

# Antibacterial Activity of Xanthenes from Guttiferaeous Plants against Methicillin-resistant *Staphylococcus aureus*

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## Abstract

Extracts of *Garcinia mangostana* (Guttiferae) showing inhibitory effects against the growth of *S. aureus* NIHJ 209p were fractionated according to guidance obtained from bioassay and some of the components with activity against methicillin-resistant *Staphylococcus aureus* (MRSA) were characterized.

One active isolate,  $\alpha$ -mangostin, a xanthone derivative, had a minimum inhibitory concentration (MIC) of 1.57–12.5  $\mu\text{g mL}^{-1}$ . Other related xanthenes were also examined to determine their anti-MRSA activity. Rubraxanthone, which was isolated from *Garcinia dioica* and has a structure similar to that of  $\alpha$ -mangostin, had the highest activity against staphylococcal strains (MIC = 0.31–1.25  $\mu\text{g mL}^{-1}$ ), an activity which was greater than that of the antibiotic vancomycin (3.13–6.25  $\mu\text{g mL}^{-1}$ ). The inhibitory effect against strains of MRSA of two of the compounds when used in conjunction with other antibiotics was also studied. The anti-MRSA activity of  $\alpha$ -mangostin was clearly increased by the presence of vancomycin; this behaviour was not observed for rubraxanthone.

The strong in-vitro antibacterial activity of xanthone derivatives against both methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* suggests the compounds might find wide pharmaceutical use.

The incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in hospitals has increased world-wide and has recently become a critical problem, especially in Japan (Townsend et al 1987). The development of a new class of antibacterial agent is urgently required.

In this paper, we report the anti-MRSA activity of xanthenes from guttiferaceous plants. Some xanthenes are related to phytoalexins which are produced as a result of microbial infection in higher plants as a consequence of a passive defence system. We expect these xanthenes to have fewer side-effects and less tendency to acquire resistance compared with conventional antibiotics and synthetic antibacterial agents.

## Materials and Methods

### Growth of MRSA and sensitivity test

Each strain of MRSA and methicillin-sensitive *Staphylococcus aureus* (MSSA) isolated from the patients in the hospital attached to the Medicinal Department of Nagoya University were donated by Dr N. Arai, Nagoya University, and cultivated in Müller-Hinton broth (Difco), containing 0.5% yeast extract and 0.5% glucose, in air at 37°C for 18–48 h. The minimum inhibitory concentration (MIC) in  $\mu\text{g mL}^{-1}$  was determined by the liquid dilution method. Inocula were prepared by dilution of 18-h broth ( $10^8$ – $10^9$  cells  $\text{mL}^{-1}$ ) with buffered saline to  $1 \times 10^6$  colony-forming units  $\text{mL}^{-1}$ . The inoculated test tubes were incubated at 37°C for 18 h in aerobic culture. Acetone solutions of test samples were serially diluted with acetone and added to the medium so that the concentrations in the medium were 3.13–50.0  $\mu\text{g mL}^{-1}$ . The MIC was defined as the lowest concentration of antimicrobial agent in the liquid medium

resulting in complete inhibition of visible growth. Vancomycin and gentamicin were purchased from Sigma.

### Determination of anti-MRSA activity in the presence of other antibiotics

Each strain of MRSA and MSSA was cultivated in Müller-Hinton broth (Difco), containing 0.5% yeast extract and 0.5% glucose, in air at 37°C for 18 h. The MIC value was determined in the manner described above. Inocula were prepared by dilution of 18-h broth ( $10^8$ – $10^9$  cells  $\text{mL}^{-1}$ ) with buffered saline to  $1 \times 10^6$  colony-forming units  $\text{mL}^{-1}$ . The inoculated test tubes were incubated at 37°C for 18 h in aerobic culture. Acetone solutions of test samples were serially diluted with acetone and added to the medium so that the concentrations were 0.16–5.0  $\mu\text{g mL}^{-1}$  in the medium, to which was also added either vancomycin (0.63, 0.32 or 0.16  $\mu\text{g mL}^{-1}$ ) or gentamicin (1.6, 0.8 or 0.4  $\mu\text{g mL}^{-1}$ ).

## Chemistry

### Fractionation and isolation of xanthenes

Dried and ground pericarps (2.7 kg) of *Garcinia mangostana* L. (Guttiferae) collected in Bali, Indonesia, were successively extracted under reflux with *n*-hexane, benzene, acetone and 70% methanol. The benzene extract (8 g) was chromatographed on silica gel (Merck silica gel 60) eluted with an *n*-hexane-ethyl acetate to give eight fractions (fraction 1, *n*-hexane-ethyl acetate = 5 : 1; 2, 5 : 1; 3, 3 : 1; 4, 3 : 1; 5, 1 : 1; 6, 1 : 1; 7, 100% acetone; 8, 100% methanol). The MIC value of each fraction was determined and fractions with activity (fractions 2 and 3) were further purified to obtain the xanthenes.

Fraction 2 was subjected to vacuum liquid chromatography on silica gel (Fuji Davison silica gel BW-300) eluted with a

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similar system. An *n*-hexane-EtOAc (5 : 1) eluent was repeatedly chromatographed on Sephadex LH-20 (Pharmacia Fine Chemicals AB) eluted with acetone to give  $\beta$ -mangostin (2; 2 mg), garcinone E (4; 2 mg), 1,5-dihydroxy-2-isoprenyl-3-methoxyxanthone (5; 2 mg), 1,7-dihydroxy-2-isoprenyl-3-methoxyxanthone (6; 4 mg) and gartanin (7; 2 mg). Fraction 3 was also subjected to vacuum liquid chromatography; elution with a similar system gave  $\alpha$ -mangostin (1; 5 mg) from *n*-hexane-EtOAc (5 : 1) and  $\gamma$ -mangostin (3; 2 mg) from *n*-hexane-EtOAc (1 : 1). After confirmation of the purity of the isolates by TLC (Merck silica gel 60 F<sub>254</sub>) their MIC values were determined. Subelliptenone F (8) and 12-b-dihydroxy-des-D-garcigerin (9) were isolated from *Garcinia subelliptica* Merr.; caloxanthone A (10) and macluraxanthone (11) were from *Calophyllum inophyllum* L.; 1,7-dihydroxyxanthone (12) was from *Harungana madagascariensis* Lam ex Poir.

Rubraxanthone (13) was isolated from the bark of *Garcinia dioica* Bl. Dried and ground bark (900 g) of *G. dioica*, collected in Indonesia, was extracted under reflux with *n*-hexane, benzene, acetone and 70% methanol, successively. The benzene extract (16 g) was chromatographed on silica gel eluted with a benzene-acetone system. The 10 : 1 benzene-acetone eluate was recrystallized (*n*-hexane-EtOAc) to give 13 (2 g).

#### Structural identification of the active isolates (Figs 1, 2)

The isolated xanthenes were subjected to spectroscopic analysis, including high-resolution electron-impact (EI) MS on a Jeol JMS-D300 spectrometer (70 eV). UV and IR spectra were recorded with Shimadzu UV-2200 and Jasco IR-AI spectrometers, respectively. <sup>1</sup>H NMR and <sup>13</sup>C NMR, including 2D NMR, were performed on Jeol JNM EX-400 and GX-270 instruments.  $\alpha$ -Mangostin (1) was obtained as a yellow amorphous powder. MS *m/z*: 410 (*M*<sup>+</sup>); UV (methanol): 243, 256 (sh), 316, 346 nm; IR (KBr): 3425, 3250, 2960, 2805, 1635 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, acetone-*d*<sub>6</sub>)  $\delta$  (ppm): 1.65 (6H, s, H-14, 15); 1.78 (3H, s, H-20); 1.83 (3H, s, H-19); 3.35 (2H, d, *J* = 7 Hz, H-11); 3.80 (3H, s, OCH<sub>3</sub>-C7); 4.12 (2H, d, *J* = 7 Hz, H-16); 5.28 (2H, m, H-12, 17); 6.38 (1H, s, H-4); 6.80 (1H, s, H-5); 9.48 (2H, br s, OH-C3, C6); 13.77 (1H, s, OH-C1).

$\beta$ -Mangostin (2) was obtained as a yellow amorphous powder. MS *m/z*: 424 (*M*<sup>+</sup>); UV (methanol): 243, 258, 314, 345 (sh) nm; IR (KBr): 3400, 2925, 1640 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, acetone-*d*<sub>6</sub>)  $\delta$ : (ppm) 1.64, 1.66 (3H each, s, H-14, 15); 1.77 (3H, s, H-20); 1.83 (3H, s, H-19); 3.29 (2H, d,

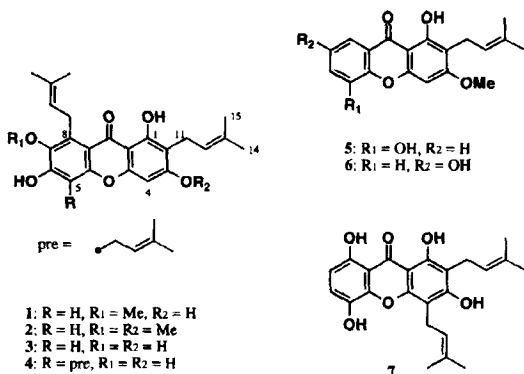


FIG. 1. Xanthenes isolated from *G. mangostana*.

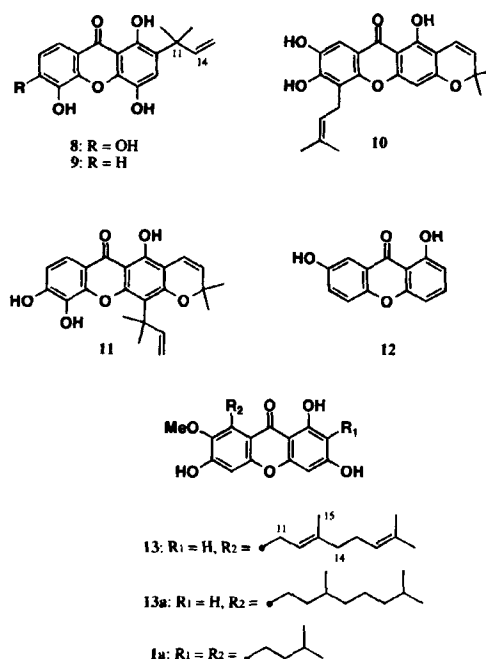


FIG. 2. The chemical structures of compounds 8–13, 1a and 13a.

*J* = 7 Hz, H-11); 3.80 (3H, s, OCH<sub>3</sub>-C7); 3.94 (3H, s, OCH<sub>3</sub>-C3); 4.10 (2H, d, *J* = 7 Hz, H-16); 5.21, 5.27 (1H each, m, H-12, 17); 6.42 (1H, s, H-4); 6.80 (1H, s, H-5); 9.38 (1H, br s, OH-C6); 13.58 (1H, s, OH-C1).

$\gamma$ -Mangostin (3) was obtained as a yellow amorphous powder. MS *m/z*: 396 (*M*<sup>+</sup>); UV (methanol): 243, 259, 317, 364 nm; <sup>1</sup>H NMR (400 MHz, acetone-*d*<sub>6</sub>)  $\delta$  (ppm): 1.65 (6H, s, H-14, 15); 1.79 (3H, s, H-20); 1.84 (3H, s, H-19); 3.36 (2H, d, *J* = 7 Hz, H-11); 4.20 (2H, d, *J* = 7 Hz, H-16); 5.29, 5.32 (1H each, m, H-12, 17); 6.36 (1H, s, H-4); 6.80 (1H, s, H-5); 9.18 (3H, br s, OH-C3, C6, C7); 13.89 (1H, s, OH-C1).

Garcinone E (4) was obtained as a yellow amorphous powder. MS *m/z*: 464 (*M*<sup>+</sup>); UV (methanol): 244, 260, 320, 361 nm; <sup>1</sup>H NMR (400 MHz, acetone-*d*<sub>6</sub>)  $\delta$  (ppm): 1.63 (3H, s, CH<sub>3</sub>); 1.65 (6H, s, CH<sub>3</sub> × 2); 1.79, 1.82, 1.88 (3H each, s, CH<sub>3</sub> × 3); 3.36, 3.60, (2H each, d, *J* = 7 Hz, H-11, 16); 4.20 (2H, d, *J* = 7 Hz, H-21); 5.27 (3H, m, H-12, 17, 22); 6.46 (1H, s, H-4); 13.90 (1H, s, OH-C1).

1,5-Dihydroxy-2-isoprenyl-3-methoxyxanthone (5) was obtained as a yellow amorphous powder. MS *m/z*: 326 (*M*<sup>+</sup>); UV (methanol): 219, 244, 253, 262 (sh), 270 (sh), 311, 357 nm; <sup>1</sup>H NMR (400 MHz, acetone-*d*<sub>6</sub>)  $\delta$  (ppm): 1.65 (3H, s, H-14); 1.78 (3H, s, H-15); 3.35 (2H, d, *J* = 7 Hz, H-11); 4.01 (3H, s, OCH<sub>3</sub>-C<sub>3</sub>); 5.23 (1H, m, H-12); 6.64 (1H, s, H-4); 7.28 (1H, t, *J* = 8 Hz, H-7); 7.36 (1H, dd, *J* = 8, 1 Hz, H-6); 7.70 (1H, dd, *J* = 8, 1 Hz, H-8); 9.12 (1H, br s, OH-C5); 13.07 (1H, s, OH-C1).

1,7-Dihydroxy-2-isoprenyl-3-methoxy-xanthone (6) was obtained as a yellow amorphous powder. MS *m/z*: 326 (*M*<sup>+</sup>); UV (methanol): 240, 263, 305, 375 nm; <sup>1</sup>H NMR (400 MHz, acetone-*d*<sub>6</sub>)  $\delta$  (ppm): 1.64 (3H, s, H-14); 1.78 (3H, s, H-15); 3.34 (2H, d, *J* = 7 Hz, H-11); 4.00 (3H, s, OCH<sub>3</sub>-C<sub>3</sub>); 5.22 (1H, m, H-12); 6.60 (1H, s, H-4); 7.35 (1H, dd, *J* = 9, 3 Hz, H-6); 7.45 (1H, d, *J* = 9 Hz, H-5); 7.59 (1H, d, *J* = 3 Hz, H-8); 8.78 (1H, br s, OH-C7); 13.09 (1H, s, OH-C1).

Gartarinin (7) was obtained as a yellow amorphous powder. MS *m/z*: 396 ( $M^+$ ); UV (methanol): 222, 240, 258, 282, 320 (sh), 351, 390 (sh) nm; IR (KBr): 3430, 1665, 1630  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz, acetone- $d_6$ )  $\delta$  (ppm): 1.66, 1.67 (3H each, s, H-19, 20); 1.79 (3H, s, H-14); 1.85 (3H, s, H-15); 3.46 (2H, d,  $J=7$  Hz, H-11); 3.67 (2H, d,  $J=7$  Hz, H-16); 5.23, 5.27 (1H each, m, H-12, 17); 6.63 (1H, d,  $J=9$  Hz, H-7); 7.31 (1H, d,  $J=9$  Hz, H-6); 8.40, 8.51 (1H each, br s, OH-C3, C4); 11.31 (1H, s, OH-C8); 12.35 (1H, s, OH-C1).

Compounds 8–12 were determined to be subelliptenone F (8), 12-b-dihydroxy-des-D-garcigerin (9), caloxanthone A (10), macluraxanthone (11) and 1,7-dihydroxyxanthone (12). Details of the structural elucidation and spectral data of these xanthenes have been reported previously (Iinuma et al 1994a, 1995a, b, c).

Rubraxanthone (13) was obtained as a yellow amorphous powder. MS, *m/z*: 410 ( $M^+$ ); IR (KBr): 3440, 2920, 1645, 1605  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz, acetone- $d_6$ )  $\delta$  (ppm): 1.52, 1.56 (3H each, s, H-19, 20); 1.83 (3H, s, H-15); 1.97 (2H, m, H-14); 2.09 (2H, m, H-16); 3.81 (3H, s,  $\text{OCH}_3$ -C7); 4.12 (2H, d,  $J=6$  Hz, H-11); 5.04, 5.28 (1H each, m, H-17, 12); 6.19 (1H, d,  $J=2$  Hz, H-2); 6.30 (1H, d,  $J=2$  Hz, H-4); 6.82 (1H, s, H-5); 9.44 (2H, br s, OH-C3, C6); 13.47 (1H, s, OH-C1).

#### Preparation of 1a by reduction of 1

An ethanol solution (5 mL) containing 1 (15 mg) was stirred with Pd-C (50 mg) under  $\text{H}_2$  for 2 h at room temperature. The

reaction mixture was purified by preparative TLC (Merck silica gel 60 F<sub>254</sub>) (*n*-hexane-ethyl acetate-methanol, 8:2:1) to give 1a (14 mg) as a pale yellow viscous oil. MS *m/z*: 414 ( $M^+$ ). Compound 13 (10 mg) was similarly converted to 13a; 13a (8 mg) was obtained as a yellow viscous oil. MS *m/z*: 414 ( $M^+$ ).

#### Results and Discussion

Although vancomycin has been widely used as first-choice agent for severe MRSA infections, side effects and the development of resistant bacteria has become a serious problem. We have previously reported the anti-MRSA activity of 1.56–6.25  $\mu\text{g mL}^{-1}$  of the flavanone exiguaflavanone D, isolated from *Sophora exigua* (Iinuma et al 1994b). To the best of our knowledge, 13 is the most active phytochemical against MRSA strains.

In continuation of our work oriented at identifying xanthenes with bioactive potency in guttiferous plants, we reported the isolation and structure determination of some xanthenes (Iinuma et al 1994c, d). After initial screening of extracts of guttiferous plants for antibacterial activity against *S. aureus* NIHJ 209p, we found strong activity in a benzene extract of the pericarps of *G. mangostana*; the MIC value was 80  $\mu\text{g mL}^{-1}$ . The active components of the extract were investigated by monitoring the antibacterial activity of different fractions; this led to the discovery of two xanthenes, 1 and 6 with anti-MRSA activity (Tables 1 and 2). The most active

Table 1. Minimum inhibitory concentrations ( $\mu\text{g mL}^{-1}$ ) against microorganisms of fractions from a benzene extract of *G. mangostana*.

Fraction <sup>a</sup>	MRSA strain			MSSA strain			<i>S. aureus</i> NIHJ 209p	<i>E. coli</i> NIHJ K 12
	1–11	1–33	25–22	24–11	26–30	1–38		
1	25	12.5	12.5	25	12.5	25	12.5	25
2	12.5	12.5	6.25	25	6.25	25	12.5	25
3	12.5	6.25	6.25	6.25	6.25	12.5	12.5	25
4	25	25	25	25	25	25	12.5	25
5	25	25	25	25	25	25	12.5	25
6	25	25	25	25	25	25	12.5	25
7	25	25	25	25	25	> 25	12.5	25
8	25	25	25	25	25	25	12.5	25
Vancomycin	6.25	6.25	6.25	3.13	6.25	6.25	0.8	> 25
Gentamicin	> 25	> 25	> 25	3.13	> 25	25	1.57	25

<sup>a</sup>A benzene extract of pericarps of *G. mangostana* was chromatographed on silica gel eluted with an *n*-hexane-EtOAc system to give eight fractions: 1, *n*-hexane-EtOAc, 5:1; 2, 5:1; 3, 3:1; 4, 3:1; 5, 1:1; 6, 1:1; 7, 100% acetone; 8, 100% methanol.

Table 2. Minimum inhibitory concentrations ( $\mu\text{g mL}^{-1}$ ) against microorganisms of xanthenes isolated from the active fractions of a benzene extract of *G. mangostana*.

Compound	MRSA strain			MSSA strain			<i>S. aureus</i> NIHJ 209p	<i>E. coli</i> NIHJ K 12
	1–11	1–33	25–22	24–11	26–30	1–38		
1	6.25	1.57	12.5	3.13	1.57	6.25	1.57	25
2	25	> 25	25	25	> 25	25	12.5	25
3 <sup>a</sup>	12.5	12.5	12.5	> 12.5	> 12.5	> 12.5	12.5	25
4 <sup>a</sup>	12.5	12.5	12.5	> 12.5	> 12.5	> 12.5	> 12.5	25
5 <sup>a</sup>	> 12.5	> 12.5	> 12.5	> 12.5	–	> 12.5	–	25
6 <sup>a</sup>	6.25	6.25	6.25	> 12.5	> 12.5	> 12.5	12.5	25
7 <sup>a</sup>	> 12.5	> 12.5	> 12.5	–	–	> 12.5	> 12.5	25
Vancomycin	6.25	6.25	6.25	3.13	6.25	6.25	0.8	> 25
Gentamicin	> 25	> 25	> 25	3.13	> 25	25	1.57	25

<sup>a</sup>The maximum concentration of these xanthenes (3–7) in the medium was 12.5  $\mu\text{g mL}^{-1}$ .

Table 3. Minimum inhibitory concentrations ( $\mu\text{g mL}^{-1}$ ) against microorganisms of xanthones (**8**–**13**, and **13a** and **1a**).

Compound	MRSA strain			MSSA strain			<i>S. aureus</i> NIHJ 209p
	1–11	1–33	25–22	24–11	26–30	1–38	
<b>8</b>	12.5	25	25	6.25	25	12.5	25
<b>9</b>	12.5	3.13	6.25	6.25	6.25	6.25	12.5
<b>10</b>	> 25	> 25	25	6.25	6.25	6.25	25
<b>11</b>	25	12.5	12.5	6.25	6.25	6.25	25
<b>12</b>	6.25	> 25	> 25	> 25	> 25	> 25	6.25
<b>13</b>	1.25	1.25	0.313	0.63	1.25	1.25	0.313
<b>13a</b>	> 25	> 25	12.5	> 25	25	25	12.5
<b>1a</b>	12.5	12.5	0.8	12.5	3.13	3.13	1.57
Vancomycin	3.13	6.25	3.13	3.13	3.13	3.13	1.57
Gentamicin	> 25	> 25	> 25	6.25	12.5	> 25	1.57

xanthone in these isolates was **1**; concentrations in the range  $1.57$ – $12.5 \mu\text{g mL}^{-1}$  inhibited the growth of MRSA. The activity of **1** against MRSA is nearly equal to that of the antibiotic vancomycin ( $3.13$ – $6.25 \mu\text{g mL}^{-1}$ ).

Because of the activity of **1**, the anti-MRSA activity was determined for thirty-six other xanthones isolated from guttiferous plants. Compounds **9**, **12** and **13** showed inhibitory effects against strains of MRSA (Table 3). The inhibitory effect of **13** was the strongest; the MIC value for **13** against both MRSA and MSSA was  $0.313$ – $1.25 \mu\text{g mL}^{-1}$ . The inhibitory strength was higher than that of vancomycin ( $3.13$ – $6.25 \mu\text{g mL}^{-1}$ ). The anti-MRSA activity of **9** ( $3.13$ – $12.5 \mu\text{g mL}^{-1}$ ) was less than that of **1**. Compounds **10** and **11**, on the other hand, had high activity (concentration  $6.25 \mu\text{g mL}^{-1}$ ) against strains of MSSA but not against each strain of MRSA. Compound **12** inhibited the growth of MRSA strain 1–11.

From the viewpoint of structure-activity relationships, both **1** and **13** have a similar oxidation pattern (both are 1,3,6-trihydroxy-7-methoxyxanthenes). Structural differences between **1** and **13** are the pattern of substitution and the type of alkyl chain. Compound **1** has a geranyl chain at C-8 and no substitution at C-2. Compound **13**, on the other hand, is substituted by two isoprenyl chains at C-2 and C-8. It is considered that the alkyl chain at C-8 plays an important role in the anti-MRSA activity. When **1** and **13** were converted to their tetra-

hydro derivatives by reduction (compounds **1a** and **13a**), the anti-MRSA activity was reduced, especially for **13a**. These findings suggested that double bonds on the alkyl chains are essential to the activity of a 1,3,6-trihydroxy-7-methoxy-xanthone derivative. Compounds **1**, **2** and **3**, on the other hand, have a closely similar structures, the only differences being the location of methoxy groups. Although **2** and **3** are the 3-methyl and 7-demethyl derivatives of **1**, they have no anti-MRSA activity. A permethyl and a perisopropyl ether of **1** also had no activity (data not shown). These results indicated that a suitable oxidation pattern for a xanthone skeleton to show anti-MRSA activity was of the 1,3,6-trihydroxy-7-methoxy type; 1,3,6-trihydroxy-7-methoxy substitution in xanthone derivatives is an effective oxidation pattern for conferring anti-MRSA activity, and the presence of an alkyl chain (C<sub>5</sub> or C<sub>10</sub>) at C-8 enhances the activity.

Xanthenes **1** and **13** were the most active principles from guttiferous plants against the MRSA strains in this study. Both xanthenes were further investigated by measurement of MIC values when the xanthenes used in combination with an antibiotic (Tables 4 and 5). The anti-MRSA activity of **1** was increased by the presence of vancomycin at a concentration of  $0.32 \mu\text{g mL}^{-1}$  (Table 4). In the presence of gentamicin (Table 4) the antibacterial activity of **1** against the MRSA strains was not increased, but that against the MSSA strains was increased. Activity against *S. aureus* NIHJ 209p was increased in the

Table 4. Minimum inhibitory concentrations ( $\mu\text{g mL}^{-1}$ ) of  $\alpha$ -mangostin (**1**), vancomycin and gentamicin used separately, and of **1** used in combination with vancomycin and gentamicin.

	MRSA			MSSA			<i>S. aureus</i> NIHJ 209P
	1–11	1–33	25–22	1–38	24–11	26–30	
<b>1</b>	2.50	1.25	0.63	2.50	2.50	1.25	1.25
Vancomycin	1.25	2.50	1.25	2.50	2.50	2.50	1.25
Gentamicin	> 25	> 25	> 25	1.25	> 25	> 25	< 0.32
<b>1</b> + vancomycin							
0.63 $\mu\text{g mL}^{-1}$	2.50	2.50	0.32	1.25	0.32	1.25	< 0.16
0.32 $\mu\text{g mL}^{-1}$	0.32	0.63	< 0.16	2.50	2.50	1.25	< 0.16
0.16 $\mu\text{g mL}^{-1}$	2.50	2.50	1.25	2.50	2.50	2.50	0.32
<b>1</b> + gentamicin							
1.6 $\mu\text{g mL}^{-1}$	2.50	2.50	1.25	< 0.16	< 0.16	1.25	< 0.16
0.8 $\mu\text{g mL}^{-1}$	2.50	2.50	1.25	< 0.16	0.32	2.50	< 0.16
0.4 $\mu\text{g mL}^{-1}$	2.50	2.50	1.25	0.32	1.25	2.50	< 0.16

Table 5. Minimum inhibitory concentrations ( $\mu\text{g mL}^{-1}$ ) of rubraxanthone (13) and vancomycin used separately, and of 13 used in combination with vancomycin.

	MRSA			MSSA			<i>S. aureus</i> NIHJ 209P
	1-11	1-33	25-22	1-38	24-11	26-30	
<b>13</b>	1.25	1.25	0.31	1.25	1.25	1.25	0.63
Vancomycin	1.25	2.50	1.25	2.50	2.50	2.50	1.25
<b>13 + vancomycin</b>							
0.63 $\mu\text{g mL}^{-1}$	1.25	1.25	1.25	1.25	1.25	0.63	0.63
0.32 $\mu\text{g mL}^{-1}$	0.63	1.25	0.63	1.25	1.25	0.63	0.63
0.16 $\mu\text{g mL}^{-1}$	1.25	1.25	1.25	1.25	1.25	0.63	0.63

presence of both antibiotics (vancomycin and gentamycin). The antibacterial activity of 13 against MRSA and MSSA strains or against *S. aureus* NIHJ 209p was not, on the other hand, increased by the presence of vancomycin (Table 5). These results suggest that 1 and 13 may have different modes of action against the staphylococcal strains.

To reduce toxicity and side-effects of antibiotics, the development of a new class of antibacterial agents is urgently required. The xanthone derivatives described above showed intense antibacterial activity against both MRSA and MSSA in the in-vitro experiment. Wide application of these xanthones to pharmaceutical uses is anticipated.

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