GC-MS of *Crinum latifolium* L. Alkaloids

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A GC-MS analysis of undervatized 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-
GC-MS of Crinum latifolium L. Alkaloids

The GC-MS analysis was performed using a capillary column 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 comparison 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 the underivatized alkaloid mixture to GC-MS analysis, encouraged by the excellent results of Kreh et al. (1995). These authors applied 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 Crinum 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 Crinum latifolium (Table I, Fig. 1). Ambelline (9), 1,2-β-epoxyambelline (14) and powelline (7) have been found in other plant parts of Crinum 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 cinamidine (12), which contain 1β,2β-epoxy ring. Other important components of the alkaloid mixtures were 6-hydroxybuphanidine (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.

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th>M⁺ and characteristic ions (%)</th>
<th>RT [min.s]</th>
<th>At flowering (%)</th>
<th>After flowering (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9–octadecenamide (1)</td>
<td>281(10), 126(21), 112(18), 98(90), 72(95), 59(100)</td>
<td>15.39</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Dihydro-oxo-demethoxyhaemantidine (2)</td>
<td>271(22), 243(100), 214(75), 186(60), 115(25)</td>
<td>16.03</td>
<td>0.7</td>
<td>3.5</td>
</tr>
<tr>
<td>Augustamine (3)</td>
<td>301(66), 300(100), 244(72), 215(30), 201(27)</td>
<td>16.43</td>
<td>1.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Oxosassoanine (4)</td>
<td>281(100), 266(12), 250(8), 238(20)</td>
<td>16.66</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Crinane-3c-ol(5)</td>
<td>273(100), 256(27), 229(42), 201(50), 185(32), 115(25)</td>
<td>16.98</td>
<td>–</td>
<td>1.1</td>
</tr>
<tr>
<td>Buphanidine (6)</td>
<td>315(100), 300(31), 284(34), 260(45), 245(63), 231(37), 130(46)</td>
<td>18.78</td>
<td>2.2</td>
<td>–</td>
</tr>
<tr>
<td>Powelline (7)</td>
<td>301(100), 258(20), 246(20), 220(61), 217(40)</td>
<td>19.31</td>
<td>&lt;0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Undulatine (8)</td>
<td>331(100), 258(41), 205(62), 189(43), 173(39)</td>
<td>20.68</td>
<td>19.9</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Ambelline (9)</td>
<td>331(98), 299(38), 287(100), 260(97), 257(90), 211(75)</td>
<td>20.92</td>
<td>6.2</td>
<td>3.0</td>
</tr>
<tr>
<td>6-Hydroxybuphanidine (10)</td>
<td>331(55), 276(100), 261(20), 229(78), 91(35)</td>
<td>21.08</td>
<td>8.5</td>
<td>–</td>
</tr>
<tr>
<td>6-Hydroxypowelline (11)</td>
<td>317(100), 299(34), 262(28), 244(90), 233(60)</td>
<td>21.65</td>
<td>–</td>
<td>1.5</td>
</tr>
<tr>
<td>Cinamidine (12)</td>
<td>317(60), 288(100), 244(29), 217(42), 205(38), 203(37)</td>
<td>21.75</td>
<td>14.1</td>
<td>30.0</td>
</tr>
<tr>
<td>6-Hydroxyundulatine (13)</td>
<td>347(30), 276(32), 256(29), 229(31), 219(100), 204(20)</td>
<td>22.76</td>
<td>4.8</td>
<td>0.7</td>
</tr>
<tr>
<td>1β,2β-Epoxyambelline (14)</td>
<td>347(35), 318(100), 274(32), 231(30), 205(52)</td>
<td>23.02</td>
<td>1.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Epoxy-3,7-dimethoxycrinane-11-one (15)</td>
<td>345(100), 316(46), 286(63), 270(71), 231(90)</td>
<td>23.59</td>
<td>–</td>
<td>0.8</td>
</tr>
<tr>
<td>6-Hydroxycrinamidine (16)</td>
<td>333(80), 304 (45), 286(100), 274(98), 256(58), 231(87)</td>
<td>23.84</td>
<td>–</td>
<td>2.8</td>
</tr>
</tbody>
</table>

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.
them are biogenetically related and can be produced by an oxidation or O-methylation of crinine. Two of these alkaloids are known to possess biological activities. Epoxymelline (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-hydroxybuphanidine (10), 6-hydroxyundulatine (13), ambelline (9), buphanidine (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 buphanidine (6), of 6-hydroxypowelline (11) to 6-hydroxybuphanidine (10), of crinamidine (12) to undulatine (8) and of 6-hydroxycrinamidine (16) to 6-hydroxyundulatine (13) prevails at flowering. Oxidized products of buphanidine (6): undulatine (8) (1,2-epoxidation), ambelline (9) (11-hydroxylation), 6-hydroxybuphanidine (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.


