

## Two Alkaloids from *Ephedra aphylla* growing in Egypt

Maged S. Abdel-Kader\*, Fahima F. Kassem, and Rokia M. Abdallah

Department of Pharmacognosy, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt

**Abstract** – The aerial parts of *Ephedra aphylla* afforded two alkaloids belonging to two different classes. The first alkaloid, ephedradine C, belonging to the spermine alkaloid, a group characterized by its hypotensive effect. The other alkaloid, hordenine is a phenylalkylamine alkaloid isolated for the first time from *Ephedra* Species. The structures were elucidated by spectroscopic methods and the assignment of some carbons in ephedradine C was achieved based on 2 D-NMR experiments.

**Key words** – *Ephedra aphylla*, Ephedraceae, alkaloids, isolation and identification.

### Introduction

*Ephedra* is the only genus in the family Ephedraceae. It comprises 40 species distributed worldwide. In Egypt, genus *Ephedra* is represented by five species (Boulos, 1999). Many members of the genus have been used medicinally. They have a long history of use as stimulant and for management of bronchial disorders. These plants have been used by the Chinese since more than 5,000 years to treat asthma (DerMardersian, 2001). Production of phenylethylamine alkaloids mainly ephedrine and pseudoephedrine seems to be a common feature in many *Ephedra* species (Brossia and Pecherer, 1970). However, quinoline-2-carboxylic acid alkaloids were also reported from members of the genus (Caveney *et al*, 2001; Nawwar *et al*, 1985, Starratt and Caveney, 1996; Al-Khalil *et al*, 1999). Macrocyclic spermine alkaloids designated as ephedradines were isolated from *Ephedra* root (Hikino *et al*, 1983; Tamada *et al*, 1979). Ephedradines are responsible for the hypotensive effect of the crude root; they exerted hypotensive effect in rats, at a dose of 1 mg/Kg IV, due to their ganglionic blocking effect (Hikino *et al*, 1983; Kabuto *et al*, 1980).

### Experimental

**General** – UV spectra were determined using a Shimadzu UV 1201 spectrophotometer. Optical rotation was measured on Perkin-Elmer 241 MC polarimeter. NMR spectra were recorded on a Varian Unity 400 NMR instrument at

399.951 MHz for  $^1\text{H-NMR}$  and 100.578 MHz for  $^{13}\text{C-NMR}$ . MS were taken on a VG 7070 E-HF. Melting points were determined using a Griffin melting point apparatus, and are uncorrected. Aluminium oxide neutral (Merck) was used for column chromatography, and Aluminium oxide 60 GF<sub>254</sub> neutral (Type E, Merck) was used for TLC.

**Plant materials** – *Ephedra aphylla* Frossk was collected from the sandy locations near Rosetta in 2001. The plant was identified by Prof. El-Gharib, Faculty of Science, University of Alexandria. A voucher specimen is deposited in the Herbarium of Faculty of Science, University of Alexandria, Alexandria, Egypt.

**Extraction and isolation** – The air dried powdered aerial parts of *Ephedra aphylla* (6 Kg) were extracted successively with 90% EtOH at R.T. The alcoholic extract was evaporated under vacuum to 500 ml; 500 ml water was added and the solution was acidified with 5% citric acid solution. The acidic solution was successively extracted with  $\text{C}_6\text{H}_{14}$  (3×700 ml),  $\text{CHCl}_3$  (3×700 ml) and EtOAc (3×500 ml). The aqueous solution was rendered alkaline with  $\text{NH}_4\text{OH}$  solution and extracted with  $\text{CHCl}_3$  (3×700 ml) and EtOAc (3×500 ml).

The  $\text{CHCl}_3$  soluble fraction obtained from alkaline medium (1 gm) was fractionated by VLC over alumina (150 gm) eluted with  $\text{CHCl}_3$  and  $\text{CHCl}_3/\text{MeOH}$  mixtures. Total of 22 fractions, 50 ml each were collected, concentrated and screened by TLC. Similar fractions were combined.

Fractions 10- 13 eluted with 5% MeOH in  $\text{CHCl}_3$ , were evaporated and the residue (250 mg) was rechromatographed using alumina column (70 gm, 1.5 cm diameter) eluting with 1% MeOH in  $\text{CHCl}_3$ . Fraction 4 (70 mg) was further purified by pTLC using alumina plates and  $\text{CHCl}_3/\text{MeOH}$  (9:1 v/v) as developing system. The zone with  $R_f$

\*Author for correspondence

Fax: 20-3-4948952, E-mail: mpharm101@hotmail.com

value of 0.4 detected under UV light was scrapped off, eluted with  $\text{CHCl}_3/\text{MeOH}$  (1:1 v/v) and crystallized from MeOH after the addition of few drops of 0.1N HBr to afford 30 mg of **1**.

Fractions 14 and 15 eluted with 5% MeOH in  $\text{CHCl}_3$ , were evaporated and the residue (100 mg) was refractionated over alumina column (40 gm, 1 cm diameter) eluting with 2% MeOH in  $\text{CHCl}_3$ . Fractions 6-7 (45 mg) were subjected to pTLC using alumina plates and  $\text{CHCl}_3/\text{MeOH}$  (9:1 v/v) as developing system. The zone with  $R_f$  value of 0.24 detected under UV light was scrapped off, eluted with  $\text{CHCl}_3/\text{MeOH}$  (1:1 v/v) and crystallized from MeOH to afford 20 mg of **2**.

**Ephedradine C (1)** – White crystals, mp 225- 227°C.  $[\alpha]_D^{25} = -110^\circ$  ( $c=1.0$ , MeOH). UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm: 230, 281.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data (Table 1). ES-MS (rel. int., %): 537 ( $\text{M}^+\text{H}$ ,  $\text{C}_{30}\text{H}_{40}\text{N}_4\text{O}_5$ ) (100), 507 ( $\text{M}^+\text{H}-2\text{CH}_3$ ) (78), 335 (18), 309(29), 269 (80), 229 (40), 214 (34), 203(26). HRESMS  $m/z$ : 537.308 ( $\text{M}^+\text{H}$ ), calculated for  $\text{C}_{30}\text{H}_{40}\text{N}_4\text{O}_5 + \text{H}$ , 537.307.

**Hordenine (N, N-dimethyltyramine) (2)** – Colourless crystals, mp 116-117°C. UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm: 226, 281(sh),  $\lambda_{\text{max}}^{\text{MeOH}+\text{NaOMe}}$  208, 240, 296 (sh).  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data (Table 1). EI-MS (rel. int., %): 165 ( $\text{M}^+$ , 9), 151 (5), 135 ( $\text{M}^+-2\text{CH}_3$ , 11), 133 (14), 121 ( $\text{M}^+-\text{N}(\text{CH}_3)_2$ , 30), 120 (71), 107 ( $\text{M}^+-\text{CH}_2\text{N}(\text{CH}_3)_2$ , 46), 91 (35), 77(100). CI-MS (rel. int.%): 167 ( $\text{M}^++2\text{H}$ , 53), 166 ( $\text{M}^+\text{H}$ , 100), 152 (8), 124 (6), 121 (6), 74 (32), 58 (85). HRCIMS  $m/z$ : 166.123 ( $\text{M}^+\text{H}$ ), calculated for  $\text{C}_{10}\text{H}_{15}\text{NO} + \text{H}$  166.123.

## Results and Discussion

HRESMS of **1** showed an  $\text{M}^+\text{H}$  at  $m/z$  537.308 for the molecular formula  $\text{C}_{30}\text{H}_{40}\text{N}_4\text{O}_5 + \text{H}$ . The UV maxima at 230 and 281 nm as well as six aromatic carbons, oxygenated carbon and methine in the  $^{13}\text{C}$ -NMR (Table I) were all diagnostic for a dihydrobenzofuran substituted at C-2 and C-3. The down field shift of H-2 and H-3 at  $\delta$  6.24 ( $J=10.5$ ) and 4.89 ( $J=10.5$ ) indicated phenyl and carbonyl substitutions, while the large coupling constant was diagnostic for the *trans*-orientation of the two substituents (Tamada *et al*, 1979). Both  $^1\text{H}$ -NMR (Table I), DEPT and HMQC experiments indicated that the phenyl and the benzene ring of the dihydrobenzofuran are trisubstituted with an ABX system in the proton spectrum. The chemical shift of the six phenyl carbons includes two quaternary oxygenated carbons at 148.1 and 148.4 ppm indicating that they are *meta*-oriented. These positions should be occupied by the two methoxy groups at  $\delta$  3.84, 3.85 and 55.4, 55.5 in  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR, respectively. The

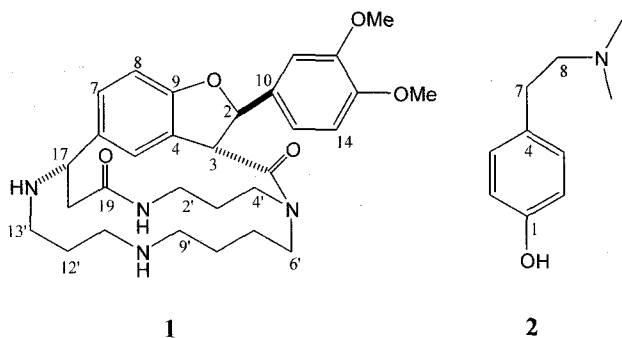
remaining two oxygen atoms in the molecule were involved in two carbonyl groups at 170.8 and 174.7 ppm as deduced from the  $^{13}\text{C}$ -NMR and their chemical shift indicated that both are involved in an amide linkage (Breitmaier and Voelter, 1987). The  $^{13}\text{C}$ -NMR also showed 12 aliphatic carbons ( $11 \times \text{CH}_2$  and one CH). Some of these carbons are attached to N atoms as indicated from their chemical shifts. The aliphatic part together with the two amide groups were assigned to a spermine moiety. The spermine part is connected to the dihydrobenzofuran at C-3 through the amide carbonyl. The down field shift of the C-17 methin at 58.5 ppm and its corresponding proton at  $\delta$  4.88 indicated that it is flanked between an aromatic ring and N atom (Breitmaier and Voelter, 1987). Consequently, C-17 is the second attachment site between the spermine

**Table 1.**  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR (ppm), J (Hz) of compounds **1** and **2**.

Position	<b>1</b> <sup>a</sup>		<b>2</b> <sup>b</sup>	
	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$
1	–	–	154.7	–
2	88.0	6.24 (1H, d, $J=10.5$ )	115.6	6.66 (2H, d, $J=8.3$ )
3	52.0	4.89 (1H, d, $J=10.5$ )	129.5	7.01 (2H, d, $J=8.3$ )
4	124.5	–	131.0	–
5	133.9	7.40 (bs)	129.5	7.01 (2H, d, $J=8.3$ )
6	126.2	–	115.6	6.66 (2H, d, $J=8.3$ )
7	120.9	7.02- 7.14	32.9	2.72 (2H, bt, $J=7.9$ )
8	111.5	7.02- 7.14	61.5	2.56 (2H, bt, $J=7.9$ )
9	159.5	–		
10	130.8	–		
11	110.6	7.02- 7.14		
12	148.4	–		
13	148.1	–		
14	109.7	7.02- 7.14		
15	119.6	7.02- 7.14		
16	170.8	–		
17	58.5	4.88 (1H, m)		
18	25.0	2.25 (1H, m), 2.44 (1H, bq, $J=13$ )		
19	174.7	–		
OMe	55.4	3.84 (3H, s)	–	–
	55.5	3.85 (3H, s)	–	–
Me	–	–	45.1	2.32 (6H, s)
2'	45.9	2.98 (1H, m), 3.05 (m)		
3'	21.2	1.56 (m)		
4'	44.3	3.05 (m), 3.99 (1H, m),		
6'	41.9	2.89 (1H, m), 3.05 (m)		
7', 8'	22.4	1.56 (m), 1.67 (m) 1.84 (1H, m), 1.94 (m)		
9'	41.3	2.85 (1H, m), 3.25 (1H, p, $J= 5.5$ )		
11'	37.2	2.74 (1H, d, $J= 14.5$ ), 3.05 (m)		
12'	25.1	7.02- 7.14		
13'	37.7	3.45 (1H, bq, $J= 11$ ), 3.72 (1H, bd, $J= 14$ )		

<sup>a</sup>Spectra were measured in  $\text{D}_2\text{O}$ ,  $^{13}\text{C}$ -NMR was ref. by one drop of  $\text{CD}_3\text{OD}$ .

<sup>b</sup>spectra were measured in  $\text{CDCl}_3$ .



moiety and the dihydrobenzofuran.

The data of **1** were in complete agreement with those reported for ephedradine C (Tamada *et al.*, 1979). However, the assignment of C-18 next to the carbonyl group at 21.4 ppm (Tamada *et al.*, 1979) was exchanged to 25.0 ppm based on COSY and HMQC experiments. Those experiments also allowed the assignments of the carbons of the spermine part tentatively (Table 1). The HMQC revealed the presence of two overlapped carbons at 22.4 ppm assigned for C-7' and C-8' since they have almost same environment. These values could be exchanged with those of C-3' and C-12'. The higher chemical shift values were then given to those carbons next to N atoms.

Spermine alkaloids were isolated from the roots of *Ephedra* as well as the whole plants of *Chaenorhinum* species and were designated as ephedradines that have hypotensive effect (Tamada *et al.*, 1979; Kabuto *et al.*, 1980; Breitmaier and Voelter, 1987; Zhu and Hess, 1988). This study is the first to report isolation of spermine alkaloid from the aerial parts of *Ephedra aphylla*. Therefore, the aerial parts of *Ephedra aphylla* may cause hypotension rather than hypertension reported for other *Ephedra* aerial parts (DerMardersian, 2001), especially with non-detection of ephedrine or pseudoephedrine in the studied species parts. This result is concomitant with the recent reported data concerning the absence of ephedrine and presence of other nitrogen-containing secondary metabolites with known neuropharmacological activity in Asian and New world-*Ephedra* species (Caveney *et al.*, 2001).

HRCIMS of **2** showed an  $M^+ + H$  at  $m/z$  166.123 calculated for the molecular formula  $C_{10}H_{15}NO + H$ . Positive reaction with  $FeCl_3$  and UV shift with NaOMe indicated its phenolic nature.  $^1H$ -NMR (Table 1) showed two aromatic doublets, two triplets and one singlet with the ratio 1:1:1:1:3. To fulfill the number of protons as indicated from MS each signal should represent two protons. The two doublets ( $\delta$  6.66 and 7.01,  $J = 8.3$  Hz) were assigned for a *p*-disubstituted benzene ring bearing a carbon substitution and a phenolic OH. The two triplets

in the  $^1H$ -NMR ( $\delta$  2.56 and 2.72,  $J = 7.9$  Hz) were assigned to two  $CH_2$ . This assignment was supported by DEPT and HMQC experiments. Chemical shift of the two  $CH_2$  indicated that one is next to the aromatic system and the other to a N atom (Breitmaier and Voelter, 1987). The six proton singlet at  $\delta$  2.32 correlated to carbon signal at 45.1 ppm was assigned to  $N(CH_3)_2$ . The above data were closely similar to that reported for the phenylalklyamine alkaloid hordenine (N, N-dimethyltyramine). Although hordenine was previously isolated from several plants and red alga (McLaughlin, 1969; Keller, 1981; Barwell and Blunden, 1981; Pummangura and McLaughlin, 1981; Schroeder and Stermitz, 1984; Chao *et al.*, 1987) it is the first time to be isolated from *Ephedra* species.

## References

- Al-Khalil, S., Alkofahi, A., El-Eisawi, D. and Al-Shibib, A., Transthorine, a new quinoline alkaloid from *Ephedra transitoria*. *J. Nat. Prod.*, **62**, 1214 (1999).
- Barwell, C. J. and Blunden, G., Hordenine from the red alga *Gigartina stellata*. *J. Nat. Prod.*, **44** (4), 500-502 (1981).
- Boulos, L., "Flora of Egypt", Al Hadara Publishing, Cairo, Egypt, Vol. 1, 1999.
- Breitmaier, E. and Voelter, W., "Carbon-13 NMR Spectroscopy", 3<sup>rd</sup> Ed., VCH, Weinheim, Germany (1987).
- Brossi, A. and Pecherer, B. Alkaloids containing a simple aromatic moiety in "Chemistry of the Alkaloids", Pelletier S. W., Ed., Van Nostrand Reinhold, New York, 1970.
- Caveney, S., Charlet, D. A., Freitag, H., Maier-Stolte, M. and Starratt, A. N., New observations on the secondary chemistry of world *Ephedra* (Ephedraceae). *Am. J. Bot.*, **88**(7), 1199-1208 (2001).
- Chao, L. R., Seguin, E., Tillequin, F. and Koch, M., New alkaloid glycosides from *Selaginella doederleinii*. *J. Nat. Prod.*, **50**(3), 422-426 (1987).
- DerMardersian, A., "The Review of Natural Products", Reference Publications, Inc. St. Louis, Missouri, USA, 2001.
- Hikino, H., Ogata, K., Konno, C. and Sato, S., Hypotensive actions of Ephedradines, macrocyclic spermine alkaloids of *Ephedra* roots. *Planta Med.*, **48**(4), 290-293 (1983).
- Kabuto, C., Tamada, M., Endo, K. and Hikino, H., Structure of ephedradine C, a hypotensive principle of *Ephedra* roots. *Heterocycles*, **14**(3), 295-298 (1980).
- Keller, W. J., Hordenine from *Stapelia gigantean*. *J. Nat. Prod.*, **44**(3), 366-367 (1981).
- McLaughlin, J. L., Cactus alkaloids VI. Identification of hordenine and N-methyltyramine in *Ariocarpus fissuratus* varieties *fissuratus* and *lloydii*. *Lloydia*, **32**(3), 392-394 (1969).
- Nawwar, M. A. M., Barakat, H. H., Buddrus, J. and Linscheid, M., Alkaloidal, lignan and phenolic constituents of *Ephedra alata*. *Phytochemistry*, **24**(4), 878-879 (1985).

- Pummangura, S. and McLaughlin, J. L., Cactus alkaloids XLVII.  $\beta$ -phenethylamines from the "Missouri Pincushion" *Coryphantha (Neobessya) missouriensis*. *J. Nat. Prod.*, **44**(5), 614-616 (1981).
- Schroeder, D. R. and Stermitz, F. R., Hordenine and N-methyl-4-methoxyphen ethylamine from *Eriogonum* species. *J. Nat. Prod.*, **47**(3), 555-556 (1984).
- Staratt, A. N. and Caveney, S., Quinoline-2-carboxylic acids from *Ephedra* species. *Phytochemistry*, **42**(5), 1477-1478 (1996).
- Tamada, M., Endo, K., Hikino, H. and Kabuto, C., Structure of ephedradine A, a hypotensive principle of *Ephedra* roots. *Tetrahedron lett.*, **10**, 873-876 (1979).
- Zhu, J. and Hesse, M., The spermine alkaloids of *Chaenorhinum minus*. *Planta Med.*, **54**(5) 430-433 (1988).

(Accepted May 7, 2003)