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CAPILLARY GAS CHROMATOGRAPHY-MASS SPECTROMETRY OF AMARYLLIDACEAE ALKALOIDS

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Key Word Index—*Narcissus/pseudonarcissus*; Amaryllidaceae; daffodil; GC-MS; alkaloids; haemanthamine.

Abstract—An alkaloid extract of *Narcissus/pseudonarcissus* was examined by means of gas chromatography-mass spectrometry (GC-EIMS and GC-CIMS). GC-MS of underivatized Amaryllidaceae alkaloids was performed for the first time. Most of the alkaloids seem to be suitable for GC, although haemanthamine and lycorenine decompose partly under the GC conditions used.

INTRODUCTION

Capillary gas chromatography (GC), often coupled with a mass spectrometer as a detector (GC-MS), is a well-established technique for analysing complex mixtures of alkaloids with M_rs up to *ca* 450. There are a large number of publications dealing with GC or GC-MS of underivatized alkaloids. Analyses of pyrrolizidine [1], quinolizidine [2], tropane [3, 4], tobacco [5, 6], diterpenoid [7], *Lycopodium* [8], quinazoline [9], indole [10] and steroidal alkaloids [11] have been described. There are only two publications dealing with GC-MS of Amaryllidaceae alkaloids (AmAs) derivatized as trimethylsilyl ethers [12, 13]; there are no reports on free alkaloids of this type.

We cannot think of any reason why AmAs should not be suitable for GC and GC-MS analysis even as underivatized compounds. Some exceptions may apply for quaternary alkaloids and alkaloid *N*-oxides that have been reported as constituents of Amaryllidaceae [14, 15]. Compounds bearing such structural elements are involatile and, thus, only decomposition products can be found by GC analysis. However, alkaloids of these types are very rare, so GC seems to be a suitable separation method for nearly all of the reported AmAs.

In our research group, several Narcissi and other Amaryllidaceae are currently under investigation. Therefore, we decided to apply GC-MS analysis to an alkaloid extract of the bulbs of *Narcissus pseudonarcissus* subsp. *pseudonarcissus* cv. Carlton, in addition to conventional separation of the compounds by preparative HPLC and their identification by NMR, mass spectrometry, UV and IR.

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RESULTS AND DISCUSSION

FAB-MS and CI-MS of unseparated alkaloid mixture

As the first step, we analysed the unseparated alkaloid extract by fast atom bombardment mass spectrometry (FAB-MS) and chemical ionization mass spectrometry (CIMS, ammonia as reactant gas), using a direct insertion probe. Analyses of the mixture gave quasimolecular ions (as $[M + H]^+$) of all alkaloids present. Both methods gave similar results with some deviations within the intensities. The M_rs ranged from 269 to 349. The results showed that the molecular masses of the alkaloids present do not exceed the limits of the GC-MS capabilities. AmAs with such M_rs should be eluted within the temperature limit of the capillary column used.

GC-MS of underivatized alkaloids

The GC-MS of the alkaloid extract on a DB-1 column showed *ca* 30 compounds, nearly all of which were well-separated. Some problems with overlapping peaks could be eliminated by the use of a DB-5 column as an alternative phase. However, in two cases (retention indices (RI) 2359/2361 and RI 2460 on DB-1 in Table 1) it was not possible to separate the compounds. Some of the alkaloids could be identified by comparison of their mass spectra with literature data [16–19]. Galanthamine, lycoramine, *N*-demethyl-galanthamine, *epi*-norlycoramine, *O*-methyloduline, vittatine, narwedine, 1-*O*-acetyl-10-*O*-demethylpluviine, 10-*O*-demethylpluviine, oduline, lycorenine, haemanthamine (**1**), masonine, homolycorine and *N*-demethyl-masonine were isolated by liquid chromatographic methods, and their structures were elucidated by NMR, mass spectrometry, UV and IR. These alkaloids were used as further reference material (Table 1); their

Table 1. GC-MS data of the alkaloid mixture from *Narcissus* under the described conditions

Alkaloid	$[M]^+$ and characteristic ions (%)	%GC-MS †	RI DB-1 ‡	RI DB-5 ‡	RI_{TMSI} DB-1 §	CI
Galanthamine*	287 (97), 286 (100), 244 (30), 216 (45), 174 (43)	58	2315	2380	2407	+
Lycoramine*	289 (63), 288 (100), 232 (11), 202 (17), 115 (26)	4	2335	2398	2400	+
N-Demethyl-galanthamine*	273 (100), 272 (92), 230 (38), 202 (30), 174 (13)	< 0.1	2344	2418	2445	+
	287 (86), 286 (100), 218 (17), 202 (26), 187 (14)	2	2350	2420		+
epi-Norlycoramine*	275 (86), 274 (100), 202 (15), 188 (29), 175 (19)	< 0.1	2357	2431		+
Isomer of galanthamine	287 (86), 286 (100), 244 (17), 216 (66), 174 (20)	0.3	2359	2430		+
Isomer of lycoramine	289 (56), 288 (100), 202 (21), 177 (21), 161 (24)	< 0.1	2361	2430		+
O-Methyloduline*	315(< 1), 284 (1.6), 175 (9), 109 (100)	0.3	2370	2422	2370	+
Vittatine*	271 (100), 228 (25), 199 (79), 187 (63), 115 (20), 56 (35)	< 0.1	2378	2445	2397	+
Narwedine*	285 (95), 284 (100), 216 (30), 199 (29), 174 (52)	0.8	2383	2458	2383	+
Isomer of *O*-methyl-lycorenine	331 (−), 299 (1), 191 (6), 109 (100)	< 0.1	2399	2467	2423	+
1-*O*-Acetyl-10-*O*-Demethylpluviine*	315 (38), 314 (43), 254 (100), 252 (44), 228 (27)	0.1	2410			+
O-Methyllycorenine	331 (< 1), 300 (1.7), 191 (7), 109 (100)	3	2416	2480	2476	+
10-*O*-Demethylpluviine*	273 (78), 272 (100), 256 (16), 244 (27), 228 (42)	0.6	2440	2513		+
Oduline*	301 (< 1), 283 (3), 222 (0.9), 175 (1), 109 (100)	< 0.1	2460	2517	2450	+
O-Ethyllycorenine?	345 (−), 300 (2.3), 191 (7), 109 (100)	< 0.1	2460	2518		+
	253 (54), 252 (100), 237 (19), 209 (23)	< 0.1	2460			+
11-Oxo-haemanthamine?	299 (2), 271 (99), 238 (11), 211 (25), 181 (100), 153 (24)	0.2	2481	2558		+
Lycorenine*	317 (< 1), 299 (2.5), 191 (0.7), 179 (0.6), 109 (100)	0.4	2512	2587	2478	+
Haemanthamine* (1)	301 (78), 269 (42), 257 (53), 227 (100), 225 (85)	21	2530	2598	2500	+
Decomp. product of haemanthamine	301 (15), 272 (100), 240 (18), 211 (20), 181 (40)		2532	2600		+
	331 (−), 300 (3), 175 (100), 96 (23)	< 0.1	2560		2570	+
O-Demethyl-maritidine?	303 (8), 274 (100), 242 (13), 216 (15), 181 (18), 115 (11)	< 0.1	2570			+
Masonine*	299 (−), 190 (0.3), 162 (0.9), 152 (0.4), 109 (100)	0.5	2580	2657	2580	+
Homolycorine*	315 (< 1), 206 (0.4), 178 (1.2), 150 (0.7), 109 (100)	4.7	2646	2728	2646	+
N-Demethyl-masonine*	285 (−), 162 (1.5), 134 (0.9), 115 (0.8), 95 (100)	< 0.1	2660			+
	301 (−), 109 (100)	1.3	2695	2777		+
Haemanthamine derivate	349 (−), 271 (100), 240 (9), 211 (14), 181 (48)	0.1	2742	2814		
	315 (−), 162 (2), 125 (100), 96 (32)	0.4	2755	2847	2715	+
	331 (−), 125 (100), 96 (24)	< 0.1	2815		2820?	+

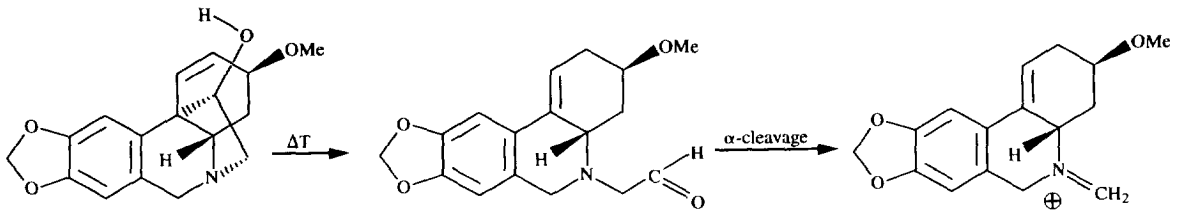
*Alkaloid obtained as reference compound from prep. HPLC.
†GC peak area of total peak area (%).
‡GC retention index for specified column.
§GC retention index for trimethylsilylated alkaloid.

isolation and the chemical data of *O*-methyloduline, 1-*O*-acetyl-10-*O*-demethylpluviine, 10-*O*-demethylpluviine and *N*-demethyl-masonine, so far unknown AmAs, will be described elsewhere (publication in preparation).

A problem arose during the identification of **1** (RI = 2531, *m/z* 301 $[M]^+$). This alkaloid was identified as haemanthamine (**1**) by NMR, mass spectrometry, UV and IR, but the mass spectrum of **1** obtained during GC-MS showed considerable differences to that obtained by direct insertion of the isolated alkaloid. Literature data of **1** [16, 17, 20] also show significant differences in the fragmentation pattern. Only during the first ascending part of the GC-peak were the mass spectra identical to that of **1** (*m/z* 301 $[M]^+$), then the fragmentation pattern drastically changed.

The explanation must be thermal decomposition of **1** under the GC conditions used. To check if the decomposition takes place within the injection port, we injected the sample without thermal stress using an on-column injector, but the decomposition still remained. Thus, **1** seemed to decompose during chromatography on the GC-column; the elution temperature was 260°. Although the decomposition process is unknown, we presume the process to be analogous to that described for the formation of the ion at *m/z* 272 from **1** [20] (Scheme 1).

For the alkaloid lycorenine, we also found hints that suggest a decomposition, a relatively broad GC peak and an additional ion in GC-CIMS with even stronger intensity at *m/z* 316 than the $[M + H]^+$ at *m/z* 318. However, it was not possible to get further information due to very



(1)

Scheme 1. Presumed decomposition reaction of 1.

low intensities of all fragments besides *m/z* 109 (Table 1), typical for EI mass spectra of lycorenine-type alkaloids. A $[M]^+$ peak always has very low intensities and is often absent. CI-MS in nearly all cases leads to a clearly discernible $[M + H]^+$, so we decided to perform GC-CIMS with the alkaloid mixture. As a result, we obtained mass spectra with $[M + H]^+$ peaks even for alkaloids of the lycorenine-type and we could identify even *O*-alkylated alkaloids of this type.

GC-MS of trimethylsilylated alkaloids

We found that derivatized alkaloids did not give a better separation by GC. The trimethylsilylated alkaloids did not give more intense $[M]^+$ peaks than the underivatized alkaloids (Table 1). In addition, we found that only half of the 30 alkaloids in the mixture could be identified after derivatization (lit. data of trimethylsilylated alkaloids are very sparse [12, 13]). Reliable identification was only possible for substances for which reference material exists. As a result, we recommend performing GC-MS with underivatized AmA mixtures.

Our investigation has shown that GC-MS of AmAs provides much structural information in a short time. The characteristic fragmentation pattern of the different structural types and the high sensitivity of GC allow a fast identification even of minor compounds of an AmA mixture without laborious isolation procedures. However, even under optimal GC conditions, some decomposition reactions may occur. A properly deactivated GC column, as well as mild GC conditions (use of on-column injection) are recommended for optimal results.

EXPERIMENTAL

Plant material. Bulbs of *N. pseudonarcissus* L. subsp. *pseudonarcissus* cv. Carlton from a culture in Sint Maarten (The Netherlands) were collected in August 1992. The plant was identified by Prof. Dr V. Melzheimer (Faculty of Biology, University of Marburg) and a voucher specimen (no. 0992 Kreh) has been deposited at the Faculty of Pharmacy, University of Marburg.

Extraction and isolation. Fr. bulbs (12 kg) were crushed and extracted ×3 by maceration with 10 l of MeOH (room temp.). After evapn of MeOH, the residue was dissolved in H_2O, acidified with 1% HCl (pH 1.5) and filtered. After removal of the neutral material with *n*-hexane (4 × 500 ml) and CH_2Cl_2 (4 × 500 ml), the aq. soln was made basic with a $NaHCO_3$–Na_2CO_3-buffer (pH 9) and extracted once more with CH_2Cl_2 (5 × 500 ml). The organic phase was washed with H_2O and evapd to dryness, affording 30.08 g of alkaloids (0.25% fr. wt).

Derivatization of alkaloids. A small part of the original alkaloid extract (20 μg) was dissolved in 50 μl of MSTFA and heated to 80° for 15 min. The reagent was removed under a stream of N_2 and the sample dissolved in CH_2Cl_2 for GC injection.

Direct insertion (FAB-MS and CI-MS). FAB-MS were measured on an instrument equipped with an Ion Tech saddle field gun using Xe as bombarding gas and glycerol as matrix. CI-MS (70 eV) were measured with the same instrument using NH_3 as reactant gas. Samples were slowly heated up to 250° during measurement.

GC-MS. The system consisted of a GC equipped with a 30 m × 0.32 mm fused silica capillary column coated with the stationary phases DB-1 and DB-5 (J&W); He was used as carrier gas. Conditions during split injection: injector 250°, split 1:20, temp. prog. 150–300° at 6° min^{-1}. Conditions during on-column-injection: injection at 70° oven temp., rapid heating to 180°, then 6° min^{-1} to 300°. The capillary column was directly coupled to a quadrupole mass spectrometer (45 eV). GC-CIMS: reactant gas NH_3 (100 eV).

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