Structural Study of Alkaloids from *Securidaca Longipedunculata* Roots. II. Isolation and Characterization by Supercritical Fluid Chromatography/Mass Spectrometry

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Various alkaloids obtained from the methanol extracts of the roots of *Securidaca Longipedunculata Fres.* were studied using supercritical fluid chromatography/mass spectrometry. This hyphenated method and parallel unimolecular and collisionally activated decomposition experiments on electron impact-generated molecular ions gave information on the structure of compounds. The presence of the ergoline skeleton in some of them was assigned.

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Introduction.

Mass spectrometry is one of the most powerful methods for structure elucidation in natural product chemistry. It has been successfully employed for this purpose since its early days [2], and the further development of ionization techniques, apart from electron ionization, and hyphenated methods has strongly increased its capabilities and applicabilities in the field.

Of the different classes of natural compounds, alkaloids have received much attention, due to their importance from both phytochemical and biomedical points of view, and extensive reviews on related mass spectrometric applications have been published [3-8].

In previous papers [9-11] we reported some preliminary studies on alkaloid fractions from methanol extracts of the roots of *Securidaca Longipedunculata Fres. (Polygalaceae).* Aqueous extracts of the roots of this West African species are used by the Balanta people of Guinea Bissau in their religious rites, possibly due to their psychotropic effects. It was in view of these properties that we undertook this study, with the final aim of isolating and characterizing possible active principles. Using both electron impact (EI) and fast atom bombardment (FAB) technique on fractions containing alkaloid species obtained by column chromatography, some indications emerged on the presence of an ergoline-type skeleton [10,11], together with the multicomponent nature of every fraction. A hyphenated method was used to test the various alkaloid-containing fractions.

Direct analysis of such fractions is generally quite difficult. In particular, the gas chromatography/mass spectrometry (gc/ms) approach is ineffective due to the high polarity of such compounds, and the problem cannot usually be overcome by simple derivatization.

Supercritical fluid chromatography/mass spectrometry (sfc/ms) [12] has recently received particular attention, being complementary to both gc and high performance liquid chromatography (hplc). Supercritical fluids are gases above their critical temperature and pressure, and are thus placed between low pressure gases and ordinary liquids. As the physical properties of a supercritical fluid are intermediate between the gaseous and liquid phases, it is a good mobile phase for chromatography [12]. Both capillary and packed column [13] can be used: the former has the advantage over gc that the sample does not have to be vaporised and the use of pressure/density programming enables high- and low-volatile compounds to be analysed. Moreover, higher chromatographic efficiency can be achieved. The packed columns offer faster analysis than hplc, a faster method of development and better detection limits.

A wide range of organic compounds, *e.g.* thermally unstable, low-volatility, and polar, have been analysed with this technique [14-16].

The further combination of sfc with mass spectrometry provides a system with higher specificity, selectivity and sensitivity [12].

For all these reasons sfc/ms was chosen in order to investigate the mixture of unknown alkaloids, available in very small sample quantities.

The sfc separation initially involved studies with various chromatographic columns (*e.g.*, amino, silica and cyano).

The solid phases gave different interactions with the functional groups present, producing varying separation of the components in the mixture. The effects of column length (10, 15 and 25 m) and mobile phase flow rates (2, 3 and 4 ml/minute) were also studied. Both electron and chemical ionization (EI and CI) were employed to gain structural information.

In parallel with the sfc/ms measurements on the various fractions, unimolecular and collisionally activated decomposition [17] of selected species was undertaken, for further structural data.

EXPERIMENTAL

Plant Materials.

Fractions 1 and 2 were obtained from the methanol extracts of *Securidaca Longipedunculata Fres. (Polygalaceae)* roots, after silica gel column chromatography, as reported in our previous papers [9-11].

Mass Spectrometric Techniques.

The scf/ms measurements were performed using a VG 70-70H (VG Analytical, Manchester, U.K.), double-focusing mass spectrometer equipped with a moving belt interface [18]. An EI/CI source, operating at 5 kV of acceleration voltage (source temperature 200°, electron energy 70 eV) was used. Under CI conditions, CH_5^+ ions were used as reactant species and obtained by a methane pressure of 5 x 10^{-5} Torr.

For sfc measurements, Gilson 302 and 303 pumps were used with an Apple 2 as the system controller. Several chromatographic packed columns of various lengths (10, 15 and 25 m) were employed (Spherisorb amino, silica and cyano, 3 μm, 0.46 cm i.d.). A T-piece at the end of the chromatographic column split the eluent to the uv detector and to the spray deposition device of the moving belt interface.

As the mobile phase, methanol or methoxyethanol and carbon dioxide at various percentages and flow rates were used. Carbon dioxide was laboratory grade, supplied in cylinders with a dip tube.

Methanol and methoxyethanol were studied as modifiers, but the best separation was achieved under the following experimental conditions: column, amino (25 x 0.46 cm, 3 μm); mobile phase, initially carbon dioxide/methanol with a gradient from 5 to 12% of CH_3OH in 2 minutes, then isocratic carbon dioxide/methanol 88/12; flow rate, 3 ml/minute; pressure, 4000 psi; oven temperature, 70°.

Metastable ion studies and collisionally activated decomposition (CAD) mass analysed ion kinetic energy (MIKE) spectra [17] were performed on a VG ZAB 2F instrument [19] operating in EI conditions (70 eV, 200 μA, ion source temperature 200°). The same instrument was used to perform accurate mass measurements on the most significant ionic species present in each fraction. This was achieved by the peak matching technique at 10,000 resolving power (10% peak valley definition).

Results and Discussion.

The sfc/ms studies were initially performed by means of a moving belt interface, which allows the use of a number of ionization techniques [18]. Both gas phase EI and CI methods, but also desorption methods such as FAB [20], can be used. Although the moving belt system, introduced in the mid-1970s, was the first successful commercially available liquid chromatography-mass spectrometry (lc/ms) interface [21], the basic principle of current models remains unchanged and still uses a continuous polyimide belt on to which the eluate is deposited [14].

The EI spectra of the whole fractions, without any chromatographic separation, were recorded for preliminary information about the possible molecular species present in each alkaloid fraction by comparison with the data previously obtained [10,11]. Each fraction (methanol solution) was spotted on to the belt and the resulting spectra were very similar to those obtained by direct insertion probe [11], with abundant ionic species at m/z 222, 302 and 230 for fraction 1 and at m/z 252 for fraction 2.

The sfc/ms separation was performed by an amino column, this phase being highly effective for the chromatographic resolution of polar compounds. Preliminary experiments on a silica column led to a lower chromatographic resolution. The reconstructed total ion current (ric) traces obtained by EI sfc/ms on the two fractions are shown in Figure 1. Both fractions are multicomponent. For unequivocal molecular weight assignment of the different components, CI experiments were also undertaken.

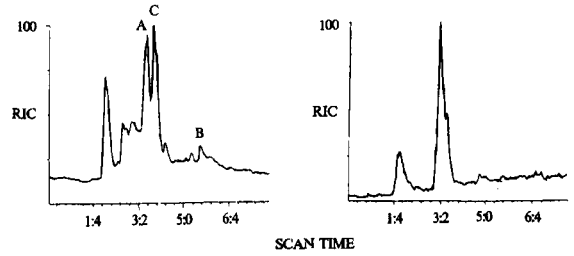


Figure 1. Reconstructed total ion current trace (ric) obtained by EI sfc/ms (moving belt) of fraction 1 (left) and fraction 2 (right); column, amino 3 μm (25 x 0.46 cm), mobile phase, carbon dioxide/methanol with gradient.

Table 1

Accurate Mass Measurements of the Most Significative Ions of Fraction 1 and 2 Respectively

m/z	Measured mass	Calculated mass	Elemental composition
	Fraction 1		
302	302.1058 (±0.002)	302.1052	$C_{19}H_{14}N_2O_2$
259	259.0875 (±0.002)	259.0869	$C_{17}H_{11}N_2O$
230	230.1410 (±0.002)	230.1415	$C_{14}H_{18}N_2O$
222	222.1159 (±0.002)	222.1154	$C_{15}H_{14}N_2$
215	215.1193 (±0.002)	215.1181	$C_{13}H_{15}N_2O$
197	197.1069 (±0.002)	197.1076	$C_{13}H_{13}N_2$
191	191.0732 (±0.002)	191.0733	$C_{14}H_9N$
187	187.1237 (±0.002)	187.1232	$C_{12}H_{15}N_2$
	Fraction 2		
252	252.1267 (±0.002)	252.1259	$C_{16}H_{16}N_2O$
237	237.1022 (±0.002)	237.1025	$C_{15}H_{13}N_2O$
221	221.1071 (±0.002)	221.1076	$C_{15}H_{13}N_2$
209	209.1075 (±0.002)	209.1076	$C_{14}H_{13}N_2$

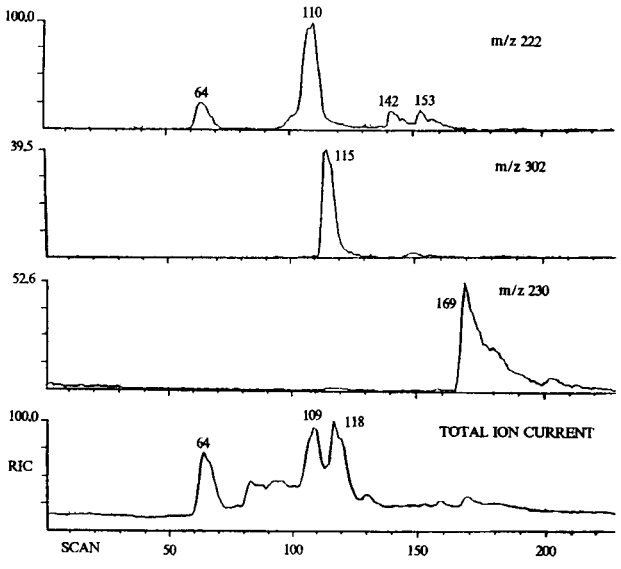


Figure 2. EI sfc/ms traces of fraction 1: ric and m/z 222, 302 and 230 traces.

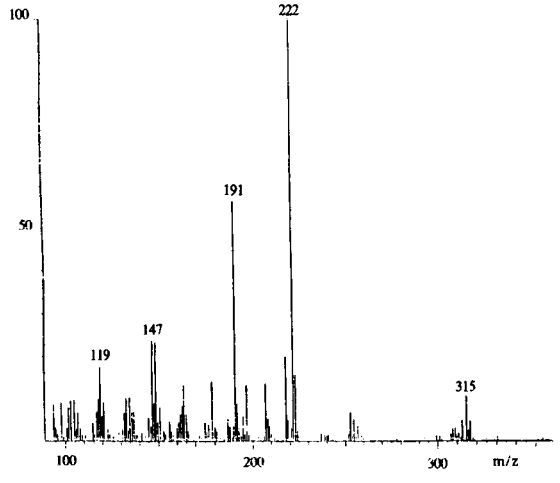


Figure 3. EI mass spectrum of peak A as obtained by sfc separation of fraction 1.

We focused our interest on the same ions already revealed by direct probe introduction of the whole fractions into the EI source [11] and for which the accurate masses reported in Table 1 were found. For fraction 1, the three most abundant components of the mixture lead to $M^{+\cdot}$ at m/z 222, 230 and 302. These results are clearly evidenced by the corresponding mass chromatogram (Figure 2). The related EI spectra are shown in Figures 3-5. CI measurements confirmed that these ions are molecular species as, in these experimental conditions, their mass values shift to m/z 223, 231 and 303.

Fraction 2 was studied with the same approach and, in EI conditions, the most abundant component shows a molecular ion at m/z 252 (Figure 6). In the CI spectrum, this turned out to be shifted to m/z 253.

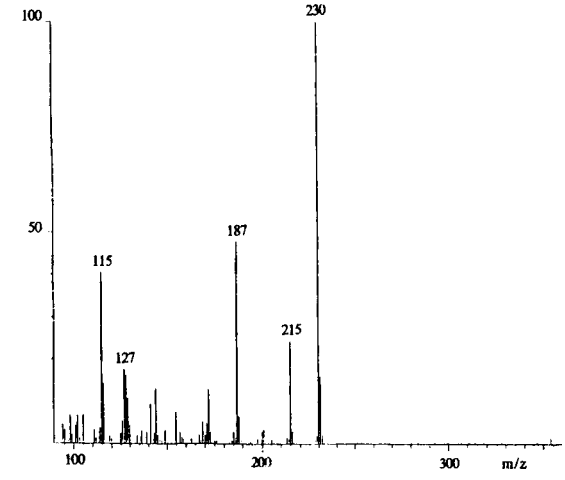


Figure 4. EI mass spectrum of peak B as obtained by sfc separation of fraction 1.

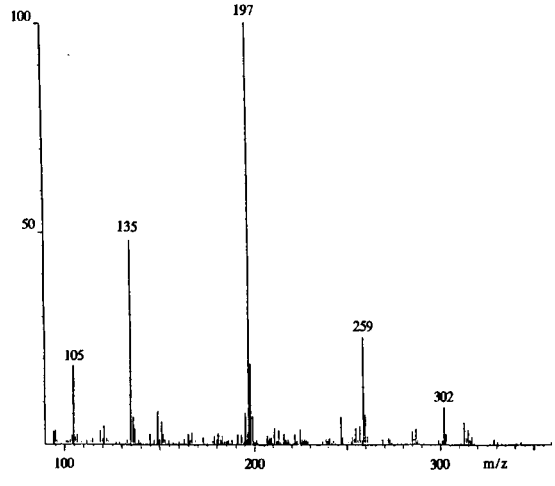


Figure 5. EI mass spectrum of peak C as obtained by sfc separation of fraction 1.

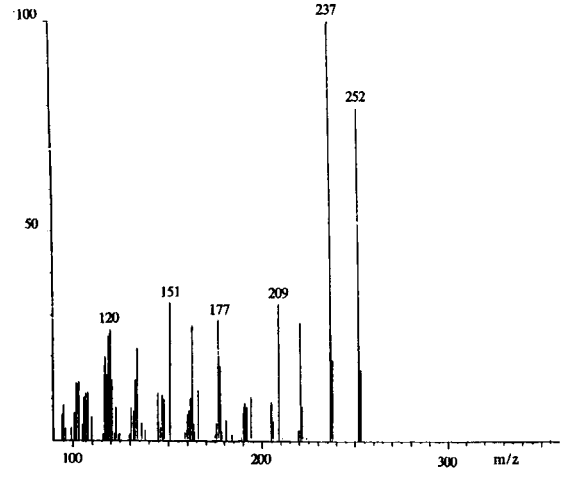
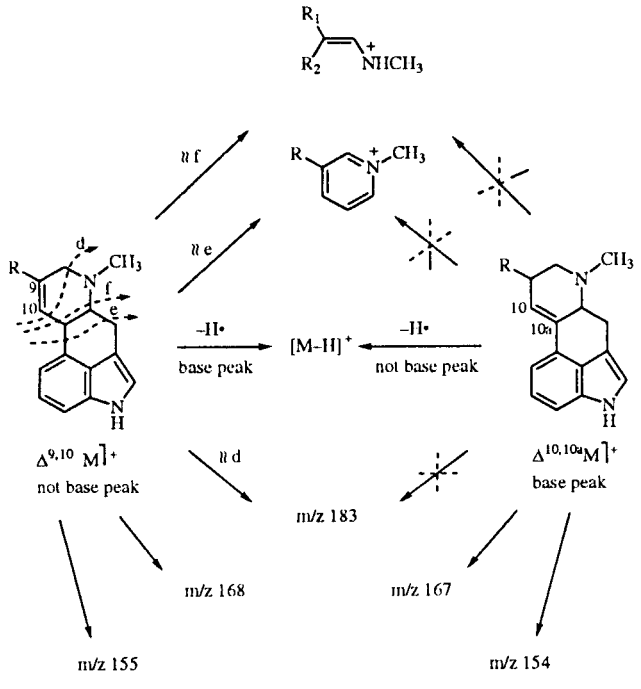


Figure 6. EI mass spectrum of compound with molecular ion at m/z 252 (Rt = 3.2 minutes) from fraction 2.

Schmidt and Maier [22] studied the mass spectra of a wide range of alkaloids and proposed some diagnostic ions characteristic for the ergoline skeleton. Comparing their data with others on ergot alkaloids [23,24], a general fragmentation pathway is proposed for clavine-type ergot alkaloids [11] (Scheme 1). Some diagnostic conclusions can

Scheme 1



be drawn from it, as follows:

1. If the base peak is $[M-H]^+$ ion, the double bond is usually in C(9)-C(10) position.
2. Fragment ions at m/z 155 and 168 are present only when ring D in the ergoline skeleton is in an open form, whereas ions at m/z 154 and 167 are present when all four rings are present.
3. Another diagnostic ion for the ergoline group (rings A, B, C) is m/z 183, frequently present when ring D is an open form.
4. Ions involving the R group may have variable values. They are common in clavine alkaloids with a double bond at C(9)-C(10).

This general information about diagnostic ions for ergot alkaloids may be successfully used to interpret mass spectra for both the fractions analysed and described here. The sfc/ms data indicate that the ergoline skeleton can be considered only for a few components of the mixture.

In particular:

Fraction 1.

We focused on the three main components (A, B and C) evidenced by the supercritical fluid chromatogram, *i.e.* those leading to peaks A, B and C in Figure 2. In particular, component A leads to the mass spectrum reported in Figure 3. The corresponding CI spectrum mainly shows an abundant ion at m/z 223, so that a molecular weight of 222 Da can be assigned to component A. Consequently, an even number of nitrogen atoms must be present in the structure. As confirmed by CAD MIKE data (Figure 7), ions at m/z 207 and 191 originate from molecular ions at m/z 222 through losses of neutral species of 15 and 31 Da. Interestingly, in the EI mass spectrum of pure lysergol (Figure 8), abundant ions at m/z 223, 207 and 192 are present. This analogy suggests that component A has a different structure, but close to the fragment at m/z 223 described in the EI mass spectrum of lysergol the difference possibly being in the presence of a double bond between C(8)-(9). This proposed structure fits the observed fragmentation pattern, which is different from but similar to that of ionic species at m/z 223 from lysergol, as described in Scheme 2.

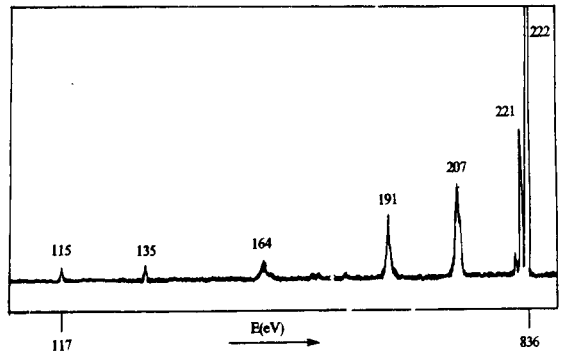


Figure 7. CAD MIKE spectrum of ionic species at m/z 222.

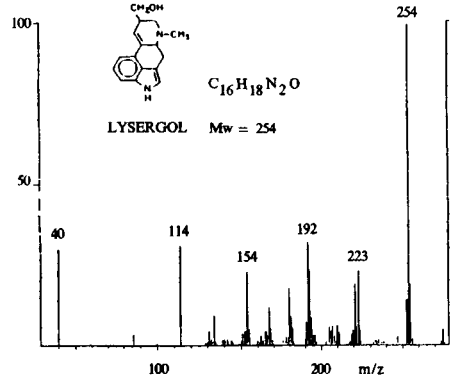
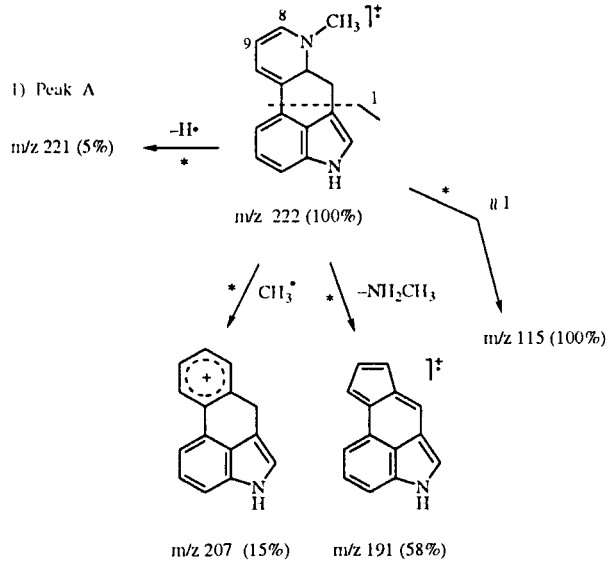
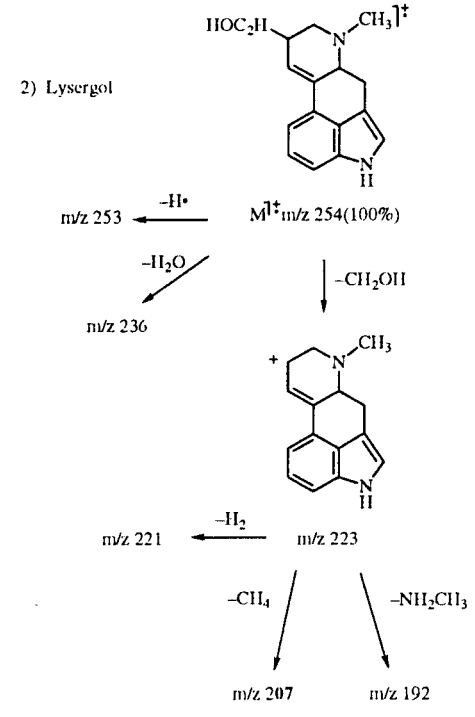


Figure 8. EI mass spectrum of lysergol standard and related fragmentation pathway.

The EI mass spectrum of component C is reported in Figure 5. Again, its comparison with CI data leads to identification of molecular weight at m/z 302. Accurate mass measurements gave a value of 302.1058 (±0.002 Da), in agreement with an elemental formula $C_{19}H_{14}N_2O_2$ (calculated mass: 302.1052 Da). Its MIKE spectrum (Figure 9) shows the main unimolecular fragmentation products at m/z 287 and 285, together with less abundant ionic species

Scheme 2





at m/z 272, 258, 242, 228 and 207. Ions at m/z 135 and 197, the most abundant species in the EI spectrum (Figure 5), are completely absent in the MIKE spectrum of $M^{+•}$, and consequently must be considered either as products of secondary fragmentation processes or due to other species present in the unresolved sfc peak.

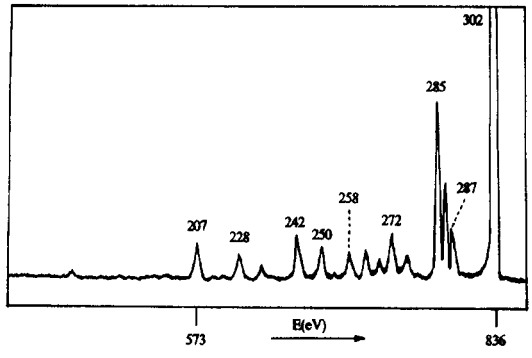


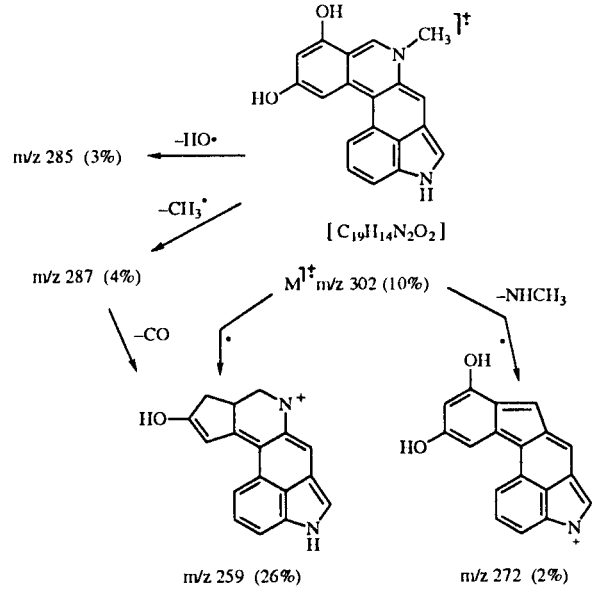
Figure 9. CAD MIKE spectrum of ionic species at m/z 302.

The higher elution time of C with respect to A further suggests its higher polarity.

On the basis of these experimental data, for C we propose the tentative structure shown in Scheme 3, which fits the observed fragmentation pathways and the diagnostic behaviour described above for ergot alkaloids.

The same approaches were employed in the structural assignment of component B. The related EI mass spectrum (Figure 4) and CI measurements led to a molecular weight assignment of 230 Da. Observed losses of 15 and 43

Scheme 3

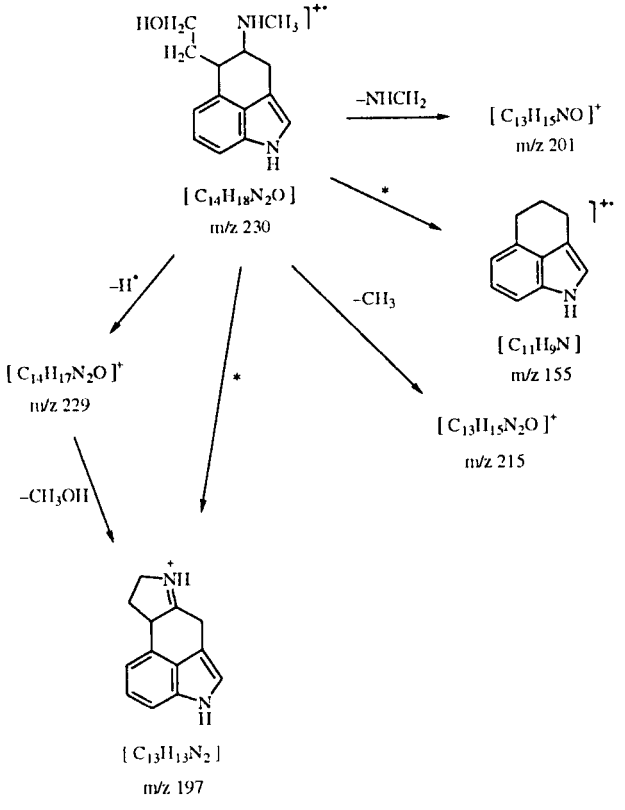


Da, confirmed by MIKE, strongly suggest the presence of an acetyl moiety, absent in components A and C. The proposed structure, together with the relative fragmentation pattern, is shown in Scheme 4.

Fraction 2.

As may be seen from the Figure 1, fraction 2 mainly consists of only one component, whose EI spectrum is reported in Figure 6. CI data confirmed the species at m/z

Scheme 4



252 as the molecular ion. However, in this case, there are no ions diagnostic for the presence of the ergoline skeleton, throwing doubt on whether the compound belongs to this class of alkaloids.

In conclusion, the use of sfc/ms provided more information on the class of alkaloids present in the methanol extracts of the roots of *Securidaca Longipedunculata Fres.*, confirming preliminary data from both EI and FAB mass spectrometric measurements only, and giving structural information on another alkaloid component.

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REFERENCES AND NOTES

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[2] H. Budzikiewicz, D. Djerassi and D. H. Williams, Structure Elucidation of Natural Products by Mass Spectrometry, Vol **1**, Alkaloids, Holden-Day, San Francisco, 1964, pp 233.

[3] S. D. Sastry, *Biochem. Appl. Mass Spectrom.*, 655 (1972).

[4] M. Hesse, Fortschritte der Massenspektrometrie: Indolalkaloide, Bd 1 and Bd 2, Verlag Chemie, Weinheim, 1974.

[5] M. Hesse and H. O. Bernhard, Fortschritte der Massenspektrometrie, Bd 3, Alkaloide ausser Indol-, Triterpen- und Stereoidalkaloide, Verlag Chemie, Weinheim, 1975.

[6] S. D. Sastry and K. M. Madyastha, *Biochem. Appl. Mass Spectrom.*, 1st Suppl Vol, 751 (1980).

[7] M. Hesse, *Biochem. Appl. Mass Spectrom.*, 1st Suppl Vol, 797 (1980).

[8] H. Budzikiewicz, *Mass Spectrom. Rev.*, **1**, 125 (1982).

[9] C. Costa, A. Bertazzo, M. Biasiolo, G. Allegri, O. Curcuruto and P. Traldi, *Org. Mass Spectrom.*, **27**, 255 (1992).

[10] C. Costa, A. Bertazzo, O. Curcuruto and P. Traldi, *IL Farmaco*, **47**, 121 (1992).

[11] C. Costa, A. Bertazzo, G. Allegri, O. Curcuruto and P. Traldi, *J. Heterocyclic Chem.*, **29**, 1641 (1992).

[12] R. M. Smith, Supercritical Fluid Chromatography, RSC Chromatography Monographs, R. M. Smith, ed, London, 1988, pp 238.

[13] A. J. Berry, D. E. Games, I. C. Mylchreest, J. R. Perkins and S. Pleasance, *Biomed. Environ. Mass Spectrom.*, **15**, 105 (1988).

[14] D. E. Games, A. J. Berry, I. C. Mylchreest, J. R. Perkins and S. Pleasance, *Europ. Chromat. News*, **11**, 10 (1987).

[15] A. J. Berry, D. E. Games and J. R. Perkins, *J. Chromatogr.*, **363**, 147 (1986).

[16] A. J. Berry, D. E. Games and J. R. Perkins, *Anal. Proc.*, **23**, 451 (1986).

[17] R. G. Cooks, in Collisional Spectroscopy, R. G. Cooks, ed, Plenum Press, New York, 1978, pp 458.

[18] T. Van der Greef, A. C. Tas, M. C. Ten Noever De Brauw, M. Hohn, G. Meijerhoff and U. Rapp, *J. Chromatogr.*, **323**, 81 (1985).

[19] R. P. Morgan, J. H. Beynon, R. A. Bateman and B. N. Green, *Int. J. Mass Spectrom. Ion Phys.*, **28**, 171 (1978).

[20] M. Barber, R. S. Bordoli, R. D. Sedgwick and A. N. Tyler, *J. Chem. Soc., Chem. Commun.*, 325 (1981).

[21] P. Arpino, *Mass Spectrom. Rev.*, **8**, 35 (1989).

[22] J. Schmidt and W. Maier, *Biomed. Mass Spectrom.*, **11**, 290 (1984).

[23] C. Eckers, D. E. Games, D. N. B. Mallen and B. P. Swann, *Biomed. Mass Spectrom.*, **9**, 162 (1982).

[24] J. Schmidt, R. Kraft and D. Voigt, *Biomed. Mass Spectrom.*, **5**, 674 (1978).