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PHYTOCHEMICAL STUDY AND BIOASSAY OF SOME CONSTITUENTS FROM *HELIOTROPIUM BACCIFERUM* FORSSK

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ABSTRACT

Heliotropium bacciferum forssk (Boraginaceae) was found to contain heleurine N-oxide, heliotrine N-oxide and europine N-oxide as well as β-amyrin, β-sitosterol, β-sitosterol-O-glucoside. A new preparative HPLC method for the isolation of PAs N-oxides was developed. The expected cytotoxic activities were determined by the brine shrimp bioassay to measure their toxic activities and potential cytotoxicity.

INTRODUCTION

Pyrrolizidine alkaloids of which over 300 are known, have been isolated from over 450 plant species from 14 different plant families worldwide[1]. pyrrolizidine alkaloids (PAs) have received considerable attention over the last 30 years, largely on account of their biological activities, which include hepatotoxic, mutagenic and /or anti-cancer properties[2-4]. It has been considered for along time that PAs occur in Plants as mixtures of tertiary bases and the corresponding N-oxides [2-4]. However, because the N-oxides are much polar and water soluble than the tertiary bases and so more difficult to isolate, it has been of common practice to subject aqueous acidic plant extracts to a mild reduction (designed to reduce N-oxides to tertiary amines) before isolating the PAs[3,5]. Although convenient, this procedure is disadvantageous in as much as it may change, both qualitatively and quantitatively, the composition of the alkaloid mixture from that present in the plant .

For biological screening of activity of PAs, the brine shrimp bioassay which has been widely used as prescreen for anti- tumor compounds[6], was employed. Its value over other *in- vitro* methods lies in the low cost, simplicity, applicability in laboratories lacking cell culture facilities and the correlation to cytotoxicity and antitumor assays make this bioassay a convenient routine in-house prescreen of antitumor activities.

Although many techniques have been used for the isolation of PAs viz: Column chromatography [7-8], Flash CC[3], DCC [9,10], PTLC [11,12] . HPLC has not been exploited for this purpose. It was used for the Identification of PAs [13,14] using reversed-phase C_{18} columns and the aqueous buffered solvent system required made this technique not optimum for preparative work . On the other hand, normal phase HPLC systems would require buffered systems besides organic solvents that are not transparent to permit detection of PAs at 200 nm, and bence this technique is unsuitable for HPLC of PAs.

A new reversed - phase HPLC techinque was developed for the resolution of PAs (N-oxides) using a preparative μ-Bondapack (CN) column. This type of columns are more efficient for N-containing compounds as it is packed with silica particles (4μ) capped with cyano-propyl functions. This column permitted separation of closely related N-oxide of PAs (e.g., heliotrine N-oxide and europine N-oxide) using simple and cheap mixture of H_2O and MeOH in an isocratic mode, and no undesired buffer system were needed . However, the addition of 1% NH_4OH to the mobile phase improved peak shapes. The transparency of the employed mobile phase (MeOH and H_2O) permitted easy detection of PAs (N- oxides) at 200 nm.

MATERIALS AND METHODS

General Procedures :

MPs are uncorrected and were determined on a Mettler FP5 electrothermal unit; specific rotation was determined by the use of a Perkin- Elmer Polarimeter, Model 241 MC; IR spectra were recorded (in KBr) using Perkin-Elmer 580 B IR Spectrophotometer . ^1H and ^{13}C NMR spectra were run at 200 and 50 MHz, respectively (varian XL-200) or at 300 and 75 MHz, respectively (Varian VSR-3000). Standard Varian pulse sequences were used for APT, DEPT, COSY and HETCOR.

Mass spectra (low resolution EI) were determined using Finnigan 5100 or Schimadzu QP 1000 Ex instruments (70 eV ionization potential). Chemical ionization (CI) masss spectra were obtained using ammonia as ionizing gas. TLC was carried out on Si gel plates (kiselgel 60, GF 254), developed with $CHCl_3$-MeOH-Me_2CO-NH_4OH (S_1, 48:18:26:8), $CHCl_3$-MeOH (S_2, 92:8) and silica C_{18}-UV 254 (Macherey and Nagel, Germany) de-

veloped with H_2O-MeOH- acetonitrile- NH_4OH (S_3, 70:15:12:3).

HPLC system consisted of Waters Modular system (Waters Assc. Inc., Milford, MA, USA) fitted with a model M-45 solvent delivery system attached to an automated controller model 680; model U6K injector, with a 2ml sample loop' a Lambda-Max model 481 LC spectrophotometer on a data modular module 730.

Brine shrimp eggs *(Artemia salina leach)* [San Francisco Bay Brand Inc., New York, California, USA]; artificial sea water (Aquarium System of Instant Oceans, Ohio, USA) and double distilled water.

Plant Meterial :

Heliotropium bacciferum Forssk was collected in flowering stage from the vicinity of Belbis City (Cairo - Belbis desert road)-Egypt, during May 1987 and 1988 and authenticated by Dr. N. El-Hadidi, Professor of Taxonomy, Faculty of Science, Cairo University). A voucher specimen is deposited in the Pharmacognosy Department, Faculty of Pharmacy, Zagazig University, Egypt.

Extraction and Isolation :

Air- dried powdered aerial parts of ***Heliotropium bacciferum*** Forssk (2 Kg) was defatted with N-hexane to yield 22 g (fraction A), then exhaustively extracted with EtOH 95% . The alcohol free extract was further extracted with ether (4 x 150 ml) to give alkaloids- free fraction (8g, fraction B) . The aqueous phase was alkalinized to PH 10 by NH_4OH and then extracted with $CHCl_3$ (6 x 150 ml) to yield the tertiary alkaloids. The aqueous layer was then extracted with ethyl acetate (10 x 150 ml) using a mechanical shaker. The ethyl acetate extract (1.5 L) on concentration gave 7.1 g of a brown viscous residue of the crude N-oxide alkaloids (fraction C) .

Chromatography :

The residue (fraction C, 7.1 g) of the crude N-oxide was column chromatographed using neutral alumina (5 x 60 cm, 800g), eluted with $CHCl_3$- MeOH-NH_4OH (55: 40: 5) gave 3.8g crude N-oxides. The purified N-oxides mixture was rechromatographed on silica gel column (65x3 cm, 750g). Gradient elution was performed by ($CHCl_3$ -Me_2CO- MeOH- NH_4OH). The separated fractions (200 ml each) were concentrated and monitored by TLC using systems (S_1 and S_3) visualized by iodine vapour and Dragendorff's reagent followed by 1% HCl. Similar fractions were combined. Three compounds were detected **4, 5** and **6** .

Isolation of N-oxides:

Fractions 5-10 showed one major spot (R_f 0.42, S_1), trial for crystallization after PTLC on silica gel plates using $CHCl_3$ - MeOH-NH_4OH (90:9:1) showed a band of (R_f 0.35), yielded 265 mg of white gummy residue **4.**

Fractions 14 - 18 showed two major alkaloidal spots (**5 and 6**) with (R_f 0.24, 0.20, S_1) and (R_f 0.62 and 0.75, S_3) using RP-C_{18} plates. The previous mixture was subjected to preparative HPLC method, dissolved in methanol and injected onto μ-Bondapak CN (RP) column (7.8 x 30 cm); using an isocratic mixture of H_2O-MeOH-NH_4OH (60:39:1) at a flow rate of 4 ml/min and UV Detector at 220nm. This afforded 420 mg of **6** (R_t 2.6 min) and 810 mg of **5** (R_t 3.7 min) both were crystallized as colorless needles form $CHCl_3$-EtOAc and MeOH-Me_2CO, respectively.

Isolation of Non-Alkaloidal Fractions :

The hexane extract (Fraction A, 20g) was chromatographed on a silica gel CC(80 x 5 cm, 750 g) using hexane, chloroform and methanol in gradient manner. Fractions (250 ml, each) were collected, similar fractions complied, afforded three compounds (**1,2 and 3**).

Compound [1] : Fraction(**16-20**) showed a major spot (R_f 0.8, S_2) visualized with anisaldehyde H_2SO_4 reagent. Trial for crystallization using $CHCl_3$ - MeOH mixture afforded 315 mg of crystalline needles mp 196-197 °C showed co-chromatographic spot identical with β - amyrin. It showed white needles ($CHCl_3$-MeOH), gave positive results with Liebermann's and Salkowiski tests. IR (KBr) : 3300 (OH), 2940 (C-H, aliphatic) and 1620 (C=C). EIMS (DE), m/z (rel. int. %) : 411 (26), 393 (0.9), 257 (32), 218 (100%), 207 (8.3), 205 (4.3), 203 (56), and 189 (19.5). ^{1}HNMR spectrum was obtained at 200 MHz . It showes 8 methyl signals at (δ0.79 ,0.83, 0.87 x 2, 0.94, 0.97, 0.99 and 1.13); an olefinic proton (H-12) at δ5.2 t and a multiplet of one proton at δ3.22 . The remaining skeleton appeared between δ 0.7-2.2. The ^{13}C NMR data are identical with those of β-amyrin .

Compound [2]: Fractions (21-26) on concentration after trials of crystallization using $CHCl_3$-MeOH, gave 350 mg of white needles . It showed R_f 0.67 (S_2) and mp (136 - 138 °C) .

Compound [3]: The pooled fractions (35-40) on concentration yielded (900 mg) dissolved in EtOH, clarified using charcoal, crystallization (MeOH) afforded 750 mg of white granular material, showed (R_f 0.26, S_2) and mp 280° - 282 °C. It was confirmed by comparison of the (IR and MS) spectra with authentic sample of β-sitosterol glucoside and also by acid hydrolysis . The hydrolized products were proved to be β-sitosterol and glucose by all aspects (mmp, ir and co-chromatography)

Compound [4] : It showed $[\alpha]_D^{22}$ +13° (EtOH, C=.02). IR (Thin film) cm^{-1} : 3450 , 2970 , 1730 , 1650 , 1230, 995. PICI-MS, m/z (rel. int. %) : 313 $[M+1]^+$(75), 289 (20), 194 (13), 138 (18), 137 (7), 136

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(55), 120 (100). the ^1H (300 MHz) and ^{13}C (75 MHz) are summarized in Tables (1 and 2) . The APT and DEPT spectra, the ^1H-^1H COSY and - ^1H ^{13}C HETCOR experiments were obtained using the pulse sequences.

The alkaloid **4** (80 mg) was reduced using 2N H_2SO_4 and Zn dust, alkalinized with NH_4OH and extracted using $CHCL_3$. Trial fo crystallization of the residue after extraction yielded 40 mg of colourless needles . The mp, OR, R_f (TLC, S_1) MS, ^1H and ^{13}C NMR data of this reduced base coincides with those reported for the alkaloid heleurine [19,20] .

Compound [5] : Colourless needles (MeOH-Me_2C=O) mixture, mp 170°-171°C, $[\alpha]_D^{22}$ + 22° [EtOH, C 0.2] . IR (KBr) cm^{-1} 3500-3000 , 1735 , 1235 , 960. EI-MS, m/z (rel. int. %) : 313 $[M-16]^+$ (0.7), 295 $[M-16-18]^+$ (3.8), 255 (0.1), 214 (1.1), 197 (0.2), 156(2.5), 139 (7.8), 138 (8.6), 136 (9.3), 120 (100). ^1H and ^{13}C NMR spectra were determined at 200 and 50 MHz respectively, results are presented in Tables (1 and 2) . The ^1H - ^1H (HOMOCOR) and ^1H-^{13}C (HETCOR) experiments were done .

The alkaloid **5** (100 mg) was reduced using 2N H_2SO_4 and Zn dust, extracted with CH_2Cl_2, trial of crystallization of the dried extract with (MeOH - Me_2CO) mixture gave 65 mg of colourless crystals . The mp, OR, R_f (TLC, S_1), IR, MS, ^1H and ^{13}C NMR data of this reduced product were identical to those of the alkaloid heliotrine.

Compound [6]: Colourless, micro - needle crystals ($CHCl_3$ - EtoAC), mp 166° - 168°, $[\alpha]_D^{22}$ + 25° (EtOH, C= 0.2). IR (KBr) cm^{-1}: 3540 (OH), 1730 (C =O, satd. CO_2R), 1630 (C=C), 1230 (C -O), 980 (N-O). The ^1H (200 MHz) and ^{13}C NMR at (50 MHz) are summarized in Tables (1 and 2). The HOMOCOR and HETCOR pulse sequences are used to determine the ^1H / ^1H and ^1H/ ^{13}C correlations .

Reduction of the alkaloid 6 : About 70 mg of [6] were reduced with H_2SO_4/Zn dust. The obtained tertiary alkaloid (yellowish-white gum) was identical in all aspects [(R_f, TLC, S_1), OR, IR, ^1H and ^{13}CNMR]with those reported of europine alkaloid [15-17] .

Bioassay : The brine shrimp bioassay described by Meyer et al[1982][6] was employed. Three doses (200, 50 and 1 ppm) were used for each drug (Heleurine N-oxide, Heliotrine N-oxide, Europine N-oxide and the tertiary alkaloids of the previous N-oxides Heleurine, heliotrine and Europine). Three separate tests were used for each dose of the previous drugs. The number of deaths out of 30 shrimps per dose was recorded after 24 hr and LC_{50} values with 95% confidence intervals were determined for each compound by a Finney computer program[18] . LC_{50} values greater than 200 ppm were considered inactive . The results of brine shrimp bioassy are shown in Table (3) .

RESULTS AND DISCUSSION

The alkaloid heleurine N-oxide **4** was obtained as water soluble gum. The ^{13}C NMR data of compound **4** Table (1) revealed the presence of 16 carbon atoms; the APT alongside with DEPT experiments classified them as 3 methy1, one methoxy, 5 methylene, 4 methine and 3 quaternary carbon atoms. These data are similar to those of the corresponding free base heleurine. Picims showed $[M+1]^+$ m/z 314 consistent with the molecular formula C_{16} H_{27} NO_5. This mass was higher than heleurine by 16 mu suggesting the presence of a N-oxide group. Other Ms fragments at m/z 298. 138, 136 and 120 (base peak) siginified the presence of a supinidine ester N- oxide[19]. This was supported by the IR spectrum at 995 cm^{-1} (N-O); the downfield ^1HNMR shifts of the necine protons H-3 D, 3U, 5D, 5U and H-8 (δ4.74, 4.65, 3.73,2.46 and 4.67; respectively) and by the down field ^{13}C NMR shifts of the C-3 (δ 60.46), C-5 (δ.70.66) and C-8 (δ88.89) in comparison to those of heleurine. The ^{13}C signals at δ136.33 and 120.98 indicated a double bond between C-I and C-2. the COSY spectrum showed strong coupling between the H-2 proton and the two H-9 protons each appeared as unequal pair of doublets duc to splitting by each other ($J_{9U,D}$ = 16Hz) and further fine splitting by H-2 ($J_{9U,2}$ =1.5Hz, $J_{9D,2}$ =1.9Hz) indicating that the supinidine is esterified at C-9 .

The ^1HNMR showed signals characteristic for the acid moiety at δ0.88, 0.91, 1.12 (three methyl groups at C-3`, 3` and 4`); δ3.26 (3H, s,4`-OMe), δ1.96 (1H, m, H-3`) and one proton quartet (4` - H, J= 6Hz) coupled with 4` methyl protons. These data indicated the presence of heliotrine ester [20] ^1H /^1H and ^1H/^{13}C 2D experiments, both were in complete accordance with the structural assignments (Table 1) of the alkaloid [4] which is concluded to be the N-oxide of heleurine. Confirmation done, by reducing the alkaloid [4] to give a reduced base identical (mp, OR, co-chromatograpby) with heleurine[21]. Constantinidis et al 1993[19] reported alkaloid **4** in *H. hirsutissimum.* However, the previous ^{13}C assignments of C-3 and C-9 of helurine N-oxide [4] were not correct as reported (δ 76.7 and 60.5 respectively) .

The HETCOR done in this work showed clear correlation between the ^{13}C signal at δ76.31t and ^1H signals of H-9 indicating that the downfield ^{13}C signal is that of C-9. Correlation between the upfield signal at 60.64 (C-3) and the ^1H signals at δ4.65 (3U) and δ4.47 (3D) was also evident . Accordingly, the reported values for C-3 and C-9 need to be reversed [19] .

The ^1HNMR spectrum of the alkaloid heliotrine N-oxide **5** showed strong resemblance with that of the alkaloid heliotrine and clearly indicated the presence of a heliotrinic acid moiety. Differences in the mp and specific rotation [heliotrine, mp 125°-8,$[\alpha]_D^{22}$ + 6° [$CHCl_3$,

C= 0.2], besides the IR spectrum which showed an ester (1735 cm^{-1}) and N-O bond (960 cm^{-1})suggested the probable presence of heliotrine N-oxide.

The MS displayed, in addition, to ions corresponding to $[M-16]^+$ (m/z 313) and $[M-16-H_2O]^+$ (m/z 295), diagnostic peaks at m/z 255, 214, 197, 138, 136 and 120 which parallel fragments ions reported for heliotrine N-oxide[22]. The results of elemental analysis were identical to heliotrine N-oxide and confirmed the molecular formula $C_{16}H_{27}NO_5$.

The ^{13}C NMR spectrum showed the presence of 16 carbon atoms. The multiplicities were determined using APT and DEPT experiments. These revealed the presence of 4 methyl, 4 methylene, 5 methine and 3 quaternary carbon atoms. When compared to heliotrine the ^{13}C data showed significant differences in the value for C-3 (δ 68.69), C-5 (δ 61.03) and C-8 (δ 96.59) indicating that the difference between heliotrine and heliotrine N-oxide 5 is located at this structure fragment and verify that 5 has to be heliotrine N-oxide. Confirmation of the structure was done by chemical correlation. Reduction of heliotrine N-oxide 5 with Zn dust and H_2SO_4 afforded a reduced base identical in physical and spectral data with those of heliotrine .

The assignments of NMR data Tables (1 and 2) were based on 2 D NMR experiments (^1H - ^1H HOMOCOR and ^1H- ^{13}C HETCOR) and comparison with related compounds[16] .

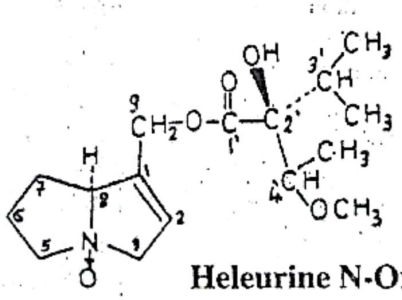
Heliotrine N-oxide was previously isolated from ***Heliotropium*** species[22] but this is the first report for its isolation from ***Heliotropium bacciferum*** Forssk.

In addition, compound **6** showed IR data (3450 and 1730 cm^{-1}) indicated that it is a monoester with several hydroxyl groups [23,10] . The ^{13}C signals at δ134.5 and 123.4 indicated a double bond between C - 1 and C-2 [15,16, 20].

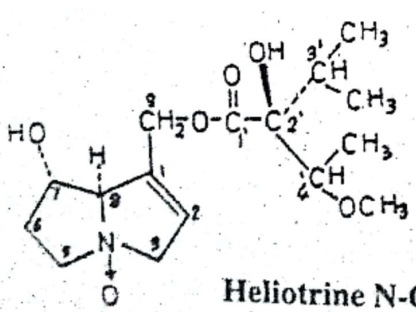
The $^{13}CNMR$ spectrum showed 16 carbon atoms and the ^1HNMR spectrum revealed 24 protons (those of the 3 OH groups were missing). The APT and DEPT spectra revealed 3 methyl, one methoxy, 4 methylene, 4 methine and 4 quaternary carbon atoms. The chemical shifts value of these carbons verified the presence of two oxygenated methine carbons resonating at δ72.99 (C-7) and δ 80.33 (C-4); and two oxygenated quaternary carbons at δ 86.09 (C-2) and δ 74.23 (C-3) besides that of C-O resonating at δ174.82 (C-1`). These data showed a great similarity of the alkaloid europine N-oxide **6** and europine .

Differences in the $^{13}CNMR$ spectrum of europine alkaloid and the alkaloid **6** were observed in the chemical shifts of C-3, C-5, C-7 and C-8. Those of C-8 (δ 98.38), C-5 (δ 61.81) and C-3 δ 69.13) were strong shifted down field, while those of C-7 (δ72.99) and C -2 (δ 123.4) showed a small upfield shifts, when compared to those of europine. These observations strongly suggested the presence of the N-oxide form of europine [16,17] .

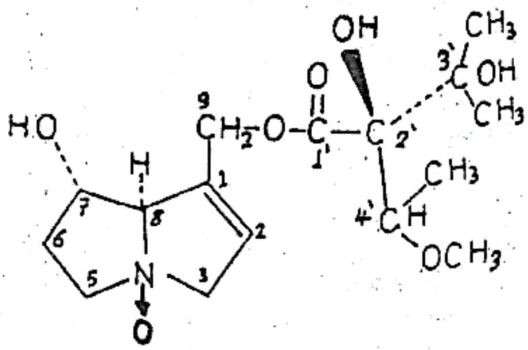
The ^1HNMR data (Table 2) signified that the acid moiety is lasiocarpic acid, and hence, the presence of europine N-Oxide[17] was considered . The MP, OR, IR ^1H and $^{13}CNMR$ data Tables (1 and 2) of the alkaloid **6** were identical to those reported for europine N-oxide [15,17,19] Reduction of the alkaloid 6 with H_2SO_4/Zn yielded a tertiary alkaloid which was identical (MP, OR, IR and ^1H and ^{13}C NMR) with those of europine alkaloid, and provided a concrete evidence that alkaloid **6** is europine N-oxide . This is the first report about the isolation of europine N-oxide from ***H. bacciferum***.



4 Heleurine N-Oxide



5 Heliotrine N-Oxide



6 Europine N-Oxide

β-amyrin **1** gave positive Liebermann's and Salkowiski's tests, suggested a triterpene or steroid nucleus. The ^{13}CNMR spectrum revealed 30 carbon atoms and the IR spectrum indicated C = C (1620 cm^{-1}) and OH (3300 cm^{-1}) groups . The MS showed a molecular ion $[M]^+$ at m/z 426 and characteristic fragments of β - amyrin (27) ; m/z at 411, 393, 218 (base peak) , 207, 205 and 203 . TLC of **1** alongside with β-amyrin reference sample, strongly suggested that cmpound **1** is βamyrin.

Also, compound **2** was proved by co-chromatography, mp, mmp, ir and ms to be similar with authentic sample of β-sitosterol which was previously reported in *H. digynum* (27) and *H. ovalifolium* (28) . This is the first report for the presence of βsitosterol in *H.bacciferum*.

The NMR data of **1** were indistinguishable from those of β- amyrin. β-amyrin was previously isolated from *H. digynum* (13) and *H. ovalifolium* (24) . This is the first report for the presence of β-amyrin in *H.bacciferum*.

The β-sitosterol -O- glucoside **3** was proved by co- chromatography, mp, mmp, ir and ms to be similar with authentic sample of -β- sitosterol -O- glucoside. This the first report of the presence of β- sitosterol -O- glucoside in the genus *Heliotropium* .

The shrimp bioassay tests of the PAs isolated from *H.bacciferum* only those with 7 -OH group were strongly toxic (i.e. heliotrine, europine, heliotrine N-oxide and europine N-oxide) while those lacking OH group at C-7 (heleurine and supinine) were considered inactive . The most powerful toxic effects were shown by europine and europine N-oxide (LC_{50} =47 and 42.8 ppm, respectively). The later has strong structure similarity to the well known antitumor indicine N-oxide. It was also noted that the tertiary alkaloids (heliotrine and europine) are less active than their corresponding N-oxides, Table (3) .

Table 1 : ^{13}Cnmr (50 and 75 MHz) of Isolated Pyrrolizidine Alkaloids.

Protons	Compound		
	4	5	6
1	136.33 (s)	134.45 (s)	134.5 (s)
2	120. 89 (d)	120. 20 (d)	123.4 (d)
3	[60.46(t)] [a]	68.69 (t)	69.13 (t)
5	70.66 (t)	61.03 (t)	61.81 (t)
6	24.54 (t)	33.35 (t)	34.11 (t)
7	27.39 (t)	71.87 (d)	72.99 (d)
8	88.89 (d)	96.59 (d)	98.38 (d)
9	[76.31 (t)] [a]	77.76 (t)	78.6 (t)
1'	174.30 (s)	174.29 (s)	174.82 (s)
2'	83.16 (s)	82.23 (s)	86.1 (s)
3'	32.67 (d)	33.09 (d)	74.23 (s)
4'	78.59 (d)	78.97 (d)	26.6 (q)
3' - Me	17.07 (q)	17.14 (q)	25.73 (q)
3' - Me	17.07 (q)	17.14 (q)	80.3 (d)
4' - Me	11.45 (q)	11.66 (q)	13.2 (q)
4'-OMe	56.60 (q)	56.75 (q)	56.86 (q)

[a] Values in parenthesis are interchangeable indicated by the superscript : Constantinidis et al (19) Constantinidis, T.; Harvala , C.and Skaltsounis, A.L.; **Phytochemistry** 32 (5), 1335 (1993) .

Table 2 : ^{1}Hnmr (200 and 300 MHz) of Isolated pyrrolizidine Alkaloids.

Protons	Compound		
	4	5	6
2	5.73 brs	5.72 brs	5.9 brs
3D	4.74d (J = 14Hz)	4.08m	4.6 m
3U	4.65d (J = 14Hz)	3.60m	3.85 m
5D	3.73 m	-	-
5U	2.46 m	*4.86	*3.8 m
6U	2.47 m	2.42 m	-
7D	1.96 m	2.07 m	*2.2 m
7U	2.47 m	-	H - 7 4.4 m
8	1.96 m	*4.46 brs	-
9D	4.67 brs	4.88 m	4.4 m
9D	4.49dd (J=16, 1.9Hz)	4.43AB[a]	4.8 d[b]
9U	4.41dd(J=16, 1.5Hz)	4.35AB[a]	4.8 d[b]
⟍3	1.96 m	1.99 Sept (J = 6.5 Hz)	-
⟍4	3.66q (J = 6Hz)	3.69q (J = 6.5 Hz)	3.85d (J = 7Hz)
⟍3 - Me	0.91d (J = 6Hz)	0.91d (J = 6.5 Hz)	1.23 s
⟍3 - Me	0.88d (J = 6Hz)	0.94d (J = 6.5 Hz)	1.31 s
⟍4 - Me	1.12d (J - 6Hz)	1.15d (J = 6.5 Hz)	1. 23d (J = 7Hz)
⟍4 - OMe	3.26 s	3.29 s	3.27 s

U = upfield ; D = downfield * U + D

[a]AB = Two doublets forming AB system (J value could not be determined).
b = Coupling Constant could not be measured, signals were distorted by solvent signal

Table 3 : Brine Shrimp Bioassay Results of Some Isolated Compounds.

Compound	LC_{50} (ppm)
Heleurine	1560
Heliotrine	97
Supinine	> 2000
Europine	47
Heleurine N - oxide	1199
Heliotrine N - oxide	62.7
Europine N - Oxide	42.8
Strychnine Sulfate	62 (77.2) *

* Strychnine sulfate was used for comparison [25]
LC_{50} value is shown in parenthesis .
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دراسة كيميائية وتقييم حيوى لبعض مركبات تم فصلها من نبات هليوتروبيوم باكسيفيرم (فورسك)

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فى هذه الدراسة وجد أن نبات الهليوتربيوم باكسيفيرم (فورسك) (بوراجينيسى) يحتوى على هليورين-إن-أوكسيد ، يوروبين -إن-أوكسيد وهيليوترين -إن-أوكسيد، كذلك بيتا سيتوستيرول وبيتا سبتوستيرول جلوكوزيد. تم استحداث طريقة جديدة لفصل بعض مركبات البيروليزيدين- إن أوكسيد التى يحتمل أن يكون لها تأثير مضاد للخلايا السرطانيه بواسطة جهاز كوروماتوجرافيا الضغط العالى وكذا تم فحص هذه المركبات باستخدام طريقة الجمبرى للمعايرة الحيوية لتعبئ مدى سمية هذه المركبات وكذا خاصيتها كمواد مضادة للخلايا السرطانية .