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BENZOYL ESTERS AND FLAVONES FROM THE LEAVES OF *POLYALTHIA PARVIFLORA*

Nguyen Thi Minh Hang*, Nguyen Thi Tu Oanh, Cao Thi Hue, To Hai Tung, Ha Thi Thoa, Le Nguyen Thanh, Tran Huu Giap, Nguyen Van Hung, Chau Van Minh

Institute of Marine Biochemistry, Vietnam Academy of Science and Technology

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Abstract

Two benzoyl esters, benzyl 2,6-dimethoxybenzoate (**1**), benzyl 2,5-dihydroxybenzoate (**2**), together with three flavones, 5-hydroxy-3,4',7-trimethoxyflavone (**3**), 3,5-dihydroxy-4',7-dimethoxyflavone (**4**) and 5,6-dihydroxy-3,4',7-trimethoxyflavone (**5**), were isolated from the ethyl acetate extract of *Polyalthia parviflora* leaves. Their structures were elucidated on the basis of spectroscopic analysis and comparisons with related known compounds.

Keywords. *Polyalthia parviflora*, benzoyl ester, flavone.

1. INTRODUCTION

There are about 120 species of *Polyalthia* in the world, of which only 28 species are distributed in Vietnam and *Polyalthia parviflora* is one specie growing in Cao Bang Province, Vietnam. Previous investigations on *Polyalthia* genus [1] resulted in the isolation of various types of secondary metabolites, including alkaloids, acetogenins, clerodane diterpenes, triterpenes, benzopyran derivatives, flavonoids, and polyacetylenes. The previous researches reported the isolation of *p*-coumarate, *p*-hydroxyphenylethyl ferulate, dehydrodiscretamine, (-)-discretamine [2] and styryllactones [3] from *P. parviflora*. The purpose of searching bioactive compounds from Vietnamese *Polyalthia* species prompted us to investigate the constituents of this plant. In this paper, we report the isolation and structure elucidation of two benzoyl esters named benzyl 2,6-dimethoxybenzoate (**1**), benzyl 2,5-dihydroxybenzoate (**2**), and three flavones named 5-hydroxy-3,4',7-trimethoxyflavone (**3**), 3,5-dihydroxy-4',7-dimethoxyflavone (**4**), 5,7-dihydroxy-3,4',6-trimethoxyflavone (**5**) from the leaves of *P. parviflora.*

2. EXPERIMENTAL

2.1. General Experimental Procedures

Melting points were measured with a Model Themo Scientific Mel-Tem 3.0 apparatus. ESI-MS and APCI-MS were measured with a AGILENT 6120 mass spectrometer. NMR spectra were recorded by a Bruker Avance 500 MHz instrument using TMS as Internal Standard.

2.2. Plant material

The leaves of *Polyalthia parviflora* were collected at Tra Linh District, Cao Bang Province, Viet Nam, in June 2012 and the plant material was identified by Dr. Nguyen Quoc Binh, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology where a voucher specimen (VN-0123) was deposited.

2.3. Extraction and Isolation

The air-dried and powdered material (1.16 kg) was xiliviated with MeOH at room temperature (4 times, 1 day/time). The extracts were combined and evaporated *in vacuo* and the residue was suspended in H_2O. The suspension was successively extracted with *n*-hexane and ethyl acetate to obtain *n*-hexane residue (**PH**, 30 g) and ethyl acetate residue (**PE**, 67 g). The **PE** residue was subjected to column chromatography on silica gel using gradient elution with CH_2Cl_2-MeOH to afford fourteen fractions (**PE1-PE14)**. By repeated column chromatography on silica gel and Sephadex LH-20 together with recrystallization, **1** (3.5 g), **2** (50 mg) and **3** (28 mg) were obtained from fraction PE4, **4** (0.5 g) from fraction PE7 and **5** (1.01 g) from fraction PE8.

Benzyl 2,6-dimethoxybenzoate (1): colorless needles, mp. 98-99 ^{o}C.

^{1}H NMR (500 MHz, $CDCl_3$) δ_H ppm: 3.80 (6H, s, 2-OC$\underline{H}_3$ and 6-OC$\underline{H}_3$), 5.39 (2H, s, H-1'), 6.54 (2H, d, *J* = 8.5 Hz, H-3 and H-5), 7.26 (1H, t, *J* = 8,5 Hz, H-4), 7.31 (1H, d, *J* = 8.0 Hz, H-5'), 7.37 (2H, t, *J* = 8.0 Hz, H-4' and H-6'), 7.45 (2H, t, *J* = 8.0 Hz, H-3' and H-7').

^{13}C NMR (125 MHz, $CDCl_3$) δ_C ppm: 55.91 (2-O$\underline{C}H_3$ and 6-O$\underline{C}H_3$), 66.77 (C-1'), 103.95 (C-3 and C-5), 113.03 (C-1), 127.91 (C-5'), 128.02 (C-4' and C-6'), 128.31 (C-3' and C-7'), 131.09 (C-4), 136.09 (C-2'), 157.42 (C-2 and C-6), 166.39 (C-7).

ESI-MS *m/z*: 273 $[M+H]^+$.

Benzyl 2,5-dihydroxybenzoate (2): colorless needles (*n*-hexane - CH_2Cl_2), mp. 104-105 ^{o}C.

^{1}H NMR (500 MHz, $CDCl_3$) δ_H ppm: 4.65 (1H, 5-OH), 5.36 (2H, H-1'), 6.88 (1H, d, *J* = 9.0 Hz, H-3), 7,00 (1H, dd, *J* = 3.0, 9.0 Hz, H-4), 7.31 (1H, d, *J* = 3.0 Hz, H-6), 7.36-7.44 (5H, m, H3'→H7'), 10.32 (2-OH).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C ppm: 67.01 (C-1'), 112.19 (C-1), 114.79 (C-3), 118.56 (C-4), 124.19 (C-6), 128.31 (C-3' and C-7'), 128.61 (C-5'), 128.73 (C-4' and C-6'), 135.19 (C-2'), 147.70 (C-5), 156.00 (C-2), 169.50 (C-7).

APCI-MS *m/z*: 243 $[M-H]^-$.

5-hydroxy-3,4',7-trimethoxyflavone (**3**): yellow crystal (CH_2Cl_2 - MeOH), mp. 145-146 ^{o}C.

^{1}H NMR (500 MHz, $CDCl_3$) δ_H ppm: 3.85 (3H, s, 3-OC$\underline{H}_3$), 3.87 (3H, s, 4'-OC$\underline{H}_3$), 3.89 (3H, s, 7-OC$\underline{H}_3$), 6.35 (1H, d, *J* = 2.0 Hz, H-6), 6.44 (1H, d, *J* = 2.0 Hz, H-8), 7.02 (2H, d, *J* = 9.0 Hz, H-3' and H-5'), 8.07 (2H, d, *J* = 9.0 Hz, H-2' and H-6'), 12.64 (1H, 5-O$\underline{H}$).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C ppm: 178.81 (C-4), 165.44 (C-7), 162.06 (C-4'), 161.71 (C-5), 156.78 (C-9), 156.00 (C-2), 138.90 (C-3), 130.18 (C-2' and C-6'), 122.85 (C-1'), 114.08 (C-3' and C-5'), 106.09 (C-10), 97.83 (C-6), 92.19 (C-8), 60.15 (3-O$\underline{C}H_3$), 55.80 (4'-O$\underline{C}H_3$), 55.44 (7-O$\underline{C}H_3$).

APCI-MS *m/z*: 329 $[M+H]^+$

3,5-dihydroxy-4',7-dimethoxyflavone (4, PPEE2): yellow crystal (CH_2Cl_2 - MeOH), mp. 179-180 ^{o}C.

^{1}H NMR (500 MHz, DMSO-d_6) δ_H ppm: 3.79 (3H, s, 4'-OC$\underline{H}_3$), 3.85 (3H, s, 7-OC$\underline{H}_3$), 5.74 (1H, s, 3-OH), 6.35 (1H, d, *J* = 2.0 Hz, H-6), 6.71 (1H, d, *J* = 2.0 Hz, H-8), 6.94 (2H, d, *J* = 9.0 Hz, H-3' and H-5'), 7.96 (2H, d, *J* = 9.0 Hz, H-2' and H-6'), 12.65 (1H, s, 5-OH).

^{13}C NMR (125 MHz, DMSO-d_6) δ_C ppm: 178.04 (C-4), 165.09 (C-7), 160.92 (C-4'), 160.26 (C-5), 156.27 (C-9), 155.92 (C-2), 137.81 (C-3), 130.18 (C-2' and C-6'), 120.47 (C-1'), 115.64 (C-3' and C-5'), 105.71 (C-10), 97.72 (C-6), 92.31 (C-8), 59.67 (7-O$\underline{C}H_3$), 55.98 (4'-O$\underline{C}H_3$).

APCI-MS *m/z*: 315 $[M+H]^+$.

***5,6-dihydroxy-3,4',7-trimethoxyflavone (5)*:** yellow solid (MeOH).

^{1}H NMR (500 MHz, DMSO-d_6) δ_H ppm: 3.80 (3H, s, 3-OC$\underline{H}_3$), 3.85 (3H, s, 4'-OC$\underline{H}_3$), 3.90 (3H, s, 7-OC$\underline{H}_3$), 6.88 (1H, s, H-8), 7.13 (2H, d, *J* = 9.0 Hz, H-3' and H-5'), 8.04 (2H, d, *J* = 9.0 Hz, H-2' and H-6'), 8.72 (1H, s, 6-OH), 12.30 (1H, s, 5-OH).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C ppm: 178.15 (C-4), 161.31 (C-4'), 155.27 (C-2), 154.60 (C-7), 148.89 (C-9), 145.65 (C-5), 137.79 (C-3), 129.93 (C-2' and C-6'), 129.66 (C-6), 122.33 (C-1'), 114.20 (C-3' and C-5'), 105.60 (C-10), 90.94 (C-8), 59.71 (3-O$\underline{C}H_3$), 56.31 (7-O$\underline{C}H_3$), 55.42 (4'-O$\underline{C}H_3$).

APCI-MS *m/z*: 345 $[M+H]^+$

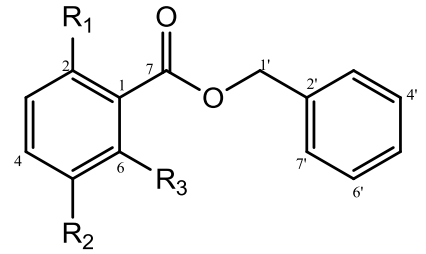
3. RESULTS AND DISCUSSION

Successive chromatographies of the EtOAc crude extract of *P. parviflora* leaves on silica gel, Sephadex LH-20 yielded five compounds: the benzoyl esters **1**-**2** and flavones **3**-**5**. Their structures were determined based on NMR studies in combination with the literatures.

Compound **1**, the major component of the EtOAC crude extract, was obtained as white solid, mp 98-99 ^{o}C. The ^{1}H NMR spectrum indicated the presence of a phenyl group at δ 7.31 (1H, d, *J* = 8.0 Hz), 7.37 (2H, t, *J* = 8.0 Hz) and 7.45 (2H, t, *J* = 8.0 Hz), a AB_2 coupling system of three aromatic protons at δ 6.54 (2H, d, *J* = 8.5 Hz) and 7.26 (1H, t, *J* = 8,5 Hz), an isolated oxymethylene group at δ 5.39 (2H, s) and two methoxy groups at δ 3.80 (6H, s). The ^{13}C NMR spectrum showed the resonated signals of 12 aromatic carbons including 8 methine groups at δ 103.95, 127.91, 128.02, 128.31 and 131.09 together with 4 quaternary carbons at δ 113.03, 136.09 and 157.42. The remaining signals were belonged to one oxymethylene carbon at δ 66.77 and two methoxy carbons at δ 55.91. Specially, the signal of a carboxyl group at δ 166.39 showed that **1** was a ester compound. The ESI-MS spectrum exhibited a protonated molecular ion at *m/z* 273 $[M+H]^+$, so the molecular formula was determined to be $C_{16}H_{16}O_4$. The above spectra data in combination with the literature [4], the structure of **1** could be unambiguously confirmed as benzyl 2,6-dimethoxybenzoate.

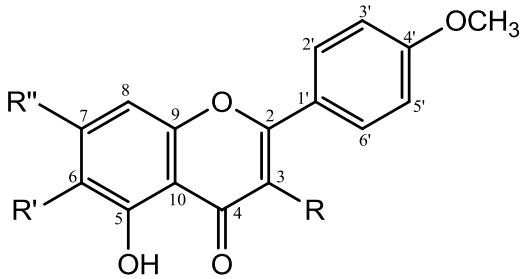
Compound **2**, isolated as colorless needles, mp 104-105 ^{o}C, gave the *pseudo*-molecular ion at *m/z* 243 $[M-H]^-$ in the APCI-MS spectrum, that indicated the molecular formula of $C_{14}H_{12}O_4$. The ^{1}H NMR

and ^{13}C NMR were similar to those of **1**. In the ^{1}H NMR spectrum, the presence of a phenyl group at δ 7.36-7.44 (5H, m), an ABX coupling system of three aromatic protons at δ 6.88 (1H, d, *J* = 9.0 Hz), 7.00 (1H, dd, *J* = 3.0, 9.0 Hz) and 7.31 (1H, d, *J* = 3.0 Hz), one oxymethylene group at δ 5.36 suggested that the structure of **2** was very close to **1**. The most significant differences observed in the ^{1}H NMR spectrum were the replacement of two methoxy groups at δ 3.80 (δ_C 55.91) of **1** by two hydroxyl group at δ 10.32 and 4.65 of **2**. The signal at δ 169.50 in the ^{13}C NMR spectrum was specific of one carboxy group. Based on the spectra data and the literature [4] the structure of **2** could be identified as benzyl 2,5-dihydroxybenzoate. It was also named trichocarpinine [5].



1: $R_1 = R_3 = OCH_3$; $R_2 = H$

2: $R_1 = R_2 = OH$, $R_3 = H$



3: $R = OCH_3$; R' = H; R" = OCH_3

4: R = OH; R' = H, R" = OCH_3

5: R = R" = OCH_3; R' = OH

Figure 1: Structures of **1**-**5**

Compound **3** was isolated as yellow crystals, mp 145-146 ^{o}C. The ^{1}H NMR spectrum showed the presence of an AX coupling system of two aromatic protons at δ 6.35 (1H, d, *J* = 2.0 Hz) and 6.44 (1H, d, *J* = 2.0 Hz), and an AB coupling system of four protons in a *para*-substituted benzene ring at δ 7.02 (2H, d, *J* = 9.0 Hz) and 8.07 (2H, d, *J* = 9.0 Hz). The remaining signals were assigned for three methoxy groups at δ 3.85 (3H, s), 3.87 (3H, s) and 3.89 (3H, s) and a chelated proton of one hydroxyl group at δ 12.64. The ^{13}C NMR spectrum had signals of 14 aromatic carbons and one carbonyl group at δ 178.81 that was clearly approved of flavone skeleton for **3**. Three carbons of methoxy groups were resonated at δ 60.15, 55.80 and 55.44. The *pseudo*-molecular ion at *m/z* 329 $[M+H]^+$ in the APCI-MS spectrum together with the above spectra data, one could be determined a molecular formula of $C_{18}H_{16}O_6$ of **3**. The signal of the chelated proton at δ 12.64 indicated that C-5 was linked to a hydroxyl group and **3** could be 5-hydroxy-3,4',7-trimethoxyflavone. The coincidence of the ^{13}C NMR spectra data of **3** with those of 5-hydroxy-3,4',7-trimethoxyflavone in the literature [6] was undoubtedly confirmed the structure of **3** as 5-hydroxy-3,4',7-trimethoxyflavone.

Compound **4,** isolated as yellow crystal, mp 179 - 180 ^{o}C, exhibited a *pseudo*-molecular ion at *m/z* 315 $[M+H]^+$ in the APCI-MS spectrum. The NMR spectra were very similar to that of **3**. The main differences were the replacement of one methoxy group (δ_H 3.85 and δ_C 60.15) in the NMR spectra of **3** by one hydroxyl group (δ 5.74) in the ^{1}H NMR of **4**. Based on these spectra analysis, **4** also was a flavone compound as **3**. In the ^{1}H NMR spectrum, the signal of the chelated proton at δ 12.65 showed that the C-5 of flavone skeleton was still linked to one hydroxyl group and **4** was a 5-hydroxy flavone compound. The ^{1}H NMR and ^{13}C NMR data were compared with those of 3,5-dihydroxy-4',7-dimethoxyflavone in the literature [6]. The coincidences of these data were confirmed the structure of **4** as 3,5-dihydroxy-4',7-dimethoxyflavone.

Compound **5** was yielded as yellow powder. The ^{1}H NMR spectrum indicated the presence of five aromatic protons belonging to a *para*-substituted benzene ring at δ 7.13 (2H, d, *J* = 9.0 Hz) and 8.04 (2H, d, *J* = 9.0 Hz), and one isolated proton of another benzene ring at δ 6.88 (1H, s), three methoxyl groups at δ 3.80 (3H, s), 3.85 (3H, s) and 3.90 (3H, s) together with two hydroxyl groups at δ 8.72 (1H, s) and 12.30 (1H, s). The ^{13}C NMR spectrum had fourteen signals of aromatic carbons at δ 90.94-161.31 and a carbonyl group at δ 178.15, which were very specific of a polysubstituted flavone compound. Three methoxy groups were resonated at δ 59.71, 56.31 and 55.42. The signal at δ_H 12.30 indicated the existence of a hydroxyl group at C-5 and therefore **5** was also a 5-hydroxyflavone compound. The coincidence of the ^{13}C NMR data of **5** with those of 5,6-dihydroxy-3,4',7-trimethoxyflavone in the literature [7] was clearly confirmed the structure of **5** as 5,6-dihydroxy-3,4',7-trimethoxyflavone.

4. CONCLUSION

The chemical constituents of *Polyalthia parviflora* leaves were studied on the first time. Five compounds, including two benzoyl esters, benzyl 2,6-dimethoxybenzoate (**1**), benzyl 2,5-dihydroxybenzoate (**2**), together with three flavones, 5-hydroxy-3,4',7-trimethoxyflavone (**3**), 3,5-dihydroxy-4',7-dimethoxyflavone (**4**) and 5,6-dihydroxy-3,4',7-trimethoxyflavone (**5**), were isolated from the EtOAc crude extract of *P. parviflora* leaves by repeated chromatographies on silica gel and Sephadex LH-20. These compounds were isolated from *Polyalthia parviflora* for the first time.

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Corresponding author: **Nguyen Thi Minh Hang**
Institute of Marine Biochemistry, Vietnam Academy of Science and Technology
18 Hoang Quoc Viet, Cau Giay, Hanoi.
E-mail: minhhang@imbc.vast.vn
Tel. 04. 37916337.