Zagazig J. Pharm. Sci., June 2004
Vol.13, No. 1, pp. 1-5

ISSN 1110-5089

PYRROLIZIDINE ALKALOIDS OF *CERINTHE GLABRA* MILLER (BORAGINACEAE)

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ABSTRACT:

The pyrrolizidine alkaloids of *Cerinthe glabra* Miller (total yield 0.3 mg/g dry weight) was investigated by capillary GLC and GLC-MS in combination with retention indices. Six pyrrolizidine alkaloids were identified for the first time in this plant, of which 5 unambiguously identified by comparing their specific retention indices and mass fragmentation with those of authentic alkaloids and literature data. Lycopsamine, 7-acetyllycopsamine and 3'-acetyllycopsamine were the prominent alkaloids in the reduced extract. Supinine, 3',7-diacetyllycopsamine and the tentatively identified 7-hydroxymethylbutyryl-9-viridifloryl-retronecine were detected as minor constituents.

INTRODUCTION

The pyrrolizidine alkaloids-containing plants has received extensive chemical and biological study mainly because their hazard to livestock, wildlife and humans. Cases of human poisoning most often are a result of food contamination or when pyrrolizidine alkaloid containing plants are used for medicinal purposes as herbal teas or in traditional medicines[1,2]. The toxicological features include: hepatotoxicity, pneumotoxicity, mutagenicity, carcinogenicity, cytotoxicity as well as exhibiting antimitotic and genotoxic effects[3-9]. The toxicity is due to the conversion of pyrrolizidine alkaloids to the corresponding pyrrole derivatives which are highly reactive and have the ability to form DNA cross-linkage and interrupt DNA replication[10-12]. The ring nucleus (necines) with a double bond at the 1:2 position is essential for toxic effects of theses alkaloids. However, some saturated pyrrolizidine alkaloids have interesting pharmacological and biological effects e.g. spasmolytic, antihistaminic, glucosidases inhibitor, anti-HIV and antiviral activities[13-15].

About 10 species of the genus *Cerinthe* are known, mainly distributed in Europe and the Mediterranean region[16]. Only *C. minor* L. has been studied for its pyrrolizidine alkaloid contents and one alkaloid (intermedine) was recorded[17,18].

Cerinthe glabra Miller [19] is a perennial or biennial plant with a hight of 15-50 cm. The plant grows in damp or shady places of C. and S. Europe. The leaves are obtuse; the lower being oblong-cuneate, tapering to a petiole; others are ovate, sessile and cordate at the base. The alkaloidal composition of this plant had not been studied before. Thus, in the present study, we report on the pyrrolizidine alkaloids profile of *C. glabra* Miller in the form of tertiary bases (free) and N-oxide form (through reduction with Zn/H^+) determined by capillary GLC and GLC-MS.

EXPERIMENTAL

Plant material:

The plant material of *Cerinthe glabra* Miller (Boraginaceae) was kindly supplied by the staff of the Botanical Garden of Heidelberg University, Germany. The aerial parts were collected in August 2000 after full blooming. Identity of the plant was verified by Dr. K. Kramer (Botanical Garden, Heidelberg). A voucher specimen is deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Egypt.

Alkaloids isolation:

The fresh aerial parts of *C. glabra* (50 g) was homogenized with an Ultra turrax in 0.5 N HCl (1L) and kept for 1 h. Half of the acidic aqueous extract was adjusted to 2 N HCl and the N-oxides were reduced with Zn dust aided by stirring overnight at room temperature[3,4]. Excess Zn was removed by filtration and filtrate was washed with CH_2Cl_2 (2 x 150 ml). Basification of the washed aqueous acidic solution was accomplished with NH_4OH, followed by extraction with CH_2Cl_2 (3 × 350 ml), drying over anhydrous Na_2SO_4 and evaporation of the solvent to leave a residue (8.1 mg; 0.032%) constitutes total pyrrolizidine alkaloids as bases (tertiary pyrrolizidine alkaloids + pyrrolizidine N-oxides). The remaining half of the acidic extract was treated in the same manner, but without Zn/H^+ reduction. The alkaloid residue (3 mg; 0.012%) represents the free tertiary pyrrolizidine alkaloids. Concerning the dry plant material the yield of alkaloids were 0.08 and 0.03 % of the total and free 3^{rd} pyrrolizidine, respectively. Both alkaloid fractions were subjected to capillary GLC and GLC-MS analyses.

Capillary gas chromatography:

A Carlo Erba ICU 600 gas chromatograph equipped with FID and fused silica capillary column (DB1-30 W, 15 m, 0.317 mm ID). Condition: carrier gas He (2 ml/min.); detector temp. 300°C; injector temp. 250°C; oven temp. program: initial 150°C 2 min isothermal, 150-250°C at 15 min^{-1}, 250-300°C at 25 min^{-1}, 300°C, 5 min isothermal, 1 mg alkaloid fraction was dissolved in 1 ml methanol and 1µl was injected.

Gas chromatography -mass spectrometry:

A Carlo Erba HRGC 4160 gas chromatograph equipped with a fused silica capillary column (OV1; 30 m. 0.3 mm ID) was employed. The column was directly coupled to a quadruple mass spectrometer (Finnigan MAT 4500). Condition: injector 250°C; temp. program: 120-138°C at 3 min^{-1}, 138-300°C at 6 min^{-1}; Split ratio 1:10; carrier gas He 0.5 bar.

EI-Mass spectra were recorded at 40 eV. CI mass spectra were recorded with the same GLC-MS system using NH_3 as reagent gas. Retention index (RI): Kovats indices were calculated with reference to a set of co-injected hydrocarbons (C_{14} - C_{28}). 2 µl volume was injected.

RESULTS AND DISCUSSION

The combination of capillary GLC and MS is the method of choice for the separation and identification of complex pyrrolizidine alkaloid mixtures. Retention index (RI) data, molecular weight [M^+], and group-specific MS fragmentation provide sufficient information for an unequivocal identification of most pyrrolizidine alkaloids especially which are present as trace components or of geometrical isomers[20-30]. The alkaloid contents of aerial parts of *C. glabra* Miller were analysed in this way. It has long been recognized that the pyrrolizidine alkaloids occur in plants as a mixtures of tertiary bases and the corresponding N-oxides[3,5]. However, because of the N-oxides are more polar and water soluble than the tertiary bases, it would be better to subject the aqueous acidic plant extract to mild reduction (Zn/H^+) to convert the N-oxides into the corresponding tertiary basic alkaloids, before isolation and analysis.

Capillary GLC and GLC-MS analyses of the crude tertiary pyrrolizidine alkaloids of *C. glabra* Miller revealed the presence of six alkaloids. All were found in the form of tertiary alkaloid and corresponding N-oxides (Table 1). The identified components represent 94.6 and 92.92 % of the tertiary and total pyrrolizidine alkaloids, respectively. While high lycopsamine (mainly as N-oxide) concentration (49.6%) is found in the reduced extract, only minor percentage (4.2%) in the tertiary form has been detected (Table 1).

Table 1: Pyrrolizidine alkaloid profile of *Cerinthe glabra* Miller as determined by capillary GLC and GLC-mass spectrometry

Alkaloid	RI†	Alkaloid relative abundance	
		Tertiary alkaloid*	Total alkaloid**
1 Supinine	1984	10.14	3.03
2 Lycopsamine	2153	4.23	49.61
3 3'-Acetyllycopsamine	2216	6.41	13.88
4 7-Acetyllycopsamine	2233	35.03	19.43
5 3',7-Diacetyllyco-psamine	2304	11.52	3.84
6 7-Hydroxymethyl-butyryl-9-viridifloryl-retronecine☼	2560	27.31	3.13
Total pyrrolizidine alkaloid (fresh weight %)		0.012	0.032

* = before reduction
** = free bases + N-oxides
† = retention index relative to C_{14}-C_{28} *n* alkanes on OV-1 column.
☼ = tentative identification

Table 2: GLC-MS analysis of the pyrrolizidine alkaloids in *Cerinthe glabra* Miller.

Alkaloid	Formula	EI-MS [M^+]	CI-MS [$M^+ + 1$]	Characteristic ion m/z (relative abundance)
1 Supinine	$C_{15}H_{25}NO_4$	283	--	140(5), 123(22), 122(100), 121(41), 120(55), 108(10), 93(20), 80(5), 70(12), 53(5), 43(19).
2 Lycopsamine	$C_{15}H_{25}NO_5$	299	300	284(0.1), 254(0.2), 156(8), 139(35), 138(100), 137(15), 136(10), 120(8), 108(3), 95(15), 94(50), 93(78), 80(10), 67(8), 53(3), 43(17).
3 3'-Acetyl lycopsa-mine	$C_{17}H_{27}NO_6$	341	342	298(6), 255(14), 139(20), 138(100), 137(12), 136(11), 120(10), 99(8), 94(30), 93(70), 80(10), 67(5), 43(20).
4 7-Acetyl lycopsamine	$C_{17}H_{27}NO_6$	341	342	296(3), 281(7), 198(9), 181(30), 180(100), 136(18), 121(25), 120(65), 119(15), 101(18), 94(25), 93(50), 80(10), 73(5), 67(5), 43(28).
5 3',7-Diacetyl lyco-psamine	$C_{19}H_{29}NO_7$	383	384	340(0.3), 297(2), 268(27), 253(1), 211(18), 181(20), 180(100), 136(15), 121(8), 120(45), 119(20), 101(9), 99(10), 94(20), 93(40), 85(10), 80(3), 71(15), 57(18), 43(34).
6 7-Hydroxy butyryl-9-viridifloryl-retronecine (or closely related isomer)*	$C_{26}H_{33}NO_7$	399	400	384(2), 354(0.4), 296(0.3), 282(2), 256(6), 239(21), 238(55), 138(15), 136(21), 121(35), 120(100), 108(2), 101(5), 95(10), 94(20), 93(42), 83(5), 80(6), 73(5), 67(3), 59(12), 55(0.5), 43(25).

* = tentative identified on bases of MS.

Five alkaloids could be unambiguously identified (Table 2) by direct comparison of their retention indices and mass spectra with authentic alkaloids and literature data[21-27,29,30]; supinine (**1**), lycopsamine (**2**), 3′-acetyllycopsamine (**3**), 7-acetyllycopsamine (**4**) and 3′,7-diacetyllycopsamine (**5**). The remaining alkaloid was tentatively identified on the basis of MS spectra and biogenetic consideration as 7-hydroxymethyl-butyryl-9-viridifloryl-retronecine (**6**) or closely related isomer.

The GLC-EI mass spectrum of compound **1** showed a molecular ion [M^+] at 283; this corresponds to $C_{15}H_{25}NO_4$. The base peak at m/z 122 [M – $C_7H_{13}O_4$]$^+$ results by cleavage of the weak allylic ester bond at C-9 confirms the presence of supinidine necine esterified at C-9. Comparison of mass fragmentation and retention index of this compound with reported data [22,24], it could be concluded that this compound is supinine.

Alkaloid **2** showed a molecular ion at m/z 299, consistent with the formula $C_{15}H_{25}NO_5$. The base peak at m/z 138 [M- $C_7H_{13}NO_4$]$^+$ due to cleavage of the weak allylic ester bond at C-9 which gives strong evidence for the presence of free OH at C-7[31,32]. Comparison of the mass fragmentation and retention index with literature data[22,27], it was clear that alkaloid **2** is lycopsamine.

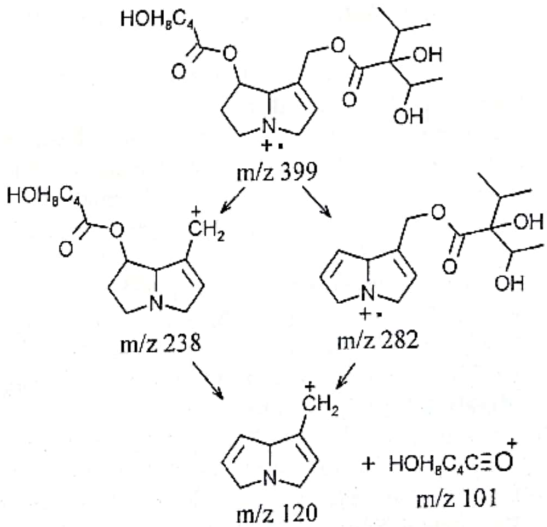
Compound **3** exhibited a molecular ion peak at m/z 341, consistent with formula $C_{17}H_{27}NO_6$. The presence of free OH at C-7 was established through the base peak at m/z 138 [M- $C_9H_{15}NO_5$]$^+$. Forty-two mass units was the difference between the M^+ of alkaloid **3** and lycopsamine (**2**) beside full similarity of fragments of both compounds. Thus, an extra acetate moiety esterified in C-9 hydroxyl. Previous reports on the dihydroxyl-acid at C-9 (C-2′ and C-3′) indicated only 3′-acetylation as a sole product[5,30]. Based upon GLC-MS data and biogenetic consideration, alkaloid **3** was identified as 3′-acetyllycopsamine[21].

The mass spectrum of alkaloid **4** showed an [M^+], more than that of lycopsamine (**2**) by 42 mass units as well as the base peak at m/z 180 (C-7 acetylated) as a result of cleavage of C-9 ester (allylic ester bond) and the ion at m/z 281 is due to loss of acetic acid attached at C-7 (M^+-CH_3COOH). Thus, the presence of an acetyl group at C-7 was established [32]. Comparison of the mass spectra and retention index of this compound with that reported in literature [25,26,30], indicated clearly that **4** is 7-acetyllycopsamine.

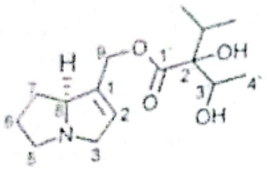
Alkaloid **5** showed [M^+] at m/z 383 (corresponding to $C_{19}H_{29}NO_7$), together with ion series at m/z 136, 120, 93 and 80, which are characteristic for 1,2-unsaturated diester necine[31,32]. The base peak at m/z 180 is due to allylic fission of the esterified acid at C-9 (M^+- $C_9H_{15}O_5$), also confirm C-7 acetyl moiety. One the other hand, this alkaloid has 42 mass units more than of 7-acetyllycopsamine (**4**), again extra acetyl group is present. Based upon GLC-MS data and biogenetic consideration, alkaloid **5** was identified as 3′,7-diacetyllycopsamine[21].

The MS spectral fragmentation of alkaloid **6** showed a molecular ion at m/z 399, which corresponds to formula $C_{20}H_{33}NO_7$. The ion series at m/z 136, 119, 120, 94, 93 and 80 are characteristic for 1,2-unsaturated diester pyrrolizidine alkaloid. The fragment at m/z 282 is due to loss of the acid attached at C-7 [M-$C_5H_9O_3$]$^+$ and the peak at 238 is due to the cleavage of the weak allylic ester bond at C-9 (M^+-viridifloric acid or its isomer). From the fragments at m/z 117, 101, 83, 59 and 55 the presence of saturated acid at C-7 with the formula $C_5H_9O_3$ is presumed; the fragment 73 and 55 are assumed to derived from m/z 101 ($C_5H_9O_2$) through subsequent loss of CO and H_2O, respectively. However, the ion at m/z 83 resulting from the same radical through direct loss of one molecule of H_2O and ion m/z 59 is corresponding to CH_3CHCH_2OH. Proposed fragmentation pattern is illustrated in Scheme 1. Thus, based upon GLC-MS spectra and biogenetic consideration, the structure of **6** is proposed as retronecine necine base esterified at C-9 with viridifloric acid and hydroxymethylbutanoic acid at C-7 (or closely related isomers). A suggested structure of this alkaloid is shown in Fig. 1. Unfortunately, there is no sufficient material to perform NMR and confirm this identification and specify the optical form of the five-carbon acid at C-7. To the best of our knowledge, this is the first record about the presence of these alkaloids in *C. glabra* and in genus *Cerinthe*. Our result could be of chemotaxonomical interest in this genus as the pyrrolizidine alkaloids have been used as a chemotaxonomic marker in the Boraginaceae[33,34].

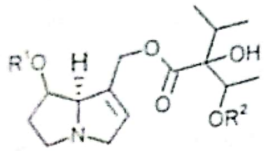
Finally, as confirmed experimentally, *C. glabra* contains 1,2-unsaturated pyrrolizidine alkaloids as main secondary metabolites. Thus, it is suggested that human should not ingest foods or herbal teas that doubtedly contain any plant material (including *C. glabra*) contain this type of alkaloids.



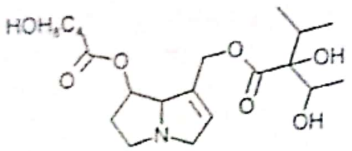
Scheme 1: Mass fragmentation of alkaloid **6**.



1



2 R^1 = H, R^2 = H
3 R^1 = H, R^2 = Ac
4 R^1 = Ac, R^2 = H
5 R^1 = Ac, R^2 = Ac



6

Fig. 1: Structures of pyrrolizidine alkaloids found in *Cerinthe glabra*.

Acknowledgement:

Thanks are due to Prof. M. Wink (Institut für Pharmazie und Molekulare Biotechnologie, Heidelberg University, Germany) for providing analysis facilities. We thank also Dr. K. Kramer (General Supervisor) and the staff of the Botanical Garden, Heidelberg for supplying plant material.

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Zagazig J. Pharm. Sci., June 2004
Vol.13, No. 1, pp. 1-5

القلوانيات البيروليذدينية لنبات سيرنزا جلابرا (الفصيلة البوراجينية)

عاصم محمد الشاذلى

قسم العقاقير – كلية الصيدلة – جامعة الزقازيق – مصر

فى هذا البحث تم دراسة القلوانيات البيروليذدينية لنبات سيرنزا جلابرا بواسطة كروماتوجرافيا الغاز الشعرية والمتصلة بمطياف الكتلة. وقد أمكن التعرف على خمسة قلوانيات لأول مرة فى هذا النبات وكذا التعرف المبدئى على قلوانى أخر.

كما تم التعيين الكمى للقلوانيات سواء كانت ثلاثية القاعدة (الحرة) أو المجموع الكلى للقلوانيات (ثلاثية القاعدة الحرة + ن–أوكسيد بعد اختزالها) ووجدت بنسبة ٠,٠٣% ؛ ٠,٠٨ % على التوالى فى النبات الجاف. ونتيجة لهذه الدراسة وجد أن مركبات الليكوبسمين ، ٧–استيل ليكوبسمين ، ٣–استيل ليكوبسمين بتركيزات عالية فى الخلاصة المختزلة. أما المركبات الباقية فقد وجدت بنسبة ضئيلة.

وقد أوصى البحث بعدم استخدام هذا النبات طبيا نظرا للسمية الشديدة لنوعية هذه القلوانيات للإنسان والحيوان على حد سواء.

Received: March 2, 2004
Accepted: May 15, 2004