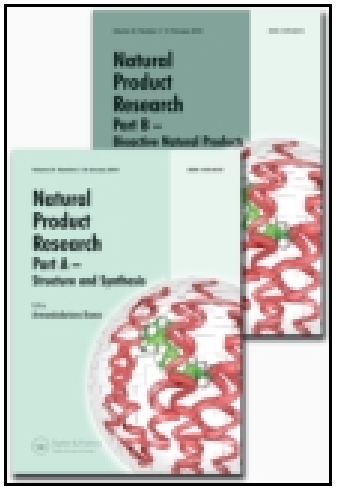


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# A new antioxidant flavonoid from the lianas of Gnetum macrostachyum

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# A new antioxidant flavonoid from the lianas of *Gnetum macrostachyum*

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A new flavonoid, named 5,7,2′-trihydroxy-5′-methoxyflavone (**1**), was isolated from the acetone extract of the lianas of *Gnetum macrostachyum* together with 5,7,4′-trihydroxy-3′-methoxyflavanone (**2**) and seven known stilbenoids (**3**–**7**). The structure of the new compound was determined by interpretation of its spectral data and chemical transformation. The isolated compounds showed radical scavenging activity against DPPH with $IC_{50}$ values in the range of 0.21–19.90 mM.

**Keywords:** *Gnetum macrostachyum*; Gnetaceae; 5,7,2′-trihydroxy-5′-methoxyflavone; antioxidation



## 1. Introduction

The genus *Gnetum* (Gnetaceae) consists of approximately 40 species, distributed in South America (Amazon region), Southwest Africa, and the tropical and subtropical zones of Asia (Ali et al., 2003). The family Gnetaceae has been recognised as an abundant source of stilbene oligomers, in addition to the families Cyperaceae, Dipterocarpaceae, Leguminosae and Vitaceae (Sotheeswaran & Pasupathy, 1993). Resveratol is one of the prominent stilbenoids, which possess various intriguing biological activities, such as antioxidant, antimutagenic, being an inducer of phase II drug-metabolising enzymes, antifungal, anti-inflammatory, antiviral, antibacterial and antiplatelet aggregation (Huang, Lin, & Cheng, 2001; Huang, Tsai, Shen, & Chen, 2005; Kim, Saleem, Seo, Jin, & Lee, 2005; Pryce & Langcake, 1977; Villano, Fernandez-Pachon, Moya, Troncoso, Garcia-Parrilla, 2007). In our continuing search for antioxidant compounds, the $CH_2Cl_2$ extract of *Gnetum macrostachyum* lianas exhibited potent radical scavenging towards DPPH. This article deals with the isolation and structure elucidation of a new flavonoid, **1**, including antioxidant activity of the isolated compounds (Figure 1).

## 2. Results and discussion

5,7,2′-Trihydroxy-5′-methoxyflavone (**1**) was isolated as an amorphous yellow solid [m.p. 269°C (dec)]. The molecular formula was established to be $C_{16}H_{12}O_6$, based on the

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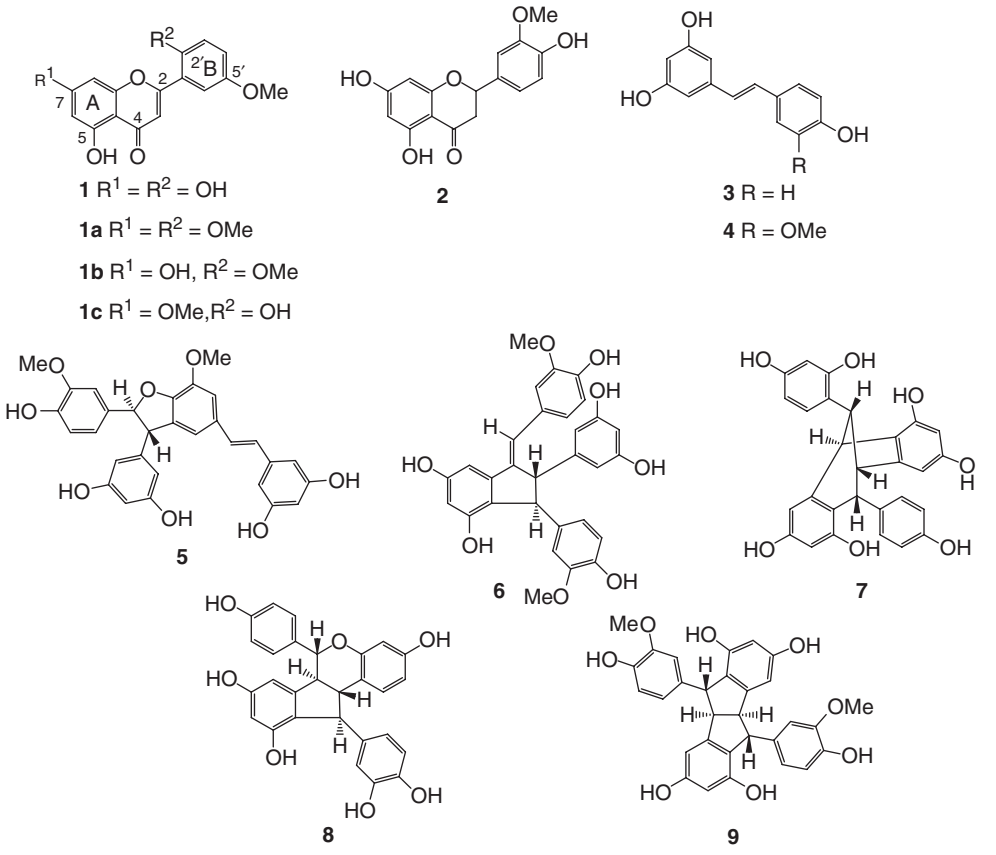


Figure 1. Flavonoids and stilbenoids from *G. macrostachyum*.

$[M + Na]^+$ ion peak at $m/z$ 323.0517 and $^{13}C$ NMR data. The UV spectrum of **1** exhibited maximal absorption bands at 270 and 345 nm, which are typical of a flavonoid moiety (Mabry, Markham, & Thomas, 1970).

The IR spectrum showed absorptions of a hydroxyl group (3347 $cm^{-1}$), a carbonyl group (1647 $cm^{-1}$), a carbon ether bond (1347 $cm^{-1}$), and an aromatic moiety (1600–1400 $cm^{-1}$). The $^1H$ NMR spectrum (Table 1) revealed the singlet resonance of a chelated hydroxyl proton at $\delta_H$ 12.90 (5-OH), in addition to a pair of exchangeable hydroxyl protons ($\delta_H$ 10.42 and 9.30). The signals in the aromatic region ($\delta_H$ ca 6.0–7.8) were assigned as two isolated spin systems, 6.20 and 6.46 (each 1H, d, $J = 2.0$ Hz), for an AB system, as well as 6.95 (1H, d, $J = 8.4$ Hz), 7.51 (1H, d, $J = 8.4$ Hz) and 7.53 (1H, brs) for an ABX system. The $^{13}C$ NMR spectrum showed 16 signals, three of which ($\delta_C$ 182.1, 164.0 and 103.3) were indicative of a flavone moiety. The overall structure of **1** was deduced mainly based on HMBC data (Figure 2). In ring A, the correlations of 5-OH/C-5, C-6 and C-10 and 7-OH/C-6, C-7 and C-8 indicated that C-5 and C-7 were accommodated by two hydroxyl groups. The methoxy group ($\delta_H$ 3.95) was placed in ring B at C-5′ ($\delta_C$ 149.3), which was in turn coupled to H-3′ and H-6′. This assignment was further confirmed by NOESY cross peaks of 5′-OMe/H-4′ and H-6′/5′-OMe, in addition to those of H-3′/H-4′ and H-3/H-6′. The presence of the hydroxyl group was also supported by the

Table 1. $^{1}H$ and $^{13}C$ NMR spectral data (DMSO-$d_6$) of **1**.

| Position | $\delta_H$ (mult, $J$ in Hz) | $\delta_C$ | HMBC |
|---|---|---|---|
| 1 | | | |
| 2 | | 164.0 | |
| 3 | 6.65 (s) | 103.3 | C-2, 4, 10, 1′ |
| 4 | | 182.1 | |
| 5 | | 162.0 | |
| 6 | 6.20 (d, 2.0) | 99.0 | C-5, 7, 8, 10 |
| 7 | | 164.2 | |
| 8 | 6.46 (d, 2.0) | 93.9 | C-6, 7, 9, 10 |
| 9 | | 157.8 | |
| 10 | | 104.0 | |
| 1′ | | 122.4 | |
| 2′ | | 150.9 | |
| 3′ | 6.95 (d, 8.4) | 115.7 | C-1′, 2′, 5′ |
| 4′ | 7.51 (d, 8.4) | 120.3 | C-2′, 6′ |
| 5′ | | 149.3 | |
| 6′ | 7.53 (s) | 109.7 | C-2, 1′, 2′, 4′, 6′ |
| 5-OH | 12.90 (s) | | C-5, 6, 10 |
| 7-OH | 10.42 (s) | | C-6, 7, 8, |
| 2′-OH | 9.30 (s) | | |
| 5′-OMe | 3.93 (s) | 55.7 | C-5′ |

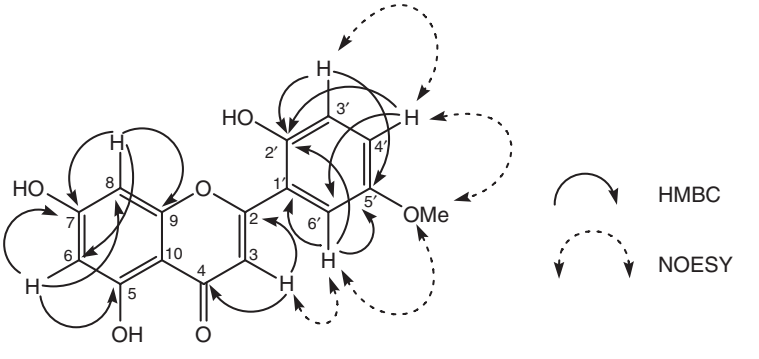


Figure 2. Key HMBC and NOESY correlations of **1**.

formation of methyl ethers of **1**. Treatment of **1** with trimethylsilyldiazomethane ($TMSCHN_2$) yielded **1a** as the major product, together with two minor methyl ethers, **1b** and **1c**. Two additional methoxy groups newly generated in **1a** indicated the presence of 7-OH and one hydroxyl group on ring B, in addition to chelated 5-OH. Therefore, the structure of 5,7,2′-trihydroxy-5′-methoxyflavone was depicted for **1**.

All isolated compounds were subjected to an examination of their radical scavenging against DPPH (Table 2). Compound **1** showed slightly weak activity with an $IC_{50}$ value of 19.9 mM. Conversely, resveratol (**3**) and other stilbenoids (**4**–**9**) displayed stronger inhibition, with $IC_{50}$ values in the range of 0.21–4.23 mM: of which, gnetulin (**6**) was the most active. Stilbenes have been recognised as potent oxygen radical scavengers, possibly

Table 2. DPPH radical scavenging.

| Test compounds | DPPH radical scavenging ($IC_{50}$, mM) |
|---|---|
| **1** | 19.9 |
| **2** | 1.48 |
| **3** | 0.40 |
| **4** | 0.30 |
| **5** | 0.78 |
| **6** | 0.21 |
| **7** | 2.90 |
| **8** | 4.23 |
| **9** | 0.53 |
| Ascorbic acid | 0.11 |



through an oxidative coupling reaction forming stable oligostilbenes. A recent investigation has shown that stilbenes undergo oxidation by nitrite ions ($NO_2^-$), a key mediator in the inflammatory response and in carcinogenesis (Panzella et al., 2006). A comprehensive study on radical scavenging of stilbenes towards causative reactive species would provide an insight into the prevention of pathological diseases.

## 3. Experimental

### 3.1. *General experimental procedure*

Melting points were determined on a Fisher–John apparatus and are uncorrected. UV spectra were taken on a UV-160A spectrometer (SHIMADZU). IR spectra were recorded on a Nicolet Impact 410 spectrometer. HRESIMS and LCMS were obtained by a Micromass LCT mass spectrometer and a Micromass Quattro Micro$^{TM}$ API. NMR spectra were recorded on a Variant Mercury 400 spectrometer and chemical shifts were reported in parts per million reference to solvent residues ($\delta_H$ 2.49 and $\delta_C$ 39.7 ppm for DMSO-$d_6$ and $\delta_H$ 7.25 and $\delta_C$ 77.0 ppm for $CDCl_3$).

### 3.2. *Plant material*

The lianas of *G. macrostachyum* were collected in April 2006 from Nakornphanom. The plant material was identified by the Plants of Thailand Research Unit, Department of Botany, Faculty of Science, Chulalongkorn University, and the voucher specimen (BKF 108547) has been deposited in the Herbarium of the Royal Forest Department, Bangkok, Thailand.

### 3.3. *Extraction and isolation*

The dried and powdered lianas of *G. macrostachyum* (2.5 kg) were extracted with $CH_2Cl_2$, acetone and MeOH, respectively, in a Soxhlet extractor. A part of the acetone extract was stirred with acetone, yielding acetone soluble and insoluble fractions. The acetone soluble fraction (47.4 g) was fractionated on vacuum liquid chromatography (VLC) eluted with

$CH_2Cl_2$, MeOH : $CH_2Cl_2$ (10 : 90 → 50 : 50), MeOH, yielding seven fractions labelled 'F1' to 'F7'. Fraction F2 was purified on silica gel CC using $CH_2Cl_2$, MeOH : $CH_2Cl_2$, (5 : 95 → 50 : 50) and MeOH, to afford six fractions, labelled 'G1' to 'G6'. Fraction G3 was subsequently purified on silica gel CC using MeOH : $CH_2Cl_2$, (0 : 100 → 100 : 0) to afford 5,7,2′-trihydroxy-5′-methoxyflavone (**1**, 30 mg). A portion of fraction G4 (21 g) was purified on silica gel CC using $CH_2Cl_2$, MeOH : $CH_2Cl_2$, (0 : 100 → 100 : 0) and further purified on Sephadex LH 20 using MeOH : $CH_2Cl_2$ (50 : 50) to give resveratol (**3**, 200 mg) and 3-methoxyresveratol (**4**, 600 mg). Fraction G5 was purified on silica gel CC using $CH_2Cl_2$, MeOH : $CH_2Cl_2$, (0 : 100 → 100 : 0) and further purified on Sephadex LH 20 using MeOH : $CH_2Cl_2$ (50 : 50) to give 5,7,4′-trihydroxy-3′-methoxyflavanone (**2**, 12 mg) and shegansu B (**5**, 80 mg). Fraction F3 was purified on silica gel CC using $CH_2Cl_2$, MeOH : $CH_2Cl_2$, (5 : 95 → 50 : 50), MeOH to afford seven fractions, labelled 'H1' to 'H7'. Fraction H4 was purified on Sephadex LH 20 using MeOH : $CH_2Cl_2$ (50 : 50) to afford gnetulin (**6**, 25 mg), gnetuhainin C (**7**, 35 mg), parvifolol B (**8**, 20 mg) and pallidol (**9**, 11 mg).

**5,7,2′-Trihydroxy-5′-methoxyflavone (1):** Amorphous yellow solid; m.p. 269°C (dec); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 227 (3.09), 270 (2.93), 345 (3.06); IR (KBr) 3347, 3082, 1647, 1621, 1517, 1347, 1269, 1204, 1165, 826 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 400 MHz) and $^{13}C$ NMR (DMSO-$d_6$, 100 MHz), see Table 2; HRESIMS $m/z$ $[M + Na]^+$ 323.0517 (Calcd for $C_{16}H_{12}O_6Na$, 323.0526).

### 3.4. *Preparation of methyl ethers of 1*

Compound **1** (10 mg), dissolved in MeOH (2 mL), was added drop wise to 2.0 M trimethylsilyldiazomethane ($TMSCHN_2$) in hexane until yellow solution persisted. The mixture was stirred at room temperature for 1 h. After the reaction mixture was evaporated to dryness, the residue was purified on preparative TLC developed with 5 : 95 MeOH : $CH_2Cl_2$ to afford **1a** (7 mg), **1b** (2 mg) and **1c** (2 mg).

**1a:** Yellow powder; $^1H$ NMR ($CDCl_3$) $\delta$ 12.90 (1H, 5-OH), 3.86 (3H, s), 3.84 (3H, s), 3.79 (3H, s), 6.13 (1H, d, 2.0), 6.43 (1H, d, 2.0), 6.60 (1H, s), 7.03 (1H, d, 8.4), 7.55 (1H, d, 8.4), 7.50 (1H, s).

**1b:** Yellow powder; $^1H$ NMR ($CDCl_3$) $\delta$ 12.90 (1H, 5-OH), 3.80 (3H, s), 3.82 (3H, s), 6.20 (1H, d, 2.0), 6.60 (1H, d, 2.0), 6.65 (1H, s), 7.05 (1H, d, 8.4), 7.60 (1H, d, 8.4), 7.50 (1H, s).

**1c:** Yellow powder; $^1H$ NMR ($CDCl_3$) $\delta$ 12.90 (1H, 5-OH), 3.80 (3H, s), 3.85 (3H, s), 6.20 (1H, d, 2.0), 6.59 (1H, d, 2.0), 6.65 (1H, s), 6.90 (1H, d, 8.4), 7.50 (1H, d, 8.4), 7.55 (1H, s).

### 3.5. *DPPH radical scavenging*

Radical scavenging activity of the isolated compounds was validated using DPPH colorimetric method (Phuwapraisirisan, Udomchotphruet, Surapinit, & Tip-pyang, 2006; Wiboonpun, Phuwapraisirisan, & Tip-pyang, 2004). Briefly, a sample solution (0.25 mM, 0.5 mL) was added to 1 mL methanolic solution of DPPH (final concentration of DPPH was 0.3 mM). The mixture was vigorously shaken and kept in the dark for 30 min. The absorbance of the resulting solution was measured at 518 nm with a UV spectrometer.

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