

PYRROLIZIDINE ALKALOIDS FROM *HELIOTROPIUM* *ARBAINENSE* AND *H. OVALIFOLIUM*

By

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ABSTRACT

Investigation of the pyrrolizidine alkaloids of *Heliotropium arbainense* resulted in the isolation and identification of heliotrine, europine and 7-angelylheliotrine. From *H. ovalifolium* three alkaloids were isolated: supinine, 7-angelylheliotridine and an alkaloid which was shown to be of the heliotridine monoester type with a dehydrated viridifloric acid.

INTRODUCTION

Pyrrolizidine alkaloids have been so far isolated from more than 30 *Heliotropium* species (Boraginaceae) (Mattocks, 1986, Rizk, 1990). Consumption of these plants by livestock leads to a fatal disease, usually with the main lesion in the liver, the disease was also reported in several human cases. The largest documented incident of accidental human pyrrolizidine alkaloid toxicity occurred in Afghanistan (Smith and Culvenor, 1981; Anand and Atal, 1986). The poisoning was caused by the consumption of bread from wheat contaminated with seeds of *Heliotropium popovii*. Hepatotoxic pyrrolizidine alkaloids are esters of unsaturated amino alcohols (necines) e.g. retronecine, heliotridine, otonecine, and supinidine, usually with branched-chain hydroxy acids (McLean, 1970; Riz and Kamel, 1990). The carcinogenicity of some pyrrolizidine alkaloids viz. retrorsine (and its N-oxide isatidine), lasiocarpine, and monocrotaline has been reported (Mattocks, 1986; Rizk and Kamel, 1990).

Previous investigation of the studied species revealed the presence of heliotrine, lasiocarpine and europine from *H. arbainense* (Zalkow *et al.* 1979), and heliofoline and retronecine from *H. ovalifolium* (Mohanraj *et al.* 1981).

EXPERIMENTAL

Plant Material

Heliotropium arbainense Fres. and *H. ovalifolium* Forssk. were collected in March from Wadi Hof (near Cairo) and Aswan (upper Egypt) respectively. The plants were kindly identified by Prof. Dr. M.N. El-Hadidi, Faculty of Science, Cairo University. The plants were separately, air dried and powdered.

TLC:

Aluminium oxide G: solvent: benzene-methanol (90:10) + 5 drops ammonium hydroxide; detection: Dragendorff's and Ehrlich reagents (Rizk and Hussiney 1990).

GLC:

Perkin-Elmer apparatus applying the following conditions: glass column 6 ft. x 4 mm: 3% OV-101 on chromosorb WAM (80-100 mesh); temp. initial 180°C, final 240°C (rate 5°C/min).

HPLC:

Water Associate Analytical liquid chromatography, model ALG/GPC 244 applying the following two conditions: Condition 1: u Bondapack-C₁₈ (Waters) column, 30 cm x 4 mm; solvent: 61% MeOH, 39% phosphate buffer (pH 6.4); detector: UV at 220 nm. Condition II: u Bondapack-CN (Waters) column, 30 cm x 4 mm; solvent: 50% MeOH + 50% phosphate buffer (pH 7.8); detector: UV at 235 nm.

Extraction of the Alkaloids:

About 1 kg of the powdered plant was percolated with methanol till exhaustion. The methanolic extract was concentrated and the residue was taken up in dil. HCl, defatted with ether, reduced with zinc dust (4 hours) and filtered. The filtrate was rendered alkaline (pH 10) with conc. ammonium hydroxide and extracted with chloroform as previously described (Rizk *et al.* 1983).

In another experiment, the methanolic extract of 100 gm of *H. arbainense*, after treating with HCl and defatting, was rendered alkaline with ammonium hydroxide and extracted with chloroform (free bases). The aqueous alkaline solution was made acidic, reduced with zinc dust (to reduce N-oxides), filtered and the filtrate was further treated according to the above procedure. The tertiary bases (extracted before reduction of the N-oxides) amounted to 22.6% of the total alkaloids of *H. arbainense*.

Fractionation of the Alkaloids of *H. arbainense*:

The alkaloidal mixture (7.4 gm obtained from *H. arbainense*) was treated with boiling petroleum ether (b.p. 60-80°C). Concentration and cooling of the petroleum ether extract afforded heliotrine. Dry column chromatographic fractionation of the total alkaloids (aluminium oxide G. grade II developed with benzene-methanol 90:10 + drops of ammonium hydroxide) succeeded in separation of heliotrine and europine. 7-Angelylheliotrine and europine were obtained by liquid column chromatographic fractionation of the total alkaloids (aluminium oxide G. developed with benzene-methanol 99:1), followed by preparative TLC.

Heliotrine:

The alkaloid has m.m.p. 127-128°C. IR showed absorption bands at 3300-2400 cm^{-1} and strong bands at 1830 cm^{-1} characteristic for stretching frequency of C=O and of α , β -unsaturated ester groups. MS showed (M^+) at m/e 313 ($\text{C}_{16}\text{H}_{27}\text{O}_5\text{N}$), a base peak at m/e 138 in addition to the ion peaks at 295, 255, 214, 197, 156, 120, 93, 80 and 59. Hydrolysis of the alkaloid (2 N NaOH for 30 minutes) gave heliotridine (TLC, m.p.) and heliotric acid (identified as brucine salt, m.p. 119-123°C (Hammouda *et al.* 1984).

Europine:

MS of the alkaloid (oily) showed M^+ at m/e 329 ($\text{C}_{16}\text{H}_{27}\text{O}_6\text{N}$) and fragments at 271, 138 (base peak), 93, 80 and 59. Treatment of the alkaloid with H_2O_2 (3 days at room temperature) gave europine N-oxide, m.p. 170°C; reported 170°C and 171°C (Hammouda *et al.* 1984; Rizk, 1990).

7-Angelylheliotrine:

MS of the alkaloid exhibited a molecular ion peak (M^+) at m/e 395 ($\text{C}_{12}\text{H}_{33}\text{O}_6\text{N}$) and fragment ions at m/e 295, 220 (base peak), 136, 120, 119, 93, 83, 71, 59 and 43 (Suri *et al.* 1975, Hammouda *et al.* 1984).

Fractionation of the Alkaloids of *H. ovalifolium*:

About 0.5 gm. of the total alkaloidal mixture was subjected to column chromatographic fractionation (aluminium oxide G, developed with benzene-methanol 99:1 and 98:2). Fractionation as well as further purification of the alkaloids were achieved by preparative TLC. Three alkaloids were isolated: 7-angelyl-heliotridine, supinine and a heliotridine monoester.

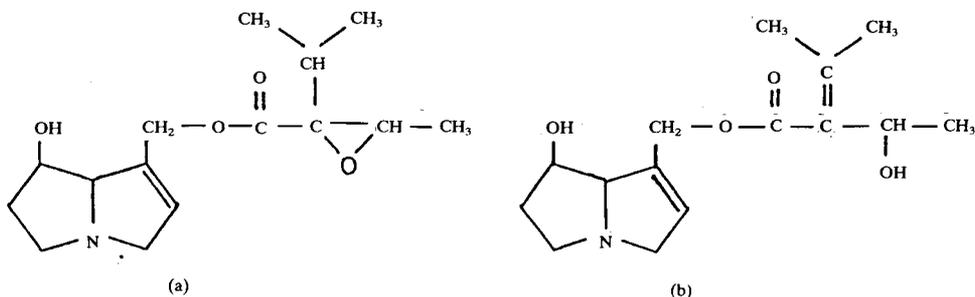
7-Angelylheliotridine:

MS of the alkaloid showed M^+ at m/e 237 ($\text{C}_{13}\text{H}_{19}\text{NO}_3$) and fragments at m/e 154, 137, 124, 111, 106, 94 and 80 which are characteristic of 7-angelylheliotridine (Pedersen and Larsen 1970).

Supinine:

MS of the alkaloid showed M^+ at m/e 383 ($C_{15}H_{25}NO_4$) and fragments at m/e 239, 121, 93 and 45 which are characteristic of supinine (Lüthy *et al.* 1984).

A third alkaloid was isolated in small amounts. The alkaloid showed a molecular ion (M^+) at 181 and fragments which are characteristic of the heliotridine monoesters type. The acid part is likely a dehydrated viridifloric acid of either structures a or b.



RESULTS AND DISCUSSION

Pyrrolizidine alkaloids occurred in *H. arbainense* (0.75%) in both free (tertiary) and N-oxide from in 22.7 and 77.4% respectively. On the other hand, *H. ovalifolium* contains a lower percentage of alkaloids (0.1%). TLC revealed that *H. arbainense* contains the same alkaloids in both the free and the N-oxide form. Several physico-chemical techniques were applied for the fractionation and identification of the alkaloids *viz.* dry and liquid column chromatography, TLC, GLC, HPLC supplemented by IR, MS, hydrolysis and preparation of N-oxides. Six alkaloids were isolated and identified *viz.* 7-angelylheliotrine, heliotrine, europine, 7-angelylheliotridine, supinine and an alkaloid tentatively identified as heliotridine monoester with a dehydrated viridifloric acid. Moreover, TLC, GLC and HPLC revealed the probable presence of heliotridine in *H. arbainense*.

The mass spectrum of 7-angelylheliotridine (not previously isolated from *H. arbainense*) (M^+ 395) exhibited the base peak at m/e 220 which is referred to the cleavage of the weakly allylic ester band (heliotric acid) and the ion m/e 295 due to the loss of angelic acid from C-7 of the parent ion. The ion peaks at m/e 71, 59 and 43 are due to the radical $C_3H_7C=O^+$, $CH_3-CH-OCH_3$ and $C_3H_7^+$ respectively and thus emphasizing the presence of heliotric acid. Ehrlich test gave a magenta colour characteristic of the unsaturated pyrrolizidine alkaloids. This alkaloid has

been first isolated from *H. eichwaldii* (Suri *et al.*, 1975) and was later identified from *H. digynum* (Hammouda *et al.*, 1984). 7-Angelyl-heliotridine (isolated from *H. ovalifolium*) has been identified from several species of Boraginaceae and Compositae (Riz, 1990). The heliotridine monoester (with a dehydrated viridifloric acid), isolated from *H. ovalifolium* was not previously identified from any *Heliotropium* species. However, the presence of related heliotridine esters has been reported in *H. supinum* (Crowley and Culvenor, 1959).

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القلويدات البيروليزيدينية لنباتي هليوتروبيوم أربينينز ، هليوتروبيوم أوقا ليفوليم

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أسفرت دراسة نبات الهليوتروبيوم أربينينز عن فصل والتعرف على الهليوترين ،
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