Indole Alkaloids from the Roots of an African Plant *Securidaca Longipedunculata*. I. Isolation by Column Chromatography and Preliminary Structural Characterization by Mass Spectrometry

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Received April 13, 1992

The extraction and the isolation of some alkaloids from the roots of an African plant (*Securidaca Longipedunculata Fres*) are reported. The structural characterization was performed by mass spectrometry. Alternative ionization methods (FAB and EI), collisionally-induced decomposition experiments and accurate mass measurements revealed the presence of elymoclavine and dehydroelymoclavine and the presence of a new ergoline alkaloid.

J. Heterocyclic Chem., **29**, 1641 (1992).

Introduction.

We recently reported the isolation of some compounds from the roots of an African plant used in traditional medicine [1-3]. The plant was identified by the Royal Botanic Gardens, Kew, England, as *Securidaca longipedunculata Fres (Polygalaceae)*, a species native to Africa, and called "Tchúnfki" by the local people. Aqueous extracts of the roots are widely used by the Balanta people of Guinea Bissau in religious rites, due to their psychotropic effects [4]. The fresh roots, which exale a very pungent and disagreeable smell, are also placed in huts to repel snakes and rodents.

In view of the psychotropic effects of this plant, we thought it of interest to analyse the methanol extracts of the roots with the aim to isolate the active principles. Separation of compounds was performed by silica gel column chromatography of extracts. Some fractions thus obtained gave positive reaction to alkaloid reagents. Since many alkaloids have an effect on the central nervous system [5], our investigation was focused on these fractions.

In order to obtain structural information about the compounds, we undertook a preliminary study by mass spectrometry. As a first analytical approach, electron impact (EI) and fast atom bombardment (FAB) [6] ionization systems, followed by collisional experiments on molecular species, were employed to investigate on molecular structures.

Such a strategy led to the unequivocal identification of the molecular ion, together with precious information on the decomposition pathways which can be highly diagnostic from the structural point of view.

Scheme 1

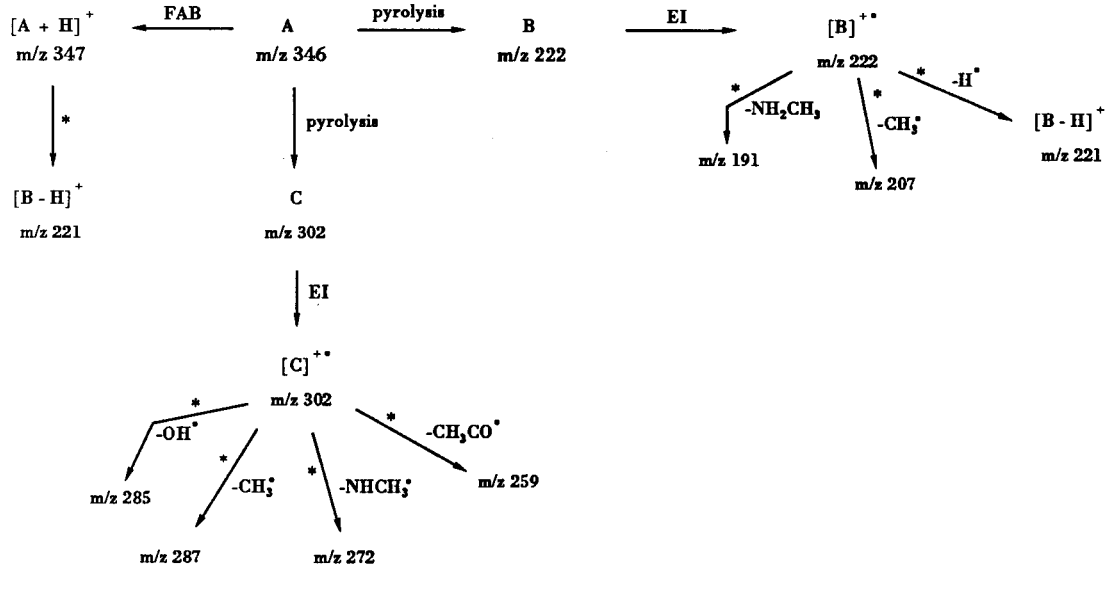


Table 1

70 eV EI mass spectra of fraction **1** recorded at different probe temperatures

Ionic Species	115	181	187	191	197	207	215	222	230	302
Temperature (C°)	Relative Intensity									
80	33	15	25	47	-	25	17	100	47	17
100	30	25	34	52	9	18	35	100	90	30
120	40	8	48	7	10	9	42	14	100	5
140	35	5	38	12	10	8	43	14	100	-
160	50	-	22	15	-	15	33	100	42	-

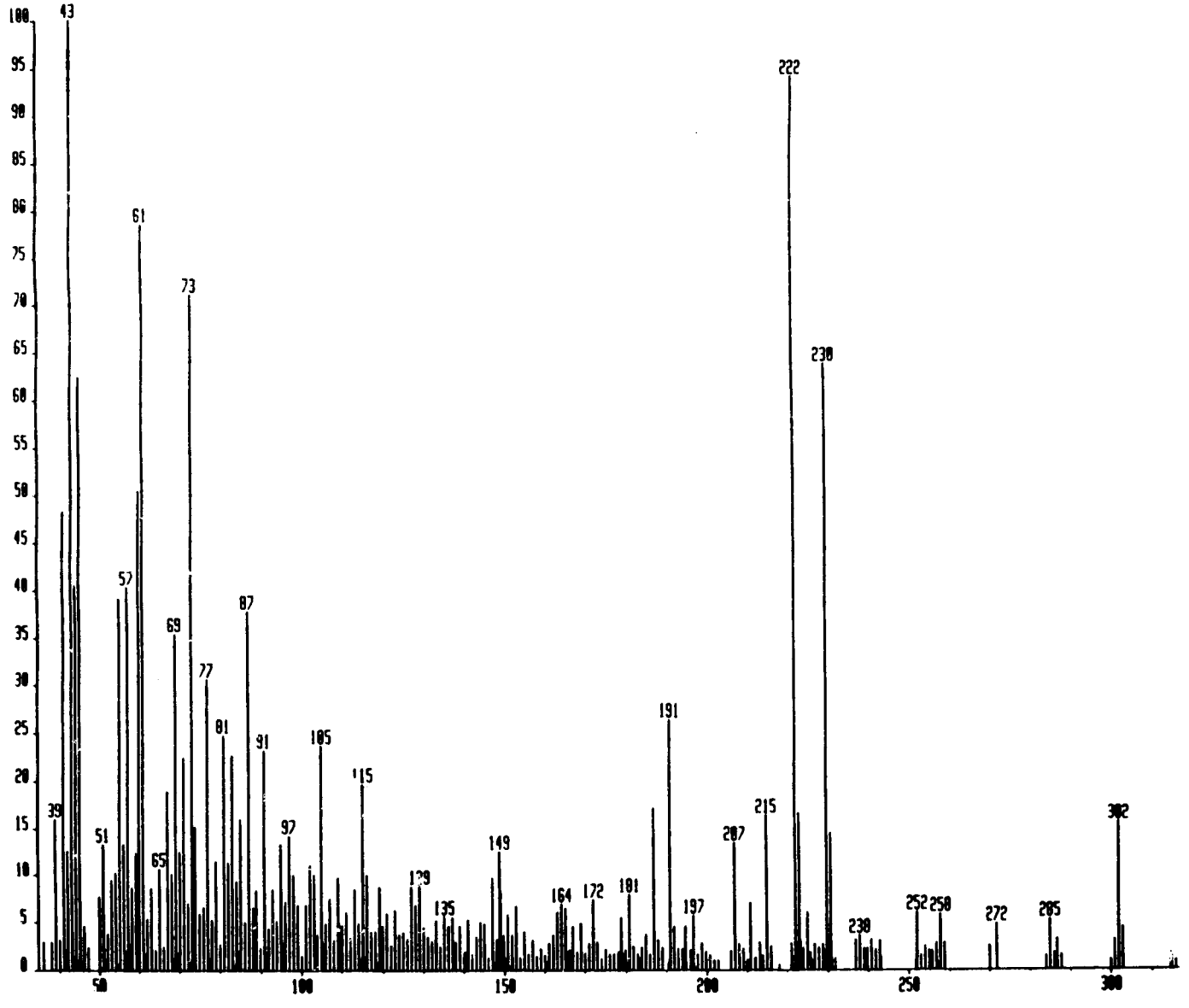


Figure 1. 70 eV EI mass spectrum of fraction **1**.

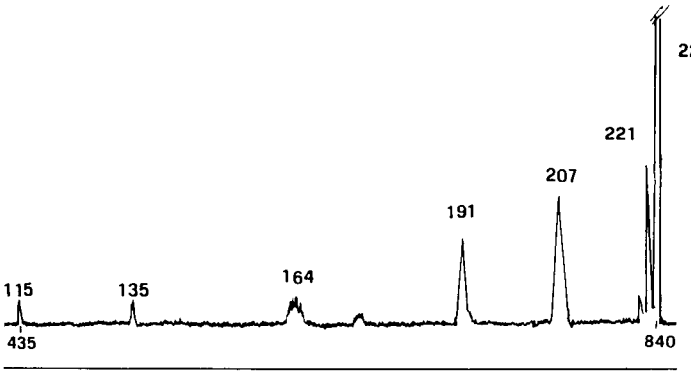


Figure 2. CAD MIKE spectrum of ionic species at m/z 222 EI-generated on fraction I.

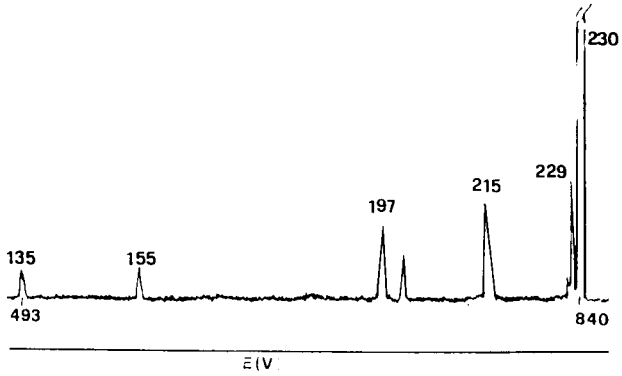


Figure 3. CAD MIKE spectrum of ionic species at m/z 230 EI-generated on fraction I.

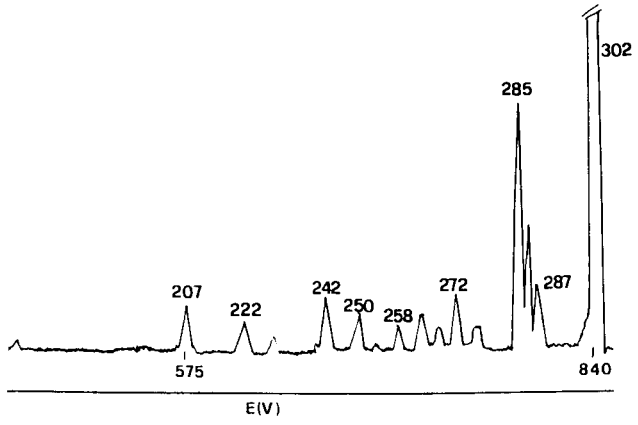


Figure 4. CAD MIKE spectrum of ionic species at m/z 302 EI-generated on fraction I.

EXPERIMENTAL

Materials.

Analytical thin layer chromatography (tlc) was carried out on silica gel (Kiesigel 60 F_{254}-Merck) developed with cyclohexane-chloroform-acetic acid 45:45:10. Deactivated silica gel (20%, w/w) (Kiesigel 60, 70-200 mesh, Merck) was used for column chromatography. All solvents used were of analytical grade.

Plant Material.

Collection of roots of *Securidaca longipedunculata Fres.* was made in winter during the dry season in the proximity of Bissau (Guinea Bissau, West Africa). The roots, after being cleaned with a stainless steel brush, were cut into small pieces, placed in a mortar and then triturated to obtain a coarse powder.

Extraction Procedure and Column Chromatography.

The powdered roots were extracted exhaustively with methanol. Evaporation of the extract on a rotary evaporator under reduced pressure to dryness gave 17 g of a residue from 1173.6 g of powdered roots. This residue was mixed thoroughly with 20 ml of 10% aqueous ammonia solution, then extracted with ethyl ether.

The ethereal extract was concentrated under vacuum and 3 g of the residue were applied to the top of a glass column of silica gel (6 cm diameter, 70 cm long). The components were eluted with the following solvent series: hexane (750 ml), benzene (500 ml), a gradient of benzene:chloroform (2000 ml), chloroform (750 ml), ethyl ether (500 ml), ethyl acetate (500 ml), acetone (500 ml), methanol (1000 ml).

Collected fractions (100 ml each) were analyzed by tlc (developed with cyclohexane-chloroform-acetic acid 45:45:10) and recombined on the basis of similarity of patterns observed under uv light at 254 and 365 nm and after spraying with alkaloid reagents (Dragendorff and iodoplatinate [7]). Only the fractions eluted with benzene:chloroform exhibited a positive reaction to the alkaloid reagents, giving brown or orange spots on spraying.

Unfortunately, the tlc showed that all fractions were mixtures of several compounds, difficult to isolate. The residues of two of these fractions were selected for identification by mass spectrometric analysis.

Mass Spectrometric Measurements.

Electron ionization (EI) and Fast Atom Bombardment (FAB) mass spectrometric measurements were performed on VG ZAB 2F [8] double focussing, reversed geometry, mass spectrometer (VG Analytical, Manchester, UK).

The EI mass spectra were obtained at 70 eV (200 μA, source temperature 200°). The samples were introduced by the direct inlet system and warmed slowly up to 200°, in order to obtain a possible fractional distillation of the various components.

FAB [9] spectra were achieved by 8 keV Xe atoms bombarding glycerol solutions of the samples.

Metastable transitions were detected by linked-scan techniques at B/E = cost and B^2/E = cost [10] and by mass analysed ion kinetic energy (MIKE) spectroscopy [11], using the same instrument.

Collisional spectroscopy [12] was performed by 8 keV ions colliding with air in the second free field region. The gas pressure in the collision cell was such as to reduce main beam intensity to 40% of its usual value.

Accurate mass measurements were obtained by the peak matching technique at 10000 resolution (10% valley definition).

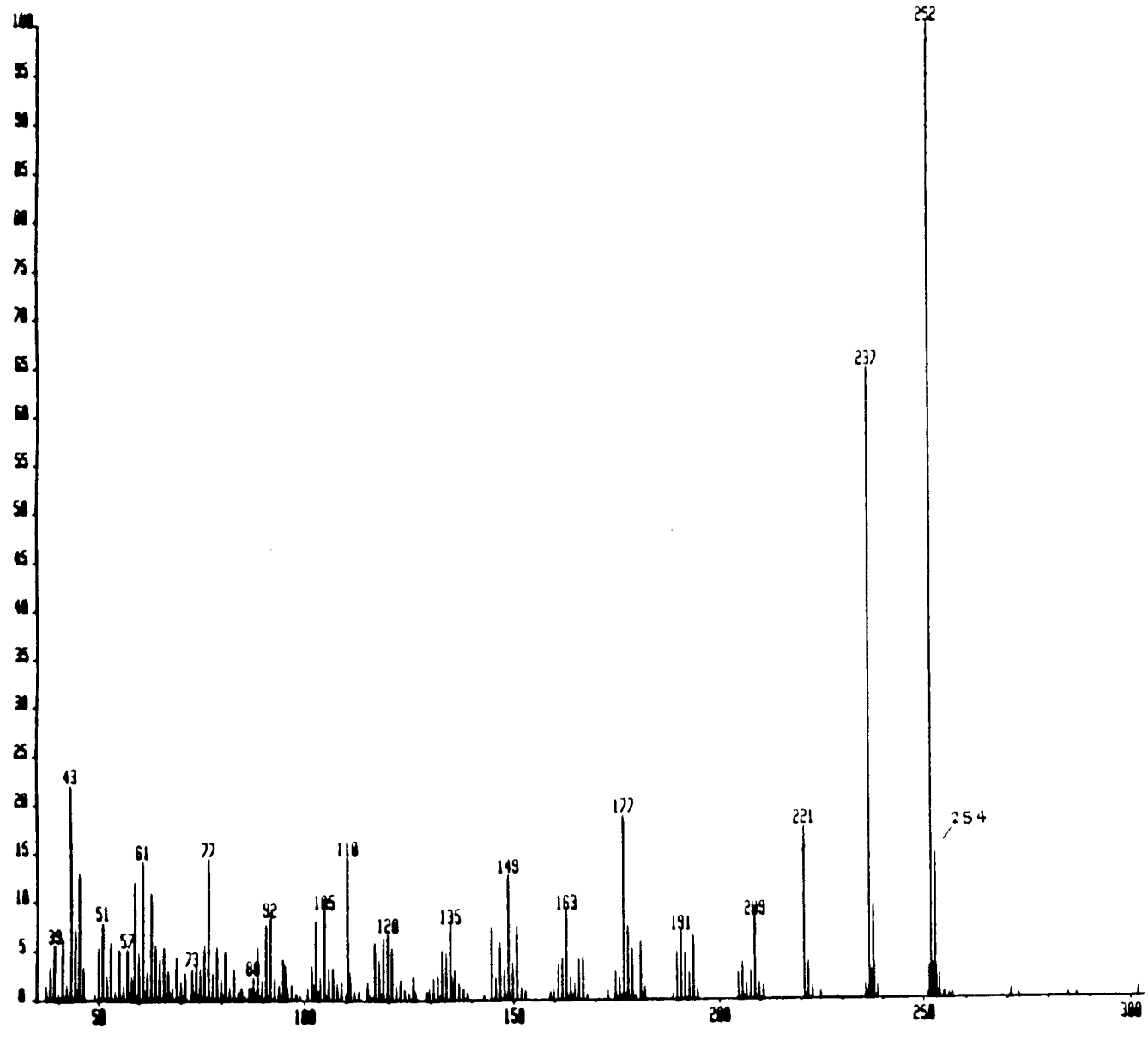


Figure 5. 70 eV EI mass spectrum of fraction **2**.

Results and Discussion.

Ten fractions, exhibiting a positive reaction to the alkaloids reagents, were obtained. In particular, two fractions (named **1** and **2**) were more abundant in alkaloid components. For this reason we firstly focused our attention on them, applying the analytical approaches offered by mass spectrometry in different experimental conditions.

Electron Impact Mass Spectrometry.

Data obtained by EI mass spectra revealed that these two fractions were mixtures of two or more alkaloids.

For the fraction **1** (Figure 1) this approach allowed us to distinguish possible molecular species present in the mixture, according to their different volatility. The spectrum showed different ratios in relative intensities of two ionic species at m/z 222 and 230 with respect to probe temperature (Table 1). As an example the [222]/[230] ratio was 100:47 with a probe temperature of 80°, but it becomes 17:100 at 120°. It is reasonable to assume that these two ions are molecular species of two compounds of different volatility. Linked scans at B^2/E = cost (parent ion scan) demonstrated that the ions at m/z 222 and 230 were both molecular species. In fact no precursor ions were detected for them.

Furthermore, the spectrum of fraction 1 showed another interesting ion at m/z 302, which B^2/E linked scans proved to be molecular species. For gaining information on the fragmentation pathways and the structural identity of these three ions, accurate mass measurements and collision-activated decomposition (CAD) MIKE experiments were undertaken. Accurate mass measurements gave for the ions at m/z 222 the elemental formula $[C_{15}H_{14}N_2]$ (Calcd: 222.1154; Found: 222.1198 ±0.005). The CAD

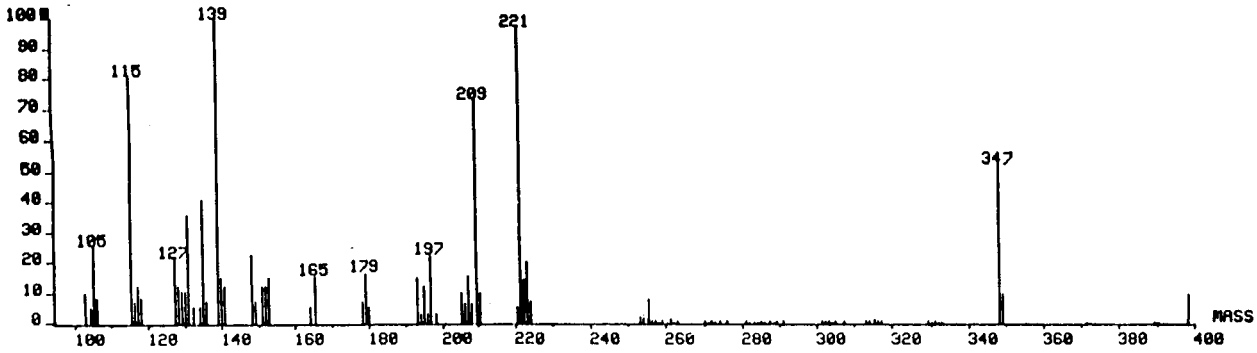


Figure 6. FAB mass spectrum of fraction **1**.

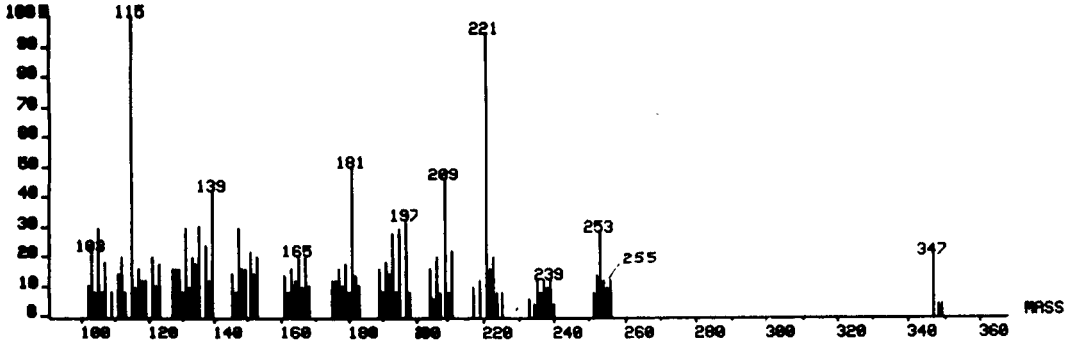
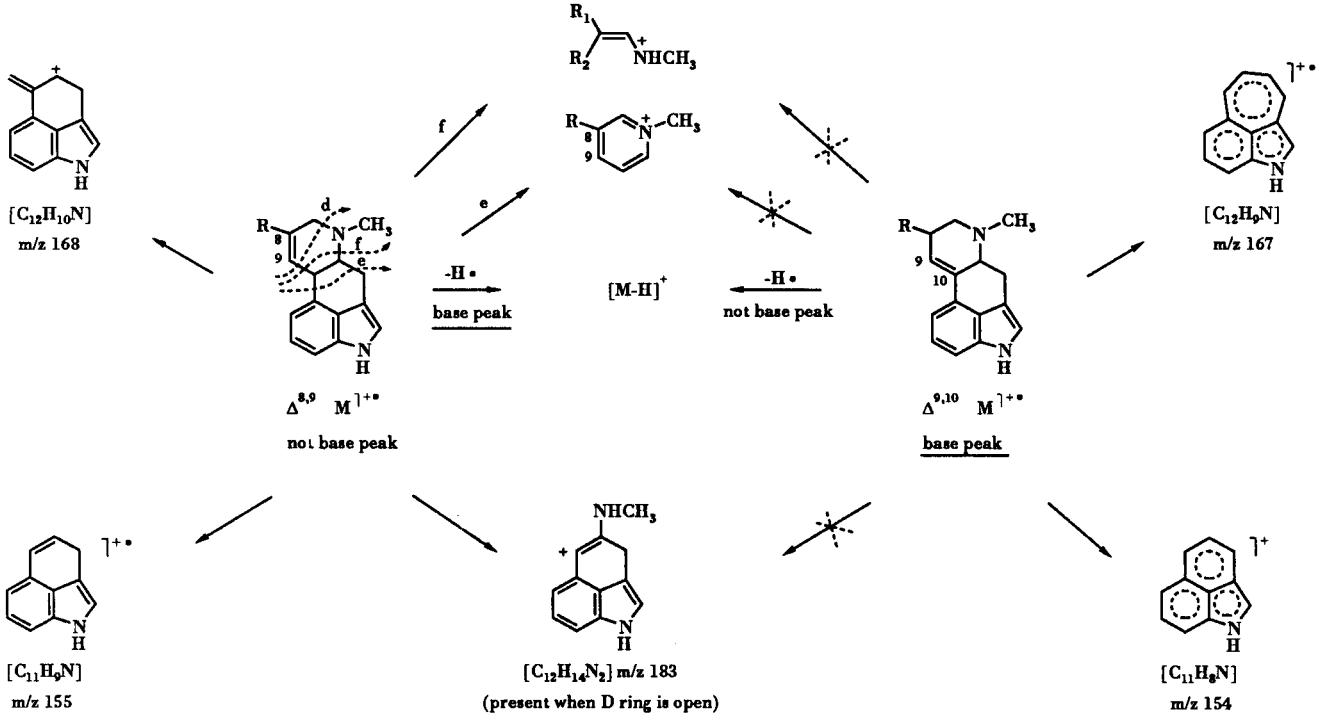


Figure 7. FAB mass spectrum of fraction **2**.

Scheme 2



MIKE spectrum of such species (Figure 2), showed fragment ions at m/z 221, 207 and 191. The first two species, due to H· and CH_3· losses, are isobaric with those already described in the fragmentation pattern of lysergic acid [13], thus suggesting that the selected ion could contain the skeleton of lysergic acid.

For the ion at m/z 230 ([$C_{14}H_{18}N_2O$]; Calcd: 230.1415; Found: 230.1438 ±0.005), the CAD MIKE spectrum showed neutral losses of 15 and 33 u (Figure 3), leading to ionic species at m/z 215 and 197 respectively. The primary H· loss is responsible for the formation of ions at m/z 229.

An interesting ion at m/z 302 was detected in the EI spectra at low probe temperature. The CAD MIKE spectrum of this ion showed numerous fragments (Figure 4). Together with losses of neutral moieties of 15 and 17 u (reasonable CH_3· and OH· respectively) typical fragments were seen as before, in particular for ions at m/z 222 and 207. This fact suggests that the components with molecular species at m/z 222 and 302 may be structurally related. Accurate mass measurements for ions at m/z 302 gave the elemental formula [$C_{19}H_{14}N_2O_2$] (Calcd: 302.1052; Found: 302.1106 ±0.005), indicating that the difference with respect to ions at m/z 222 lies in a [C_4O_2] moiety.

For fraction **2**, electron impact mass spectrum, recorded at different probe temperatures, showed the same fragment ions. In particular, two molecular species at m/z 252 ([$C_{16}H_{16}N_2O$]; Calcd: 252.1259; Found: 252.1214 ±0.005) and 254 ([$C_{16}H_{18}N_2O$]; Calcd: 254.1415; Found: 254.1483 ±0.005) were always found (Figure 5). The ions at m/z 254 and their related fragmentation patterns have already been described by Eckers *et al.* [14] in a study on ergot alkaloids. By mean of this comparison, these ions were attributed to elymoclavine molecular ion.

For the ion at m/z 252, both accurate mass measurements and daughter ion spectroscopy agree with the structure of dehydroelymoclavine.

Fast Atom Bombardment.

By EI different molecular species were revealed in fractions **1** and **2**; more precisely, ions at m/z 222, 230 and 302 were shown as molecular ions in fraction **1**, and molecular ions of elymoclavine (m/z 254) and dehydroelymoclavine (m/z 252) in fraction **2**. However, as is well known, EI can sometimes give misleading results, mainly due to thermally induced decomposition processes related to sample vaporization and/or ion source heating. In other words what is sometimes detected does not represent the real molecule but its thermal degradation products.

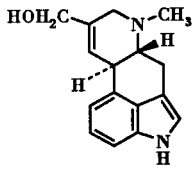
Hence, in order to be more confident about the identification of the molecular species present in the alkaloid fractions, mass spectrometric measurements in FAB conditions were undertaken.

The positive ion FAB mass spectrum of fraction **1** appears in Figure 6. Quite surprisingly, it shows abundant ionic species at m/z 221 and 347. Ions at m/z 223, 231 and 303, to be expected on the basis of EI data, are completely absent. This should be ascribed to the choice of the unsuitable matrix, in which the compounds of interest are insoluble. But even when the matrix was varied (glycerol, thioglycerol, nitrobenzyl alcohol) ions at m/z 223, 231 and 303 were never detected. These results must necessarily mean that the ions at m/z 222, 230 and 302 detected in EI conditions represent pyrolysis products of an undetected species.

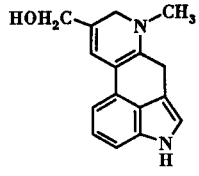
Daughter ion spectroscopy of FAB-generated ions at m/z 347 mainly consists of ions at m/z 221 and 209.

The molecular species at m/z 302 present in the EI spectrum of fraction **1** may also be related with the ion at m/z 347 revealed in FAB conditions; it is also a pyrolysis product from the latter ion, resulting from a CO_2 loss. These results confirm the hypothesis that the ions at m/z 222 and 302 in the EI spectra are structurally related and also that they both originate from pyrolysis of the species at m/z 346.

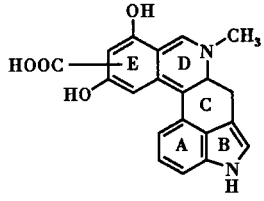
The above data on fraction **1** are summarized in Scheme 1. Fraction **1** is mainly composed of molecular species **A** which, in FAB conditions, leads to abundant $[M+H]^+$ species at m/z 347, further decomposing to give rise to ions at m/z 221. In EI conditions, the same molecular species undergoes extensive pyrolysis, leading to molecular ions at m/z 222 and 302 of elemental formula [$C_{15}H_{14}N_2$] and [$C_{19}H_{14}N_2O_2$], respectively. The structure of the species may be suggested in terms of structures **B** and **C**. In fact, while the collisionally-induced fragmentation pattern suggests the structure of 7,8-9,10-didehydroergoline for ion **B**, both accurate mass measurements and collisional experiments give evidence for the structure of 4,5-7,8-9,10-tridehydro-8,9-resorcinolergoline for ion **C**. Ion **C** differs from **A** of 44 u, reasonably a CO_2 molecule. This moiety must necessarily be present in ring E, otherwise the fragment $[B-H]^+$ observed in FAB conditions would shift by 44 u (m/z 267 instead of 221). Hence, we propose the following structure for species **A**:



Elymoclavine



Dehydroelymoclavine



Structure A

The positive FAB spectrum of fraction **2** (Figure 7) shows ions at m/z 253 and 255 and the fragment ions characteristic of the already described elymoclavine and dehydroelymoclavine, thus confirming the structures attributed to these molecular species in EI conditions.

Conclusions.

In the present preliminary study on the alkaloids contained in roots of *Securidaca longipedunculata Fres.*, some interesting results were achieved.

First of all, the presence of elymoclavine and dehydroelymoclavine was unequivocally proved. Secondly, alternative ionization methods (EI and FAB), collisionally-induced decomposition experiments and accurate mass measurements revealed the presence of a new ergoline alkaloid.

Ergoline alkaloids consisting of the derivatives of lysergic acid occur in a plant of the Polygalaceae family, al-

though alkaloids of similar structure to that of lysergic acid are only rarely found in higher plants [15].

Hofmann and Tscherter [16], discovered some alkaloids of lysergic acid structure in seeds of two *Convolvulaceae: Ipomea violaceae L.*, and *Rivea corymbosa L.*, used by the Indians of Central America in religious cerimonies for their psychotropic effects. Similar alkaloids have been found in several Ipomea species [17-22].

Schmidt and Maier [23] studied the mass spectra of a wide range of alkaloids and proposed some diagnostic ions characteristic for the ergoline skeleton. Using these data, together with those of other mass spectral studies on ergot alkaloids [13,14,23] and our own data, a general fragmentation pathway can be proposed for clavine-type ergot alkaloids (see Scheme 2), in agreement with the data discussed here.

Acknowledgments.

This work was supported by M.U.R.S.T.-Rome.

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* Author to whom correspondence should be addressed.

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