Alkaloids and Aromatics of *Cyathobasis fruticulosa* (Bunge) Aellen

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A *β*-carboline-, a tryptamine-, and two phenylethylamine-derived alkaloids and three known aromatic compounds were isolated from the aerial parts and roots of *Cyathobasis fruticulosa* (Bunge) Aellen, and their structures were elucidated by spectroscopic techniques. The one new alkaloid, *N*-methyl-*N*-formyl-4-hydroxy-*β*-phenylethylamine (**1**), showed marginal antifungal activity.

The genus *Cyathobasis* is represented by only one species in the flora of Turkey, namely, *Cyathobasis fruticulosa* (Bunge) Aellen, which grows most commonly in Central Anatolia. This species was formerly included in the genus *Girgensohnia* as "*Girgensohnia fruticulosa* Bunge", but was later separated as a different monotypic genus and was renamed as "*Cyathobasis fruticulosa* (Bunge) Aellen".[1] Therefore, *C. fruticulosa* is a monotypic "single species/single genus" endemic plant for Turkey. *C. fruticulosa* is included in the tribe "Salsoleae" of the family Chenopodiaceae, in which alkaloid-containing plants are present. Tryptophan- and lysine-derived alkaloids have been reported from closely related *Girgensohnia* species.[2] The main constituents of *Cyathobasis* and *Girgensohnia* and other plants of the Chenopodiaceae family are phenylethylamine-,[3] *β*-carboline-,[4] and tryptophan (tryptamine)-[5]derived alkaloids. Many of these alkaloids are known hallucinogens; in particular, harmine alkaloids (*β*-carboline alkaloids) are consumed as hallucinogenic drinks and snuffs in the Amazon basin.[6] This is the first report on the secondary metabolites of *C. fruticulosa*.

From the aerial parts and roots of the plant a *β*-carboline-, a tryptamine-, and two phenylethylamine-type alkaloids and three simple aromatics were isolated, and their structures were elucidated as *N*-methyl-*N*-formyl-4-hydroxy-*β*-phenylethylamine (**1**), hordenine, *N*-methyl-*N*-formyltryptamine, *N*-methyltetrahydro-*β*-carboline, *p*-methoxybenzoic acid (*p*-anisic acid), *p*-hydroxybenzaldehyde, and *p*-aminobenzoic acid by HRMS, 1D and 2D NMR, UV, and IR techniques. In this study, *N*-methyl-*N*-formyl-4-hydroxy-*β*-phenylethylamine (**1**) was isolated as a new compound. It is noteworthy that hordenine[7,8] was isolated from Chenopodiaceae family plants for the first time, while the known compounds *N*-methyltetrahydro-*β*-carboline[9−11] and *N*-methyl-*N*-formyltryptamine[10,12] were isolated from a plant of the *Cyathobasis* genus for the first time. The former was previously isolated from two *Arthrophytum* species[9] and *Gymnacranthera paniculata* (A.D.C.) Wab. var. *zippeliana* (Miq). J. Sinclair,[11] the latter was isolated from *Testulea gabonensis* and *Virola sebifera*, and both were isolated from *Acacia simplicifolia* and some other plant species.[10]

1
(E Form)

1
(Z Form)
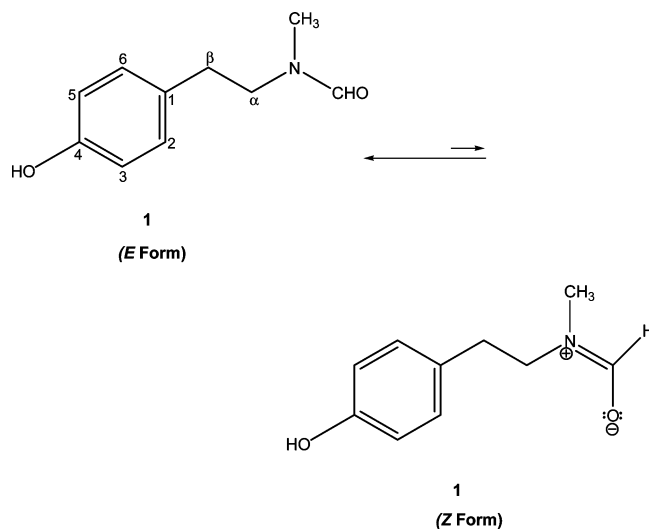
Compound **1**, mp 112−114 °C, was isolated as white needles. HREIMS gave a molecular ion peak at *m/z* 179.0951 corresponding to $C_{10}H_{13}NO_2$ (calcd 179.0946), which has five double-bond equivalents accounted for by one aromatic ring and one formyl carbonyl. It gave a positive Dragendorff test. Its UV spectrum exhibited maxima at 270 (ϵ 2.8) and 226 nm (ϵ 2.8). The IR spectrum showed a formyl carbonyl at 1660 cm^{-1}, aromatic bands at 1625, 1605, 1582, and 1502 cm^{-1}, and an aromatic hydroxyl at 3165 cm^{-1}.

In the ^{1}H NMR spectrum of **1** (Table 1), two aromatic doublets of doublets at δ 6.87 and 6.68 (J = 8.5 and 2.0 Hz) were attributed to the AA′ and BB′ protons of a *p*-disubstituted benzene ring. A three-proton signal at δ 2.82 was assigned to an *N*-methyl group. Triplets at δ 3.36 (J = 7.5 Hz) and 2.67 (J = 7.5 Hz) were assigned to the methylene protons α and β to a nitrogen, respectively. In addition, a singlet at δ 7.60 was assigned to a formamide proton. A ^{1}H NMR spectrum was also recorded in d_6-acetone, and on addition of D_2O the signal at δ 8.27 disappeared, indicating the presence of a hydroxyl group. Irradiation of the signals of each methylene group collapsed the signals for the adjacent methylene triplet to a singlet. The ^{13}C NMR (APT) spectrum revealed eight signals corresponding to 10 carbon atoms, since the signals at δ 130.67 and 116.19 each corresponded to two carbons (2,6 and 3,5) on the phenolic ring. An *N*-methyl carbon was

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Table 1. ^{1}H and ^{13}C NMR and HMBC Data of Compound **1** in CDCl$_3$ (*J* values (Hz) in parentheses)^a

position	^{13}C	^{1}H	HMBC
1	129.94 (129.94)		C-3, C-5
2, 6	130.67 (130.49)	6.87 (6.97) dd (8.5, 2.0)	C-4, C-β
3, 5	116.19 (116.19)	6.68 (6.66) dd (8.5, 2.0)	C-1
4	157.00 (156.98)		C-2, C-6
α	51.73 (46.57)	3.36 (3.43) t (7.5)	C-1
β	34.33 (32.91)	2.67 (2.69) t (7.5)	C-2, C-6
N-CH$_3$	34.83 (34.83)	2.82 (2.79) s	*N*-CHO
N-CHO	163.21 (163.08)	7.60 (7.88) brs	*N*-CH$_3$

^{a} *E* and *Z* forms of compound **1** were observed in a 2:1 ratio, respectively; the numbers in parentheses refer to the minor (*Z*) form.

observed at δ 34.83, while the formyl carbon appeared at δ 163.21. The HMQC spectrum exhibited correlations between protons and carbons that verified that the signal at δ 7.60 belongs to a formamide proton correlating with the carbon signal at δ 163.21. In the HMBC spectrum, three-bond correlations were observed between the β-methylene carbon at δ 34.33 and the C-2 and C-6 protons at δ 6.87, as well as between the α-methylene carbon at δ 51.73 and the formamide proton at δ 7.60 and the *N*-methyl proton at δ 2.82. In fact, observation of duplicate resonances for all signals in both ^{1}H and ^{13}C NMR (Table 1) showed the formation of the *Z* form of the compound besides the *E* form; however the *Z* form was less preferable due to its higher energy than the *E* form.

In the EIMS spectrum, the molecular ion peak was observed at *m/z* 179. As informative fragments, an ion at *m/z* 150 generated by the loss of CHO as well as a base peak at *m/z* 120 arising from the loss [CH$_3$NCHO + H]$^+$ from the molecular ion provided additional structural information. The spectroscopic/spectrometric data confirmed the structure of **1** as the new alkaloid *N*-methyl-*N*-formyl-4-hydroxy-β-phenylethylamine (**1**).

Cytotoxicity testing was carried out on the MeOH extracts of the aerial parts and roots against a panel of cell lines [LU1 (human lung cancer), COL-2 (human colon cancer), KB (human epidermoid carcinoma), LNCaP (hormone-dependent human prostate cancer), and P-388 (mouse leukemia)], but no activity was found. Since Towers and Abramowski showed that some β-carboline- and tryptophan-derived alkaloids inhibit mitosis and cause chromosomal damage,[13] *N*-methyltetrahydro-β-carboline and hordenine were investigated for their activity against the A 2780 mammalian ovarian cell line and were found to be weakly active, exhibiting 38% and 39% inhibition, respectively, at a dose of 50 μg/mL.

Antifungal activity for hordenine, *N*-methyltetrahydro-β-carboline, and *N*-methyl-*N*-formyl-4-hydroxy-β-phenylethylamine (**1**) was determined by a dose-dependent microtiter assay against three yeast strains. Only the new compound showed marginal antifungal activity against the two genetically modified yeasts RS321NYCp50 and RS321NpRAD52.

Experimental Section

General Experimental Procedures. UV spectra were recorded in MeOH and CHCl$_3$ on a Varian DMS 90 spectrophotometer. IR spectra were recorded in CHCl$_3$ on a Perkin-Elmer Model 983 spectrophotometer. Melting points were recorded on a Kofler apparatus (Reichert) and are uncorrected. ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) were recorded on a Bruker AC 200L instrument. EIMS and HRMS were recorded on a VG ZabSpec instrument (Micromass). Chromatographic separations were carried out on silica gel (Merck,

Art. 7733 and 7734), aluminum oxide neutral (Merck, Art. 1077), and Sephadex LH-20 (Pharmacia) columns.

Chromatotron rotors coated with 1 mm thick layers of aluminum oxide neutral 60 PF$_{254}$ (Merck, Art. 1092) were used for the separation of combined fractions. Final purifications were achieved on 0.25 mm thick preparative TLC plates (Merck, Art. 5554).

Plant Material. The aerial parts and roots of *C. fruticulosa* were collected from Central Turkey by A. Küçükosmanoğlu Bahçeevli in July 1998 and were identified by Prof. Mecit Vural. A voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy, Gazi University (AEF 19952).

Extraction and Isolation. Dried and powdered aerial parts and roots of *C. fruticulosa* (Bunge) (1.5 kg) were macerated with MeOH (4 L) for one week at room temperature. The macerate was stirred with a mixer (Heidolph type 743) for 6 h per day and then filtered. The solvent was evaporated in vacuo, and a 150 g extract was obtained. H$_2$O was added and the extract was acidified with 1 N H$_2$SO$_4$ to pH 2.5 and extracted with petroleum ether (5 × 500 mL). The remaining aqueous MeOH extract was basified with 5 N NaOH to pH 10 and extracted with chloroform (10 × 500 mL). After evaporating the solvent, 74 g of crude alkaloidal mixture was obtained.

The crude extract was dissolved in MeOH and mixed with silica gel, dried at room temperature, and added to a silica gel column (Merck, Art. 7733). Fractions eluting with petroleum ether (fraction **A**, 26 g), petroleum ether/CHCl$_3$ (50:50) (fraction **B**, 7 g), petroleum ether/CHCl$_3$ (75−100:25−0) (fraction **C**, 2 g), CHCl$_3$/MeOH (90:10) (fraction **D**, 2 g), and a CHCl$_3$/MeOH gradient up to 100% (fraction **E**, 3 g) were collected. Fractions **B**, **D**, and **E** yielded more alkaloids than the others. For further isolation of compounds, silica gel (Merck, Art. 7734), neutral aluminum oxide (Merck, Art. 1077), and Sephadex LH-20 (Pharmacia) columns were used. Hordenine was obtained from fraction **B** by using a Chromatotron apparatus (PE/CHCl$_3$/EtOH) on neutral aluminum oxide plates (Merck, Art. 1092). Final purifications were carried out on preparative TLC plates (Merck, Art. 5554) using different solvent systems: hordenine was purified using petroleum ether/ether (25:75; 60 mg); *p*-methoxybenzoic acid from fraction **C** with 100% CHCl$_3$ (10 mg); and *N*-methyltetrahydro-β-carboline from fraction **D** with CHCl$_3$/MeOH (90:10; 32 mg); from fraction **E**, *N*-methyl-*N*-formyl-4-hydroxy-β-phenylethylamine (**1**) and *N*-methyl-*N*-formyltryptamine, using CHCl$_3$/MeOH (10:90; 50 and 12 mg, respectively), and *p*-hydroxybenzaldehyde and *p*-aminobenzoic acid using 100% MeOH (8 and 10 mg, respectively) were purified. The known compounds were identified by comparing their spectroscopic data to those of authentic compounds and by TLC with standards.

***N*-Methyl-*N*-formyl-4-hydroxy-β-phenylethylamine (1):** white needles (in MeOH), mp 112−114 °C; UV (MeOH) λ_{max} (log ϵ) 270 (2.8), 226 (2.8) nm; IR (CHCl$_3$) ν_{max} 3165 (OH), 1660 (HC=O), 1625, 1605, 1582, 1502 (aromatic ring), 1460,-1440,1380, 1250, 1160, 1100, 1070, 1020, 950, 850, 830, 760, 650 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); EIMS *m/z* (rel int) 179.1 [M]$^+$ (23), 150 [M − CHO]$^+$ (3), 120 [150 − N − CH$_3$]$^+$ (100), 107 [M − CH$_2$CH$_3$NCHO]$^+$ (82), 91 (15), 77 (34), 72 (73), 65 (8), 60 (6); HREIMS *m/z* 179.0951 (calcd for C$_{10}$H$_{13}$NO$_2$, 179.0946).

Cytotoxicity Assays. Both the MeOH extracts of the aerial parts and roots of the plant were evaluated for their cytotoxic activity against a panel of cell lines (LU1, COL-2, KB, LNCaP, and P-388).[14]

Hordenine and *N*-methyltetrahydro-β-carboline were evaluated against the A2780 human ovarian cancer cell line.[15]

Microtiter Yeast Assay. The assay was carried out as previously described; streptonigrin was used as a positive control.[16]

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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