# Tetrahydroisoquinoline and β-Carboline Alkaloids from *Haloxylon articulatum* (Cav.) Bunge (Chenopodiaceae)

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Salsolidine and 2-methyl,1,2,3,4-tetrahydro-β-carboline were isolated from *Haloxylon articulatum* (growing in Egypt) besides the known carnegine and *N*-methylisosalsoline. 2-methyl,1,2,3,4-tetrahydro-β-carboline was recorded for the first time in the genus *Haloxylon.* The alkaloids were identified by spectroscopic methods (MS, 1D and 2D NMR).

*Key words: Haloxylon articulatum*, Chenopodiaceae, Tetrahydroisoquinoline Alkaloids

## Introduction

*Haloxylon articulatum* (Cav.) Bunge (family Chenopodiaceae) is a glabrous, grey-brown, woody, dwarf shrub usually turning darker or blackish when dried. The plant grows wild in dry habitats of the Mediterranean region and the Near East. Carnegine and *N*-methylisosalsoline have been previously isolated from this plant (Carling and Sandberg, 1970). *Haloxylon (Hammada) articulatum* ssp. *scoparium* from Algeria has been reported to contain the alkaloids carnegine and *N*-methylisosalsoline as major tetrahydroisoquinoline alkaloids in addition to isosalsoline, salsolidine, dehydrosalsolidine, isosalsolidine, *N*-methylcorydaldine, tryptamine and *N*-methyltryptamine as minor alkaloids (Benkrief *et al.*, 1990). Simple tetrahydroisoquinoline alkaloids are common in the Chenopodiaceae (Shamma, 1972). Furthermore, simple tetrahydroisoquinoline alkaloids can be formed in humans and animals after alcohol consumption since acetaldehyde (derived from ethanol) can react with corresponding biogenic amines. The tetrahydroisoquinoline and β-carboline alkaloids affect the vegetative nervous system (Schütte and Liebisch, 1985; Vetulani *et al.*, 2001; Wink *et al.*, 1998). Simple isoquinoline and β-carboline alkaloids display potent, and often selective cytotoxicity or exhibit potential antimicrobial, antimalarial, antiviral and anti-HIV activities (Baker, 1996; Iwasa *et al.*, 2001).

We have investigated the alkaloidal constituents of this desert plant from Egypt and describe the isolation and structural determination of three tetrahydroisoquinoline alkaloids (carnegine, salsolidine, 1-methylcorypalline) and for the first time in genus *Haloxylon* a β-carboline alkaloid: 2-methyl-1,2,3,4-tetrahydro-β-carboline.

## Materials and Methods

### *Plant material*

Aerial plant parts of *Haloxylon articulatum* (Cav.) Bunge [= *Hammada scoparia* (Pomel) Iljin., *Arthrophytum scoparium* (Pomel) Iljin., *Salsola articulata* Cav., *Haloxylon scoparium* Pomel] (Zohary, 1966; Täckholm, 1974; Boulos, 1999) were collected from the vicinity of El-Aresh, North Sinia, Egypt in March 2000. The plant was kindly identified by Dr. H. Abdel Baset, Faculty of Science, Zagazig University. A voucher specimen was deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University.

### *Extraction and isolation of the alkaloids*

About 500 g of the dried plant material were ground to a fine powder and extracted with 80 % EtOH in a percolator. The EtOH extract was concentrated *in vacuo* and acidified with HCl (pH 3) and then defatted by extraction with $CH_2Cl_2$. The

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defatted mother liquor was made alkaline with an $NH_4OH$ solution (pH 10) and immediately extracted with $CH_2Cl_2$ to exhaustion. The latter $CH_2Cl_2$ extract was concentrated to yield 20.1 g of reddish-brown residue (total alkaloid). A sample of this residue (5 g) was fractionated on a column of neutral $Al_2O_3$ (100 g) which was packed in $CH_2Cl_2$ and eluted with $CH_2Cl_2$- 2 % $NH_4OH$ (25 %) in MeOH mixture adopting the gradient elution technique to afford four alkaloids. TLC [silica gel $F_{254}$, $CHCl_3$ – MeOH – $NH_4OH$ (25 %), 9:1:0.5)] for the isolated alkaloids (**1–4**) showed $R_f$, 0.7, 0.5, 0.48 and 0.34, respectively.

$^1$H- and $^{13}$C-NMR spectra were performed on an Ac Bruker Instrument (in $CDCl_3$ for compounds **1–3** and $CD_3OD$ for compound **4**) at 300 and 75 MHz, respectively (for data see Tables I and II). EIMS was recorded at 70 eV by direct inlet in JEOL (Japan). Melting points were recorded by a Gallenkamp digital apparatus (Belton Park, Loughborough, UK) and were uncorrected.

Carnegine (**1**), yellowish-white oil (2.1 g), UV $\lambda_{max}$ (MeOH) nm 289, 354. EIMS, *m/z* (rel. int.): [M$^+$] 221 (30), 220 (34), 207 (80), 206 (100), 191 (60), 190 (76), 178 (40), 162 (50), 148 (30), 103 (35), 91 (20), 42 (14).

*N*-Methylisosalsoline (1-Methylcorypalline) (**2**), white rosette crystals (MeOH) (20 mg), mp 170–171 °C, UV $\lambda_{max}$ (MeOH) nm 235, 283. EIMS, *m/z* (rel. int.): [M$^+$] 207 (15), 193 (30), 192 (100), 177 (45), 164 (10), 149 (15), 121 (5), 96 (6), 91 (5), 77 (5), 57 (5), 42 (4).

Salsolidine (**3**), white needle-shaped crystals ($CHCl_3$-MeOH) (19 mg), mp 198–200 °C, UV $\lambda_{max}$ (MeOH) nm 236, 281. EIMS, *m/z* (rel. int.): [M$^+$] 207 (78), 206 (79), 193 (80), 192 (100), 190 (60), 178 (55), 177 (60), 176 (75), 163 (30), 160 (25), 134 (23), 131 (25), 118 (20), 96 (24), 91 (25), 77 (20). 65 (10), 36 (17).

2-Methyl-1,2,3,4-tetrahydro-$\beta$-carboline (**4**), white shiny needle crystals (MeOH) (50 mg), mp 213 °C, UV $\lambda_{max}$ (MeOH) nm 290, 294. EIMS, *m/z* (rel. int.): [M$^+$] 186 (26), 171 (6), 144 (100), 143 (63), 115 (10), 94 (5), 77 (4), 42 (4).

## Results and Discussion

Alkaloid **1** was obtained as a yellowish-white oil. The structure of **1** was established through a combination of NMR and MS analysis. The EIMS showed an [M]$^+$ ion at 221 (for $C_{13}H_{19}NO_2$) together with fragment ion peaks at *m/z* 206 (100 %) [M–Me]$^+$ and 190 (76 %) [M–OMe]$^+$. The $^1$H-NMR spectral data show signals for two aromatic *para*-oriented protons ($\delta$ 6.51 and 6.54) and four methyl peaks; one doublet at $\delta$ 1.32 and three singlet of which one at $\delta$ 2.42 and the other two at $\delta$ 3.79 (integrated for 6 protons). In addition to four protons (two methylene) at $\delta$ 2.58, 2.95, 2.73 (2H) and one proton resonated at $\delta$ 3.48 coupled with methyl group at $\delta$ 1.32. The $^{13}$C-NMR spectrum of **1** showed 13 signals. The type of these carbons was identified from the chemical shift and attached proton test (APT) spectrum; four methyl peaks, two methylene peaks, three methine peaks and four quaternary carbons peaks. The chemical shifts and multiplicities indicated that the compound has a benzene ring with four substituents and two methyl carbons adjacent to oxygen ($\delta$ 55.6 and 55.8), one methine ($\delta$ 58.5) and one methylene resonated at $\delta$ 27.4. Also, there are one methyl and one methylene adjacent to nitrogen with $\delta$ 42.8 and 48.8, respectively. From the above mentioned data it was assumed that compound **1** is a tetrahydroisoquinoline (Hughes *et al.*, 1976; 1981). Comparing the MS, $^1$H- and $^{13}$C-NMR data of this compound with the published data (Carling and Sandberg 1970; Khalil *et al.*, 1992), we concluded that this compound is carnegine. Noteworthy, carnegine has so far previously been isolated from a *Cactus* species (Brown *et al.*, 1972; Menachery *et al.*, 1986).

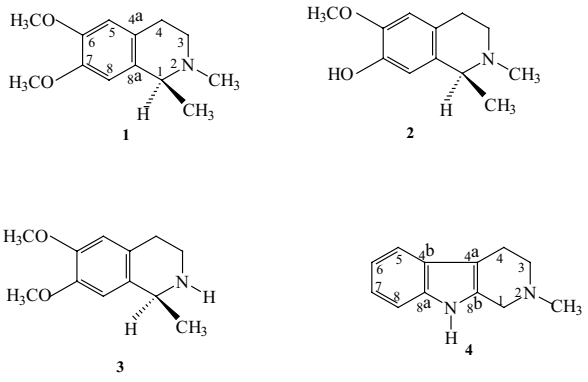


Fig. 1. Structures of alkaloids found in *Haloxylon articulatum*: Carnegine (**1**), *N*-methylisosalsoline (1-methylcorypalline) (**2**), salsolidine (**3**), and 2-methyl-1,2,3,4-tetrahydro-β-carboline (**4**).

Table I. $^1$H-NMR chemical shifts of **1–4** alkaloids.*

| H | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | 3.48, 1H, q, *J* = 6.54 Hz | 3.48, 1H, q, *J* = 6.54 Hz | 4.52, 1H, q, *J* = 6.75 Hz | 2.84, 2H, s. |
| 3α | 2.95, 1H, ddd, *J* = 11.7, 7.1, 5 Hz. | 2.99, 1H, ddd, *J* = 11.7, 7.1, 5 Hz. | 3.48, 1H, ddd, *J* = 11.7, 7.1, 5 Hz. | |
| 3β | 2.58, 1H, ddd, *J* = 11.7, 7.1, 5 Hz. | 2.63, 1H, ddd, *J* = 11.7, 7.1, 5 Hz. | 3.32, 1H, ddd, *J* = 11.7, 7.1, 5 Hz. | {3.66, 2H, m. |
| 4 | 2.73, 2H, m. | 2.76, 2H, m. | 3.09, 2H, m. | 2.84, 2H, m. |
| 5 | 6.54, 1H, s. | 6.53, 1H, s. | 6.58, 1H, s. | 7.37, 1H,d, *J* = 7.59 |
| 6 | – | – | – | 6.96, 1H, m. |
| 7 | – | – | – | 7.03, 1H, m. |
| 8 | 6.51, 1H, s. | 6.65, 1H, s. | 6.56, 1H, s. | 7.25, 1H, d, *J* = 7.95 |
| 1-$CH_3$ | 1.32, 3H, d, *J* = 6.57 Hz. | 1.34, 3H, d, *J* = 6.57 Hz. | 1.78, 3H, d, *J* = 6.57 Hz. | – |
| N-$CH_3$ | 2.42, 3H, s. | 2.45, 3H, s. | – | 2.51, 3H, s. |
| 6-$OCH_3$ | 3.79, 3H, s. | 3.84, 3H, s. | 3.84, 3H, s. | – |
| 7-$OCH_3$ | 3.79, 3H, s. | | 3.84, 3H, s. | – |

* Spectra of **1–3** were performed in $CDCl_3$ and **4** was performed in $CD_3OD$.

Table II. $^{13}$C-NMR chemical shifts of **1–4** alkaloids.*

| C | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | 58.5 *d* | 58.6 *d* | 50.7 *d* | 54.1 *t* |
| 3 | 48.7 *t* | 49.2 *t* | 38.9 *t* | 53.2 *t* |
| 4 | 27.4 *t* | 27.7 t | 25.3 *t* | 22.2 *t* |
| 4a | 125.8 s | 125.2 *s* | 123.4 *s* | 107.6 *s* |
| 4b | – | – | – | 128.2 *s* |
| 5 | 109.7 *d* | 110.5 *d* | 108.7 *d* | 118.5 *d* |
| 6 | 147.2 *s* | 145.0 *s* | 148.4 *s* | 119.7 *d* |
| 7 | 147.0 *s* | 143.8 *s* | 148.8 *d* | 122.0 *d* |
| 8 | 111.0 *d* | 112.7 *d* | 111.4 *d* | 111.8 *d* |
| 8a | 131.5 *s* | 132.4 *s* | 125.1 *s* | 138.0 *s* |
| 8b | – | – | – | 132.3 *s* |
| 1-$CH_3$ | 19.6 *q* | 19.7 *q* | 20.2 *q* | – |
| N-$CH_3$ | 42.8 *q* | 42.9 *q* | – | 45.6 *q* |
| 6-$OCH_3$ | 55.8 *q* | 55.9 *q* | 56.1 *q* | – |
| 7-$OCH_3$ | 55.6 *q* | – | 55.9 *q* | – |

* Spectra of **1–3** were performed in $CDCl_3$ and **4** was performed in $CD_3OD$.

Alkaloid **2** had white needle-shaped crystals with 14 mass units less than in **1** and showed a fragmentation pattern ($M^+$, 207) and a base peak of *m/z* 192 ($M^+ - 15$). In order to corroborate the structure of **2**, its $^1$H- and $^{13}$C-NMR spectra were recorded and compared with those of structurally related compounds, such as carnegine **1**. $^1$H- and $^{13}$C-NMR general features were similar to those of **1**, except for the absence of one methyl peak adjacent to oxygen. Therefore, alkaloid **2** must have one methoxy group and one free hydroxyl group instead of two methoxy groups in **1**. Comparing the MS, $^1$H- and $^{13}$C-NMR spectra of alkaloid **2** with the reported data (Khalil *et al.*, 1992) , it is evident that **2** is *N*-methylisosalsoline (1-methylcorypalline). The results showed that **1** and **2** were present confirming an earlier report (Carling and Sandberg, 1970) of their occurrence in Egyptian *H. articulatum* species.

Alkaloid **3** was obtained as white needle-shaped crystals and its mass spectrum showed a $[M]^+$ at *m/z* 207 (14 mass units less than in **1**), corresponding to the formula $C_{12}H_{17}NO_2$. The comparison of $^1$H- and $^{13}$C-NMR spectrum with that of **1** (carnegine) revealed that **3** is *N*-demethylated carnegine (1-methyl, 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline). The MS, $^1$H- and $^{13}$C-NMR data (Menachery *et al.*, 1986; Khalil, 1994) of **3** were identical with that reported for salsolidine.

The structure of **4** was determined from the EIMS (which revealed a $[M]^+$ at *m/z* 186, consistence with $C_{12}H_{14}N_2$) and a series of 1D and 2D NMR spectroscopic experiments. The $^1$H-NMR spectrum of **4** contained signals for 14 non-exchangeable protons, of which four were aromatic while the other ten were 3 methylene (6 H), *N*-methyl and a nitrogen-adjacent proton. The $^{13}$H-NMR spectrum of **4** contained twelve carbon resonances, of which eight arose from protonated carbons. The chemical shifts together with the HETCOR experiments suggested a $\beta$-carboline nucleus with a 1,2,3,4-tetrahydro substitution (Prinsep *et al.*, 1991). The MS and $^{13}$C-NMR were identical to those reported in the literature (Gander *et al.*, 1976; Poupat *et al.*, 1976) for 2-methyl-1,2,3,4-tetra-

hydro-$\beta$-carboline. This $\beta$-carboline alkaloid has not been reported previously as a constituent of *H. articulatum* and also the first evidence for the presence of this alkaloidal class in genus *Haloxylon.*

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