Alkaloids in Bulgarian *Pancratium maritimum* L.

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A GC/MS analysis of alkaloids from leaves, bulbs and roots of *Pancratium maritimum* was performed. From the identified 16 alkaloids, 5 alkaloids were reported for the first time for this plant. Several compounds with pharmacological activity were found. Haemanthamine was main alkaloid in the leaves and bulbs whereas galanthane was found to be main alkaloid in roots.

Key words: Amaryllidaceae Alkaloids, GC/MS, *Pancratium maritimum*

Introduction

Amaryllidaceae have attracted attention as a source of valuable biologically active alkaloids. The genus *Pancratium* includes about 15 species distributed in the Mediterranean, Africa and Asia (Willis, 1973). The alkaloid composition of only a few of them have been investigated in detail.

Pancratium maritimum L. is characteristic for sandy coastal habitats of the Mediterranean. The plant is endangered and protected in Bulgaria. Bulb and leaf extracts of *P. maritimum* have purgative (Iordanov, 1964), acaricidal, insecticidal (Abbassy *et al*., 1998) and antifungal activities (Sur-Altiner *et al*., 1999). About 40 alkaloids have been reported for this species: dihydrolycorine, norpluviine (Sandberg and Michel, 1968), lycorine, 6-*O*-methylhaemanthidine, *O,N*-dimethylnorbelladine, hippeastrine, hordenine, harbanthine, ungiminorine, ungiminorine-*N*-oxide, vittatine (Tato *et al*., 1988), tazettine, pancracine, lycorenine, galanthamine, sickernbergine, homolycorine, hemanthidine, hippadine, trispheridine, haemanthamine, pseudolycorine, 9-*O*-demethylhomolycorine, 11-hydroxyvittatine, ungeremine, zefbetaine, narciclasine-4-*O*-β-D-glucopyranoside (Abou-Donia *et al*., 1991), 3,11-dihydroxy-1,2-dehydrocrinane (Sener *et al*., 1993), buphanisine, crinine, 3-methoxy-6-dihydroxy-3-methoxy-1,2-dehydrocrinane, 6,11-dihydroxy-3-methoxy-1,2-dehydrocrinane, 6,11-dihydroxy-1,2-dehydrocrinane, 8-hydroxy-9-methoxycrinine (Sener *et al*., 1994), pancratistatine (Pettit *et al*., 1995), *N*-demethylgalanthamine, 2-*O*-demethylmonthanine (Sener *et al*., 1998), marithidine, lycoramine (Youssef and Frahm, 1998); pancritamine, acetyllycoramine, *N*-demethyllycoramine (Youssef, 1999). Some of these alkaloids have interesting pharmacological properties such as anti-tumor (pancratistatine and ungiminorine; Pettit *et al*., 1995), anti-viral (lycorine), anti-cholinesterase (galanthamine) and analgesic activities (lycorine and galanthamine; Bastida and Viladomat, 2002).

Gas-chromatography/mass-spectrometry (GC/MS) proved to be an useful method for investigation of complex mixtures of different alkaloid groups (Wink *et al*., 1983; Witte *et al*., 1987; Kreh *et al*., 1995). In order to increase the volatility of the alkaloids and make them suitable for GC/MS investigation the alkaloid mixtures can be silylated before analyses, but the spectra obtained gave limited information (Kreh *et al*., 1995). Much more informative appeared to be the spectra of underivatized alkaloids. There are only a few reports on GC/MS of underivatized alkaloid mixtures from Amaryllidaceae plants which showed that the alkaloids retain their characteristic EIMS fragmentation pattern under GC/MS conditions (Kreh *et al*., 1995; Tram *et al*., 2002).

The alkaloid composition of *P. maritimum* plants from the Bulgarian seacoast has not been studied and we performed GC/MS analysis of the alkaloid fractions from leaves, bulbs and roots of *P. maritimum* growing in Bulgaria.

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Experimental

Plant material

Samples of *P. maritimum* were collected in May, 2002 form the Black Sea coast near Kavatsite camping, Bulgaria. A voucher specimen (COM-Co 974) is deposited at the herbarium of Institute of Botany, Bulgarian Academy of Sciences.

Isolation of the alkaloid fractions

Fresh plant tissues were cut into small pieces and extracted tree times (48 h each) with ethanol. The extracts were concentrated *in vacuo*, acidified with 3% sulfuric acid to pH 1–2 and defatted with chloroform (3×). After that, the acidic aqueous phase was alkalized with 25% NH_4OH to pH 10–11 and the alkaloids were extracted three times with chloroform. The chloroform extracts were combined, dried over anhydrous Na_2SO_4 and than evaporated. The residues obtained were dissolved in methanol and subjected to GC/MS analysis.

GC/MS analysis

The GC/MS were recorded on a Hewlett Packard 5890/MSD 5972A instrument operating in EI mode at 70 eV. A HP5 MS column (30 m × 0.25 mm × 0.25 μm) was used. The temperature program was 80 to 280 °C at 10 °C · min^{-1} and 10 min hold at 280 °C. Injector temperature was 280 °C. The flow rate of carrier gas (He) was 0.8 ml · min^{-1}. The identification of the alkaloids was confirmed by comparing the mass spectral data with those of authentic compounds from database NIST 98 (a Hewlett Packard Mass Spectral Library, Hewlett Packard, Palo Alto, CA, USA) or with data obtained from the literature.

Results and Discussion

We analyzed the alkaloid composition of roots, bulbs and leaves from *P. maritimum* in order to establish the presence of biologically active alkaloids in different tissues and to obtain some data for the alkaloid metabolism.

More than 30 compounds from the investigated alkaloid mixtures showed the characteristic mass spectral fragmentation of Amaryllidaceae alkaloids. Almost all of them produce well separated GC peaks. Sixteen compounds were identified (Table I, Fig. 1). To the best of our knowledge, five alkaloids, namely graciline (**2**), 6α-deoxytazettine (**8**), galanthane (**9**), *N*-formylgalanthamine (**15**),

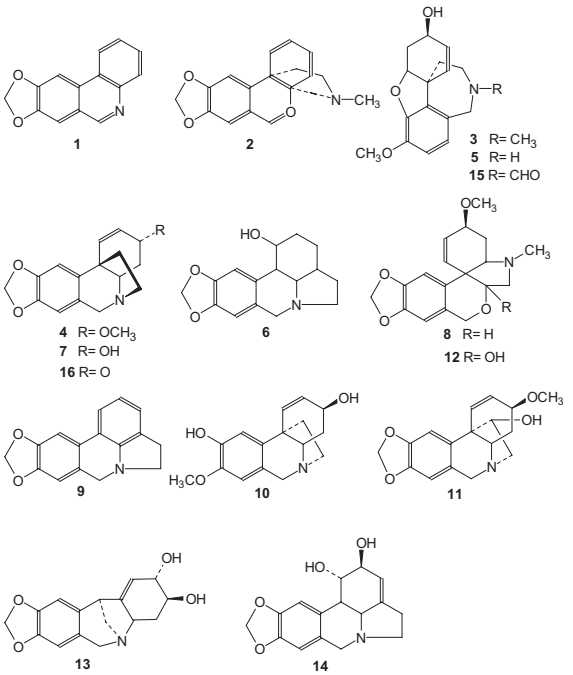

Fig. 1. Structures of alkaloids identified in *P. maritimum.* Numbers are identical with the numbers of Table I.

and crinane-3-one (**16**) are reported for the first time for *P. maritimum.* Alkaloids **2**, **6**, **8** and **16** were present in trace amounts in the alkaloid mixtures and it seems that the only method for their identification is GC/MS. The GC/MS spectra of eleven other compounds (P-1–P-11) with mass spectral fragmentation characteristic for the Amaryllidaceae alkaloids are listed in Table I. We did not identify them because of the absence of similar spectra in the available literature or database. Alkaloids P-1 and P-2 show mass spectral fragmentation typical for lycorenine type alkaloids – no molecular ion peak and very low intensities of all fragments besides the base peak at *m/z* 109. For this type of alkaloids, M^+ ion can not be determined unambiguously by electron impact mass detector (Kreh *et al.*, 1995). Alkaloid P-6 possesses fragments at *m/z* 185, 199, 214 and 270 as well as intensive M^+ ion characteristic for pancracine derivatives (Wildman and Brown, 1968). Alkaloid P-10 shows very similar fragmentation to those of 6α-deoxytazettine (**8**), only with differences in the relative intensities of some ion fragments and they must be isomers. Alkaloid P-11 shows M^+, M^+-15 and base peaks with two mass units lower than those of tazettine (**12**) as

Table I. Alkaloids of *Pancratium maritimum* L.

Compound	[M$^+$]	*m/z* (rel. int.)	Roots	Bulbs	Leaves	MS ref.
Trispheridine (**1**)	223(100)	222(39), 167(10), 165(10), 164(16), 138(22)	2.1	3.05	3.14	Ali *et al*., 1986
Graciline (**2**)	283(4)	282(4), 264(5), 254(6), 240(5), 227(2), 226(20), 225(100), 139(7)	0.43	0.22	–	Noyan *et al*., 1998
Galanthamine (**3**)	287(82)	286(100), 244(24), 230(12) 216(35), 174(31)	1.56	1.37	2.65	Kreh *et al*., 1995
Buphanisine (**4**)	285(100)	270(32), 254(33), 230(18), 215(82), 201(22), 187(10), 185(18), 172(18), 157(20), 115(31)	1.01	1.32	3.06	Viladomat *et al*., 1995
N-Demethylgalanthamine (**5**)	273(98)	272(100), 230(33) 202(27) 174(12)	–	2.11	1.34	Kreh *et al*., 1995
α-Dihydrocaranine (**6**)	273(35)	272(100), 254(6), 242(2), 226(2), 214(5), 200(3), 188(2), 174(3), 162(4),	0.8	–	–	NIST 98
Crinine (**7**)	271(100)	270(14), 254(10) 228(23) 214(12), 199(65) 187(57), 173(18) 115(22)	6.27	8.91	14.16	Viladomat *et al*., 1995
6α-Deoxytazettine (**8**)	315(27)	300(49), 231(100), 217(7), 211(4), 197(8), 185(12), 159(6), 152(6), 141(5), 128(8), 115(11), 70(51)	0.91	–	–	NIST 98
Galanthane (**9**)	251(45)	250(100), 220(4), 204(2), 192(14), 191(12), 165(6), 152(2), 139(4), 96(7), 95(9)	15.2	–	4.81	NIST 98
Demethylmarithidine (**10**)	273(100)	230(25), 201(86), 189(54), 175(22) 157(16), 128(19), 115(20)	–	2.04	–	Bastida *et al*., 1988
Haemanthamine (**11**)	301(15)	272(100), 240(18), 211(16), 181(23)	4.93	19.53	38.2	Kreh *et al*., 1995
Tazettine (**12**)	331(30)	316(14), 298(22), 260(5), 247(100), 227(13), 211(12), 201(14), 181(12), 152(10), 141(9), 128(10), 115(16)	7.02	6.38	1.65	Duffield *et al*., 1965
Pancracine (**13**)	287(100)	270(20), 243(18), 223(20), 214(14), 199(18), 185(29), 141(8), 128(8), 115(10)	0.89	2.43	–	Wildman and Brown, 1968
Lycorine (**14**)	287(27)	286(25), 268(20), 250(10), 227(61), 226(100), 212(5), 147(8), 135(4), 119(8)	1.88	3.36	0.51	Likhitwitayawuid *et al*., 1993
N-Formylgalanthamine (**15**)	301(100)	272(2), 243(6), 230(8), 225(15), 211(16) 128(11), 115(10)	2.9	4.72	2.02	Bastida *et al*., 1987
Crinane-3-one (**16**)	271(100)	270(41), 240(14), 238(14), 226(8), 211(22), 181(65), 153(15), 152(15), 115(9)	–	–	0.36	NIST 98
P-1	–	250(4), 238(3), 209(4), 190(1), 152(3), 135(3), 110(8), 109(100), 94(5), 82(5)	0.24	–	–	–
P-2	–	207(2), 199(1), 164(4), 152(3), 135(3) 110(8), 109(100), 108(25), 94(6), 82(6)	0.47	2.90	–	–
P-3	253(49)	252(100), 224(38), 181(7), 166(16), 152(11), 128(3), 115(6)	0.23	0.32	0.59	–
P-4	265(12)	227(100), 199(34), 128(5), 115(9)	–	0.38	–	–

Table I. (cont.)

Compound	[M^+]	*m/z* (rel. int.)	Roots	Bulbs	Leaves	MS ref.
P-5	301(49)	286(15), 272(14), 245(100), 229(51), 128(7), 115(20)	tr.	–	–	–
P-6	299(100)	284(22), 270(33), 244(35), 227(83), 214(7), 199(33), 185(20), 141(19), 128(15), 115(26),	tr.	–	–	–
P-7	271(100)	254(19), 238(16), 211(16), 181(16), 165(15), 128(11), 115(17)	–	0.42	–	–
P-8	273(60)	272(65), 257(43), 224(100), 212(10), 199(44), 166(10), 141(19), 128(1), 115(15)	–	2.12	1.34	–
P-9	277(100)	211(21), 181(70), 153(31), 152(31), 128(5), 115(11)	0.72	–	4.81	–
P-10	315(19)	300(16), 231(100), 217(3), 211(15), 197(11), 185(5), 159(4), 152(8), 141(8), 128(11), 115(11), 70(9)	1.43	2.04	–	–
P-11	329(21)	314(25), 295(25), 245(100), 227(14), 211(18), 181(9), 152(10), 141(9), 128(5), 115(10)	tr.	–	–	–

* The ion current generated depends on the characteristics of the compound and is not a true quantification.

well as fragments at *m/z* 227, 211, 181, 152, 141 which are present in the mass spectrum of **12**. Evidently, P-11 is a dehydroderivative of tazettine. Alkaloids P-3 and P-8 have intensive M^+-H peaks characteristic for lycorine, phenantridine and galanthamine type alkaloids. The M^+ ions in the spectra of alkaloids P-6, P-7 and P-9 form the most prominent peaks which are characteristic for many alkaloids of crinane type.

Crinane type alkaloids haemanthamine and crinine appeared to be the main alkaloids in the Bulgarian *P. maritimum*. Major alkaloid in roots was galanthane and in bulbs and leaves haemanthamine. Tazettine was also present in relatively high levels in the alkaloid fractions from roots and bulbs. Crinane-3-one might be produced by crinine oxidation in the leaves.

Several alkaloids with pharmacological activity were found. The most interesting was the intensively studied acetylcholinesterase inhibitor galanthamine. This compound was found at higher concentrations in the leaves whereas the galanthamine precursor *N*-demethylgalanthamine (Bastida and Viladomat, 2002) was accumulated mainly in the bulbs. The further transformation of galanthamine to *N*-formylgalanthamine probably proceeds in the bulbs. Other compound of interest is lycorine. Previous study of Tato *et al*. (1988) on Spanish *P. maritimum* showed that lycorine is the main component of the alkaloid fraction from bulbs. Contrary to them, we found that this compound is accumulated as a minor component in the plant tissues. The major alkaloid of bulbs and leaves, haemanthamine, exhibits cytotoxic and hypertensive properties (Bastida and Viladomat, 2002).

Taking into account the complexity of the alkaloid fractions, GC/MS is the method of choice for a rapid analysis of *Pancratium* alkaloids. It requires minimum of plant material and allows the identification of numerous compounds, some of them of pharmacological interest.

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