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Ellagic acid derivatives and cytotoxic cucurbitacins from *Elaeocarpus mastersii*

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Abstract

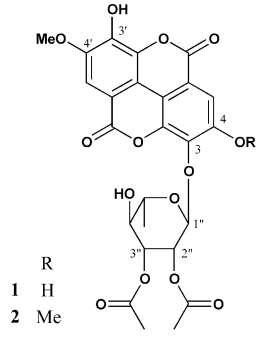
Bioassay-guided investigation of the bark of *Elaeocarpus mastersii* using KB (human oral epidermoid carcinoma) cells as a monitor led to the isolation of two cucurbitacins, cucurbitacin D and cucurbitacin F as cytotoxic principles, together with two ellagic acid derivatives, 4′-*O*-methylellagic acid 3-(2″,3″-di-*O*-acetyl)-α-L-rhamnoside (**1**) and 4,4′-*O*-dimethylellagic acid 3-(2″,3″-di-*O*-acetyl)-α-L-rhamnoside (**2**). These compounds were evaluated against a panel of human tumor cell lines.

Keywords: *Elaeocarpus mastersii*; Elaeocarpaceae; Cytotoxicity; Ellagic acid derivatives; Cucurbitacins

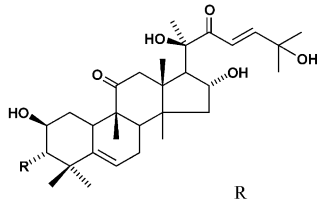
1. Introduction

Elaeocarpus mastersii King (Elaeocarpaceae) is a tree indigenous to Malaysia and Indonesia. In the only previous phytochemical report on *E. mastersii*, an extract of young-leaf pigments afforded several anthocyanins (Lowry, 1970). The crude extracts of some species of this genus have shown biological activity, such as a central nervous system depressant effect and with a direct musculotropic action (Bhattacharya et al., 1975). Alkaloids (Johns et al., 1969a–c; Ray et al., 1979), cucurbitacins (Fang et al., 1984), and flavonoids (Ray et al., 1976) have been isolated from other species in this genus.

As a part of our ongoing program for the discovery of new anticancer agents from plants (Kinghorn et al., 1999), a chloroform-soluble extract of the bark of *E. mastersii* was found to exhibit significant cytotoxic activity when evaluated against a panel of human cancer cell lines. Bioassay-guided phytochemical investigation of this extract, using a human oral epidermoid carcinoma cell line (KB) to monitor fractionation, led to the isolation of two new ellagic acid derivatives, 4′-*O*-methylellagic acid 3-(2″,3″-di-*O*-acetyl)-α-L-rhamnoside (**1**) and 4,4′-*O*-dimethylellagic acid 3-(2″,3″-di-*O*-acetyl)-α-L-rhamnoside (**2**), together with cucurbitacin D (**3**) and cucurbitacin F (**4**) as cytotoxic principles. The structures of compounds **1** and **2** were determined based on various 1D and 2D NMR spectroscopic experiments. Isolates **1–4** were evaluated for their cytotoxicity against a human cancer cell line panel.



R
1 H
2 Me



R
3 =O
4 OH

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2. Results and discussion

The HRFABMS of compound **1** showed a protonated molecular ion at m/z 547.1061, indicating an elemental formula of $C_{25}H_{22}O_{14}$. In the ^{1}H NMR spectrum of **1**, signals for a methoxyl group at δ_H 3.87, two sets of methyl protons at δ_H 2.10 and δ_H 2.04, two aromatic singlets (δ_H 7.98 and 7.84), and five oxygen-bearing methine protons were observed (δ_H 6.52–4.44), along with a doublet methyl signal (δ_H 1.87), indicating the presence of a deoxy sugar. The configuration of the sugar was determined as α from the coupling constant of 1.6 Hz for the anomeric proton (Marzouk et al., 1999). In its ^{13}C NMR spectrum, 25 signals were observed, including those for the two 7-carbon units of an ellagic acid skeleton (δ_C 159.7–107.5), as well as for a deoxy sugar unit (δ_C 100.7, 70.6, 73.1, 70.5, 72.2, 18.4), a methoxy at δ_C 56.6, and two acetyl groups (δ_C 170.6, 170.3, 20.9, and 20.7). The HMBC spectrum showed cross-peaks between H-5 and the C-3, C-4, C-6, and C-7 signals, as well as between the analogous H-5′ signal and the C-3′, C-4′, C-6′, and C-7′ resonances. These interactions gave evidence for the presence of the ellagic acid moiety. Further correlations in the HMBC spectrum between H-1″ and C-3, H-2″ (δ_H 6.52) and the C-2″ acetyl carbonyl (δ_C 170.3), H-3″ (δ_H 6.30) and the C-3″ acetyl carbonyl (δ_C 170.6), the C-2″ acetyl methyl proton (δ_H 2.10) and the C-2″ acetyl carbonyl carbon (δ_C 170.3), and C-3″ acetyl methyl proton (δ_H 2.04) and the C-3″ acetyl carbonyl carbon (δ_C 170.6), supported the presence of acetate groups at C-2″ and C-3″, and the linkage of the sugar unit to the C-3 position of the ellagic acid moiety. Also, a HMBC correlation between the methoxy methyl proton signal and C-4′ was observed. The glycoside was determined as α-rhamnose through analysis of the chemical shifts and coupling patterns of its proton signals (Marzouk et al., 1999), which were confirmed by the ^{1}H–^{1}H COSY spectrum. In addition, in a NOESY experiment, a correlation was observed between H-5′ and OMe-4′, although no correlation was observed for H-5 to any proton of the rhamnose unit. These results confirmed the attachment of the methoxyl group and rhamnose at C-4′ and C-3 of the ellagic acid unit, respectively. Thus, compound **1** was determined as a new ellagic acid glycoside, 4′-*O*-methylellagic acid 3-(2″,3″-di-*O*-acetyl)-α-L-rhamnoside.

Compound **2** exhibited a molecular formula of $C_{26}H_{24}O_{14}$ from its HRFABMS. The ^{1}H NMR spectrum of **2** was very similar to that of **1**, except for the presence of signals consistent with an additional methoxy group at δ_H 3.83 (3H, *s*) at C-4. In the ^{13}C NMR spectrum of **2**, when compared with that of **1**, one more methoxyl carbon at δ_C 56.7 was observed, indicating methylation of the hydroxy signal at C-4. The structure of **2** was characterized therefore as 4,4′-*O*-dimethylellagic acid 3-(2″,3″-di-*O*-acetyl)-α-L-rhamnoside.

Compounds **3** and **4** were identified as cucurbitacin D and cucurbitacin F, respectively, on the basis of their physical and spectral data comparison with literature values (Kupchan et al., 1972; Fang et al., 1984; Konoshima et al., 1993; Fujita et al., 1995).

As summarized in Table 1, compounds **1–4** were evaluated against a panel of human tumor cell lines (Likitwitayawuid et al., 1993; Seo et al., 2001). Cucurbitacin D (**3**) and cucurbitacin F (**4**) showed significant cytotoxicity against all of the cell lines in which they were tested. These compounds have not been previously evaluated against the hTERT-RPE1 and HUVEC cell lines. In contrast, the two ellagic acid derivatives did not exhibit strong cytotoxic effects, although **1** mediated a weak response against all of the cell lines. Cucurbitacin D (**3**) has been found to exhibit significant cytotoxicity against human tumor cells (Konopa et al., 1974; Ryu et al., 1994; Kim et al., 1997), antagonizes the action of insect steroid hormones (Dinan et al., 1997; Sarker et al., 1999), and affects the growth in vitro of symbiotic bacteria of entomopathogenic nematodes (Barbercheck and Wang, 1996). Cucurbitacin F (**4**) also has been found to be cytotoxic against human tumor cells (Fang et al., 1984; Mata et al., 1990; Kim et al., 1997) and to antagonize the action of insect steroid hormones (Dinan et al., 1997, 2001; Sarker et al., 1999).

3. Experimental

3.1. General

Melting points were determined using a Fisher-Johns melting point apparatus, and are uncorrected. Optical rotations were obtained on a Perkin-Elmer model 241 polarimeter. UV spectra were measured on a Beckman DU-7 spectrometer. IR spectra were taken on a JASCO FT/IR-410 spectrophotometer. ^{1}H and ^{13}C NMR data (including DEPT, HMQC, HMBC, and ^{1}H–^{1}H COSY spectra) were measured on a Bruker DRX-500 instrument operating at 500.1 and 125.7 MHz, respectively.

Table 1
Cytotoxic activity of compounds **1–4**[a,b]

Compound	Lu1	Col2	KB	LNCaP	hTERT-RPE1	HUVEC
1	18.4	14.7	13.6	12.9	8.2	13.8
2	> 20	> 20	> 20	> 20	> 20	> 20
3	0.06	0.02	0.01	0.02	0.01	0.02
4	0.2	1.9	0.1	0.2	0.2	0.1

[a] Results are expressed as ED_{50} values (μg/mL).

[b] Key to cell lines used: Lu1 = human lung cancer; Col2 = human colon cancer; KB = human oral epidermoid carcinoma; LNCaP = hormone-dependent human prostate cancer; hTERT-RPE1 = human telomerase reverse transcriptase—retinal pigment epithelial cells; HUVEC = human umbilical vein endothelial cells.

Compounds were analyzed in $CDCl_3$, with tetramethylsilane (TMS) as internal standard. ^{13}C NMR multiplicity was determined using DEPT experiments. FABMS and HRFABMS were recorded on a Finnigan MAT-90 spectrometer.

3.2. Plant material

The bark of *E. mastersii* King was collected at Kalteng, in Indonesia, in October 1999 and identified by S.R. A voucher specimen (A4740, TWH020) has been deposited at the Field Museum of Natural History, Chicago, IL.

3.3. Extraction and isolation

The dried bark of *E. mastersii* (970 g) was extracted three times with MeOH at room temperature. The resultant extracts were combined, concentrated under vacuum, dissolved in MeOH (500 ml), and washed with hexane (3×500 ml). The lower layer was concentrated to dryness under reduced pressure and partitioned between 5% MeOH/H_2O (500 ml) and $CHCl_3$ (3×500 ml). The $CHCl_3$-soluble extract [2.9 g, ED_{50} 1.3 μg/ml against the KB cell line (human oral epidermoid carcinoma)] was subjected to Si gel (150 g) column chromatography and eluted with a gradient mixture of hexane–Me_2CO–MeOH (8:1:0.1→2:1:0.1, 50 ml per fraction) to give 14 pooled fractions. Fractions 6, 8, and 9 were active when tested against the KB cell line (ED_{50} 0.1, 0.7, and 0.4 μg/ml, respectively). Compound **1** (20 mg) was isolated from fraction 8, using Si gel column chromatography eluted with hexane–Me_2CO–MeOH (5:1:0.1). Additional chromatographic separation of active fraction 6 over reversed-phase Si gel with 50% MeOH–H_2O afforded compound **2** (1 mg) and cucurbitacin D (**3**, 127 mg) (Kupchan et al., 1972; Fujita et al., 1995). Further chromatography of fraction 9 over reversed-phase Si gel with 50% MeOH–H_2O afforded cucurbitacin F (**4**, 61 mg) (Fang et al., 1984; Konoshima et al., 1993).

3.3.1. Compound *1*

4′-*O*-Methylellagic acid 3-(2″,3″-di-*O*-acetyl)-α-L-rhamnoside. Needles (MeOH); mp 217–218 °C. $[\alpha]_D$ −24.0° (MeOH; *c* 0.1). UV (MeOH) λ_{max} nm (log ε): 215 (4.54), 276 (4.45), 360 (4.06). IR ν_{max}^{NaCl} cm^{-1}: 1743, 1609, 1490, 1362, 1246, 1079. ^1H NMR (500 MHz, pyridine-d_5): δ 7.98 (1H, *s*, H-5), 7.84 (1H, *s*, H-5′), 6.52 (1H, *dd*, *J* = 3.3, 1.6 Hz, H-2″), 6.47 (1H, *d*, *J* = 1.6 Hz, H-1″), 6.39 (1H, *dd*, *J* = 9.9, 3.3 Hz, H-3″), 5.53 (1H, *dd*, *J* = 9.9, 6.1 Hz, H-5″), 4.44 (1H, *dd*, *J* = 9.9, 9.9 Hz, H-4″), 3.87 (3H, *s*, OMe-4′), 2.10 (3H, *s*, OAc-3″), 2.04 (3H, *s*, OAc-2″), 1.87 (3H, *d*, *J* = 6.1 Hz, H-6″). ^{13}C NMR (75.6 Hz, pyridine-d_5): δ 170.6 (*s*, OAc-2″), 170.3 (*s*, OAc-3″), 159.7 (*s*, C-7), 159.5 (*s*, C-7′), 154.4 (*s*, C-4), 151.0 (*s*, C-4′), 143.9 (s, C-3′), 142.8 (*s*, C-2′), 137.7 (*s*, C-3), 136.9 (*s*, C-2), 115.3 (*s*, C-1), 114.6 (*s*, C-6′), 112.5 (*d*, C-5), 112.2 (*s*, C-6), 107.7 (*d*, C-5′), 107.5 (*s*, C-1′), 100.7 (*d*, C-1″), 73.1 (*d*, C-3″), 72.2 (*d*, C-5″), 70.6 (*d*, C-2″), 70.5 (*d*, C-4″), 56.6 (*q*, OMe-4′), 20.9 (*q*, OAc-2″), 20.7 (*q*, OAc-3″), 18.4 (*q*, C-6″). FABMS *m*/*z* (rel. int.): 547 $[M+H]^+$ (32), 460 (21), 308 (50), 289 (100), 231 (52). HRFABMS *m*/*z* 547.10608 (calcd for $C_{25}H_{23}O_{14}$, 547.10878).

3.3.2. Compound *2*

4,4′-*O*-Dimethylellagic acid 3-(2″,3″-di-*O*-acetyl)-α-L-rhamnoside. Amorphous powder; $[\alpha]_D$ −21.6° (*c* 0.1, MeOH); UV (MeOH) λ_{max} nm (log ε): 205 (4.47), 254 (4.64), 276 (4.48), 322 (4.12), 359 (4.11). IR ν_{max}^{NaCl} cm^{-1}: 1700, 1576, 1356, 1316, 1090. ^1H NMR (pyridine-d_5, 500 MHz): δ 7.86 (1H, *s*, H-5), 7.82 (1H, *s*, H-5′), 6.37 (1H, *dd*, *J* = 3.4, 1.6 Hz, H-2″), 6.23 (1H, *dd*, *J* = 9.9, 3.4 Hz, H-3″), 6.15 (1H, *d*, *J* = 1.6 Hz, H-1″), 5.38 (1H, *dd*, *J* = 9.9, 6.2 Hz, H-5″), 4.42 (1H, *dd*, *J* = 9.9, 9.9 Hz, H-4″), 3.88 (3H, *s*, OMe-4′), 3.83 (3H, *s*, OMe-4), 2.12 (3H, *s*, OAc-3″), 2.06 (3H, *s*, OAc-2″), 1.85 (3H, *d*, *J* = 6.2 Hz, H-6″). ^{13}C NMR (pyridine-d_5, 75.6 Hz): δ 170.7 (*s*, OAc-2″), 170.3 (*s*, OAc-3″), 159.7 (*s*, C-7), 159.2 (*s*, C-7′), 154.8 (*s*, C-4), 151.4 (*s*, C-4′), 143.2 (*s*, C-3′), 142.9 (*s*, C-2′), 137.8 (*s*, C-3), 136.0 (*s*, C-2), 115.2 (*s*, C-1), 114.1 (*s*, C-6′), 113.9 (*d*, C-5), 113.9 (*s*, C-6), 107.8 (*s*, C-1′), 107.8 (*d*, C-5′), 101.1 (*d*, C-1″), 72.9 (*d*, C-3″), 72.2 (*d*, C-5″), 70.5 (*d*, C-2″), 70.4 (*d*, C-4″), 56.7 (*q*, OMe-4), 56.6 (*q*, OMe-4′), 20.9 (*q*, OAc-2″), 20.7 (*q*, OAc-3″), 18.3 (*q*, C-6″). FABMS *m*/*z* $[M+H]^+$ 561. HRFABMS *m*/*z* 583.10648 $[M+Na]^+$ (calcd for $C_{26}H_{24}O_{14}Na$, 583.10638).

3.3.3. Bioassay evaluation

Compounds **1**–**4** were evaluated for cytotoxicity against a panel of human cancer cell lines, according to established protocols (Likitwitayawuid et al., 1993; Seo et al., 2001). ED_{50} values of > 5 μg/ml are regarded as inactive.

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