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Benzophenones of *Garcinia pseudoguttifera* (Clusiaceae)

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Abstract

Four biogenetically related benzophenones have been isolated from the Fijian *Garcinia pseudoguttifera*. They are: 6-hydroxy-2,4-dimethoxy-3,5-bis(3-methyl-2-butenyl)benzophenone (myrtiaphenone-A); 2,2-dimethyl-8-benzoyl-7-hydroxy-5-methoxy-6-(3-methyl-2-butenyl)benzopyran (myrtiaphenone-B); 2,6-dihydroxy-4-methoxy-3,5-bis(3-methyl-2-butenyl)benzophenone (vismiaphenone-C) and a new benzophenone, 2,2-dimethyl-8-benzoyl-3,7-dihydroxy-5-methoxy-6-(3-methyl-2-butenyl)-3,4-dihydrobenzopyran (pseudoguttiaphenone-A). Pseudoguttiaphenone-A could be biogenetically derived from vismiaphenone-C. The major component of *G. pseudoguttifera* was identified as eupha-8,24-dien-3β-ol. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Garcinia pseudoguttifera*; Clusiaceae; Heartwood; Benzophenones; Myrtiaphenones; Vismiaphenone-C; Pseudoguttiaphenone-A; Triterpene; Eupha-8,24-dien-3β-ol

1. Introduction

Six *Garcinia* species are recorded in Fiji (Parham, 1972). Out of the six, only two species, *G. pseudoguttifera* and *G. sessilis*, are recorded as medicinal plants in the South Pacific traditional system of medicine (Sotheeswaran, Doyle & Aalbersberg, 1998; Cambie & Ash, 1994). An extract of the leaves of *G. pseudoguttifera* is mixed with coconut oil and used to relieve pain in the limbs (Cambie & Ash, 1994). In this current investigation, a phytochemical study of *G. pseudoguttifera* was undertaken and the benzophenones: vismiaphenone-C (**1**), myrtiaphenone-A (**2**), myrtiaphenone-B (**3**); a new benzophenone named pseudoguttiaphenone-A (**4**), and a triterpene, eupha-8,24-dien-3β-ol were isolated from the heartwood extracts.

2. Results and discussion

Vismiaphenone-C (**1**), myrtiaphenone-A (**2**) and myrtiaphenone-B (**3**) were previously isolated by Spino, Lal, Sotheeswaran and Aalbersberg (1995) from another *Garcinia* species (*G. myrtifolia*) from the South Pacific.

The hexane extract of the heartwood of *G. pseudoguttifera* had a new benzophenone (pseudoguttiaphenone-A) in an overall yield of 0.026% and was separated by combined chromatographic techniques (vacuum liquid chromatography (VLC) and repeated preparative thick layer chromatography (PTLC)). The high resolution-electron ionization mass spectra (HR-EIMS) (M^+, m/z 396.1934) indicated pseudoguttiaphenone-A to have the formula, $C_{24}H_{28}O_5$.

Intense peaks in the mass spectrum at m/z 77 $[Ph]^+$ and at m/z 105 $[Ph\text{-}CO]^+$ indicated the new natural product to be a benzophenone with a monosubstituted benzene ring (Locksley & Murray, 1971).

The ^{13}C- and ^1H-NMR signals at [δ_H 7.54–7.50 (*m*, 2H) and 7.48–7.26 (*m*, 3H), δ_C 141.8 (*s*, 1C), 130.8 (*d*,

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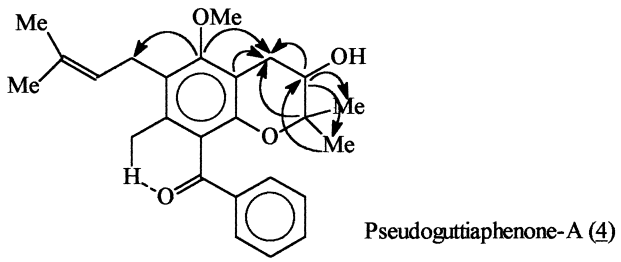


Fig. 1. Partial HMBC (long range) correlations.

1C), 127.8 (*d*, 2C (symmetrical)) and 127.4 (*d*, 2C (symmetrical))] confirmed the presence of the mono-substituted aromatic ring (Chaudhuri, Zymalkowski & Fraha, 1837).

The Infrared (IR), ^{13}C- and ^{1}H-NMR spectra were consistent with the presence of a conjugated carbonyl group [ν_{max} 1641 cm^{-1}, δ_C 198.9 (*s*, 1C)] and a hydrogen bonded phenolic hydroxyl group [ν_{max} 3700–2800 cm^{-1}, δ_C 161.2 (*s*, 1C) and δ_H 12.21 (*s*, 1H)], a methoxy group attached to an aromatic ring [δ_C 58.9 (*q*, 1C), δ_H 3.94 (*s*, 3H)], an isoprenyl moiety also attached to an aromatic ring [δ_C 112.7 (*s*, 1C), 122.9 (*d*, 1C), 25.8 (*q*, 1C), 22.1 (*t*, 1C) and 17.8 (*q*, 1C), δ_H 5.22 (*tm*, 1H, J = 6.9 Hz), 3.30 (*dm*, 2H, J = 6.9 Hz), 1.79 (*d*, 3H, J = 1.2 Hz) and 1.70 (*d*, 3H, J = 1.2 Hz)] and a 2,2-dimethylchroman ring with a hydroxyl substituent [(δ_C 90.5 (*d*, 1C), 71.2 (*s*, 1C), 29.3 (*t*, 1C), 23.7 (*q*, 1C,), and 25.6 (*q*, 1C); δ_H 5.01 (*s*, 1H), 4.32 (*t*, 1H), 3.15 (*d*, 2H), 1.04 (*s*, 3H) and 0.87 (*s*, 3H)].

The ^{13}C-NMR chemical shifts also showed the presence of a fully substituted aromatic ring [δ_C 162.9 (*s*), 161.2 (*s*), 160.9 (*s*), 112.7 (*s*), 105.8 (*s*) and 102.7 (*s*)].

These spectroscopic data, coupled with consideration of the structures of benzophenones isolated from the genus *Garcinia*, particularly vismiaphenone-C (**1**), myrtiaphenone-A (**2**) and myrtiaphenone-B (**3**) were consistent with pseudoguttiaphenone-A having a ben-

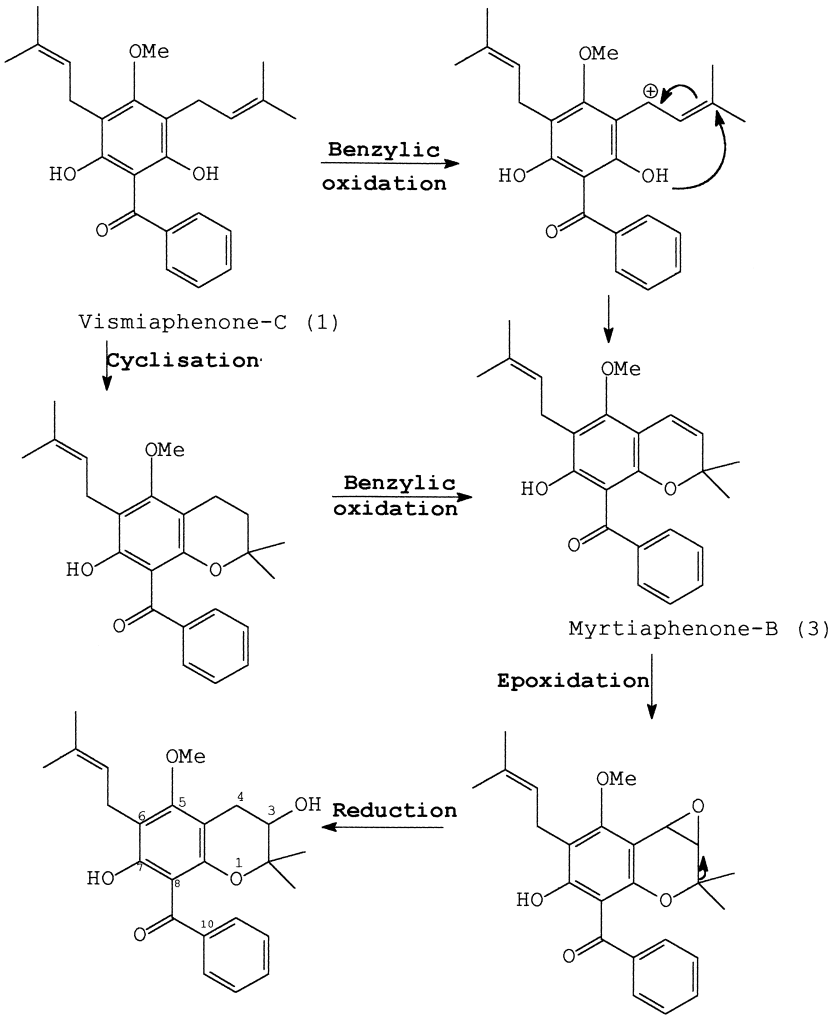
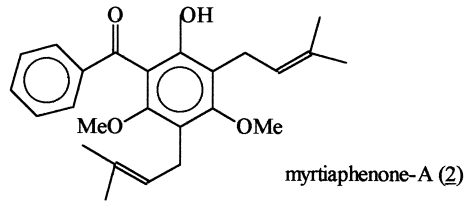


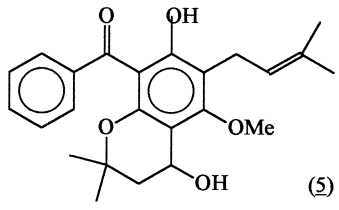
Fig. 2

zophenone structure with a hydroxyl substituted 2,2-dimethylchroman ring. Thus, two structures (**4**) and (**5**) were possible for pseudoguttiaphenone-A. Structure (**4**) is likely to be correct since the calculated ^{13}C-NMR spectral chemical shift of the hydroxyl substituted methine carbon was 89.2 ppm, which was closer to the observed value [δ_C 90.5 (*d*, 1C)] when compared with the calculated (Silverstein, Bassler & Morril, 1981) value of 59.2 ppm for the methine carbon in (**5**). A similar calculation for the CMR shifts of the carbon containing the benzylic proton(s) of the 2,2-dimethyl chroman ring gave a value of 31.4 ppm for structure **4** and a value of 56.2 ppm for structure **5**. The experimental value (29.3 ppm) agreed with the structure **4** for pseudoguttiaphenone-A. A 2D long-range CH experiment (HMBC) was conducted to confirm the structure of pseudoguttiaphenone-A. The partial HMBC correlations relevant to the assignment of the OH group are given in Fig. 1. The hydroxyl function introduces an asymmetry in the molecule whereby both the gem-dimethyl groups were different by 0.2 ppm in the proton NMR and 2 ppm in the carbon NMR chemical shifts. Though the gem-dimethyls were diastereotopic, the benzylic methylene protons of the 2,2-dimethyl chromanol ring were found to be equivalent by coincidence. The stereochemistry at the methine carbon was not determined.

The name pseudoguttiaphenone-A was derived from the botanical name of the plant, *G. pseudoguttifera*, from which the new metabolite was isolated. From a biosynthetic point of view, pseudoguttiaphenone-A could be formed from vismiaphenone-C via a cyclisation between a hydroxyl and isoprenyl group and epoxidation in the 2,2-dimethylchroman ring as shown in Fig. 2.



myrtiaphenone-A (2)



(5)

3. Experimental

The heartwood of *G. pseudoguttifera* was collected and identified by Mr. Saula Vodonaivalu of the South Pacific Regional Herbarium, Suva, Fiji. A voucher specimen was deposited at the Herbarium. IR spectra were determined on a Shimadzu 470 infrared spectrophotometer. The 1601 and 906.7 cm^{-1} absorptions of polystyrene were used as reference peaks. Fourier transformation infrared spectra were obtained using Perkin–Elmer paragon 1000. All proton nuclear magnetic resonance (^{1}H-NMR) and carbon nuclear magnetic resonance (^{13}C-NMR) spectra were taken on a Bruker AC-300 spectrophotometer (^{1}H-NMR at 300 MHz and ^{13}C-NMR at 75 MHz) using tetramethylsilane as an internal standard. The signals observed are described in terms of chemical shift (δ), multiplicity, coupling constant(s) where applicable, number of protons and assignment. The abbreviations *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartlet), *m* (multiplet), *dm* (doublet of multiplet), and *tm* (triplet of multiplet) have been used. EIMS were measured at 70 eV on a VG Micromass ZAB-2F spectrophotometer. Kofler hot stage microscope melting point apparatus was used to determine melting points (mp) and are uncorrected. Chromatotron model 7924T (Harrison Research serial number T21 Patented) was used for centrifugal thin layer chromatography (TLC). Silica gel PF_{254} 2 mm thick plates were used in this separation. VLC was carried out using silica gel type 60 (Merck) and for column chromatography silica gel 60, particle size 230–400 mesh ASTM (Merck) were used. PTLC utilized silica gel 60 PF_{254} spread on 20 × 20 cm glass plates, 1 mm thick. Analytical TLC was performed on 20 × 20 cm pre-coated plates (E. Merck, Germany, Art. 5554 kieselgel 60 F_{254}) of 0.20 mm thickness.

Dried and powdered heartwood (800 g) of *G. pseudoguttifera* was extracted with *n*-hexane (2 l) followed by dichloromethane (1 l). Evaporation of solvent yielded 73 g of *n*-hexane and 10 g of CH_2Cl_2 extracts. The components in both extracts were found to be identical when examined on TLC. The *n*-hexane extract (1 g) was chromatographed on a column (gravity) over Merck silica gel and was eluted with *n*-hexane and *n*-hexane/EtOAc. The consecutive fractions (each 40 ml) were eluted in order with the indicated solvents: 1–4 (hexane), 5–8 (95 : 5 *n*-hexane/EtOAc), 9–12 (90 : 10 *n*-hexane/EtOAc), 13–18 (85 : 15 *n*-hexane/EtOAc), 19–23 (70 : 30 *n*-hexane/EtOAc), 24–29 (40 : 60 *n*-hexane/EtOAc), 30–37 (EtOAc).

PTLC of combined fractions 6–7 (35 mg) on silica gel using Merck with EtOAc/*n*-hexane (10 : 90) as the developing solvent gave a yellow gum (23 gm) which on TLC examination showed two spots, myrtiaphenone-A (**2**) (major) and myrtiaphenone-B (**3**). Repeated chromatographic attempts failed to separate the two benzophenones; however, the ^{1}H- and ^{13}C-NMR signals were well separated and identifiable for

each benzophenone except for the phenyl protons. PTLC of fractions 13–17 (120 mg) using silica gel and with EtOAc/*n*-hexane (10 : 90) as the eluent gave crude vismiaphenone-C (**1**) (90 mg) which was further purified using PTLC with EtOAc/*n*-hexane (15 : 85) as the eluent. Pure vismiaphenone-C (**1**) (41 mg) was isolated as a yellow gum. A portion of the hexane extract (2 g) was subjected to VLC on silica gel to yield 12 fractions (150 ml each) with the following indicated solvents: 1–5 (20 : 80), 6–7 (40 : 60), 8–9 (50 : 50), 10–12 (60 : 40) CH_2Cl_2/*n*-hexane. On extended purification using VLC, the residue from combined fractions 8 and 9 (95 mg) gave 34 50 ml subfractions: *n*-hexane (1–10), 99 : 1 *n*-hexane/CH_2Cl_2 (11–14), 98 : 2 *n*-hexane/CH_2Cl_2 (15–18), 95 : 5 in-hexane/CH_2Cl_2 (19–22), 50 : 50 *n*-hexane/CH_2Cl_2 (23–34). Part of the residue from combined subfractions 26–28 (16 mg) on further purification using PTLC and developing solvent 20 : 80 EtOAc/*n*-hexane, yielded yellow, gummy pseudoguttiaphenone-A (**4**) (6 mg).

About 18 g of *n*-hexane extract was subjected to VLC on silica gel to yield 20 (100 ml each) fractions, 1–8, 9–18, 19–20, on successive elution with *n*-hexane, 95 : 5 *n*-hexane/EtOAc, and 90 : 10 *n*-hexane/EtOAc, respectively. Purification of fraction 12 (0.2 g) on chromatotron using 95 : 5 *n*-hexane/EtOAc gave a crude triterpene (72 mg). PTLC of 10 mg of the crude triterpene using silica gel and 2 : 98 EtOAc/*n*-hexane as eluent gave the pure triterpene (8 mg), which was isolated as a white solid, mp 117–119°C and was identified (Spino et al., 1995) as eupha-8,24-dien-3β-ol.

The spectral data for vismiaphenone-C (**1**), myrtiaphenone-A (**2**) and myrtiaphenone-B (**3**) were identical to those published previously (Spino et al., 1995). Complete ^{1}H- and ^{13}C-NMR data for pseudoguttiaphenone-A (**4**) are given in Section 2.

3.1. Pseudoguttiaphenone-A (*4*)

EIMS (probe) 70 eV, *m/z* (relative intensity): 396 $[M]^+$ (100); 381 $[M - 15]^+$ (15); 363 (32); 341 $[M - 55]^+$ (31); 323 (10.6); 309 (7.5); 149 (79); 105 $[PhCO]^+$ (65); 84 (61); 77 $[Ph]^+$ (26). $[\alpha]_D^{25} = +2.48$ (*c* 1.1 $CHCl_3$).

Molecular formula: $C_{24}H_{28}O_5$; exact mass calculated: 396.1937, found: 396.1934.

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