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CAPILLARY GAS CHROMATOGRAPHY/POSITIVE AND NEGATIVE ION CHEMICAL IONIZATION MASS SPECTROMETRY ON PYRROLIZIDINE ALKALOIDS OF *SENECIO INAEQUIDENS* USING AMMONIA AND HYDROXYL IONS AS THE REAGENT SPECIES

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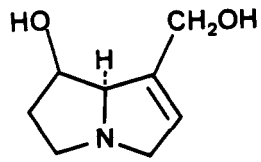
ABSTRACT.—The composition of the pyrrolizidine alkaloid (PA) fraction of *Senecio inaequidens* was studied by capillary gc and ms. Different ionization modes were used: electron impact (ei), positive ion chemical ionization (pici) with NH_3, and negative ion chemical ionization (nici) with NH_3 and hydroxyl ions as reagent species. Nineteen constituents were characterized and sixteen of them identified by these techniques. A new structure, desacetyl doronine [**3**] (mol wt 417), was elucidated. The application of NH_3 picims and OH^- nicims to otonecine PA derivatives and NH_3 nicims to retronecine and otonecine PA derivatives is reported and discussed for the first time.

Pyrrolizidine alkaloids (PAs) have known hepatotoxic properties (1,2). They are present in several plant families worldwide, e.g., the Boraginaceae, Compositae (Senecioneae and Eupatorieae tribes), and Leguminosae (genus *Crotalaria*) (3). *Senecio inaequidens* DC. (Compositae), native of South Africa, naturalized in Italy after World War II and is now so widely diffused in Italy as to be a potential danger, both as a food



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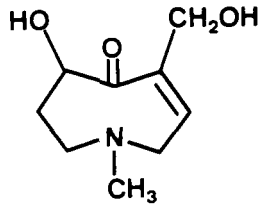

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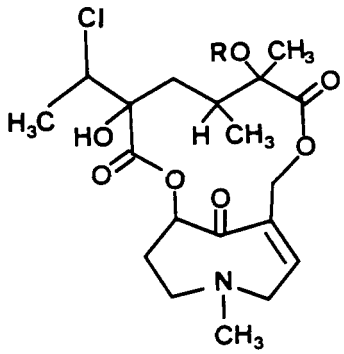

3 R=H
4 R=Ac

contaminant and directly for cattle. Senecivernine, senecionine, integerrimine, and retrorsine were previously identified by gc-ms on the PA fraction of *S. inaequidens* (4,5); an ongoing ontogenic study has isolated a very rich PA fraction at the beginning of the plant's vegetative period.

We investigated the use of capillary gc-ms with different ionization modes [electron impact (ei), positive and negative ion chemical ionization (pici/nici)], to identify PAs, and we report on the otonecine-derived PA in pici and nicims and on the behavior of PAs with different necinic bases under nicims.

RESULTS AND DISCUSSION

Figure 1 shows the capillary gc pattern of the *S. inaequidens* PA fraction: 19 components were characterized by ms techniques; 16 of them were completely identified, being two homologous and phytochemically consistent series of PAs deriving from retronecine [**1**] and otonecine [**2**]. The retronecine derivatives are senecivernine (mol wt 335), senecionine (mol wt 335), seneciphylline (mol wt 333), spartioidine (mol wt 333), integerrimine (mol wt 335), retrorsine (mol wt 351), and usaramine (mol wt 351). The otonecine series consists of senkirkine (mol wt 365), neosenkirkine (mol wt

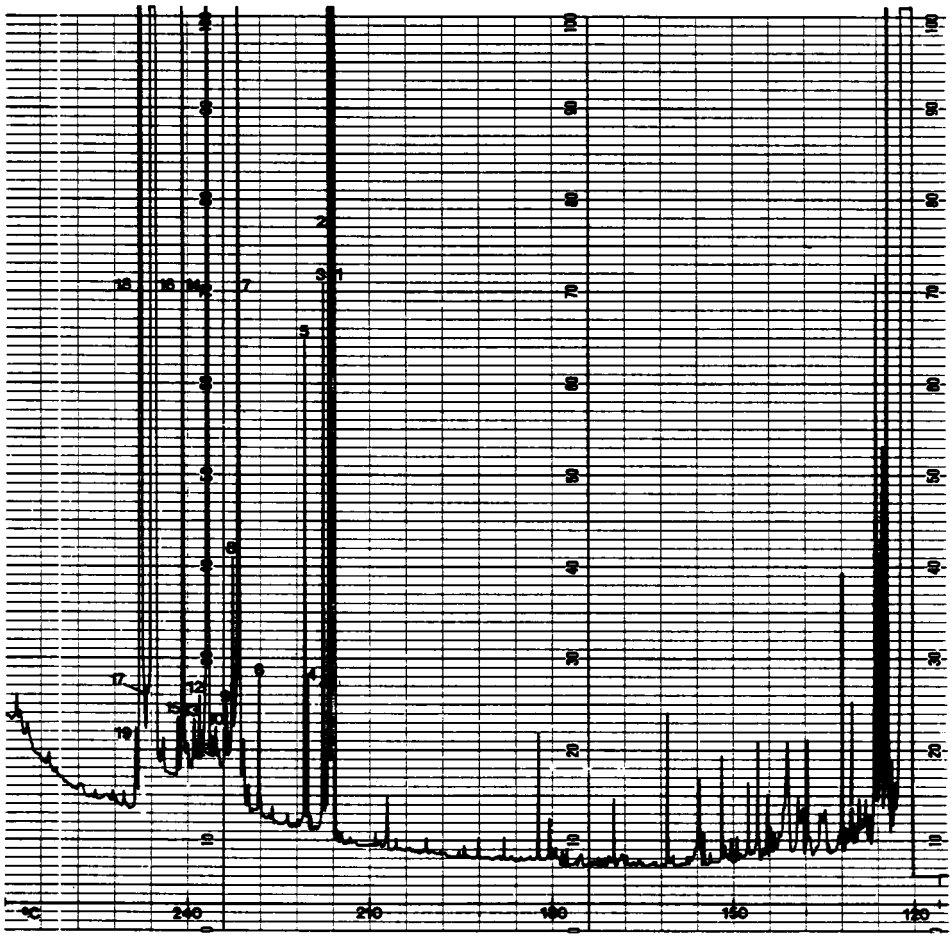

FIGURE 1. Capillary gc/fid pattern of the PA fraction of *Senecio inaequidens*. Senecivernine = 1, senecionine = 2, seneciphylline = 3, spartioidine = 4, integerrimine = 5, senkirkine = 6, retrorsine = 7, compound x = 8, neosenkirkine = 9, usaramine = 10, otosenine = 11, compound y = 12, *O*-acetylsenkirkine = 13, desacetyl doronine [**3**] = 14, compound z = 15, florosenine = 16, floridanine = 17, doronine [**4**] = 18, floricaline = 19.

365), otosenine (mol wt 381), *O*-acetylsenkirkine (mol wt 407), desacetyl doronine (mol wt 417), florosenine (mol wt 423), floridanine (mol wt 441), doronine (mol wt 459), and floricaline (mol wt 483). The structure elucidation of three unknown components (compounds x, y, and z) is still in progress. Figures 2 and 3 show the NH_3 pici, OH^- nici, and NH_3 nici mass spectra of seneciphylline and florosenine, respectively.

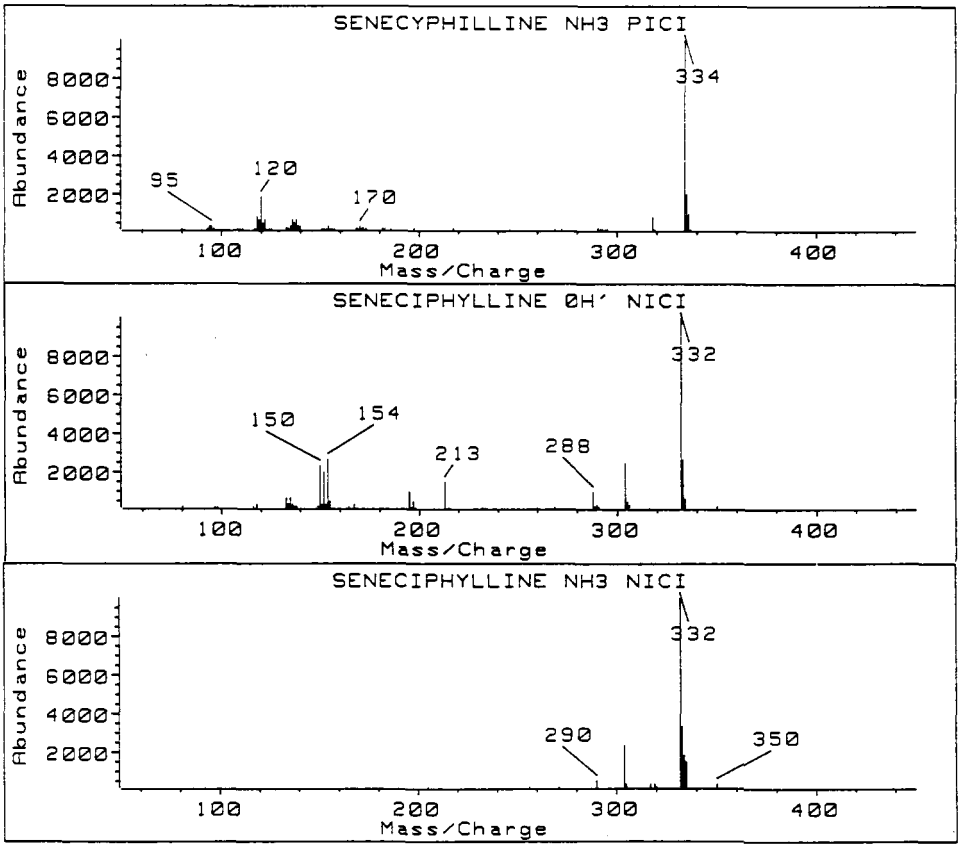

FIGURE 2. NH_3 positive ion ci, OH^- negative ion ci and NH_3 negative ion ci mass spectra of seneciphylline.

The combination of picims and nicims, together with eims, furnishes complementary data for the structural identification of each alkaloid, giving useful information not only on the quasimolecular or molecular ion but also on the necic acid and the necine that constitute them.

NH_3 picims (Table 1) produces an intense and significant quasimolecular peak at *m/z* $[M + 1]^+$ together with fragment ions that can characterize the necine (6,7). The retronecine-derived PAs show a diagnostic peak at *m/z* 120, which is the C-6, C-7 dehydrogenated retronecine-free base $[C_8H_{10}N]^+$, resulting from a pyrolytic elimination of the C-7 ester moiety prior to, or concurrent with, ionization of the molecule at the C-9 ester moiety (7). A minor peak at *m/z* 138 $[C_8H_{12}NO]^+$ corresponds to loss of the diester moiety as its anhydride. Some other necinic-base-related minor diagnostic peaks, at *m/z* 94, 95, 106, 108, 110, and 112, are also produced (7). The otonecine-derived PAs show an intense and diagnostic peak at *m/z* 152 corresponding to the otonecine-free base $[C_9H_{14}NO]^+$, together with a minor peak at *m/z* 168 $[C_9H_{14}NO_2]^+$ due again to loss of the diester moiety as its anhydride.

TABLE 1. NH_3 Positive Ion ci Mass Spectra of *Senecio inaequidens* PAs—*m/z* (percent relative abundance).

Compound	$[M+1]^+$	$[M+H-R(CO)_2O]^+$	$[M+H-R(CO_2H)_2-H_2]^{+a}$ $[M+H-R(CO_2H)_2]^{+b}$	Some other diagnostic peaks
Senecivernine	336(100)	138	120(7)	93, 106, 136(5), 220, 248, 291
Senecionine	336(100)	138	120(9)	93, 106, 136(6), 220, 246, 292
Seneciphylline	334(100)	138(6)	120(20)	94, 136(8), 218, 246, 288, 318(8)
Spartioidine	334(100)	138(15)	120(41)	95(5), 110(8), 136(19), 196, 288, 318(11)
Integerrimine	336(100)	138(8)	120(25)	93, 138(6), 220, 248, 292, 320
Senkirkine	366(40)	168(18)	152(100)	94(8), 110(8), 120(18), 138(25)
Retrorsine	352(90)	138(19)	120(100)	95(7), 136(21), 220, 246, 292, 320
Compound x	364(100)	138(6)	120(39)	93, 136(10), 220, 292, 318
Neosenkirkine	366(42)	168(35)	152(100)	
Usaramine	352(36)	138(25)	120(100)	95(7), 136(23), 222(5), 292, 318(5)
Otosenine	382(58)	168(15)	152(100)	96, 124, 232, 266, 338
Compound y	396(100)	138(15)	120(85)	93, 140(12), 248, 292, 320
O-Acetylsenkirkine	408(15)	168	152(100)	
Desacetyl doronine [3]	418(90)	168(30)	152(100)	232, 250(11), 382(37), 420(29)
Compound z	410(88)	138(32)	120(100)	154(29), 244, 274, 318, 350
Florosenine	424(54)	168(7)	152(100)	213, 250, 304, 366, 408
Floridanine	442(100)	168(19)	152(80)	336(7), 354(5), 384(5), 424(23)
Doronine [4]	460(100)	168(9)	152(60)	213, 250, 336, 424(54), 462(35)
Floricaline	non-significant spectrum[c]			

[a]Retronecine derivatives.

[b]Otonecine derivatives.

[c]Spectrum not sufficiently clear to characterize the compound.

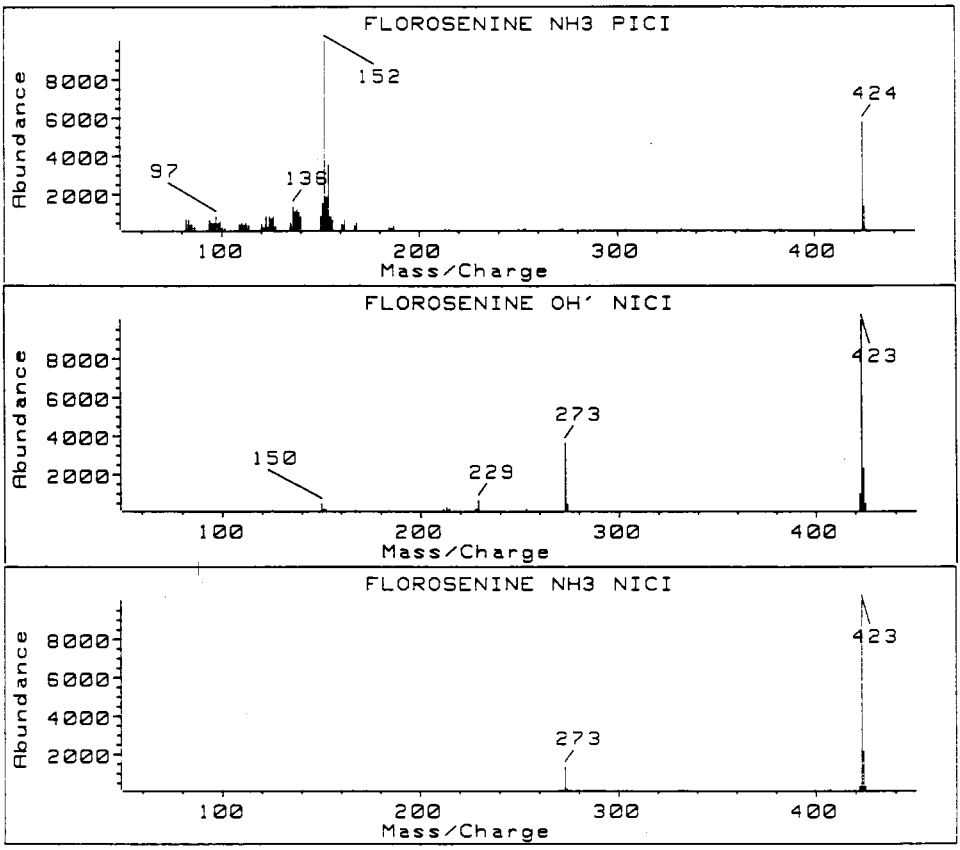

FIGURE 3. NH_3 positive ion ci, OH^- negative ion ci and NH_3 negative ion ci mass spectra of florosenine.

OH^- nici has already been applied to PA structure elucidation by other authors, both on the compounds as such (8) and on their trimethylsilyl derivatives (9,10). To our knowledge NH_3 nicims has not yet been applied to PAs.

OH^- as nici reactant gas is a strong Bronsted base that produces either the pseudomolecular ion $[M-H]^-$ or the molecular ion $[M]^-$ with other substitution and elimination reactions such as the cleavage of the ester bond with formation of carboxylate anion in the gas phase equivalent to a base hydrolysis of an ester (11,12). OH^- nicims on retronecine PA derivatives (Table 2) produces an intense quasimolecular ion together with other important diagnostic peaks: $[M-120]^-$, consistent with the singly ionized dibasic necic acid; $[M-138]^-$, corresponding to the loss of H_2O molecule from the necic acid. The highly significant very low intensity peak at $[M+17]^-$ is an $[M+OH]^-$ adduct produced by a nucleophilic attack with subsequent cleavage (8,13). The retronecine anion $[C_8H_{12}NO_2]^-$ at *m/z* 154 is always produced (8), intensity varying with PA. OH^- nicims on otonecine PA derivatives produces two prevailing diagnostic peaks: the ions at *m/z* $[M]^-$ and $[M-150]^-$ related to the negatively charged molecular ion and to the singly ionized necic acid.

Fragmentation in NH_3 nici mass spectra is much more limited than in OH^- nici. Most PA spectra present only a high intensity peak corresponding to either the quasimolecular ion $[M-H]^-$ for the retronecine derivatives or the molecular ion $[M]^-$ for the otonecine derivatives. In NH_3 nici, too, the retronecine derivatives give a significant low intensity peak at $[M+17]^-$. Florosenine, floricaline, floridanine, and doronine exhibit a low intensity peak at *m/z* $[M-150]^-$ in addition to $[M]^-$ (Table 3).

TABLE 2. OH^- Negative Ion ci Mass Spectra of *Senecio inaequidens* PAs [*m/z* (percent relative abundance)].

Compound	$[M-1]^-$	$[M]^-$	$[M-120]^-$	$[M-138]^-$	$[M-150]^-$	Some other diagnostic peaks
Senecivernine	334(60)		215(22)	197(100)		154(35), 290(78), 306(12), 352
Senecionine	334(100)		215(36)	197(36)		154(75), 290(15), 306(17), 352
Seneciphylline	332(100)		213(18)	195(10)		154(26), 288(10), 304(23), 350
Spartioidine	332(100)		213(20)	195(9)		154(60), 288(25), 304(18), 350
Integerrimine	334(96)		215(36)	197(30)		154(100), 290(57), 306(32), 352
Senkirkine		365(100)			215(16)	150, 184, 197(5), 229, 248
Retrorsine	350(15)		231(16)	213(30)		154, (50), 195(52), 320(100), 368
Compound x	362(28)		243(50)	225		154(6), 181(100), 197, 215, 288, 318, 336
Neosenkirkine		365(56)			215(100)	150(8), 184, 197(12)
Usaramine	350(5)		231	213(15)		154(44), 195(100), 320(33), 368
Otosenine		381(100)			231(64)	150, 213(13), 293, 337, 365
Compound y	394(100)		275(19)	257(16)		154(62), 195(35), 211(25), 229(16), 412(20)
O-Acetylsenkirkine		407(17)			257(100)	150(13), 184
Desacetyl doronine [3]		417(100)			267(5)	150(5), 184, 187(48), 231(36), 249(7), 381(93), 419(31)
Compound z	408(100)		289(8)	271(10)		154(40), 209(22), 225(15), 243(6), 426(12)
Florosenine		423(100)			273(35)	150(5), 184, 213, 229(6), 255
Floridanine		441(100)			291(7)	150(11), 169(22), 184, 273, (6), 423(6)
Doronine [4]		459(100)			309(6)	150, 229(88), 273(48), 423(60), 461(29)
Floricaline		483(100)			333(7)	150(24), 184

TABLE 3. NH_3 Negative Ion ci Mass Spectra of *Senecio inaequidens* PAs [*m/z* (percent relative abundance)].

Compound	$[M-H]^-$	$[M]^-$	Some other diagnostic peaks
Senecivernine	334(100)		197, 290(64), 306(8), 352
Senecionine	334(100)		290(20), 306(8), 352
Seneciphylline	332(100)		304(23), 350
Spartioidine	332(100)		304, 350(10)
Integerrimine	334(100)		290(30), 306(10), 352
Senkirkine		365(100)	
Retrorsine	350(100)		320(16), 368
Compound x		non-significant spectrum[a]	
Neosenkirkine		365(100)	
Usaramine		non-significant spectrum[a]	
Otosenine		381(100)	
Compound y		394(100)	320(12), 350(9), 412
O-Acetylsenkirkine		non-significant spectrum[a]	
Desacetyl doronine [**3**]		417(100)	381(7), 419(36)
Compound z	408(100)		392(6), 426
Florosenine		423(100)	273(13)
Floridanine		non-significant spectrum[a]	
Ddoronine [**4**]		459(100)	273, 423(6), 461(30)
Floricaline		483(100)	333

[a]Spectrum not sufficiently clear to characterize the compound.

Three of the unidentified PAs (compounds x, y, and z) have been characterized by ms techniques as retronecine derivatives showing presumed mol wts of 363, 395, and 409 amu, respectively. Ms techniques have identified the fourth unknown PA as desacetyl doronine [**3**] ($C_{19}H_{28}NO_7Cl$): its mass spectra (Figure 4) are strictly related to those of doronine [**4**] ($C_{21}H_{30}NO_8Cl$), previously isolated from *Doronicum macrophyllum* (14) and *Senecio abrotanifolius* (15). The ei fragmentation pattern of desacetyl doronine [**3**] is very similar to that of doronine [**4**], showing the characteristic otonecine ions at *m/z* 110, 122, 123, 140, 151, and 168, together with the ions resulting from the molecular fragmentation at *m/z* 238, 254, 278, 340, and 354 and a low intensity molecular ion at *m/z* 417. All these fragments are consistent with the fragmentation pattern of the otonecine macrocyclic diesters (16). Greater and equally significant similarities between the spectra of the two compounds are shown in ci mode. In NH_3 pici, together with the intense peak at *m/z* 152 characterizing the otonecinic ring, both doronine and desacetyl doronine have high-intensity quasimolecular peaks (at *m/z* 460 and 418, respectively), and medium-intensity diagnostic peaks (at *m/z* 424 and 382, respectively) corresponding to the loss of HCl from the quasimolecular ion $[M+1-HCl]^+$. The presence of a chlorine atom is confirmed by the two ions at *m/z* 462 and 420. In OH^- nici, both compounds **3** and **4** present the molecular ions as base peak, at *m/z* 459 and 417, respectively, and a further intense peak at *m/z* 423 and 381 corresponds to the loss of HCl $[M-HCl]^-$. Two other significant pairings of peaks are present, the very low-intensity *m/z* 309 and 267 fragments, i.e., $[M-150]^-$ consistent with the singly ionized dibasic necic acid, and the medium intensity *m/z* 273 and 231 fragments that are derived by loss of an HCl molecule from the previous ones. Two very intense peaks at 229 and 187, corresponding to the loss of 44 amu from both the previous fragments, are also produced (16). The presence of chlorine is confirmed by

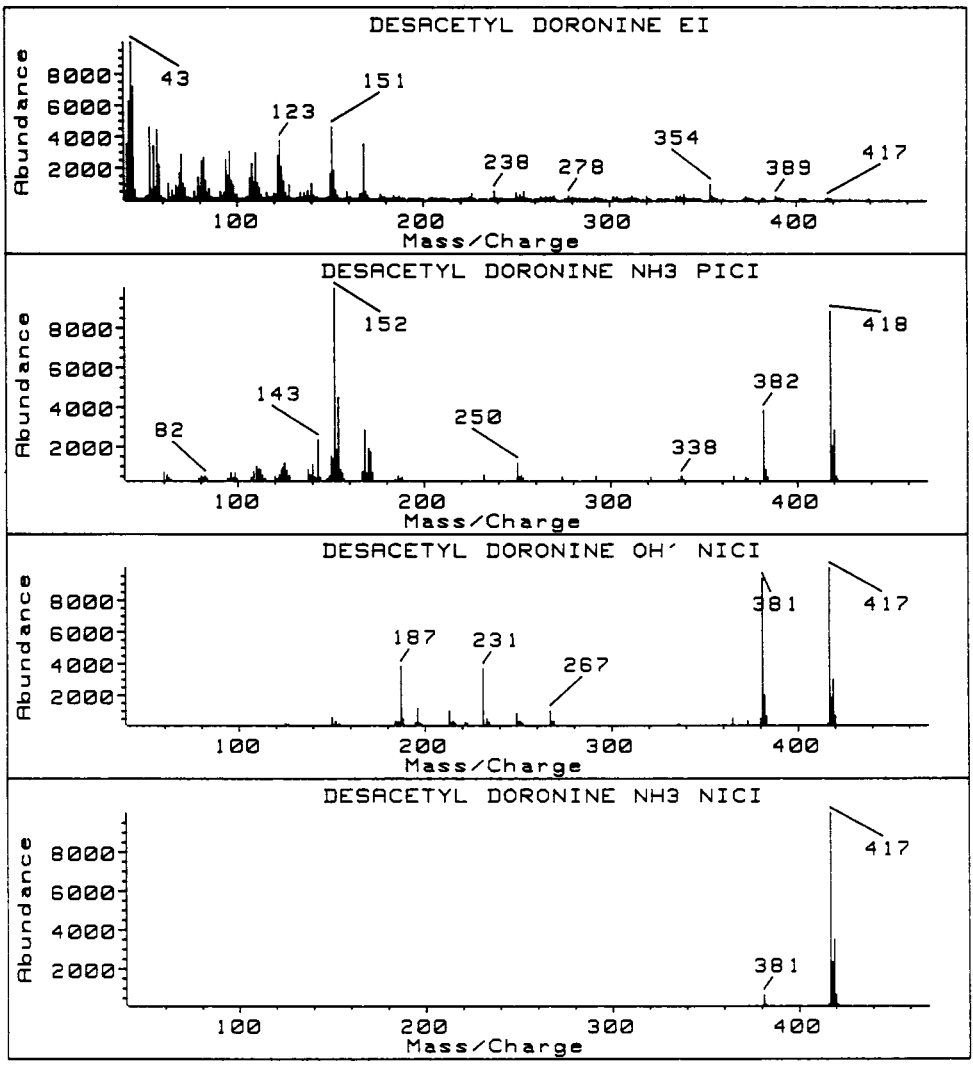

FIGURE 4. Ei, NH_3 positive ion ci, OH^- negative ion ci and NH_3 negative ion ci mass spectra of desacetyl doronine [3].

two intense peaks at *m/z* 461 and 419, respectively. NH_3 nici mass spectra confirm the previous results.

Retronecine and otonecine macrocyclic diesters behave differently under nici conditions both with OH^- and NH_3 as reactant species; while the retronecine derivatives give a quasimolecular ion at $[M-H]^-$ because of a proton abstraction and an $[M+OH]^-$ adduct, the otonecine derivatives present the molecular ion $[M]^-$ due to an electron capture process. The different extent of fragmentation of retronecine derivatives in NH_3 and OH^- nici may be due to various factors: the difference in proton affinities of the anions NH_2^- (1670 kJ/mol) and OH^- (1634 kJ/mol) or the occurrence of other processes, e.g., electron capture dissociation, favored if the mixture CH_4/N_2O is used to produce the OH^- reagent species (17). For otonecinic derivatives the different extent of fragmentation is due to the different electron affinity between the ionizing species (NH_2^- 72.0 kJ/mol, OH^- 176.0 kJ/mol) (17). This agrees with the general theory of chemical ionization (18).

EXPERIMENTAL

PLANT MATERIAL.—Plant material was collected in March 1986, from a roadside on the outskirts of Padua, Italy, and identified by Dr. R. Caniato of Dipartimento di Biologia Vegetale, Università di Padova, Padova, Italy. Voucher specimens (DTA 6122) are deposited at Dipartimento di Biologia Vegetale, Università di Padova.

REAGENTS.—All the chemicals used were analytical-reagent grade (E. Merck, Darmstadt, F.R.G.). PS 264 and PS 122 are commercially available from Petrarch System, Inc., Bristol, Massachusetts.

SAMPLE PREPARATION.—Air-dried plant material (25 g) was extracted in a Soxhlet apparatus with MeOH for 4 h. The extract was evaporated to dryness under vacuum, and the residue suspended in 2.5% HCl and washed with Et_2O and $CHCl_3$. Half of the aqueous phase was basified by 25% NH_3 solution and extracted with CH_2Cl_2. The organic layer was again treated with 2.5% HCl, then with 25% NH_3 solution and again extracted with CH_2Cl_2. The resulting CH_2Cl_2 solution was dried over anhydrous Na_2SO_4 and evaporated to dryness. To investigate the presence of PA-*N*-oxides, the second aliquot of the aqueous phase was reduced with Zn dust overnight, filtered, and subsequently treated as above. The dried residues were weighed and dissolved in suitable amounts of CH_2Cl_2 for capillary gc and capillary gc-ms analysis. PAs as free bases (69.7 mg) and as *N*-oxides (22.6 mg) were present in the sample.

CAPILLARY GC ANALYSIS.—Capillary gc analyses were performed by introducing 1 μl PA extract on a Carlo Erba Mega 5360 instrument under the following conditions: carrier gas H_2; flow rate 3 ml/min; injection system split-split ratio 1:30; injector temperature 300°; detector fid; temperature 300°; column temperature program 120° (1 min)–180° (20 min) at 3°/min. A 30 m Duran 50 glass capillary column coated with 0.3 μm of PS 264 (polydimethyl siloxane, 7% diphenyl, 1% vinyl) was used. To obtain a highly inactive column, persilylation was carried out with a PS 122 (polimethylhydroxylane) solution in CH_2Cl_2 at 320° for 4 h (19).

CAPILLARY GC-MS ANALYSIS.—Capillary gc-ms analyses were carried out on a Nermag R 30-10 mass spectrometer equipped with a Digital PDP 11/75 computer. Pici mass spectra were obtained by using NH_3 as reactant gas at a 1×10^{-1} atm source pressure. Nicims was carried out with both NH_3 (source pressure 5×10^{-2} atm) and a 1:1 mixture of N_2O and CH_4 (total source pressure of 5×10^{-2} atm). The source temperature was kept at 150°. Capillary gc separations were carried out on a Dani 6500 gas chromatographic unit. Carrier gas He; flowrate 3 ml/min. A fused silica column as above was used. Capillary gc conditions were as above. PAs were identified by comparison with spectra of pure samples and spectra reported in literature. All the mass spectra were recorded on-line to the capillary gc separation.

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