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Phytochemistry 66 (2005) 2577–2580
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Anti-babesial ellagic acid rhamnosides from the bark of *Elaeocarpus parvifolius*

A. Elkhateeb [a], Subeki [a], K. Takahashi [a], H. Matsuura [a,*], M. Yamasaki [b], O. Yamato [b], Y. Maede [b], K. Katakura [b], T. Yoshihara [a], K. Nabeta [a]

[a] *Laboratory of Bioorganic Chemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan*
[b] *Laboratory of Internal Medicine, Department of Veterinary Clinical Science, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan*

Received 16 May 2005; received in revised form 21 July 2005
Available online 14 October 2005

Abstract

Bioassay-guided investigation of the bark of *Elaeocarpus parvifolius* led to the isolation of three new ellagic acid derivatives, 4-*O*-methylellagic acid 3′-α-rhamnoside (**2**), 4-*O*-methylellagic acid 3′-(3″-*O*-acetyl)-α-rhamnoside (**3**), and 4-*O*-methylellagic acid 3′-(4″-*O*-acetyl)-α-rhamnoside (**4**) in addition to the known ellagic acid derivative, 4-*O*-methylellagic acid 3′-(2″,3″-di-*O*-acetyl)-α-rhamnoside (**1**). Their structures were elucidated on the basis of analysis of ^{1}H NMR, ^{13}C NMR, HMQC, HMBC and MS spectroscopic data. Compounds **1**–**4** were evaluated for their growth-inhibitory effect on *Babesia gibsoni* in vitro. Compounds **2** and **4** showed very weak activity, while compounds **1** and **3** showed moderate activity, with IC_{50} values of 28.5 and 52.1 μg/ml, respectively.

Keywords: *Elaeocarpus parvifolius*; Elaeocarpaceae; Ellagic acid derivatives; Anti-babesial activity; *Babesia gibsoni*

1. Introduction

The infection of dogs with the parasite *Babesia gibsoni* is a worldwide problem and in recent years the geographic range of the infection has spread. *B. gibsoni* proliferates within erythrocytes by lysing the cells, which results in the induction of anemia in infected animals. The drug diminazene aceturate (Ganaseg) has been shown to be effective against *B. gibsoni* infection (Grovies and Vanniasingham, 1970; Farewell et al., 1982), but causes side effects such as weakness, irritability, paralysis, non-responsiveness to stimuli and fatal central nervous system hemorrhage (Breitschwerd, 1990). Because of this, production of the drug was recently stopped, and an alternative chemotherapeutic agent with few side effects is urgently needed for the treatment of *B. gibsoni* infection.

It is possible that plant extracts may provide an affordable treatment. In Indonesia, medicinal plants are traditionally used for curing various diseases, forming the basis of a system of medicine called ''Jamu''. The bark of the plant *Elaeocarpus parvifolius* (Elaeocarpaceae), which is found in Indochina, Thailand, Peninsular Malaysia, Singapore and Borneo (Sosef et al., 1998), is often used as a ''Jamu'' constituent, especially in the treatment of malarial infection. The modes of action and replication of the malaria parasite are similar to those of *B. gibsoni*. The bark extract of this plant shows moderate anti-babesial activity against *B. gibsoni* in vitro (IC_{50} 12.6 μg/ml) (Subeki et al., 2004), but no chemical studies of this plant have yet been reported. The aim of this study, therefore, was to isolate and characterize anti-babesial compounds from *E. parvifolius*.

2. Results and discussion

An extract of the stem bark of *E. parvifolius* was obtained using boiling water and was partitioned into H_2O and

* Corresponding author. Tel.: +81 11 706 2495; fax: +81 11 706 2505.
E-mail address: matsuura@chem.agr.hokudai.ac.jp (H. Matsuura).

0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.08.020

EtOAc layers. The EtOAc-soluble layer exhibited anti-babesial activity, and successive column chromatography on silica gel followed by preparative TLC and semi-preparative HPLC yielded four ellagic acid rhamnosides (**1**–**4**).

Compound **1** was obtained as a yellow amorphous solid. Analysis of the COSY, NOE, HMQC and HMBC spectra established that the compound was 4-*O*-methylellagic acid 3′-(2″,3″-di-*O*-acetyl)-α-rhamnoside (Fig. 1). This compound has previously been isolated from *Elaeocarpus mastersii*, and its structure was firmly established using ID- and 2D-NMR spectroscopic techniques (Ito et al., 2002). The spectroscopic data and optical rotation of **1**, which was isolated in this study, was in good accordance with previously reported data (Ito et al., 2002).

Compound **2** was obtained as pale yellow needle-shaped crystals. The ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **2** were almost the same as those of **1** in the aglycone regions, indicating that the aglycone moiety of **2** is 4-*O*-methylellagic acid. The position of the 4-*O*-methyl moiety was substantiated by NOE experiments with irradiation at δ 3.85 (Fig. 2). However, the spectroscopic data for the sugar moiety differed from the corresponding data for **1**. The ^{1}H NMR spectrum of **2** in the sugar regions exhibited six signals corresponding to five methine protons and a methyl group. The connectivity of each proton was established by COSY spectra and analysis of coupling constants in the ^{1}H NMR spectrum revealed the sugar moiety to be rhamnose. As the value of the coupling constant of H-1″ was less than 7 Hz, it was determined to be α-rhamnose. The evidence of a cross-peak between H-1″ and C-3′ in the HMBC spectrum (Fig. 2) allowed identification of **2** as 4-*O*-methylellagic acid 3′-α-rhamnoside. Full ^{1}H and ^{13}C NMR spectroscopic assignments are given in Tables 1 and 2.

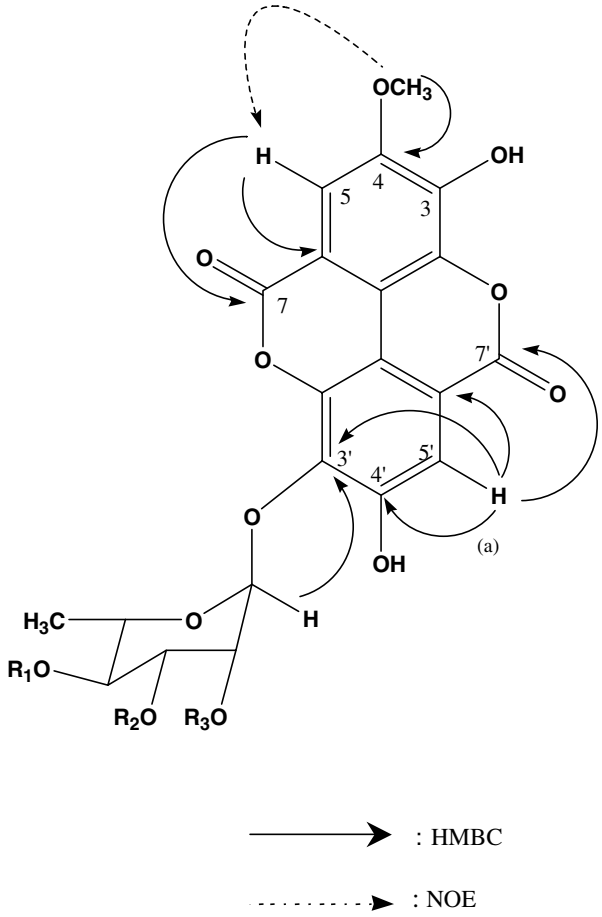
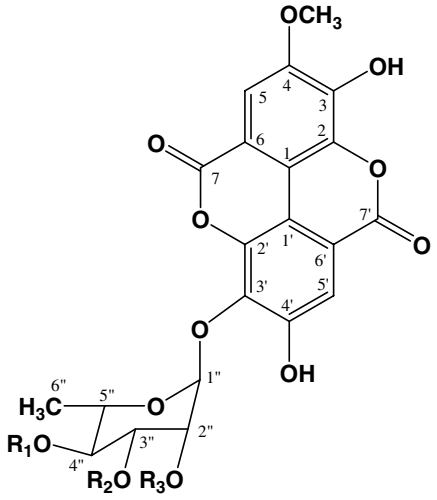


Fig. 2. Important HMBC correlations and NOE experiments for compounds **1**–**4**. (a) Observed only for compounds **1** and **4**.



	R_1	R_2	R_3
1	H	CH_3CO	CH_3CO
2	H	H	H
3	H	CH_3CO	H
4	CH_3CO	H	H

Fig. 1. Chemical structures of compounds **1**–**4**.

In the same manner, the structure of compound **3** was established. The ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **3** were almost the same as those of **1** and **2** in the aglycone regions, indicating that the aglycone moiety was 4-*O*-methylellagic acid. The position of the 4-*O*-methyl moiety was reconfirmed by NOE experiments with irradiation at δ 3.98 (Fig. 2). However, the spectroscopic data of the sugar moiety were different from those of the previous compounds. The results obtained from ^{1}H NMR (δ 2.16) and ^{13}C NMR (δ 172.9 and 21.1) spectroscopic analyses, and the difference between the FAB-MS *m*/*z* values of **2** and **3**, suggested that an acetyl group was present in **3**. The ^{1}H NMR spectrum of **3** in the sugar regions also exhibited six signals corresponding to a methyl group and five methine protons, one of which showed downfield shifting. The connectivity of each proton was established by analyses of its COSY spectra. The position of the acetyl group in the sugar moiety was determined to be at C-3″ due to the downfield shift of the H-3″ signal (δ 5.30) when compared with the spectrum of **2**. Analysis of coupling

Table 1
^{1}H NMR spectroscopic data of compounds **2**–**4** in CD_3OD[a]

Position	**2**[b]	**3**	**4**
5	7.20 (1H, *s*)	7.55 (1H, *s*)	7.56 (1H, *s*)
5′	7.30 (1H, *s*)	7.60 (1H, *s*)	7.60 (1H, *s*)
4-OMe	3.85 (3H, *s*)	3.98 (3H, *s*)	3.99 (3H, *s*)
1″	5.52 (1H, *br.s*)	5.70 (1H, *d*, 1.7)	5.70 (1H, *br.s*)
2″	4.20 (1H, *br.s*)	4.47 (1H, *dd*, 1.7, 3.3)	4.35 (1H, *br.s*)
3″	3.96 (1H, *dd*, 2.5, 9.8)	5.28 (1H, *dd*, 3.0, 9.8)	4.20 (1H, *dd*, 3.6, 9.8)
4″	3.41 (1H, *t*, 9.8)	3.69 (1H, *t*, 9.8)	5.05 (1H, *t*, 9.8)
5″	4.25 (1H, *dd*, 6.1, 9.8)	4.56 (1H, *dd*, 6.2, 9.8)	4.56 (1H, *dd*, 6.1, 9.8)
6″	1.14 (3H, *d*, 6.1)	1.26 (3H, *d*, 6.2)	1.10 (3H, *d*, 6.1)
OAc-2″			
OAc-3″		2.16 (3H, *s*)	
OAc-4″			2.16 (3H, *s*)

[a] Chemical shifts in the ^{1}H NMR spectra (270 MHz) are recorded as δ (ppm) values relative to the proton signal (δ 3.30) of CD_3OD. Number of protons, signal multiplicity and coupling constants (Hz) are shown in parentheses.
[b] ^{1}H NMR (500 MHz, CD_3OD).

Table 2
^{13}C NMR spectroscopic data of compounds **2**–**4** in CD_3OD[a]

Position	**2**	**3**	**4**
1[c]	113.1	113.5	113.2
2	138.2	138.1	138.2
3	152.0	N.O[b]	153.0
4	154.0	154.0	154.0
5	107.6	107.4	107.5
6[c]	115.2	115.3	115.3
7	161.0	161.5	161.2
1′[c]	113.1	113.5	113.2
2′	143.8	143.9	143.9
3′	138.2	138.1	138.2
4′	154.0	154.0	154.0
5′	112.6	112.7	112.7
6′[c]	115.2	115.4	115.5
7′	160.8	161.3	161.1
4-OMe	57.1	56.8	56.9
1″	103.7	103.4	103.6
2″	71.9	69.7	71.9
3″	72.1	75.4	70.1
4″	73.6	71.1	75.2
5″	71.9	71.9	69.7
6″	17.9	17.9	17.7
OAc-3″		172.9	
OAc-4″			172.7
OAc-3″		21.1	
OAc-4″			21.1

[a] Chemical shifts in the ^{13}C NMR spectra (125 MHz) are recorded as δ (ppm) values relative to carbon signal (δ 49.0) of CD_3OD.
[b] Not observed.
[c] Assignments may be interchangeable within each column.

constants in the ^{1}H NMR spectrum revealed the sugar moiety to be rhamnose; the coupling constant of H-1″ (1.7 Hz) showed that it was α-rhamnose. Because its HMBC spectrum showed a long-range correlation between H-1″ (δ 5.70) and C-3′ (δ 138.1) (Fig. 2), it was deduced that compound **3** was 4-*O*-methylellagic acid 3′-(3″-*O*-acetyl)-α-rhamnoside (Fig. 1). Full ^{1}H and ^{13}C NMR spectroscopic assignments are given in Tables 1 and 2.

In the same manner, the structure of compound **4** was established. The ^{1}H, ^{13}C NMR and HMBC spectroscopic data of **4** were almost the same as those of **1**–**3** in the aglycone regions, indicating that the aglycone moiety of **4** was 4-*O*-methylellagic acid. The position of the 4-*O*-methyl moiety was substantiated by NOE experiments with irradiation at δ 3.99 (Fig. 2). However, the spectroscopic data for the sugar moiety were not same as those for **1**–**3**. The data obtained from ^{1}H NMR (δ 2.16) and ^{13}C NMR (δ 172.7 and 21.1) spectroscopic analyses, and the difference between the FAB-MS *m*/*z* values of **2** and **4** again suggested that an acetyl group existed in the structure of **4**. The connectivity of each proton was established by analyses of its COSY spectra, and the coupling constants in the ^{1}H NMR spectrum revealed the sugar moiety to be rhamnose. As the value of the coupling constant of H-1″ was less than 7 Hz, the form of rhamnose was deduced to be α-rhamnose. Taking into account the downfield shift of H-4″ (δ 5.05) and the long-range correlation of H-4″ (δ 5.05) with the acetyl carbonyl (δ 172.7), the attachment of the acetyl group to C-4″ of the rhamnose moiety was established. As there was evidence of coupling between H-1″ and C-3′ (Fig. 2), compound **4** was identified as 4-*O*-methylellagic acid 3′-(4″-*O*-acetyl)-α-rhamnoside (Fig. 1). Full ^{1}H and ^{13}C NMR spectroscopic assignments are given in Tables 1 and 2.

The structures of compounds **2**–**4** were found to be very similar to those previously reported in a study in which various structures were elucidated from a mixture (Kim et al., 2001), the only difference being the attachment of the methoxy group. Compounds **1**–**4** were evaluated for their growth-inhibitory effect against *B. gibsoni* in vitro. The IC_{50} values of **1**–**4** against *B. gibsoni* are given in Table 3. Compounds **1** and **3** showed moderate activity, but compounds **2** and **4** showed very weak activity against *B. gibsoni* in vitro compared with the standard drug, diminazene aceturate (Ganaseg). Considering the potentially fatal side-effects of diminazene aceturate, compounds **1** and **3** are promising new candidates for the treatment of *B. gibsoni* infection. This is the first report on the chemistry of *E. parvifolius* and on the anti-babesial activities of ellagic acid rhamnoside derivatives.

Table 3
Anti-babesial activity of compounds **1**–**4** against *B. gibsoni* in vitro

Compound	IC_{50} (μg/ml)
1	28.5
2	>180
3	52.1
4	>180
Diminazene aceturate (Ganaseg)[a]	0.60

[a] Drug for *B. gibsoni*.

3. Experimental

3.1. General

Optical rotations were measured with a JASCO DIP-370 digital polarimeter in MeOH. IR spectra were recorded on a Bio-Rad FTS-50A spectrometer. UV spectra were measured on a HITACHI U-3210 spectrophotometer. FAB-MS, HRFAB-MS were measured on a JEOL JMS-SX102A and JEOL JMS-AX500 spectrometers. NMR was recorded in MeOD on a JEOL JNM-EX 270 FT-NMR and on a Bruker AM-500 FT-NMR spectrometer. Column chromatography was conducted with silica gel 60 (Kanto Chemical). Analytical thin-layer chromatography was performed on silica gel 60 F_{254}(Merck).

3.2. Plant material

The bark of *E. parvifolius* was collected from Central Kalimantan, Indonesia and identified by Dr. Irawati at the Herbarium Bogoriense, Indonesia. A voucher specimen (SA1-CK-2001) was deposited at the Department of Research and Development for Biology, Indonesian Institute of Sciences, Bogor.

3.3. Extraction and isolation

The air-dried bark of *E. parvifolius* (100 g) was extracted with boiling water (2 L), for 30 min, and the same material was re-extracted by the same manner. The extract was filtered and concentrated to ~500 ml under reduced pressure and then partitioned with EtOAc (500 ml × 4). The EtOAc layer was dried (Na_2SO_4) and then concentrated under reduced pressure. The obtained residue (2.5 g) was subjected to silica gel column chromatography eluted with $CHC1_3$, MeOH–$CHCl_3$ (3:97), MeOH–$CHCl_3$ (20:80), and MeOH successively. The MeOH–$CHCl_3$ (20:80) extract was reapplied to silica gel column with MeOH–$CHCl_3$ (20:80) as eluant to give five fractions (Fr. I-V). Fr. I (100 mg) was further purified by PTLC on silica gel. The plates were developed with MeOH–$CHCl_3$ (10:90). The bands were collected and eluted from silica gel to afford compound **1** (8.9 mg) and a mixture of compounds **2**–**4**. This mixture was separated by HPLC (CAPCELL Pak C_{18}, 15 mm × 250 mm, Shiseido), using CH_3CN–H_2O–AcOH (3.0:7.0:0.01, v/v/v) as a solvent at a flow rate of 3 ml/min, to afford compounds **2** (t_R: 17.2 min, 3.6 mg), **3** (t_R: 31.5 min, 1.3 mg), and **4** (t_R: 47.5 min, 1.9 mg).

3.3.1. 4-O-methylellagic acid 3′-(2″, 3″-di-O-acetyl)-3′-α-rhamnoside (*1*)

Yellow amorphous solid; $[\alpha]_D^{25}$ −25.9° (*c* 0.32; MeOH); FAB-MS *m/z* 545 $[M-H]^-$; HRFAB-MS *m/z* 545.0959 $[M-H]^-$ ($C_{25}H_{21}O_{14}$ requires 545.0931).

3.3.2. 4-O-methylellagic acid 3′-α-rhamnoside (*2*)

Pale yellow needle-shaped crystals; $[\alpha]_D^{25}$ −30.8° (*c* 0.036; MeOH). UV λ_{max}^{MeOH}, nm (log ε): 221.4 (5.59), 255 (*sh*, 5.35), 364 (4.96). IR ν_{max}^{KBr} (cm^{-1}): 3420, 2900, 1718, 1457, 1107, 1070. FAB-MS *m/z* 461 $[M-H]^-$; HRFAB-MS *m/z* 461.0721 $[M-H]^-$ ($C_{21}H_{17}O_{12}$ requires 461.0720): for ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

3.3.3. 4-O-methylellagic acid 3′-(3″-O-acetyl)-α-rhamnoside (*3*)

Yellow amorphous solid; $[\alpha]_D^{25}$ −57.9° (*c* 0.011; MeOH). UV λ_{max}^{MeOH}, nm (log ε): 221.2 (5.62), 255 (*sh*, 5.38), 364.6 (4.17). IR ν_{max}^{KBr} (cm^{-1}): 3438, 2915, 1717, 1465, 1113, 1052. FAB-MS *m/z* 503 $[M-H]^-$; HRFAB-MS *m/z* 503.0815 $[M-H]^-$ $C_{23}H_{19}O_{13}$ requires 503.0825; for ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

3.3.4. 4-O-methylellagic acid 3′-(4″-O-acetyl)-α-rhamnoside (*4*)

Yellow amorphous solid; $[\alpha]_D^{25}$ −20.7° (*c* 0.19; MeOH). UV λ_{max}^{MeOH}, nm (log ε): 221.2 (5.78), 255 (*sh*, 5.38), 363.8 (3.89). IR ν_{max}^{KBr} (cm^{-1}): 3430, 2920, 1718, 1473, 1457, 1110, 1060. FAB-MS *m/z* 503 $[M-H]^-$, HRFAB-MS *m/z* 503.0838 $[M-H]^-$ ($C_{23}H_{19}O_{13}$ requires 503.0825); for ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

3.4. Bioassay

The assay was performed against *B. gibsoni* in vitro according to the method of Subeki et al. (2004).

Acknowledgements

The authors thank Dr. E. Fukushi and Mr. K. Watanabe for FAB-MS and HRFAB-MS measurements. The authors are grateful to Dr. Chairul, Dr. Trimurningsih and Dr. Irawati for the help in the collection and identification of plant material in Indonesia.

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