Prenylated Xanthones from *Garcinia xanthochymus*

Wisinee CHANMAHASATHIEN,[a] Yushan LI,[a] Masayuki SATAKE,[b] Yasukatsu OSHIMA,[b] Masami ISHIBASHI,[c] Nijsiri RUANGRUNGSI,[d] and Yasushi OHIZUMI*,[a]

[a] *Graduate School of Pharmaceutical Sciences, Tohoku University; Aoba, Aramaki, Aoba-ku, Sendai 980–8578, Japan:* [b] *Graduate School of Life Sciences, Tohoku University; 1–1 Tsutsumidori-Amamiyamachi, Aoba-ku, Sendai 981–8555, Japan:* [c] *Graduate School of Pharmaceutical Sciences, Chiba University; Yayoi-cho, Inage-ku, Chiba 263–8522, Japan: and* [d] *Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University; Bangkok 10330, Thailand.* Received June 30, 2003; accepted August 13, 2003

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 μM) and 2 (10 μM) 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 xanthones.[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 E_2.[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 H_2O 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 $C_{28}H_{32}O_6$ 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 λ_{max} 256 and 331 nm indicated **1** to be a hydroxyl xanthone derivative. ^{1}H- and ^{13}C-NMR data (Table 1), aided by distortionless enhancement by polarization transfer (DEPT) and ^{1}H-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 ^{1}H-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, H_3-5′) and 1.66 (3H, s, H_3-4′), 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, H_2-1′); the second one of which has a pair of *gem*-dimethyl signals at δ 1.77 (3H, s, H_3-5″) and 1.68 (3H, s, H_3-4″), 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, H_2-1″); and the third one of which has a pair of *gem*-dimethyl signals at δ 1.77 (3H, s, H_3-5‴) and 1.68 (3H, s, H_3-4‴), 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, H_2-1‴). 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 H_2-1′/C-3 (δ_C 164.3), H_2-1′/C-4, and H_2-1′/C-4a (δ_C 155.8); H_2-1″/C-6 (δ_C 153.0), H_2-1″/C-7 and H_2-1″/C-8; and H_2-1‴/C-7, H_2-1‴/C-8 and H_2-1‴/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)] (CD_3OD, ^{1}H-NMR 600 MHz, ^{13}C-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) ^{1}H- and ^{13}C-NMR signals were assigned by ^{1}H–^{1}H COSY, DEPT, HMQC, and HMBC experiments.

* To whom correspondence should be addressed. e-mail: ohizumi@mail.pharm.tohoku.ac.jp

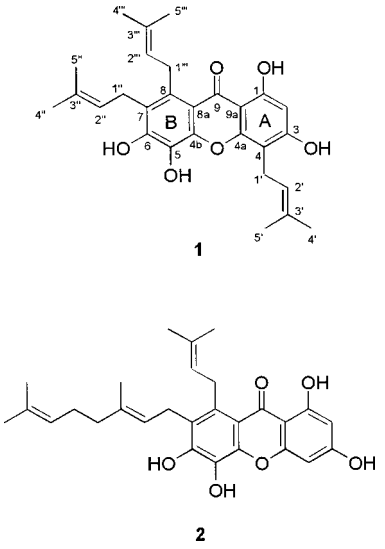

Fig. 1. Chemical Structures of **1** and **2**

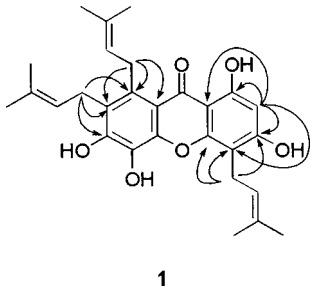

Fig. 2. Selected HMBC Correlations of **1**

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 ^{1}H- 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-prenylxanthone[9] 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-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.

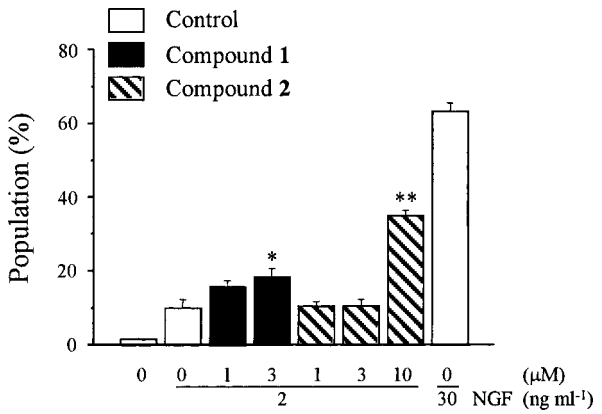

Fig. 3. Enhancement of Effects of NGF at Stimulating Neurite Outgrowth in PC12D Cells with Compounds **1** and **2**

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±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.

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 Chiangmai, 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 (4×9 l) 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 (3×800 ml) and *n*-BuOH (3×800 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→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, 300×10 mm i.d., 85% MeOH in H_2O, 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. ^{1}H- 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 $C_{28}H_{32}O_6$: 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.

Acknowledgements This research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1) Bennett G. J., Lee H. H., *Phytochemistry*, **28**, 967—998 (1989).
2) Rukachaisirikul V., Kaewno W., Koysomboon S., Phongpaichit S., Taylor W. C., *Tetrahedron*, **56**, 8539—8543 (2000).
3) Likhitwitayawuid K., Chanmahasathien W., Ruangrungsi N., Krungkrai J., *Planta Med.*, **64**, 281—282, (1998).
4) Asano J., Chiba K., Tada M., Yoshii T., *Phytochemistry*, **41**, 815—820 (1996).
5) Nakatani K., Nakahata N., Arakawa T., Yasuda H., Ohizumi Y., *Biochem. Pharmacol.*, **63**, 73—79 (2002).
6) Li Y., Matsunaga K., Kato R., Ohizumi Y., *J. Pharm. Pharmacol.*, **53**, 915—919 (2001).
7) Li Y., Matsunaga K., Kato R., Ohizumi Y., *J. Nat. Prod.*, **64**, 806—808 (2001).
8) Li Y., Matsunaga K., Ishibashi M., Ohizumi Y., *J. Org. Chem.*, **66**, 2165—2167 (2001).
9) Gonda R., Takeda T., Akiyama T., *Chem. Pharm. Bull.*, **48**, 1219—1222 (2000).
10) Iinuma M., Tosa H., Tanaka T., Shimano R., Asai F., Yonemori S., *Phytochemistry*, **35**, 1355—1360 (1994).
11) Minami H., Takahashi E., Kodama M., Fukuyama Y., *Phytochemistry*, **41**, 629—633 (1996).
12) Nagafuji S., Abe F., Okabe H., Akahane H., Kinjo K., Yaga S., "Abstract Papers of the 49th Annual Meeting of the Japanese Society of Pharmacognosy," Fukuoka, 2002, p. 213.