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

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The antiungal activity of several xanthones 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 xanthones 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.[1]

In our effort to screen constitutive antifungal substances in plants, we evaluated the natural xanthones from the fruit hulls of *Garcinia mangostana* L. (Guttiferae) and their synthetic analogues. The xanthones isolated from *G. mangostana* have been reviewed.[2,3] The fruit hulls of this species find application in native medicine as an antiinflammatory and antidiarrheal.[4] Mangostin 3,6-di-*O*-glucoside, obtained from *G. mangostana*, has been shown to have interesting pharmacological activity;[5] however, there is no information on the antifungal activities of xanthones from *G. mangostana*. The only report on antifungal activity of xanthones 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*.[6] Herein we report the isolation of xanthones 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 Me₂CO successively. The residues obtained from both the extracts were subjected to extensive chromatography to afford various xanthones. Gartanin[7,8] (3), 8-desoxygartanin,[7,8] BR-xanthone[9] (2), and mangostin[7,8] (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 CHCl₃ in order of increasing polarity. The major quantity of mangostin, however, was isolated, along with β-mangostin[10,11] (4), gartanin[7,8] (3), γ-mangostin[8,10] (5), and garcinone-D[12] (6), from the Me₂CO extract by simple VLC over Si gel and elution

with *n*-hexane–CHCl₃ (90:10). The structures of the various xanthones 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,[13] 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**.
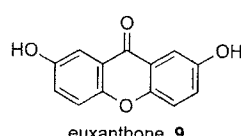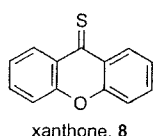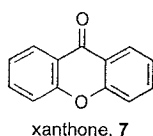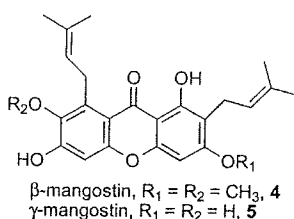
Di-*O*-acetylmangostin[10] (**17**) was obtained by acetylation of mangostin with Ac₂O and pyridine. Mangostin was subjected to acid-catalyzed cyclization using *p*-toluenesulfonic acid to yield 3-isomangostin[14] (**18**). The reaction of xanthone (**7**) with Lawesson's reagent gave xanthione (**8**) in 90% yield.

The natural xanthones **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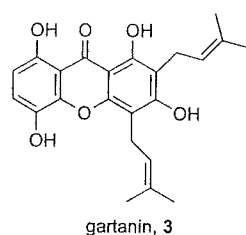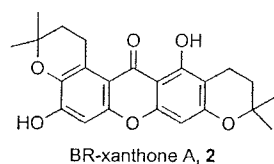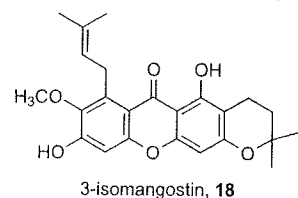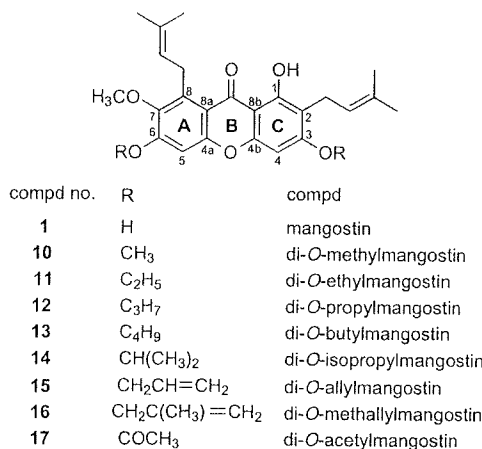 three fungi were selected because they are phytopathogens of agricultural importance. The results are presented in Tables 3–5.

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 xanthones and their derivatives. The natural xanthones 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-

compd no.	R	compd
1	H	mangostin
10	CH₃	di-O-methylmangostin
11	C₂H₅	di-O-ethylmangostin
12	C₃H₇	di-O-propylmangostin
13	C₄H₉	di-O-butylmangostin
14	CH(CH₃)₂	di-O-isopropylmangostin
15	CH₂CH=CH₂	di-O-allylmangostin
16	CH₂C(CH₃)=CH₂	di-O-methallylmangostin
17	COCH₃	di-O-acetylmangostin

3-isomangostin, **18**

BR-xanthone A, **2**

gartanin, **3**

garcinone D, **6**

β-mangostin, R₁ = R₂ = CH₃, **4**
γ-mangostin, R₁ = R₂ = H, **5**

xanthone, **7**   xanthone, **8**   euxanthone, **9**
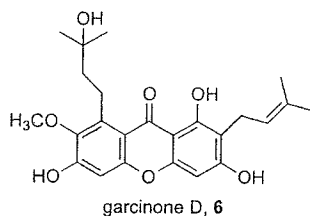
gesting that free hydroxyls are important for optimal antifungal activity. The conversion of >C=O (**7**) to >C=S (**8**) did not improve the inhibitory activity.

Among the natural xanthones, γ-mangostin (**5**) was found to be the most efffective 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[a]
diethylsulfate	**11**	82	112–114
n-propyl bromide	**12**	75	92–94
n-butyl iodide	**13**	78	76–78
isopropyl bromide	**14**	72	102–104
allyl bromide	**15**	75	78–80
methallyl chloride	**16**	78	102–104

[a] Lit.[13] 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 CCl₄, CDCl₃, DMSO-d₆, and Me₂CO-d₆ 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 F₂₅₄, 0.2-mm thickness) were used for TLC and developed in n-hexane—CHCl₃ or CHCl₃—MeOH and were visualized under UV light (λ 254 and 365 nm) or by spraying with 10% H₂SO₄ 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 Me₂CO successively. Evaporation of solvents yielded 25.8 g and 140.4 g of residues from n-hexane and Me₂CO 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, 1,60 g) to yield three fractions, A, B, and C, on successive elution with 40:60 n-hexane—CHCl₃, 20:80 n-hexane—CHCl₃, and pure CHCl₃, 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 CHCl₃ as eluents. Subfractions 1, 2, and 3 were obtained on elution with 3:2, 2:3, and 1:4 n-hexane—CHCl₃, respectively. Repeated column chromatograpy (gravity) of subfraction 1 (1.5 g) (Si gel, 230–400 mesh, 60 g) afforded 8-desoxygartanin[7,8] (50 mg, mp 155–156 °C) and BR-xanthone[9] (**2**) (191 mg, mp 180–182 °C) on elution with 1:1 C₆H₆—CHCl₃. Subfraction 2 yielded gartanin[7,8] (**3**) (370 mg, mp 164–165 °C), and subfraction 3 yielded mangostin[7,8] (**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 C₆H₆ to afford mangostin (**1**) (11 g, mp 181–182 °C).

The residue from the Me₂CO extract (140.4 g) was subjected to VLC (TLC Si gel, 400–600 mesh, 500 g) using n-hexane, n-hexane—CHCl₃, CHCl₃, CHCl₃—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% CHCl₃ in n-hexane when subjected to column chromatography (Si gel, 70–

Table 2. ¹H-NMR, Mass Spectral Data of Compounds **10–16**

compound	¹H NMR (CCl₄) δ (ppm)	molecular formula	[M]⁺ 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, ArCH₂), 3.84 (6H, s, –OMe), 3.60 (3H, s, –OMe), 3.10 (2H, d, ArCH₂), 1.8 (3H, s, Me), 1.73 (3H, s, Me), 1.6 (6H, s, Me's)	$C_{26}H_{30}O_6$	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, ArCH₂ and OCH₂), 3.72 (3H, s, OMe), 3.18 (2H, d, ArCH₂), 1.8 (6H, s, Me's), 1.6 (6H, s, Me's), 1.45 (6H, t, Me's)	$C_{28}H_{34}O_6$	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, ArCH₂ and OCH₂), 3.79 (3H, s, –OMe), 3.21 (2H, d, ArCH₂), 1.84 (6H, s, Me's), 1.81 (4H, m, CH₂'s), 1.6 (6H, s, Me's), 1.1 (6H, t, Me's)	$C_{30}H_{38}O_6$	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, ArCH₂ and OCH₂), 3.72 (3H, s, –OMe), 3.10 (2H, d, ArCH₂), 1.73–1.65 (20H, m), 0.98 (6H, t, Me's)	$C_{32}H_{42}O_6$	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, ArCH₂), 3.64 (3H, s, –OMe), 3.13 (2H, d, ArCH₂), 1.76 (6H, s, Me's), 1.56 (6H, s, Me's), 1.29 (12H, m, Me's)	$C_{30}H_{38}O_6$	494 (494)
15	13.35 (1H, s, –OH), 6.46 (1H, s, Ar-H), 5.95 (1H, s, Ar-H), 5.56 (4H, d, =CH₂), 5.17 (4H, m, –CH=), 4.54 (4H, d, –OCH₂), 4.03 (2H, d, ArCH₂), 3.72 (3H, s, –OMe), 3.21 (2H, d, ArCH₂), 1.8 (3H, s, Me), 1.73 (3H, s, Me), 1.6 (6H, s, Me's)	$C_{30}H_{34}O_6$	490 (490)
16	13.35 (1H, s, –OH), 6.46 (1H, s, Ar-H), 6.11 (1H, s, Ar-H), 5.09 (6H, m, =CH₂ and –CH=), 4.50 (4H, s, –OCH₂), 4.03 (2H, d, ArCH₂), 3.93 (3H, s, –OMe), 3.24 (2H, d, ArCH₂), 1.86 (6H, s, Me's), 1.76 (6H, s, Me's), 1.65 (6H, s, Me's)	$C_{32}H_{38}O_6$	518 (518)

Table 3. Percentage Inhibition of *Alternaria tenuis* on CDA Medium Incorporated with Xanthones (at 168 h)[a]

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

[a] Values in parentheses give the radial growth (mm) ± S.E. 0.05.

325 mesh, 150 g), yielded β-mangostin[10,11] (**4**) (20 mg, mp 178–179 °C) and gartanin (**3**) (33 mg, mp 164–166 °C), successively, on elution with 1:1 CHCl₃–*n*-hexane. Elution with pure CHCl₃ 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–CHCl₃ and pure CHCl₃.

Fraction C (5.6 g), on elution with 2% MeOH in CHCl₃, when subjected to column chromatography

Table 4. Percentage Inhibition of *Dreschlera oryzae* on CDA Medium Incorporated with Xanthones (at 168 h)[a]

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)

[a] Values in parentheses give the radical growth (mm) ± S.E. 0.05.

(gravity, Si gel, 70−320 mesh, 100 g) with $CHCl_3$ as eluent, afforded γ-mangostin[8,10] (**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 $CHCl_3$, 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−$CHCl_3$. Subfraction 1 was identified as γ-mangostin (**5**) (200 mg, mp 203−205 °C), while garcinone D[12] (**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 $CHCl_3$ 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 $CHCl_3$ (3 × 50 mL). The organic layer was washed with H_2O (2 × 50 mL) and dried (anhydrous Na_2SO_4). 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_2CO_3−Me_2CO 10, 12−16. MeI (0.42 g, 3 mmol) was added to a solution of mangostin (**1**) (0.41 g, 1 mmol) in Me_2CO (10 mL) and K_2CO_3 (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 $CHCl_3$ (3 × 50 mL). The organic layer was washed with H_2O (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 ^{1}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_2O (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 $CHCl_3$ (3 × 50 mL). The organic layer was washed with H_2O (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.[10] mp 117 °C) (0.38 g, 76%); ^{1}H NMR (TMS, 60 MHz) δ 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, $ArCH_2$), 3.64 (3H, s, −OMe), 3.3 ($ArCH_2$, 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_6H_6 (70 mL) was refluxed over a H_2O bath with a Dean-Stark apparatus. After the removal of H_2O (4.4 mL) for 1 h, C_6H_6 was removed, and the residue was extracted with $CHCl_3$ (3 × 100 mL). The organic layer was washed with H_2O (2 × 100 mL) and dried. The residue on purification by column chromatography (gravity, Si gel, 60−120 mesh, 3:2 $CHCl_3$−*n*-hexane) yielded **18** (1 g, 100%) as a pale-yellow fluffy flake: mp 164−166 °C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 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)[a]

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)

[a] 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$_2$−), 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$_3$ (10 mL). The solution turned green. Stirring continued overnight. The reaction mixture was poured onto ice pieces (10 g) and extracted with CHCl$_3$ (3 × 25 mL). The organic layer was washed well with H$_2$O (2 × 25 mL) and dried (anhydrous Na$_2$SO$_4$). Removal of the solvent yielded xanthione (**8**) as a fluorescent reddish-green needle, which was purified by crystallization from C$_6$H$_6$ (0.2 g, 90.6%), mp 157−158 °C (lit.[15] 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$_2$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$_2$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 corkborer 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.

References and Notes

(1) Grayer, R. J.; Harborne, J. B. *Phytochemistry* **1994**, *3*, 19.
(2) Sultanbawa, M. U. S. *Tetrahedron* **1980**, *36*, 1465.
(3) Mandal, S.; Das, P. C.; Joshi, P. C. *J. Indian Chem. Soc.* **1992**, *69*, 611.
(4) Kupchan, S. M.; Steelman, D. R.; Snedan, A. *J. Nat. Prod.* **1980**, *43*, 296.
(5) Pai, B. R.; Natarajan, S.; Suguna, B.; Kameswaran, L.; Shankaranarayanan, D.; Gopalakrishnan, C. *J. Nat. Prod.* **1979**, *42*, 361.

(6) Marston, A.; Hamburger, M.; Sordat-Diserens, I.; Msonthi, J. D.; Hostettmann, K. *Phytochemistry* **1993**, *33*, 809.

(7) Govindachari, T. R.; Kalyanaraman, P. S.; Muthukumaraswamy, N.; Pai, B. R. *Tetrahedron* **1971**, *2*, 3919.

(8) Govindachari, T. R.; Kalyanaraman, P. S.; Muthukumaraswamy, N.; Pai, B. R. *Indian J. Chem.* **1971**, *9*, 505.

(9) Balasubramanian, K.; Rajagopalan, K. *Phytochemistry* **1988**, 2, 1552.

(10) Jefferson, A.; Quillinan, A. J.; Schienmann, F.; Sim, K. Y. *Aust. J. Chem.* **1970**, *23*, 2539.

(11) Yates, P.; Bhat, H. B. *Can. J. Chem.* **1968**, *46*, 3770.

(12) Sen, A. K.; Sarkar, K. K.; Majumder, P. C.; Banerji, N. *Indian J. Chem.* **1986**, *25B*, 1157.

(13) Yates, P.; Stout, G. H. *J. Am. Chem. Soc.* **1958**, *80*, 1691.

(14) Wilawan, M.; Pichaet, W.; Walter, C. T. *J. Nat. Prod.* **1987**, *50*, 601.

(15) Nagarajan, K.; Kulkarni, C. L.; Venkateswarlu, A. *Indian J. Chem.* **1974**, *12*, 247.