Chemical Constituents of *Calophyllum brasiliensis*: Structure Elucidation of Seven New Xanthones and Their Cancer Chemopreventive Activity[1]

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The first study of chemical constituents of the stem bark of *Calophyllum brasilienses* collected in Brazil has led to the isolation and identification of seven new xanthones named brasixanthones A (**1**), B (**4**), C (**5**), D (**6**), E (**2**), F (**3**), and G (**10**), together with 10 known xanthones. Among the xanthones isolated in this study, **4**, **5**, **6**, and **11** were found to exhibit significant inhibitory activity against 12-*O*-tetradecanoylphorbol-13-acetate induced Epstein−Barr virus early antigen activation in Raji cells.

Continuing our search for cancer chemopreventive compounds from plant sources, we examined constituents of *Calophyllum brasilienses* (Guttiferae) collected in Brazil. Many *Calophyllum* species have been studied, and xanthones,[2−10] coumarins,[11−19] biflavonoids,[20,21] chalcones,[22−25] benzofurans,[26] and triterpenoids[27] have been identified as constituents. In a previous paper, we reported the isolation and identification of 26 natural xanthones from plants of the Guttiferae family, such as *Garcinia assigu* LANTB., *Garcinia dulcis* (ROXB.) KURZ., *Garcinia latissima* MIQ., and *Calophyllum panciflorum* A. C. SMITH.[26,28] Furthermore, in a primary screening test for novel cancer chemopreventive agents (inhibition of tumor promoters), we found that several xanthones showed potent inhibitory effects on Epstein−Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells.[29]

In this paper, we describe the isolation and identification of seven new xanthones, brasixanthones A (**1**), B (**4**), C (**5**), D (**6**), E (**2**), F (**3**), and G (**10**), from the stem bark of *Calophyllum brasiliensis* CAMB. collected in Brazil. Eleven xanthones isolated from the plant were also evaluated for inhibitory effects on EBV-EA activation.

Results and Discussion

The acetone extract of stem bark of *C. brasiliensis* was fractionated by silica gel column chromatography and preparative TLC to obtain seven new xanthones along with 10 known xanthones. The presence of a 1-hydroxyxanthone nucleus in all of the new compounds isolated in this study was suggested by their UV absorptions (see Experimental Section),[30] IR bands, and NMR signals, which were assignable to a hydrogen-bonded hydroxy (ν_{max} 3509−3588 cm^{-1}, δ_H 13.05−13.51) and a carbonyl group (ν_{max} 1645−1652 cm^{-1} and δ_C 179.9−182.2).

Brasixanthone A (**1**) was obtained as a yellow oil, and its molecular formula was determined as $C_{24}H_{24}O_6$ by HRMS. The ^{1}H NMR spectrum (Table 1) showed the presence of a methoxy, an additional hydroxy, a prenyl group, and a dimethylpyran ring together with two isolated aromatic protons (H-4 and H-8). The arrangement of these substituents on the xanthone nucleus was revealed by HMBC analysis. Long-range C−H correlation of C-2 with a hydrogen-bonded 1-OH, H-2′ on the pyran ring and a lone H-4 indicated a [3,2-*b*] orientation of the dimethylpyran ring. Further, HMBC correlation of a carbonyl carbon with H-8, which, in turn, correlated with the methylene carbon (C-1″) on the prenyl moiety, indicated the location of the prenyl group at C-7. The locations of the OH and OCH_3 groups at C-6 and C-5, respectively, were confirmed by the HMBC correlations of C-7 with the OH and of C-5 with the OH and the OCH_3. On the basis of these data, the structure of brasixanthone A was concluded to be **1**.

Brasixanthones E and F were both obtained as yellow oils, and molecular formulas were determined by HRMS as $C_{24}H_{24}O_6$ and $C_{19}H_{16}O_6$, respectively. ^{1}H NMR and HMBC analyses of these compounds also showed the presence of a [3,2-*b*]-oriented dimethylpyran ring in the molecules, the same as structure **1**. The ^{1}H NMR spectrum of brasixanthone E also showed signals due to a prenyl, an OCH_3, an additional OH group, and *para*-located protons in the molecule. Observation of a NOE between the OCH_3 and a deshielded singlet [δ_H 7.58, H-8], which had a long-range correlation with the carbonyl carbon in HMBC, confirmed the location of the OCH_3 at C-7. The position of the remaining OH at C-6 was also suggested by the HMBC correlation of C-6 with H-8. Accordingly, brasixanthone E was assigned the structure **2**.

The ^{1}H NMR spectrum of brasixanthone F was similar to that of **2**, except for the appearance of a 1H singlet at δ_H 6.33 instead of signals due to a prenyl moiety in the spectrum of **2**. The HMBC data were in agreement with structure **3** (see Experimental Section, below) for brasixanthone F.

Brasixanthone B was isolated as yellow needles, $C_{23}H_{22}O_5$. ^{1}H NMR showed the presence of a prenyl, an additional OH, and a dimethylpyran fused to the xanthone skeleton. A [3,2-*b*] orientation of the dimethylpyran ring and the location of the prenyl moiety at C-4 were confirmed by HMBC correlations of C-2 with H-2′ and of C-3 with H-1′ and methylene protons at C-1″, respectively. ABC type signals [δ_H 7.55, 7.24, 7.36], including a deshielded *meta*-coupled proton, were assigned to H-8, H-6, and H-5,

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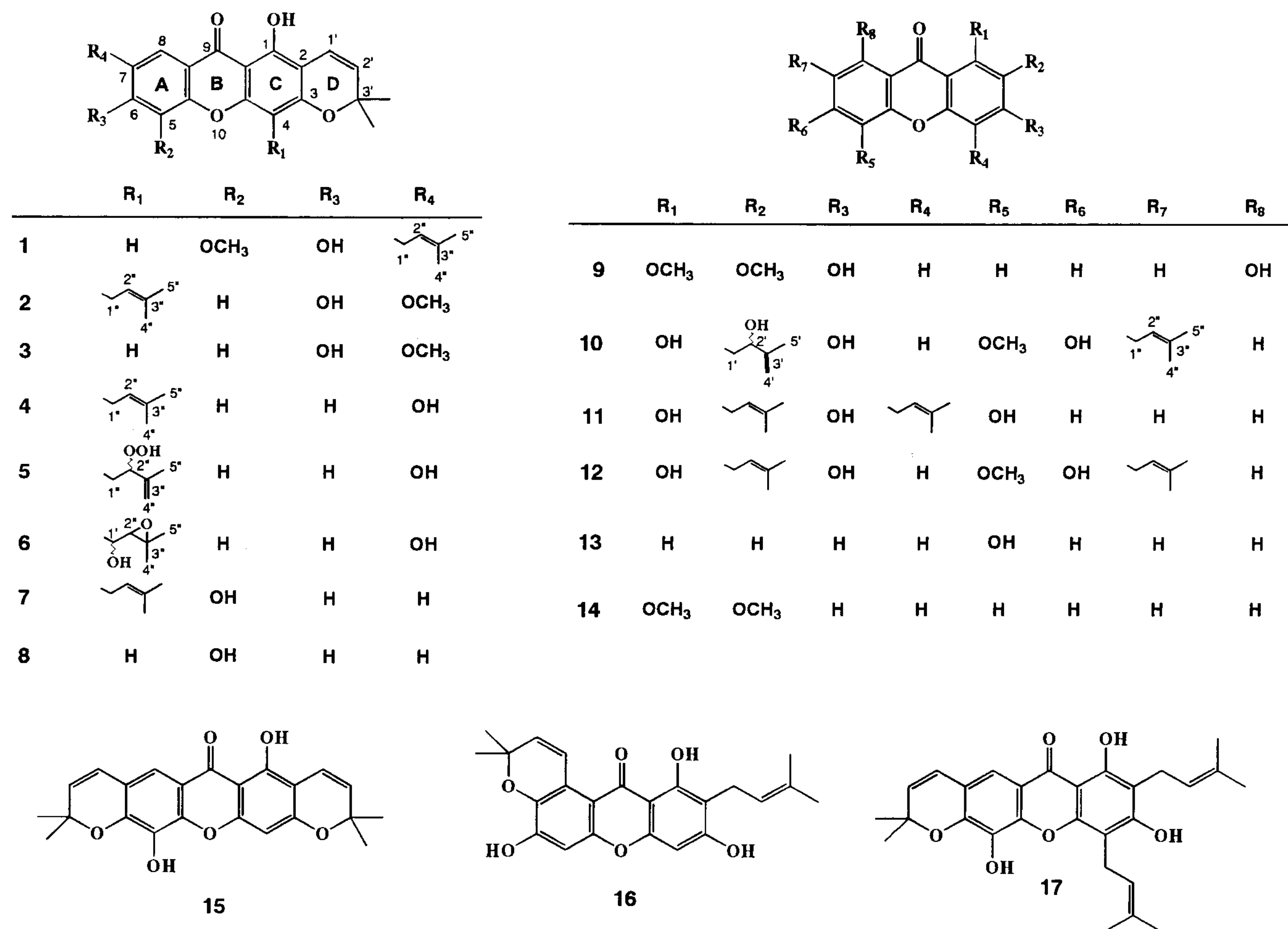
Scheme 1. Structures of Xanthones from *Calophyllum brasiliensis*



	R_1	R_2	R_3	R_4
1	H	OCH_3	OH	1″ 2″ 3″ 4″ 5″
2	1″ 2″ 3″ 4″ 5″	H	OH	OCH_3
3	H	H	OH	OCH_3
4	1″ 2″ 3″ 4″ 5″	H	H	OH
5	OOH 1″ 2″ 3″ 4″ 5″	H	H	OH
6	OH 1′ 2″ O 3″ 4″ 5″	H	H	OH
7		OH	H	H
8	H	OH	H	H

	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
9	OCH_3	OCH_3	OH	H	H	H	H	OH
10	OH	OH 1′ 2′ 3′ 4′ 5′	OH	H	OCH_3	OH	1″ 2″ 3″ 4″ 5″	H
11	OH		OH		OH	H	H	H
12	OH		OH	H	OCH_3	OH		H
13	H	H	H	H	OH	H	H	H
14	OCH_3	OCH_3	H	H	H	H	H	H

respectively, with an OH group at the remaining site at C-7. On the basis of these data, structure **4** was assigned to brasixanthone B. The HMBC data were consistent with structure **4**. Recently, Nomura and collaborators[31] reported the isolation of a new xanthone named cudraxanthone Q from *Cudrania cochinchinensis* (Moraceae). This xanthone was found to be identical with brasixanthone B by comparison with the spectral data in the literature.[31]

Brasixanthones C and D were both obtained as pale yellow oils, $[\alpha]_D$ ±0° (MeOH), and determined to have the molecular formulas $C_{23}H_{22}O_7$ and $C_{23}H_{22}O_7$ by HRFABMS and HREIMS, respectively. The NMR features (Table 1) of these compounds were similar to those of **4**, except for signals due to the side chain at C-4, indicating both compounds could have a 4-substituted 1,7-dihydroxyxanthone nucleus with a [3,2-*b*]-fused dimethylpyran ring.

In brasixanthone C, occurrence of a typical mass fragment at *m*/*z* 394 ($M^+ - O$), a 1H singlet (δ_H 9.04), and oxygen-linked methine-carbon and -proton signals (δ_C 89.4, δ_H 4.63), which were deshielded relative to those of a secondary alcohol, suggested the presence of a hydroperoxy moiety in the molecule. Furthermore, three-spin proton signals [δ_H 3.09 (H-1″) and 2.88 (H-1″), δ_H 4.63 (H-2″)] assignable to a methylene linked to a methine bearing a hydroperoxy group, a vinylmethyl, and *exo*-methylene proton signals were observed. These spectral data coupled with the observation of HMBC correlations of C-2″ with *exo*-methylene (H-4″) and vinylmethyl protons, and a MS fragment base peak at *m*/*z* 323 arising from cleavage at the benzylic position, indicated the structure of $-CH_2-CH(OOH)-C(CH_3)=CH_2$ for the side chain. On the basis of these results, structure **5** was assigned to brasixanthone C. For confirmation of this structure, treatment of **4** with O_2 in pyridine in the presence of hematoporphyrin gave a peroxygenated product, which was identified with the natural brasixanthone C.

In brasixanthone D, the presence of a 1,1-dimethyloxirane ring in the side chain was deduced by NMR signals assignable to an oxymethine group with a shielded proton (δ_C 67.7, δ_H 3.68) linked to a quaternary carbon (δ_C 58.2) bearing oxygen and to two methyls with relatively shielded protons (δ_H 1.19 and 1.17) and by HMBC correlation of C-2″ with *geminal* methyl protons. Moreover, the evaluation of the H−H COSY spectrum of the three-spin proton system at δ_H 5.08 (H-1″), 4.23 (1″-OH), and 3.68 (H-2″) led to the structure of the side chain as shown in formula **6**. The location of this side chain at C-4 and a [3,2-*b*] orientation of the dimethylpyran ring were proposed by HMBC correlation of C-3 with H-1″ and H-1′ and of C-2 with a hydrogen-bonded OH and H-2′. On the basis of these spectral data together with other HMBC data (see Experimental Section, below), structure **6** was assigned to brasixanthone D. The relative stereochemistry of **6** remains undetermined.

Brasixanthone G was isolated as a pale yellow oil, $[\alpha]_D$ ±0° (MeOH), and the molecular formula $C_{24}H_{26}O_7$ was established by HRMS. Similarity of UV absorptions of this compound with those of **12** suggested the presence of a 1,3,5,6-oxygenated xanthone skeleton in the molecule. In the ^1H NMR spectrum, one OCH_3, two OH in addition to a hydrogen-bonded OH, one prenyl moiety, and two lone aromatic proton signals were observed. Furthermore, NMR

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

	brasixanthone-A (**1**)		brasixanthone-B (**4**)		brasixanthone-C (**5**)[b]		brasixanthone-D (**6**)[b]		brasixanthone-E (**2**)		brasixanthone-F (**3**)		brasixanthone-G (**10**)	
	δH	δC	δH	δC	δH	δC	δH	δC	δH	δC	δH	δC	δH	δC
1		157.8		155.6		156.4		157.9		155.5		157.4		161.2
1-OH	13.31 (s)		13.05 (s)		13.34 (s)		13.51 (s)		13.24 (s)		13.30 (s)		13.43 (s)	
2		104.7		104.2		104.1		104.9		104.3		103.2		108.0
3		160.3		158.3		159.8		159.2		157.6		160.1		163.8
3-OH													9.31 (s)	
4	6.38 (s)	95.0		107.3		105.0		108.9		107.3	6.33 (s)	94.9	6.51 (s)	95.4
4a		156.6		154.4		157.3		155.4		154.4		157.2		156.0
5		133.1	7.36 (d, 8.8)	119.1	7.50 (d, 9.2)	120.4	7.59 (d, 9.2)	120.1	6.96 (s)	102.6	6.94 (s)	102.7		133.2
5-OCH3	4.10 (3H, s)	61.8											4.11 (3H, s)	61.9
6		152.5	7.24 (dd, 8.9, 2.2)	123.7	7.37 (dd, 9.2, 3.1)	125.8	7.38 (dd, 9.2, 2.9)	125.5		152.4		152.5		152.5
6-OH	6.51 (s)								6.37 (s)		6.39 (s)		6.47 (s)	
7		125.6		152.1		155.5		150.6		144.3		144.4		125.3
7-OH			9.04 (s)		10.63 (s)		9.04 (s)							
7-OCH3									4.10 (3H, s)	56.5	4.01 (3H, s)	56.5		
8	7.75 (s)	120.5	7.55 (d, 2.2)	109.1	7.55 (d, 3.1)	109.6	7.56 (d, 2.9)	109.1	7.58 (s)	104.4	7.58 (s)	104.4	7.76 (s)	120.6
8-OH														
8a		114.0		120.8		122.0		121.5		113.0		113.4		114.0
9		180.2		180.8		182.2		181.8		180.2		179.9		180.2
9a		103.1		103.3		110.9		103.7		103.4		104.5		102.7
10a		147.8		150.6		151.1		155.1		152.6		152.6		147.9
1′	6.72 (d, 10.0)	115.4	6.74 (d, 10.1)	115.8	6.69 (d, 9.8)	116.4	6.70 (d, 9.9)	115.7	6.74 (d, 9.9)	118.3	6.73 (d, 10.1)	115.5	3.19 (dd, 15.0, 2.2)	28.1
													2.92 (dd, 15.0, 8.1)	
2′	5.59 (d, 10.0)	127.4	5.61 (d, 10.1)	127.3	5.75 (d, 9.8)	129.0	5.78 (d, 9.9)	128.7	5.60 (d, 9.9)	127.3	5.59 (d, 10.1)	127.5	4.44 (dd, 8.1, 2.2)	77.5
3′		78.2		78.1		79.8		79.8		77.9		77.3		146.6
4′	1.48 (3H, s)	28.4	1.48 (3H, s)	28.4	1.52 (3H, s)	29.1	1.52 (3H, s)	28.4	1.48 (3H, s)	28.3	1.48 (3H, s)	28.4	5.00 (br), 4.88 (br)	110.5
5′	1.48 (3H, s)	28.4	1.48 (3H, s)	28.4	1.51 (3H, s)	29.1	1.52 (3H, s)	28.6	1.48 (3H, s)	28.3	1.48 (3H, s)	28.4	1.88 (3H, s)	18.6
1″	3.40 (2H, d, 7.3)	28.1	3.46 (2H, d, 7.3)	21.4	2.88[c]	25.7	5.08 (dd, 7.0, 7.3)	66.6	3.46 (2H, d, 7.3)	21.4			3.41 (2H, d, 7.3)	28.1
					3.09 (dd, 13.4, 7.0)									
2″	5.35 (m)	120.9	5.22 (m)	122.2	4.63 (t, 7.0)	89.4	3.68 (d, 7.3)	67.7	5.22 (m)	122.2			5.35 (m)	121.0
3″		133.9		131.4		146.2		58.2		131.4				133.8
4″	1.74 (3H, s)	17.8	1.88 (3H, s)	17.9	4.71 (br), 4.74 (br)	113.7	1.17 (3H, s)	19.8	1.88 (3H, s)	18.0			1.77 (3H, s)	25.8
5″	1.77 (3H, s)	25.8	1.68 (3H, s)	25.8	1.87 (3H, s)	17.8	1.19 (3H, s)	25.1	1.71 (3H, s)	25.8			1.74 (3H, s)	17.8
others					9.04 (br s, OOH)		4.23 (d, 7.0, 1″-OH)						2.68 (br s, 2′-OH)	

[a] Values in (δ_H and δ_C) ppm. 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 H_2O.

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

compound	EBV-EA-positive cells (% viability) compound concentration (mol ratio/32 pmol TPA) 1000	500	100	10	IC_{50}[b] (mol ratio/32 pmol TPA)
brasixanthone A (**1**)	0.0 ± 0.2 (70)	41.3 ± 1.2 (>80)	69.2 ± 0.5 (>80)	93.2 ± 0.2 (>80)	390
brasixanthone B (**4**)	0.0 ± 0.2 (70)	8.4 ± 1.6 (>80)	52.6 ± 1.1 (>80)	92.1 ± 0.5 (>80)	120
brasixanthone C (**5**)	0.0 ± 0.4 (70)	11.1 ± 1.2 (>80)	52.5 ± 1.4 (>80)	100.0 ± 0.3 (>80)	200
brasixanthone D (**6**)	0.0 ± 0.5 (70)	14.7 ± 1.3 (>80)	58.6 ± 1.3 (>80)	100.0 ± 0.4 (>80)	210
6-deoxyjacareubin (**8**)	12.6 ± 0.4 (70)	45.1 ± 1.3 (>80)	62.2 ± 1.3 (>80)	100.0 ± 0.4 (>80)	400
3,8-dihydroxy-1,2-dimethoxyxanthone (**9**)	15.7 ± 0.5 (70)	47.6 ± 1.4 (>80)	82.5 ± 1.2 (>80)	100.0 ± 0.4 (>80)	475
8-desoxygartanin (**11**)	0.0 ± 0.2 (70)	30.5 ± 1.1 (>80)	71.5 ± 1.7 (>80)	90.4 ± 0.3 (>80)	310
cudraxanthone F (**12**)	0.0 ± 0.5 (70)	39.7 ± 1.3 (>80)	80.6 ± 1.4 (>80)	97.7 ± 0.5 (>80)	420
4-hydroxyxanthone (**13**)	21.3 ± 0.4 (70)	52.8 ± 1.3 (>80)	87.4 ± 1.4 (>80)	100.0 ± 0.5 (>80)	525
1,2-dimethoxyxanthone (**14**)	20.6 ± 0.2 (70)	50.5 ± 1.2 (>80)	81.5 ± 1.3 (>80)	100.0 ± 0.3 (>80)	520
garcinone B (**16**)	9.4 ± 0.5 (70)	35.5 ± 1.2 (>80)	80.5 ± 1.3 (>80)	94.6 ± 0.4 (>80)	400
β-carotene[c]	9.1 ± 0.6 (60)	34.3 ± 1.1 (>80)	82.7 ± 1.8 (>80)	100.0 ± 0.2 (>80)	400

[a] Mole 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 viability % of Raji cells measured through Trypan Blue staining. At least 60% viability of Raji cells 2 days after treatment with the compounds is required for an accurate result. [b] IC_{50} represents mol ratio to TPA that inhibits 50% of positive control (100%) activated with 32 pmol TPA. [c] Positive control substance.

signals assignable to a methylene [δ_H 3.19 (H-1′), 2.92 (H-1′), δ_C 28.14(C-1′)] linked to a methine [δ_H 4.44 (H-2′), δ_C 77.5 (C-2′)] having a OH (δ_H 2.68), a vinylmethyl (δ_H 1.88), and an *exo*-methylene [δ_H 5.00 and 4.88 (H-4′)] group were observed. The HMBC correlations of C-2′ with H-4′, C-4′ with H-5′, and C-3′ with H-1′, together with an EIMS fragment at *m*/*z* 355 arising from the fission at the benzylic position, indicated the presence of the 2-hydroxy-3-methylbut-3-enyl side chain. The arrangement of substituents on the 1-hydroxyxanthone nucleus was clarified by HMBC and NOE data as follows: (1) correlations from C-2 to a hydrogen-bonded OH, H-2′, and H-4 indicated the location of the side chain at C-2; (2) correlations from C-3 to H-1′ and H-4 indicated the location of the OH at C-3; (3) correlations of C-6 with H-1″ on the prenyl group and the deshielded H-8 placed the location of the prenyl at C-7 and the OH at C-6; (4) correlation of C-5 with 6-OH and OCH_3 protons revealed the location of OCH_3 at C-5. From these spectral data, together with NOE and other HMBC data, the structure of brasixanthone G was determined as **10**.

Ten known xanthones, toxyloxanthone A (**7**),[32] 6-deoxyjacareubin (**8**),[33] 3,8-dihydroxy-1,2-dimethoxyxanthone (**9**),[34] 8-desoxygartanin (**11**),[35] cudraxanthone F (**12**),[36] 4-hydroxyxanthone (**13**),[37] 1,2-dimethoxyxanthone (**14**),[38] pyranojacareubin (**15**),[39] garcinone B (**16**),[40] and latisxanthone C (**17**),[28] were also isolated and were identified by comparison of their spectral data with that published in the literature. Further examination of the constituents of this plant are in progress.

Inhibitory Effects on EBV-EA Induction. Eleven natural xanthones (**1**, **4**−**6**, **8**, **9**, **11**−**14**, and **16**) 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 viabilities of Raji cells and the 50% inhibitory concentration (IC_{50}) values are shown in Table 2. All the test compounds showed inhibitory activity on the EBV-EA activation at more than 1×10^2 mol ratio/TPA (12.6−47.5%) and showed a strong inhibitory effect at high concentration (1×10^3 mol ratio/TPA) without causing a decrease in viability of the Raji cells. At 1×10^3 mol ratio/TPA, brasixanthones A (**1**), B (**4**), C (**5**), and D (**6**), 8-desoxygartanin (**11**), and cudraxanthone F (**12**), all having a five-carbon side chain (prenyl group or its analogue) in the xanthone molecule, showed 100% inhibition of activation. Among these compounds, **1**, **4**, **11**, and **12** were effective (9.6−2.3% inhibition) even at 1×10 mol ratio/TPA. The corresponding IC_{50} values of tested compounds were within the range 120−525 mol ratio/TPA. Among them, **4**, **5**, and **6**, having both a hydroxy group at the 7-position on the A ring and a hydrophobic C5 side chain on another aromatic ring in the xanthone skeleton, showed the most significant activities (IC_{50} 120−210). The 5-hydroxy analogue, **11** (IC_{50} 310), was also more potent than β-carotene (IC_{50} 400), a vitamin A precursor commonly used in cancer prevention studies.[41] In previous studies, we reported that the presence of a prenyl moiety in the xanthone molecule plays an important role in the inhibition on EBV-EA induction.[29] In view of the present findings, the relative location of a hydroxy group and a hydrophobic prenyl moiety on the xanthone nucleus might also be important factors for inhibiting the tumor-promoting effect of chemical-induced carcinogenesis. A study examining the tumor-promoting inhibitory activity of these compounds in vivo is now in progress.

Experimental Section

^1H 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 a JMS-700 (JEOL) spectrometer having a direct inlet system. UV spectra were recorded on a UVIDEC-610C double-beam spectrophotometer (JASCO) in MeOH; 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, *Calophyllum brasiliensis* Camb., were collected in the garden of the Federal University of Santa Catarina, Brazil, in March 1998. Plant materials were identified by Dr. Ademir Reis. A voucher specimen has been deposited at Barbosa Rodrigues Herbarium under number VC Filho 007.

Isolation of Brasixanthones A (1), B (4), C (5), D (6), E (2), F (3), and G (10) from *C. brasiliensis*. The dried stem bark (630 g) of *C. brasiliensis* was extracted with acetone at room temperature, and the solvent was evaporated under reduced pressure to give the acetone extract. The residue was further extracted with MeOH under reflux to give the MeOH extract. The acetone extract was subjected to Si gel column chromatography eluted with hexane−acetone (19:1, 97:3, 9:1, 17:3, 4:1, 3:1, 7:3, 2:1, 3:2, 1:1), acetone, CH_2Cl_2−MeOH (3:1), and MeOH, successively, to separate 13 fractions. Succes-

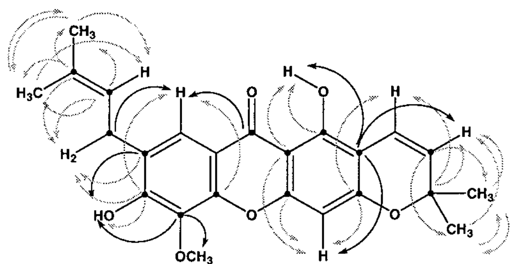


Figure 1. C−H long-range correlations in the HMBC spectrum of brasixanthone A (**1**). Bold line: more significant correlations in the structure determinations.

sive treatment of each fraction with Si gel column and preparative TLC, using appropriate combinations of solvents (hexane, EtOAc, $CHCl_3$, CH_2Cl_2, Et_2O, acetone, $^{i}Pr_2O$, benzene, and MeOH) as eluting or developing solvents, gave the following compounds. From fraction 4 (hexane−acetone, 17:3): **1** (9.2 mg), **4** (44.8 mg), **5** (8.3 mg), **6** (2.4 mg), **7** (46.2 mg), **17** (2.8 mg), **11** (9.9 mg), **15** (10.6 mg), **8** (7.5 mg), and **14** (2.1 mg). From fraction 5 (hexane−acetone, 4:1): **2** (2.0 mg), **3** (2.8 mg), **11** (6.1 mg), and **9** (8.8 mg). From fraction 6 (hexane−acetone, 3:1): **10** (2.8 mg), **16** (2.3 mg), **12** (28.0 mg), and **13** (3.6 mg).

Brasixanthone A (1): yellow oil; HRMS *m/z* 408.1563 (calcd for $C_{24}H_{24}O_6$ 408.1572); UV (MeOH) λ_{max} 224, 243, 278sh, 288, 330 nm; IR ($CHCl_3$) ν_{max} 3509, 3265br, 1650, 1605 cm^{-1}; HMBC, Figure 1; EIMS *m/z* 408 (M^+, 65), 393 ($M^+ - CH_3$, 100), 377 (73), 335 (20), 323 (11).

Brasixanthone B (4): yellow needles; mp 227−229 °C (from acetone); HRMS *m/z* 378.1454 (calcd for $C_{23}H_{22}O_5$ 378.1467); UV (MeOH) λ_{max} 234, 252sh, 294, 319sh, 344, 384 nm; IR ($CHCl_3$) ν_{max} 3588, 3312br, 1650, 1611 cm^{-1}; HMBC C−H three (or two)-bond correlations: C-1→(1-OH); C-2→1-OH, H-2′; C-3→H-1′, H-1″; C-4→(H-1″); C-4a→H-1″; C-6→H-8; C-7→H-5; C-8a→H-5; C-9a→1-OH; C-10a→H-6, H-8; C-2′→H-4′, H-5′; C-3′→(H-4′), (H-5′), H-1′, (H-2′); 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″; EIMS *m/z* 378 (M^+, 84), 363 (100), 335 (27), 323 (23), 307 (11), 295 (11), 279 (8), 265 (4).

Brasixanthone C (5): pale yellow oil; $[\alpha]_D$ ±0° (*c* 0.0565, MeOH); HR-FABMS *m/z* 411.1482 (calcd for $C_{23}H_{23}O_7$ 411.1444); UV (MeOH) λ_{max} 234, 252sh, 294, 320sh, 344, 382 nm; IR ($CHCl_3$) ν_{max} 3587, 3330br, 1650, 1612 cm^{-1}; HMBC C−H three (or two)-bond correlations: C-1→(1-OH); C-2→1-OH, H-2′; C-3→H-1′, H-1″; C-4→(H-1″); C-4a→H-1″; C-6→H-8; C-7→H-5; C-8→7-OH; C-8a→H-5; C-9→H-8; C-9a→1-OH; C-10a→H-6, H-8, (H-5); C-2′→H-4′, H-5′; C-3′→(H4′), (H-5′), H-1′, (H-2′); C-4′→H-5′; C-5′→H-4′; C-1″→(H-2″); C-2″→H-4″, H-5″, (H-1″); C-3″→H-1″, (H-5″); C-5″→H-2″, H-4″; C-4″→H-5″; EIMS *m/z* 394 ($M^+ - O$, 11), 368 (4), 323 (100), 309 (10).

Photooxygenation of Brasixanthone B (4). Oxygen gas was bubbled through a solution of brasixanthone B (**4**) (8.65 mg, 0.023 mmol) in pyridine (2 mL) containing hematoporphyrin (5.0 mg, 0.008 mmol), and the solution was irradiated with a high-pressure Hg lamp using a Pyrex glass filter for 40 min and evaporated to dryness. The residue was subjected to Si gel P-TLC [hexane−acetone (4:1), $CHCl_3$−MeOH (96:4)] to afford **5′** (2.0 mg, yield 21%). This product was found to be identical with natural brasixanthone C (**5**) by IR and ^1H NMR comparisons and co-TLC (hexane−acetone, 2:1, R_f 0.35).

Brasixanthone D (6): pale yellow oil; $[\alpha]_D$ ±0° (*c* 0.0575, MeOH); HRMS *m/z* 410.1364 (calcd for $C_{23}H_{22}O_7$ 410.1365); UV (MeOH) λ_{max} 233, 250, 289, 317sh, 339, 385 nm; IR ($CHCl_3$) ν_{max} 3568, 3330br, 1649, 1611 cm^{-1}; HMBC C−H three (or two)-bond correlations: C-1→(1-OH); C-2→1-OH, H-2′; C-3→H-1′, H-1″; C-4→(H-1″); C-4a→H-1″; C-6→H-8; C-7→H-5, (H-6), (H-8); C-8→H-6; C-8a→H-5; C-9→H-8; C-9a→1-OH; C-10a→H-8; C-2′→H-4′, H-5′; C-3′→(H-4′), (H-5′), H-1′, (H-2′); C-4′→H-5′; C-5′→H-4′; C-1″→(H-2″), (1″-OH); C-2″→H-4″,H-5″, (H-1″); C-3″→(H-4″), (H-5″); C-4″→H-5″; C-5″→H-2″, H-4″; EIMS *m/z* 410 (M^+, 23), 395 (23), 377 (7), 339 (100), 323 (29), 285 (11).

Brasixanthone E (2): yellow oil; HRMS *m/z* 408.1559 (calcd for $C_{24}H_{24}O_6$ 408.1573); UV (MeOH) λ_{max} 240, 290, 336, 371 nm; IR ($CHCl_3$) ν_{max} 3525, 3228br, 1649, 1608 cm^{-1}; differential NOE, irradiation of $O-CH_3$ (δ 4.10) gave 19% NOE at H-8 (δ 7.58); HMBC C−H three (or two)-bond correlations: C-1→(1-OH); C-2→1-OH, H-2′; C-3→H-1′, H-1″; C-4→(H-1″); C-4a→H-1″; C-5→6-OH; C-6→H-8, (H-5), (6-OH); C-7→H-5, 6-OH, 7-OCH_3, (H-8); C-8a→H-5; C-9→H-8; C-9a→1-OH; C-10a→H-8, (H-5); C-2′→H-4′, H-5′; C-3′→(H-4′), (H-5′), H-1′, (H-2′); C-4′→H-5′; C-5′→H-4′; C-2″→H-4″, H-5″, (H-1″); C-3″→(H-4″), (H-5″), H-1″; C-4″→H-5″; C-5″→H-4″; EIMS *m/z* 408 (M^+, 43), 393 (100), 365 (17), 353 (10), 322 (22), 307 (13), 279 (9).

Brasixanthone F (3): yellow oil; HRMS *m/z* 340.0916 (calcd for $C_{19}H_{16}O_6$ 340.0947); UV (MeOH) λ_{max} 219, 279sh, 289, 335, 371 nm; IR ($CHCl_3$) ν_{max} 3517, 3237br, 1652, 1609 cm^{-1}; differential NOE, irradiation of $O-CH_3$ (δ 4.01) gave 8% NOE at H-8 (δ 7.58); HMBC C−H three (or two)-bond correlations: C-1→(1-OH); C-2→1-OH, H-2′, H-4; C-3→H-1′, (H-4); C-4a→(H-4); C-5→6-OH; C-6→H-8, (H-5), (6-OH); C-7→H-5, 6-OH, 7-OCH_3, (H-8); C-8a→H-5; C-9→H-8; C-9a→1-OH; C-10a→H-8, (H-5); C-2′→H-4′, H-5′; C-3′→(H-4′), (H-5′); C-4′→H-5′; C-5′→H-4′; EIMS *m/z* 340 (M^+, 80), 325 (100), 310 (43), 282 (12).

Brasixanthone G (10): pale yellow oil; $[\alpha]_D$ ±0° (*c* 0.0765, MeOH); HRMS *m/z* 426.1671 (calcd for $C_{24}H_{26}O_7$ 426.1679); UV (MeOH) λ_{max} 212, 247, 260sh, 322, 356sh nm; IR ($CHCl_3$) ν_{max} 3510, 3200br, 1645, 1612 cm^{-1}; differential NOE, irradiation of $O-CH_3$ (δ 4.11) gave 2% NOE at H-4 (δ 6.51); irradiation of H-8 (δ 7.76) gave 4% NOE at H-1″ (δ 3.41); irradiation of H-1″ (δ 3.41) gave 9% NOE at H-8 (δ 7.76); HMBC C−H three (or two)-bond correlations: C-1→(1-OH), H-1′; C-2→1-OH, H-2′, H-4, (H-1′); C-3→H-1′, (H-4); C-4a→(H-4); C-5→6-OH, 5-OCH_3; C-6→H-8, (6-OH), H-1″; C-7→6-OH, (H-1″); C-9→H-8; C-9a→1-OH, H-4; C-10a→H-8; C-2′→-(H-1′), H-4′, H-5′; C-3′→(H-4′), (H-5′), (H-2′), H-1′; C-4′→H-5′; C-2″→H-4″, H-5″, (H-1″); C-3″→(H-4″), (H-5″), H-1″; EIMS *m/z* 426 (M^+, 17), 408 (5), 355 (100), 339 (7), 323 (5), 299 (13).

In Vitro EBV-EA Activation Experiments. The inhibition of EBV-EA activation was assayed using the same method described previously.[21,29] In brief, Raji cells were grown to a density of 10^6 cells/mL, harvested by centrifugation, and resuspended in RPMI 1640 medium (Nakalai Tesque, Kyoto, Japan) 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). Untreated cultures served as the controls. 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 observed in the case of a control without the test product. In each assay, at least 500 cells were counted and the results were read blind.

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