

## Evaluation of the Antifungal Activity of Natural Xanthenes from *Garcinia mangostana* and Their Synthetic Derivatives

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The antiungal activity of several xanthenes isolated from the fruit hulls of *Garcinia mangostana* and some derivatives of mangostin against three phytopathogenic fungi, *Fusarium oxysporum vasinfectum*, *Alternaria tenuis*, and *Dreschlera oryzae*, has been evaluated. The natural xanthenes showed good inhibitory activity against the three fungi. Substitution in the A and C rings has been shown to modify the bioactivities of the compounds.

Most of the synthetic fungicides in use today were discovered by empirical syntheses, and this approach has indeed produced compounds of high biological activity. The chances of obtaining newer fungicides that meet stringent environmental and food-safety requirements through empirical synthesis do not appear to be high. Natural products of plant origin offer a wide variety of bioactive compounds that could meet the aforesaid requirements. Constitutive antifungal substances are normally present in concentrations high enough to inhibit most fungi. An extensive survey of diverse classes of antifungal compounds from higher plants has been described in detail by Grayer and Harborne.<sup>1</sup>

In our effort to screen constitutive antifungal substances in plants, we evaluated the natural xanthenes from the fruit hulls of *Garcinia mangostana* L. (Guttiferae) and their synthetic analogues. The xanthenes isolated from *G. mangostana* have been reviewed.<sup>2,3</sup> The fruit hulls of this species find application in native medicine as an antiinflammatory and anti-diarrheal.<sup>4</sup> Mangostin 3,6-di-*O*-glucoside, obtained from *G. mangostana*, has been shown to have interesting pharmacological activity;<sup>5</sup> however, there is no information on the antifungal activities of xanthenes from *G. mangostana*. The only report on antifungal activity of xanthenes relates to 1,7-dihydroxy-4-methoxyxanthone and 1,7-dihydroxy-3,5,6-trimethoxyxanthone, isolated from the roots of *Polygala nyikensis* (Polygalaceae), inhibiting the plant pathogenic fungus *Cladosporium cucumerinum*.<sup>6</sup> Herein we report the isolation of xanthenes from *G. mangostana* and their synthetic modifications and the evaluation of their antifungal activities against three phytopathogenic fungi, *Fusarium oxysporum vasinfectum*, *Alternaria tenuis*, and *Dreschlera oryzae*.

The fruit hulls of *G. mangostana* were powdered and extracted with *n*-hexane and Me<sub>2</sub>CO successively. The residues obtained from both the extracts were subjected to extensive chromatography to afford various xanthenes. Gartanin<sup>7,8</sup> (3), 8-desoxygartanin,<sup>7,8</sup> BR-xanthone<sup>9</sup> (2), and mangostin<sup>7,8</sup> (1) were obtained from the *n*-hexane extract by column chromatography over Si gel (60–120 mesh) and elution with *n*-hexane and combinations of *n*-hexane and CHCl<sub>3</sub> in order of increasing polarity. The major quantity of mangostin, however, was isolated, along with β-mangostin<sup>10,11</sup> (4), gartanin<sup>7,8</sup> (3), γ-mangostin<sup>8,10</sup> (5), and garcinone-D<sup>12</sup> (6), from the Me<sub>2</sub>CO extract by simple VLC over Si gel and elution

with *n*-hexane–CHCl<sub>3</sub> (90:10). The structures of the various xanthenes isolated were confirmed by comparison of their spectral data (IR, NMR, UV, MS) and melting points with those already reported in the literature.

Several di-*O*-alkyl derivatives of mangostin were prepared by standard methods. Table 1 gives the various alkylating agents used and the yields of di-*O*-alkylmangostins obtained. As reported earlier,<sup>13</sup> the 1-OH in mangostin is sterically hindered and could not be alkylated. Under the conditions employed, only the di-*O*-alkylmangostins were obtained. Table 2 gives the spectral data of the compounds 10–16.

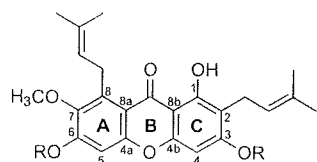
Di-*O*-acetylmangostin<sup>10</sup> (17) was obtained by acetylation of mangostin with Ac<sub>2</sub>O and pyridine. Mangostin was subjected to acid-catalyzed cyclization using *p*-toluenesulfonic acid to yield 3-isomangostin<sup>14</sup> (18). The reaction of xanthone (7) with Lawesson's reagent gave xanthione (8) in 90% yield.

The natural xanthenes 1, 2, 3, 5, and 6 from *G. mangostana*, xanthone (7), euxanthone (9), xanthione (8), and the mangostin derivatives 10–18 were tested for their antifungal activity against three phytopathogenic fungi, *F. oxysporum vasinfectum*, *A. tenuis*, and *D. oryzae*. These three fungi were selected because they are phytopathogens of agricultural importance. The results are presented in Tables 3–5.

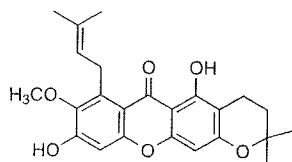
*D. oryzae* was found to be the most sensitive species, followed by *A. tenuis* and *F. oxysporum vasinfectum* in the Petri-plate bioassay, when tested against both the natural xanthenes and their derivatives. The natural xanthenes as a group were found to be more inhibitory as compared to their derivatives.

Antifungal activity profiles of xanthone (7), xanthione (8), and euxanthone (9) and correlations with their structures suggest that the hydroxyls in rings A and C are important for antifungal activity. This observation is supported by the fact that alkylating the C-3 and C-6 hydroxyls in mangostin (1) reduces the antifungal activity considerably (by about half for methyl substitution), and replacement with alkyl groups of increasing chain length correlates with decreasing inhibitory activity. In the case of *D. oryzae*, alkylation of C-3 and C-6 hydroxyls with ethyl, propyl, and butyl groups resulted in growth more than that of the control; whereas alkylation of C-3 and C-6 hydroxyls with isopropyl, allyl, methallyl, and acetyl groups reduced the inhibitory activity drastically. Gartanin (3) with C-5 and C-8 hydroxyls also has good inhibitory activity, again sug-

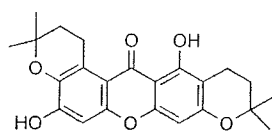
<sup>®</sup> Abstract published in *Advance ACS Abstracts*, May 1, 1997.



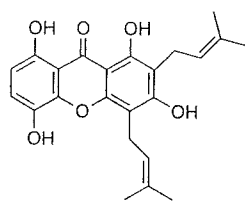
compd no.	R	compd
1	H	mangostin
10	CH <sub>3</sub>	di- <i>O</i> -methylmangostin
11	C <sub>2</sub> H <sub>5</sub>	di- <i>O</i> -ethylmangostin
12	C <sub>3</sub> H <sub>7</sub>	di- <i>O</i> -propylmangostin
13	C <sub>4</sub> H <sub>9</sub>	di- <i>O</i> -butylmangostin
14	CH(CH <sub>3</sub> ) <sub>2</sub>	di- <i>O</i> -isopropylmangostin
15	CH <sub>2</sub> CH=CH <sub>2</sub>	di- <i>O</i> -allylmangostin
16	CH <sub>2</sub> C(CH <sub>3</sub> )=CH <sub>2</sub>	di- <i>O</i> -methallylmangostin
17	COCH <sub>3</sub>	di- <i>O</i> -acetylmangostin



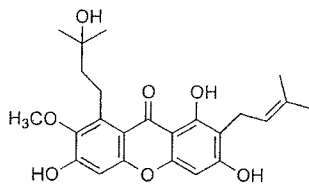
3-isomangostin, 18



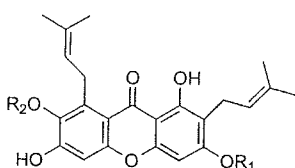
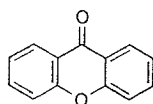
BR-xanthone A, 2



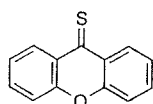
gartanin, 3



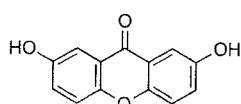
garcinone D, 6

β-mangostin, R<sub>1</sub> = R<sub>2</sub> = CH<sub>3</sub>, 4  
γ-mangostin, R<sub>1</sub> = R<sub>2</sub> = H, 5

xanthone, 7



xanthone, 8



euxanthone, 9

gesting that free hydroxyls are important for optimal antifungal activity. The conversion of >C=O (7) to >C=S (8) did not improve the inhibitory activity.

Among the natural xanthenes, γ-mangostin (5) was found to be the most effective where the C-7 has a -OH instead of -OMe, as in mangostin. A structure-activity comparison of γ-mangostin (5) with garcinone-D (6) and mangostin (1) reveals that modifications of the isoprenyl group in C-8 and the functional group at C-7 alter the activity. Cyclization of the isoprenyl groups completely reduces the activity against all three fungi as has been seen in BR-xanthone (2).

Among the compounds tested, γ-mangostin (5) was the most active against all the test fungi at 1000 ppm.

## Experimental Section

**General Experimental Procedures.** Melting points were determined on a Toshniwal melting point apparatus (Toshniwal Pvt. Ltd, India) and are uncorrected.

**Table 1.** Alkylation of Mangostin

alkylating agent	product	isolated yield(%)	mp (°C)
methyl iodide	10	79	120–122 <sup>a</sup>
diethylsulfate	11	82	112–114
<i>n</i> -propyl bromide	12	75	92–94
<i>n</i> -butyl iodide	13	78	76–78
isopropyl bromide	14	72	102–104
allyl bromide	15	75	78–80
methallyl chloride	16	78	102–104

<sup>a</sup> Lit.<sup>13</sup> mp 121–122 °C.

NMR spectra were recorded on a 60 MHz Hitachi, 90 MHz JEOL, and 400 MHz JEOL NMR instruments using TMS as internal standard and CCl<sub>4</sub>, CDCl<sub>3</sub>, DMSO-*d*<sub>6</sub>, and Me<sub>2</sub>CO-*d*<sub>6</sub> as solvents. EIMS were recorded on a Shimadzu QP1000A instrument. Radial chromatography was performed using a Chromatotron (model 7924, Harrison Research,) on a Si gel plate of 2-mm thickness. Column chromatography was performed using Si gel (60–120 mesh, 230–400 mesh, and 400–600 mesh). Precoated plates (E. Merck, Germany, Art. 5554 Kieselgel 60 F<sub>254</sub>, 0.2-mm thickness) were used for TLC and developed in *n*-hexane-CHCl<sub>3</sub> or CHCl<sub>3</sub>-MeOH and were visualized under UV light (λ 254 and 365 nm) or by spraying with 10% H<sub>2</sub>SO<sub>4</sub> in EtOH. For the preparation of the Czapek-Dox medium, reagent grade chemicals were used. Samples of xanthone (7) and euxanthone (9) were kindly provided by Prof. T. R. Govindachari, Centre for Agrochemical Research, SPIC Science Foundation, Madras, Tamil Nadu, India.

**Extraction and Isolation.** The fruit hulls of *G. mangostana* were collected in March 1993, from Madras, Tamil Nadu, India. The powdered hulls (1.8 kg) were placed in a Soxhlet apparatus and extracted with *n*-hexane and Me<sub>2</sub>CO successively. Evaporation of solvents yielded 25.8 g and 140.4 g of residues from *n*-hexane and Me<sub>2</sub>CO extracts, respectively.

The residue from the *n*-hexane extract (25.8 g) was subjected to a column chromatography (gravity) over Si gel (60–120 mesh, 160 g) to yield three fractions, A, B, and C, on successive elution with 40:60 *n*-hexane-CHCl<sub>3</sub>, 20:80 *n*-hexane-CHCl<sub>3</sub>, and pure CHCl<sub>3</sub>, respectively. Fraction A (7.2 g) was rechromatographed (gravity) over a Si gel column (60–120 mesh, 120 g) with varying proportions of *n*-hexane and CHCl<sub>3</sub> as eluents. Subfractions 1, 2, and 3 were obtained on elution with 3:2, 2:3, and 1:4 *n*-hexane-CHCl<sub>3</sub>, respectively. Repeated column chromatography (gravity) of subfraction 1 (1.5 g) (Si gel, 230–400 mesh, 60 g) afforded 8-desoxygartanin<sup>7,8</sup> (50 mg, mp 155–156 °C) and BR-xanthone<sup>9</sup> (2) (191 mg, mp 180–182 °C) on elution with 1:1 C<sub>6</sub>H<sub>6</sub>-CHCl<sub>3</sub>. Subfraction 2 yielded gartanin<sup>7,8</sup> (3) (370 mg, mp 164–165 °C), and subfraction 3 yielded mangostin<sup>7,8</sup> (1) (2.1 g, mp 184–186 °C). Fractions B (4.8 g) and C (7.6 g) were pooled together (TLC guided) and crystallized from C<sub>6</sub>H<sub>6</sub> to afford mangostin (1) (11 g, mp 181–182 °C).

The residue from the Me<sub>2</sub>CO extract (140.4 g) was subjected to VLC (TLC Si gel, 400–600 mesh, 500 g) using *n*-hexane, *n*-hexane-CHCl<sub>3</sub>, CHCl<sub>3</sub>, CHCl<sub>3</sub>-MeOH, and MeOH (solvents of increasing polarity) as eluents, to give four major fractions, A (13.3 g), B (65 g), C (5.6 g), and D (4.6 g). Fraction A (13.3 g), on elution with solvents up to 60% CHCl<sub>3</sub> in *n*-hexane when subjected to column chromatography (Si gel, 70–

Table 2. <sup>1</sup>H-NMR, Mass Spectral Data of Compounds 10–16

compound	<sup>1</sup> H NMR (CCl <sub>4</sub> ) δ (ppm)	molecular formula	[M] <sup>+</sup> obs. mass (calcd)
10	13.23 (1H, s, -OH), 6.38 (1H, s, Ar-H), 5.95 (1H, s, Ar-H), 5.13 (2H, t, -CH=), 4.05 (2H, d, ArCH <sub>2</sub> ), 3.84 (6H, s, -OMe), 3.60 (3H, s, -OMe), 3.10 (2H, d, ArCH <sub>2</sub> ), 1.8 (3H, s, Me), 1.73 (3H, s, Me), 1.6 (6H, s, Me's)	C <sub>26</sub> H <sub>30</sub> O <sub>6</sub>	438 (438)
11	13.25 (1H, s, -OH), 6.35 (1H, s, Ar-H), 5.95 (1H, s, Ar-H), 5.09 (2H, t, -CH=), 4.03 (6H m, ArCH <sub>2</sub> and OCH <sub>2</sub> ), 3.72 (3H, s, OMe), 3.18 (2H, d, ArCH <sub>2</sub> ), 1.8 (6H, s, Me's), 1.6 (6H, s, Me's), 1.45 (6H, t, Me's)	C <sub>28</sub> H <sub>34</sub> O <sub>6</sub>	466 (466)
12	13.35 (1H, s, -OH), 6.57 (1H, s, Ar-H), 6.07 (1H, s, Ar-H), 5.09 (2H, t, -CH=), 4.00 (6H, m, ArCH <sub>2</sub> and OCH <sub>2</sub> ), 3.79 (3H, s, -OMe), 3.21 (2H, d, ArCH <sub>2</sub> ), 1.84 (6H, s, Me's), 1.81 (4H, m, CH <sub>2</sub> 's), 1.6 (6H, s, Me's), 1.1 (6H, t, Me's)	C <sub>30</sub> H <sub>38</sub> O <sub>6</sub>	494 (494)
13	13.31 (1H, s, -OH), 6.49 (1H, s, Ar-H), 6.14 (1H, s, Ar-H), 5.13 (2H, t, -CH=), 4.03 (6H, m, ArCH <sub>2</sub> and OCH <sub>2</sub> ), 3.72 (3H, s, -OMe), 3.10 (2H, d, ArCH <sub>2</sub> ), 1.73–1.65 (20H, m), 0.98 (6H, t, Me's)	C <sub>32</sub> H <sub>42</sub> O <sub>6</sub>	522 (522)
14	13.35 (1H, s, -OH), 6.49 (1H, s, Ar-H), 6.07 (1H, s, Ar-H), 5.13 (2H, t, -CH=), 4.50 (2H, m, -OCH), 4.0 (2H, d, ArCH <sub>2</sub> ), 3.64 (3H, s, -OMe), 3.13 (2H, d, ArCH <sub>2</sub> ), 1.76 (6H, s, Me's), 1.56 (6H, s, Me's), 1.29 (12H, m, Me's)	C <sub>30</sub> H <sub>38</sub> O <sub>6</sub>	494 (494)
15	13.35 (1H, s, -OH), 6.46 (1H, s, Ar-H), 5.95 (1H, s, Ar-H), 5.56 (4H, d, =CH <sub>2</sub> ), 5.17 (4H, m, -CH=), 4.54 (4H, d, -OCH <sub>2</sub> ), 4.03 (2H, d, ArCH <sub>2</sub> ), 3.72 (3H, s, -OMe), 3.21 (2H, d, ArCH <sub>2</sub> ), 1.8 (3H, s, Me), 1.73 (3H, s, Me), 1.6 (6H, s, Me's)	C <sub>30</sub> H <sub>34</sub> O <sub>6</sub>	490 (490)
16	13.35 (1H, s, -OH), 6.46 (1H, s, Ar-H), 6.11 (1H, s, Ar-H), 5.09 (6H, m, =CH <sub>2</sub> and -CH=), 4.50 (4H, s, -OCH <sub>2</sub> ), 4.03 (2H, d, ArCH <sub>2</sub> ), 3.93 (3H, s, -OMe), 3.24 (2H, d, ArCH <sub>2</sub> ), 1.86 (6H, s, Me's), 1.76 (6H, s, Me's), 1.65 (6H, s, Me's)	C <sub>32</sub> H <sub>38</sub> O <sub>6</sub>	518 (518)

Table 3. Percentage Inhibition of *Alternaria tenuis* on CDA Medium Incorporated with Xanthenes (at 168 h)<sup>a</sup>

compound	concentration (ppm)				
	control	1	10	100	1000
1		17	38	39	41
	(67.3 ± 1.2)	(56.0 ± 1.2)	(41.7 ± .2)	(41.0 ± 1.2)	(39.7 ± 1.2)
2		14	18	15	14
	(62.7 ± 2.2)	(53.7 ± 2.2)	(51.7 ± 2.2)	(53.3 ± 2.2)	(54.0 ± 2.2)
3		4	20	22	29
	(62.7 ± 1.7)	(60.3 ± 1.7)	(50.3 ± 1.7)	(45.3 ± 1.7)	(44.3 ± 1.7)
5		1	9	33	58
	(47.0 ± 1.2)	(46.7 ± 1.2)	(42.7 ± 1.2)	(31.3 ± 1.2)	(19.7 ± 1.2)
6		-7	2	-11	26
	(45.0 ± 2.3)	(48.0 ± 2.3)	(44.0 ± 2.3)	(50.0 ± 2.3)	(33.3 ± 2.3)
7		-43	5	-6	-14
	(45.0 ± 6.1)	(64.3 ± 6.1)	(43.0 ± 6.1)	(47.7 ± 6.1)	(51.3 ± 6.1)
8		-6	-12	-22	-32
	(45.0 ± 5.9)	(47.7 ± 5.9)	(50.7 ± 5.9)	(54.7 ± 5.9)	(59.3 ± 5.9)
9		5	16	22	27
	(45.0 ± 1.6)	(42.7 ± 1.6)	(37.7 ± 1.6)	(35.0 ± 1.6)	(32.7 ± 1.6)
10		14	22	16	16
	(62.7 ± 2.0)	(53.7 ± 2.0)	(48.7 ± 2.0)	(52.7 ± 2.0)	(52.7 ± 2.0)
11		-4	4	-11	-1
	(55.3 ± 3.4)	(57.3 ± 3.4)	(53.3 ± 3.4)	(61.3 ± 3.4)	(56.0 ± 3.4)
12		-6	-3	-2	1
	(55.3 ± 1.2)	(58.7 ± 1.2)	(57.3 ± 1.2)	(56.3 ± 1.2)	(54.7 ± 1.2)
13		1	4	-2	-5
	(55.3 ± 0.6)	(54.7 ± 0.6)	(53.0 ± 0.6)	(56.3 ± 0.6)	(58.0 ± 0.6)
14		-10	2	-1	1
	(47.0 ± 1.6)	(51.7 ± 1.6)	(46.3 ± 1.6)	(47.3 ± 1.6)	(46.7 ± 1.6)
15		-22	-13	8	42
	(45.0 ± 5.5)	(54.7 ± 5.5)	(50.7 ± 5.5)	(48.7 ± 5.5)	(63.7 ± 5.5)
16		-8	-2	4	-1
	(47.0 ± 1.6)	(50.7 ± 1.6)	(48.0 ± 1.6)	(45.0 ± 1.6)	(47.3 ± 1.6)
17		8	6	6	-6
	(47.0 ± 1.6)	(43.3 ± 1.6)	(44.0 ± 1.6)	(45.3 ± 1.6)	(50.0 ± 1.6)
18		11	15	28	31
	(50.0 ± 2.6)	(44.7 ± 2.6)	(42.7 ± 2.6)	(36.0 ± 2.6)	(34.3 ± 2.6)

<sup>a</sup> Values in parentheses give the radial growth (mm) ± S.E. 0.05.

325 mesh, 150 g), yielded β-mangostin<sup>10,11</sup> (**4**) (20 mg, mp 178–179 °C) and gartanin (**3**) (33 mg, mp 164–166 °C), successively, on elution with 1:1 CHCl<sub>3</sub>-*n*-hexane. Elution with pure CHCl<sub>3</sub> yielded mangostin (**1**) (5.6 g, mp 181–182 °C).

Major quantities of mangostin (**1**) (56 g, mp 181–184 °C) were obtained from fraction B (65 g) on elution with 1:4 *n*-hexane-CHCl<sub>3</sub> and pure CHCl<sub>3</sub>.

Fraction C (5.6 g), on elution with 2% MeOH in CHCl<sub>3</sub>, when subjected to column chromatography

**Table 4.** Percentage Inhibition of *Dreschlera oryzae* on CDA Medium Incorporated with Xanthenes (at 168 h)<sup>a</sup>

compd	concentration (ppm)				
	control	1	10	100	1000
1		55	51	54	53
	(50.0 ± 3.9)	(22.3 ± 3.9)	(24.7 ± 3.9)	(23.0 ± 3.9)	(23.3 ± 3.9)
2		27	28	31	36
	(75.7 ± 1.9)	(55.3 ± 1.9)	(54.7 ± 1.9)	(52.3 ± 1.9)	(48.7 ± 1.9)
3		18	54	75	71
	(75.7 ± 2.9)	(62.3 ± 2.9)	(35.0 ± 2.9)	(18.7 ± 2.9)	(21.7 ± 2.9)
5		5	35	81	85
	(87.0 ± 1.7)	(83.0 ± 1.7)	(46.0 ± 1.7)	(37.7 ± 1.7)	(23.7 ± 1.7)
6		27	30	43	64
	(65.7 ± 3.0)	(83.7 ± 3.0)	(46.0 ± 3.0)	(37.7 ± 3.0)	(23.7 ± 3.0)
7		19	29	34	3
	(65.7 ± 3.0)	(53.3 ± 3.0)	(46.7 ± 3.0)	(43.7 ± 3.0)	(64.0 ± 3.0)
8		11	5	-11	10
	(65.7 ± 4.1)	(73.0 ± 4.1)	(62.3 ± 4.1)	(73.3 ± 4.1)	(59.0 ± 4.1)
9		16	61	59	73
	(65.7 ± 1.7)	(55.3 ± 1.7)	(25.7 ± 1.7)	(26.7 ± 1.7)	(17.7 ± 1.7)
10		7	9	27	41
	(75.7 ± 2.6)	(70.3 ± 2.6)	(68.7 ± 2.6)	(55.3 ± 2.6)	(45.0 ± 2.6)
11		-72	-34	-31	-57
	(47.7 ± 3.9)	(82.0 ± 3.9)	(64.0 ± 3.9)	(62.3 ± 3.9)	(75.0 ± 3.9)
12		8	-25	-36	-10
	(47.7 ± 8.2)	(44.0 ± 8.2)	(59.7 ± 8.2)	(64.7 ± 8.2)	(52.7 ± 8.2)
13		-38	-38	-66	-69
	(47.7 ± 8.4)	(65.7 ± 8.4)	(66.0 ± 8.4)	(79.0 ± 8.4)	(80.7 ± 8.4)
14		-1	4	1	9
	(87.0 ± 1.8)	(88.0 ± 1.8)	(83.3 ± 1.8)	(86.3 ± 1.8)	(79.7 ± 1.8)
15		-19	-18	-4	-34
	(65.7 ± 4.4)	(78.0 ± 4.4)	(77.7 ± 4.4)	(68.0 ± 4.4)	(88.0 ± 4.4)
16		-1	-1	-1	17
	(87.0 ± 0.5)	(88.0 ± 0.5)	(88.0 ± 0.5)	(88.0 ± 0.5)	(72.0 ± 0.5)
17		11	51	11	14
	(87.0 ± 1.4)	(77.7 ± 1.4)	(42.3 ± 1.4)	(77.3 ± 1.4)	(75.3 ± 1.4)
18		29	45	58	61
	(73.3 ± 5.3)	(52.3 ± 5.3)	(40.3 ± 5.3)	(31.0 ± 5.3)	(28.7 ± 5.3)

<sup>a</sup> Values in parentheses give the radical growth (mm) ± S.E. 0.05.

(gravity, Si gel, 70–320 mesh, 100 g) with CHCl<sub>3</sub> as eluent, afforded  $\gamma$ -mangostin<sup>8,10</sup> (**5**) (668 mg, mp 203–205 °C) and mangostin (3.8 g, mp 181–182 °C).

Fraction D (4.6 g), on elution with 5% MeOH in CHCl<sub>3</sub>, was subjected to column chromatography (gravity, Si gel, 60–120 mesh, 100 g) to afford subfractions 1 and 2 on elution with 1:4 *n*-hexane–CHCl<sub>3</sub>. Subfraction 1 was identified as  $\gamma$ -mangostin (**5**) (200 mg, mp 203–205 °C), while garcinone D<sup>12</sup> (**6**) (355 mg, mp 223–225 °C) was obtained by a repeated chromatography of subfraction 2 (1.5 g) on a Chromatotron using 6% MeOH in CHCl<sub>3</sub> as eluent.

**Di-*O*-ethylmangostin (11).** Diethylsulfate (0.46 g, 3 mmol) was added dropwise over a period of 10 min to a stirred solution of mangostin (**1**) (0.41 g, 1 mmol) in 50% KOH solution (0.2 g, 28 mmol) and absolute EtOH (10 mL). Stirring continued overnight. Then the reaction mixture was poured onto ice pieces (10 g) and extracted with CHCl<sub>3</sub> (3 × 50 mL). The organic layer was washed with H<sub>2</sub>O (2 × 50 mL) and dried (anhydrous Na<sub>2</sub>SO<sub>4</sub>). Removal of the solvent yielded di-*O*-ethylmangostin (**11**) as a pale yellow solid that was purified by crystallization (*n*-hexane, mp 112–114 °C, 0.35 g, 81%).

**General Procedure for the *O*-Alkylation of Mangostin Using Alkyl Halide and K<sub>2</sub>CO<sub>3</sub>–Me<sub>2</sub>CO **10**, **12**–**16**.** MeI (0.42 g, 3 mmol) was added to a solution of mangostin (**1**) (0.41 g, 1 mmol) in Me<sub>2</sub>CO (10 mL) and K<sub>2</sub>CO<sub>3</sub> (0.14 g, 10.9 mmol) and was refluxed over a waterbath for 6 h. The solvent was removed, and the residue obtained was poured onto ice pieces (10 g) and extracted with CHCl<sub>3</sub> (3 × 50 mL). The organic layer

was washed with H<sub>2</sub>O (2 × 50 mL) and dried. Removal of the solvent yielded **10** as pale yellow needles after crystallization (*n*-hexane, mp 120–122 °C, 0.35 g, 78%). Compounds **12**–**16** were obtained using appropriate alkyl halides (Table 1). For details of <sup>1</sup>H-NMR and mass spectral data, see Table 2.

**Di-*O*-acetylmangostin (17).** A solution of mangostin (**1**) (0.41 g, 1 mmol) in Ac<sub>2</sub>O (1 g, 10 mmol) and pyridine (1 g, 10 mmol) was refluxed over a waterbath for 1 h. The reaction mixture was poured onto ice pieces (10 g) and extracted with CHCl<sub>3</sub> (3 × 50 mL). The organic layer was washed with H<sub>2</sub>O (2 × 50 mL) and dried. Removal of the solvent yielded **17** as a yellow solid after crystallization using *n*-hexane: mp 113–114 °C (lit.<sup>10</sup> mp 117 °C) (0.38 g, 76%); <sup>1</sup>H NMR (TMS, 60 MHz)  $\delta$  13.62 (1H, s, –OH), 7.13 (1H, s, Ar-H), 6.93 (1H, s, Ar-H), 5.01 (2H, t, –CH=), 3.95 (2H, d, ArCH<sub>2</sub>), 3.64 (3H, s, –OMe), 3.3 (ArCH<sub>2</sub>, d, 2H), 2.39 (3H, s, –OCOMe), 2.28 (3H, s, –OCOMe), 1.68 (6H, s, Me's), 1.81 (6H, s, Me's).

**3-Isomangostin (18).** A solution of mangostin (**1**) (1 g, 2.44 mmol) and *p*-toluenesulfonic acid (0.1 g, 0.58 mmol) in dry C<sub>6</sub>H<sub>6</sub> (70 mL) was refluxed over a H<sub>2</sub>O bath with a Dean-Stark apparatus. After the removal of H<sub>2</sub>O (4.4 mL) for 1 h, C<sub>6</sub>H<sub>6</sub> was removed, and the residue was extracted with CHCl<sub>3</sub> (3 × 100 mL). The organic layer was washed with H<sub>2</sub>O (2 × 100 mL) and dried. The residue on purification by column chromatography (gravity, Si gel, 60–120 mesh, 3:2 CHCl<sub>3</sub>–*n*-hexane) yielded **18** (1 g, 100%) as a pale-yellow fluffy flake: mp 164–166 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 90 MHz)  $\delta$  13.60 (1H, s, –OH), 11.26 (1H, s, –OH), 6.79 (1H, s,

Table 5. Percentage Inhibition of *Fusarium oxysporum vasinfectum* on CDA Medium Incorporated with Xanthones (at 144 h)<sup>a</sup>

compd	concentration (ppm)				
	control	1	10	100	1000
1	(88.0 ± 2.6)	11 (78.3 ± 2.6)	22 (68.3 ± 2.6)	29 (62.7 ± 2.6)	24 (66.7 ± 2.6)
2	(75.0 ± 0.8)	3 (72.7 ± 0.8)	0 (75.0 ± 0.8)	0 (74.7 ± 0.8)	3 (72.7 ± 0.8)
3	(75.0 ± 0.5)	3 (72.7 ± 0.5)	4 (72.0 ± 0.5)	9 (68.0 ± 0.5)	12 (66.3 ± 0.5)
5	(75.7 ± 0.9)	0 (75.7 ± 0.9)	6 (84.7 ± 0.9)	50 (38.3 ± 0.9)	64 (27.3 ± 0.9)
6	(77.0 ± 0.9)	-1 (78.0 ± 0.9)	10 (69.7 ± 0.9)	12 (67.7 ± 0.9)	41 (45.7 ± 0.9)
7	(77.0 ± 0.6)	-2 (78.3 ± 0.6)	8 (71.0 ± 0.6)	1 (76.0 ± 0.6)	6 (72.7 ± 0.6)
8	(77.0 ± 1.5)	2 (75.7 ± 1.5)	4 (74.3 ± 1.5)	4 (74.0 ± 1.5)	6 (72.3 ± 1.5)
9	(77.0 ± 0.9)	1 (76.3 ± 0.9)	13 (67.3 ± 0.9)	17 (64.0 ± 0.9)	20 (61.7 ± 0.9)
10	(75.0 ± 0.6)	5 (71.3 ± 0.6)	12 (66.3 ± 0.6)	16 (63.3 ± 0.6)	17 (62.7 ± 0.6)
11	(71.0 ± 1.1)	-3 (73.0 ± 1.1)	-2 (72.3 ± 1.1)	-3 (73.3 ± 1.1)	-1 (72.0 ± 1.1)
12	(71.0 ± 1.2)	-4 (73.7 ± 1.2)	5 (67.3 ± 1.2)	-6 (75.3 ± 1.2)	-2 (72.3 ± 1.2)
13	(71.0 ± 0.9)	-7 (75.7 ± 0.9)	-4 (73.7 ± 0.9)	-4 (74.0 ± 0.9)	-3 (73.3 ± 0.9)
14	(75.7 ± 0.7)	3 (73.7 ± 0.7)	1 (75.0 ± 0.7)	4 (72.3 ± 0.7)	3 (73.3 ± 0.7)
15	(77.0 ± 0.4)	0 (77.0 ± 0.4)	1 (76.3 ± 0.4)	4 (73.7 ± 0.4)	1 (76.3 ± 0.4)
16	(75.7 ± 0.8)	1 (74.7 ± 0.8)	2 (74.0 ± 0.8)	3 (73.7 ± 0.8)	9 (69.0 ± 0.8)
17	(75.7 ± 0.7)	6 (71.3 ± 0.7)	5 (72.3 ± 0.7)	4 (73.0 ± 0.7)	3 (73.3 ± 0.7)
18	(75.0 ± 1.1)	4 (72.0 ± 1.1)	8 (69.3 ± 1.1)	19 (60.7 ± 1.1)	18 (61.7 ± 1.1)

<sup>a</sup> Values in parentheses give the radial growth (mm) ± S.E. 0.05.

Ar-H), 6.21 (1H, s, Ar-H), 5.5 (1H, t, -CH=), 4.1 (2H, d, ArCH<sub>2</sub>-), 3.79 (3H, s, -OMe), 2.79 (2H, t), 1.82-1.69 (8H, m), 1.37 (6H, m).

**Xanthione (8).** Lawesson's reagent (0.1 g, 0.25 mmol) was added to a stirred solution of xanthone (7) (0.2 g, 1 mmol) in CHCl<sub>3</sub> (10 mL). The solution turned green. Stirring continued overnight. The reaction mixture was poured onto ice pieces (10 g) and extracted with CHCl<sub>3</sub> (3 × 25 mL). The organic layer was washed well with H<sub>2</sub>O (2 × 25 mL) and dried (anhydrous Na<sub>2</sub>SO<sub>4</sub>). Removal of the solvent yielded xanthione (8) as a fluorescent reddish-green needle, which was purified by crystallization from C<sub>6</sub>H<sub>6</sub> (0.2 g, 90.6%), mp 157-158 °C (lit.<sup>15</sup> mp 156-158 °C).

**Evaluation of Antifungal Activity.** Cultures of *F. oxysporum vasinfectum*, *Alternaria tenuis*, and *Dreschlera oryzae* were kindly provided by Prof. R. Balasubramanian, C.A.S. in Botany, University of Madras. The stock cultures were maintained on Czapek-Dox medium at 25 ± 1 °C.

The natural xanthones and their derivatives in Me<sub>2</sub>CO-MeOH were incorporated into the molten medium (ca. 45 °C) in aseptic conditions to have concentrations of 1, 10, 100, or 1000 ppm in the medium. Into each Petri dish (88 mm in diameter), 15 mL of the medium was distributed. Me<sub>2</sub>CO-MeOH devoid of the compound was appropriately incorporated into the medium to serve as a control. Circular blocks of mycelia from stock culture (5 mm in diameter, grown on normal Czapek-Dox medium) were punched using a sterile cork-borer and were centrally placed onto the medium incorporated with the compound in the Petri plate.

Triplicates were maintained for each concentration, compound, and fungus. Growth in diameter (in mm) of each fungus was measured for 240 h at 24-h intervals. The data were subjected to analysis variance, and Neumann-Keuls means were arrived at using COSTAT in an IBM PC/AT Computer. Using the Neumann-Keuls mean values, the percentage of inhibition was calculated using the formula

$$\% \text{ inhibition} = 100 - \frac{\text{growth in treated}}{\text{growth in control}} (100)$$

Percentage of inhibition of *D. oryzae* (at 168 h), *A. tenuis* (at 168 h), and *F. oxysporum vasinfectum* (at 144 h) in media incorporated with different xanthones are presented in Tables 3-5.

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