# An Antibacterial Biphenyl Derivative from *Garcinia bancana* Miq.

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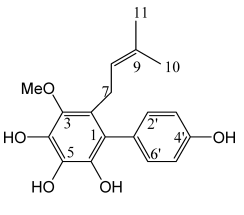
**From the methanol extract of the twigs and leaves of *Garcinia bancana* Miq., one new biphenyl derivative (1), was isolated and characterized along with nine known compounds; garcinol, isogarcinol, (−)-mellein, 8-hydroxy-6-methoxy-3-*n*-pentylisocoumarin, blumenol C *O*-β-D-glucoside, quercetin 3-*O*-α-L-rhamnoside, kaemferol 3-*O*-α-L-rhamnoside, lupeol and stigmasterol. Their structures were determined by analysis of 1D and 2D NMR data and comparison of spectral data and physical data with those previously reported. The antibacterial activity against methicillin-resistant *Staphylococcus aureus* was evaluated. Garcinol showed the lowest minimum inhibition concentration (MIC) at 16 μg/ml while compound 1 exhibited weaker activity with MIC value of 64 μg/ml.**

**Key words** *Garcinia bancana*; biphenyl; prenylated benzophenone; isocoumarin; flavone rhamnoside; antibacterial activity

*Garcinia bancana* Miq., belonging to the Guttiferae family, is distributed throughout Southern Thailand, Malaysia and Indonesia.[1] In our continuing phytochemical investigation of *Garcinia* plants found in Southern Thailand, we have examined the twigs and leaves of *G. bancana*. To our knowledge, no chemical investigation of *G. bancana* has been reported. This investigation led to the isolation and structural elucidation of one new biphenyl derivative (**1**) and seven known compounds from the twigs; two prenylated benzophenones [garcinol and isogarcinol],[2—5] two isocoumarins [(−)-mellein (**2**)[6] and 8-hydroxy-6-methoxy-3-pentylisocoumarin (**3**)[7]], blumenol C *O*-β-D-glucoside (**4**),[8] lupeol, stigmasterol, and two flavone rhamnosides from the leaves; quercetin 3-*O*-α-L-rhamnoside and kaemferol 3-*O*-α-L-rhamnoside.[9] This is the first report on the isolation of compounds **2** and **3** from plant. The structures of known compounds were identified by comparison of physical data and NMR spectral data with those previously reported. The structure of **4** was assigned on the basis of spectroscopic data of a tetraacetate derivative.

Coumpound **1** was found to have the molecualr formula of $C_{18}H_{20}O_5$ by HR-MS. The UV absorption bands at $\lambda_{max}$ 208 and 250 nm indicated the presence of benzene chromophore. The IR spectrum exhibited an absorption band due to a hydroxyl group at 3391 $cm^{-1}$. The $^1$H-NMR spectrum (Table 1) showed characteristic peaks for one *p*-disubstituted benzene unit [$\delta$ 7.08 (2H, d, $J$=8.0 Hz) and 6.78 (2H, d, $J$=8.0 Hz)], one prenyl group [$\delta$ 5.02 (1H, qqt, $J$=1.5, 1.5, 7.0 Hz), 3.16 (2H, d, $J$=7.0 Hz), 1.58 (3H, d, $J$=1.5 Hz) and 1.43 (3H, d, $J$=1.5 Hz)] and one methoxyl group ($\delta$ 3.81, 3H, s). HMBC correlations between the methylene protons ($H_2$-7, $\delta$ 3.16) and a quaternary carbon (C-9, $\delta$ 130.5) and those of the olefinic proton (H-8, $\delta$ 5.02) with two vinylic methyl carbons [C-10 ($\delta$ 17.7) and C-11 ($\delta$ 25.7)] confirmed the presence of the prenyl side chain. The position of the hydroxyl group at C-4′ of the *p*-disubstituted benzene unit was deduced from HMBC cross peaks between the *ortho* coupled protons ($\delta$ 7.08, H-2′, H-6′) with an oxygenated carbon ($\delta$ 155.2) assigned to C-4′. Futhermore, an HMBC correlation of H-2′ and H-6′ with C-1 ($\delta$ 134.0) of other fully substituted benzene unit established the biphenyl moiety. The prenyl group was determined to be at C-2 ($\delta$ 124.8) of the second benzene ring, *ortho* position to the *p*-hydroxybenzene substituent at C-1, by HMBC cross peaks between $H_2$-7 with C-1, C-2 and C-3 ($\delta$ 146.1). The methoxyl group was placed at C-3, adjacent to the prenyl group due to its HMBC correlation with C-3. Since no other proton signals were observed, the remaining substituents in the second benzene ring were hydroxyl groups. Signal enhancement of the methoxy protons and H-2′ and H-6′ of the *p*-disubstituted benzene ring, in the NOE difference spectrum, after irradiation of $H_2$-7 confirmed the location of these subtiutents. Thus, compound **1** was deter-




**1**

Table 1. $^1$H-, $^{13}$C-NMR and DEPT Spectral Data of Compound **1** (500 MHz and 125 MHz in $CDCl_3$)

| Position | **1** | | DEPT |
|---|---|---|---|
| | $^1$H | $^{13}$C | |
| 1 | — | 134.0 | C |
| 2 | — | 124.8 | C |
| 3 | — | 146.1 | C |
| 3-OMe | 3.81, s | 60.7 | $CH_3$ |
| 4 | — | 113.0[a)] | C |
| 5 | — | 114.9[a)] | C |
| 6 | — | 136.3 | C |
| 7 | 3.16, d, 7.0 Hz | 26.3 | $CH_2$ |
| 8 | 5.02, qqt, 1.5, 1.5, 7.0 Hz | 124.5 | CH |
| 9 | — | 130.5 | C |
| 10 | 1.43, d, 1.5 Hz | 17.7 | $CH_3$ |
| 11 | 1.58, d, 1.5 Hz | 25.7 | $CH_3$ |
| 1′ | — | 133.5 | C |
| 2′, 6′ | 7.08, d, 8.0 Hz | 130.6 | CH |
| 3′, 5′ | 6.78, d, 8.0 Hz | 114.7 | CH |
| 4′ | — | 155.2 | C |

*a*) Interchangeable.

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mined as [1,1′-biphenyl]-2-(3-methyl-2-butenyl)-3-methoxy-4,4′,5,6-tetraol.

All compounds other than compound **3** were tested for antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) as some partially-purified fractions exhibited interesting activity against MRSA. Garcinol showed the most remarkable activity with minimum inhibition cocentration (MIC) value of 16 $\mu$g/ml while isogarcinol and the biphenyl derivative (**1**) exhibited weaker activity with MIC values of 32 and 64 $\mu$g/ml, respectively. Others gave weak activity with the same MIC value (>128 $\mu$g/ml). These results were in agreement with the previous investigation which indicated that garcinol gave lower MIC value than isogarcinol against MRSA.[11]

## Experimental

**General Experimental Procedures** Infrared spectra (IR) were obtained on a Perkin Elmer Spectrum GX FT-IR system. $^1$H- and $^{13}$C-NMR spectra were recorded on a Varian UNITY INOVA 500 MHz spectrometer using tetramethylsilane (TMS) as internal standard. EI and HR-EI mass spectra were measured on ThermoFinnigan MAT95XL spectrometer. Thin-layer chromatography (TLC) and precoated TLC were performed on silica gel 60 $GF_{254}$ (Merck). Light petroleum had bp 40—60 °C.

**Plant Material** The leaves and twigs of *G. bancana* were collected at the Pru Tao Daeng Wildlife Sanctuary, Naratiwath, Thailand, in June 2000. The plant (a voucher specimen No. VR0001) was identified by Professor Puangpen Siriraksa, Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla.

**Isolation** The crude MeOH extract (88 g) obtained from the twigs of *G. bancana* was subjected to column chromatography eluted with a gradient system of $CHCl_3$–MeOH to afford 12 fractions. Fraction 2 (520 mg, eluted with 100% $CHCl_3$) was further purified on Sephadex LH20 column chromatography using MeOH as eluent to yield 5 subfractions. The third subfraction (83.7 mg) gave **2** (4.2 mg) and **3** (4.4 mg) after column chromatography on silica gel using a gradient system of EtOAc–light petroleum. Lupeol (13.5 mg) was obtained from fraction 4 (205.6 mg, eluted with 0.2% MeOH–$CHCl_3$), upon column chromatography on silica gel with solvent mixtures of increasing polarity ($CHCl_3$–light petroleum), while stigmasterol (24.0 mg) precipitated from fraction 5 (662.8 mg, eluted with 0.2—0.7% MeOH–$CHCl_3$), upon standing at room temperature. Fraction 7 (2.03 g, eluted with 3% MeOH–$CHCl_3$) was subjected to column chromatography on reverse phase C-18 silica gel using a gradient system of MeOH–$H_2O$, starting from 80% MeOH–$H_2O$ up to 100% MeOH, to afford garcinol (106 mg). Isogarcinol (20.5 mg) precipitated from fraction 8 (7.6 g, eluted with 5% MeOH–$CHCl_3$), upon standing at room temperature. Further separation of fraction 10 (1.8 g, eluted with 10% MeOH–$CHCl_3$) on Sephadex LH20 column chromatography using MeOH as eluent afforded 3 subfractions. The second subfraction (21.2 mg, eluted with 3% MeOH–$CHCl_3$) was subjected to column chromatography on silica gel with a gradient system of $CHCl_3$–MeOH to yield **1** (5.7 mg). Purification of fraction 12 (950.6 mg, eluted with 20—50% MeOH–$CHCl_3$) on column chromatography using reverse phase C-18 silica gel and solvent mixtures of MeOH–$H_2O$ (starting from 50% MeOH–$H_2O$ up to 100% MeOH) yielded 3 subfractions. The second fraction (45 mg, eluted with 60% MeOH–$H_2O$) was subjected to column chromatography on reverse phase C-18 silica gel using 60% MeOH–$H_2O$ as eluent to afford 3 subfractions. Acetylation of the second subfraction (6.1 mg) with acetic anhydride in pyridine at room temperature gave tetraacetate of **4** (3.5 mg) after purification on silica gel column chromatography using $CHCl_3$ as eluent. The crude MeOH extract (48.4 g) obtained from the leaves of *G. bancana* was divided into two portions by dissolving in *n*-hexane. The *n*-hexane insoluble portion (46.74 g) was further dissolved in $CHCl_3$ to afford the $CHCl_3$ insoluble part (44.20 g). This insoluble part (2 g) was then subjected to column chromatography on silica gel using a mixture of $CHCl_3$, MeOH and water in a ratio of 65 : 25 : 3 as eluent to yield 4 fractions. The third fraction (220 mg) was subjected to Semi-preparative HPLC ($\mu$Bondapak® C-18 precoated column, 10 $\mu$m, 25×10 mm, Waters) with gradient elution (20% MeOH–$H_2O$ up to 100% MeOH within 35 min, flow rate 10 ml/min, UV 254 nm) to yield 7 subfractions. Quercetin 3-*O*-$\alpha$-L-rhamnoside (68.5 mg) and kaemferol 3-*O*-$\alpha$-L-rhamnoside (2.9 mg) were obtained from the fourth and sixth subfractions, respectively.

Compound **1**: Colourless viscous liquid. $^1$H-NMR ($CDCl_3$, 500 MHz) and $^{13}$C-NMR ($CDCl_3$, 125 MHz), see Table 1. IR (neat) cm$^{-1}$: 3391, 1600. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 208 (4.64), 250 (4.09). HR-EI-MS *m/z* 316.1311 (Calcd for $C_{18}H_{20}O_5$ [M]$^+$: 316.1346). EI-MS *m/z* 316 [M]$^+$, 301, 300, 286, 245, 243, 230, 229, 225, 213, 69.

**Antibacterial Activity** Minimum inhibitory concentrations (MICs) were determined by agar microdilution method.[10] The test substances were dissolved in DMSO (Merck, Germany). Serial two-fold dilutions of the test substances were mixed with melted Mueller–Hinton agar (Difco) in the ratio of 1 : 100 in microtiter plates with flat-bottomed wells (Nunc, Germany). Final concentration of the test substances in agar was ranged from 128—0.03 $\mu$g/ml. Methicillin-resistant *Staphylococcus aureus* (MRSA) was used as test microorganism which was isolated from the Songklanakarin Hospital, Thailand. The strain was maintained in the laboratory of the Department of Microbiology, Faculty of Science, Prince of Songkla University. Inoculum suspensions of $10^6$ CFU/ml (10 $\mu$l) were spotted on agar-filled wells. The inoculated plates were incubated at 35 °C for 18 h. MICs were recorded by reading the lowest substance concentration that inhibited visible growth. Vancomycin was used as positive control drug. Growth controls were performed on agar containing DMSO.

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