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Canarosine: A new guanidine alkaloid from *Canavalia rosea* with inhibitory activity on dopamine D1 receptors

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A new acyclic guanidine alkaloid, canarosine (**1**), together with five known compounds, β-sitosterol (**2**), stigmasterol (**3**), daucosterol (**4**), *epi*-inositol 6-*O*-methyl ether (**5**), and rutin (**6**), were isolated from the aerial parts of *Canavalia rosea*. Their structures were established on the basis of their spectroscopic data. In the radioligand receptor binding assay, canarosine (**1**), at a concentration of 100 μg/ml, caused 91% inhibition of the dopamine D1 receptor binding with an IC_{50} value of 39.4 ± 5.8 μM.

Keywords: *Canavalia rosea* DC; Fabaceae; guanidine alkaloid; dopamine D1 receptors

1. Introduction

Canavalia rosea DC. (or *C. maritima* Thouars) is a ground plant of the family Fabaceae commonly found in the coastal sand of many tropical countries. Its roots have been used in the western Pacific for the treatment of ciguatera fish poisoning [1]. In Mexico, the plant is smoked as a marijuana substitute, although no psychoactive substance has been isolated. Several studies have been conducted on the lectins from its seeds [2–5]. During our initial assay on the inhibitory activity of a number of plant extracts on dopamine D1 receptors, the CH_2Cl_2 extract of the aerial parts of *C. rosea* displayed notable receptor binding inhibition. Phytochemical investigation of this plant yielded a new acyclic guanidine alkaloid, named canarosine (**1**), and five known compounds including β-sitosterol (**2**) [6], stigmasterol (**3**) [6], daucosterol (**4**) [7], *epi*-inositol 6-*O*-methyl ether (**5**) [8], and rutin (**6**) [9].

2. Results and discussion

Chromatographic separation of the CH_2Cl_2-soluble fraction of the EtOH extract from the aerial parts of *C. rosea* yielded compound **1** as pale yellow needles. The molecular formula of **1** was determined to be $C_{20}H_{30}N_4O_3$ on the basis of its HR-ESI-MS, which showed a quasi-molecular ion peak at *m/z* 375.2409 $[M + H]^+$. An IR absorption band at 1657 cm^{-1} and a signal at δ 157.5 in the ^{13}C NMR spectrum suggested that **1** contained a guanidine group [10]. The ^1H NMR spectrum revealed the presence of an isoprene moiety, including two *gem*-dimethyl doublets at δ 1.72 (3H, d, $J = 0.5$ Hz, H-9) and 1.76 (3H, d, $J = 0.5$ Hz, H-10), an olefinic proton signal of a trisubstituted double bond at δ 5.15 (1H, m,

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H-7), and a methylene proton resonance at δ 3.94 (2H, d, $J = 6.5$ Hz, H-6). The downfield shift of the latter signal suggested its proximity to a heteroatom. Typical resonances of a ferulic amide residue, including aromatic proton resonances at δ 7.10 (1H, d, $J = 1.8$ Hz, H-2′), 6.78 (1H, d, $J = 7.8$ Hz, H-5′), and 7.01 (1H, dd, $J = 7.8, 1.8$ Hz, H-6′); olefinic proton signals of the unsaturated amide trans-double bond at δ 7.43 (1H, d, $J = 15.6$ Hz, H-7′) and 6.40 (1H, d, $J = 15.6$ Hz, H-8′); and a methoxyl singlet at δ 3.86 (3H, s, 3′-OCH_3) could also be observed. The $^1H-^1H$ COSY spectrum revealed the spin system of a four methylene chain (C_1-C_4) between δ 1.57 and 3.34, indicating that the chain could be located between two nitrogen atoms, one of which was the ferulic amide NH. Another spin system of an isoprene unit, from the olefinic H-7 to both the methylene H-6 and the *gem*-dimethyl H-9 and H-10, was also confirmed by the COSY spectrum.

The ^{13}C NMR spectrum exhibited 20 carbon signals including those of 3 methyl, 5 methylene, 6 methine, and 6 quaternary carbons. The most downfield carbon signal at δ 169.2 represented the amide carbonyl (C-9′), while an imine carbon resonating at δ 157.5 (C-5) was characteristic of a guanidine group [11]. The chemical shifts of C-2′ (δ 111.5) and C-5′ (δ 116.4) indicated that the methoxyl group was at C-3′ (δ 149.1) of the aromatic ring [12]. HMBC correlations (Table 1) of H-2′ and methoxyl proton with C-3′ further confirmed the presence of this ferulyl moiety. The HMBC correlations also revealed the linkage between the isoprene unit and the guanidine nitrogen at one end of the C_1-C_4 methylene chain, as evidenced by cross-peaks of H-4 (δ 3.33, 2H, m) with C-5 and C-6 (δ 47.5), and of H-6 with C-4 (δ 49.0) and C-5. The correlations between H-1 at δ 3.34 (2H, t, $J = 6.3$ Hz) and C-2 (δ 28.0), C-3 (δ 25.4), and C-9′ (δ 169.2), and between H-2 at δ 1.57 (2H, quintet, $J = 6.3$ Hz) and C-1 (δ 39.5), C-3, and C-4 in the HMBC spectrum confirmed the linkage of the ferulic amide moiety to the other end of the C_1-C_4 chain (Figure 2). From the above spectral data, the structure of compound **1** was elucidated as a new acyclic guanidine alkaloid named canarosine (Figure 1).

Table 1. ^1H NMR, ^{13}C NMR, and HMBC spectral data of **1**[a].

No.	δ_C	δ_H (J)	HMBC (H → C)
1	39.5	3.34 t (6.3)	C-2, 3, 9′
2	28.0	1.57 quintet (6.3)	C-1, 3, 4
3	25.4	1.65 m	C-2, 4
4	49.0	3.33 m	C-2, 3, 5, 6
5	157.5		
6	47.5	3.94 d (6.5)	C-4, 5, 7, 8
7	118.6	5.15 m	C-9, 10
8	139.3		
9	18.1	1.72 d (0.5)	C-7, 8, 10
10	25.9	1.76 d (0.5)	C-7, 8, 9
1′	127.9		
2′	111.5	7.10 d (1.8)	C-3′, 4′, 6′, 7′
3′	149.1		
4′	149.9		
5′	116.4	6.78 d (7.8)	C-1′, 3′, 4′
6′	123.1	7.01 dd (7.8, 1.8)	C-2′, 4′, 7′
7′	142.1	7.43 d (15.6)	C-1′, 2′, 6′, 8′, 9′
8′	118.3	6.40 d (15.6)	C-1′, 9′
9′	169.2		
3′-OCH_3	56.4	3.86 s	C-3′

[a] Recorded in CD_3OD at 500 (^1H NMR) and 125 MHz (^{13}C NMR).

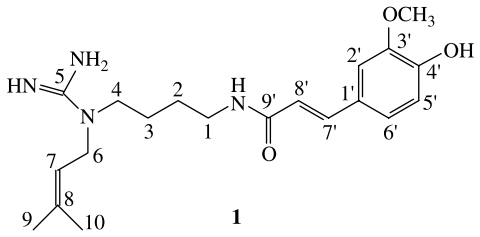

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Figure 1. Structure of canarosine (**1**).

A limited number of guanidine alkaloids have been reported from higher plants, mostly from the families Compositae, Fabaceae, Euphorbiaceae, and Gramineae. Several acyclic guanidine alkaloids from the leaves of *Verbesina caracasana*, given intravenously, were able to lower blood pressure in rats [10]. In our study, when canarosine was subjected to radioligand receptor binding assay [13,14], the compound, at a concentration of 100 μg/ml, caused 91% inhibition of the dopamine D1 receptor binding with an IC_{50} value of 39.4 ± 5.8 μM.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Fisher-John instrument and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR Spectrum One spectrometer. ^{1}H NMR, ^{13}C NMR, and 2D-NMR spectra were recorded on either a 500 MHz Varian Unity INOVA or a 300 MHz Bruker Avance DPX-300 NMR spectrometer, in CD_3OD or DMSO-d_6 with TMS as an internal standard. The HR-ESI mass spectra were taken on a Micromass LCT mass spectrometer. Silica gel 60 (70–230 and 230–400 mesh, Merck) was used for column chromatography.

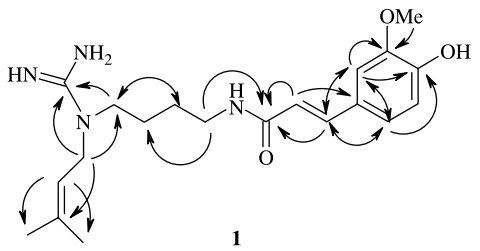

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Figure 2. Important HMBC correlations of compound **1**.

3.2 Plant material

The aerial parts of *C. rosea* DC. were collected from Rayong Province, Thailand, in June 2003, and were identified by one of the authors (R. Suttisri). A voucher specimen (No. RS03061) has been deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

3.3 Extraction and isolation

The dried aerial parts (940 g) of *C. rosea* were ground, macerated with EtOH (41 × 3), and then the solvent was evaporated under reduced pressure to afford ethanolic extract (133 g). The extract was dissolved with 70% EtOH and partitioned with CH_2Cl_2 (41 × 3). The CH_2Cl_2 fraction was further partitioned with *n*-hexane (41 × 3) to give *n*-hexane extract (23 g) and CH_2Cl_2 extract (6 g), whereas the aqueous ethanolic fraction was partitioned with BuOH (41 × 3) to afford BuOH extract (20 g). The *n*-hexane extract was chromatographed on a silica gel column (10 × 10 cm), eluted stepwise with a gradient mixture of *n*-hexane–EtOAc (1:0 → 0:1), and then EtOAc–MeOH (1:0 → 0:1), to give eight fractions (A1–A8). Canarosine (**1**, 7 mg) crystallized from fraction A8, while fraction A6, left at room temperature overnight, yielded a precipitate of daucosterol (**4**, 90 mg). Fraction A4 was separated on a silica gel column (2.5 × 20 cm), using an *n*-hexane–EtOAc gradient (9:1 → 0:1) as eluent, to give β-sitosterol (**2**) and stigmasterol (**3**) as a 2:1 mixture (41 mg). The CH_2Cl_2 extract was subjected to a silica gel column, eluted with a gradient of CH_2Cl_2–MeOH (1:0 → 0:1), to give four fractions (B1–B4). *epi*-Inositol 6-*O*-methyl ether (**5**, 16 mg) was obtained after recrystallization of fraction B4 in a mixture of CH_2Cl_2–MeOH (2:1). Rutin (**6**, 2.1 g) was precipitated as a yellow powder from the BuOH extract.

3.3.1 Canarosine (*1*)

$C_{20}H_{30}N_4O_3$. Pale yellow needles, mp 235–237°C; IR (KBr) ν_{max} (cm^{-1}): 3429, 1657,

1613, 1575, 1409, 651 cm^{-1}; ^1H NMR and ^{13}C NMR spectral data: see Table 1; HR-ESI-MS (*m/z*) 375.2409 $[M + H]^+$ (calcd for $C_{20}H_{31}N_4O_3$, 375.2396).

3.4 Dopamine D1 receptor binding assay

Inhibitory activity on dopamine D1 receptors was determined using the radioligand receptor binding assay [13–14]. The assay was performed at 25°C in a glass tube containing 750 μg of membrane protein, prepared from rat brain striata, in binding buffer containing 50 mM Tris–HCl (pH 7.4), 20 mM $MgCl_2$, and 120 mM NaCl. The binding was initiated by adding 100 μl of 5 nM [^{3}H] SCH23390, a dopamine D1 receptor antagonist. Non-specific binding was measured in the presence of 50 μl of 1.0×10^{-4} M (+)-butaclamol or the solution of test compound in DMSO. After an incubation period of 30 min, the suspension was immediately filtered under vacuum through Whatman GF/B glass fiber filters previously soaked for 1 h in 0.3% polyethyleneimine. The filters were washed rapidly twice with 3 ml of ice-cold washing buffer and placed in scintillation vials containing 5 ml of scintillation cocktail (Ultima Gold®, Perkin Elmer). Radioactivity was determined by liquid scintillation spectrometry. Total binding was determined in DMSO solvent control experiment. SCH23390 was used as the reference compound (at 0.005 μM, it caused 47% binding inhibition). Percent inhibition was obtained as follows:

$$\text{Specific binding} = \text{total binding} - \text{non} - \text{specific binding},$$

$$\%\,\text{Inhibition} = 100 - [(\text{specific binding})_{\text{test}}/(\text{specific binding})_{\text{control}}] \times 100.$$

IC_{50} value was calculated using Microcal Origin® software to obtain the concentration of tested compound that caused 50% inhibition of the binding of [^{3}H] SCH23390 to dopamine D1 receptors.

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