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An antimalarial stilbene from *Artocarpus integer*

Choosak Boonlaksiri[a], Worrapoj Oonanant[a], Palangpon Kongsaeree[a,*], Prasat Kittakoop[b], Morakot Tanticharoen[b], Yodhathai Thebtaranonth[a,b]

[a]*Department of Chemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand*
[b]*National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 73/1, Rama VI Road, Bangkok 10400, Thailand*

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Abstract

Antimalarial activity-guided study of the aerial parts of *Artocarpus integer* led to the isolation of the prenylated stilbene, *trans*-4-(3-methyl-*E*-but-1-enyl)-3,5,2′,4′- tetrahydroxystilbene with an EC_{50} of 1.7 μg/ml against *Plasmodium falciparum* in culture. The known stilbenes, *trans*-4-isopentenyl-3,5,2′,4′-tetrahydroxystilbene and 4-methoxy-2,2-dimethyl-6-(2-(2,4-dihydroxy)phenyl-*trans*-ethenyl)chromene, were also isolated. Structures of these compounds were deduced on the basis of their spectral data.

Keywords: *Artocarpus integer*; Moraceae; Jumpada; 3,5,2′,4′-Tetrahydroxystilbene; Antimalarial activity; *Plasmodium falciparum*

1. Introduction

Plants of the genus *Artocarpus* are widely distributed throughout Thailand. While some are sources of highly valuable heartwood, others are used as folk medicines. For example, *A. lakoocha* and *A. chaplasha* are effectively used for the treatment of tapeworm infection (*Taeniasis saginata*) (Charoenlarp et al., 1981). *Artocarpus integer* Merr. (Moraceae) is commonly found in the South of Thailand and its edible fruits are popular among Thais. So far, only a few constituents from this plant, including catechin (Yamazaki et al., 1987) and lectin derivatives have been reported (Hashim et al., 1992, Lim et al., 1998). In a continuation of our research program on biologically active substances from plants and micro-organisms, the crude extract of the aerial parts of *A. integer* showed moderate in vitro antimalarial activity against *Plasmodium falciparum* with an EC_{50} of 6.8 μg/ml. We therefore investigated the antimalarial constituents of this plant and herewith present data on a new prenylated stilbene, together with two known related compounds. Though stilbenoids were found to exhibit various biological activities (Gorham, 1995), to our knowledge, this is the first report of their antimalarial activity.

2. Results and discussion

Separation of the CH_2Cl_2–MeOH extracts of *A. integer* on a Sephadex LH-20 column and reverse-phase HPLC yielded three stilbenes, **1**–**3**. Compounds **1** and **3** were identified by comparison of their NMR spectra with previously reported literature data (Shimizu et al., 1997; Takasugi et al., 1978).

The composition of **2** was deduced to be $C_{19}H_{20}O_4$ from the HR-EIMS mass spectrum and ^{1}H- and ^{13}C-NMR data. The structure of **2** was elucidated with ^{1}H- and ^{13}C-NMR spectral data by comparison with those of **1**, as well as 2D-NMR techniques (HMQC and HMBC). The ^{1}H-NMR spectrum of **2** suggested a structure similar to that of **1**, differing only in the

* Corresponding author. Tel.: +662-246-1358 X 1302; fax: +662-247-7050.

E-mail address: scpks@mahidol.ac.th (P. Kongsaeree).

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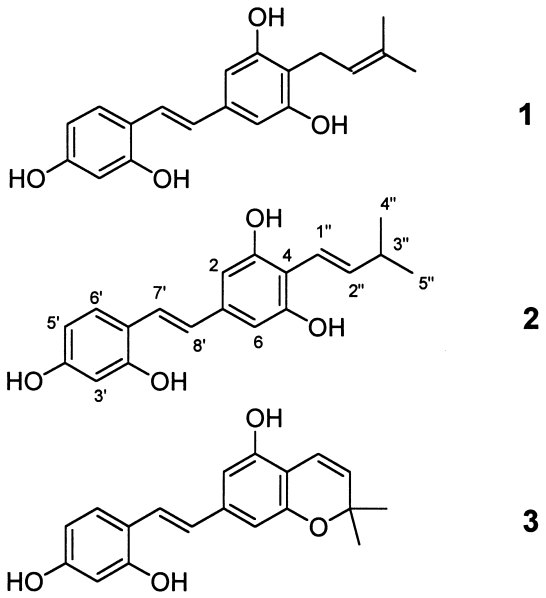
prenylated part. Notably, the chemical shifts of H-1″ (δ 3.25, *d*, 2H) and H-2″ (δ 5.23, *t*, 1H) were absent in **2**, while two methyl groups at C-4″ and C-5″ appeared as a doublet at δ 1.09 (J = 6.7 Hz) and the additional H-3″ signal appeared at δ 2.38. The coupling constant of 16.2 Hz (in DMSO-d_6) between H-1″ and H-2″ revealed the *trans* geometry of the C-1″–C–2″ double bond. From these differences, **2** was deduced to be *trans*-4-(3-methyl-*E*-but-1-enyl)-3,5,2′,4′-tetrahydroxystilbene. The ^{1}H–^{13}C long range correlation (HMBC) spectrum of **2** showed the correlations of H-2, H-6 and H-2″ to C-4, in agreement with the proposed structure.

Compounds **1**–**3** exhibited in vitro antimalarial activity against *P. falciparum* (K1, multidrug resistant strain) with the EC_{50} values of 8.2, 1.7 and 9.4 μg/ml, respectively.



3. Experimental

3.1. General

^{1}H-, ^{13}C-NMR, DEPT, HMQC and HMBC spectra were recorded on a Bruker DRX 400 spectrometer, operating at 400.1 MHz for proton and 100.6 MHz for carbon, with TMS as an internal standard. EIMS spectra were obtained from a Finnigan MAT (INCOS 50) mass spectrometer. UV spectra were taken on a Varian CARY1E spectrophotometer. HR-EIMS spectrum of **2** (70 eV) was obtained using a VG 7070E-HF mass spectrometer.

3.2. Plant material and isolation

Artocarpus integer Merr. was collected from Nakhon Srithammarat, in southern Thailand, in January 1999 and identified by comparison with an authentic herbarium specimen No. BKF 8475 deposited at the Royal Forest Department, Forest Botany Division, Ministry of Agriculture and Cooperatives, Bangkok. The plant material (208.7 g) was shade-dried, ground, and extracted by soaking in CH_2Cl_2–MeOH (9:1, 1 l) at room temperature for two days. The filtrate was evaporated to dryness under vacuum at room temperature, yielding a crude extract (1.64 g). The crude extract was subjected to chromatographic separation on a Sephadex LH-20 column (5 × 20 cm) using MeOH as an eluent to give 46 fractions (50 ml each) from which those fractions showing similar TLC patterns were pooled. Subsequent purification of fractions 34–42 (20 mg) by HPLC (Waters 600 pump, Waters 996 photodiode array UV detector) using a C-18 reverse-phased column (Prep Nova-Pak) with acetonitrile/H_2O (35:65) as eluent yielded compounds **1** (6 mg), **2** (8 mg) and **3** (2 mg). Under the described HPLC conditions, compounds **1**–**3** had retention times of 9, 12, and 14 min, respectively.

3.3. In vitro antimalarial assays

The parasite *P. falciparum* (K1, multidrug resistant strain) was cultured continuously according to the method of Trager and Jensen (Trager and Jensen, 1976). Quantitative assessment of antimalarial activity in vitro was determined by means of the microculture radioisotope technique based on the method described by Desjardins et al. (1979). Effective concentration (EC_{50}) represents the concentration which causes 50% reduction in parasite growth as indicated by the in vitro uptake of [^{3}H]-hypoxanthine by *P. falciparum*. An EC_{50} value of 0.16 μg/ml (3.1 μM) was observed for the standard sample, chloroquine diphosphate, in the same test system.

3.4. Trans-4-isopentenyl-3,5,2′,4′-tetrahydroxystilbene, *1*

EC_{50}: 8.2 μg/ml; MS and NMR data were in accordance with literature data (Shimizu et al., 1997; Takasugi et al., 1978).

3.5. Trans-4-(3-methyl-E-but-1-enyl)-3,5,2′,4′-tetrahydroxystilbene, *2*

$C_{19}H_{20}O_4$, EC_{50}: 1.7 μg/ml; pale orange powder: mp 191–193°C; λ_{max}^{MeOH} nm (logε) 217 (4.1), 243 (3.9), 311 (4.0), 345 (4.2) nm; ^{1}H-NMR spectral data (400 MHz, DMSO-d_6): δ 1.03 (6H, *d*, J = 6.7 Hz, H-4″, H-5″), 2.38 (1H, *m*, H-3″), 6.24 (1H, *dd*, J = 2.3, 8.5 Hz, H-

5′), 6.31 (1H, *d*, *J* = 2.3, H-3′), 6.44 (2H, *s*, H-2, H-6), 6.51 (1H, *d*, *J* = 16.2 Hz, H-1′), 6.58 (1H, *dd*, *J* = 6.3, 16.2 Hz, H-2″), 6.70 (1H, *d*, *J* = 16.4 Hz, H-8′), 7.13 (1H, *d*, *J* = 16.4 Hz, H-7′), 7.35 (1H, *d*, *J* = 8.5 Hz, H-6); ^{13}C-NMR spectral data (100 MHz; DMSO-d_6): δ 23.3 (C-4″ and C-5″), 34.3 (C-3″), 103.4 (C-3′),105.7 (C-2, C-6), 108.3 (C-5′), 112.6 (C-4′), 117.9 (C-1), 119.4 (C-1′), 124.2 (C-7′), 126.2 (C-8′), 128.2 (C-6), 138.9 (C-1), 141.2 (C-2), 157.3 (C-3, C-5, C-2), 159.1 (C-4), EIMS (*m*/*z*): 312 $[M]^+$ (100), 297 $[M - Me]^+$ (50), 268 (5), 257 $[M - C_4H_7]^+$ (53), 251 (9), 213 (4), 187 (4), 161 (5), 147 (9), 135 (8), 123 (6), HR-EIMS (*m*/*z*): 312.1360 $[M]^+$ (Calcd. for $C_{19}H_{20}O_4$ 312.1362).

3.6. 4-Methoxy-2,2-dimethyl-6-(2-(2,4-dihydroxy)phenyl-trans-ethenyl)chromene, ***3***

EC_{50}: 9.4 μg/ml; MS and NMR data were in accordance with literature data (Shimizu et al., 1997).

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