GC-MS of *Crinum latifolium* L. Alkaloids

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A GC-MS analysis of underivatized alkaloids from leaves of *Crinum latifolium* was performed. From the identified 15 alkaloids, 9 were found for the first time in this plant. Almost all alkaloids belonged to the crinane type. Substantial changes in the methylation and oxidation pattern of the alkaloids at and after flowering were observed.

Introduction

The plants of the genus *Crinum* (Amaryllidaceae) are used in Asian folk and traditional medicine as rubefacient, tonic and for treatment of allergic disorders and tumor diseases (Ghosal *et al.,* 1985). These activities are attributed to the presence of Amaryllidaceae alkaloids known to possess moderate antitumor and immunostimulating activities (Ghosal, Saini, & Razdan 1985). Aqueous extracts of *Crinum latifolium* L. leaves are used in Vietnamese folk medicine as an anticancer remedy (Tram *et al.,* 1999). Recently, aqueous extracts from *C. latifolium* leaves from Vietnam showed *in vitro* and *in vivo* T-lymphocyte activation (Tram, *et al.,* 1999) and retarded growth of chemically induced tumors (sarcomas) in rats (Tram *et al.*, 2000).

Till now, the chemical investigations on *C. latifolium* alkaloids were concentrated mainly on bulbs (Ghosal and Singh, 1986; Ghosal *et al.*, 1984; Ghosal *et al.,* 1983). The present paper deals with the GC-MS analysis of the alkaloid fraction from *C. latifolium* leaves (water extract). The leaves were investigated at and after flowering, because significant changes in the alkaloid content of *C. latifolium* during different stages of plant growth have been observed (Ghosal *et al.,* 1985).

Experimental

Plant material

Leaves from *Crinum latifolium* L. at and after flowering were collected in April and July respectively, at Dist. Go Vap, Ho Chi Min City, Vietnam. A voucher specimen is kept in the Nguyen Thi Ngoc Tkam Herbarium. The identity of the plant was confirmed by Prof. Dr. Sc. Tkan Cong Khanh, Department of Botany – Hanoi College of Pharmacy, Center for Research and Development of Ethnomedicinal Plants (CREDEP).

Isolation of the alkaloid fractions

250 ml boiling water was added to ground *C. latifolium* leaves (30 g), after 30 min the extract was filtered and acidified to pH 4 with acetic acid. The acidic solution was extracted successively with light petroleum and chloroform. The acidic aqueous phase was made alkaline (pH 9) with 25% aqueous ammonia. It was extracted with chloroform (3×). The chloroform extract (0.02 g, the same at and after flowering) was subjected to GC-MS investigation.

GC-MS analysis

Total alkaloids were investigated by GC/MS on a Hewlett Packard gas chromatograph 5890 Series II Plus linked to Hewlett Packard 5972 mass spec-

trometer system equipped with a 30 m long, 0.25 mm id, 0.25 μm film thickness HP1-MS capillary column. The temperature was programmed from 150 °C to 270 °C at a rate of 5 °C.min^{-1} with a 10 min hold. Helium was used as a carrier gas with a constant flow at 0.9 ml.min^{-1}. The ionization voltage was 70 eV.

Identification of compounds

The alkaloid identification was performed by comparisons of RT and mass spectra with authentic samples. When such samples were not available tentative structures were proposed on the basis of the mass spectral fragmentation.

Results and Discussion

We subjected to GC-MS analysis the underivatized alkaloid mixture, encouraged by the excellent results of Kreh *et al.* (1995). These authors applied for the first time GC-MS to underivatized Amaryllidaceae alkaloids (from *Narcissus pseudonarcissus*) and demonstrated its advantages over the analysis of silylated samples, especially in identifying minor components. Using this method, we identified 16 alkaloids (one of them tentatively) (Table I). Some components remained unidentified due to the lack of reference substances and library spectra.

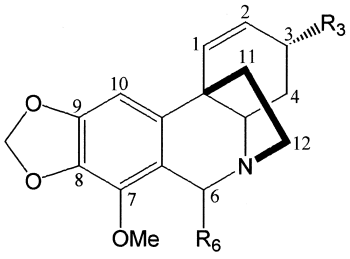
Until now, 7 alkaloids have been isolated and identified in *C. latifolium* leaves (Kobayashi *et al.,* 1984; Kobayashi *et al.*, 1984; Jeffs. *et al.*, 1985; Vo, 1997). In our samples, we found only 3 of them: 6-hydroxycrinamidine (**16**), 6-hydroxypoweline (**11**), undulatine (**8**). From the remaining 12 alkaloids, 9 are found for the first time in *C. latifolium* (Table I, Fig. 1). Ambelline (**9**), 1,2-β-epoxyambelline (**14**) and powelline (**7**) have been found in other plant parts of *C. latifolium*.

Contrary to other reports on *C. latifolium* leaves (Kobayashi *et al.,* 1984; Kobayashi *et al.,* 1984; Jeffs. *et al.*, 1985; Vo, 1997), in leaves of Vietnamese *C. latifolium* we identified almost exclusively alkaloids of the crinane type. The main alkaloids appeared to be undulatine (**8**) and crinamidine (**12**), which contain 1β,2β-epoxy ring. Other important components of the alkaloid mixtures were 6-hydroxybuphanidrine (**10**), ambelline (**9**) and 6-hydroxyundulatine (**13**). According to the structures of the identified alkaloids most of

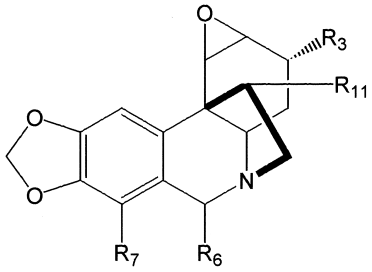
Table I. GC-MS data of the alkaloid mixture from *C. latifolium* leaves.

Alkaloid	M^+ and characteristic ions (%)	RT [min.s]	At flowering (%)[a]	After flowering (%)[a]
9–0ctadecenamide[b](**1**)	281(10), 126(21), 112(18), 98(90), 72(95), 59(100)	15.39	0.5	0.2
Dihydro-oxo-demethoxy haemanthamine[b](**2**)	271(22), 243(100), 214(75), 186(60), 115(25)	16.03	0.7	3.5
Augustamine[b](**3**)	301(66), 300(100), 244(72), 215(30), 201(27)	16.43	1.8	0.2
Oxoassoanine[b](**4**)	281(100), 266(12), 250(8), 238(20)	16.66	0.3	0.7
Crinane-3α-ol[b](**5**)	273(100), 256(27), 229(42), 201(50), 185(32), 115(25)	16.98	–	1.1
Buphanidrine[b](**6**)	315(100), 300(31), 284(34), 260(45), 245(63), 231(37), 130(46)	18.78	2.2	–
Powelline (**7**)	301(100), 258(20), 246(20), 220(61), 217(40)	19.31	<0.1	0.3
Undulatine (**8**)	331(100), 258(41), 205(62), 189(43), 173(39)	20.68	19.9	<0.1
Ambelline (**9**)	331(98), 299(38), 287(100), 260(97), 255(70), 211(75)	20.92	6.2	3.0
6-Hydroxybuphanidrine[b](**10**)	331(55), 276(100), 261(28), 229(78), 91(35)	21.08	8.5	–
6-Hydroxypowelline (**11**)	317(100), 299(34), 262(28), 244(90), 233(60)	21.65	–	1.5
Crinamidine[b](**12**)	317(60), 288(100), 244(29), 217(42), 205(38), 203(37)	21.75	14.1	30.0
6-Hydroxyundulatine[b](**13**)	347(30), 276(32), 256(29), 229(31), 219(100), 204(20)	22.76	4.8	0.7
1*β*,2*β*-Epoxyambelline(**14**)	347(35), 318(100), 274(32), 231(30), 205(52)	23.02	1.6	1.8
Epoxy-3,7-dimethoxycrinane-11-one[c](**15**)	345(100), 316(46), 286(63), 270(71), 231(90)	23.59	–	0.8
6-Hydroxycrinamidine (**16**)	333(80), 304 (45), 286(100), 274(98), 256(58), 231(87)	23.84	–	2.8

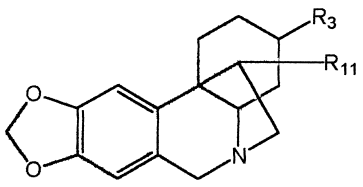
[a] % of the total ion current. The area of the GC/MS peaks depends not only on the concentration of the corresponding compounds, but also on the intensity of their mass spectral fragmentation, so the data given in the table is not a true quantitation but can be used for comparisons between the two samples, which is the objective of this work.
[b] New for *C. latifolium.*
[c] Tentative structure.



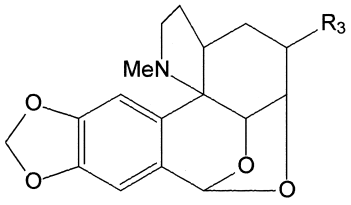
	R_3	R_6	R_{11}
6	OMe	H	H
7	OH	H	H
9	OMe	H	OH
10	OMe	α-OH	H
11	OH	OH	H



	R_3	R_6	R_7	R_{11}
8	OMe	H	OMe	H
12	OH	H	OMe	H
13	OMe	OH	OMe	H
14	OMe	H	OMe	OH
15	OMe	H	OMe	O
16	OH	OH	OMe	H



	R_3	R_{11}
2	H	O
5	α-OH	H



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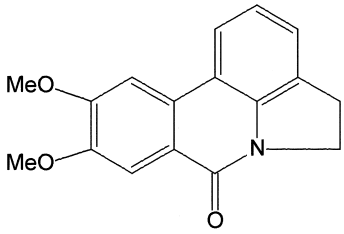

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Fig. 1. Alkaloids in *C. latifolium* leaves: dihydro-oxo-demethoxyhaemanthamine (**2**), augustamine (**3**), oxoassoanine (**4**), crinane-3α-ol (**5**), buphanidrine (**6**), powelline (**7**), undulatine (**8**), ambelline (**9**), 6-hydroxy-buphanidrine (**10**), 6-hydroxypowelline (**11**), crinamidine (**12**), 6-hydroxyundulatine (**13**), 1β,2β-epoxyambelline (**14**), epoxy-3,7-dimethoxycrinane-11-one (tentative) (**15**), 6-hydroxycrinamidine (**16**).

them are biogenetically related and can be produced by an oxidation or O-methylation of crinine. Two of these alkaloids are know to possess biological activities. Epoxyambelline (**14**) moderately activated mouse spleen lymphocytes; a mixture of epoxyambelline (**14**) and ambelline (9) (1:1) produced pronounced activation of the spleen lymphocytes (Ghosa *et al.,* 1984).

Significant differences in the alkaloid composition at flowering and after flowering were observed (Table I). Some components considerably prevail at flowering (undulatine (**8**), 6-hydroxybuphanidrine (**10**), 6-hydroxyundulatine (**13**), ambelline (9), buphanidrine (**6**), and others after flowering (6-hydroxycrinamidine (**16**), crinamidine (**12**), 6-hydroxypowelline(**11**).

According to Table I it is evident that there are substantial changes in the methylation and oxidation of alkaloids at different ontogenetic stages. The 3-O-methylation of powelline (**7**) to buphanidrine (**6**), of 6-hydroxypowelline (**11**) to 6-hydroxybuphanidrine (**10**), of crinamidine (**12**) to undulatine (**8**) and of 6-hydroxycrinamidine (**16**) to 6-hydroxundulatine (**13**) prevails at flowering. Oxidized products of buphanidrine (**6**): undulatine (**8**) (1,2-epoxidation), ambelline (**9**) (11-hydroxylation), 6-hydroxybuphanidrine (**10**) (6-hydroxylation) and 6-hydroxyundulatine (**13**) (1,2-epoxidation and 6-hydroxylation) are present in significantly higher concentrations at flowering. On the other hand, the oxidized products of powelline (**7**) (6-hydroxypowelline (**11**), crinamidine (**12**), 6-hydroxycrinamidine (**16**) dominate after flowering.

The results obtained here differ from previous results on *C. latifolium* alkaloids (Ghosal *et al.,* 1985). This could be caused by presence of plant varieties or hybridization, or to specificities of the

collection site. The ontogenetic stage at the moment of collection of plant material is also of importance. Taking into account the low concentration of alkaloids in leaves (lower than 0.1%) and the complexity of the alkaloid mixture, GC-MS of underivatized samples is the method of choice for rapid analysis of *Crinum* alkaloids. It requires minimum amount of plant material and allows the identification of numerous compounds.

Ghosal Sh., Saini K. S. and Frahm A. W. (1983), Alkaloids of *Crinum latifolium. Phytochemistry* 22, 2305–2309.

Ghosal Sh., Saini K. S and Arora V. K. (1984), 1,2-β-Epoxyambelline, an immuno-stimulant alkaloid from *Crinum latifolium.* J. Chem. Research *(S),* 232–233.

Ghosal Sh., Saini K. S. and Razdan S. (1985), *Crinum* alkaloids: their chemistry and biology. Phytochemistry **24**, 2141–2156.

Ghosal Sh. and Singh S. K. (1986), Chemical constituents of Amaryllidaceae. Part 24. Crinafoline and crinafolidine, two anti-tumor alkaloids from *Crinum latifolium.* J. Chem. Research *(S),* 312–313.

Jeffs. P. W., Abou-Donia A., Campau D and Staiger D. (1985), Structures of 9-O-demethylhomolycorine and 5α-hydroxyhomolycorine. Alkaloids of *Crinum defixum, C. scabrum* and *C. latifolium.* Assingment of aromatic substitution patterns from ^{1}H-coupled ^{13}C spectra. J. Org. Chem. **50**, 1732–1737.

Kobayashi Sh., Tokumoto T., Kihara M., Imakura Y., Shingu T. and Taira. Z. (1984), Alkaloidal constituents of *Crinum latifolium and Crinum bulbispermum* (Amaryllidaceae). Chem. Pharm. Bul. **32**, 3015–3022.

Kobayashi Sh., Tokumoto T. and Taira Z. (1984), Latifine, a biogenetic isomer of cherylline, from *Crinum latifolium* L. J. Chem. Soc., Chem Commun.,1043–1044.

Kreh M., Matusch R. and Witte L. (1995), Capillary gas chromatography-mass spectrometry of Amaryllidaceae alkaloids. Phytochemistry **38**, 773–776.

Tram Ng. Th. Ng., Zvetkova E., Nikolova E., Katzarova E., Kostov G., Yanchev I. and Baicheva, O. (1999), A novel in vitro and in vivo T-lymphocyte activating factor in *Crinum latifolium* (L.) aqueous extracts. Exp. Pathol. Parasitol. **3**, 21–26.

Tram Ng. Th. Ng., Yanchev I., Zvetkova E., Katzarova E., Kostov G., Svilenov D. and Shalamanov P. (2000), Retarded growth of chemically induced with 20 methylcholanthrene in rats under the action of cold-hot aqueous extracts (decoctions) from Vietnamese plant *Crinum latifolium* (L.). Exp. Pathol. Parasitol., in press.

Vo, T. B. H., Nguyen, K.,Q.,C. and Ngo, V. Th (1997), Hydroxycrinamidine, a new alkaloid from leaves of *Crinum latifolium*(L.). Tap Chi Duoc Hoc **11**, 9–10 (Vietnamese), from CA128: 292716u.