Prenylated Xanthenes from *Garcinia xanthochymus*

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A new prenylated xanthone, 1,3,5,6-tetrahydroxy-4,7,8-tri(3-methyl-2-butenyl)xanthone (1), was isolated from the wood of *Garcinia xanthochymus* together with a known xanthone, garciniaxanthone E (2). Their structures were determined by spectroscopic analysis. Compounds 1 (3 μ M) and 2 (10 μ M) elicited marked enhancement of nerve growth factor-mediated neurite outgrowth in PC12D cells.

Key words *Garcinia xanthochymus*; Guttiferae; xanthone; nerve growth factor (NGF)-potentiating activity; PC12D cell

The medicinal plants of the genus *Garcinia*, which belongs to the family Guttiferae, are known to be rich in prenylated xanthenes.¹⁾ Xanthone constituents have been reported to possess several biological activities, such as antibacterial activity,²⁾ antimalarial activity,³⁾ cytotoxicity,⁴⁾ and inhibition of cyclooxygenase and prostaglandin E₂.⁵⁾ As part of our search for natural products that possess nerve growth factor (NGF)-potentiating activity or neurotrophic activity from medicinal plants,^{6–8)} we found that the methanol extract of *G. xanthochymus* significantly enhanced NGF-mediated neurite outgrowth in PC12D cells. The extract was partitioned into EtOAc, *n*-BuOH, and H₂O fractions. The EtOAc-soluble residue was chromatographed by a series of bioassay-directed chromatographic separations including silica gel and Sephadex LH-20 column chromatography, and reverse-phase semipreparative HPLC to yield 1,3,5,6-tetrahydroxy-4,7,8-tri(3-methyl-2-butenyl)xanthone (**1**, 0.0016%) and garciniaxanthone E (**2**, 0.0025%).

Compound **1** was shown to have the molecular formula C₂₈H₃₂O₆ by high-resolution electron impact mass spectrometry (HR-EI-MS) measurement (m/z 464.2176, [M⁺], Δ –2.3 mmu). The IR spectrum exhibited strong bands due to the phenolic hydroxyl (3450 cm^{–1}) and chelated carbonyl (1645 cm^{–1}) group. The UV absorptions (MeOH) at $\lambda_{\max}$ 256 and 331 nm indicated **1** to be a hydroxyl xanthone derivative. ¹H- and ¹³C-NMR data (Table 1), aided by distortionless enhancement by polarization transfer (DEPT) and ¹H-detected heteronuclear multiple quantum coherence (HMQC) experiments, disclosed the presence of a carbonyl, 14 *sp*² quaternary carbons (six of which were oxygen bearing), four *sp*² methine, three *sp*³ methylene, and six methyl groups. The initial analysis of the NMR spectral data of **1** indicated that the molecule consisted of a xanthone skeleton and three prenyl moieties.

The ¹H-NMR spectrum of **1** revealed the proton signals of three prenyl moieties, the first one of which has a pair of *gem*-dimethyl signals at δ 1.83 (3H, s, H₃-5') and 1.66 (3H, s, H₃-4'), a methine signal at δ 5.32 (1H, t, J =7.2 Hz, H-2'), and a methylene signal at δ 3.56 (2H, d, J =7.2 Hz, H₂-1'); the second one of which has a pair of *gem*-dimethyl signals at δ 1.77 (3H, s, H₃-5'') and 1.68 (3H, s, H₃-4''), a methine signal at δ 5.05 (1H, t, J =6.0 Hz, H-2''), and a methylene signal at δ 3.41 (2H, d, J =6.0 Hz, H₂-1''); and the third one

of which has a pair of *gem*-dimethyl signals at δ 1.77 (3H, s, H₃-5''') and 1.68 (3H, s, H₃-4'''), a methine signal at δ 5.05 (1H, t, J =5.4 Hz, H-2'''), and a methylene signal at δ 4.03 (2H, d, J =5.4 Hz, H₂-1'''). The locations of three prenyl moieties were placed at the C-4 (δ_C 108.1), C-7 (δ_C 127.3), and C-8 (δ_C 136.8) positions base on the correlations of H₂-1'/C-3 (δ_C 164.3), H₂-1'/C-4, and H₂-1'/C-4a (δ_C 155.8); H₂-1''/C-6 (δ_C 153.0), H₂-1''/C-7 and H₂-1''/C-8; and H₂-1'''/C-7, H₂-1'''/C-8 and H₂-1'''/C-8a (δ_C 112.9) in the heteronuclear multiple bond connectivity (HMBC) spectrum of **1** (Fig. 2), respectively. Furthermore, an aromatic proton at δ_H 6.16 (1H, s, H-2) showed a definite cross peak to the carbon signal at

Table 1. NMR Spectral Data of **1**^{a)} (CD₃OD, ¹H-NMR 600 MHz, ¹³C-NMR 150 MHz)

Position	δ_C	δ_H (J =Hz)
1	163.3 (s)	
2	99.0 (d)	6.16 (1H, s)
3	164.3 (s)	
4	108.1 (s)	
4a	155.8 (s)	
4b	149.5 (s)	
5	131.9 (s)	
6	153.0 (s)	
7	127.3 (s)	
8	136.8 (s)	
8a	112.9 (s)	
9	184.9 (s)	
9a	104.8 (s)	
1'	23.0 (t)	3.56 (2H, d, 7.2)
2'	125.0 (d)	5.32 (1H, t, 7.2)
3'	132.7 (s)	
4'	26.8 (q)	1.66 (3H, s)
5'	19.2 (q)	1.83 (3H, s)
1''	26.4 (t)	3.41 (2H, d, 6.0)
2''	125.1 (d)	5.05 (1H, t, 6.0)
3''	133.0 (s)	
4''	26.7 (q)	1.68 (3H, s)
5''	18.9 (q)	1.77 (3H, s)
1'''	30.2 (t)	4.03 (2H, d, 5.4)
2'''	126.6 (d)	5.05 (1H, t, 5.4)
3'''	131.6 (s)	
4'''	26.7 (q)	1.68 (3H, s)
5'''	19.0 (q)	1.77 (3H, s)

a) ¹H- and ¹³C-NMR signals were assigned by ¹H–¹H COSY, DEPT, HMQC, and HMBC experiments.

Fig. 1. Chemical Structures of **1** and **2** Fig. 2. Selected HMBC Correlations of **1**

δ_C 99.0 (C-2) in the HMQC spectrum, which was supported by the HMBC correlations of H-2/C-1 (δ_C 163.3), H-2/C-3, H-2/C-4, and H-2/C-9a (δ_C 104.8). Comparing the 1H - and ^{13}C -NMR data of **1** with those of the known xanthones having the same partial structure, the substituted pattern of ring A was similar to that of 1,3,6-trihydroxy-5-methoxy-4-prenylxanthone⁹ and ring B was similar to that of subelliptinone A.¹⁰ Therefore, four hydroxyl groups were located to the C-1, C-3, C-5 (δ_C 131.9), and C-6 by analysis of the DEPT, HMQC and HMBC data. Thus, compound **1** was determined to be 1,3,5,6-tetrahydroxy-4,7,8-tri(3-methyl-2-butenyl)xanthone.

Compound **2** was identified as garciniaxanthone E by comparison of its NMR spectral data with the literature values.¹¹

The ability of **1** and **2** to enhance the effects of NGF at stimulating neurite outgrowth in PC12D cells was assessed utilizing methodology previously reported.⁶ In control experiments, the percentage of neurite-bearing cells was 9.9 and 63.1% following incubation with NGF 2 and 30 ng/ml after 48 h, respectively. Compounds **1** (1–3 μM) and **2** (1–10 μM) did not induce neurite outgrowth from PC12D cells in the absence of NGF, but as shown in Fig. 3, **1** (3 μM) and **2** (10 μM) significantly increased the NGF-induced (2 ng/ml) proportion of neurite-bearing cells by 8.3 and 24.9%, respectively. **1** (10 μM) and **2** (30 μM) exhibited cytotoxicity towards PC12D cells.

Although there is a report on 1,3,5,6-tetrahydroxy-4,7,8-

Fig. 3. Enhancement of Effects of NGF at Stimulating Neurite Outgrowth in PC12D Cells with Compounds **1** and **2**

Cells were incubated in the presence of NGF (2 or 30 ng/ml) alone and in the presence of compounds **1** and **2** plus NGF (2 ng/ml) for 48 h before being fixed with 2% glutaraldehyde (37 °C, 1 h). The ratio of neurite-bearing cells was determined and expressed as a mean $\pm$ S.E. ($n=12$). Statistically significant differences (* $p < 0.01$ or ** $p < 0.001$) from the control (2 ng/ml NGF) in the absence of compounds **1** and **2** were apparent.

tri(3-methyl-2-butenyl)xanthone (**1**),¹² which was isolated from the medicinal plant *G. subelliptica*, the present study is the first to report the isolation and potentiating effects of NGF at stimulating neurite outgrowth in PC12D cells in a scientific journal.

Experimental

General Procedures Melting points were determined on a Yanaco micro-melting point apparatus and were uncorrected. UV spectra were recorded on a Shimadzu UV-260 spectrophotometer. IR spectra were measured on an IR-408 spectrometer. One-dimensional (1D) and 2D NMR spectra were measured on a JEOL-600 spectrometer. Chemical shifts were reported using residual MeOH (δ_H 3.30 and δ_C 49.0) as an internal standard. EI-MS and HR-EI-MS were recorded on a JEOL JMS AX500 and JEOL JMS DX303 spectrometer.

Plant Material The wood of *G. xanthochymus* was collected at Chiang-mai, Thailand in July 1999. A voucher specimen has been deposited in the herbarium of Chulalongkorn University, Faculty of Pharmaceutical Sciences (Bangkok, Thailand).

Extraction and Isolation Dried and ground wood of *G. xanthochymus* (10 kg) was extracted with MeOH (4 $\times$ 91) at room temperature and concentrated under vacuum to yield a brown gummy extract (694 g). The extract was suspended in water, and partitioned with EtOAc (3 $\times$ 800 ml) and *n*-BuOH (3 $\times$ 800 ml) to yield EtOAc (380 g), *n*-BuOH (153 g), and water (124 g) fractions. Of these residues, only the EtOAc-soluble materials showed significant enhancement of nerve growth factor-mediated neurite outgrowth from PC12D cells. The EtOAc-soluble residue (24 g) was subjected to vacuum liquid chromatography on silica gel and eluted with *n*-hexane–EtOAc (50:50 $\rightarrow$ 0:100) and 100% MeOH to give fr. I–VII. Fr. II (3.1 g) was further chromatographed on a Sephadex LH-20 column (eluted with MeOH) to give five subfractions. The fourth subfraction was purified by reverse-phase semipreparative HPLC (YMC-AM 324, ODS, 300 $\times$ 10 mm i.d., 85% MeOH in H₂O, 1 ml/min) to afford compounds **1** (10.3 mg, t_R = 137 min) and **2** (15.7 mg, t_R = 225 min).

1,3,5,6-Tetrahydroxy-4,7,8-tri(3-methyl-2-butenyl)xanthone (1) Yellow powders (MeOH), mp. 190–192 °C. UV λ_{max} (MeOH) nm (log ϵ): 256 (5.23), 331 (4.96). IR ν_{max} (neat) cm^{-1} : 3450, 2425, 1645, 1490, 1435, 1270. 1H - and ^{13}C -NMR data see Table 1. EI-MS m/z (rel. int.): 464 [M^+] (43), 449 (14), 421 (96), 409 (33), 393 (31), 367 (100), 352 (58), 337 (32). HR-EI-MS m/z : 464.2176 [M^+] (Calcd for C₂₈H₃₂O₆: 464.2197).

Bioassay Procedure The enhancement of NGF-mediated neurite outgrowth on PC12D cells was examined using a published method.⁶ All test compound stock solutions were prepared at 100 mM in DMSO and diluted to the test concentrations (1, 3, 10, 30 μM). Neurite processes with a length equal to or greater than the diameter of the neuron cell body were scored as neurite-bearing cells. The ratio of the neurite-bearing cells to total cells

(with at least 100 cells examined/viewing area; three viewing areas/well; six wells/sample) was determined and expressed as a percentage. The data were analyzed using the Student *t*-test.

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