Chemical Constituents of *Garcinia fusca*: Structure Elucidation of Eight New Xanthones and Their Cancer Chemopreventive Activity[1]

Chihiro Ito,[†] Masataka Itoigawa,*[,‡] Tomoko Takakura,[†] Nijisiri Ruangrungsi,[§] Fumio Enjo,[⊥] Harukuni Tokuda,[⊥] Hoyoku Nishino,[⊥] and Hiroshi Furukawa[†]

Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468-8503, Japan, Tokai Gakuen University, Miyoshi, Aichi 470-0207, Japan, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand, and Department of Biochemistry, Kyoto Prefectural University of Medicine, Kamigyou-ku, Kyoto 602-0841, Japan

Received June 26, 2002

We describe the isolation and spectrometric structure elucidation of eight new xanthones, fuscaxanthone A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), F (**6**), G (**7**), and H (**8**), together with eight known xanthones from the stem bark of *Garcinia fusca* collected in Thailand. All the new xanthones were shown to have a terpenoid (prenyl and/or geranyl) side chain(s) in their molecules. We also present the results of a primary screening of the inhibitory effects of eight xanthones (**9**−**16**) isolated as major components of this plant on 12-*O*-tetradecanoylphorbol-13-acetate induced Epstein−Barr virus early antigen activation in Raji cells.

Introduction

Previously we reported the constituents of some *Garcinia* plants (Guttiferae) collected in Papua New Guinea[2] and/or New Caledonia.[3] In those papers, we reported the first isolation of depsidones from a higher plant along with new xanthones, benzophenone, chromone, and biflavanone derivatives.[2] This paper describes the isolation and identification of eight new xanthones from another *Garcinia* plant, *Garcinia fusca* Pierre, collected in Thailand. Furthermore, in a primary screening test for novel cancer chemopreventive agents (anti-tumor promoters), we found that several xanthones[4,5] and depsidones[3] showed potent inhibitory effects on Epstein−Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells. In the course of our continuing search for active cancer chemopreventive compounds from higher plants, we also carried out a primary screening of eight xanthones we isolated in this study by examining their possible inhibitory effects on EBV-EA activation.

Results and Discussion

The acetone extract of stem bark of *G. fusca* was fractionated by silica gel column chromatography and preparative TLC to obtain eight new xanthones named fuscaxanthone A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), F (**6**), G (**7**), and H (**8**), along with eight known xanthones. The following spectral data showed that all the new xanthones (except **5**) have the 1-hydroxyxanthone nucleus with prenyl or geranyl groups at C-8: (1) UV absorptions of these compounds, except **1**, showed two strong bands at λ_{max} 234−244 and 256−264 nm, a medium band at λ_{max} 308−318 nm, and a weak broad band at λ_{max} 350−380 nm. Some bathochromic shifts of absorption bands in the spectrum of **1** (λ_{max} 278sh, 290, 328 nm) suggested the presence of another conjugated system in **1**. (2) IR ν_{max} 3200−3300 cm^{-1}, NMR δ_H 12.93−13.79 and IR ν_{max} 1640−1650 cm^{-1}, NMR δ_C 181.5−182.0 due to hydrogen-bonded 1-OH and 9-C=O groups, respectively. (3) Appearance of characteristic NMR signals assignable to a prenyl or geranyl group (Table 1). The lower chemical shift values at δ_H 4.07−4.18 of the methylene protons on the side chain, except for the spectrum of **5**, indicated a deshielding by the adjacent 9-C=O and allowed the assignment of the location of the terpenoid moiety to C-8. (4) In the case of the presence of a geranyl group in the molecule, the typical fragment peak corresponding to loss of 69 mass units (C_5H_9) from the molecular ion appeared.

Structures of Fuscaxanthone A (1), B (2), C (3), D (4), G (7), and H (8). These compounds showed analogous ^1H NMR spectra having signals of OCH_3 (δ_H 3.79−3.80), a lone aromatic H (δ_H 6.75−6.85), and a geranyl in **1**, **2**, **7**, and **8** (Table 1), and a prenyl moiety in **3** and **4** (Table 1). In addition to the lower chemical shift of CH_2 in the terpenoid side chain, HMBC correlations from C-8a to H-5 and H-1′ and from C-7 to H-1′, H-5, and OCH_3 protons indicated the locations of a geranyl (or prenyl), OCH_3, and a lone aromatic H at C-8, 7, and 5, respectively. A remaining C-6 substituted with an OH (OCH_3 in **3**) was also demonstrated by HMBC correlations from C-5 and C-7 to 6-OH in **1**, **2**, **4**, **7**, and **8**, and by NOE between OCH_3 and H-5 in the spectrum of **3**. The structure of another aromatic ring C of each molecule will be discussed below.

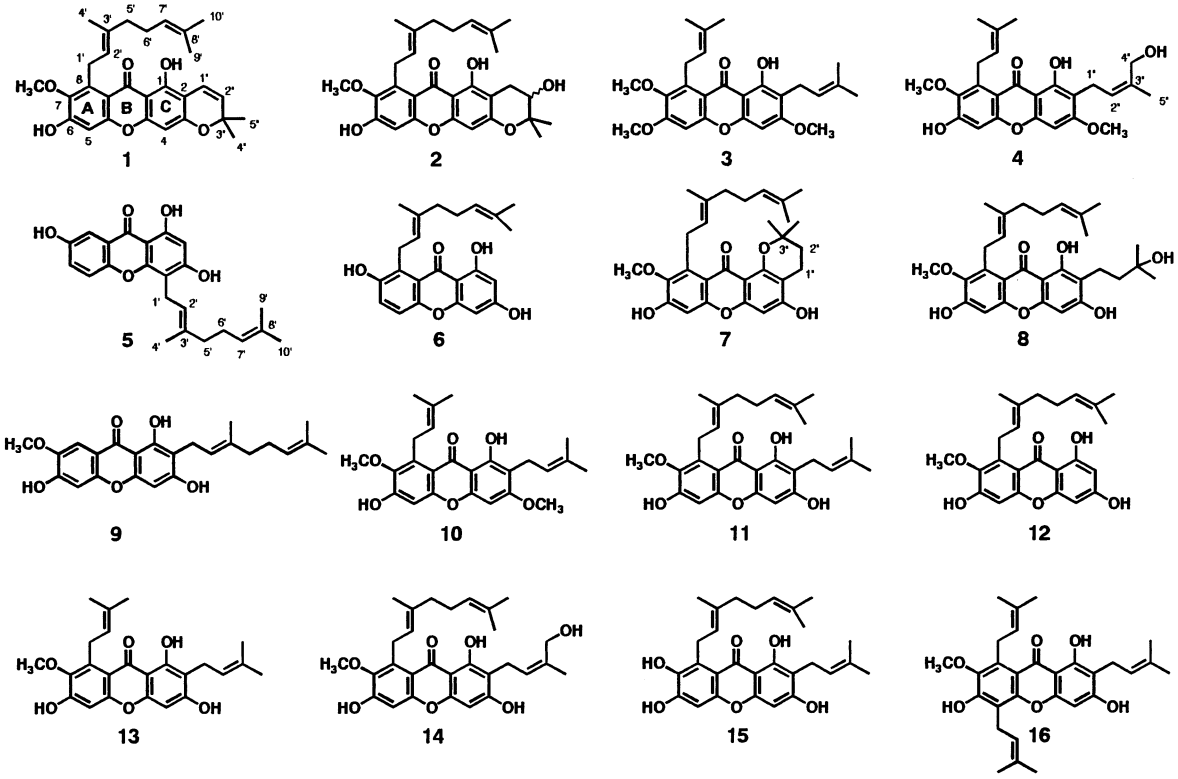
The molecular formula of fuscaxanthone A (**1**) was determined as $C_{29}H_{32}O_6$ by HRMS. Besides the aforementioned ^1H NMR signals (8-geranyl, 7-OCH_3, 6-OH, and H-5) due to substituents on ring A, signals due to a 2,2-dimethylpyran ring and an additional lone aromatic proton were observed. C−H long-range correlations in the HMBC spectrum from C-2 to a hydrogen-bonded OH and H-2″ and from C-3 to H-4 and H-1″ indicated that the orientation of the dimethylpyran ring is [3,2-*b*]. On the basis of these data, the structure of fuscaxanthone A was determined to be **1**.

The molecular formula of fuscaxanthone B was established as $C_{29}H_{34}O_7$. The ^1H NMR spectrum showed ABC-type signals at δ_H 2.76 (1H, dd, J = 17.2, 5.5 Hz), 2.97 (1H, dd, J = 17.2, 5.1 Hz), and 3.88 (1H, dd, J = 5.5, 5.1 Hz) assignable to the [−CH_2−CH(OH)−] moiety along with two methyls attached to an oxygenated carbon and a 1H singlet (H-4) together with signals of a 8-geranyl, 7-OCH_3, 6-OH, and H-5 on ring A. HMBC correlations from C-2 to a

* Please address correspondence to Masataka Itoigawa. Tel: +81-5613-6-5555. Fax: +81-5613-6-6757. E-mail: itoigawa@tokaigakuen-u.ac.jp.
† Meijo University.
‡ Tokai Gakuen University.
§ Chulalongkorn University.
⊥ Kyoto Prefectural University of Medicine.

10.1021/np020290s CCC: $25.00
Published on Web 01/01/2003

Scheme 1. Structures of Xanthones from *Garcinia fusca*



hydrogen-bonded OH, H-4, and H-1″, and from quaternary C-3″ to H-1″ and two CH_3, which further correlated with C-2″, suggested the presence of a [3,2-*b*]-oriented 2,2-dimethyl-3-hydroxydihydropyran ring in **2**. On the basis of these results coupled with appearances of an M−69 fragment in EIMS and other HMBC data, we propose structure **2** for fuscaxanthone B. The absolute stereochemistry of **2** remains undetermined.

Fuscaxanthone C was shown to have the molecular formula $C_{26}H_{30}O_6$ by HRMS. The ^{1}H NMR spectrum showed signals due to three OCH_3, two prenyl groups, and two lone aromatic H. Locations of substituents on ring C were established by HMBC correlations from C-2 to a hydrogen-bonded OH and a lone H-4, and from C-1 to H″-1 of a prenyl group, which correlated to C-3 having a OCH_3, together with an observation of NOE enhancements between 3-OCH_3 and a lone H-4. On the basis of these spectral data, coupled with another NOE observation between OCH_3 (δ_H 3.96) and a lone H-5 at δ_H 6.75, we propose structure **3** for fuscaxanthone C.

Fuscaxanthone D (**4**) was shown to have the molecular formula $C_{25}H_{28}O_7$ by HRMS analysis. The ^{1}H NMR features were similar to those of **3**, except for the appearance of a 2H singlet (δ_H 4.27) due to a hydroxymethylene group instead of a vinylmethyl and the lack of one OCH_3 in the spectrum of **3**. The presence of a (*Z*)-3-hydroxymethyl-2-butenyl moiety at C-2 was suggested by 5% NOE enhancement between H-4″ and H-1″ and HMBC correlations from C-2″ to H-4″ and H-5″ and from C-3″ to H-1″, from C-2 to a hydrogen-bonded OH, H-4, and H-1″, and from C-1 to H-1″. On the basis of these data coupled with other HMBC correlations (see Experimental Section), we propose structure **4** for fuscaxanthone D.

The molecular formula of fuscaxanthone G (**7**) was indicated as $C_{29}H_{34}O_6$ by HRMS. The ^{1}H NMR spectrum showed the presence of two methyls (δ_H 1.40, 6H, s) attached to an oxygenated quaternary carbon, vicinally located methylenes (δ_H 1.79 and 2.65, each 2H, t, J = 6.6 Hz), and a lone aromatic H (δ_H 6.54, 1H, s), as well as the aforementioned signals due to 8-geranyl, 7-OCH_3, and H-5 on ring A. Considering the lack of a typical lower field hydrogen-bonded OH signal in the NMR spectrum and the appearance of a strong fragment peak at *m*/*z* 353, which occurred by initial fission of the C-5′/C-6′ bond in a geranyl group followed by a retro-Diels−Alder collapse of the 2,2-dimethyldihydropyran ring, we propose structure **7** for fuscaxanthone G.

The molecular formula $C_{29}H_{36}O_7$ for fuscaxanthone H (**8**) was indicated by HRMS. Its ^{1}H NMR spectrum was shown to be quite similar to that of **7**, except for the appearance of a lower field hydrogen-bonded OH signal (Table 1), together with signals due to two methyls and a methylene, both bonded to an oxygenated carbon, and a benzylic methylene indicating the presence of the 3-hydroxy-3-methylbutyl moiety attached at C-2. These MS data also supported the presence of this side chain along with a geranyl moiety: (1) Observation of MS fragments at *m*/*z* 409 assignable to $[M^+ - {}^\bullet CH_2CH_2C(CH_3)_2(OH)]$. (2) A base fragment peak at *m*/*z* 353 produced by cleavage at a benzylic bond of this side chain after loss of $[{}^\bullet CH_2CH{=}C(CH_3)_2]$ in a geranyl moiety from the molecular ion. These results indicated the structure of fuscaxanthone H to be **8**.

Structure of Fuscaxanthone E (5). This compound was obtained as a pale yellow oil. The molecular formula $C_{23}H_{24}O_5$ was indicated by HRMS. The ^{1}H NMR spectrum showed a set of ABC type signals and a lone singlet in the aromatic proton region, together with signals due to a hydrogen-bonded OH and a geranyl moiety, suggesting the presence of an additional two OH groups as remaining substituents. HMBC correlations from C-2 bearing a lone H to a hydrogen-bonded OH, both from C-3 and C-4a to H-1′ in the geranyl moiety, revealed the 1,3-dihydroxy-4-geranyl moiety on ring C. Further, among ABC type signals, a lower field signal at δ_H 7.56 was assignable to H-8, which was affected by deshielding due to 9-C=O,

Table 1. ^{1}H and ^{13}C NMR Spectral Data of Fuscaxanthones[a]

	fuscaxanthone A (**1**)		fuscaxanthone B (**2**)		fuscaxanthone C (**3**)		fuscaxanthone D (**4**)		fuscaxanthone E (**5**)[b]		fuscaxanthone F (**6**)[b]		fuscaxanthone G (**7**)	fuscaxanthone H (**8**)
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_H
1		157.9		161.3		159.8		159.5		162.2		165.0		
1-OH	13.70 (s)		13.75 (s)		13.48 (s)		13.79 (s)		12.93 (s)		13.40 (s)			13.71 (s)
2		104.5		101.5		111.5		110.4	6.33 (s)	98.2	6.20 (d, 2.2)	98.5		
3		159.8		159.4		163.4		163.3		163.6		158.8		
3-OCH_3					3.91 (3H, s)		3.93 (3H, s)							
4	6.24 (s)	94.1	6.29 (s)	94.0	6.33 (s)	88.6	6.37 (s)	89.2		107.1	6.32 (d, 2.2)	93.7	6.54 (s)	6.35 (s)
4a		156.2		155.0		155.2		155.4		156.0				
5	6.83 (s)	101.6	6.84 (s)	101.6	6.75 (s)	98.2	6.85 (s)	101.5	7.49 (d, 8.8)	119.9	7.37 (d, 8.8)	123.9	6.77 (s)	6.83 (s)
6		154.5		154.5		158.0		155.7	7.36 (br d, 8.8)	125.0	7.25 (d, 8.8)	116.8		
6-OH	6.35 (s)		6.33 (s)											
6-OCH_3					3.96 (3H, s)	56.0								
7		142.7		142.5		144.0		142.7		154.7		151.6		
7-OCH_3	3.80 (3H, s)	62.0	3.80 (3H, s)	62.1	3.80 (3H, s)	60.9	3.81 (3H, s)	62.1					3.79 (3H, s)	3.80 (3H, s)
8		137.0		137.1		137.0		137.1	7.56 (br s)	109.2		119.5		
8a		122.2		111.2		112.1		112.2		121.6		*d*		
9		181.9		182.0		182.0		182.0		181.5		181.5		
9a		103.7		103.3		104.0		103.9		103.6		104.2		
10a		155.7		155.9		155.4		154.7		151.0		*d*		
1′	4.09 (2H, d, 7.3)	26.5	4.09 (2H, d, 5.9)	26.5	4.13 (2H, d, 6.6)	26.2	4.07 (2H, d, 6.4)	26.6	3.52 (2H, d, 7.3)	22.1	4.18 (2H, d, 7.0)	26.1	4.07 (2H, d, 6.6)	4.10 (2H, d, 6.2)
2′	5.26 (m)	123.2	5.25 (m)	123.2	5.24 (m)	123.2	5.24 (m)	123.0	5.30 (m)	123.3	5.30 (m)	124.3	5.38 (m)	5.27 (m)
3′		135.6		135.6		131.8		132.2		135.4		135.4		
4′	1.82 (3H, s)	16.5	1.83 (3H, s)	16.5	1.85 (3H, s)	18.2	1.83 (3H, s)	18.2	1.88 (3H, s)	16.3	1.83 (3H, s)	16.6	1.79 (3H, s)	1.83 (3H, s)
5′	2.01 (2H, m)	39.7	2.00 (2H, m)	39.7	1.68 (3H, s)	25.8	1.69 (3H, s)	25.8	1.95 (2H, m)	40.4	1.94 (2H, m)	40.6	1.94 (2H, m)	2.00 (2H, m)
6′	2.04 (2H, m)	26.4	2.04 (2H, m)	26.6					2.04 (2H)	27.2	2.04 (2H)[c]	27.4	2.01 (2H, m)	2.05 (2H, m)
7′	5.02 (m)	124.2	5.02 (m)	124.3					5.00 (m)	124.9	5.03 (m)	125.2	4.99 (m)	5.07 (m)
8′		131.2		131.3						131.6		131.4		
9′	1.54 (3H, s)	17.6	1.55 (3H, s)	17.7					1.48 (3H, s)	17.6	1.51 (3H, s)	17.6	1.51 (3H, s)	1.55 (3H, s)
10′	1.60 (3H, s)	25.6	1.60 (3H, s)	25.6					1.50 (3H, s)	25.7	1.54 (3H, s)	25.7	1.57 (3H, s)	1.60 (3H, s)
1"	6.72 (d, 10.0)	115.7	2.76 (dd, 17.2, 5.5) 2.97 (dd, 17.2, 5.1)	25.5	3.36 (2H, d, 7.0)	21.4	3.44 (2H, d, 7.8)	21.0					2.65 (2H, t, 6.6)	2.79 (2H, t, 7.7)
2"	5.56 (d, 10.0)	127.1	3.88 (dd, 5.5, 5.1)	69.0	5.25 (m)	122.3	5.36 (m)	125.5					1.79 (2H, t, 6.6)	1.82 (2H, t, 7.7)
3"		77.9		78.4		131.7		135.0						
4"	1.46 (3H, s)	28.3	1.41 (3H, s)	22.1	1.80 (3H, s)	17.8	4.27 (2H, s)	61.7					1.40 (3H, s)	1.31 (3H, s)
5″	1.46 (3H, s)	28.3	1.36 (3H, s)	24.8	1.68 (3H, s)	25.9	1.79 (3H, s)	22.2					1.40 (3H, s)	1.31 (3H, s)

[a] Values in ppm (δ_H and δ_C). All signals correspond to 1H, unless otherwise stated. Figures in parentheses are coupling constants (J) in Hz. [b] Spectra were taken in acetone-d_6. [c] Overlapped with solvent. [d] No signal was detected for overlapping or broadening of the signal.

Table 2. Inhibitory Effects of Xanthones on TPA-Induced EBV-EA Activation[a]

	EBV-EA-positive cells (% viability)				
	compound concentration (mol ratio/32 pmol TPA)				IC_{50}[b]
compound	1000	500	100	10	(mol ratio/32 pmol TPA)
cowaxanthone (**9**)	0.0 ± 0.4 (70)	40.6 ± 1.8 (>80)	76.6 ± 2.2 (>80)	97.5 ± 0.7 (>80)	398
β-mangostin (**10**)	0.0 ± 0.3 (70)	20.5 ± 1.4 (>80)	62.6 ± 2.4 (>80)	90.4 ± 0.7 (>80)	270
cowanin (**11**)	0.0 ± 0.4 (70)	33.6 ± 1.8 (>80)	71.6 ± 1.2 (>80)	95.8 ± 0.7 (>80)	320
rubraxanthone (**12**)	0.0 ± 0.3 (70)	39.5 ± 1.2 (>80)	74.1 ± 2.5 (>80)	96.8 ± 0.5 (>80)	340
α-mangostin (**13**)	0.0 ± 0.5 (70)	19.1 ± 1.1 (>80)	60.0 ± 2.2 (>80)	89.2 ± 0.3 (>80)	220
cowanol (**14**)	0.0 ± 0.3 (70)	30.5 ± 1.3 (>80)	69.6 ± 2.5 (>80)	93.1 ± 0.5 (>80)	310
norcowanin (**15**)	0.0 ± 0.5 (70)	31.9 ± 1.4 (>80)	70.1 ± 1.9 (>80)	94.2 ± 0.3 (>80)	315
7-*O*-methylgarcinone (**16**)	0.0 ± 0.8 (70)	16.3 ± 1.1 (>80)	59.2 ± 1.9 (>80)	83.8 ± 0.9 (>80)	210
β-carotene[c]	9.1 ± 0.5 (60)	34.3 ± 1.1 (>80)	82.7 ± 1.8 (>80)	100.0 ± 0.2 (>80)	400

[a] Mol ratio/TPA (32 pmol = 20 ng/mL), 1000 mol ratio = 32 nmol, 500 mol ratio = 16 nmol, 100 mol ratio = 3.2 nmol, and 10 mol ratio = 0.32 nmol. Values are EBV-EA activation (%) ± SD in the presence of the test compound relative to the positive control (100%). Values in parentheses represent the surviving Raji cells measured with Trypan Blue staining. At least 60% surviving Raji cells 2 days after treatment with the compounds is required for an accurate result. [b] IC_{50} represents the mol ratio to TPA that inhibits 50% of positive control (100%) activated with 32 pmol of TPA. [c] Positive control substance.

indicating the presence of 7-OH as a lone substituent on the A ring. Thus, the structure of fuscaxanthone E was determined to be that shown in formula **5**.

Structure of Fuscaxanthone F (6). This compound, $C_{23}H_{24}O_5$, was also obtained as a yellow oil. The ^{1}H NMR spectrum showed signals due to a hydrogen-bonded OH, a geranyl group, and both *meta*- and *ortho*-coupled aromatic H. HMBC correlations from C-2 having one proton *meta*-coupled (δ_H 6.20, d, J = 2.2 Hz) to a hydrogen-bonded OH and another *meta*-coupled H (δ_H 6.32, d, J = 2.2 Hz) indicated the presence of 1,3-disubstituted ring C. On the other hand, correlations from an oxygenated C-7 to one of the *ortho*-coupled H (δ_H 7.37, d, J = 8.8 Hz) and deshielding of H-1′ by 9-C=O indicated an 8-geranyl-7-hydroxy structure on ring A. On the basis of these data and other HMBC results, we propose **7** as the structure of fuscaxanthone F.

Nine known xanthones, cowaxanthone (**9**),[6] *β*-mangostin (**10**),[7] cowanin (**11**),[6] rubraxanthone (**12**),[8] α-mangostin (**13**),[9] cowanol (**14**),[6] norcowanin (**15**),[6] 7-*O*-methylgarcinone (**16**),[10] and garbogiol,[11] were isolated and identified by comparison of their spectral data with those published in the literature.

Inhibitory Effects on EBV-EA Induction. Eight xanthones (**9**−**16**) isolated as major components of *Garcinia fusca* Pierre were tested for their tumor-promoting inhibitory activity by using a short-term in vitro assay of TPA-induced EBV-EA activation in Raji cells. Their inhibitory effects on the activation of the virus-genome and the viability of Raji cells and the 50% inhibitory concentration (IC_{50}) values are shown in Table 2. All the test compounds showed inhibitory activity on EBV-EA activation even at 1 × 10 mol ratio/TPA (2.5−16.2%) and fully blocked EBV-EA activation at high concentration (1 × 10^3 mol ratio/TPA) without causing a decrease in viability (>70%) of the Raji cells. The corresponding IC_{50} values of tested compounds were within the range of 210−398 mol ratio/TPA and were lower than that of *β*-carotene (IC_{50} 400), a vitamin A precursor commonly used in cancer prevention studies.[12] Of the other compounds, 7-*O*-methylgarcinone (**16**), having three prenyl side chains at C-2, C-5, and C-8 of the xanthone nucleus, exhibited the most potent inhibitory activity (IC_{50} 210; 100, 83.7, 40.8, and 16.2% inhibition of activation at 1 × 10^3, 5 × 10^2, 1 × 10^2, and 1 × 10 mol ratio/TPA, respectively). Furthermore, *β*-mangostin (**10**) and α-mangostin (**13**), having two prenyl side chains at both C-2 and C-8 of the xanthone nucleus, showed significant inhibitory activity (IC_{50} 270 and 220, respectively). The inhibitory activity of cowanin (**11**), cowanol (**14**), and norcowanin (**15**), replacing the C5 side chain (prenyl group) with a C10 side chain (geranyl group) at C-8, was weaker (IC_{50} 310−320) than that of compounds **10**, **13**, and **16**. The corresponding IC_{50} values of cowaxanthone (**9**) and rubaxanthone (**12**), with only one geranyl group at C-2 or C-8 in the molecule, were 389 and 340 mol ratio/TPA, respectively. From the viewpoint of structure−activity relationships, an essential feature for the activity of the xanthones examined in the present study is the presence of the two C5 side chains (prenyl groups) at the 2- and 8-positions in a xanthone skeleton that has oxygen-linked substituents at positions 1, 3, 6, and 7. In previous studies, we reported that the presence of a prenyl moiety in the 1,3-dihydroxyxanthone molecule plays an important role in producing inhibitory effects on EBV-EA induction.[4,5] In view of the present findings taken together, the relative location of a hydroxy group and a hydrophobic prenyl moiety on the xanthone nucleus might be important factors in producing the observed chemopreventive effect against chemical-induced carcinogenesis. A study examining the tumor-promoting inhibitory activity of these compounds in vivo is now in progress.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR, COSY, HMQC, HMBC (J = 8 Hz), and NOE were measured on JNM A-400, A-600, and/or ECP-500 (JEOL) spectrometers. Chemical shifts are shown in δ (ppm) with tetramethylsilane (TMS) as an internal reference. All mass spectra were taken under EI conditions, unless otherwise stated, using a HX-110 (JEOL) and/or JMS-700 (JEOL) spectrometer having a direct inlet system. UV spectra were recorded on a UVIDEC-610C double-beam spectrophotometer (JASCO) in MeOH, and IR spectra on an IR-230 (JASCO) in $CHCl_3$. Preparative TLC was done on Kieselgel 60 F_{254} (Merck).

Plant Materials. The plant materials used in this study, *Garcinia fusca* Pierre, were collected in Ubon-rachathani Province, in 1996. Authentication was achieved by comparison with the herbarium specimen in the Forest Herbarium, Department of Forestry, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. The herbarium specimen (CUNR-09251) has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Isolation of Fuscaxanthone A (1), B (2), C (3), D (4), E (5), F (6), G (7), and H (8) from *G. fusca*. The dried stem bark (605 g) of *G. fusca* was extracted with acetone at room temperature, and the solvent was evaporated under reduced pressure to give the acetone extract (124.2 g). The residue was further extracted with MeOH under reflux to give the MeOH extract. The acetone extract (15 g) was subjected to silica gel column chromatography eluted with hexane−acetone (9:1, 4:1,

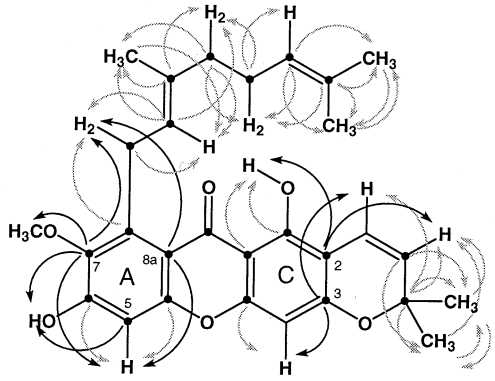


Figure 1. C−H long-range correlations in the HMBC spectrum of fuscaxanthone A (**1**). Bold lines: more significant correlations in the structure determinations.

7:3, 3:2, 1:1), acetone, and MeOH, successively to separate seven fractions. Successive treatment of each fraction with silica gel column and preparative TLC using appropriate combinations of solvents (hexane, $CHCl_3$, acetone, iPr_2O, benzene, and MeOH) as eluting or developing solvents gave the following compounds: from fraction 2 (hexane−acetone, 4:1) **1** (16.7 mg), **3** (4.3 mg), **16** (4.8 mg); from fraction 3 (hexane−acetone, 7:3) **10** (53.8 mg); from fraction 4 (hexane−acetone, 3:2) **13** (101.9 mg), **11** (467.9 mg), **5** (3.8 mg), **6** (1.2 mg), **9** (23.0 mg), **2** (2.5 mg), and **12** (17.4 mg); from fraction 5 (hexane−acetone, 1:1) **15** (11.4 mg), **4** (3.6 mg), **7** (1.0 mg), **8** (1.0 mg), and **14** (609.7 mg).

Fuscaxanthone A (1): yellow oil; HRMS *m/z* 476.2218 (calcd for $C_{29}H_{32}O_6$: 476.2199); UV (MeOH) λ_{max} 204, 212, 238, 290, 328, 360sh nm; IR ($CHCl_3$) ν_{max} 3510, 1649, 1603 cm^{-1}; HMBC, Figure 1; EIMS *m/z* 476 (M^+, 27), 461 ($M^+ - CH_3$, 11), 407 (100), 365 (9), 339 (7), 323 (6), 295 (4).

Fuscaxanthone B (2): pale yellow oil; $[\alpha]_D$ +14° (*c* 0.076, MeOH); HRMS *m/z* 494.2315 (calcd for $C_{29}H_{34}O_7$: 494.2304); UV (MeOH) λ_{max} 204, 242, 256, 318, 356 nm; IR ($CHCl_3$) ν_{max} 3510, 3200br, 1646, 1604 cm^{-1}; HMBC C−H three (or two)-bond correlations, C-1→(1-OH), H-1″; C-2→1-OH, (H-1″), H-4; C-3→H-1″, (H-4); C-4a→(H-4); C-5→6-OH; C-6→(H-5), (6-OH); C-7→H-5, 7-OCH_3, H-1′; C-8→(H-1′); C-8a→H-5, H-1′; C-9a→1-OH, H-4; C-10a→(H-5); C-2″→(H-1″), H-4″, H-5″; C-3″→(H-4″), (H-5″), H-1″; C-4″→H-5″; C-5″→H-4″; C-2′→H-4′, H-5′, (H-1′); C-3′→H-1′, (H-4′), (H-5′); C-4′→H-2′, H-5′; C-5′→H-2′, H-4′, (H-6′); C-6′→(H-5′), (H-7′); C-7′→H-9′, H-10′, (H-6′); C-8′→H-6′, (H-9′), (H-10′); C-9′→H-10′; C-10′→H-9′; EIMS *m/z* 494 (M^+, 68), 426 (78), 425 (100), 383 (30), 372 (21), 353 (16), 323 (15), 297 (9).

Fuscaxanthone C (3): yellow oil; HRMS *m/z* 438.2053 (calcd for $C_{26}H_{30}O_6$: 438.2042); UV (MeOH) λ_{max} 204, 244, 260, 310, 350 nm; IR ($CHCl_3$) ν_{max} 3568, 1646, 1599 cm^{-1}; differential NOE, irradiation of O−CH_3 (δ 3.96) gave 15% NOE at H-5 (δ 6.75); irradiation of O−CH_3 (δ 3.91) gave 17% NOE at H-4 (δ 6.33); HMBC C−H three (or two)-bond correlations, C-1→(1-OH), H-1″; C-2→1-OH, (H-1″), H-4; C-3→H-1″, (H-4), 3-OCH_3; C-4a→(H-4); C-6→(H-5), 6-OCH_3; C-7→H-5, 7-OCH_3, H-1′; C-8→(H-1′); C-8a→H-5, H-1′; C-9a→1-OH, H-4; C-10a→(H-5); C-2″→(H-1″), H-4″, H-5″; C-3″→(H-4″), (H-5″), (H-2″); C-4″→H-1″, H-5″; C-5″→H-1″, H-4″; C-2′→H-4′, H-5′, (H-1′); C-3′→H-1′, (H-4′), (H-5′); C-4′→H-2′, H-5′; C-5′→H-2′, H-4′; EIMS *m/z* 438 (M^+, 100), 395 (62), 383 (51), 382 (48), 367 (79), 351 (22), 339 (25), 313 (13).

Fuscaxanthone D (4): yellow oil; HRMS *m/z* 440.1818 (calcd for $C_{25}H_{28}O_7$: 440.1835); UV (MeOH) λ_{max} 204, 244, 258, 316, 356 nm; IR ($CHCl_3$) ν_{max} 3510, 1646, 1600 cm^{-1}; differential NOE, irradiation of O−CH_3 (δ 3.93) gave 15% NOE at H-4 (δ 6.37); irradiation of H-4″ (δ 4.27) gave 5% NOE at H-1″ (δ 3.44); HMBC C−H three (or two)-bond correlations, C-1→(1-OH), H-1″; C-2→1-OH, (H-1″), H-4; C-3→H-1″, (H-4), 3-OCH_3; C-4a→(H-4); C-6→(H-5); C-7→H-5, 7-OCH_3, H-1′; C-8→(H-1′); C-8a→H-5, H-1′; C-9a→1-OH, H-4; C-10a→(H-5); C-2″→(H-1″), H-4″, H-5″; C-3″→(H-4″), (H-5″), H-1″; C-4″→H-2″, H-5″; C-5″→H-4″, H-2″; C-2′→H-4′, H-5′, (H-1′); C-3′→H-1′, (H-4′), (H-5′); C-4′→H-2′, H-5′; C-5′→H-2′, H-4′; EIMS *m/z* 440 (M^+, 67), 422 (24), 407 (100), 379 (29), 369 (50), 353 (38), 313 (14).

Fuscaxanthone E (5): pale yellow oil; HRMS *m/z* 380.1633 (calcd for $C_{23}H_{24}O_5$: 380.1623); UV (MeOH) λ_{max} 234, 264, 316, 380 nm; IR ($CHCl_3$) ν_{max} 3300br, 1647, 1615 cm^{-1}; differential NOE, irradiation of H-1′ (δ 3.52) gave 6% NOE at H-4′ (δ 1.88); HMBC C−H three (or two)-bond correlations, C-1→(1-OH), (H-2); C-2→1-OH; C-3→H-1′; C-4→H-2, (H-1′); C-4a→H-1′; C-6→H-8; C-7→H-5; C-8→H-6; C-8a→H-5; C-9→H-8; C-9a→1-OH, H-2; C-10a→H-6, H-8; C-1′→(H-2′); C-2′→(H-1′), H-4′, H-5′; C-3′→(H-4′), (H-5′), H-1′; C-4′→H-2′, H-5′; C-5′→H-4′, H-2′, (H-6′); C-6′→(H-5′); C-7′→H-5′, (H-6′), H-9′, H-10′; C-8′→H-6′, (H-9′), (H-10′); C-9′→H-10′, H-7′; C-10′→H-9′, H-7′; EIMS *m/z* 380 (M^+, 37), 311 (74), 297 (15), 269 (18), 257 (100), 244 (14), 228 (7).

Fuscaxanthone F (6): yellow oil; HRMS *m/z* 380.1609 (calcd for $C_{23}H_{24}O_5$: 380.1623); UV (MeOH) λ_{max} 238, 262, 314, 380 nm; IR ($CHCl_3$) ν_{max} 3263br, 1650, 1611 cm^{-1}; differential NOE, irradiation of H-1′ (δ 4.18) gave 5% NOE at H-4′ (δ 1.83); HMBC C−H three (or two)-bond correlations, C-1→(1-OH); C-2→1-OH, H-4; C-3→(H-2), (H-4); C-4→H-2; C-7→H-5, (H-6), H-1′; C-8→H-6, (H-1′); C-9a→1-OH, H-2, H-4; C-2′→(H-1′), H-4′, H-5′; C-3′→(H-4′), (H-5′); C-5′→H-4′, H-2′; C-7′→H-9′, H-10′; C-8′→(H-9′), (H-10′); C-9′→H-10′; C-10′→H-9′; EIMS *m/z* 380 (M^+, 61), 311 (100), 295 (36), 269 (91), 258 (51).

Fuscaxanthone G (7): yellow oil; HRMS *m/z* 478.2359 (calcd for $C_{29}H_{34}O_6$: 478.2356); UV (MeOH) λ_{max} 204, 210sh, 242, 256sh, 308, 350sh nm; IR ($CHCl_3$) ν_{max} 3275br, 1640, 1602 cm^{-1}; EIMS *m/z* 478 (M^+, 40), 409 (100), 353 (85), 321 (13), 297 (15).

Fuscaxanthone H (8): yellow oil; HRMS *m/z* 496.2437 (calcd for $C_{29}H_{36}O_7$: 496.2461); UV (MeOH) λ_{max} 204, 242, 260sh, 318, 358sh nm; IR ($CHCl_3$) ν_{max} 3256br, 1641, 1605 cm^{-1}; EIMS *m/z* 496 (M^+, 46), 481 ($M^+ - CH_3$, 7), 427 (20), 423 (8), 422 (12), 409 (59), 367 (9), 353 (100), 297 (17).

In Vitro EBV-EA Activation Experiments. The inhibition of EBV-EA activation was assayed according to Ito et al.[3–5] In brief, Raji cells were grown to a density of 10^6 cells/mL, harvested by centrifugation, and resuspended in RPMI 1640 medium (Sigma, St. Louis, MO) with 10% FCS containing 4 mM *n*-butyric acid as inducer, 32 pmol of TPA (20 ng/mL in DMSO), and 32, 3.2, or 0.32 nmol of the test compound (DMSO solutions). The cells were incubated at 37 °C for 48 h. Cell number and viability were determined after 48 h by means of a hemocytometer (Trypan Blue staining method). EBV-EA inhibitory activity of the test compounds was estimated on the basis of the percentage of the number of positive cells compared to that of a control without the test product. In each assay, at least 500 cells were counted and the results were read blind. Concerning the effects of xanthones on this assay, the IC_{50} values were estimated by the probit transformation technique.

Acknowledgment. This work was supported in part by Grants-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science, High-Tech Research Center Project from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

References and Notes

(1) A part of this paper was presented at the 48th Annual Meeting of the Japanese Society of Pharmacognosy (Kanazawa, September, 2001) and International Symposium of the Phytochemical Society of Europe 2001 (Lausanne, September, 2001).
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NP020290S