



A new natural pyrrolone from the Egyptian *Ageratum* species

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ABSTRACT

Ageratum conyzoides L., is an annual herb with a long history of traditional medicinal uses in many countries in the world, especially in the tropical and subtropical regions. A wide range of chemical compounds including alkaloids, flavonoids, chromenes, benzofurans and terpenoids have been isolated from this species. Extracts and metabolites from this plant have been found to possess pharmacological and insecticidal activities. In continuation of our interest in the Egyptian medicinal plants, the reinvestigation of the methylenechloride extract of the air-dried aerial parts of *Ageratum conyzoides* afforded a new natural compound **1**, pyrrolone, 5-ethoxy-1H-pyrrol-2(5H)-one, together with a known flavonoid. The structures of the compounds were determined by comprehensive NMR studies, including DEPT, COSY, HMQC, HMBC, and MS.

1. Introduction

The genus *Ageratum* consists of approximately 30 species but only a few species have been phytochemically investigated [1]. Some members of the genus *Ageratum* (family Asteraceae, tribe Eupatorieae) are used in folk medicine for the treatment of various diseases and as an insecticide [2,3]. Recently, the essential oils of *Ageratum fastigiatum* were tested for their antimicrobial activity by agar-diffusion method and minimal inhibitory concentration measurements (MIC) [4]. Several constituents, chromenes, benzofurans, highly methoxylated flavonoids, farnesene derivatives, daucanolides triterpenoids, sterols and alkaloids have been reported from this genus [5-12]. Moreover, the *n*-hexane extract of *A. conyzoides*, collected in Colombia, showed insecticidal activity against the *Musca* domestic larvae. This extract afforded several highly methoxylated flavonoids and chromene derivatives [13,14]. *Ageratum conyzoides* (Asteraceae) is folklorically used to treat diseases associated with bleeding; a study was conducted to determine the efficacy of *Ageratum conyzoides* in treating caecal coccidiosis of broilers [15]. The essential oil of *A. conyzoides* showed an inhibitory effect on *Aspergillus flavus* growth, inhibited aflatoxin biosynthesis, and caused irreversible cellular changes [16,17]. A wide range of pyrrolizidine alkaloids (PAS) have been isolated from *Ageratum conyzoides* [18,19]. Recent communication reported on the isolation, identification and antiprotozoal evaluation of constituents isolated from *Ageratum conyzoides* [20]. Reinvestigation of the CH₂Cl₂ extract of the air-dried aerial parts of *Ageratum conyzoides* yielded a new natural compound (**1**), 5-ethoxy-1H-pyrrol-2(5H)-one, together with a known compound (**2**) (Figure 1).

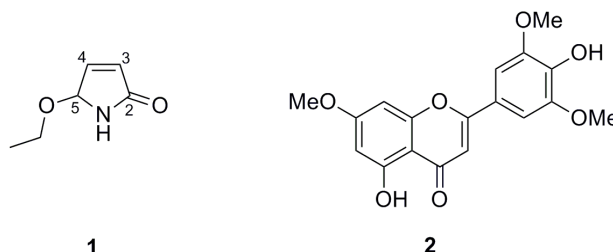


Figure 1. Structures of compounds **1** and **2**.

2. Experimental

2.1. General

NMR spectra were measured with a Bruker AMX-400 spectrometer, with TMS as an internal standard. CC: Silica gel (Merck, 60-120 mesh) and Sephadex LH-20 (Pharmacia). TLC and Preparative TLC: Silica gel 60 GF₂₅₄ (Merck). The TLC developer was ether/petroleum ether. The compounds were visualized either by spraying with reagent (Sulphuric acid/ethanol) or under UV lamp.

2.2. Plant materials

The aerial parts of *A. conyzoides* were collected from Aswan (Upper Egypt), in 2005. A voucher specimen has been deposited in the Herbarium of the Department of Botany, Faculty of Science, South Valley University, Aswan, Egypt.

2.3. Extraction and Isolation

Air-dried aerial parts (300 g) were extracted with CH₂Cl₂ at room temperature. The extract was concentrated *in vacuo* giving a residue (40 g), which was fractionated by flash chromatography over a silica gel column (6 x 80 cm, 1 kg), eluting first with *n*-hexane (2 L) then followed by a gradient of *n*-hexane-CH₂Cl₂ up to 100 % CH₂Cl₂ (2 L each of the solvent mixture). The first fraction *n*-hexane (100%) contained hydrocarbons and waxes. The *n*-hexane-CH₂Cl₂ fraction (1:1) was carefully chromatographed on a Sephadex LH-20 (4 x 35 cm), eluting with *n*-hexane-CH₂Cl₂-MeOH (7:4:0.25). Further purification of each subfraction through repeated chromatography using ether/petroleum ether as a developer, yielded compound **2** (12 mg). The CH₂Cl₂ fraction (100%) was chromatographed on a Sephadex LH-20 column eluted with *n*-hexane-CH₂Cl₂-MeOH (7:4:0.5), followed by further purification through PTLC chromatography using ether/petroleum ether as a developer, afforded compound **1** (12 mg).

5-ethoxy-1H-pyrrol-2(5H)-one: IR (cm⁻¹): 3350 (NH) and 1706 (CO). ¹H NMR (CDCl₃) δ_H: 6.15 (d, *J* = 7.6 Hz, 1H, CH, H-3), 6.90 (d, *J* = 7.6 Hz, 1H, CH, H-4), 5.48 (s, 1H, CH, H-5), 3.46 (m, 2H, CH₂, H-1'), 1.18 (t, 3H, CH₃, H-2'). ¹³C NMR (CDCl₃) δ_C: 172.21 (C-2), 128.65 (C-3), 146.03 (C-4), 85.08 (C-5), 61.10 (C-1'), 14.75 (C-2'). MS, *m/z* (*I*_r/%) : 128 (100) (M+H), 114 (27), 111 (70), 105 (19).

3. Results and Discussion

The methylenechloride extract of the air-dried aerial parts of *Ageratum conyzoides* was chromatographed on silica gel and Sephadex LH-20 columns to give a new natural product (**1**), for which the name pyrrolone was given. The structure of compound **1** was deduced from ¹H-NMR, ¹³C-NMR, DEPT, ¹H-¹H COSY, ¹H-¹³C COSY, HMBC, and chemical ionization mass spectrum (CIMS). Compound **1** was isolated as a yellowish oily material [*R*_F = 0.40; in TLC (Et₂O-petrol, 2:1)]. Its IR spectrum showed absorption bands at 3350 cm⁻¹ (NH) and 1706 cm⁻¹ (CO). The CIMS showed a molecular ion peak at *m/z* 128 (M+H)⁺ (100%), in accordance with the molecular formula of C₆H₉O₂N. The structure of (**1**) was determined from careful investigation of the 1D and 2D NMR experiments. The ¹³C-NMR spectrum revealed the presence of six carbon atoms and their multiplicities (by DEPT analysis) confirmed the number of hydrogen atoms of the formula given above. The carbon atoms were assigned as one methyl carbon at δ_C = 14.75 (C-2'), one carbonyl carbon at δ_C = 172.2 which is characteristic for the carbonyl of α,β-unsaturated-γ-lactone (C-2), one methylene carbon (bearing oxygen) at δ_C = 61.10 (C-1'), and three methine carbons, of which one oxygen bearing carbon at δ_C = 85.0 (C-5), and the other two at δ_C = 128.7 and 146.0 for C-3 and C-4, respectively.

The ¹H-NMR spectrum of compound **1** showed a triplet signal at δ_H 1.18 integrated for three protons and was assigned to H-2'. These methyl protons showed clear correlation in the ¹H-¹H COSY spectrum with a multiplet two proton signals at δ_H 3.46 that could be assigned to H-1'. The latter protons H-1' correlated with a carbon signal at δ_H 61.10 in ¹H-¹³C COSY spectrum indicating the presence of the O-CH₂ moiety. All those couplings and correlations proved the presence of the ethoxy part in compound **1**. Moreover, the ¹H-NMR spectrum revealed the presence of a doublet signal appeared at δ_H 6.9 that could be assigned to H-4 (*J* = 7.6 Hz), which showed AB coupling in the ¹H-¹H COSY spectrum with proton doublet signal at δ_H 6.15 (*J* = 7.6 Hz), H-3. Another proton singlet signal appeared at δ_H 5.48 in the ¹H-NMR that showed a coupling with a carbon signal at δ_C 85.08 in the ¹H-¹³C COSY spectrum which could be assigned to C-5. All previous data proved that compound **1** was

5-ethoxy-1H-pyrrol-2(5H)-one, which is synthetically known [21,22] and a new natural product.

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