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Note

Ammonium positive-ion and hydroxide negative-ion chemical ionization gas chromatography–mass spectrometry for the identification of pyrrolizidine alkaloids in *Eupatorium rotundifolium* L. var. *ovatum*

H. HENDRIKS*

Laboratory of Pharmacognosy, State University Groningen, Antonius Deusinglaan 2, 9713 AW Groningen (The Netherlands)

H.J. HUIZING

Foundation for Agricultural Plant Breeding, P O. Box 117, 9700 AW Wageningen (The Netherlands)

and

A.P. BRUINS

Department of Pharmacy, State University Groningen, Antonius Deusinglaan 2, 9713 AW Groningen (The Netherlands)

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Hepatotoxic and carcinogenic pyrrolizidine alkaloids (PAs) in phytopharmaceuticals, herbal teas, food and cattle forage are a cause of poisoning, disease and even cattle losses. On the other hand, the N-oxide of indicine has been used in trials for use as an anti-neoplastic agent [1]. Other PAs are used as markers in chemotaxonomical studies [2].

The combination of positive-ion and negative-ion chemical ionization (PICI and NICI) in gas chromatographic–mass spectrometric (GC–MS) analyses of trimethylsilyl derivatives of mono- and diester PAs (Fig. 1) offers a rapid tentative structure elucidation [3].

PICI with ammonium ions gives abundant MH^+ ions; cleavage of the ester bond at C-9 produces a fragment that further decomposes by elimination of the

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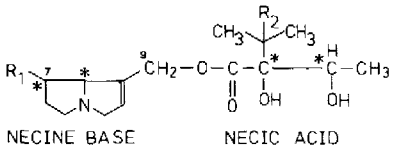

Fig. 1 General structure of mono- and diester PAs. $R_1 = R_2 = H$, supinine; $R_1 = OH$, $R_2 = H$, echinatine; $R_1 = C_4H_7COO$, $R_2 = OH$, echimidine.

functional group at C-7, i.e. loss of trimethylsilyl alcohol (TMSOH), acetic acid, angelic acid, tiglic acid, etc.

NICI with hydroxide ions cleaves ester bonds, thus generating carboxylate anions that provide a direct indication of the esterifying acids present in PAs. Identification of macrocyclic PAs is more difficult, because cleavage of one ester bond opens the macrocyclic ring. In this case the $(M+OH)^-$ ion can often be observed [4].

Within the scope of a study of antitumour compounds in higher plants, the native *Eupatorium cannabinum* L. has been submitted to an extensive examination into the presence of PAs. A great number of such-like compounds could be identified using combined PICI and NICI GC–MS. Some of those have not been described before [5]. In this communication the results of a screening for PAs in roots of *E. rotundifolium* L. var. *ovatum*, a North American species, are given.

EXPERIMENTAL

Plant material has been grown from seeds obtained from A.O. Tucker and C.E. Phillips (Herbarium Delaware, State College Dover, DE, U.S.A.). A voucher specimen is available in the collection of our laboratory.

Air-dried root material was used to prepare a methanolic alkaloid extract, according to ref. 6. A suitable amount of the methanolic extract was treated with 1 ml of dimethoxypropane (Janssen, Beerse, Belgium) and submitted to a nitrogen stream in order to remove traces of water. The residue was derivatized with Trisil-TBT (Pierce, Rockford, IL, U.S.A.), a mixture of trimethylsilylimidazole, bis(trimethylsilyl)acetamide and trimethylchlorosilane (3:3:2, v/v/v). Of this reaction mixture, 1 μl was used for further GC–MS analyses. Retention indices were calculated using a mixture of *n*-alkanes as reference.

The GC conditions were: column, 25 m × 0.32 mm I.D. coated with polydimethylsiloxane (Cp-Sil 5; Chrompack, Middelburg, The Netherlands); temperature programme, 150 to 325 °C at 6 °C/min.

The mass spectrometer was a modified Finnigan 3300 quadrupole mass spectrometer equipped with a standard chemical ionization source [7]. The source temperature was 250 °C and the pressure 0.40 mbar. The reactant gas for PICI was ammonia, and for NICI it was nitrous oxide–methane (1:1).

Data acquisition was started immediately after injection; 800 mass spectra were recorded during one GC analysis.

RESULTS AND DISCUSSION

The total ion current profiles ("gas chromatograms") obtained after PICI and NICI GC–MS of the alkaloid extract are presented in Fig. 2. After evaluation of the mass spectra, the peaks a–j were identified as trimethylsilyl (TMS) derivatives of PAs. These are listed in Table I, as are the retention indices of some reference compounds.

The presence of TMS derivatives of echinatine (a), β-angelyl/tiglyl trachelanthamine (c), β-isovaleryl echinatine (d) and β-angelyl/tiglyl echinatine (e), or one of their stereoisomers, could be shown very easily by comparing the mass

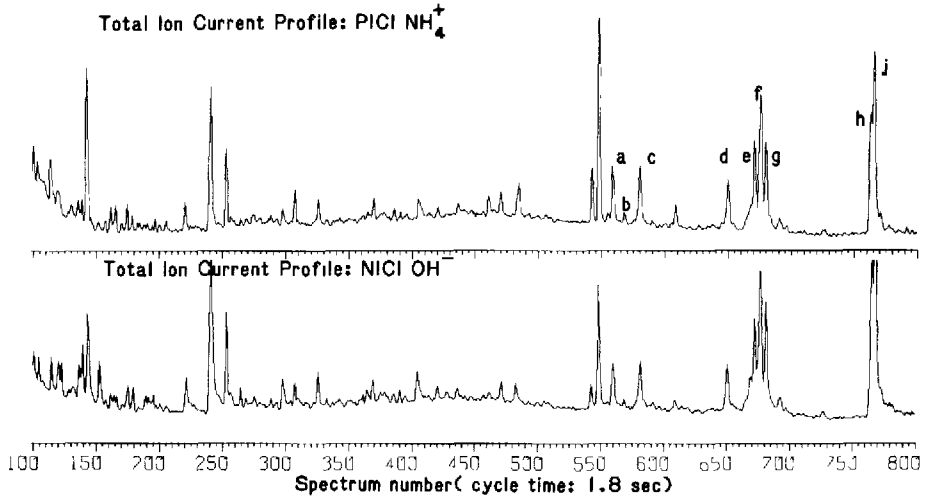

Fig. 2. Total ion current profile obtained after PICI and NICI GC–MS analyses of the alkaloid extract from *E. rotundifolium* L. var. *ovatum*.

TABLE I

RETENTION INDICES OF TMS DERIVATIVES OF SOME REFERENCE COMPOUNDS AND THE PAs FOUND IN *E. ROTUNDIFOLIUM* VAR. *OVATUM* FOR Cp-Sil 5

Derivative	MW	Index
References		
1. Supinine-$(TMS)_2$	427	2220
2 Echinatine-$(TMS)_3$	515	2415
3. Echimidine-$(TMS)_3$	613	2823
PAs in *E. rotundifolium* var. *ovatum*		
a. Echinatine-$(TMS)_3$	515	2415
b 7-Acetyl echinatine-$(TMS)_2$	485	2428
c. β-Angelyl/tiglyl trachelanthamine-(TMS)	439	2475
d. β-Isovaleryl echinatine-$(TMS)_2$	527	2610
e. β-Angelyl/tiglyl echinatine-$(TMS)_2$	525	2655
f. 7-Angelyl/tiglyl echinatine-$(TMS)_2$	525	2664
g. 7-Angelyl/tiglyl echinatine-$(TMS)_2+2H$	527	2673
h. An acetyl ester of echimidine-$(TMS)_2$	583	2870
j. An echimidine-$(TMS)_3$ isomer	613	2874

spectra with those of the same compounds present in *E. cannabinum* [5]. In this case the β-carbon atom of the viridifloric/trachelanthic acid moiety of the molecule is esterified with angelic/tiglic acid or isovaleric acid.

More striking is the presence of 7-acetyl echinatine (b), 7-angelyl/tiglyl echinatine (f) and a 7-angelyl/tiglyl ester of a dihydro derivative of echinatine (g) in the same extract. This has been deduced by the characteristic differences in the PICI and NICI mass spectra of the derivatized β-esters and C-7 esters, as has been extensively described for 7-acetyl lycopsamine-$(TMS)_2$ and its structural isomer β-acetyl echinatine-$(TMS)_2$ [3]. Except for 7-angelyl heliotridine, which occurs in *E. altissimum* L. [8], no other C-7 esters have been reported in *Eupatorium* species.

Most of the alkaloids present in the genus *Eupatorium* are esterified with trachelanthic or viridifloric acid at C-9. Compound j (MW 613), however, could be identified as a TMS derivative of an echimidine isomer, based on molecular weight information and on the fragmentation pattern in the PICI and NICI mass spectra (Fig. 3). This is elucidated by the fragmentation scheme (Fig. 4). Echimidine itself, present in an alkaloid extract of *Symphytum asperum* Lepechin [2], elutes somewhat earlier than compound j from the GC column (see Table I); the mass spectra were identical.

Compound h, which was not fully separated from compound j, represents an acetyl derivative of compound j (or another stereoisomer) in which a hydroxy

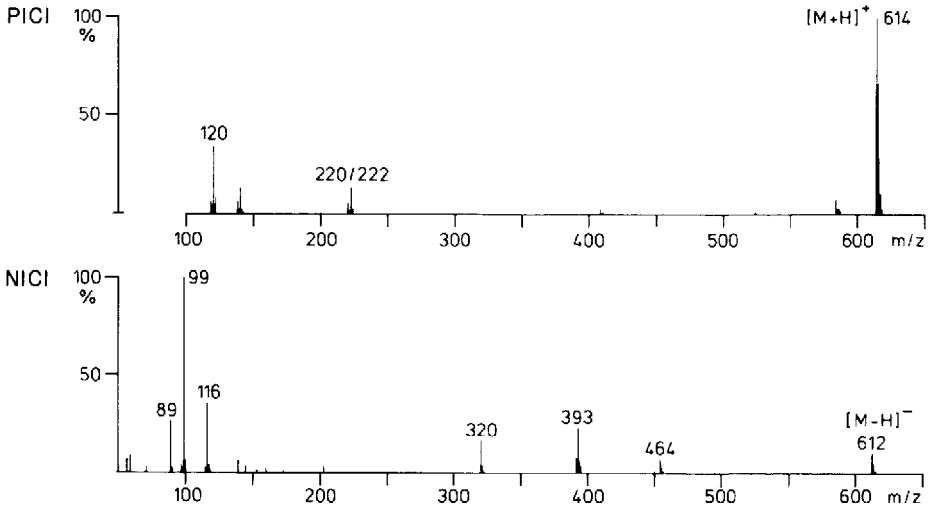

Fig. 3. PICI and NICI mass spectra of compound j (an echimidine stereoisomer).

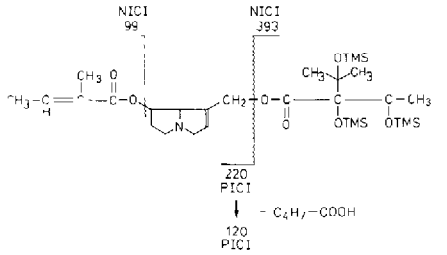

Fig 4 Fragmentation scheme of compound j.

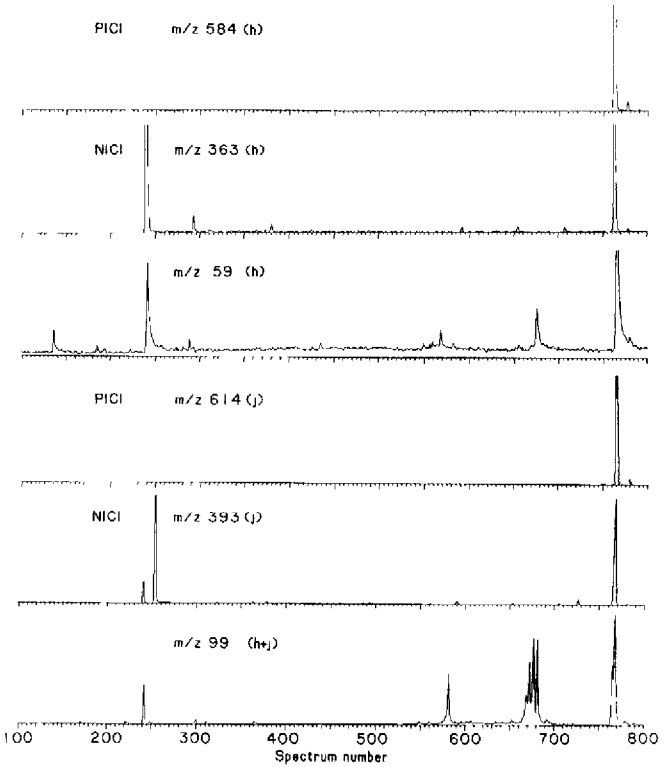

Fig 5. Some extracted ion current profiles of compounds h and j obtained after PICI and NICI GC-MS analyses of the alkaloid extract.

group of the echimidinic acid moiety is esterified with acetic acid. This can be concluded from the presence of *m/z* 363 and 59, representing the acetate anion. Fig. 5 gives the extracted ion current profiles (EICPs) of *m/z* 614 (PICI), 393 and 99 and 584 (PICI), 363 and 59. Such EICPs are very useful to unravel gas chromatograms with overlapping peaks. In this case it can be seen that the *m/z* values 614 and 393 and *m/z* 584 and 363 belong to compounds j and h, respectively, having *m/z* 99 in common.

This is the first time that echimidine-like compounds have been reported in the genus *Eupatorium*.

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