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Key fragmentation patterns of aporphine alkaloids by electrospray ionization with multistage mass spectrometry

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This work reports a detailed study of the fragmentations of aporphine alkaloids by electrospray ionization with multistage mass spectrometry (ESI-MS^n) in positive mode. In a first step the loss of the amino group and its substituent is observed. Further steps display the loss of the peripheral groups. Losses of methanol and CO are observed if an OH is vicinal to an OCH_3 on the aromatic ring. Otherwise the spectra show radical losses of $CH_3^{\cdot}$ or $CH_3O^{\cdot}$ as the main fragmentations. If a methylenedioxy group is present losses of formaldehyde followed by CO are observed. These fragmentations yield important information on the structures of aporphines. Copyright © 2004 John Wiley & Sons, Ltd.

The resurgence of interest in the plant kingdom as a possible source of new therapeutic compounds has prompted the need for modern mass spectrometric methods which enable a rapid characterization of these compounds. Alkaloids are a rich source of bioactive products, and isoquinoline compounds exhibit a wide range of biological activities.[1] Among these isoquinoline derivatives, aporphinoids appear to be increasingly investigated for biological activities.[2] Recently, we demonstrated that neolitsine, dicentrine, cassythine and actinodaphnine, which were all isolated from *Cassytha filiformis*, showed cytotoxic activities against different cancer cell lines *in vitro*, but these molecules also possess other properties.[3,4]

Electron impact (EI) ionization mass spectrometry has been extensively used for structural elucidation of aporphine alkaloids.[5,6] To our knowledge, no publication deals with fast atom bombardment (FAB) analysis of isoquinoline alkaloids of this type, and only one publication reports on chemical ionization (CI).[7] Electrospray ionization (ESI) is currently one of the most used ionization methods because it allows analysis of non-volatile compounds, and produces multiply charged ions from high molecular weight compounds. Furthermore, as an atmospheric pressure source, it is easy to couple directly to liquid chromatography (LC). ESI spectra usually display a low degree of fragmentation and thus the technique is best used with tandem mass spectrometry (MS^n).

Fragmentations observed by ESI-MS^n may be different from those observed with other mass spectrometric methods, and it is important to understand these fragmentation mechanisms for the identification of such compounds. This work analyzes fragmentations of aporphine alkaloids by ESI-MS^n. The results obtained could help in identifying pure aporphine alkaloids or those separated and analyzed by LC/MS from crude extracts. To our knowledge the only report on LC/ESI-MS of these compounds is due to Fabre *et al.*,[8] who analyzed a semi-purified methanolic extract of *Eschscholtzia californica* for a direct and rapid characterization of isoquinoline alkaloids that included two aporphines. The aim of the present work is to propose general rules for the fragmentations of aporphines as a basis for the elucidation of their structures by ESI-MS^n.

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EXPERIMENTAL

Samples

Five *S*-aporphine alkaloids (**1**–**5**, Fig. 1) were isolated from *Cassytha filiformis* L. The isolation and structural determinations of four of them (**2**–**5**) have been described in detail previously.[3] The isolation of norneolitsine (**1**) followed the same purification procedure; high-speed counter-current chromatography (HSCCC) fractions containing **1** were purified on a Toyopearl® HW-40S (TosoHaas) column with ethanol and finally on a Merck Lobar® LiChroprep® RP-18 column with MeOH/CH_3CN/H_2O (3:2:1) as mobile phase. The structure was established by combining MS and NMR spectral analysis and comparison with literature data.[9]

Four additional *S*-aporphine alkaloids (**6**–**9**, Fig. 1) were purchased: bulbocapnine·HCl and isocorydine·HCl were obtained from ICN (Aurora, OH, USA); boldine from Federa (Brussels, Belgium); and glaucine·HCl and *R*-dicentrine from Sequoia Research (Oxford, UK).

Apparatus

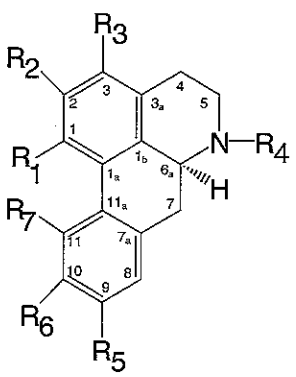
Mass spectra were acquired using an LCQ DECA XP^{Plus} ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an ESI source in the positive ion mode. The operating parameters were as follows: the spray needle voltage was set at 4.5 kV and the spray was stabilized with a nitrogen sheath gas (35 psi). The ES capillary voltage was 15 V and the capillary temperature was 200°C. For MS^n, collisional activation was obtained by setting the secular RF amplitude to 33% of its maximum (5 V). A syringe pump delivering 3 μL min^{-1} was used for the direct loop injections of pure compounds, tested in the same concentration range (about 0.1 mg/mL) dissolved in a mixture of 10% CH_2Cl_2 and 90% MeOH. High-resolution mass accurate mass measurements for cassythine (**5**) were made using a MAT95XP-TRAP double-focusing/ion trap hybrid (ThermoFinnigan MAT, Bremen, Germany) in ESI mode. Exact mass measurements were performed by peak matching using polyethyleneimine as reference compound, with a resolution of 24 000 (10% valley definition).

RESULTS AND DISCUSSION

All ESI spectra showed $[M+H]^+$ ions as the main peak (Table 1). Dimers were sometimes observed but we focused on fragmentations. The sole important fragment ion in the ESI mass spectra was always observed at either $[M+H-17]^+$ or $[M+H-31]^+$ depending on the nature of substituent of the amino group. These fragments were attributed to the loss of NH_3 (17 Da) or CH_3NH_2 (31 Da). These assignments were confirmed by the loss of 17 Da observed for norneolitsine (**1**) that lacks an OH group, and by high-resolution accurate mass measurement of the $[M+H-17]^+$ peak for cassythine (**5**) that does contain an OH group (Table 2). Proposed fragmentation pathways for this step are described in Scheme 1. Pathway **a** seems more favorable since the intermediate so obtained has a more extended π-conjugation.

Except for the molecular mass and the nature of the N-substituent, the ESI-MS spectra failed to give other structural information, as no other major fragmentations were observed. In order to further characterize aporphines we performed MS^n analyses to obtain more structural information. MS^2 spectra of the $[M+H]^+$ ions also indicate the loss of 17 or 31 Da as in the MS^1 spectra. In addition, depending on the substituents, minor losses of 30, 32, 15 or 31 Da may be observed.

MS^3 spectra of $[M+H-RNH_2]^+$ displayed loss patterns that are dependent on the type of substituents on the aromatic rings, which can lead to the proposal of general rules for the



Compounds	Mw	R_1	R_2	R_3	R_4	R_5	R_6	R_7
1 NORNEOLITSINE	309	-O-CH_2-O-		H	H	-O-CH_2-O-		H
2 NEOLITSINE	323	-O-CH_2-O-		H	CH_3	-O-CH_2-O-		H
3 DICENTRINE	339	-O-CH_2-O-		H	CH_3	O-CH_3	O-CH_3	H
4 ACTINODAPHNINE	311	-O-CH_2-O-		H	H	OH	O-CH_3	H
5 CASSYTHINE	341	-O-CH_2-O-		O-CH_3	H	OH	O-CH_3	H
6 BOLDINE	327	O-CH_3	OH	H	CH_3	OH	O-CH_3	H
7 BULBOCAPNINE	325	-O-CH_2-O-		H	CH_3	H	O-CH_3	OH
8 ISOCORYDINE	341	O-CH_3	O-CH_3	H	CH_3	H	O-CH_3	OH
9 GLAUCINE	355	O-CH_3	O-CH_3	H	CH_3	O-CH_3	O-CH_3	H

Figure 1. Structures of the alkaloids.

Table 1. Major ions observed by positive ESI-MS of aporphines and main fragment ions observed in MS^n from the base peak or major fragment MS^{n-1}

Compounds [Mw]	MS	MS^2	MS^3	MS^4	MS^5	MS^6
1	310 (100)	310 (1)	293 (4)	263 (31)	235 (42)	205 (0)
NORNEOLITSINE	293 (7)	293 (100)	263 (100)	235 (100)	205 (100)	177 (100)
[309]				233 (60)	177 (12)	
∇				205 (10)		
2	324 (100)	324 (0)	293 (6)	263 (29)	235 (25)	205 (44)
NEOLITSINE	293 (6)	293 (100)	263 (100)	235 (100)	205 (100)	177 (100)
[323]				233 (52)	177 (5)	
∇				205 (7)		
3	340 (100)	340 (1)	309 (6)	279 (39)	251 (100)	236 (67)
DICENTRINE	309 (6)	309 (100)	279 (100)	264 (24)	236 (53)	235 (16)
[339]		279 (13)		251 (100)	220 (52)	221 (100)
∇○						208 (36)
						207 (31)
4	312 (100)	312 (0)	295 (7)	265 (37)	237 (34)	205 (18)
ACTINODAPHNINE	295 (6)	295 (100)	265 (100)	250 (8)	222 (14)	177 (100)
[311]			263 (4)	237 (100)	205 (100)	
∇◆				233 (58)	177 (10)	
				205 (10)		
5	342 (100)	342 (4)	325 (9)	295 (100)	263 (79)	235 (100)
CASSYTHINE	295 (7)	325 (100)	310 (9)	280 (33)	235 (100)	220 (59)
[341]			295 (100)	279 (12)	248 (7)	207 (15)
∇○◆			267 (11)	267 (59)		205 (11)
				263 (75)		179 (11)
				235 (10)		
6	328 (100)	328 (6)	297 (1)	265 (23)	237 (48)	205 (19)
BOLDINE	297 (2)	297 (100)	265 (100)	237 (100)	222 (28)	177 (100)
[327]		265 (63)		233 (10)	205 (100)	
◆				205 (5)	177 (6)	
7	326 (100)	326 (0)	295 (11)	263 (78)	235 (100)	205 (11)
BULBOCAPNINE	295 (3)	295 (100)	265 (47)	235 (100)	205 (70)	177 (100)
[325]		263 (13)	263 (100)		177 (4)	
∇◆		265 (5)	235 (55)			
			233 (9)			
8	342 (100)	342 (2)	311 (1)	279 (5)	264 (100)	236 (100)
ISOCORYDINE	311 (2)	311 (100)	296 (13)	264 (100)	247 (7)	206 (43)
[341]		279 (57)	279 (100)	251 (9)	236 (82)	178 (7)
○◆				248 (18)	234 (19)	
					233 (9)	
9	356 (100)	356 (1)	325 (2)	294 (36)	279 (12)	251 (100)
GLAUCINE	325 (2)	325 (100)	310 (68)	279 (100)	251 (100)	236 (71)
[355]		294 (14)	294 (100)	251 (5)	236 (18)	220 (13)
○		310 (9)			223 (9)	219 (12)

Values are *m/z*, relative abundances are in parentheses.
∇—Compound containing a methylenedioxy group.
◆—Compound containing both a hydroxy group and a methoxy in vicinal positions.
○—Compound containing one or more methoxy groups non vicinal to an OH or lacking an OH substituent

main fragmentations. Nevertheless, other minor fragmentations may also be observed (Table 1).

- Compounds with a methylenedioxy group always lose a CH_2O followed by a CO loss.
- Compounds containing both a hydroxy and a methoxy group in vicinal positions lose CH_3OH followed by CO.
- Compounds containing one or more methoxy groups non-vicinal to an OH or lacking an OH substituent will display, after the loss of the amino group, radical losses of $CH_3^{\bullet}$ or $OCH_3^{\bullet}$ in competition with the previously described fragmentation rules. These radical losses may also be observed as minor fragmentations when a methoxy group is vicinal to an OH.

Table 2. High-resolution accurate mass data for cassythine (**5**)

	Mw
$[M+H-17]^+$ observed	325.1074
$[M+H-17]^+$ calculated for $-NH_3$	325.1076
$[M+H-17]^+$ calculated for $-OH$	325.1314

All these losses were observed only from the $[M+H-RNH_2]^+$ ion. These fragmentations may be parallel or consecutive, but a CO loss was never observed directly from the $[M+H-RNH_2]^+$ fragment. Further fragmentations following the same general rules were observed in MS^n spectra of the major fragments from MS^{n-1}.

An example of a compound containing methylenedioxy groups is norneolitsine (**1**), for which a fragmentation mechanism is proposed in Scheme 2. The first loss observed is that of NH_3, then formaldehyde and CO as the main pathway.

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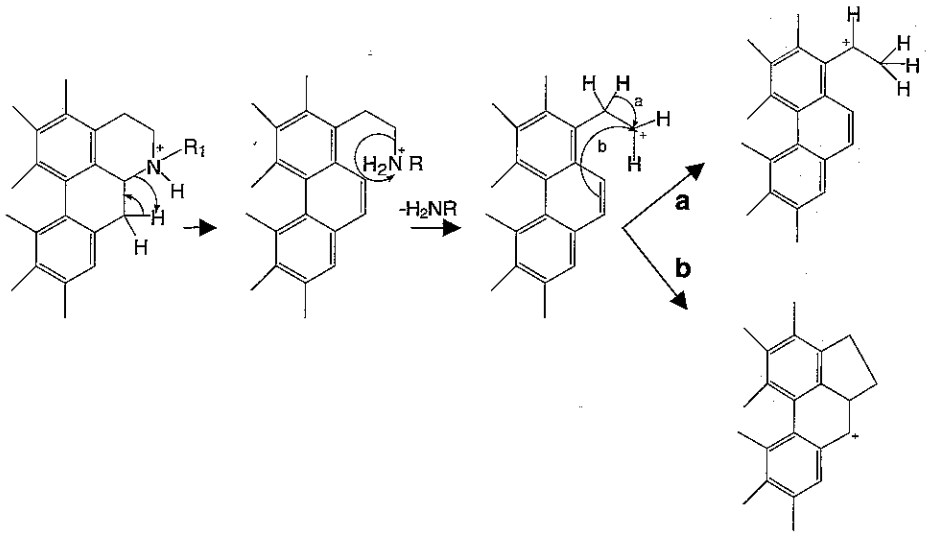
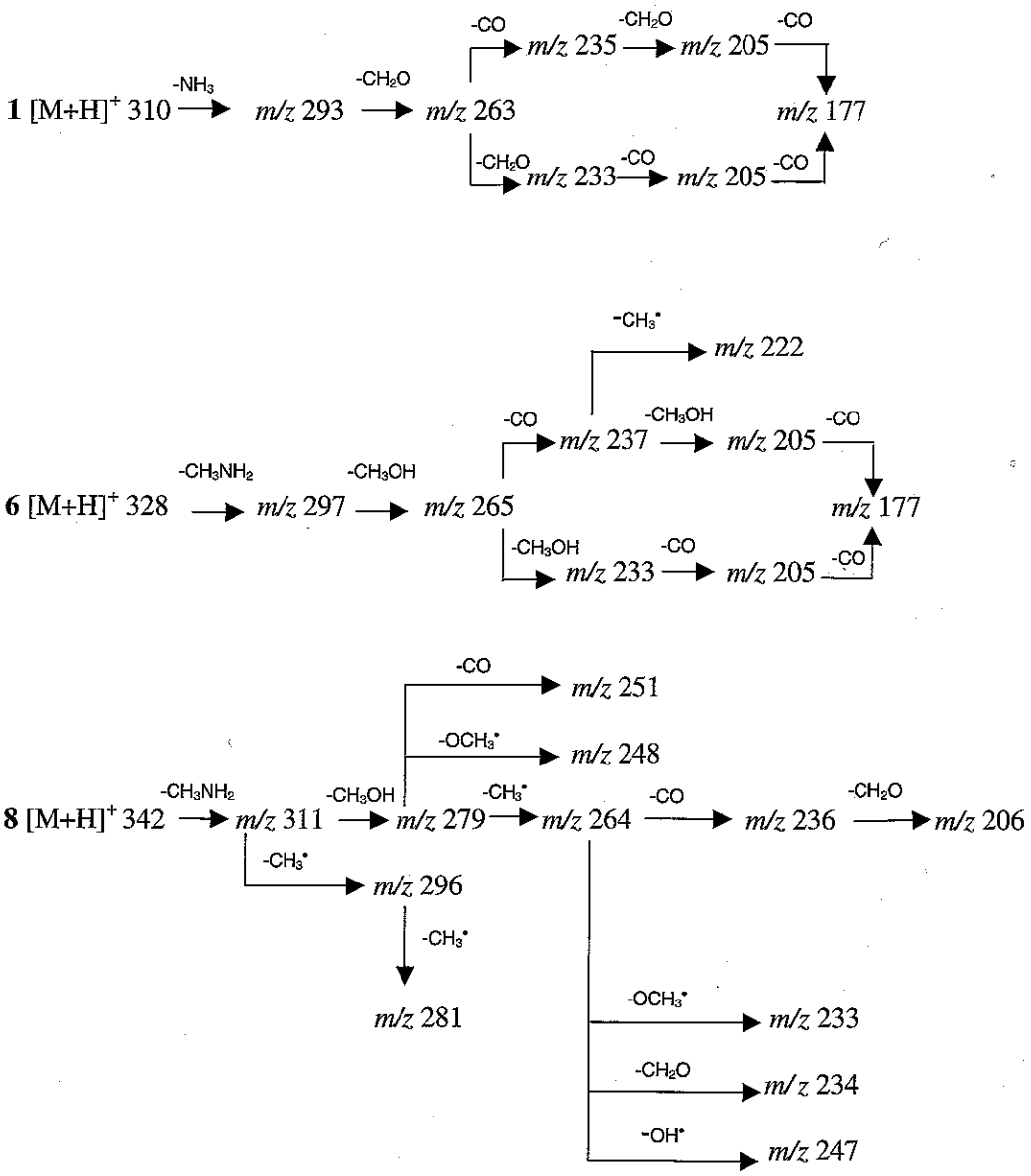


Scheme 1. Fragmentation pathways proposed for the loss of RNH_2.

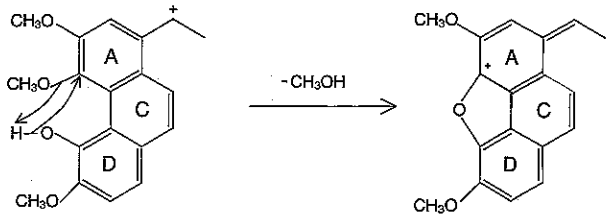


Scheme 2. Most important observed fragmentations for compounds **1**, **6** and **8**; arrows indicate fragmentations (MS^n) of the previous fragment (MS^{n-1}).

The loss of the second molecule of formaldehyde from $[M+H-NH_3-CH_2O]^+$ is observed as a minor pathway competing with the first loss of CO. Fragments obtained followed the general rules with sequential losses of formaldehyde and/or CO leading to an ion at *m/z* 177. This fragment at *m/z* 177 is the result of the loss of all the peripheral groups and is frequently observed for the aporphines we analyzed in this work. Nevertheless, a fragment at *m/z* 177 may also be observed for other benzylisoquinolines such as pavine.[8] Proposed mechanistic pathways are described in Scheme 3. The same pathways were observed for neolitsine (**2**).

Another typical example is a compound containing both a hydroxy and a methoxy group in vicinal positions, e.g., boldine (**6**) (Scheme 2). The $[M+H-CH_3NH_2]^+$ fragment ion lost a molecule of methanol, then CO, another methanol, and finally another CO, in the major pathway. Here again the loss of a second methanol molecule instead of the first CO loss is observed as an alternative fragmentation.

Examples of compounds possessing, among their substituents, methoxy groups non-vicinal to a hydroxy group or without an OH substituent are dicentrine (**3**), isocorydine (**8**), glaucine (**9**) and cassythine (**5**) (Table 1). A particular behavior is observed for isocorydine (**8**): the fragment obtained after the loss of CH_3NH_2 can lose MeOH by two different pathways. The first one is the pathway described above, i.e., loss of MeOH from a methoxy group vicinal to an OH. However, the accompanying abundant loss of CO is not observed. Another fragmentation pathway seems to be more important with the formation of an assigned furan-type intermediate, as shown in Scheme 4. CO cannot now be lost as the oxygen atom is now included in an aromatic ring. This intermediate possesses two isolated methoxy groups and

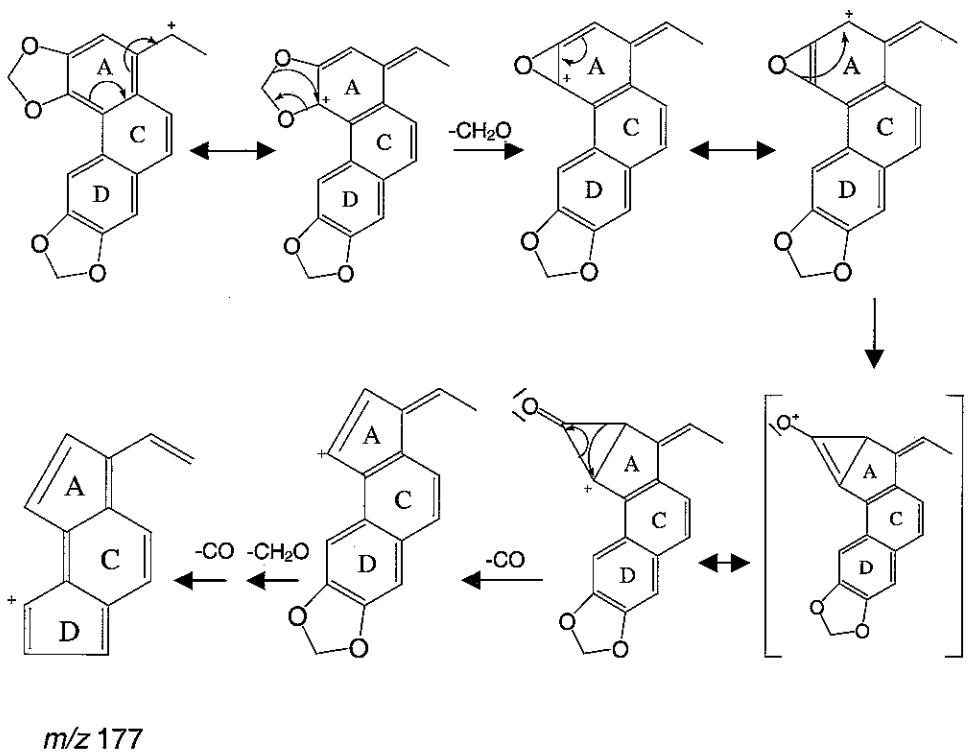


Scheme 4. Proposed MS^n mechanistic pathway for isocorydine (**8**).

will undergo typical fragmentations with losses of $CH_3^{\bullet}$ and $CH_3O^{\bullet}$ radicals. This second pathway is predominant as indicated by the major loss of $CH_3^{\bullet}$ from the fragment at *m/z* 279 (Table 1, Scheme 2). This unexpected radical loss from an even-electron precursor may be rationalized by the higher stability of the furan intermediate. For glaucine (**9**), after the loss of methylamine we observed only competition between losses of $CH_3^{\bullet}$ or $CH_3O^{\bullet}$ with relative abundances of 68 and 100%, respectively (Table 1).

Fragmentations of bulbocapnine (**7**) and actinodaphnine (**4**) also followed the general rules in their main fragmentation pathways. Moreover the fragmentations of dicentrine (**3**), possessing a methylenedioxy and two vicinal methoxy groups, showed the competition between the loss of CO and radical losses from the fragment ion $[M+H-CH_3NH_2-CH_2O]^+$, as described above. The spectra of the two enantiomers of dicentrine (**3**) give similar results, as expected, indicating that fragmentation is independent of the configuration of C_{6a}.

The fragmentations reported by Fabre *et al.*[8] for two aporphines are consistent with our rules, described above.



Scheme 3. Proposed ESI-MS^n mechanistic pathways for fragmentation of $[1+H-NH_3]^+$.

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CONCLUSIONS

A comparison of EI and ESI mass spectra shows that ESI always produce $[M+H]^+$ as the main peak while, in EI, the molecular species is the radical cation which leads to abundant fragmentations (losses of $H^\bullet$, $CH_3^\bullet$, $CH_3O^\bullet$ and $OH^\bullet$ are important reactions). The loss of $H^\bullet$ often provides the base peak of the EI spectrum.[10] The presence of methylenedioxy groups induces the loss of formaldehyde, and fragments corresponding to $[M-CH_2NR]^+$ (R=CH_3 or H) are also observed and, when identified, give information about the substitution of the amine group.[5,6]

In contrast, the ESI mass spectrum (MS^1) displays only the loss of the amino group with its substituent as low-intensity fragment ion peaks. MS^n must be used to obtain more information, and identifies the nature of the substituent on the amino group and the peripheral substituents on the aromatic rings. Thus ESI-MS^n provided valuable information about the substitution of the amine, the presence (or not) of a methylenedioxy group, the presence (or not) of aromatic OCH_3 substituents, and the presence (or not) of an OH vicinal to OCH_3 on the aromatic ring. No information on the correct positions of these substituents on the aromatic rings and on the stereochemistry of C_{6a} can be deduced from ESI-MS^n. Nevertheless, our work can be used to obtain structural information during analysis of aporphines or other benzylisoquinolines by LC/MS in crude plant extracts.

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REFERENCES

1. Bruneton J. *Pharmacognosie-Phytochimie-Plantes médicinales* (3rd edn). Tec & Doc: Paris, 1999; 890–947.
2. Boustie J, Stigliani JL, Montanha J, Amoros M, Payard M, Girre L. *J. Nat. Prod.* 1998; **61**: 480.
3. Stévigny C, Block S, De Pauw-Gillet MC, de Hoffmann E, Llabrès G, Adjakidjé V, Quetin-Leclercq J. *Planta Med.* 2002; **68**: 1042.
4. Yang-Chang W, Fang-Rong C, Ya-Chieh C, Che-Ming T. *Phytother. Res.* 1998; **12**: S39.
5. Jackson HA, Martin JA. *J. Chem. Soc. C* 1966; 2181.
6. Ba Hung P, Seguin E, Tillequin F, Koch M. *Phytochemistry* 1994; **35**: 1363.
7. Fales HM, Lloyd HA, Milne WA. *J. Am. Chem. Soc.* 1970; **92**: 1590.
8. Fabre N, Claparols C, Richelme S, Angelin ML, Fourasté I, Moulis C. *J. Chromatogr. A* 2000; **904**: 35.
9. Johns SR, Lamberton JA. *Aust. J. Chem.* 1966; **19**: 297.
10. Shamma M. *The Isoquinoline Alkaloids*. Academic Press: New York, 1972; 219.