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A NEW LIPOXYGENASE INHIBITORY FLAVONOID FROM THE KINO OF *Eucalyptus citriodora*

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*A new lipoxygenase inhibitory flavonoid, 8-[1-(*p*-hydroxyphenyl)ethyl]rhamnocitrin (**1**), together with four known compounds, cinnamic acid (**2**), p-coumaric acid (**3**), caffeic acid (**4**), and gallic acid (**5**), was isolated from the kino of* Eucalyptus citriodora*, and their structures were elucidated on the basis of spectroscopic methods including 2D NMR spectra. These compounds were tested for inhibitory activity against 15-lipoxygenase. Compound **1** showed stronger inhibitory activity (IC_{50} 27.6 ± 0.7 μM) than the positive control quercetin (IC_{50} 37.5 ± 0.8 μM).*

Keywords: *Eucalyptus citriodora*, kino, lipoxygenase, flavonoid.

Eucalyptus, a diverse genus of flowering trees and shrubs, belongs to the Myrtaceae family and comprises over 900 species and subspecies [1, 2]. Many species of eucalyptus produce kino in response to wounding or disease [3]. *Eucalyptus citriodora*, a tree native to Australia, is found mainly in the tropical and temperate world, which is also commonly cultivated in Taiwan. This plant, rich in essential oils, phenolics, and flavonoids [4], is traditionally used as analgesic, anti-inflammatory, and antipyretic remedies [5]. The kino of *E. citriodora* has been discovered as a botanical origin for propolis [6, 7]. Phytochemical studies on *E. citriodora* kino have described the isolation of triterpenoids, phenolics, and flavonoids [7–9]. The cytotoxic effect on HepG 2 cells [10] and the antiproliferative activity and apoptosis induction of *E. citriodora* kino in melanoma cells have been investigated [11]. In previous studies, rhamnocitrin [12], 6-[1-(*p*-hydroxyphenyl)ethyl]rhamnocitrin [9], kaempferol [9], 7-*O*-methylaromadendrin [9], and 6-[1-(*p*-hydroxyphenyl)ethyl]-7-*O*-methylaromadendrin [11], previously isolated from this plant, exhibited antiatherogenic, lipoxygenase inhibitory, and antiproliferative activity, respectively.

As part of our search for bioactive constituents from natural sources, the ethyl acetate (EtOAc) extract of *E. citriodora* kino was further investigated to afford a new flavonoid, 8-[1-(*p*-hydroxyphenyl)ethyl]rhamnocitrin (**1**), together with four known compounds, cinnamic acid (**2**) [13], *p*-coumaric acid (**3**) [14], caffeic acid (**4**) [15], and gallic acid (**5**) [16]. Their structures were elucidated on the basis of spectroscopic methods including HR-EI-MS and 2D NMR spectra and comparison with the data reported in the literature. The 15-lipoxygenase inhibitory activity of compounds **1**–**5** was evaluated.

Compound **1** was isolated as a yellow amorphous powder. The molecular formula of **1** was determined to be $C_{24}H_{20}O_7$ by HR-EI-MS. The IR spectrum of **1** displayed absorption bands at 3500 and 3250 (OH), 1660 (conjugated C=O), and 3050, 1605, 1580, and 1525 (phenyl group) cm^{-1}. The UV spectrum of **1** showed the λ_{max} at 271 and 370 nm, suggesting the existence of a flavonoid nucleus. The ^1H NMR (Table 1) of **1** displayed a pair of A_2B_2 aromatic system protons at δ 7.66 (2H, d, J = 8.4 Hz, H-2′, 6′) and 6.81 (2H, d, J = 8.4 Hz, H-3′, 5′) due to a *p*-substituted phenyl group, a singlet aromatic proton at δ 6.56 (1H, s, H-6), a chelating hydroxyl proton at δ 12.72 (1H, s, 5-OH), and methoxy protons at δ 3.85 (3H, s, 7-OCH_3). A pair of A_2B_2 aromatic system protons at δ 7.02 (2H, d, J = 8.4 Hz, H-3″, 7″) and 6.64 (2H, d, J = 8.4 Hz, H-4″, 6″), together with substructure CH_3CH-protons at δ 4.78 (1H, q, J = 7.2 Hz, H-1″) and 1.61 (3H, d, J = 7.2 Hz, H-8″) due to a *p*-hydroxyphenylethyl moiety linked to ring A, was observed. The EI-MS of **1** showed a fragment ion at *m/z* 300 due to the rhamnocitrin moiety and a base peak signal at *m/z* 120 due to the *p*-hydroxyphenylethyl moiety. These data revealed that compound **1** was a rhamnocitrin derivative with a *p*-hydroxyphenylethyl moiety linked to ring A. The ^1H and ^{13}C NMR data of **1** were closely similar to those of 6-[1-(*p*-hydroxyphenyl)ethyl]rhamnocitrin [9], except for the signals due to the A-ring.

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TABLE 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **1** (DMSO-d_6, TMS, δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
2	–	147.7 (qC)	4′	–	159.3 (qC)
3	–	135.6 (qC)	1″	4.78 (1H, q, J = 7.2)	31.4 (CH)
4	–	176.6 (qC)	2″	–	134.7 (qC)
5	–	159.5 (qC)	3″/7″	7.02 (2H, d, J = 8.4)	127.6 (CH)
6	6.56 (s)	95.1 (CH)	4″/6″	6.64 (2H, d, J = 8.4)	114.9 (CH)
7	–	162.6 (qC)	5″	–	155.3 (qC)
8	–	112.0 (qC)	8″	1.61 (3H, d, J = 7.2)	18.3 (CH_3)
9	–	152.8 (qC)	OCH_3	3.85 (3H, s)	56.4 (CH_3)
10	–	104.1 (qC)	3-OH	9.36 (s)	
1′	–	121.8 (qC)	5-OH	12.72 (s)	
2′/6′	7.66 (2H, d, J = 8.4)	129.8 (CH)	4′-OH	10.04 (s)	
3′/5′	6.81 (2H, d, J = 8.4)	115.4 (CH)	5″-OH	9.06 (s)	

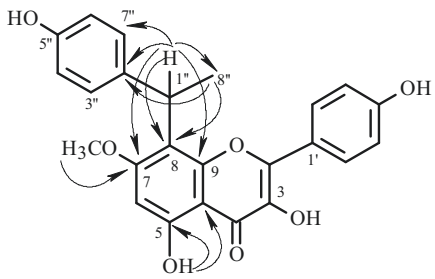

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Fig. 1. Key HMBC correlations of compound **1**.

Therefore, the location of the *p*-hydroxyphenylethyl moiety was different in these two compounds. The location of the *p*-hydroxyphenylethyl moiety at C-8 in compound **1** was confirmed on the basis of the following HMBC correlations (Fig. 1): δ 4.78 (H-1″) with δ 162.6 (C-7), 112.0 (C-8), 152.8 (C-9), 134.7 (C-2″), 127.6 (C-3″, 7″), and 18.3 (C-8″), and δ 1.61 (H-8″) with δ 112.0 (C-8), 31.4 (C-1″), and 134.7 (C-2″). The optical rotation of compound **1** was 0° and no Cotton effect was observed in the CD spectrum, implying that compound **1** was a racemate. Therefore, the structure of **1** was elucidated to be 8-[1-(*p*-hydroxyphenyl)ethyl]rhamnocitrin.

The lipoxygenase inhibitory activity of compounds **1–5** along with quercetin (positive control) were evaluated. Compound **1** showed stronger inhibitory activity (IC_{50} 27.6 ± 0.7 μM) than the positive control quercetin (IC_{50} 37.5 ± 0.8 μM), while the four known compounds (**2–5**) showed no inhibitory activity against lipoxygenase.

EXPERIMENTAL

General Experimental Procedures. Column chromatography (CC) was performed on silica gel 60 (Merck, 70–230 mesh) and Sephadex LH-20 (Pharmacia). Thin-layer chromatography (TLC) and preparative TLC were performed on precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm and 1.00 mm). UV spectra were obtained on a Cary 50 UV-visible spectrophotometer. Optical rotations were measured on a JASCO DIP-360 digital polarimeter. IR spectra were taken on a PerkinElmer 781 infrared spectrophotometer. CD spectra were obtained on a Jasco J-720 spectropolarimeter. NMR spectra were obtained on a Bruker AV-500 NMR spectrometer. EI-MS and HR-EI-MS were recorded on a JEOL JMS-700 mass spectrometer. 15-Lipoxygenase (Type I, from soybean) and linoleic acid were purchased from Sigma Chemical Co. (St. Louis, MO).

Plant Material. The kino of *Eucalyptus citriodora* was collected in Yung Kang, Tainan, Taiwan, and identified by Prof. Chang-Sheng Kuoh, Department of Biology, National Cheng Kung University. A voucher specimen (CNUNP0605) was deposited in the Natural Product Laboratory of the Department of Medicinal Chemistry, Chia Nan University of Pharmacy and Science.

Extraction and Isolation. The extraction and fractionation of *E. citriodora* kino has been reported previously [9]. Fraction 3 was further purified by silica gel CC using *n*-hexane–EtOAc–MeOH (3:1:0.1) as eluent to afford compounds **2** (8.0 mg) and **3** (11.0 mg). Fraction 6 was further purified by repeated Sephadex LH-20 CC using EtOH–H_2O (1:1) and preparative TLC using *n*-hexane–EtOAc–MeOH (1:1:0.1) as eluent to afford compound **1** (1.2 mg). Fraction 8 was further purified by silica gel CC using *n*-hexane–EtOAc–MeOH (1:1:0.3) as eluent, followed by recrystallization, to afford compounds **4** (16.0 mg) and **5** (89.0 mg).

8-[1-(*p*-Hydroxyphenyl)ethyl]rhamnocitrin (1). Yellow amorphous powder, mp > 300°C. $[\alpha]_D^{20}$ ±0° (*c* 0.01, MeOH). CD (*c* 8.7×10^{-5} M, MeOH): no Cotton effect. UV (MeOH, λ_{max}, nm) (log ε): 370 (3.92), 271 (4.28). IR (KBr, ν_{max}, cm^{-1}): 3500, 3250, 3050, 1660, 1605, 1580, 1525. HR-EI-MS *m/z* 420.1207 $[M]^+$ (calcd for $C_{24}H_{20}O_7$, 420.1205). EI-MS (*m/z*, I_{rel}, %): 420 (30), 405 (45), 300 (31), 286 (29), 120 (100). For ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6), see Table 1.

Measurement of 15-Lipoxygenase Inhibitory Activity. 15-Lipoxygenase inhibitory activities were measured according to the method of Lyckander [17] with minor modification. Borate buffer (200 μL, pH 9.0) was added to 50 μL of different concentrations of compounds **1–5** along with quercetin (positive control) and enzyme (500 μL, 400 units/mL in borate buffer). The compounds and quercetin were added as DMSO solutions. After incubation of the test solution for 4 min, 250 μL linoleic acid was added, and the change in the absorbance was measured for 60 s at 234 nm. The enzyme solution was kept in ice, and controls were measured at intervals throughout the experimental periods to ensure that the enzyme activity was constant. Each compound was tested in triplicate, and the values are expressed as means ± SD. Quercetin, a well-known inhibitor of 15-lipoxygenase [17], was employed as a positive control.

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REFERENCES

1. J. J. W. Coppen, *Eucalyptus*: *the Genus Eucalyptus*, Taylor and Francis, London, 2002, p. 261.
2. C. J. Williams, *Medicinal Plants in Australia*, Vol. 2, Rosenberg publishing, Australia, 2011, p. 70.
3. S. von Martius, K. A. Hammer, and C. Locher, *J. Ethnopharmacol*., **144**, 293 (2012).
4. Y. Vaghasiya, R. Nair, and S. Chanda, *Nat. Prod. Res*., **22**, 754 (2008).
5. J. Silva, W. S. M. Abebe, S. M. Sousa, V. G. Duarte, M. I. L. Machado, and F. I. A. Matos, *J. Ethnopharmacol*., **89**, 277 (2003).
6. M. I. Moreno, M. I. Isla, A. R. Sampietro, and M. A. Vattuone, *J. Ethnopharmacol*., **71**, 109 (2000).
7. M. O. Freitas, F. A. F. Ponte, M. A. S. Lima, and E. R. Silveira, *J. Braz. Chem. Soc*., **19**, 532 (2008).
8. M. O. Freitas, M. A. S. Lima, and E. R. Silveira, *Magn. Res. Chem*., **45**, 262 (2007).
9. S. W. Lee, W. J. Hung, and Z. T. Chen, *Nat. Prod. Res*., **26**, 609 (2017).
10. K. H. Shen, Z. T. Chen, and P. D. Duh, *Am. J. Chin. Med*., **40**, 399 (2012).
11. P. D. Duh, Z. T. Chen, S. W. Lee, T. P. Lin, Y. T. Wang, W. J. Yen, L. F. Kuo, and H. L. Chu, *J. Agric. Food Chem*., **60**, 7866 (2012).
12. Y. C. Tu, T. W. Lian, J. H. Yen, Z. T. Chen, and M. J. Wu, *J. Agric. Food Chem*., **55**, 9969 (2007).
13. C. L. Lin, M. H. Perng, W. J. Li, H. T. Li, and C. Y. Chen, *Chem. Nat. Compd*., **54**, 628 (2018).
14. J. Y. Cho, J. H. Moon, K. Y. Seong, and K. H. Park, *Biosci. Biotechnol. Biochem*., **62**, 2273 (1998).
15. Y. Lu and L. Y. Foo, *Food Chem*., **59**, 187 (1997).
16. V. A. Kurkin, T. K. Ryazanova, E. D. Daeva, and V. I. Kadentsev, *Chem. Nat. Compd*., **54**, 278 (2018).
17. I. M. Lyckander and K. E. Malterud, *Acta Pharm. Nord*., **4**, 159 (1992).