Two 5-HT_{1A} Receptor-Interactive Tryptamine Derivatives from the Unripe Fruit of *Evodia rutaecarpa*

Li-Li Yu, Li-Kang Ho, Jyh-Fei Liao,* and Chieh-Fu Chen

Department and Institute of Pharmacology, National Yang-Ming University, and National Research Institute of Chinese Medicine, Taipei 11221, Taiwan

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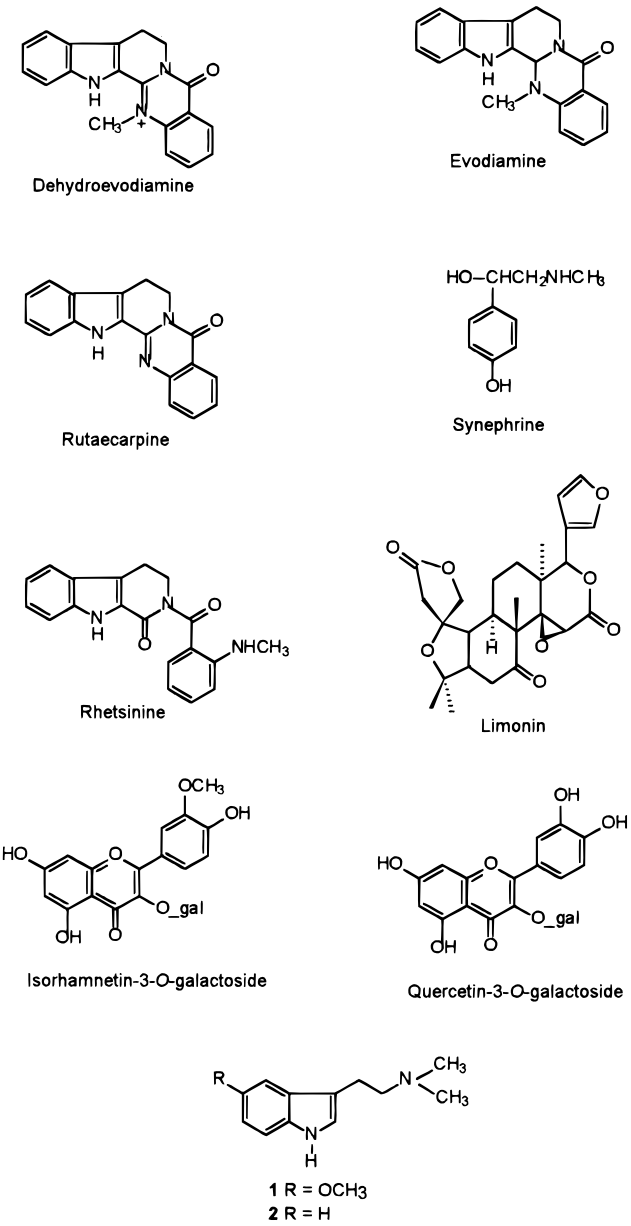
5-Methoxy-*N*,*N*-dimethyltryptamine (**1**) and *N*,*N*-dimethyltryptamine (**2**) isolated from the dried, unripe fruit of *Evodia rutaecarpa* interacted with 5-HT_{1A} receptors with K_i values of 28 nM and 0.41 μM, respectively. Compound **2** was found in the *E. rutaecarpa* fruit and the genus *Evodia* for the first time.

Evodiae Fructus (the dried, unripe fruit of *Evodia rutaecarpa* Hooker f. et Thomas (Rutaceae)) is an herbal drug recommended for the treatment of abdominal pain, nausea, diarrhea, hernia, and dysmenorrhea in Chinese medicine.[1] Previous studies suggest that the pharmacological actions of *E. rutaecarpa* fruit on cardiovascular, gastrointestinal, or central nervous systems may be due to the effect on the adrenergic, muscarinic, histamine, or 5-hydroxytryptamine (5-HT) receptors or the L-type Ca^{2+} channel.[1−4] In order to explain the pharmacological actions of *E. rutaecarpa* fruit at the receptor level, it must be demonstrated that *E. rutaecarpa* fruit indeed contains the active principle(s), capable of action on specific receptors or binding sites. Therefore, this study was conducted to evaluate whether *E. rutaecarpa* fruit extract can interact with these receptors or binding sites by radioligand receptor binding assays and to isolate the responsible principles. The interaction of *E. rutaecarpa* fruit extract with the 5-HT_{1A} receptors and the subsequent isolation of two active principles, 5-methoxy-*N*,*N*-dimethyltryptamine (**1**) and *N*,*N*-dimethyltryptamine (**2**), were reported.

The ethanol−water (1:1) extract of *E. rutaecarpa* fruit was evaluated with radioligand receptor binding assays, and the most potent interaction was found with the 5-HT_{1A} receptors having a K_i value of 18.4 μg dry weight of *E. rutaecarpa* fruit per mL (Table 1). This extract was also active on the α_2-, β_1-, and β_2-adrenoceptors, muscarinic acetylcholine, histamine H_1, dopamine D_1, D_2, and 5-HT_{1A} receptors, and the dihydropyridine (DHP) site of the L-type Ca^{2+} channel but had no significant interaction with the α_1-adrenoceptors and 5-HT_2 receptors. Since the 5-HT_{1A} receptors are specific in the control of mood, appetite, motor behavior, nociception, endocrine secretion, thermoregulation, and cardiovascular function,[5] it was interesting to isolate the 5-HT_{1A} receptor-interactive principle(s) from *E. rutaecarpa* fruit for further pharmacological study.

To facilitate this isolation, the EtOH extract of *E. rutaecarpa* fruit, which also interacted with the 5-HT_{1A} receptors with a K_i value of 1.64 μg extract weight per mL (Table 2), was used. This K_i value is equivalent to 21.8 μg dry weight of *E. rutaecarpa* fruit per mL, which is comparable to that of the ethanol−water (1:1) extract. The EtOH extract was then separated into H_2O-soluble and H_2O-insoluble fractions, of which the former fraction significantly interacted with the 5-HT_{1A} receptors. Further separation of the H_2O-soluble fraction led to the isolation of two active principles, 5-methoxy-*N*,*N*-



* To whom correspondence should be addressed. Tel: 886-2-8267095. Fax: 886-2-8264372. E-mail: jfliao@ym.edu.tw.

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Table 1. K_i Values of the Ethanol−Water (1:1) Extract of *E. rutaecarpa* Fruit for Various Receptors or Binding Sites[a,b]

type	K_i value (mg/mL)
α_1-adrenoceptor	>50
α_2-adrenoceptor	1.7 ± 0.6
β_1-adrenoceptor	1.1 ± 0.3
β_2-adrenoceptor	3.1 ± 0.2
muscarinic acetylcholine receptor	9.2 ± 1.5
histamine H_1 receptor	9.6 ± 0.6
dopamine D_1 receptor	3.9 ± 0.9
dopamine D_2 receptor	6.0 ± 1.6
5-HT_{1A} receptor	0.018 ± 0.003
5-HT_2 receptor	>50
DHP site	7.9 ± 0.4

[a] The data are expressed as mean ± SEM of three experiments with duplicate determinations. [b] DHP site = dihydropyridine binding site of L-type Ca^{2+} channel.

Table 2. K_i Values of the EtOH Extract, the H_2O-Soluble Fraction, and Isolates from *E. rutaecarpa* Fruit for the 5-HT_{1A} Receptors[a,b]

drug	K_i (μg/mL)	K_i (μM)
EtOH extract	1.64 ± 0.18	
H_2O-soluble fraction of EtOH extract	0.24 ± 0.03	
5-methoxy-*N*,*N*-dimethyltryptamine (**1**)	0.0062 ± 0.0004	0.028 ± 0.002
N,*N*-dimethyltryptamine (**2**)	0.078 ± 0.006	0.41 ± 0.03
dehydroevodiamine		36 ± 8
evodiamine		>100
rutaecarpine		>100
synephrine		63 ± 17
rhetsinine		32 ± 3
limonin		>100
isorhamnetin 3-*O*-galactoside		>100
quercetin 3-*O*-galactoside		>100

[a] The data are expressed as mean ± SEM of three experiments with duplicate determinations. [b] [^{3}H]-8-OH-DPAT = 0.85 ± 0.05 nM.

dimethyltryptamine (**1**) and *N*,*N*-dimethyltryptamine (**2**). Compounds **1** and **2** interacted with the 5-HT_{1A} receptor with K_i values of 28 nM (0.0062 μg/mL) and 0.41 μM (0.078 μg/mL), respectively (Table 2).

For comparison, the interactions with the 5-HT_{1A} receptors of a number of compounds previously isolated from *E. rutaecarpa* fruit [6] were also investigated. Table 2 shows that only dehydroevodiamine, synephrine, and rhetsinine interacted moderately with the 5-HT_{1A} receptors with a K_i value greater than 30 μM. Evodiamine, rutaecarpine, limonin, isorhamnetin 3-*O*-galactoside, and quercetin 3-*O*-galactoside did not show significant interactions even at a concentration of 10^{-4} M (Table 2).

Compound **1** was previously found in a number of plants, including *E. rutaecarpa* fruit,[7] *Phalaris tuberosa* (Gramineae),[8] and *Desmodium pulchellum* (Leguminosae).[9] Compound **2** has been found in *Phalaris tuberosa*,[8] *Desmodium pulchellum*,[9] *Zanthoxylum arborescens*,[10] and many other species. Compound **2** was found in *E. rutaecarpa* fruit and the genus *Evodia* for the first time during the course of this investigation.

Experimental Section

General Experimental Procedures. ^{1}H-NMR and ^{13}C-NMR spectra were taken on a Bruker AC-300 (Analytik GmbH, Bremen, Germany) or a Varian Gemini 200 spectrometer (Varian-Associates Inc., Taloalco, CA). EIMS spectra were recorded on a JEOL JMS-HX100 spectrometer (Japan).

Plant Material. The dried, unripe fruits of *E. rutaecarpa* Hooker f. et Thomas were purchased from a Chinese herbal drug store in Taipei and identified by Mr. Jun-Chih Ou, National Research Institute of Chinese Medicine, where voucher specimens (#LC1) are maintained.

Ethanol−Water (1:1) Extract. *E. rutaecarpa* fruit (400 g) was extracted with 1 L of 50% ethanol/H_2O three times at room temperature, and then the filtered solutions were concentrated and subjected to lyophilization (freezemobile 12, The Virtis Company, Gardiner, NY). The yield was 16.2%. The lyophilized powder extract was dissolved in twice-filtered H_2O to give a solution containing 1 g dry weight of the drug raw material per 1 mL. This solution was centrifuged, and then the supernatant was used in the receptor binding assays.

Extraction and Isolation of Pure Principles. *E. rutaecarpa* fruit (3 kg) was ground and extracted with 95% ethanol (3 L × 3). The extracts were filtered, combined, and concentrated. The crude extract (224 g, 7.5%) was then divided into an H_2O-insoluble fraction (143 g, 4.8%) and an H_2O-soluble fraction (81 g, 2.7%). The H_2O-soluble fraction was basified with concentrated ammonia water and extracted with $CHCl_3$ (200 mL × 3). The $CHCl_3$ extract (250 mg) was subjected to Si gel column chromatography (Kieselgel 60, 230−400 mesh, E. Merck), eluting with hexane, hexane−$CHCl_3$, $CHCl_3$, and $CHCl_3$−MeOH to give 19 fractions. Fraction 8 (33.8 mg, 0.00113%) was further separated by Si gel column chromatography and HPTLC (precoated plates, Si gel 60 F254) to give **1** (15 mg, 0.0005%) and **2** (7.8 mg, 0.00026%). The identities of **1** and **2** were verified through NMR experiments.[10]

Pure dehydroevodiamine, evodiamine, rutaecarpine, rhetsinine, limonin, isorhamnertin 3-*O*-galactoside, and quercetin 3-*O*-galactoside were isolated and purified from *E. rutaecarpa* fruit according to a previous work.[6]

Receptor Binding Assays. The receptor binding assays were similar to previously established protocol.[4,11−15] In brief, binding assays were initiated by the addition of a receptor membrane preparation in an appropriate buffer containing the specific radioligand for the tested receptor or binding site. Reactions were incubated, and bound ligands were separated from free ligands by vacuum filtration through a 24 mm glass fiber filter (Whatman GF/C). The radioactivity of bound radioligand was then counted. The crude extract and pure principles were tested on the listed receptors in various concentrations from 0.001 to 50 mg/mL and from 10^{-10}−10^{-4} M, respectively. The competition binding curve was analyzed to determine the IC_{50} (concentration of the tested drug to compete 50% of specifically bound radioligand) using the computer software GraFit (Erithacus Software Limited, Staines, Middlessex, U.K.). The K_i value was calculated from the IC_{50} value using the Cheng−Prusoff equation.[16]

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