A New Triterpenoid Ester from the Fruits of *Bruguiera parviflora*

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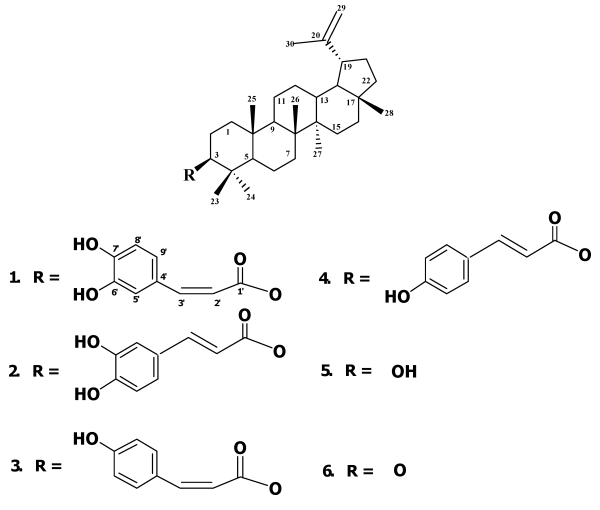
A new lupane caffeoyl ester (1), 3-(*Z*)-caffeoyllupeol, together with five known triterpenoids, lupeol caffeate (2), 3-(*Z*)-coumaroyllupeol (3), dioslupecin A (4), lupeol (5), and lupenone (6), were isolated from the fruits of *Bruguiera parviflora*. Their structures were elucidated by spectroscopic methods. Compound 1 exhibited antimalarial activity with an EC_{50} value of 8.6 μg/ml, but compound 2 was inactive.

Key words *Bruguiera parviflora*; rhizophoraceae; triterpenoid ester; antimalarial

Bruguiera parviflora (Rhizophoraceae), a mangrove plant, is widely distributed in the coastal areas of Southeast Asia and the Indian Ocean. This plant was used by the local Thai people as a folk medicine for treatment of marrow and as nourishment.[1)] As part of our continuing search for bioactive constituents from Thai medicinal plants,[2—4)] we have isolated and elucidated a new triterpenoid ester (**1**) along with five known triterpenoids, lupeol caffeate (**2**),[5)] 3-(*Z*)-coumaroyllupeol (**3**),[6)] dioslupecin A (**4**),[7)] lupeol (**5**),[8)] and lupenone (**6**),[8)] from the fruits of *B. parviflora*. The structures of the known compounds were elucidated by comparison of their physical and spectral data with literature values.

Compound **1** was obtained as an amorphous powder and its molecular formula was determined to be $C_{39}H_{56}O_4$ by negative HR-FAB-MS ($[M-H]^-$ *m/z* 587.4180, calcd 587.4101). The IR spectrum exhibited absorption bands at 3300 (hydroxy) and 1686 cm^{-1} (carbonyl), and the UV spectrum exhibited absorption bands at λ_{max} 234, 293, and 317 nm. The ^{1}H-NMR spectrum revealed the presence of seven singlet methyls (δ_H 0.78, 0.81, 0.85, 0.88, 0.94, 1.03, 1.68), and methylene protons at δ_H 4.69 (d, J=2.5 Hz) and 4.57 (m), for H-29a and H-29b, respectively, as well as a doublet of doublet of an oxymethine proton at δ_H 4.51 (J=4.5, 11.0 Hz) for H-3. The assignment of the signals of the methyl groups and the remaining ^{1}H and ^{13}C signals was performed by analysis of heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC) and correlated spectroscopy (COSY) experiments, and the results were consistent with a lupane-type triterpene.[9,10)] The presence of a caffeoyl ester was shown by the carbon signals at δ 114.7, 117.0, and 124.7, as well as the quaternary carbons at δ 127.5, 143.6, and 145.4, and a carbonyl carbon at δ 167.0 in the ^{13}C-NMR spectrum. Also, two olefinic signals that are characteristic of a *cis* double bond at δ_H 5.82 (1H, d, J=13.0 Hz, H-2′) and 6.80 (1H, d, J=13.0 Hz, H-3′); and three aromatic protons at δ_H 7.02 (1H, dd, J=2.0, 8.5 Hz, H-6″), 7.54 (1H, d, J=2.0 Hz, H-5″), and 6.81 (1H, d, J=8.5 Hz, H-2″) were found in the ^{1}H-NMR spectrum. To confirm the position of the *O*-caffeoyl group, the 2D NMR spectra were measured. In the HMBC spectrum, the oxymethine proton at δ_H 4.51 (δ_C 81.3, C-3) showed long-range correlation with the carbon signal at δ 167.0 (C-1′). Thus, the *O*-caffeoyl group was confirmed to be at C-3. Due to the correlation of H-3 and H-5 shown in the nuclear Overhauser enhancement (NOE) spectrum, the relative stereochemistry of the *O*-caffeoyl group was indicated to be 3β. From the above evidence, the structure of compound **1** was determined to be 3-(*Z*)-caffeoyllupeol.

Both compounds **1** and **2** were evaluated for their antimalarial activity, but only compound **1** significantly inhibited *Plasmodium falciparum* with an EC_{50} value of 8.6 μg/ml.



Experimental

General Experimental Procedures Specific rotations were determined with an Autopol automatic II polarimeter. UV spectra were measured with a UV SPECCORD S100, and IR spectra were recorded on a Perkin–Elmer 1750 FTIR spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker Avance DPX-300 MHz and 500 MHz Varian Unity INOVA spectrometers. Chemical shifts were recorded in parts per million (δ) in $CDCl_3$. FAB-MS and HR-FAB-MS were performed using a Thermofinnigan MAT 95 XL mass spectrometer. Column chromatography was carried out on silica gel 60 GF_{254} (Merck). Silica gel 60 F_{254} precoated aluminium plates (0.2 mm, Merck) were used for TLC analysis; detection was performed by

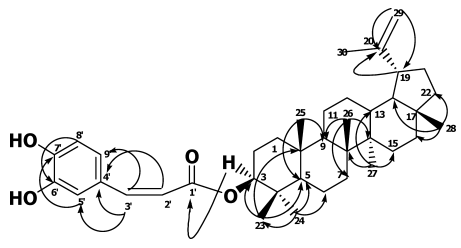


Fig. 1. Key HMBC Correlations of Compound **1**

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

Position	δ_C	δ_H
1	38.4	1.65 (2H, m)
2	23.7	1.62 (2H, m)
3	81.3	4.51 (1H, dd, J=11.0, 4.5 Hz)
4	38.0	—
5	55.4	0.78 (1H, m)
6	18.2	1.50 (1H, m), 1.40 (1H, m)
7	34.2	1.42 (1H, m)
8	40.8	—
9	50.3	1.30 (1H, m)
10	37.1	—
11	20.9	1.40 (1H, m), 1.22 (1H, m)
12	25.1	1.66 (1H, m), 1.08 (1H, m)
13	37.8	0.98 (1H, m)
14	42.8	—
15	27.4	1.58 (1H, m), 1.01 (1H, m)
16	35.5	1.49 (1H, m), 1.36 (1H, m)
17	42.9	—
18	48.3	1.38 (1H, m)
19	47.9	2.38 (1H, m)
20	150.9	—
21	29.8	1.92 (1H, m), 1.26 (1H, m)
22	39.9	1.38 (1H, m), 1.20 (1H, m)
23	27.9	0.88 (3H, s)
24	16.5	0.81 (3H, s)
25	16.1	0.85 (3H, s)
26	15.9	1.03 (3H, s)
27	14.5	0.94 (3H, s)
28	17.9	0.78 (3H, s)
29	109.3	4.69 (1H, d, J=2.5 Hz)
		4.57 (1H, m)
30	19.3	1.68 (1H, s)
Caffeoyl moiety		
1′	167.0	—
2′	117.5	5.82 (1H, d, J=13.0 Hz)
3′	142.8	6.80 (1H, d, J=13.0 Hz)
4′	127.6	—
5′	114.8	6.81 (1H, d, J=8.5 Hz)
6′	143.7	—
7′	145.5	—
8′	117.1	7.54 (1H,d, J=2.0 Hz)
9′	124.8	7.02 (1H, dd, J=8.5, 2.0 Hz)

spraying with 5% H_2SO_4 in ethanol and 1% vanillin in ethanol, followed by heating at 100—110 °C for 5 min.

Plant Material The fruits of *Bruguiera parviflora* were collected at Phang-nga province, Thailand, in August 2002. A voucher specimen (number WU-0014) was deposited in the Herbarium of the Institute of Science, Walailak University, Thasala, Nakhon Si Thammarat, Thailand.

Extraction and Isolation Air-dried fruits of *Bruguiera parviflora* were extracted with hexane, methylene chloride, and methanol, successively. The hexane extract (40.0 g) was subjected to column chromatography (CC) over silica gel and eluted with acetone–hexane (1 : 20, v/v) to give twenty fractions. Fraction f9 (6.07 g) was washed with acetone to yield compound **5** (4.0 g). Fraction f6 (0.87 g) was rechromatographed on a silica gel column and eluted with methylene chloride–hexane (1 : 10, v/v) to afford compound **6** (20.2 mg). The methylene chloride extract (20.0 g) was separated by Sephadex LH-20 using methylene chloride–hexane (10 : 3, v/v) as an eluent to afford six fractions. Fraction f5 (2.0 g) was purified by CC over silica gel using ethyl acetate–hexane (1 : 5, v/v) as an eluent to give compounds **3** (4.0 mg) and **4** (20.4 mg). Fraction f6 (0.61 g) was further purified by CC over silica gel using ethyl acetate–hexane (1 : 5, v/v) as an eluent to give compounds **2** (25.3 mg) and **1** (14.0 mg).

Biological Evaluation The malarial parasite, *Plasmodium falciparum* (K1, multidrug resistant strain), was cultured according to the method of Trager and Jensen.[11] Quantitative assessment of malarial activity *in vitro* was determined by means of the microculture radioisotope technique based upon the method described by Desjardins.[12] The inhibitory concentration (IC_{50}) represents the concentration that 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 1—3 ng/ml was observed for the standard compound, artemisinin, in this test system.

3-(*Z*)-Caffeoyllupeol (**1**): Yellow amorphous powder, $[\alpha]_D^{25}$ +10° (c=0.014, $CHCl_3$), UV λ_{max} ($CHCl_3$) nm (log ε): 234 (2.94), 293 (3.06), and 317 (3.08), IR (neat) cm^{-1}: 3300 (OH) and 1686 (C=O). Negative HR-FAB-MS m/z: 587.4180 $[M-H]^-$ (Calcd for $C_{39}H_{55}O_4$: 587.4101). ^{1}H-NMR ($CDCl_3$) (δ, ppm) (500 MHz) and ^{13}C-NMR ($CDCl_3$) (δ, ppm) (125 MHz): see Table 1.

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