biological Resources.

General procedures

The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded by a Bruker AM500 FT-NMR spectrometer (Institute of Chemistry, VAST) using TMS as an internal standard. The electrospray ionization mass spectra (ESI-MS) were obtained on an Agilent 1260 series single quadrupole LC/MS system. Column chromatography (CC) was performed on silica gel (Merck, 230-400 mesh), reversed-phase silica gel (YMC, RP-18) or Sephadex LH-20 (Sigma). Thin layer chromatography used precoated silica gel plates (Merck 60 F_{254}). Compounds were visualized by spraying with 10% sulfuric acid. The optical rotations were measured on the JASCO P-2000 Polarimeter (Institute of Marine Biochemistry, VAST).

Extraction and isolation of compounds (Fig. 1)

The leaves of *R. harmandiana* were dried in the shade and crushed into a powder. The dried leaves powder (2.0 kg) was extracted with MeOH at room temperature for 24 hours (10 l × 3 times). The extracts were combined and concentrated under reduced pressure to obtain MeOH residues. MeOH residue was suspended with 1 l of distilled water, and extracted with ethyl acetate (500 ml × 3 times). After removal of the organic solvent, ethyl acetate residue (44 g) was obtained.

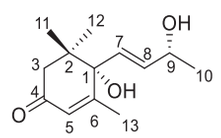
The ethyl acetate residue was subjected to a silica gel column chromatography (CC) and eluted with a gradient solvent of *n*-hexane/acetone (0-100% acetone) to afford 13 fractions (E1-E13). The E7 fraction (4.1 g) was purified on a silica gel CC using dichloromethane/ethyl acetate (19/1) as eluant to afford 10 sub-fractions denoted as E7.1-E7.10. The E7.7 sub-fraction (210 mg) was separated on silica gel CC and eluted with dichloromethane/acetone (9/1) to obtain **1** (3.2 mg). The sub-fraction E7.10 (324 mg) was purified on a reversed-phase silica gel CC, and eluted with MeOH/water (3/1) to give **2** (4.2 mg), and **3** (3.7 mg). The E8 fraction (2.5 g) was fractionated on a Sephadex LH-20 CC using dichloromethane/MeOH (1/4) as eluant to give 3 sub-fractions denoted as E8.1-E8.3. The sub-fraction E8.3 (371 mg) was purified on a reversed-phase silica gel CC, and eluted with acetone/water (1/3) to give **4** (7.1 mg).

Vomifoliol (1): colorless oil; $[\alpha]^{25}_{D}$ + 190^{o} (*c* 0.13, MeOH); ESI-MS *m/z*: 225 $[M+H]^{+}$. ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm) 5.89 (1H, m, H-5), 5.83 (1H, m, H-8), 5.80 (1H, m, H-7), 4,34 (1H, m, H-9), 2.53 (1H, d, *J*=17 Hz, H-3), 2.18 (1H, d, *J*=17 Hz, H-3), 1.94 (3H, s, H-13), 1.26 (3H, d, *J*=6.5 Hz, H-10), 1.06 (3H, s, H-11), 1.04 (3H, s, H-12). ^{13}C-NMR (125 MHz, CD_3OD): δ_C (ppm) 201.2 (C-4), 167.4 (C-6), 136.9 (C-8), 130.1 (C-7), 127.1 (C-5), 79.9 (C-1), 68.7 (C-9), 50.7 (C-3), 42.4 (C-2), 24.5 (C-11), 23.8 (C-10), 23.4 (C-12), 19.5 (C-13).

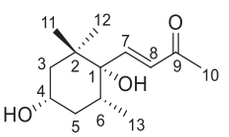
Boscialin (2): white solid, $[\alpha]^{25}_{D}$ -17^{o} (*c* 0.12, MeOH); ESI-MS *m/z*: 229 $[M+H]^{+}$. ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm) 6.90 (1H, d, *J*=16 Hz, H-7), 6.37 (1H, d, *J*=16 Hz, H-8), 3.85 (1H, m, H-4), 2.29 (3H, s, H-10), 2.11 (1H, m, H-6), 1.75-1.67 (2H, m, H-3 and H-5), 1.46-1.38 (2H, m, H-3 and H-5), 1.06 (3H, s, H-11), 0.88 (3H, s, H-12), 0.83 (3H, d, *J*=7 Hz, H-13). ^{13}C-NMR (125 MHz, CD_3OD): δ_C (ppm) 201.2 (C-9), 154.3 (C-7), 131.6 (C-8), 78.9 (C-1), 67.2 (C-4), 45.7 (C-3), 40.9 (C-2), 39.5 (C-5), 35.3 (C-6), 27.4 (C-10), 25.9 (C-11), 25.1 (C-12), 16.4 (C-13).

Abscisic acid (3): white solid, $[\alpha]^{25}_{D}$ + 225^{o} (*c* 0.13, MeOH); ESI-MS *m/z*: 265 $[M+H]^{+}$. ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm) 7.78 (1H, d, *J*=16 Hz, H-8), 6.23 (1H, d, *J*=16 Hz, H-7), 5.94 (1H, s, H-5), 5.78 (1H, s, H-10), 2.54 (1H, d, *J*=17 Hz, H-3), 2.19 (1H, d, *J*=17 Hz, H-3), 2.05 (3H, s, H-12), 1.95 (3H, s, H-15), 1.08 (3H, s, H-13), 1.05 (3H, s, H-14). ^{13}C-NMR (125 MHz, CD_3OD): δ_C (ppm) 201.0 (C-4), 170.5 (C-11), 166.6 (C-9), 150.1 (C-6), 137.5 (C-7), 129.6 (C-8), 127.5 (C-5), 120.5 (C-11), 80.6 (C-1), 50.7 (C-3), 42.8 (C-2), 24.6 (C-12), 23.5 (C-14), 21.1 (C-15), 19.6 (C-13).

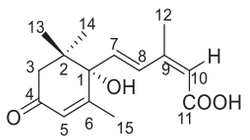
p-coumaric acid (4): white solid. ESI-MS *m/z*: 265 $[M+H]^{+}$. ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm) **7**.62 (1H, d, *J*=16.0 Hz, H-7), 7.45 (1H, d, *J*=8.5 Hz, H-2 and H-6), 6.82 (2H, d, *J*=8.5 Hz, H-3 and H-5), 6.29 (1H, d, J=16.5 Hz, H-8). ^{13}C-NMR (125 MHz, CD_3OD): δ_C (ppm) 171.0 (C-9), 161.0 (C-4), 146.6 (C-7) 131.0 (C-2 and C-6), 127.2 (C-1), 116.7 (C-3 and C-5), 115.6 (C-8).



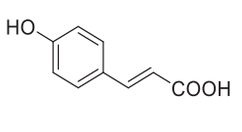
Vomifoliol (1)



Boscialin (2)



Abscisic acid (3)



***p*-coumaric acid (4)**

Fig. 1. Chemical structures of isolated compounds 1-4.

Results and discussion

Vomifoliol (1)

Compound 1 was obtained as colorless oil. The ESI-MS showed a *quasi*-molecular ion peak *m/z* 225 $[M+H]^+$, that suggests the molecular formula of **1** is $C_{13}H_{20}O_3$ (M=224). The ^1H and ^{13}C-NMR spectra showed typical signals of a megastigman compound. The ^1H NMR spectrum shown three olefinic protons at δ_H 5.89 (1H, m, H-5), 5.83 (1H, m, H-8), and 5.80 (1H, m, H-7); an oxymethine group signal at δ_H 4.34 (1H, m, H-9) and signals of 4 methyl groups including 3 singlets at δ_H 1.94 (3H, s, H-13), 1.06 (3H, s, H-11) and 1.04 (3H, s, H-12), and a doublet at δ_H 1.26 (3H, d, *J*=6.5 Hz, H-10). The ^{13}C-NMR spectrum displayed 13 carbon signals including a carbonyl group at δ_C 201.2 (C-4), 4 olefinic carbons at δ_C 167.4 (C-6), 136.9 (C-8), 130.1 (C-7), and 127.1 (C-5), 2 oxygen-bonded carbons at δ_C 79.9 (C-1) and 68.7 (C-9) and 4 methyl groups at δ_C 24.5 (C-11), 23.8 (C-10), 23.4 (C-12) and 19.5 (C-13). From the MS, NMR spectra together with optical rotation value [6], **1** was determined as vomifoliol. The analytical NMR data of **1** are in accordance with those of a previous report [7].

Boscialin (2)

Compound **2** was obtained as a white solid. The ESI-MS showed a *pseudo*-molecular ion peak *m/z* 227 $[M+H]^+$, combined with the NMR spectra, that suggests the molecular formula of **2** is $C_{13}H_{22}O_3$ (M=226). The NMR data of **2** also exhibited the signals of a megastigman compound with signals of four methyl groups including three singlets at δ_H 2.29 (3H, s, H-10), 1.06 (3H, s, H-11) and 0.88 (3H, s, H-12) and a doublet at δ_H 0.83 (3H, d, *J*=7 Hz, H-13); an oxymethine signal at δ_H 3.85 (1H, m, H-4) and 2 *trans*-olefinic protons at δ_H 6.90 (1H, d, *J*=16 Hz, H-7) and 6.37 (1H, d, *J*=16 Hz, H-8). The singlet of methyl group at δ_H 2.29 (3H, s, H-10) suggested that a ketone group was positioned at C-9. Similar to **1**, the ^{13}C-NMR spectrum also gave signals of 13 carbons including a carbonyl group at δ_C 201.2 (C-9), two olefinic carbons at δ_C 154.3 (C-7) and 131.6 (C-8), two oxygen-bonded signals at δ_C 78.9 (C-1) and 67.2 (C-4) and 4 methyl groups at δ_C 27.4 (C-10), 25.9 (C-11), 25.1 (C-12) and 16.4 (C-13). Based on the NMR spectral data analysis above, along with comparison of the optical rotation, **2** was identified as boscialin [8].

Abscisic acid (3)

Compound **3** was isolated from ethyl acetate extract as a white solid. The ESI-MS showed a *quasi*-molecular ion peak *m/z* 265 $[M+H]^+$, that corresponds to a molecular formula of $C_{15}H_{20}O_4$ (M=264). The ^1H-NMR spectrum displayed proton signals similar to those of **1**. On the spectrum, there appeared signals of two olefinic protons in *trans* form at δ_H 7.78 (1H, d, *J*=16 Hz, H-8) and 6.23 (1H, d, *J*=16 Hz, H-7) and two other singlet olefinic protons at δ_H 5.94 (1H, s, H-5) and 5.78 (1H, s, H-10). Different from compound **1**, four methyl groups of **3** appeared as four singlets at δ_H 2.05 (3H, s, H-12), 1.95 (3H, s, H-15), 1.08 (3H, s, H-13) and 1.05 (3H, s, H-14). The ^{13}C-NMR spectrum gave of 15 carbon signals including a carbonyl group at δ_C 201.0 (C-4), a carboxylic group at δ_C 170.5 (C-11); six olefinic carbons at δ_C 166.6 (C-9), 150.1 (C-6), 137.5 (C-7), 129.6 (C-8), 127.5 (C-5) and 120.5 (C-11), an oxygen-bonded carbon at δ_C 80.6 (C-1) and four methyl groups at δ_C 24.6 (C-12), 23.5 (C-14), 21.1 (C-15) and 19.6 (C-13). From the analysis of the spectral data above, combined with previous literature [9], compound **3** was identified as abscisic acid.

p-coumaric acid (4)

Compound **4** was isolated as a white solid. The ESI-MS showed a *quasi*-molecular ion peak *m/z* 165 $[M+H]^+$, that corresponds to a molecular formula of $C_9H_8O_3$ (M=164). The ^1H-NMR spectrum showed signals of an A_2B_2 system at δ_H 7.45 (1H, d, *J*=8.5 Hz, H-2 and H-6) and 6.82 (2H, d, *J*=8.5 Hz, H-3 and H-5). In addition, signals of 2 *trans*-olefinic protons were observed at δ_H **7**.62 (1H, d, *J*=16.0 Hz, H-7) and 6.29 (1H, d, J=16.5 Hz, H-8). The ^{13}C-NMR spectrum revealed 9 carbon signals including a carboxylic acid at δ_C 171.0 (C-9), and 8 olefinic carbon signals at δ_C 161.0 (C-4), 146.6 (C-7), 131.0 (C-2 and C-6), 127.2 (C-1), 116.7 (C-3 and C-5), and 115.6 (C-8). Therefore, **4** was elucidated as *p*-coumaric acid. The NMR data of **4** are in agreement with those of a previous study [10].

These compounds **1-4** were the first chemical substances discovered from *R. harmandiana* as well as from the genus *Rourea*. Especially, in the previous studies of *Rourea* species, only one megastigman compound dihydrovomifoliol-9-β-D-glucopyranoside was discovered from *R. minor* [11]. Vomifoliol (**1**) showed antimicrobial against *Aspergillus niger* and *Fusarium oxysporum* strains with MIC values of 100 and 50 µg/ml, respetively [6]. Boscialin (**2**) revealed activity against various strains including *Corynebacterium minutissimum*, *Candida albicans*, against *Trypanosoma brucei rhodesiens*e and also revealed cytotoxicity against HT-29 human cancer cell [12]. Abscisic acid (**3**) exhibited antibacterial activity against *Helicobacter pylori* [13] and antifungal activity. Compound **4** demonstrates an antibacterial activity against three Gram-positive bacteria (*Streptococcus pneumonia*, *Staphylococcus aureus* and *Bacillus subtilis*; all MIC_{50}=20 µg/ml) and three Gram-negative bacteria (*Escherichia coli*, MIC_{50}=80 µg/ml; *Shigella dysenteriae*, MIC_{50}=10 µg/ ml; and *Salmonella typhimurium*, MIC_{50}=20 µg/ml) [14]. The antibacterial activity of the isolated compounds **1-4** possibly made contributions to

antibacterial activity of the MeOH extract of *R. harmandiana* leaves.

Conclusions

From the ethyl acetate extract of *R. harmandiana* leaves, four compounds were isolated and identified as vomifoliol (**1**), boscialin (**2**), abscisic acid (**3**) and *p*-coumaric acid (**4**). This is the first study about *R. harmandiana* in the world. These compounds were firstly discovered from *R. harmandiana* as well as from the *Rourea* genus.

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The authors declare that there is no conflict of interest regarding the publication of this article.

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