6-Methoxy-*N*-methyl-1,2,3,4-tetrahydro-β-carboline from Evodiae Fructus

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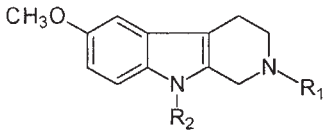
Abstract: **A receptor binding assay directed separation has led to the identification of a minor alkaloid 6-methoxy-*N*-methyl-1,2,3,4-tetrahydro-β-carboline (1) from the Chinese herbal drug Evodiae Fructus [EF, the dried, unripe fruit of *Evodia rutaecarpa* Look. f. et Thomas (Rutaceae)]. The structure of compound 1 was elucidated by means of spectroscopic methods and a comparison with synthetic materials. Compound 1 interacted with 5-HT_{1A} and 5-HT_2 receptors with a moderate K_i value of 78 and 1.2 μM, respectively. Compound 1 is found in EF and the genus Evodiae for the first time.**

Evodiae Fructus [EF, the dried, unripe fruit of *Evodia rutaecarpa* Look. f. et Thomas (Rutaceae)] has been recommended for treatment of abdominal pain, acid regurgitation, nausea, diarrhea, hernia, and dysmenorrhea, and used as analgesic, antiemetic, astringent, and antihypertensive agents in Chinese medicine (1). Previous studies suggested that an action on the adrenergic, muscarinic, histamine, 5-hydroxytryptamine (5-HT) receptors, or L-type Ca^{2+} channel might be involved in the pharmacological actions of EF on cardiovascular, gastrointestinal, or central nervous systems (1–4). To explain the pharmacological actions of EF at the receptor level, a study was carried out to evaluate whether EF extract can interact with these receptors or binding sites by radioligand receptor binding assays. The results indicated that EF can interact with these receptors with various affinities and the affinity for 5-HT_{1A} receptors is the highest (data not shown). Therefore, it is interesting to isolate the receptor-interactive principle(s). Previous studies showed that EF is rich in alkaloids such as rutaecarpine, evodiamine, and dehydroevodiamine, etc. (1). In our present study, we used the 5-HT_{1A} receptor binding assay to direct the isolation of a known alkaloid 6-methoxy-*N*-methyl-1,2,3,4-tetrahydro-β-carboline (**1**) (5). The structure of **1** with a molecular formula of $C_{13}H_{16}N_2O$ [217, FAB-MS $(M + H)^+$] was verified by NMR experiments. To further confirm the structure of **1**, methylation of 6-methoxy-1,2,3,4-tetrahydro-β-carboline (**2**) (purchased from Aldrich) was done and the chemical properties were compared. Furthermore, methylation of **1** gave 6-methoxy-*N*,*N*-dimethyl-1,2,3,4-tetrahydro-β-carboline (**3**). Both **1** and **3** were found in *Phalaris tuberosa* (5) and many other species (6–9). However, **1** is found in EF and the genus Evodiae for the first time. As shown in Table **1**, **1** was more potent in interaction with 5-HT_2 receptors, followed by **2** and **3**; **2** was more potent in interaction with 5-HT_{1A} receptors, followed by **3** and **1**. These results are consistent with the report that several β-carboline derivatives were able to compete with [^3H]5-HT and [^3H]ketanserin for binding to 5-HT_1 and 5-HT_2 receptors (10). Although **1** is found for the first time in EF and in the genus Evodiae, **1** was not the most potent principle in EF to interact with the 5-HT_{1A} receptors. A more potent principle 5-methoxy-*N*,*N*-dimethyltryptamine (0.0026%) was previously found in EF (11), which was also confirmed by us in another study with a K_i value of 24 nM for 5-HT_{1A} receptors.



1 $R_1 = CH_3$ $R_2 = H$
2 $R_1 = H$ $R_2 = H$
3 $R_1 = CH_3$ $R_2 = CH_3$

Table 1 The K_i values of 6-methoxy-*N*-methyl-1,2,3,4-tetrahydro-β-carboline (**1**), 6-methoxy-1,2,3,4-tetrahydro-β-carboline (**2**), and 6-methoxy-*N*,*N*-dimethyl-1,2,3,4-tetrahydro-β-carboline (**3**) for 5-HT_{1A} and 5-HT_2 receptors.

Compound	K_i (μM)	
	5-HT_{1A}	5-HT_2
1	78 ± 8	1.2 ± 0.6
2	2.6 ± 0.1	12 ± 1
3	5.4 ± 0.7	148 ± 3

The data are expressed as mean ± S.E.M. of 3 experiments with duplicate determinations. [^3H]-8-OH-DPAT = 0.85 ± 0.05 nM for 5-HT_{1A} receptors; [^3H]ketanserin = 2.04 ± 0.08 nM for 5-HT_2 receptors.

Materials and Methods

General experimental procedures: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectrum were taken on a Bruker AC-300 spectrometer. FAB-MS and EI-MS were recorded on a JEOL JMS-HX100 spectrometer.

Plant material: The dried, unripe fruits of *E. rutaecarpa* Look. f. et Thomas (EF) 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 (4).

Extraction and isolation: EF (3 kg) was ground and extracted with 95% EtOH (10 l × 3). The filtered extracts were combined and concentrated. The EtOH extract (224 g) was further partitioned into *n*-hexane layer (19.5 g), $CHCl_3$ layer (60.3 g), ethyl acetate layer (1.3 g), BuOH layer (3.8 g, 0.19%), and H_2O layer (0.26 g). The BuOH-layer was then separated by a reversed phase C18 column (Lobar, Fertigsäule Größe B, 310 × 25 mm, 40–63 μm, Merck) and eluted with 0.1% acetic acid in H_2O and CH_3CN (0 → 100% in 120 min). The active fraction was further separated and purified by HPLC (column: 8 × 250 mm, COSMOIL 5C18-AR; solvent: A: 0.1% acetic acid in H_2O, B: CH_3CN; gradient elution: 0 → 50% for B in 0 → 20 min; 50 → 100% in 20 → 30 min; flow rate: 2 ml/min) to give **1** (6.6 mg, 0.00033%). The structure of **1** with a molecular formula of $C_{13}H_{16}N_2O$ [217, FAB-MS $(M + H)^+$] was verified by NMR experiments (6).

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Synthesis of **1** *and 6-methoxy-*N,N*-dimethyl-1,2,3,4-tetrahydro-β-carboline* (**3**): An MeOH solution (2 ml) of 6-methoxy-1,2,3,4-tetrahydro-β-carboline (**2**, 215 mg, 1.06 mmol) was stirred with a 37% formaldehyde solution (0.2 ml) for 1 h at room temperature and excess $NaBH_4$ (100 mg, 2.6 mmol) was added. The resulting solution was washed with iced water and extracted with $CHCl_3$. The $CHCl_3$ extracts were combined, dried over $MgSO_4$, and concentrated. The residue was subjected to column chromatography on silica gel (Kieselgel 60, 230 – 400 mesh, Merck). Elution with a solution of 10% MeOH in $CHCl_3$ furnished the synthetic **1** (180 mg, 0.83 mmol, 78 % yield), which is identical to the isolated **1** in comparison with NMR data. A CH_2Cl_2 solution (2 ml) of **1** (28 mg, 0.13 mmol) was stirred with CH_3I (0.2 ml) for 1 h at room temperature. The resulting solution was concentrated and the residue was purified by column chromatography on silica gel (Kieselgel 60, 230–400 mesh, Merck). Elution with a solution of 5% MeOH in $CHCl_3$ yielded **3** (26 mg, 0.11 mmol, 85% yield). The structure of **3** with a molecular formula of $C_{14}H_{18}N_2O$ [230, EI-MS (M^+)] was verified by NMR experiments (12).

Receptor binding assays: The 5-HT_{1A} and 5-HT_2 receptors in the cortex membrane preparations of ICR mice (25–30 g) were measured by steady binding of [^3H]-8-OH-DPAT and [^3H]ketanserin, respectively, as described in detail in the previous study (13). The density of receptor sites (B_{max}) and the dissociation constant (K_d) of [^3H]-8-OH-DPAT (111 ± 2 fmol/mg protein and 0.74 ± 0.03 nM) and [^3H]ketanserin (1110 ± 20 fmol/mg protein and 1.45 ± 0.03 nM) were determined by data fitting of saturation binding data (usually 5–6 concentrations of the radioligand) using the computer software GraFit (Erithacus Software Limited). In competition experiments, various concentrations of drugs (1 nM–1 mM) were added with a fixed concentration of the radioligand ([^3H]-8-OH-DPAT = 0.85 ± 0.05 nM; [^3H]ketanserin = 2.04 ± 0.08 nM) for receptor binding. 5-HT (10 μM for 5-HT_{1A}) or ritanserin (10 μM for 5-HT_2) was used to determine nonspecific binding. The IC_{50} value was determined by data fitting using the computer software GraFit. The K_i value was calculated from the IC_{50} value using the Cheng-Prusoff equation (14).

Acknowledgements

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Evaluation of four *Narcissus* Cultivars as Potential Sources for Galanthamine Production

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Abstract: **Galanthamine, an alkaloid present in the Amaryllidaceae is currently undergoing clinical trials for the treatment of Alzheimer's. Common daffodils, *Narcissus* spp., contain galanthamine and other alkaloids. Four commercial *Narcissus* cultivars were evaluated as potential sources of galanthamine. Planting depths, planting densities, bulb size or flower bud removal did not affect galanthamine content.**

Galanthamine is a drug for the treatment of Alzheimer's disease and is currently undergoing clinical trials in the USA. Pharmaceutically acceptable forms of the drug are the hydrobromide, the hydrochloride, the methylsulfate or the methiodide. A typical dosage is 50 to 300 mg per day for a patient of a body weight of 40 to 100 kg (1). Alzheimer's afflicts as many as four million people in the USA, 1.3 million of whom they categorize as having severe cases (2). Supplies of *Leucojum aestivum* L., the current commercial source of galanthamine, are insufficient to meet this potential pharmaceutical demand. In contrast, some cultivars of *Narcissus* are readily a available and they have

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