Antibacterial Activity of Some *Garcinia* Benzophenone Derivatives against Methicillin-Resistant *Staphylococcus aureus*

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Benzophenone derivatives, garcinol (1) and isogarcinol (2) isolated from the pericarps of *Garcinia purpurea* (Guttiferae), and xanthochymol (3) and a mixture of isoxanthochymol (4) cycloxanthochymol (5) from the pericarps of *G. subelliptica* were evaluated for their antibacterial activity against methicillin-resistant *Staphylococcus aureus*. Among them, 3 showed the lowest minimum inhibitory concentration at 3.1—12.5 μg/ml. This concentration is nearly equal to that of the antibiotic, vancomycin.

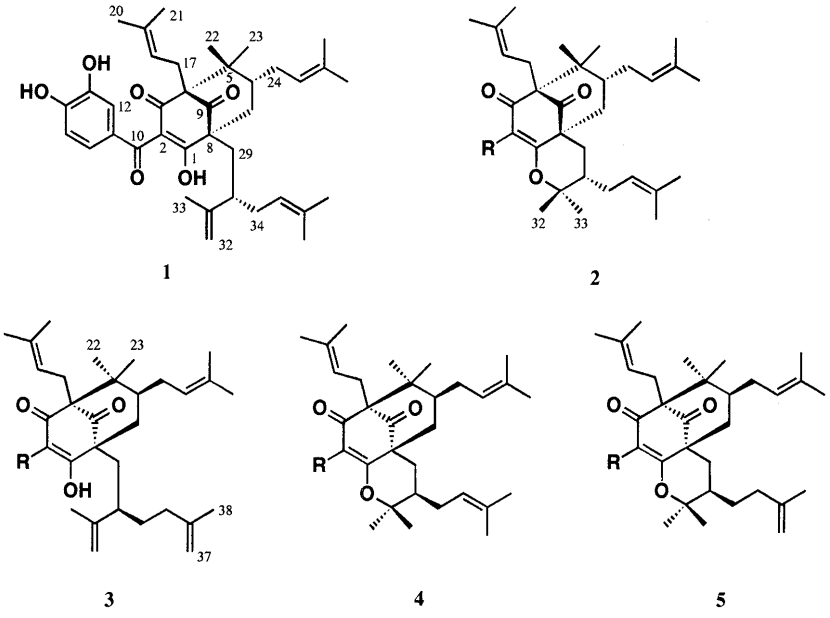
Key words antibacterial activity; methicillin-resistant *Staphylococcus aureus*; benzophenone; *Garcinia subelliptica*; *Garcinia purpurea*

The incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in hospitals has increased world-wide and has now become a critical problem, especially in Japan.[1,2] Although antibiotics are usually used for treating MRSA infections, their use evokes inevitable problems with the appearance of side-effects and eventually resistant bacteria. In continuation of our phytochemical search for biologically active compounds in Guttifereous plants, we investigated the chemical constituents of *Garcinia subelliptica* MERR.[3-5] and *G. mangostana* L.[6] (Guttiferae). In the present paper, we report the effect of polyisoprenylated benzophenone derivatives isolated from the pericarp of *G. subelliptica* and *G. purpurea* on the inhibition of MRSA.

RESULTS AND DISCUSSION

In our preceding paper on inhibitory activity with respect to MRSA, the xanthone derivative α-mangostin from *Garcinia mangostana* and rubraxanthone from *G. dioica* were characterized as active.[7] Bioassay-guided fractionation of pericarp extracts of two other *Garcinia* species (*G. purpurea* and *G. subelliptica*) resulted in the isolation and characterization of five benzophenone derivatives and four flavonoid compounds. The benzophenones isolated from two *Garcinia* species in the present experiment were mutual stereo- or regio-isomers re presented by the molecular formula $C_{38}H_{50}O_6$.

Compound **1**, garcinol,[8-10] pale yellow needles, exhibited $[\alpha]_D$ −138° and a molecular ion peak at *m/z* 602 in the MS. It reacted positively to $FeCl_3$. The ^1H- and ^{13}C-NMR spectra showed that the multiplicity of peaks was caused by a keto-enol equilibrium, and in CD_3OD/0.1% trifluoroacetic acid (TFA) the multiplicity disappeared and a single set of signals was observed,[11] supporting the hypothesis that **1** was a polyisoprenylated benzophenone derivative related to xanthochymol[12,13] or garcinol.[8-10] Taking into account the values of the



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optical rotation and 2D-NMR spectral data [H–H and C–H correlation spectroscopy (COSY), correlation spectroscopy of long-range coupling (COLOC) and phase-sensitive nuclear Overhauser effect spectroscopy (PSNOESY)], **1** was identified as garcinol.

Compound **2**, isogarcinol, a colorless amorphous powder, exhibited $[\alpha]_D$ −224° and a molecular ion peak at *m/z* 602 in the MS. The differences in the ^{1}H- and ^{13}C-NMR spectral data, compared with **1**, were the absence of the signals based on a chelated hydroxyl at C-1 and a terminal methylene group, indicating that a C_{10} side-chain attached at C-8 was cyclized with the chelated hydroxyl group to form a new dihydropyrane ring. The UV absorption bands at 233 and 277 nm were similar to those of isoxanthochymol,[11] and **2** was suggested to be isogarcinol. Examination of the 2D-NMR spectra and optical rotation data[8] led to the conclusion that **2** was isogarcinol, an enantiomer of isoxanthochymol (**4**) as described below.

Compound **3**, xanthochymol, pale yellow needles, exhibited $[\alpha]_D$ +138° and a molecular ion peak at *m/z* 602, and reacted positively with $FeCl_3$. The UV and IR absorptions closely resembled those of **1**. The ^{1}H-NMR spectrum was also similar to that of **1** except for a methine proton due to an isoprenyl group. In the spectrum of **3**, the methine proton observed in **1** was absent and an *endo*-methylene signal [δ 4.63 (2H, br s)] had appeared, suggesting that **3** was xanthochymol. The 2D-NMR spectra [H–H and C–H COSY, heteromolecular multiple bond coherence (HMBC) and PSNOESY] supported this structure. The complete ^{1}H- and ^{13}C-NMR spectra assigned in the present study are shown in the Materials and Methods section.

Isoxanthochymol (**4**) and a new compound (**5**), named cycloxanthochymol, were obtained as a colorless amorphous powder in an inseparable mixture (*ca.* 3:2 ratio), and exhibited $[\alpha]_D$ +158°. The mixture gave a single molecular ion peak at *m/z* 602.3588 in the high-resolution MS. The UV and IR absorptions of the mixture were closely similar to those of **2**. In the ^{1}H- and ^{13}C-NMR spectra, the resonances corresponding to **4** coincided with those of **2**. These results and the optical rotation data[11] suggested that **4** is isoxanthochymol. The NMR spectral data due to another compound (**5**) fundamentally resemble those of **4**. The differences between **4** and **5** in their ^{1}H-NMR spectra were the absence of a methine proton signal [δ 5.17 (1H, m)] and vinyl methyl signals [δ 1.51 and 1.74 (3H each, s)] based on an isoprenyl chain in **4** and the appearance of a terminal methylene signal [δ 4.73 (2H, br s)] in **5**. These findings suggested that **5** was a regio-isomer of **4** caused by the position of a double-bond. All protonated carbons of **5** were assigned from the C–H COSY spectrum. In the ^{1}H-NMR spectrum, the presence of an isobutenyl chain [δ 1.02, 1.47 (1H each, m, $-CH_2-CH_2-C=$), 1.68 (3H, s, Me), 2.06, 2.20 (1H each, m, $-CH_2-CH_2-C=$) and 4.73 (2H, br s, terminal methylene)] was shown, which was clearly supported by H–H COSY and COLOC spectral data (Fig. 1). In the H–H COSY spectrum, the methine proton at δ 1.24 was correlated to both the methylene protons (δ 1.01 and 2.90) at C-29 and the methine protons (δ 1.02 and 1.47)

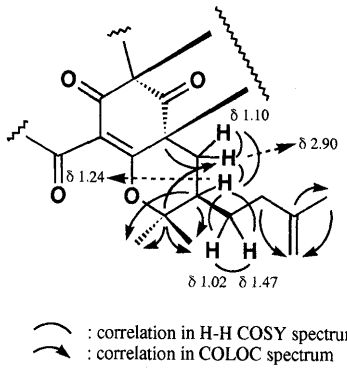

Fig. 1. H–H COSY and COLOC (J=8 Hz) Spectrum of **5**

Table 1. MIC Values of **1**—**5**, and Vancomycin and Gentamycin against MRSA and MSSA

	Staphylococcus aureus						*S. aureus*	*E. coli*
	MRSA			MSSA			NIHJ	NIHJ
	1-11	1-33	25-22	24-11	26-30	1-38	209p	K 12
1	6.25	25	6.25	6.25	12.5	25	12.5	25
2	25	25	12.5	>25	>25	25	12.5	25
3	3.13	12.5	6.25	3.13	12.5	25	12.5	25
4 and **5**[a]	25	25	25	25	>25	25	25	25
Vancomycin	6.25	6.25	6.25	3.13	6.25	6.25	0.8	>25
Gentamycin	>25	>25	>25	3.13	>25	25	1.57	25

a) Assayed as a mixture.

of the isobutenyl chain, which indicated that the isobutenyl group was attached at C-30. Thus, the structure of cycloxanthochymol was elucidated as **5**. The structure was substantiated by other correlations of H-H COSY, COLOC spectrum and PSNOESY experiments.

Following reduction (Pd–C, H_2) of the mixture, a hexahydro-derivative (**4a**) was exclusively obtained. Comparison of the ^{1}H-NMR spectral data of **4a** with a hexahydro-derivative derived from isogarcinol (**2a**) using a similar reduction, showed that both spectra were superimposable, which also supported the structures of **4** and **5**. Although the absolute stereochemistry of **4** and **5** has not been completely defined, they will have the same absolute stereochemistry as **4** following biosynthetic information and optical rotation data.

Compounds **6**—**9** were determined to be vitexin (**6**), apigenin 7-*O*-(6″-methyl ester)-glucuronide (**7**), luteolin 7-*O*-(6″-methyl ester) glucuronide (**8**) and podocarpusflavone A (**9**), respectively, by spectroscopic analysis including 2D-NMR techniques.

The minimum inhibitory concentration (MIC) of **1**—**5** against MRSA and methicillin-sensitive *S. aureus* (MSSA) was determined as well as that of two antibiotics (vancomycin and gentamycin), and the results are shown in Table 1. Among these compounds, **1** and **3** showed strong anti-MRSA activity. The MIC value of **3** ranged from 3.13 to 12.5 μg/ml against MRSA and is nearly equal to that of the antibiotic, vancomycin (6.25 μg/ml) which is currently used to treat MRSA infections. Compound **2** and a mixture of **4** and **5** were less active than **1** and **3**, which suggests that a chelated hydroxyl group at C-1 is involved in the inhibitory activity.

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MATERIALS AND METHODS

Apparatus The following instruments were used: melting points, Büchi melting point apparatus and are uncorrected; MS spectra, JOEL JMS-D300 (70 eV) instrument; ^{1}H- and ^{13}C-NMR spectra, JOEL JNM EX-400 tetramethylsilane (TMS) as internal standard), IR spectra (on KBr pellets), JASCO IR-AI spectrometer; UV (methanol solutions), Shimadzu UV-2200 spectrometer. The following adsorbents were used for purification: analytical TLC, Merck Kieselgel 60 F_{254}; column chromatography, Merck Kieselgel 60, Fuji Davison, Silica-gel BW-300, and Pharmacia Fine Chemicals AB, Sephadex LH-20 .

Plant Materials Dry pericarps of *G. purpurea* were donated by Ichimaru Pharcos Co., Ltd., Gifu, Japan and fruits of *G. subelliptica* were collected in Okinawa, Japan, in November, 1992. Specimens have been deposited in the herbarium of Gifu Pharmaceutical University, Japan.

Extraction and Purification *G. purpurea*: The dried pericarps (850 g) of *G. purpurea*, cut into small segments, were extracted with MeOH (3 l × 5) for 48 h at room temperature. After removal of the solvent, the residue (300 g) was suspended in water (1.5 l) and partitioned with benzene (1.5 l × 5, 45 g), ethyl acetate (EtOAc, 1.5 l × 5, 10 g) and *n*-BuOH (1.5 l × 5, 50 g), successively. The benzene-soluble extract (30 g) was subjected to vacuum liquid chromatography (VLC) on silica-gel (1.5 kg) with *n*-hexane–EtOAc system to give **1** (5 g) from a *n*-hexane–EtOAc (10 : 1) and **2** (800 mg) following *n*-hexane–EtOAc (5 : 1) elution. The EtOAc-soluble extract (5 g) was also subjected to VLC on silica-gel with a $CHCl_3$–MeOH system to give six fractions. The second fraction (10 : 1, 50 mg) was further purified by Sephadex LH-20 with MeOH to give **6** (5 mg) and **7** (7 mg). The third fraction was recrystallized from acetone to give **8** (5 mg).

G. subelliptica: The fresh fruits of *G. subelliptica* were separated their pericarps and seeds. The fresh pericarps (24 kg), cut into small pieces, were extracted with MeOH (18 l × 3) for 48 h at room temperature. After evaporation of the solvent, part (1 kg) of the crude residue (2.2 kg) was suspended in water (3 l) and partitioned as mentioned above to give benzene (3 l × 5, 25 g), EtOAc (3 l × 5, 18 g), *n*-BuOH (3 l × 5, 220 g) extracts, respectively. The benzene-soluble extract (20 g) was chromatographed on silica-gel (2 kg) with an *n*-hexane–EtOAc system to give six fractions. The second fraction (5 : 1) was recrystallized from *n*-hexane to give **3** (9 g). The fourth fraction (5 : 1, 1.3 g) was further purified by VLC on silica-gel (100 g) with *n*-hexane–EtOAc (5 : 1) to give a crude amorphous powder (200 mg) composed of **4** and **5**. To separate the mixture of **4** and **5**, repeated purification by chromatography including HPLC and recrystallization, and derivatization (methylation and acetylation) were tried, but all were unsuccessful. The EtOAc-soluble extract (15 g) was chromatographed on silica-gel (1 kg) in the $CHCl_3$–MeOH system to give **9** (15 mg).

Garcinol (**1**): Pale yellow needles, mp 123—124°C (*n*-hexane). $[\alpha]_D^{24}$ −138° (c=0.1, $CHCl_3$). IR ν_{max} cm^{-1}: 3560, 2960, 2920, 1730, 1635, 1605, 1530, 1450, 1295, 1195. UV λ_{max} nm (log ε): 233 sh, 278 (4.43), 348 sh. MS *m/z* (rel. int. %): 602 (M^+, 42), 533 (19), 465 (100), 449 (14), 411 (15), 341 (64), 231 (63), 137 (47), 69 (65).

Isogarcinol (**2**): A colorless amorphous powder from *n*-hexane–EtOAc (50 : 1). $[\alpha]_D^{24}$ −224° (c=0.1, MeOH). IR ν_{max} cm^{-1}: 3470, 3370, 2990, 2930, 1720, 1680, 1640, 1605, 1520, 1445, 1365, 1300, 1185. UV λ_{max} nm (log ε): 233 (4.34), 277 (4.41), 311 sh. MS *m/z* (rel. int. %): 602 (M^+, 34), 574 (41), 465 (100), 449 (34), 410 (10), 397 (10), 341 (80), 231 (46), 137 (50), 69 (55).

Xanthochymol (**3**): Pale yellow needles, mp 130—131 °C (*n*-hexane). $[\alpha]_D^{24}$ 138° (c=0.1, $CHCl_3$). IR ν_{max} cm^{-1}: 3290, 2920, 1725, 1625, 1605, 1520, 1440, 1295, 1190. UV λ_{max} nm (log ε) : 230 sh, 276 (4.30), 351 sh. MS *m/z* (rel. int. %): 602 (M^+, 5), 533 (3), 465 (42), 449 (8), 411 (5), 341 (34), 231 (34), 137 (58), 69 (100). ^{1}H-NMR [400 MHz, CD_3OD containing 0.1% TFA] δ: 1,00 (3H, s, H-23), 1.16 (3H, s, H-22), 1.46 (2H, m, H-34), 1.48 (1H, m, H-6), 1.50 (3H, s, H-28), 1.58 (3H, s, H-38), 1.61 (3H, s, H-33), 1.65 (3H, s, H-27), 1.69 (3H, s, H-21), 1.73 (3H, s, H-20), 1.85 (2H, m, H-35), 1.92 (1H, dd, J=13.9, 5.6 Hz, H-29), 2.02 (1H, m, H-29), 2.03 (1H, m, H-24), 2.05 (1H, m, H-7), 2.11 (1H, m, H-24), 2.25 (1H, br d, J=14.2 Hz, H-7), 2.55 (1H, m, H-30), 2.58 (1H, m, H-17), 2.70 (1H, dd, J=13.9, 8.8 Hz, H-17), 4.51 (2H, brs, H-32), 4.63 (2H, br d, J=6.4 Hz, H-37), 4.86 (1H, m, H-25), 5.03 (1H, m, H-18), 6.71 (1H, d, J=8.3 Hz, H-15), 6.98 (1H, dd, J=8.3, 2.0 Hz, H-16), 7.19 (1H, d, J=2.0 Hz, H-12). ^{13}C-NMR (100 MHz, CD_3OD/0.1% TFA) δ: 17.8 (C-33), 18.2 (C-28), 18.3 (C-21), 22.8 (C-38), 23.2 (C-22), 26.0 (C-27), 26.4 (C-20), 27.1 (C-17), 27.4 (C-23), 30.3 (C-24), 32.8 (C-34), 36.8 (C-35), 37.7 (C-29), 43.8 (C-7), 44.7 (C-30), 48.0 (C-6), 50.2 (C-5), 59.8 (C-8), 69.6 (C-4), 110.4 (C-37), 113.5 (C-32), 115.1 (C-15), 117.4 (C-12), 117.9 (C-2), 121.3 (C-18), 125.2 (C-16), 125.6 (C-25), 129.6 (C-11), 133.6 (C-26), 135.8 (C-19), 147.0 (C-13), 148.9 (C-31), 149.5 (C-36), 152.5 (C-14), 194.3 (C-3), 196.0 (C-10), 196.3 (C-1), 210.6 (C-9).

Isoxanthochymol (**4**) and Cycloxanthochymol (**5**): An inseparable colorless amorphous powder from *n*-hexane–EtOAc (50 : 1), composed of a *ca.* 3 : 2 mixture. $[\alpha]_D^{24}$ 158° (c=0.1, MeOH). IR ν_{max} cm^{-1}: 3475, 3350, 2965, 2930, 1715, 1680, 1640, 1605, 1520, 1445, 1375, 1295, 1180. UV λ_{max} nm (log ε): 233 (4.35), 278 (4.42), 310 sh. High-resolution MS Calcd for $C_{38}H_{50}O_6$: 602.3607. Found: 602.3588. MS *m/z* (rel. int. %): 602 (M^+, 35), 574 (37), 533 (11), 465 (100), 449 (36), 410 (11), 397 (9), 341 (73), 231 (37), 137 (48), 69 (51). Compound **5**: ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.78 (3H, s, H-32), 0.91 (3H, s, H-23), 1.01 (1H, br d, J=13.1 Hz, H-29), 1.02 (1H, m, H-34), 1.05 (3H, s, H-22), 1.17 (3H, s, H-33), 1.24 (1H, m, H-30), 1.46 (1H, m, H-6), 1.47 (1H, m, H-34), 1.52 (3H, s, H-21), 1.60 (6H, s, H-20, 28), 1.65 (3H, s, H-27), 1.68 (3H, s, H-38), 1.97 (1H, m, H-7), 2.04, 2.56 (1H each, m, H-24), 2.06, 2.20 (1H each, m, H-35), 2.13 (1H, br d, J=14.6 Hz, H-7), 2.33, 2.49 (1H each, m, H-17), 2.90 (1H, dd, J=14.2, 3.4 Hz, H-29), 4.73 (2H, br s, H-37), 4.78 (1H, m, H-18), 4.90 (1H, m, H-25), 6.68 (1H, d, J=6.8 Hz, H-15), 6.84 (1H, dd, J=8.3, 2.0 Hz, H-16), 7.17 (1H, d, J=2.0 Hz, H-12). ^{13}C-NMR (100 MHz, DMSO-d_6) δ:

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17.8 (C-21, 28)[a], 20.8 (C-33), 21.9 (C-22, 38)[b], 24.9 (C-17), 25.6, 25.7 (C-20, 27)[c], 26.1 (C-23), 27.0 (C-29), 27.6 (C-34), 28.0 (C-32), 28.8 (C-24), 34.8 (C-35), 38.9 (C-7), 41.1 (C-30), 45.2 (C-6), 45.6 (C-5), 50.9 (C-8), 67.5 (C-4), 86.6 (C-31), 110.7 (C-37), 114.6 (C-15), 114.7 (C-12), 120.3 (C-18), 122.2 (C-16), 125.1 (C-25), 124.8 (C-2), 128.9 (C-11), 131.8 (C-19), 132.7 (C-26), 144.5 (C-36), 145.2 (C-13), 150.8 (C-14), 170.4 (C-1), 191.3 (C-10), 193.2 (C-3), 206.3 (C-9) (a, b: overlapping; c: interchangeable).

Reduction of 4 and 5 to Give 4a An ethanol solution (4 ml) containing a mixture of **4** and **5** (15 mg) was stirred with Pd–C (50 mg) under an H_2 atmosphere for 2 h at room temperature. The reaction mixture was recrystallized from *n*-hexane to give a hexahydro-derivative of isoxanthochymol (**4a**) (12 mg). Compound **4a**: a pale red amorphous powder. $[\alpha]_D^{22}$ +49° ($c=0.1$, MeOH). UV λ_{max} nm (log ε): 232 (4.17), 279 (4.26), 310 sh. High-resolution MS Calcd for $C_{38}H_{56}O_6$: 608.4076. Found: 608.4091. Another identical hexahydro-derivative (**2a**) was obtained from **2** (10 mg) by the same method. Compound **2a**: a pale red amorphous powder from *n*-hexane; $[\alpha]_D^{22}$ −72° ($c=0.1$, MeOH). High-resolution MS Calcd for $C_{38}H_{56}O_6$: 608.4076. Found: 608.4048. The structures of **2a** and **4a** are identical except for their optical rotation.

Growth of MRSA and Sensitivity Testing Strains of MRSA and MSSA isolated from patients in the hospital attached to 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 MIC were determined by the liquid dilution method, essentially as described by the Association of Chemotherapy Japan.[14] Inocula were prepared by dilution of 18 h broth (10^8—10^9 cells/ml) with buffered saline to 1×10^6 colony-forming units/ml. The inoculated test-tubes were incubated at 37 °C for 18 h in aerobic culture. The acetone solutions of test samples were serially diluted with acetone and added to the medium so that concentrations of 3.13—50.0 μg/ml were obtained in the medium. The MIC was defined as the lowest concentration of antimicrobial agent in the liquid medium resulting in complete inhibition of visible growth. Vancomycin and gentamycin were purchased from Sigma.

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