SHORT REPORT

A C G publications

Rec. Nat. Prod. 6:3 (2012) 288-291

records of natural products

Chemical Constituents and Antioxidant Activity from the Stems of *Alyxia reinwardtii*

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(Received March 14, 2011; June 22, 2011; July 8, 2011)

Abstract: Eight compounds were isolated from the stems of *Alyxia reinwardtii*, namely coumarin (**1**), 3-hydroxycoumarin (**2**), 6-hydroxycoumarin (**3**), 8-hydroxycoumarin (**4**), scopoletin (**5**), (+)-pinoresinol (**6**), zhebeiresinol (**7**) and *p*-hydroxybenzoic acid (**8**). The structures of all compounds were characterized by means of NMR, MS, chemical analysis and comparison with the literature data. The structure of compound **7** was also confirmed by X-ray crystallography. To the best of our knowledge, compounds **2-3**, **5** and **7-8** have been isolated for the first time from this species. In terms of antioxidant activity, the isolated compounds were evaluated by various *in vitro* model assays, which include the DPPH radical scavenging activity, xanthine oxidase-related activity (superoxide scavenging activity and inhibitory effect on xanthine oxidase) and lipid peroxidation inhibitory activity.

Keywords: *Alyxia reinwardtii;* Apocynaceae; DPPH; xanthine oxidase-related activity; lipid peroxidation inhibitory activity.

1. Plant Source

Alyxia reinwardtii (Apocynaceae) contains 60-70 species in Eastern Asia, Australia and the Pacific. *A. reinwardtii* is one of 4 species in Thailand and is widely distributed. It has been locally known as 'Chalood' [1] and used as a traditional Thai medicinal plant. The leaves and fruits of this plant can be used to reduce fever, the flowers are effective in treating mental confusion and hallucination associated with high fever, and the stems are used to treat fainting, heart failure and abdominal discomforts due to gaseous distention or other unspecified causes [2].

The stems of *A. rewardtii* were collected from Nakornpratom Province, Thailand in January 2002. The specimens were identified by Associate Professor Dr. Obchan Thaithong, Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 03 /25/2012 EISSN:1307-6167

2. Previous Studies

Iridoids, coumarins and lignans were isolated from the stems, bark, leaves and inner bark of *A. reinwardtii* [3-6]. There are no reports on the xanthine oxidase-related activity (superoxide scavenging activity and inhibitory effect on xanthine oxidase) and lipid peroxidation inhibitory activity of this plant.

3. Present Study

The dried stems (4.8 kg) of *A. reinwardtii* were pulverized and then macerated with hexane, dichloromethane and ethyl acetate thrice for each solvent at room temperature. The extracts of each solvent were filtrated and evaporated under reduced pressure to afford 49.06 g of hexane crude extract, 82.24 g of dichloromethane crude extract and 33.9 g of ethyl acetate crude extract. The CH_2Cl_2 extract (55.0 g) was subjected to vacuum liquid chromatography (VLC) over silica gel (Merck Art 7730), using hexane, CH_2Cl_2, EtOAc and MeOH with increasing polarity. A total seven fractions were collected (A-G). From VLC fraction C was chromatographed on silica gel column using EtOAc-CH_2Cl_2 (4:6 to 6:4) to yield the white powder of **1** (2.15 g), which was identified as coumarin [7]. Fraction D was chromatographed on silica gel column using a stepwise gradient elution of hexane and CH_2Cl_2 (7:3 to 8:2) to furnish **2** (2.55 g), which was identified as 3-hydroxycoumarin [6]. Similarly, fraction F was also subjected to column chromatography over silica gel using a stepwise gradient of hexane, CH_2Cl_2 and EtOAc to give **3** (1.2 g), **4** (0.7 g) and **5** (0.018 g), which were identified as 6-hydroxycoumarin, 8-hydroxycoumarin [6] and scopoletin [8], respectively. Fraction G was recrystallized from EtOAc-CH_2Cl_2 (1:1) to yield the white needles of **7** (0.010 g), which was identified as zhebeiresinol [9]. The mother liquor of this fraction was further purified with chromatotron using a stepwise gradient of EtOAc-CH_2Cl_2 (2:8 to 1:1) to give **6** (0.057 g), which was identified as (+)-pinoresinol [10].

The EtOAc extract (10 g) was similarly chromatographed on silica gel VLC using a stepwise gradient elution of MeOH in CH_2Cl_2, yielding three fractions (H-J). Repeated column chromatography of J, eluting with EtOAc-CH_2Cl_2 (0:10 to 6:4) afforded **8** (0.013 g), which was identified as *p*-hydroxybenzoic acid [8].

The identification of all isolated compounds (Figure 1) was determined by means of spectroscopic methods (MS, ^1H, ^{13}C NMR and 2D NMR) as well as comparison with literature data. The exact molecular structure of compound **7** was also confirmed by X-ray crystallography (Figure 2).

Antioxidant activity of the isolated compounds were evaluated using assays for DPPH radical scavenging activity [11], scavenging activity of $O_2^{\bullet-}$ by xanthine oxidase, inhibitory activity against xanthine oxidase [12] and ferric thiocyanate assay [13]. The details of these assays are described in the supporting information.

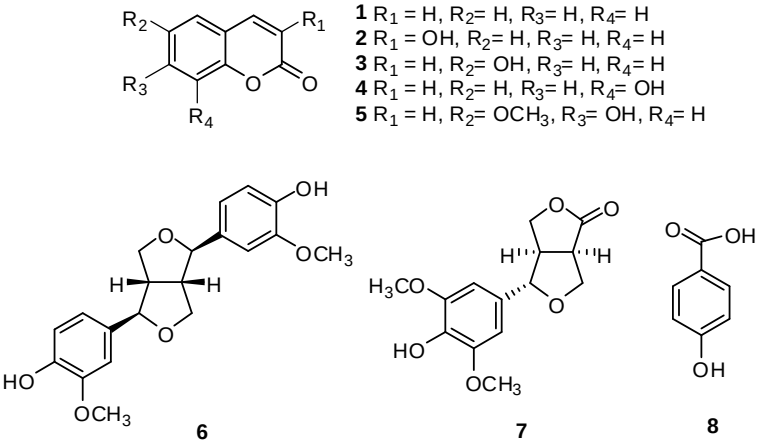

Figure 1. Compounds **1**-**8** isolated from *A. rewardtii* stems

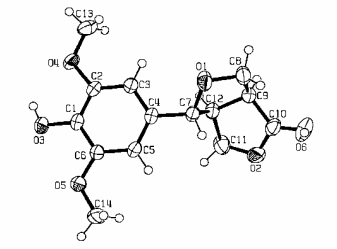

Figure 2. ORTEP view of x-ray molecular structure of compound **7**

Table 1. Antioxidant activity of all isolated compounds.

Compound	IC_{50} (mM)			
	DPPH	Xanthine		Lipid peroxidation
		Superoxide scavenging	Xanthine oxidase inhibition	
1	>100	>100	No activity	67.64 ± 1.46
2	0.61 ± 0.08	4.55 ± 0.05	No activity	69.07 ± 0.81
3	>100	19.23 ± 0.17	No activity	67.45 ± 0.75
4	>100	13.35 ± 1.11	No activity	58.13 ± 1.17
5	3.17 ± 0.31	-	-	-
6	0.31 ± 0.02	4.51 ± 0.41	No activity	3.37 ± 0.13
7	0.19 ± 0.02	3.38 ± 0.29	No activity	2.08 ± 0.06
8	>100	>100	No activity	>100
BHA[a]	0.18 ± 0.03	-	-	0.25 ± 0.01
Gallic acid[a]	0.50 ± 0.03	0.65 ± 0.02	-	-
Allopurinol[a]	-	-	0.0044 ± 0.07	-

[a] Standard references

Results and Discussion

From the stems of *A. wardtii*, eight compounds (**1-8**) were isolated and characterized. All isolated compounds were evaluated for their antioxidant activity. The DPPH test indicated that compound **7** (IC_{50} = 0.19 mM) showed the best activity, followed by **6** (IC_{50} = 0.31 mM), **2** (IC_{50} = 0.61 mM) and **5** (IC_{50} = 3.17 mM), which showed moderate to weak activity, while compounds **1**, **3**, **4** and **8** were regarded as inactive (IC_{50}> 100 mM). In addition, compounds **2** (IC_{50} = 4.55 mM), **6** (IC_{50} = 4.51 mM) and **7** (IC_{50} = 3.38 mM) exhibited moderate superoxide scavenging activity while compounds **1** and **8** were inactive (IC_{50} >100 mM). However, all compounds displayed no inhibitory activity against xanthine oxidase. On the other hand, in the lipid peroxidation test, compounds **6** and **7** showed potent activity (IC_{50} = 3.31 and 2.08 mM, respectively), while compounds **1**, **2**, **3** and **4** showed very weak activity (IC_{50} = 67.64, 69.07, 67.45 and 58.13 mM, respectively) (Table 1).

As a conclusion, *A. reinwardtii* might be a natural source of plant antioxidants [5] due to the presence of compounds **2**, **6** and **7**, especially zhebeiresinol (**7**) showed good potential antioxidant activity with fairly low IC_{50} values in antioxidant tests.

Acknowledgments

The authors are grateful to the Graduate School of Chulalongkorn University for financial support. We also thank the Center for Petroleum, Petrochemical and Advanced Materials, Chulalongkorn University and the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission (FW645A) for partially supporting this project.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/RNP

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