CHEMICAL CONSTITUENTS FROM THE ROOTS OF *BOMBAX ANCEPS*JIRAPAST SICHAEM^{1,4}, PONGPUN SIRIPONG², SUTTIRA KHUMKRATOK³, SANTI TIP-PYANG^{1*}¹Natural Products Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand.²Natural Products Research Section, Research Division, National Cancer Institute, Bangkok 10400, Thailand.³Walai Rukhavej Botanical Research Institute, Mahasarakham University, Mahasarakham 44000, Thailand.⁴Center for Petroleum, Petrochemical, and Advanced Materials, Chulalongkorn University, Bangkok 10330, Thailand.

(Received: July 27, 2009 - Accepted: April 22, 2010)

ABSTRACT

The phytochemical investigation of the roots of *Bombax anceps* (Bombacaceae) led to the isolation of one new dihydrobenzodioxine derivative, bombaxoin (**1**) along with ten known compounds. All structures of the isolated compounds were established by means of spectral analyses. Compound **2** showed the highest antioxidant activity (DPPH), while compound **8** showed the highest potential inhibitors activity toward AChE and BChE. In addition, all of the compounds were inactive to cytotoxic activity (KB and HeLa cell lines).

Key words: *Bombax anceps*, Bombacaceae, Bombaxoin, dihydrobenzodioxine derivative

INTRODUCTION

There is a growing interest in the use of natural products, as consumer awareness of their possible beneficial health effects increases. Thai medicinal plants have attracted considerable interest in view of their claimed beneficial effects¹, being sources for new compounds showing antioxidant, anticancer and antialzheimer activity. Thus, the identification of new active compounds remains a highly active research area and is be useful for developing new therapeutic strategies.

The Bombacaceae family is comprised of 28 genera and 200 species², and is widely distributed in tropical regions of the world. The genus *Bombax*, comprising some 60 species of trees, is distributed mainly in both the old and new world tropics³. Several previous reports have investigated the phytochemistry of this genus, and these have resulted in the isolation of several sesquiterpenoids⁴⁻⁶. *Bombax anceps* Pierre, locally known as “Ngiu paa”, is a deciduous tree distributed in mixed deciduous and dry dipterocarp forests of Thailand, and to this point no phytochemical study of this species has been undertaken. In our investigation we examined the chemical constituents from the roots of *B. anceps*. Bioassay-guided fractionation of the active extract resulted in the isolation of one new dihydrobenzodioxine derivative, bombaxoin (**1**) along with one naphthoquinone (**2**), two sesquiterpenoids (**3-4**), four flavonoids (**5-8**) and three steroids (**9-11**). These isolated compounds were elucidated on the basis of spectral analyses such as ¹H, ¹³C-NMR, 2D NMR, IR and MS, and also were evaluated for antioxidant activity (DPPH), cytotoxic activity (KB and HeLa cell lines) and AChE and BChE activity. This, to our knowledge, is the first report describing the chemical investigation of *B. anceps*.

EXPERIMENTAL

General experimental procedures

NMR spectra were recorded with a Varian model Mercury⁺ 400 spectrometer. The chemical shift in δ (ppm) with TMS as an internal standard. Adsorbents such as silica gel 60 Merck cat. No. 7729 and 7734, and 7731, were used for column chromatography and radial chromatography was done using a Chromatotron (model 7924 T, Harrison Research). Merck silica gel 60 F254 plates were used for TLC. ESIMS and HRESIMS were obtained using a mass spectrometer model VG TRIO 2000 and a Micromass LCT mass spectrometer. UV-visible absorption spectra were recorded on a UV-2552PC UV-Vis spectrometer (Shimadzu, Kyoto, Japan) and UV-spectrometer, with microtiter plate reader, model Sunrise (Tecan, Austria GmbH). Optical rotations were measured on a Jasco P-1010 polarimeter. Melting points were determined with a Fisher-Johns Melting Point Apparatus. IR data was obtained from a Nicolet 6700 FT-IR spectrometer.

Plant Material

The roots of *B. anceps* were collected from Mahasarakham province of Thailand in October 2008 and identified by Ms. Suttira Khumkratok, a botanist

at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, where a voucher specimen (Khumkratok no. 1-09) is deposited.

Extraction and separation of compounds

The air-dried and powdered roots (2.0 kg) of *B. anceps* were successively extracted in a soxhlet with CH₂Cl₂. The extract was concentrated under vacuum to yield 50.0 g of crude residue. This material was fractionated by vacuum liquid chromatography (VLC) over silica gel (Merck Art 7730), using hexane, CH₂Cl₂, EtOAc and MeOH with increasing polarity. A total of seven fractions (A-G) were collected. The VLC fraction A was chromatographed on silica gel column using a stepwise gradient elution of hexane, CH₂Cl₂ and EtOAc to furnish compounds **9** (238.4 mg)⁷, **10** (214.9 mg)⁸, **11** (284.6 mg)⁹, **1** (4.7 mg) and **2** (134.6 mg)¹⁰. Similarly, VLC fraction C was chromatographed on silica gel column using a stepwise gradient elution of EtOAc in hexane to afford six fractions (C-1-6). Fraction C-2 was separated by chromatotron eluting with a gradient between hexane and EtOAc to afford compounds **3** (115.3 mg), **4** (93.7 mg)⁶ and **5** (13.6 mg)¹¹. Similarly, fraction C-3 was separated by chromatotron eluting with a gradient between hexane and EtOAc to yield compound **6** (14.8 mg)¹². The VLC fraction F was rechromatographed on silica gel, using a gradient system of hexane, CH₂Cl₂ and MeOH as eluents to yield five fractions (F-1-5). Fraction F-4 was further purified by chromatotron using a stepwise gradient of EtOAc in hexane to give compounds **7** (10.6 mg)¹³ and **8** (12.3 mg)¹⁴.

Figure 1. Isolated compounds from *B. anceps*

All the isolated compounds were identified by means of spectroscopic methods (^1H , ^{13}C -NMR, 2D-NMR and MS) as well as comparison with literature data.

DPPH Radical Scavenging Assay

All of the isolated compounds were quantified using this assay¹⁵. Briefly, test sample solution (0.25 mM, 0.5 mL) was added to a 1 mL methanolic solution of DPPH (0.2 mM). The mixture was vigorously shaken and kept in the dark for 30 min. The absorbance of the resulting solution was measured at 518 nm with a UV spectrometer.

Cytotoxic Activity Assay

All of the isolated compounds were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma) and HeLa (human cervical carcinoma) cell lines employing the MTT colorimetric assay. Adriamycin was used as a standard antibiotic antitumor agent which exhibits activity against KB and HeLa cell lines according to the method of Kongkathip *et al.*¹⁶.

AChE and BChE Activities Assay

Microtitre assay used for measuring AChE (acetylcholinesterase) and BChE (butyrylcholinesterase) activities was modified from the method of Ellman *et al.*¹⁷ and Ingkaninan *et al.*¹⁸

Spectroscopic data

Bombaxoin (3-methyl-3,4-dihydrobenzo[c][1,2]dioxine-5-carbaldehyde) (**1**): Colourless oil; $[\alpha]_D^{25} = -18.0^\circ$ (c 0.05, CHCl_3); UV/Vis (MeOH): λ_{max} (log ϵ): 225 (3.93), 251 (3.71), 317 (3.40) nm; IR $\nu_{\text{max}}^{\text{KBr}}$: 2580 (CHO), 1667 (C=O), 1610 (C=C), 1243-1457 (C-O) cm^{-1} ; HRESIMS m/z 201.0523 $[\text{M}+\text{Na}]^+$ (Calcd for $\text{C}_{10}\text{H}_{10}\text{O}_3\text{Na}$: 201.0528). ^1H , ^{13}C -NMR and HMBC data are shown in Table 1.

RESULTS AND DISCUSSION

Bombaxoin (**1**) was obtained as colorless oil. Its molecular formula was established as $\text{C}_{10}\text{H}_{10}\text{O}_3$ from analysis of the HRESIMS pseudomolecular ion peak at m/z 201.0523 $[\text{M}+\text{Na}]^+$. The ^1H NMR spectrum of compound **1** displayed aromatic proton signals downfield for the doublet of doublet at δ 7.34 (1H, dd, $J=8.4$, 7.6 Hz), and the presence of two doublets at δ 6.82 (1H, d, $J=8.4$ Hz) and δ 6.62 (1H, d, $J=7.6$ Hz) confirmed that they were three adjacent aromatic protons substituted on an aromatic ring. The signal at δ 4.66 (1H, tq, $J=7.2$, 6.4 Hz) suggested the presence of the α -methine proton to an oxygen atom in dioxine ring, which showed coupling with the methylene protons at δ 2.87 (2H, d, $J=7.2$ Hz) and the methyl protons at δ 1.46 (3H, d, $J=6.4$ Hz). In addition, the proton signal at δ 1.46 (3H, d, $J=6.4$ Hz) and carbon at δ 20.8 in the ^{13}C spectrum confirmed the presence of the methyl group, which was also proved by the HMBC correlation of C-3 with the methyl protons (Table 1). The HMBC spectrum also displayed correlation of peak between the methylene protons at δ 2.87 (2H, d, $J=7.2$ Hz) and carbons at δ 76.8 (C-3) and δ 108.0 (C-10) which suggested that C-3 connected between C-4 and C-11. The aldehyde proton signal at δ 10.96 (1H, s) also correlated with the aromatic carbons (C-5, C-6, C-9 and C-10) which proved that C-12 substituted at C-5 on aromatic ring.

Figure 2. Key of HMBC correlation of bombaxoin (**1**).

According to the above evidence, the structure of compound **1** was elucidated as bombaxoin, which was further confirmed by COSY and HSQC experiments.

Table 1. NMR data of bombaxoin (**1**) in CDCl_3 (400 MHz for ^1H , 100 MHz for ^{13}C).

Position	δ_{C}	δ_{H} (mult, J in Hz)	HMBC
3	76.8	4.66(1H, tq, 7.2, 6.4)	C-5, C-11
4	34.6	2.87(2H, d, 7.2)	C-3, C-5, C-10, C-11
5	139.1	-	-
6	116.2	6.82(1H, d, 8.4)	C-8, C-10
7	136.8	7.34(1H, dd, 8.4, 7.6)	C-5, C-9
8	117.8	6.62(1H, d, 7.6)	C-6, C-10
9	162.6	-	-
10	108.0	-	-
11	20.8	1.46(3H, d, 6.4)	C-3, C-4
12-CHO	197.0	10.96(1H, s)	C-5, C-6, C-9, C-10

The ten known isolated compounds including 8-formyl-7-hydroxy-5-isopropyl-2-methoxy-3-methyl-1,4-naphthoquinone (**2**), isohemigossypol-2-methyl ether (**3**), isohemigossypol-1-methyl ether (**4**), 5-hydroxy-3,7,4'-trimethoxyflavone (**5**), 5-hydroxy-7,4'-dimethoxyflavone (**6**), 3,5,7-trimethoxyflavone (**7**), 5,7-dimethoxyflavone (**8**), cholesterol (**9**), cholestenone (**10**) and lupeol (**11**) were identified by spectroscopic data (^1H , ^{13}C -NMR,

2D-NMR and MS) as well as comparison with literature reports. A new compound (**1**) was isolated from *B. anceps*, it may be a useful taxonomic marker for the species.

The compounds **1-11** isolated from the roots of *B. anceps* were evaluated for antioxidant activity (DPPH), AChE and BChE activity (Table 2) and cytotoxic activity (KB and HeLa cell lines). The DPPH radical scavenging data indicated that compound **2** displayed the highest scavenging activity with the IC_{50} value of 0.84 ± 0.04 mM, and for other compounds were inactive ($\text{IC}_{50} > 2$ mM). It could be suggested that a reduced form of compound **2**, either the hydroquinone or semiquinone, is the active antioxidant, and it may scavenge free radicals (DPPH) involved in this assay¹⁹. Therefore, the phenolic OH groups in compounds **3** and **4** might not act as hydrogen donors to scavenge free radicals²⁰. The AChE and BChE data indicated that compound **8** showed the highest percentage inhibitory activity toward AChE and BChE with the percentage inhibitory activity values of 42.6 and 84.6%, respectively. Other compounds were less inhibitory (% Inhibition < 40%) at a concentration of 0.1 mg/mL. This result led us to conclude that the methoxy group at C-5 might be the source of higher inhibitory activity in flavone (**8**), compared to flavones (**5** and **6**), which bear a hydroxyl group. The presence of a methoxy group at C-3 in flavone (**7**) could also be a factor according for its reduced inhibitory activity compared with flavone (**8**)²¹. Cytotoxicity test using the MTT colorimetric assay against KB and HeLa cell lines, showed that all of the isolated compounds were inactive ($\text{IC}_{50} > 4$ $\mu\text{g/mL}$) with no significant cytotoxicity towards these cell lines.

Table 2. DPPH radical scavenging and AChE and BChE activities of isolated compounds.

Compounds	IC ₅₀ values (mM)	Percent inhibitory activity	
	DPPH	AChE	BChE
1	NA	NA	NA
2	0.84 ± 0.04	NA	NA
3	NA	NA	NA
4	NA	NA	NA
5	NA	NA	NA
6	NA	19.0 ± 4.2	22.8 ± 0.1
7	NA	NA	NA
8	NA	42.6 ± 1.0	84.6 ± 1.3
9	NA	NA	NA
10	NA	NA	NI
11	NA	NA	NI
Ascorbic acid	0.021 ± 0.10	-	-
Galanthamine	-	94.0 ± 1.5	95.5 ± 1.4

NA: No Activity

ACKNOWLEDGEMENTS

The authors are grateful to Graduate School of Chulalongkorn University for financial support. We also thank the Center for Petroleum, Petrochemical and Advanced Materials, Chulalongkorn University for partially supporting this project.

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