COX-1, COX-2 Inhibitors and Antifungal Agents from *Croton hutchinsonianus*

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Two new compounds, 3′-(4″-hydroxy-3″,5″-dimethoxyphenyl)-propyl benzoate (1) and 3′-(4″-hydroxyphenyl)-propyl benzoate (3) together with known compounds, 3′-(4″-hydroxy-3″-methoxyphenyl)-propyl benzoate (2), poilaneic acid (4), farnesyl acetone (5) and 4-hydroxybenzaldehyde (6) were isolated and identified from the branches of *Croton hutchinsonianus*. Their structures were determined by spectroscopic methods. The three phenylpropyl benzoates (1—3) were found to exhibit antifungal activity against *Candida albicans* (IC_{50} 5.36—11.41 μg/ml). Compounds 1—2 (IC_{50} 2.11—4.95 μg/ml) exhibited potent but non-selective activity against the enzymes cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) whereas 3 (IC_{50} 1.88 μg/ml) preferentially inhibited the enzyme COX-2.

Key words *Croton hutchinsonianus*; Euphorbiaceae; phenylpropyl benzoate; cyclooxygenase inhibitor; antifungal compound

Croton hutchinsonianus Hosseus belongs to the family Euphorbiaceae. It is a shrub or small tree reaching 4—5 meters high and native to Thailand,[1)] where it is commonly called "Plao phae". In this paper, we describe the isolation and structure elucidation of newly isolated 3′-(4″-hydroxy-3″,5″-dimethoxyphenyl)-propyl benzoate, 3′-(4″-hydroxyphenyl)-propyl benzoate and four known compounds [3′-(4″-hydroxy-3″-methoxyphenyl)-propyl benzoate, poilaneic acid, farnesyl acetone and 4-hydroxybenzaldehyde] from the branches of this plant. The complete NMR assignment of 3′-(4″-hydroxy-3″-methoxyphenyl)-propyl benzoate was established for the first time in this paper. The inhibitory activities against the enzymes COX-1 and COX-2 and antifungal activities against *Candida albicans* were also performed on some of the isolated products.

Results and Discussion

Compounds **1**—**6** were obtained from the ethyl acetate extract of *C. hutchinsonianus* after purification by Cosmosil C_{18}-OPN, Sephadex LH 20 and silica gel column chromatography.

HR-FAB-MS of **1** suggested a molecular formula of $C_{18}H_{20}O_5$ corresponding to nine degrees of unsaturation present in the molecule. The IR spectrum demonstrated the presence of a hydroxyl group (3446 cm^{-1}) and a carbonyl group (1708 cm^{-1}). The ^{1}H-NMR spectral data (Table 1) showed the presence of two benzene rings which was readily confirmed by analysis of the ^{1}H–^{1}H COSY, HMQC and HMBC spectral data. The ^{1}H-NMR spectral data showed three methylene groups (H-1′, H-2′, H-3′) bridging between benzoate and aromatic rings, two methoxyls at δ 3.86 (each 3H, s) and one D_2O-exchageable hydroxyl proton at δ 5.40 appearing as a broad singlet suggestive of the phenylpropyl benzoate moiety with the substituents of hydroxyl and methoxyl groups on the aromatic ring. In addition, HMBC data for **1** conclusively demonstrated the correlations of methylene protons H-1′ to C-1 and H-2′ to C-1″ and H-3′ to C-2″ and C-1′ respectively. The ^{13}C-NMR spectrum showed the signal of carbonyl ester at δ 166.6. The positions of two methoxyl groups and one hydroxyl group were established employing the HMBC technique (correlations as shown in Fig. 1). All data are consistent with the structure of 3′-(4″-hydroxy-3″,5″-dimethoxyphenyl)-propyl benzoate.

Compound **2** has already been isolated from the flower of *Gardenia taitensis* DC,[2)] however no NMR spectral data has been reported. The ^{1}H- and ^{13}C-NMR data of **2** are shown in Table 1.

Compound **3** had a molecular formula of $C_{16}H_{16}O_3$ based on HR-FAB-MS. The ^{1}H- and ^{13}C-NMR data of **3** (Table 1) was similar to those of **1** except for the absence of two methoxyl groups at C-5″ and C-3″. The ^{1}H-NMR spectral data of **3** showed the presence of the *para*-substituted benzene ring at δ 7.08 (2H, d, J=8.4 Hz, H-2″, H-6″); δ 6.77 (2H, d, J=8.4 Hz, H-3″, H-5″). The 2D NMR experiment on **3** also produced very similar results, indicating that both **1** and **3** were closely related.

Compounds **1**—**3** were tested for antifungal activity against *Candida albicans*. They showed moderate activity

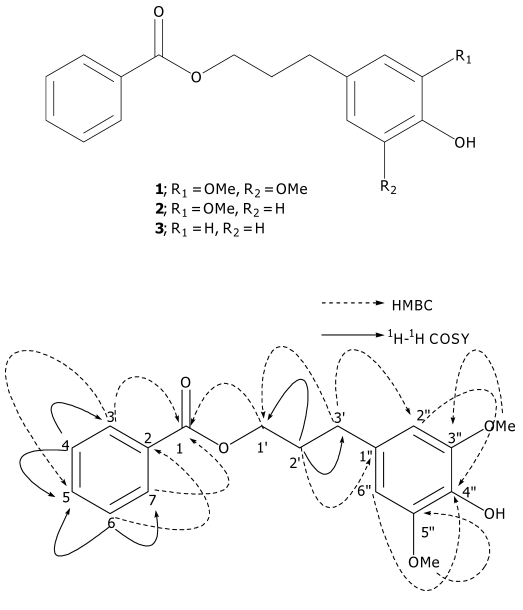

Fig. 1. ^{1}H–^{1}H COSY and HMBC Correlations of **1**

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Table 1. ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR Data of **1**—**3** ($CDCl_3$)

Position	**1**		**2**		**3**	
	δ_C	δ_H, J (Hz)	δ_C	δ_H, J (Hz)	δ_C	δ_H, J (Hz)
1	166.6	—	166.6	—	166.8	—
2	130.3	—	130.3	—	130.3	—
3	129.5	8.05, dd, 8.4, 1.3	129.5	8.04, dd, 8.4, 1.3	129.5	8.04, dd, 8.6, 1.1
4	128.3	7.45, tt, 7.4, 1.7	128.3	7.44, tt, 7.4, 1.7	128.4	7.45, tt, 7.5, 1.6
5	132.9	7.57, tt, 7.4, 1.3	132.9	7.56, tt, 7.4, 1.3	132.9	7.57, tt, 7.5, 1.1
6	128.3	7.45, tt, 7.4, 1.7	128.3	7.44, tt, 7.4, 1.7	128.4	7.45, tt, 7.5, 1.6
7	129.5	8.05, dd, 8.4, 1.3	129.5	8.04, dd, 8.4, 1.3	129.5	8.04, dd, 8.6, 1.1
1′	64.3	4.35, t, 6.5	64.2	4.34, t, 6.5	64.3	4.33, t, 6.5
2′	30.5	2.09, m	30.5	2.07, m	30.5	2.07, m
3′	32.5	2.72, br t	31.9	2.72, br t	31.3	2.72, br t
1″	132.3	—	133.0	—	133.2	—
2″	104.9	6.43, s	120.9	6.71, d, 1.3	129.5	7.08, d, 8.4
3″	146.9	—	146.4	—	115.3	6.77, d, 8.4
4″	132.9	—	143.8	—	153.9	—
5″	146.9	—	114.3	6.84, d, 8.3	115.3	6.77, d, 8.4
6″	104.9	6.43, s	110.9	6.70, dd, 8.3, 1.3	129.5	7.08, d, 8.4
3″-OMe	56.2	3.86, s	55.8	3.84, s	—	—
4″-OH	—	5.40, br s	—	5.59, br s	—	5.15, br s
5″-OMe	56.2	3.86, s	—	—	—	—

Table 2. Biological Activities of Compounds **1**—**3**

Compounds	Antifungal activity (IC_{50}, μg/ml)	Anti-inflammatory activity (IC_{50}, μg/ml)	
		COX-1	COX-2
1	11.41±1.44	4.95±0.58	2.11±0.12
2	7.05±0.61	2.14±0.19	2.11±0.18
3	5.36±0.01	Inactive	1.88±0.17
Amphotericin B	0.04±0.00	ND	ND
Aspirin	ND[a)]	4.22±0.48	13.66±0.59

a) Not determined.

with the IC_{50} values ranging from 5.36 to 11.41 μg/ml (Table 2). In addition, compounds **1**—**3** were subjected to evaluation for its inhibitory activity against the enzymes COX-1 and COX-2. Compounds **1**—**2** were potent but non-selective inhibitors of both COX enzymes (IC_{50} 2.14—4.95 μg/ml), while **3** was preferentially COX-2 (IC_{50} 1.88 μg/ml) more than COX-1. It is interesting to note that compound **3** is more active than aspirin (IC_{50} 4.22—13.66 μg/ml) as shown in Table 2.

Phenylpropyl benzoates have been previously reported as essential oil in plants such as *Wisteria floribunda*.[3)] To our knowledge, the work described here is the first report on phenylpropyl benzoates from plants in the genus *Croton*. Moreover, there has been no report on the biological activities of phenylpropyl benzoate. It is interesting to note that phenylpropyl benzoate displayed antifungal activity against *C. albicans*. Furthermore, the phenylpropyl benzoate with preferential inhibition of COX-2 over COX-1 lacks two methoxyl groups on the phenyl ring.

Experimental

General The ^{1}H, ^{13}C, DEPT, ^{1}H–^{1}H COSY, HMQC and HMBC spectra were recorded on a high resolution 400 MHz, Bruker AM 400. The FAB-MS were recorded on a Finnigan MAT 90. The IR spectra were measured on FT-IR spectrophotometer, Perkin Elmer 1760X. The UV spectra were measured on UV spectrophotometer, Shimadzu UV-2100s.

Plant Material The branches of *C. hutchinsonianus* Hosseus were collected from Karnchanaburi province, Thailand, in March 2003. Authentication was achieved by comparison with the herbarium specimen (BKF No. 2225) at the Royal Forest Department, Ministry of Agriculture and Cooperative, Thailand. A voucher specimen has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Extraction and Isolation Air-dried, powdered branches of *C. hutchinsonianus* (1.3 kg) were sequentially percolated with hexane (2×5 l), ethyl acetate (2×5 l) and then 95% ethanol (2×5 l). Removal of solvents yielded the crude extract of each solvent. The ethyl acetate extract (20.7 g) was separated by column chromatography on silica gel using hexane, with increasing concentrations of ethyl acetate to give four main fractions, A—D. Purification of fraction A (2.5 g) by silica gel column with 5% ethyl acetate in hexane yielded a colourless oil, **5** (20 mg, 0.0015%). Fraction B (8.2 g) was subjected to Cosmosil C_{18}-OPN column with 10% water in methanol to give **4** (30 mg, 0.0023%). Fraction C (6.2 g) was separated by sephadex LH-20 to give **6** (10 mg, 0.0008%) and was rechromatographed on a silica gel column with 30% hexane in ethyl acetate to furnish three compounds [**1** (40 mg, 0.0031%), **2** (25 mg, 0.0019%) and **3** (7 mg, 0.0005%)].

3′-(4″-Hydroxy-3″,5″-dimethoxyphenyl)-propyl Benzoate (**1**): A pale yellow amorphous mass; IR (KBr) ν_{max} 3446, 2921, 1708 cm^{-1}; UV (MeOH) λ_{max} (log ε) 228 (3.85), 272 (2.89); ^{1}H- and ^{13}C-NMR, see Table 1; HR-FAB-MS *m/z* 317.1395 $[M+H]^+$, Calcd for $C_{18}H_{21}O_5$, 317.1389.

3′-(4″-Hydroxy-3″-methoxyphenyl)-propyl Benzoate (**2**): A pale yellow oil; IR (neat) ν_{max} 3428, 2957, 1718 cm^{-1}; UV (MeOH) λ_{max} (log ε) 229 (4.35), 280 (3.66); ^{1}H- and ^{13}C-NMR, see Table 1; HR-FAB-MS *m/z* 287.1289 $[M+H]^+$, Calcd for $C_{17}H_{19}O_4$, 287.1284.

3′-(4″-Hydroxyphenyl)-propyl Benzoate (**3**): A pale yellow oil; IR (neat) ν_{max} 3377, 1698 cm^{-1}; UV (MeOH) λ_{max} (log ε) 228 (4.08), 279 (3.26); ^{1}H- and ^{13}C-NMR, see Table 1; HR-FAB-MS *m/z* 257.1179 $[M+H]^+$, Calcd for $C_{16}H_{17}O_3$, 257.1178.

Known compounds (**4**—**5**) were identified by comparison of physical and spectroscopic data with the literature values.[4,5)] Compound **6** was identified by comparison with available commercial authentic compound.

Antifungal Activity Compounds were evaluated in triplicate for antifungal activity against a clinical isolate of *Candida albicans*, using a method modified from the soluble formazan assay.[6)] The IC_{50} values of the tested compounds were measured in μg/ml. Amphotericin B was used as a positive control.

Cyclooxygenase Inhibitory Activity Assays were conducted in triplicate according to an established protocol.[7)] First, each of the test samples was evaluated at 10 μg/ml, and its percent inhibition was determined. Compounds exhibiting more than 80% inhibition at this concentration were further analyzed for their IC_{50} values. Aspirin was used as a positive control.

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