J. Braz. Chem. Soc., Vol. 16, No. 6B, 1431-1438, 2005.
Printed in Brazil - ©2005 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Electrospray Ionization Mass Spectrometry Screening of Piperidine Alkaloids from *Senna spectabilis* (Fabaceae) Extracts: Fast Identification of New Constituents and Co-metabolites

Marcos Pivatto[a], Antônio E. M. Crotti[b], Norberto P. Lopes[c], Ian Castro-Gamboa[a], Amanda de Rezende[a], Cláudio Viegas Jr.[a], Maria Claudia M. Young[d], Maysa Furlan[a] and Vanderlan S. Bolzani[*,a]

[a] *Instituto de Química, Universidade Estadual Paulista, CP 355, 14801-970 Araraquara - SP, Brazil*

[b] *Núcleo de Pesquisas em Ciências Exatas e Tecnológicas, Universidade de Franca, Av. Dr. Armando Salles de Oliveira 201, 14404-600 Franca - SP, Brazil*

[c] *Departamento de Física e Química, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Via do Café s/n, 14040-903 Ribeirão Preto - SP, Brazil*

[d] *Seção de Fisiologia e Bioquímica de Plantas, Instituto de Botânica, CP 4005, 01061-970 São Paulo - SP, Brazil*

O padrão de fragmentação de uma série homóloga de alcalóides piperidínicos isolados de *Senna spectabilis* foi investigado utilizando-se espectrometria de massas *tandem* com ionização por *electrospray* (ESI-EM/EM). A análise dos extratos EtOH e frações das flores e dos frutos de *S. spectabilis* por ESI-EM e ESI-EM/EM permitiu elucidar a estrutura de quatro componentes pertencentes a uma série homóloga destes alcalóides, bem como de quatro co-metabólitos inéditos. A elucidação estrutural dos co-metabólitos, com base no padrão de fragmentação de alcalóides piperidínicos previamente isolados, e confirmados pelos dados de EM de alta resolução, demonstra a importância desta técnica para a determinação do "perfil metabolômico" de uma espécie de importância farmacológica.

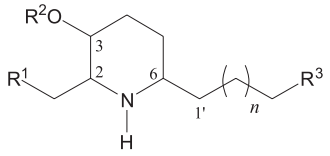
The fragmentation pattern of a homologous series of piperidine alkaloids isolated from *S. spectabilis* was investigated using electrospray ionization tandem mass spectrometry (ESI-MS/MS). The ESI-MS and ESI-MS/MS analyses of EtOH extracts and fractions from flowers and fruits of *S. spectabilis* allowed to elucidate the structures of four new compounds. The identification of these co-metabolites, based on the fragmentation patterns of previously isolated compounds, and further confirmed by accurate mass spectrometry defines this technique as a powerful tool to determine the "metabolomic profile" of species which has pharmacological importance.

Keywords*: Senna spectabilis*, piperidine alkaloids, metabolomic profile, electrospray, tandem mass spectrometry

Introduction

Senna spectabilis (synonym of *Cassia spectabilis*) is an arborous species of Fabaceae (subfamily Caesalpiniaceae). In Brazil, many species of both *Cassia* and *Senna* are used as ornamental plants due to their beautiful flowers. Some species of are also commonly used in Africa and Asia countries, mainly in India and China, as traditional medicine formulations for treatment of several diseases, alternatively to the conventional allopathic medicine.[1,2] Phenolic compounds have been proposed to be responsible for several pharmacological properties attributed to these species, such as anti-inflammatory, analgesic, laxative, antimicrobial and antiulceral.[3] Recently, leaves and flowers of *S. spectabilis* were reported to be valuable sources of bioactive piperidine alkaloids (Figure 1). Recently the cytotoxicity of these metabolites was reported using mutant strains of *Saccharomyces cerevisiae*, which is indicative of their potential antitumoral activity.[3] Interestingly, further assays with these alkaloids demonstrated significant antinociceptive activity, and also revealed them as acetylcholinesterase inhibitors.[4,5] Also, it was attributed to piperidine alkaloids, purgative,[6,7] and antimicrobial[8] activities, as well as the ability for specific inhibition of superoxide macrofages.[9] Considering

* e-mail: bolzaniv@iq.unesp.br



	R^1	R^2	R^3	*n*	MW	Configuration
1	H	H	-$COCH_3$	8	297	Not assigned
2	H	H	-$COCH_3$	10	325	2*R*, 3*R*, 6*S*
3	H	H	-$COCH_3$	10	325	2*R*, 3*R*, 6*R*
4	OH	H	-$COCH_3$	8	313	Not assigned
5	OH	H	-$COCH_3$	10	341	2*R*, 3*R*, 6*S*
6	H	-$COCH_3$	-$COCH_3$	8	339	Not assigned
7	H	-$COCH_3$	-$COCH_3$	10	367	2*R*, 3*R*, 6*S*
8	H	H	-$CH(OH)CH_3$	8	299	Not assigned
9	H	H	$CH(OH)CH_3$	10	327	2*R*, 3*R*, 6*S*
10	H	*p*-coumaroyl	-$COCH_3$	8	443	Not assigned
11	H	*p*-coumaroyl	-$COCH_3$	10	471	Not assigned
12	H	feruloyl	-$COCH_3$	8	473	Not assigned
13	H	feruloyl	-$COCH_3$	10	501	Not assigned

Figure 1. Structure of alkaloids isolated from *S. spectabilis*.

these remarkable pharmacological properties, there is an interest in searching for further piperidine alkaloids from *S. spectabilis*, including major and minor alkaloid co-metabolites. However, these alkaloids are biosynthesized by this plant species in very small amounts, and it has not been possible to obtain sufficient pure material for full structural determination using NMR techniques. Therefore, no detailed structural information for these alkaloids co-metabolites is available.

In recent years, liquid chromatography (LC) coupled to electrospray ionization mass spectrometry (ESI-MS) has been shown to be a powerful tool to separate and identify natural products.[10-12] Structural elucidation of natural co-metabolites in complex mixtures has also been possible by use of electrospray ionization tandem mass spectrometry (ESI-MS/MS), even from small quantities of plant material or other biological samples.[13,14] The use of this technique has increased substantially nowadays, and ESI-MS/MS experiments can be useful for various studies on natural product chemistry, including biosynthesis,[15-18] metabolomic profile[19,20] and dereplication.[13,21,22] The isolation and characterization of several alkaloids have been reported, however, only a handful of papers describe the use of ESI-MS/MS for the characterization of new natural products.[11,23-27] Advances in the development of high-resolution MS and the elucidation of gas-phase fragmentation chemistry is now helping in the analysis of new compounds and new organic reactions.[28-32]

In this study, we report a ESI-MS/MS spectrometric method for characterization of piperidine alkaloids from flowers and fruits extracts of *S. spectabilis.* This technique is also shown to be an excellent tool for analysis of the different alkaloid isomers co-metabolites as a homologous series, which constitutes the chemical profile or "fingerprint" in whole tissues. Additionally, a simple and rapid method for differentiation and identification of known alkaloid mixtures, and also of new piperidine analogs can be useful to describe the metabolomic design from different part of this plant species.

Experimental

Plant material

The flowers and fruits of *S. spectabilis* were collected in February 2004 from a specimen cultivated at the Institute of Chemistry in Araraquara, São Paulo State, Brazil, and identified by Dr. Inês Cordeiro. A voucher specimen (SP 370.917) was deposited at the herbarium of the Botanic Garden of São Paulo, São Paulo – SP, Brazil.

Extraction and sample preparation

Green fruits and dried flowers from *S. spectabilis* (8.0 kg and 950.0 g, respectively) were powdered and extracted (5 x 5 L) with EtOH. The ethanolic extracts (2.0 g) of both green fruits and dried flowers were re-dissolved in 5% aqueous H_2SO_4 (30 mL) and the insoluble portion was filtered off. The acid solution was extracted with hexane (3 x 20 mL) to yield a non-alkaloidal portion (14.8 from fruits and 5.7 mg from flowers). The residual acid solution was basified (pH 9) with NH_4OH, then extracted with CH_2Cl_2 (3 x 20 mL). The resulting extracts were dried over anhydrous $MgSO_4$ and concentrated, furnishing a crude alkaloidal fraction from fruits (121.6 mg) and flowers (272.7 mg).

An aliquot of each extract was dissolved in MeOH to obtain a concentration of 1 mg mL^{-1} and decanted during 12 h. The samples were filtered and clean up in a solid-liquid extraction, using a Gelman's polytetrafluorethylene membrane (PTFE) of 0.45 μm in diameter.

Mass spectrometry

Methanol (HPLC grade) and de-ionized water (Milli-Q) was used throughout the whole study. ESI mass spectra, precursor and product ions scans were acquired in positive ion mode at unit mass resolution and recorded on a Quattro-LC spectrometer (Micromass, Manchester, UK) which held a quadrupole, hexapole quadrupole configuration. The instrumental conditions used were: probe electrospray tip voltage 3 kV; cone voltage 25 V; nitrogen was used for both the bath and nebulizing gas flowing at 345 L h^{-1} and 27 L h^{-1}, respectively. Solutions were infused

into the ESI source at a flow-rate of 5 μL min^{-1}, using a Harvard Apparatus model 1746 (Holliston, MA). Compounds and cleaned up extracts were dissolved in 20% aqueous methanol to give a concentration of 0.5 mg mL^{-1} and 10 μL were injected for each analysis. The source temperature was set at 80 ^{o}C.

Electrospray ionization tandem mass spectrometry (ESI-MS/MS) experiments were carried out in positive ion mode only, using the same conditions described above. The collision cell was filled with Ar gas at pressure of 7 psi and the collision energy was fixed at 25 eV to maximize the formation of diagnostic fragments.

Accurate-mass measurements were performed on a quadrupole-time of flight instrument (UltrOTOF-Q, Bruker Daltonics, Billerica, MA).

Results and Discussion

ESI-MS/MS data of the protonated molecule **2**, **3**, **5** and **7** (Figure 1), which were previously isolated,[3,5] are shown in Table 1. The product ion mass spectra of **2** (of *m/z* 326) and **7** (of *m/z* 368), that only differ in the lateral chain linked at C-6 of the piperidine ring, are very similar. The most abundant fragment ion for both the spectra is that of *m/z* 308, which results from H_2O loss (18 Da) or CH_3CO_2H loss (60 Da), respectively from the precursor ion. Additionally, less abundant fragment ions at the low *m/z* region, of *m/z* 70, 81, 95, are also of very similar abundances for **2** and **7**. Similarly, the predominant process in the fragmentation of **5** (of *m/z* 342) is the loss of H_2O. For **5**, however, further loss of H_2O (from $[M + H - H_2O]^+$) forms the ion of *m/z* 306. This difference is likely associated with the hydroxyl group at C-7, thus allowing for the second loss of H_2O. In addition, the fragment ion of *m/z* 280 also can be considered diagnostic for the hydroxyl substituent at C-7, and it is observed only for **5**. Finally, similarities between the fragment ions of the diasteroisomers **2** and **3**, of *m/z* 326, indicate that the configuration at C-6 has little influence on their dissociation pattern. These data demonstrate that the loss of H_2O at C-3 (for **2**, **3** and **5** or CH_3CO_2H, for **7**) is the predominant process in the fragmentation of protonated piperidine alkaloids. In addition, the fragment ion $[M + H - R^2OH - H_2O]^+$, together with that of *m/z* 280, is considered to be also diagnostic of a hydroxyl group at C-7.

ESI-MS of crude alkaloidal fraction of flowers and fruits from *S. spectabilis* are shown in Figures 2 and 3, respectively. In order to investigate the structure of various alkaloid co-metabolites, a series of ions with even *m/z* relative value, that are usually characteristic for protonated molecules containing odd number of nitrogens,[32-34] were analyzed by ESI tandem mass spectrometry (ESI-MS/MS), as shown in Table 1.

Loss of H_2O is the predominant process in the fragmentation of the ion of *m/z* 298 (**1**). Other minor ions are those of *m/z* 70, 81, 95 and 109. These ESI-MS/MS data are similar to those of **2** and **3**, with a *m/z* shift of 28 regarded to the corresponding ions of *m/z* 298 and 280 (of *m/z* 326 and *m/z* 308, respectively). This shift is attributed to two methylenes units at the alkyl side chain at C-6.

Table 1. Main fragment ions of **1-13** (low accuracy). Relative abundances are given in parentheses

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
A $[M+H]^+$	298 (10)	326 (10)	326 (18)	314 (20)	342 (30)	340 (8)	368 (20)	300 (25)	328 (30)	444 (10)	472 (15)	474 (10)	502 (17)
B (A-R^2H)										298 (30)	326 (30)	298 (32)	326 (40)
C (A-R^2OH / B-H_2O)	280 (100)	308 (100)	308 (100)	296 (100)	324 (100)	280 (100)	308 (100)	282 (100)	310 (100)	280 (100)	308 (100)	280 (90)	308 (100)
D (C-H_2O)				278 (60)	306 (85)			264 (60)	292 (50)				
E ($[R^2]^+$)										147 (80)	147 (50)	177 (100)	177 (90)
Not assigned				252 (20)	280 (20)								
F (E-CH_3OH)												145 (17)	145 (10)
Not assigned	109 (10)	109 (8)	109 (5)	109 (5)	109 (3)	109 (2)	109 (3)	109 (8)	109 (3)	109 (<1)	109 (<1)	109 (<1)	109 (<1)
Not assigned	95 (15)	95 (12)	95 (7)	95 (10)	95 (5)	95 (6)	95 (10)	95 (15)	95 (5)	95 (<1)	95 (<1)	95 (<1)	95 (<1)
Not assigned	81 (15)	81 (10)	81 (7)	81 (10)	81 (5)	81 (4)	81 (5)	81 (14)	81 (5)	81 (<1)	81 (<1)	81 (<1)	81 (<1)
Not assigned	70 (35)	70 (30)	70 (13)	70 (5)	70 (10)	70 (10)	70 (20)	70 (30)	70 (12)	70 (<1)	70 (3)	70 (<1)	70 (<1)

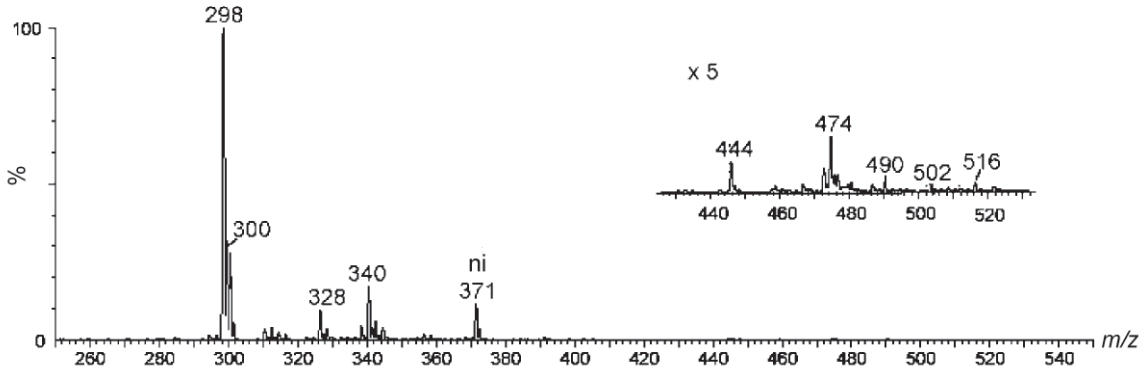


Figure 2. ESI-MS of the alkaloidal crude methanolic extract from the flowers of *S. spectabilis.* (ni = not idendified).

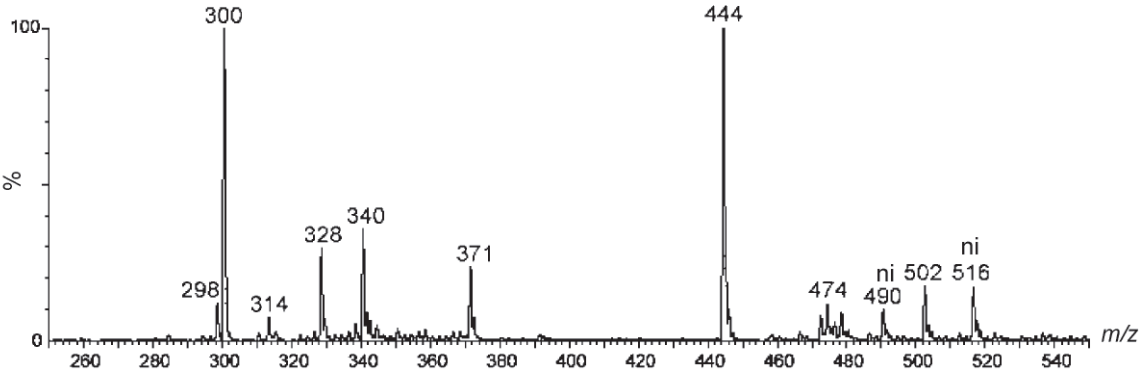


Figure 3. ESI-MS of the alkaloidal crude methanolic extract from the fruits of *S. spectabilis*. (ni = not idendified).

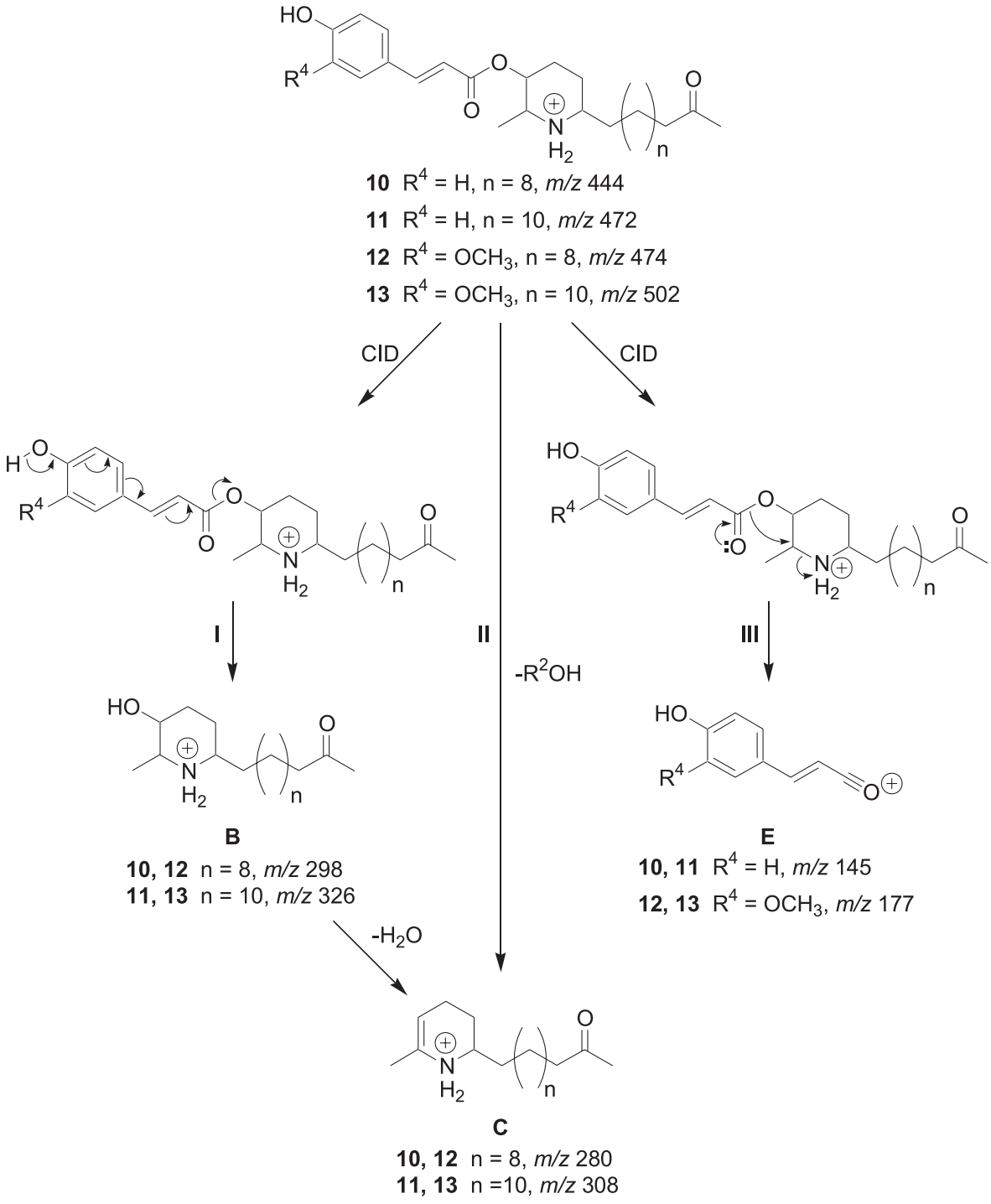
Therefore, **1** is expected to be homologue of **2** and **3** differing only in the number of CH_2 units of the substituent at C-6 (n = 8 for **1**; n = 10 for **2** and **3**). A similar alkaloid was previously isolated from *Cassia excelsa*.[35] However, the configuration of the stereocenters of the alkaloid core (C-2, C-3, and C-6) could not be unambiguously assigned as their influences on the fragmentation processes were not evident in this study.

The second most abundant fragment ion of **6,** of *m/z* 280, is resulting of loss of CH_3CO_2H (60 Da), as compared to **7.** Further similarities between the ESI-MS/MS data of **6** and **7** (*i.e.*, the low mass fragment ions series at *m/z* 70, 81, 95 and 109), that differ by 28 *m/z* units, are indicative of the lack of two methylene units for **6,** as compared to **7** (Table 1). It appears to be no previous reports on this compound or on its diasteroisomers. In the same manner, ESI-MS/MS of the ion of *m/z* 314 (**4**) shows fragment ions of *m/z* 296 (loss of H_2O) and *m/z* 278 (loss of two H_2O molecules) with a mass shift of 28 as for **5** (of *m/z* 324 and *m/z* 306, respectively). These data indicated that **4** is a $(CH_2)_8$ homologue of **5** (Figure 1), previously isolated from *Cassia leptophylla.*[5]

The two major fragment ions in the ESI-MS/MS spectrum of *m/z* 328 (**9**), of *m/z* 310 and *m/z* 292, result from two consecutive losses of H_2O (Table 1). In principle, they could be indicative of a hydroxyl substituent at C-7, similar to **5**. However, for **9** the lack of the fragment ion of *m/z* 280 (which is diagnostic for the hydroxyl substituent at C-7), as well as the two *m/z* shift units from the corresponding ion of **3**, are strong evidences for a hydroxyl group bounded in another position of the alkaloid skeleton, most probably formed by the reduction of the carbonyl group on the side chain linked at C-6. This data suggests that **9** is similar to an alkaloid previously isolated from *Cassia spectabilis*.[5] Finally, comparison between the ESI-MS/MS of **8** (of *m/z* 300) and **9** (of *m/z* 328), that also displays a *m/z* shift of 28, suggests that **8** exhibits an alkyl side chain with n = 8 instead of n = 10 (for **9**). Such compound (**8**) has been previously isolated from *C. spectabilis*.[3,5]

The fragment ion of *m/z* 280 in the ESI-MS/MS of **10** (*m/z* 444) results from the loss of a 164 Da, likely R^2OH (Table 1). An analogous fragment ion is also observed in the spectra of **1** and **6** (n = 8). However, for **10** the ion of

m/z 280 can also be formed by loss of H_2O. In addition, the relative abundance of the fragment ion of *m/z* 147 (that was expected to be diagnostic for the R^2 substituent group at C-3), is higher than those of the low mass fragment ions series of *m/z* 70, 81, 95 and 109, whose intensity is much lower than that of their corresponding ions in the spectra of **1-9,** thus suggesting a phenylpropanoid moiety linked at C-3. The fragment ions of *m/z* 147 and ([M + H - 146]$^+$ has been recently used to characterize a *p*-coumaroyl substituent (Figure 1).[35] The fragment ion of *m/z* 147 is considered to result from charge-induced fragmentation that forms an acylium ion **E** (pathway **III**, Scheme 1). This process could be initiated by proton shift from the amine group to the carbonyl of the ester group.[36] In contrast, loss of *p*-coumaric acid (pathway **II**), as well as the formation of the fragment ion



Scheme 1. Proposed fragmentation for **10**-**13**.

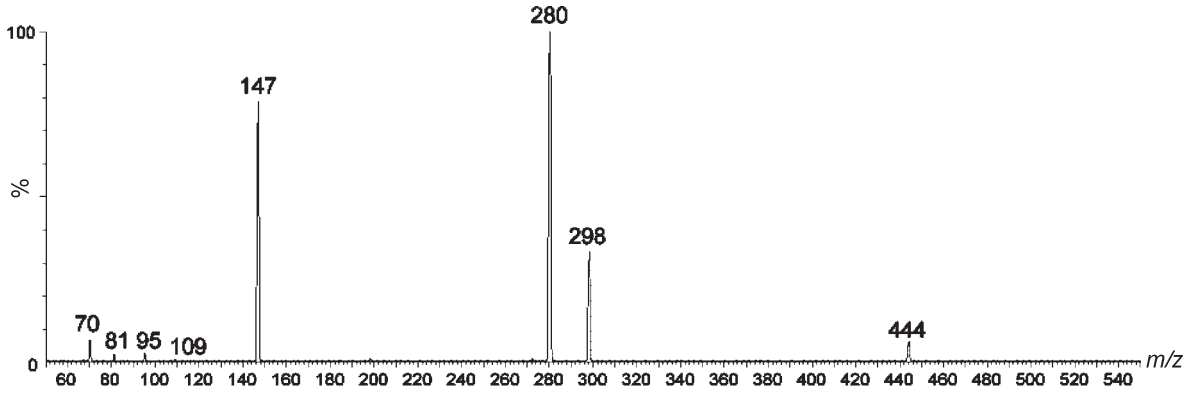


Figure 4. ESI-MS/MS of **10**.

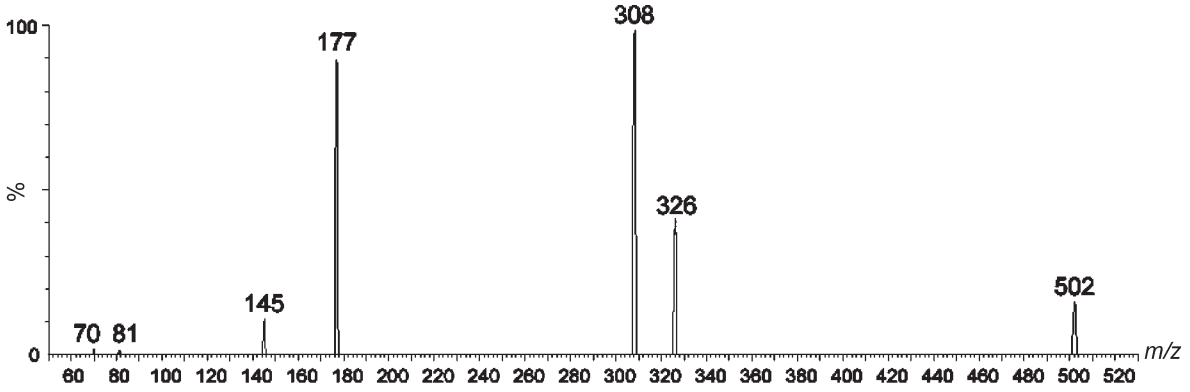


Figure 5. ESI-MS/MS of **13**.

of *m/z* 298 (pathway **I**) requires two different hydrogen shifts that do not involve the charged site.[37] A literature search indicates that there are no previous reports for alkaloid **10**.

ESI-MS/MS of the ion of *m/z* 474 (**12**), shown in Figure 4, differ from that of **10** (*m/z* 444) only by the product ion of *m/z* 177, which is 30 *m/z* units heavier than that for **10** (*m/z* 147). This difference indicates an additional methoxyl group at the aromatic moiety, that is, a feruloyl group.[23] Further evidence of a methoxyl group is given by the diagnostic ion of *m/z* 145, formed by the loss of CH_3OH from **E** (Table 1). In addition, other similarities between the spectra of **10** and **12** (*i.e.*, the low mass fragment ion series of *m/z* 70, 81, 95 and 109) are coherent with the proposed structure **12,** which appears to be novel (Figure 1).

The ESI-MS/MS of the ion of *m/z* 472 (**11**) shows the fragment ions of *m/z* 326 and *m/z* 308 with a 28 *m/z* shift as compared to the corresponding ions of **10**. However,

Table 2. Accurate mass measurements and formula assignments for **1-13**

Ion	Formula	Exact mass	Actual mass	Mass error (ppm)
[**1** + H]$^+$	$C_{18}H_{36}NO_2^+$	298.2746	298.2758	+4.0
[**2** + H]$^+$ or [**3** + H]$^+$	$C_{20}H_{40}NO_2^+$	326.3059	326.3073	+4.2
[**4** + H]$^+$	$C_{18}H_{36}NO_3^+$	314.2695	314.2676	-6.0
[**5** + H]$^+$	$C_{20}H_{40}NO_3^+$	342.3008	342.2990	-5.2
[**6** + H]$^+$	$C_{20}H_{38}NO_3^+$	340.2852	340.2864	+3.5
[**7** + H]$^+$	$C_{22}H_{42}NO_3^+$	368.3165	368.3147	-4.8
[**8** + H]$^+$	$C_{18}H_{38}NO_2^+$	300.2903	300.2914	+3.6
[**9** + H]$^+$	$C_{20}H_{42}NO_2^+$	328.3216	328.3223	+2.1
[**10** + H]$^+$	$C_{27}H_{42}NO_4^+$	444.3114	444.3129	+3.3
[**11** + H]$^+$	$C_{29}H_{46}NO_4^+$	472.3427	472.3412	-3.1
[**12** + H]$^+$	$C_{28}H_{44}NO_5^+$	474.3219	474.3198	-4.4
[**13** + H]$^+$	$C_{30}H_{48}NO_5^+$	502.3532	502.3511	-4.1

most of the fragment ions of **11** are the same as those of **10**, including that of *m/z* 147, which is indicative of the *p*-coumaroyl group. These data strongly indicate that **11** differ from **10** only by two additional CH_2 units at the side chain at C-3 (n = 10 for **11** instead of n = 8 for **10**). In fact, comparison between ESI-MS/MS of **12** and **13** (Figure 4) reveals that **13** is similar to **12** differing only in the length of the side chain at C-6 (n = 10). As for **10** and **12,** there are no reports found for **11**.

Aiming to confirm further the structures of all piperidine alkaloids, which were identified in this study, accurate-mass measurements were performed (Table 2). For **10-13**, the fragment ions of *m/z* 145 (**10**, **11**) and *m/z* 177 (**12**, **13**) corroborate the presence of a phenylpropanoid moiety (feruloyl or coumaroyl) at C-3 for these alkaloid co-metabolite series. All of the measured *m/z* are in full agreement with those calculated for the proposed formula.

Analysis of *S. spectabilis* profile's from fruits and flowers extracts revealed a predominance of alkaloids with n = 8, unlike previous results, for which the compounds with n = 10 were the predominant.[3] Our results also showed that the co-metabolites identified from flower extracts are more oxidized that those from fruits. The preferential accumulation of co-metabolites exhibiting different oxidation levels is likely to be associated with cellular differentiation in flowers tissues. We believe that this result could be of interest for further biosynthetic studies from this species.

There are, obviously, a number of structural features of the alkaloid skeleton that could not be assigned by mass spectrometry (*i.e.*, configuration of the stereocenters at C-2, C-3 and C-6). In the same manner, some minor product ions and mechanistic aspects of the ion fragmentation cannot be explained solely from ESI-MS/MS analysis. To fully elucidate the structures and to understand the ion fragmentation pathways, additional data from NMR and MS^n are required. Nevertheless, this work demonstrated the usefulness of ESI-MS and ESI-MS/MS assisting on the preliminary but very fast and considerably reliable identification of novel and potentially bioactive co-metabolite alkaloids from *Senna* species extracts.

Acknowledgments

This work was funded by grants of the State of São Paulo Research Foundation (FAPESP) within the Biota-FAPESP – The Biodiversity Virtual Institute Program (www.biotasp.org.br); Grant No. 03/02176-7 awarded to Dr. Bolzani, principal investigator. V. da S. B., I.C-G., and M. P, acknowledges CNPq, CAPES and FAPESP for fellowships.

References

1. Samy, R. P.; Ignacimuthu S.; Sen, A.; *J. Ethnopharmacol*. **1998**, *62*, 173.
2. Samy, R. P.; Ignacimuthu S.; *J. Ethnopharmacol.* **2000**, *69*, 63.
3. Viegas, Jr. C. Bolzani, V. S.; Furlan, M.; Barreiro, E. J.; Young, M. C. M.; Tomazela, D.; Eberlin, M. N.; *J. Nat. Prod*. **2004**, *67*, 908.
4. Moreira, M. S. A.; Viegas Jr., C.; Miranda, A. L. P.; Barreiro, E. J.; Bolzani, V. S.; *Planta Med.* **2003**, *69*, 795.
5. Bolzani, V. S.; Gunatilaka, A. A. L.; Kingston, D. G. I.; *Tetrahedron* **1995**, *51*, 5929.
6. Sriphong, L.; Sotanaphun, U.; Limsirichalkul, S.; Wetwitayaklung, P.; Chaichantipyuth, C.; Pummangura, S.; *Planta Med*. **2003**, *69*, 1054.
7. Mendez, A. M.; *Phytochemistry* **1971**, *10*, 2255.
8. Sansores-Peraza, P.; Rosado-Vallado, M.; Brito-Loeza, W.; Mena-Rejón, G. J.; Quijano, L.; *Fitoterapia* **2000**, *71*, 690.
9. Kamo, T.; Maehara, K.; Sato, K.; Hirota, M.; *Heterocycles* **2003**, *60*, 1303.
10. Albert, K.; Krucker, M.; Glaser, T.; Schefer, A.; Lienau, A.; Zeeb, D.; *Anal. Bioanal. Chem.* **2002**, *372*, 25.
11. Pinto, A. C.; Silva, D. H. S.; Bolzani, V. D.; Lopes, N. P.; Epifanio, R. D.; *Quim. Nova* **2002**, *25 suppl. 1*, 45.
12. Niessen, W. M. A.; *Analusis* **2000,** *28*, 885.
13. Fredenhagen, A.; Derrien, C.; Gassmann, E.; *J. Nat. Prod*. **2005**, *68*, 385.
14. Maurin, A. J. M.; Iamamoto, Y.; Lopes N. P.; Lindsay-Smith, J. R.; Bonato, P. S.; *J. Braz. Chem. Soc*. **2003**, *14*, 322.
15. Jung, W.; Yu, O.; Lau, S. M. C.; O'Keefe, D. P.; Odell, J.; Fader, G.; McGonigle, B.; *Nature Biotech.* **2000**, *18*, 208.
16. Jarvis, A. P.; Schaaf, O.; Oldham, N. J.; *Planta* **2000**, *212*, 119.
17. Hertweck, C.; Jarvis, A. P.; Xiang, L. K.; Moore, B. S.; Oldham, N. J.; *Chem. Biochem*. **2001**, *2*, 784.
18. Lange, B. M.; Ketchum, R. E. B.; Croteau, R. B.; *Plant Physiol*. **2001**, *127*, 305.
19. Kite, G. C.; Howes, M. J. R.; Simmonds, M. S. J.; *Rapid Commun. Mass Spectrom*. **2004**, *18*, 2859.
20. Hall, R.; Beale, M.; Fiehn, O.; Hardy, N.; Summer, L.; Bino, R.; *Plant Cell* **2002**, *14*, 1437.
21. Schobel, U.; Frenay, M.; Van Elswijk, V.; McAndrews, J. M.; Long, K. R.; Olson, L. M.; Bobzin, S. C.; Irth, H.; *J. Biomol. Screen* **2001**, *6*, 291.
22. Vuorela, P.; Leinonen, M.; Saikku, P.; Tammela, P.; Rauha, J. P.; Wennberg, T.; Vuorela, H.; *Curr. Med. Chem*. **2004**, *11*, 1375.
23. Lopes, N. P.; Stark, C. B. W.; Gates, P. J.; Staunton, J.; *Analyst* **2002**, *127*, 503.
24. Fonseca, T.; Lopes, N. P.; Gates, P. J.; Staunton, J.; *J. Am. Soc. Mass Spectrom.* **2004**, *15,* 325.
25. Lopes, N. P., Stark, C. B. W.; Hong, H.; Gates, P. J.; Staunton, *J. Rapid Commun. Mass Spectrom.* **2002**, *16*, 414.

26. Crotti, A. E. M.; Lopes, J. L C.; Lopes, N. P.; *J. Mass Spectrom*. **2005**, *40*, 1030.
27. Gates, P. J.; Kearney, G. C.; Jones, R.; Leadlay, P. F.; Staunton, J.; *Rapid Commun. Mass Spectrom*. **1999**, *13*, 242.
28. Kearney, G. C.; Gates, P. J.; Leadlay, P. F.; Staunton, J.; Jones, R.; *Rapid Commun. Mass Spectrom*. **1999**, *13*, 1650.
29. Lopes, N. P.; Fonseca, T.; Wilkins, J. P. G.; Gates, P. J.; *Chem. Commun*. **2003**, *1*, 72.
30. Roddis, M.; Gates, P.; Roddis, Y.; Staunton, J.; *J. Mass Spectrom*. **2002**, *13*, 862.
31. Hong, H.; Gates, P. J.; Staunton, J.; Stinear, T.; Cole, S. T.; Leadlay, P. F.; Spencer, J. B.; *Chem. Commun*. **2003**, *22*, 2822.
32. McLafferty FW, Turecek F.; *Interpretation of Mass Spectra*, 4th ed., University Science Books: Mill Valley, CA, 1993, p. 38.
33. Qin, X. Z.; *J. Am. Soc. Mass Spectrom*. **2002**, *13,* 371.
34. Highet, R. J.; *J. Org. Chem*. **1964**, *29*, 471.
35. Bily, A. C.; Burt, A. J.; Ramputh, A. I.; Livesey, J.; Regnault-Roger, C.; Philogène, B. R.; Arnason, J. T.; *Phytochem. Anal.* **2004**, *15*, 9.
36. Dongré, A. R.; Jones, J. L.; Somogyi, A.; Wysocki, V. H.; *J. Am. Chem. Soc.* **1996**, *118*, 8365.
37. McLafferty FW, Turecek F.; *Interpretation of Mass Spectra*, 4th ed., University Science Books: Mill Valley, CA, 1993, p. 80.

Received: May 24, 2005
Published on the web: December 5, 2005

FAPESP helped in meeting the publication costs of this article.