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THE FIRST HYDROPEROXYDIHYDROCHALCONE IN THE *ETLINGERA* GENUS: ETLINGLITTORALIN FROM THE RHIZOMES OF *ETLINGERA LITTORALIS*

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Abstract – Etlinglittarolin (**6**), a monoterpene-substituted hydroperoxy-dihydrochalcone, together with five known dihydrochacones including 2′, 6′-dihydroxy-4′-methoxydihydrochalcone (**1**), 2′,4′,6′-trihydroxydihydrochalcone (**2**), 2′,6′,4-trihydroxy-4′-methoxydihydrochalcone (**3**), methyllinderatin (**4**), and adunctin E (**5**), was isolated from the rhizomes of *Etlingera littoralis*. Their structures were elucidated by spectroscopic analysis.

Plants of Zingiberaceae are widely distributed throughout the tropical forests. Many of them are used for food, spices, medicines, dyes, perfume and aesthetics.[1] Some metabolites from Zingiberaceae plants have found to be interesting biological activities, for example anti-malaria,[2] anti-tumor[3] and anti-HIV-1 protease inhibitory.[4] *Etlingera littoralis* is one of the Zingiberaceae plants which are found in several parts of Thailand. Its rhizome decoction has been used for the treatment of stomachache, carminative, and heart tonic.[5] As part of our study of chemical constituents and biological activity from medicinal plants, we now report the structure elucidation of a new monoterpene-substituted dihydrochalcone,

etlinglittoralin (**6**) along with five known dihydrochalcones (Figure 1) isolated from the rhizomes of *E. littoralis*.

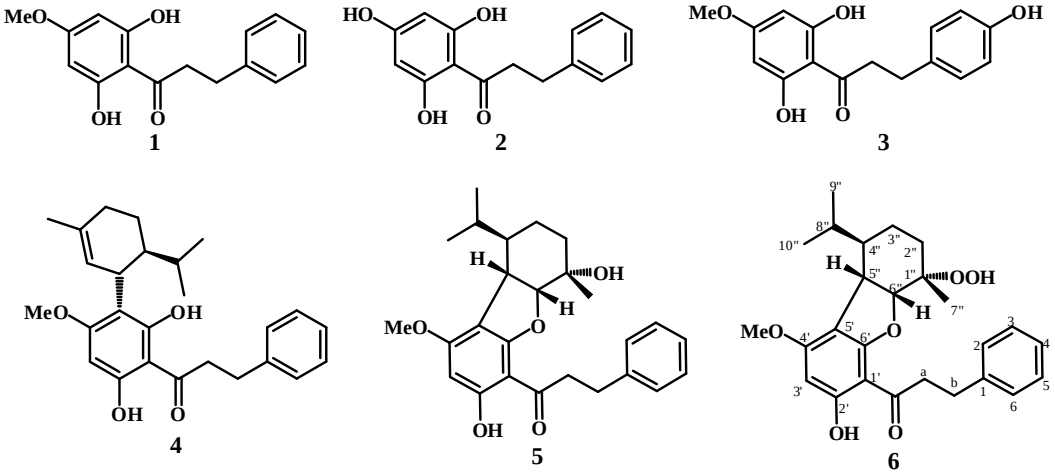

Figure 1. Structures **1-6**

Compound **6**, $[\alpha]_D^{25}$ -22 (c 0.006, $CHCl_3$), was obtained as a white amorphous powder (mp 102.0-103.6 °C). The molecular formula of $C_{26}H_{32}O_6$ was deduced from the ESITOFMS data, exhibiting the $[M+H]^+$ ion peak at *m/z* 441.2266 (calcd. for 441.2277). Its IR spectrum revealed absorption bands for hydroxy (3398 cm^{-1}) and carbonyl (1631 cm^{-1}) groups. The UV absorption bands at λ_{max} 236, 282 and 342 nm supported the presence of a conjugated carbonyl in the structure. The ^{13}C NMR and DEPT spectrum of **6** indicated the signals of the dihydrochalcone (12 aromatic carbons, 2 aliphatic carbons and one carbonyl carbon)[6] and contained ten additional resonances with three methyls [δ_C 22.2 (C-7″), 21.9 (C-10″), 15.6 (C-9″)], two methylenes [δ_C 32.0 (C-2″), 17.3 (C-3″)], four methines [δ_C 87.7 (C-6″), 46.5 (C-4″), 39.9 (C-5″), 27.3 (C-8″)] and a quaternary carbon [δ_C 81.1 (C-1″)]. The latter signals suggested that the presence of a hydroperoxy group at C-1″ was supported by the molecular formula $C_{26}H_{32}O_6$ and the downfield chemical shift of the oxygenated carbon C-1″ at δ_C 81.1.[7-9] From above data, the dihydrochalcone was substituted by a saturated cyclic monoterpene moiety (Table 1). The ^1H NMR spectroscopic data (Table 1) showed signals characteristic of isopropyl unit at δ_H 1.83 (1H, m, H-8″), 0.85 (3H, d, J = 6.8 Hz, H_3-9″), and 0.83 (3H, d, J = 6.8 Hz, H_3-10″) and a tertiary methyl group at δ_H 1.43 (3H, s, H_3-7″). This data was quite similar to a *p*-menthane unit.[10] In the ^1H NMR spectroscopic data, the signals characteristic of a 2′,6′-dihydroxy-4′-methoxydihydrochalcone derivative were clearly observed at δ_H 13.23 (1H, s, OH), 7.17-7.30 (5H, m), 6.04 (1H, s, H-3′), 3.81 (3H, s, 4′-OCH_3), 3.34 (2H, m, H-α), and 3.02 (2H, t, J = 7.6 Hz, H-β).[9]

The attachment of the *p*-menthane unit on the dihydrochalcone moiety was established with the combined results of COSY, HMQC and HMBC experiments. In the HMBC spectrum, the *p*-methine proton H-5″

correlated with C-4′, C-5′, and C-6′ suggesting that the *p*-menthane was *C*-linked to the dihydrochalcone core between C-5″ and C-5′. The deshielded oxymethine carbon C-6″ (δ_C 87.7) and the aromatic carbon C-6′ (δ_C 162.0) implied to *O*-linkage between C-6″ and C-6′. Thus, the *p*-menthane unit and the dihydrochalcone core formed a five-membered ring as in agreement with the spectral data of adunctin E, previously isolated from *Piper aduncum*.[8]

Table 1. NMR Spectral Data (400 MHz) of Etlinglittoralin (**6**) in $CDCl_3$

No.	δ_C	δ_H (mult., *J* in Hz)	HMBC ($^1H \rightarrow {}^{13}C$)
Dihydrochalcone moiety			
1	141.4	-	-
2/6	128.4	7.17 -7.30 (m)	1, 4
3/5	128.5	7.17 -7.30 (m)	-
4	126.1	7.17 -7.30 (m)	-
α	43.9	3.34 (m)	1, β, C=O
β	30.3	3.02 (t, 7.6)	1, 2/6, α, C=O
C=O	203.4	-	-
1′	102.8	-	-
2′-OH	165.4	13.23 (s)	-
3′	93.0	6.04 (s)	5′
4′	161.9	-	-
5′	113.1	-	-
6′	162.0	-	-
4′- OMe	55.6	3.81 (s)	4′
Monoterpene moiety			
1″	81.1	-	-
2″	32.0	2.09 (br s)	-
		1.57 (m)	
3″	17.3	1.33 (m)	5″
4″	46.5	1.08 (m)	-
5″	39.9	3.07 (dd,11.2, 5.6)	4′, 5′, 6′, 4″, 8″
6″	87.7	4.49 (d, 5.6)	1″, 2″,4″
7″	22.2	1.43 (s)	1″, 2″, 6″
8″	27.3	1.83 (m)	-
9″	15.6	0.85 (d, 6.8)	4″, 8″, 10″
10″	21.9	0.83 (d, 6.8)	4″, 9″, 8″

The relative location of the 4′-OMe was confirmed by an HMBC experiment in which a correlation was observed for OMe protons with C-4′ (δ_C 161.9). In addition, OMe showed the cross-peaks with H-3′ (δ_H 6.04) and H_3-10″ (δ_H 0.83) in a 2D NOESY experiment (Figure 2). The cross-peaks between H_3-7″/H-3″,

H-3″/H-5″, H-5″/H_3-10″, H-5″/H-6″ and H-6″/H_3-7″ indicated the relative configurations at C-1″, C-4″, C-5″ and C-6″ as 1″*S**, 4″*R**, 5″*S** and 6″*R**, respectively. Accordingly, the structure of **6** was determined to be (1″*S**, 4″*R**, 5″*S**, 6″*R**)-etlinglittoralin.

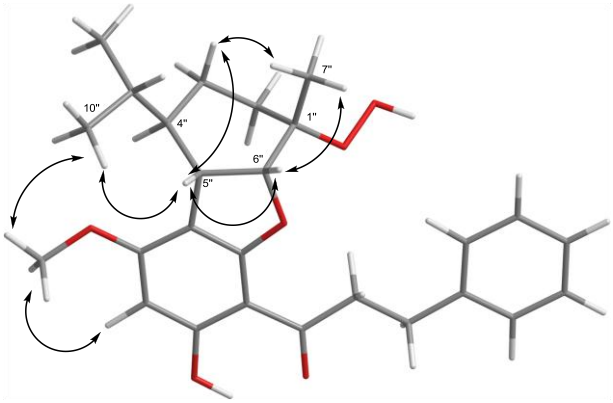

Figure 2. Selected NOESY cross-peaks for **6**

The known compounds were identified as 2′, 6′-dihydroxy-4′-methoxydihydrochalcone (**1**),[8,9] 2′,4′,6′-trihydroxydihydrochalcone (**2**),[4] 2′,6′,4-trihydroxy-4′-methoxydihydrochalcone (**3**),[11] methyllinderatin (**4**),[12] adunctin E (**5**).[6] All of them were identified by exhaustive spectral analysis (1D and 2D NMR spectra) and also comparison with their spectroscopic data with those reported in the literature. All compounds were tested for their antimalarial activity but, unfortunately, they were inactive.

EXPERIMENTAL

GENERAL

The optical rotation $[\alpha]_D$ values were determined with a Bellingham & Stanley ADP440 polarimeter. UV spectra were recorded with a Perkin-Elmer UV-Vis spectrophotometer. The IR spectra were recorded with a Perkin-Elmer FTS FT-IR spectrophotometer. The NMR spectra were recorded using 400 MHz Bruker spectrometer. Chemical shifts were recorded in parts per million (δ) in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. The ESITOFMS was obtained from a MicroTOF, Bruker Daltonics mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 H (Merck, 5-40 μm) and silica gel 100 (Merck, 63-200 μm), respectively. Precoated plates of silica gel 60 F254 were used for analytical purposes.

PLANT MATERIAL

The rhizomes of *E. littoralis* were collected in June 2009 from Surat Thani Province, southern part of Thailand. Botanical identification was made by Assistant Professor Dr. Chatchai Ngamriabsakul and a specimen (number MFU-NPR 0015) was deposited at Natural Products Research Laboratory, School of

Science, Mae Fah Luang University.

EXTRACTION AND ISOLATION

Chopped-fresh rhizomes (3.89 kg) of *E. littoralis* were extracted with CH_2Cl_2–MeOH (1:1, v/v), over the period of 3 days at room temperature. The mixture was filtered and then evaporated to dryness under reduced pressure and partition with CH_2Cl_2 to afford the CH_2Cl_2 extracted (22.80 g). A portion of CH_2Cl_2 extract (11.80 g) was subjected to quick column chromatography (QCC) over silica gel and eluted with a gradient of *n*-hexane–EtOAc (100% *n*-hexane–100% EtOAc) to afford thirteen fractions (F1-F13). Fraction F3 (755.10 mg) was resubmitted to column chromatography (CC) eluting with EtOAc–*n*-hexane (1:9, v/v) to afford four subfractions (F3A-F3D). Subsraction F3A (48.8 mg) was further purified by CC with CH_2Cl_2–*n*-hexane (1:4, v/v) to give compound **5** (3.0 mg) and three subfractions (F3A1-F3A3). Compound **4** (7.1 mg) derived from subfraction F3A1 (12.8 mg) whereas **6** (3.0 mg) obtained from subfraction F3A3 (8.7 mg) by prep. TLC developed with EtOAc–*n*-hexane (1:4, v/v) and prep. TLC with acetone–*n*-hexane (1:9, v/v), respectively. Fraction F8 (322.4 mg) was washed with CH_2Cl_2–*n*-hexane (1:4, v/v) yielding a pale-yellow solid which was further separated by CC with CH_2Cl_2–*n*-hexane (7:3, v/v) to give compound **1** (50.1 mg). Fraction F12 (1.83 g) was resubmitted to QCC over silica gel eluting with a gradient of *n*-hexane–EtOAc (1:5-3:5, v/v) to afford seven subfractions (F12A-F12G). Subfraction F12E (220.0 mg) was purified by CC eluting with EtOAc–CH_2Cl_2 (1:100, v/v) to give compound **2** (108.6 mg). Subfraction F12G (98.0 mg) was purified by CC on silica gel eluting with EtOAc–CH_2Cl_2 (1:100, v/v) to give compound **3** (47.5 mg).

Etlinglittoralin (6): White amorphous powder. Mp 102.0-103.6 °C. $[\alpha]_D^{25}$ -22 (c 0.006, $CHCl_3$). UV ($CHCl_3$) (log ε): 236 (4.23), 282 (4.40), 342 (3.36) and 337 (3.32) nm. IR (neat) ν_{max}: 3398, 2954, 2927, 1631, 1602 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) see Table 1; ESITOFMS (*m/z*): $[M+H]^+$ *m/z* 441.2266 (calc. for $C_{26}H_{33}O_6$, 441.2277).

ANTI-MALARIAL ASSAY

Anti-malarial activity was evaluated against the parasite *Plasmodium falciparum* (K_1 strain, multidrug resistant), using the method of Trager and Jensen (1976).[13] Quantitative assessment of *in vitro* malarial activity was determined by means of the microculture radioisotope technique based on the method described by Desjardins *et al*. (1979).[14] The inhibitory concentration (IC_{50}) represented the concentration that caused 50% reduction in parasite growth which was indicated by the *in vitro* uptake of [^3H]-hypoxanthine by *P. falciparum*. The standard compounds were dihydroartemisinin (IC_{50} 1.58 nM) and mefloquine (IC_{50} 0.0282 μM).

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REFERENCES

1. P. Sirirugsa, International Conference on Biodiversity and Bioresources–Conservation and Utilization, Phuket, Thailand, 1997. http://www.iupac.org/symposia/proceedings/phuket97/sirirugsa.html
2. B. Portet, N. Fabre, V. Roumy, H. Gornitzka, G. Bourdy, S. Chevalley, M. Sauvain, A. Valentin, and C. Moulis, *Phytochemistry*, 2007, **68**, 1312.
3. A. Murakami, A. M. Ali, K. Mat-Salleh, K. Koshimizu, and H. Ohigashi, *Biosci. Biotechnol. Biochem.*, 2000, **64**, 9.
4. S. Cheenpracha, C. Karalai, C. Ponglimanont, S. Subhadhirasakul, and S. Tewtrakul, *Bioorg. Med. Chem.*, 2006, **14**, 1710.
5. W. Chuakul and A. Boonpleng, *Medicinal Plants*, 2003, **10**, 33 (In Thai).
6. M. Yamashita, N. D. Yadav, Y. Sumida, I. Kawasaki, A. Kurume, and S. Ohta, *Tetrahedron Lett.*, 2007, **48**, 5619.
7. Y. Mimaki, A. Kameyama, Y. Sashida, Y. Miyata, and A. Fujii, *Chem. Pharm. Bull.*, 1995, **43**, 893.
8. J. Orjala, A. D. Wright, C. A. J. Erdelmeier, O. Sticher, and T. Rali, *Helv. Chim. Acta*, 1993, **76**, 1481.
9. B. Portet, N. Fabre, V. Roumy, H. Gornitzka, G. Bourdy, S. Chevalley, M. Sauvain, A. Valentin, and C. Moulis, *Phytochemistry*, 2007, **68**, 1312.
10. Y. Senda and S. Imaizumi, *Tetrahedron*, 1975, **31**, 2905.
11. J. Orjala, A. D. Wright, H. Behrends, G. Folkers, and O. Sticher, *J. Nat. Prod.*, 1994, **57**, 18.
12. K. Ichino, *Phytochemistry*, 1989, **28**, 955.
13. W. Trager and J. B. Jensen, *Science*, 1976, **193**, 673.
14. R. E. Desjardins, C. J. Canfield, J. D. Haynes, and J. D. Chulay, *Antimicrob. Agents Chemother.*, 1979, **16**, 710.