Antioxidative Constituents of *Etlingera elatior*

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Phytochemical studies on the rhizomes of *Etlingera elatior* have resulted in the isolation of 1,7-bis(4-hydroxyphenyl)-2,4,6-heptatrienone (**1**), demethoxycurcumin (**2**), 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (**3**), 16-hydroxylabda-8(17),11,13-trien-15,16-olide (**4**), stigmast-4-en-3-one, stigmast-4-ene-3,6-dione, stigmast-4-en-6β-ol-3-one, and 5α,8α-epidioxyergosta-6,22-dien-3β-ol. Compounds **1** and **4** are new, and their structures were elucidated by analysis of spectroscopic data. Diarylheptanoids **1**−**3** were found to inhibit lipid peroxidation in a more potent manner than α-tocopherol.

Etlingera elatior (Jack) R. M. Smith (Zingiberaceae) (previously known as *Nicolaia speciosa* Horans or *Phaeomeria speciosa* Horans) is a species native to Sumatra, Indonesia, and it has been found in many places throughout Southeast Asia. In Peninsular Malaysia the plant is cultivated in gardens for its young flower shoots, which can be eaten raw and are sometimes used for flavoring in local dishes. A decoction of the fruits has been used to treat earache, and the leaves are used to clean wounds.[1] There have not been many phytochemical studies conducted on species of this genus except those that have dealt with the analysis of the essential oils of the young flower shoots of *E. elatior*[2] and the rhizomes of *E. cevuga.*[3] An aqueous ethanol extract from the flower shoots of *E. elatior* was reported to possess antimicrobial and cytotoxicity activity against HeLa cells[4] and antitumor-promoting activity.[5] Dichloromethane and methanol extracts of *E. elatior* rhizomes have shown potent antioxidant activity as compared to α-tocopherol in a ferric thiocyanate assay (FTC).[6]

Eight compounds were isolated from *E. elatior* after chromatography of the initial crude extracts. Six known compounds were identified as demethoxycurcumin (**2**),[7,8] 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (**3**),[9] stigmast-4-en-3-one,[10] stigmast-4-ene-3,6-dione,[10] stigmast-4-en-6β-ol-3-one,[10] and 5α,8α-epidioxyergosta-6,22-dien-3β-ol,[11] based on the measurement of their spectroscopic data (UV, MS, IR, ^{1}H NMR, and ^{13}C NMR) and comparison with literature values.

A new diarylheptanoid (**1**) was isolated as a yellow powder and exhibited a molecular ion peak in the HREIMS at *m/z* 292.1113, corresponding to a molecular formula of $C_{19}H_{16}O_3$. The IR spectrum indicated the presence of carbonyl as well as olefinic or aromatic functionalities from the absorption bands at 3300 and 1653 cm^{-1}, respectively. The presence of a conjugated enone was indicated by the absorption band at 395 nm in the UV spectrum. The presence of a carbonyl group was further substantiated by the peak at δ 187.9 in the ^{13}C NMR spectrum of this compound, in addition to six other olefinic carbons at δ 124.9 (C-2), 144.1 (C-3), 130.8 (C-4), 143.1 (C-5), 126.5 (C-6), and 137.6 (C-7). The ^{1}H NMR spectrum showed olefinic proton signals at δ 6.80 (d, J = 15.0 Hz), 6.92 (dd, J = 15.0, 11.0 Hz), 6.94 (dd, J = 15.0, 11.0 Hz), 6.64 (dd, J = 15.0, 11.0 Hz), 7.46 (dd, J = 15.0, 11.0 Hz), and 7.20 (d, J = 15.0 Hz), which were assigned to H-7, H-6, H-5, H-4, H-3, and H-2, respectively. The ^{1}H−^{1}H COSY NMR spectrum showed a correlation between the proton at δ 6.80 (H-7) and that at δ 6.92 (H-6), which in turn correlated with signals at δ 6.94 (H-5), 6.64 (H-4), 7.46 (H-3), and 7.20 (H-2). In the HMBC NMR spectrum, correlations were observed between the carbonyl carbon signal at δ 187.9 and proton signals at δ 7.46 (H-3) and 7.20 (H-2), in addition to the aromatic proton signals at δ 7.96 (H-2′, H-6′) (Table 1). Further, correlations were also observed between the proton signal at δ 7.20 (H-2) and an unsubstituted aromatic carbon at δ 131.2 (C-1). These correlations indicated that the unsaturated carbonyl (2,4,6-heptatrienone moiety) is directly attached to an aromatic ring in **1**. The four *ortho*-coupled doublets at δ 6.85 (2H, d, H-3″, 5″, J = 8.5 Hz), 7.41 (2H, d, H-2″, 6″, J = 8.5 Hz), 6.95 (2H, d, H-3′, 5′, J = 8.8 Hz), and 7.96 (2H, H-2′, 6′, J = 8.8 Hz) in the ^{1}H NMR spectrum suggested the presence of two 1,4-substituted phenyl groups. Since one of the aromatic rings is attached to the carbonyl enone, the other ring (ring B) is therefore attached to the olefinic end-carbon (C-7), and this

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Table 1. NMR Data of 1,7-Bis(4-hydroxyphenyl)-2,4,6-heptatrienone (**1**)

position	^{1}H NMR mult.	^{13}C NMR	HMBC	^{1}H−^{1}H COSY
1′		131.3		
2′, 6′	7.96 (2H, d, J = 8.8 Hz)	131.5	C-1, C-1′, C-4′	H-3′, H-5′
3′, 5′	6.95 (2H, d, J = 8.8 Hz)	116.1	C-2′, C-4′	
4′		162.5		
2″, 6″	7.41 (2H, d, J = 8.5 Hz)	129.3	C-1″, C-4″	H-3″, H-5″
3″, 5″	6.85 (2H, d, J = 8.5 Hz)	116.5	C-4″, C-6″	
4″		158.9		
1″		129.5		
1		187.9		
2	7.20 (1H, d, J = 15.0 Hz)	124.9	C-1, C-1′	H-3
3	7.46 (1H, dd, J = 15.0, 11.0 Hz)	144.1	C-1, C-5	H-4
4	6.64 (1H, dd, J = 15.0, 11.0 Hz)	130.8	C-6	
5	6.94 (1H, dd, J = 15.0, 11.0 Hz)	143.1	C-7	H-4
6	6.92 (1H, dd, J = 15.0, 11.0 Hz)	126.5		H-7
7	6.80 (1H, d, J = 15.0 Hz)	137.6	C-5, C-6, C-1″	

was evidenced by the HMBC correlations between the signal at δ 7.41 (H-2″, 6″) and that at δ 137.6 (C-7), as well as between the signal at δ 6.80 (H-7) and δ 129.5 (C-1″) (Table 1). The EIMS showed a base peak at *m/z* 121, which confirmed the presence of a *p*-hydroxybenzoyl moiety, and a peak at *m/z* 171 (fragmentation at C-1 and C-2) further supported the presence of a 4-hydroxyphenyl-2,4,6-heptatrienone moiety. The structure of **1** was therefore assigned as 1,7-bis(4-hydroxyphenyl)-2,4,6-heptatrienone. Usually, the carbonyls of diarylheptanoids are located either at C-3 or C-5, or both at C-3 and C-5.[7−9,12] Compound **1** is the first C-1 oxygenated diarylheptanoid from a natural source.

Compound **4** was obtained as a gummy solid and was shown to have a molecular formula of $C_{20}H_{28}O_3$, based on the molecular ion peak [M$^+$] at *m/z* 316.2030 observed in the HREIMS. The IR spectrum indicated the presence of an α,β-unsaturated γ-lactone from the absorption band at 1750 cm^{-1}, as well as exo-methylene absorptions at 3090 and 892 cm^{-1}. The ^{13}C NMR spectrum showed 20 signals, with several signals duplicated due to the occurrence of **4** as a mixture of two isomers.[13] The ^{1}H NMR spectrum indicated the presence of three quaternary methyl groups at δ 0.85 (3H, s, H-19), 0.87 (3H, s, H-20), and 0.90 (3H, s, H-18), as well as two exo-methylene protons [δ 4.38 (d, J = 1.5 Hz, H-17a), 4.47 (d, J = 1.5 Hz, H-17b), and 4.79 (br s, H-17a, H-17b)] (Table 2). These characteristic signals suggested that compound **4** has a labdane diterpenoid skeleton but exists as an epimeric mixture at C-16.[13−15] The labdane-type skeleton of **4** was further supported by the base peak at *m/z* 137 in the EIMS.[15] In the ^{1}H NMR spectrum, two sets of *trans*-olefinic proton signals were observed at δ 6.31 (1H, d, J = 16.0 Hz, H-12) and 6.58 (dd, J = 16.0, 10.0 Hz, H-11$_a$), as well as at δ 6.59 (dd, J = 16.0, 10.0 Hz, H-11$_b$). ^{1}H−^{1}H COSY and HMBC NMR correlations as well as a NOESY experiment showed that this olefinic group was directly attached to the decalin nucleus. In addition, the ^{1}H NMR spectrum showed the singlet signals of epimeric hemiacetal methine protons at δ 6.25 (1H, s, H-16a) and at δ 6.27 (1H, s, H-16b). In the ^{1}H−^{1}H COSY spectrum, this proton did not show any correlation with other protons, but NOESY interactions were observed from H-16 to H-11 and H-12. It was also observed that the singlet olefinic proton signal at δ 5.85 (H-14) did not correlate with any protons in the ^{1}H−^{1}H COSY spectrum but showed correlations with the carbonyl at δ 171.2 as well as with the signals at δ 161.0 (C-13a, C-13b), 122.6 (C-12a), 122.7 (C-12b), 97.5 (C-16a), and 97.6 (C-16b) in the HMBC spectrum. On the basis of these observations, it was concluded therefore that the olefinic group is attached directly to the lactone carbonyl. It was also observed that the H-14 proton showed NOESY interactions with H-11 and H-12. On the basis of the HMQC and ^{13}C NMR spectra, the chemical shifts at 115.5, 97.5, and 97.6 ppm were assigned to C-14, C-16a, and C-16b, respectively. From these data, the partial structure of the hydroxy-lactone group of compound **4** was similar to 6,16-dihydroxy-8(17),11,13-labdatrien-15,16-olide isolated from *Hedychium yunnanense*,[12] rather than 15-hydroxylabda-8(17),11,13-trien-16,15-olide from *Alpinia chinensis*[13] and zerumin B from *Alpinia zerumbet*.[16] The long-range correlations deduced from the HMBC spectrum further confirmed the presence of this hemiacetal lactone group, which apparently was directly attached to the olefinic group. The structure of **4** was therefore assigned as an isomeric mixture of 16-hydroxylabda-8(17),11,13-trien-15,16-olide, which has not been reported previously from a natural source.

Table 2. NMR Data of 16-Hydroxylabda-8(17),11,13-trien-15,16-olide (**4**)

position	^{1}H NMR mult.	^{13}C NMR[a]
1a/1b	α 1.04 (1H, ddd, J = 13.2, 13.2, 3.7 Hz) β 1.38 (1H, m)	40.9/41.0
2a/2b	α 1.40 (1H, m) β 1.53 (1H, m)	18.9/19.0
3	α 1.18 (1H, m) β 1.42 (1H, m)	42.1
4		33.5
5a/5b	1.10 (1H, dd, J = 12.5, 2.7 Hz)	54.4/54.5
6	α 1.39 (1H, m) β 1.72 (1H, ddddd, J = 12.9, 2.7, 2.7, 2.7, 2.7 Hz)	23.2
7	α 2.08 (1H, m) β 2.44 (1H, m)	36.6
8a/8b		148.7/148.9
9a/9b	2.47 (1H, dd, J = 10.0 Hz)	62.1/62.0
10a/10b		39.5/39.6
11a/11b	6.58 (dd, J = 16.0, 10.0 Hz) 6.59 (dd, J = 16.0, 10.0 Hz)	144.0/144.1
12a/12b	6.31 (1H, d, J = 16.0 Hz)	122.6/122.7
13a/13b		161.0/161.0
14	5.85 (1H, s)	115.5
15		171.2
16a/16b	6.25 (s) 6.27 (s)	97.5/97.6
17a/17b	4.38 (d, J = 1.5 Hz) 4.79 (1H, brs) 4.47 (d, J = 1.5 Hz) 4.79 (1H, brs)	108.5/108.9
18	0.90 (3H, s)	33.6
19	0.85 (3H, s)	21.9
20a/20b	0.87 (3H, s)	15.1/15.2

[a] Double signals due to epimers (1:1) at C-16.

Diarylheptanoids have also been isolated from other genera of Zingiberaceous plants such as *Curcuma*, *Alpinia*,

Table 3. Percentage Inhibition of Lipid Peroxidation by Diarylheptanoids **1**−**3** from *E. elatior* Using a Ferric Thiocyanate Method

compound[a]	% inhibition
1	93.69 ± 2.1
2	92.49 ± 1.7
3	92.09 ± 1.3
α-tocopherol	69.53 ± 1.9

[a] Concentration of 300 μM.

and *Zingiber*. Compounds **2** and **3** have previously been isolated from *C. domestica* and *C. xanthorrhiza*.[8,15] Labdanes are the only type of diterpene known to occur in Zingiberaceous plants, and recently a number of related compounds have been isolated from the genus *Alpinia* (tribe Alpinieae),[13,15,16] as well as *Hedychium* and *Curcuma* (tribe Hedychieae).[14,17] From a chemotaxonomic point of view, the isolation of 16-hydroxylabda-8(17),11,13-trien-15,-16-olide (**4**) supports the relationship of *E. elatior* as a member of the tribe Alpinieae.

Although the antioxidant property of diketo-diarylheptanoids has been well established,[18] the antioxidant activity of monoketo-diarylheptanoids does not appear to have been reported. Evaluation of the antioxidant activity of the three diarylheptanoids isolated in this investigation using a ferric thiocyanate (FTC) method has shown that all were more active than α-tocopherol, but no significant differences were observed between the mono- and diketo-diarylheptanoid compounds tested (Table 3). While a conjugated 3,5-dione system is already known to be important for the antioxidant activity of diarylheptanoids, fully conjugated diarylheptanoids having only one ketone functionality thus may also exhibit similarly potent activity.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Ultraviolet (UV) and infrared (IR) spectra were obtained on Shimadzu UV−vis 160 and Perkin-Elmer 1650 FTIR spectrometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 500 spectrometer, and TMS was used as internal standard. EIMS and HREIMS were determined using a Hitachi M2000 mass spectrometer. Column chromatography was performed with Merck 7734 and Merck 9385 silica gel and TLC with Merck silica gel DC-Plastikfolien 60 F_{254} plates.

Plant Material. Fresh *E. elatior* rhizomes were collected in Klang and Banting, Selangor, Malaysia, in October 1999. The rhizomes were cleaned, chopped into small pieces (3−5 mm thickness), and dried in the shade. A voucher specimen (No. SK 80/01) was deposited at the Herbarium of the Laboratory of Natural Products (LNP), Universiti Putra Malaysia.

Extraction and Isolation. Sixteen kilograms of the dried powdered rhizomes was successively extracted with $CHCl_3$, acetone, and MeOH to give 120, 50, and 8 g of each crude extract, respectively. The $CHCl_3$-soluble extract was triturated with hexane and filtered to give a hexane extract (60 g) and a residual $CHCl_3$ extract (60 g). The acetone extract was triturated with ethyl acetate to give 8 g of an ethyl acetate-soluble extract. Column chromatography of the $CHCl_3$ extract (40 g) on silica gel (5 × 40 cm), eluted with hexane/diethyl ether, diethyl ether/ethyl acetate, and ethyl acetate/MeOH, gave combined fractions A, B, and C, respectively. Repeated column chromatography of fraction B (3 g) on silica gel using diethyl ether in hexane (1:9) gave stigmast-4-en-6β-ol-3-one (20 mg) and 5α,8α-epidioxyergosta-6,22-dien-3β-ol (8 mg). Column chromatography of the hexane extract (20 g) on silica gel (5 × 40 cm), eluted with hexane/diethyl ether, afforded eight fractions (A−H). Repeated column chromatography of fraction F (3 g) afforded four fractions (F1−F4), from which stigmast-4-en-3-one (65 mg) was isolated from fraction F2 (138 mg) after recystallization with MeOH. Stigmast-4-ene-3,6-dione (50 mg) was isolated from fraction F4 (77.3 mg) after preparative TLC (20% diethyl ether in hexane). Compound **4** (11.9 mg) was isolated from fraction H (80 mg) after repeated column chromatography on silica gel eluted with 10% ethyl acetate in $CHCl_3$. Column chromatography of the ethyl acetate-soluble extract (8 g) on Sephadex LH-20 (2.5 × 40 cm), eluted with MeOH, afforded 14 fractions (fractions A−N). Repeated column chromatography of fraction K (160 mg) on silica gel, with 10% ethyl acetate in $CHCl_3$ as the eluent, followed by column chromatography on Sephadex LH-20, using MeOH as eluent, gave **2** (5 mg). Column chromatography of fraction I (126 mg) on silica gel and eluted with 10% ethyl acetate in $CHCl_3$ followed by reversed phase HPLC [Waters PrepPak Cartridge C_{18} HPLC column (25 × 10 cm) using 30% methanol in water as a solvent system, flow rate 5 mL/min] afforded 4 mg of **1** and 5 mg of **3**.

1,7-Bis(4-hydroxyphenyl)-2,4,6-heptatrienone (1): yellow powder (Me_2CO); UV (MeOH) λ_{max} (log ϵ) 395 (4.51) nm; IR (KBr) ν_{max} 3300, 1653, 1578, 1511 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 500 MHz), see Table 1; ^{13}C NMR (CD_3COCD_3, 125 MHz), see Table 1; EIMS *m/z* 292 $[M]^+$ (94), 171 (38), 121-(100); HREIMS *m/z* 292.1113 (calcd for $C_{19}H_{16}O_3$, 292.1099).

Demethoxycurcumin (2): yellow powder, mp 170−172 °C; EIMS *m/z* 337.8 (M^+, $C_{19}H_{18}O_5$); ^{1}H NMR and ^{13}C NMR in agreement with literature values.[7,8]

1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (3): yellow powder, mp 168−170 °C; EIMS *m/z* 291.9 (M^+, $C_{19}H_{16}O_3$); ^{1}H NMR and ^{13}C NMR in agreement with literature value.[9]

16-Hydroxylabda-8(17),11,13-trien-15,16-olide (4): gummy solid ($CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 260 (4.51) nm; IR ν_{max} (KBr) 1750 (α,β-unsaturated γ-lactone), 3090, 892 (exo-methylene) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; EIMS *m/z* 316 $[M]^+$ (13), 180 (30), 162 (14), 137 (100), 123 (35); HREIMS *m/z* 316.2030 (calcd for $C_{20}H_{28}O_3$, 316.2038).

Antioxidant Activity. An antioxidant assay was carried out using established protocols.[19] Thus, the percentage of lipid peroxidation inhibition was evaluated based on the absorbance (ABS) on the last day of the experiment (usually on the 7th day, at which time the absorbance of control reached maximum and begin to decrease) and calculated as follows: % inhibition = $(ABS_{control} - ABS_{sample})/ABS_{control} \times 100\%$.

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Supporting Information Available: Table of HMBC, ^{1}H−^{1}H COSY, and NOESY NMR data for **4**. This information is available free of charge via the Internet at http://pubs.acs.org.

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