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ESI/MS/MS in characterization of diastereomeric intermediates in the stereocontrolled synthesis of protoberberines

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Abstract

The mass spectrometric behaviour of two couples of stereoisomeric compounds, intermediates in the stereocontrolled synthesis of protoberberines, has been studied by means of electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and collisional experiments of the protonated molecules. In both ESI and mass spectrometry (MS)/MS spectra, some specific fragments are detectable, diagnostic for the different stereoisomer structures. A clear difference has been observed between the fragments observed in ESI conditions and those generated either by APCI or by collision of ESI-generated $[M + H]^+$ species. This behaviour has been justified by the occurrence, in the ESI source, of some oxidative phenomena.

Keywords: Electrospray ionization; Product ion spectra; Diastereoisomers; Breakdown curves

1. Introduction

Tetrahydroisoquinolines are among the most commonly synthesized natural products, mostly because of their own biological activity and as precursor to other more complex alkaloids. Isoquinolines are found abundantly in the plant kingdom, comprising the largest family of alkaloids [1].

Among these alkaloids, 1-benzyl-1,2,3,4-tetrahydroisoquinolines (called benzylisoquinolines) occupy a central place from which a multitude of structural groups are derived, typified briefly into protoberberines, bis(benzylisoquinolines), aporphines, phtalide isoquinolines, morphine, etc. [2]. Protoberberines have been the subject of extensive chemical and pharmacological investigations. They have been found to exert physiological activities of diverse nature (for example, antimicrobial, antitumour, etc.) [3].

Many of these alkaloids provide a basis for the development of useful therapeutic medicines possessing antihypertensive, hemostatic, smooth or skeletal muscle relaxant, antispasmodic, antitussive, antimalarial, narcotic, analgesic, or antipyretic activities [3]. Much interest has been focused on their antileukemic action [2].

The biological activity attached to the isoquinoline nucleus has provided a great deal of interest in the synthesis of isoquinolines. The methods of choice for the preparation of isoquinolines derivatives are the

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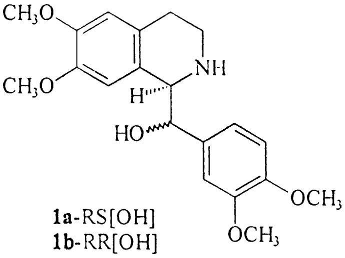
Bischler–Napieralski [4,5], Pictet–Spengler [5,6] and Pomeranz–Fritsch reactions [6,7].

Bischler–Napieralski and/or a modified Pictet–Spengler reaction were successfully applied for the preparation of diastereomers of important benzylisoquinolines: (*R*)[(1*S*)-6,7-dimethoxy-1,2,3,4-tetrahydro-1-isoquinolinyl](3,4-dimethoxyphenyl)methanol **1a** [8,10,11] and (*R*)[(1*R*)-6,7-dimethoxy-1,2,3,4-tetrahydro-1-isoquinolinyl](3,4-dimethoxyphenyl)-methanol **1b** [8,9,11].

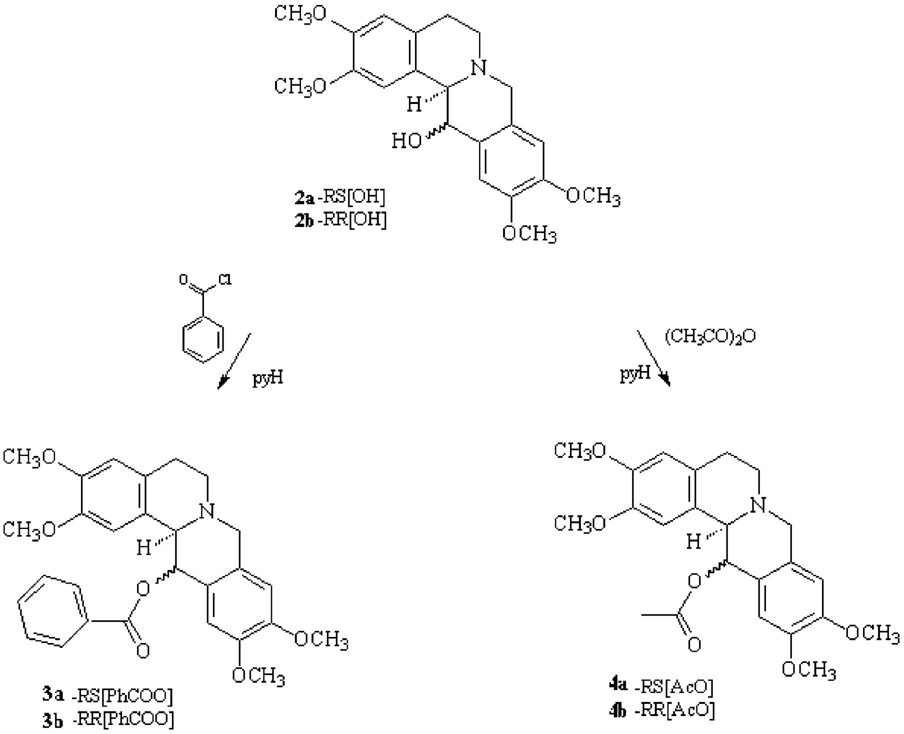


1a-RS[OH]
1b-RR[OH]

We have already shown that the electrospray ionization (ESI), accurate mass measurement and multistage mass spectrometry experiments were valuable tools for rapid and reliable stereochemistry assignment for compounds not only of type **1**, but also of their amide–ester derivatives.

When benzylisoquinolines **1a** or **1b** were treated with formaldehyde under Mannich reaction conditions, protoberberines **2a** [8,10,11] ((13*S*,13*aR*)-2,3,10,11-tetramethoxy-13*a*-methyl-5,8,13,13*a*-tetrahydro-6*H*-isoquino[3,2-*a*]isoquinolin-13-ol) and **2b** [8,10,11] ((13*R*,13*aR*)-2,3,10,11-tetramethoxy-13*a*-methyl-5,8,13,13*a*-tetrahydro-6*H*-isoquino[3,2-*a*]isoquinolin-13-ol) were formed respectively in good yield (see Scheme 1).

Seeking a suitable crystals for X-ray measurements, we prepared two pairs of ester derivatives of diastereomers **2a** and **2b**. Thus, treatment of compound **2a** with benzoyl chloride or acetic anhydride in pyridine brought about the formation of compounds



Scheme 1.

ester derivatives **3a** (13*S*,13*aR*)-2,3,10,11-tetramethoxy-5,8,13,13*a*-tetrahydro-6*H*-isoquino[3,2-*a*]isoquinolin-13-yl benzoate and **4a** (13*S*,13*aR*)-2,3,10,11-tetramethoxy-5,8,13,13*a*-tetrahydro-6*H*-isoquino[3,2-*a*] isoquinolin-13-yl acetate, respectively. On the other hand, compound **3b** upon analogous esterification gave (13*R*,13*aR*)-2,3,10,11-tetramethoxy-5,8,13,13*a*-tetrahydro-6*H*-isoquino[3,2-*a*]isoquinolin-13-yl benzoate **3b** and (13*R*,13*aR*)-2,3,10,11-tetramethoxy-5,8,13,13*a*-tetrahydro-6*H*-isoquino[3,2-*a*]isoquinolin-13-yl acetate **4b** (Scheme 1).

We report here the characterization of these stereoisomers as obtained by ESI and product ion spectrometry of the related protonated molecules.

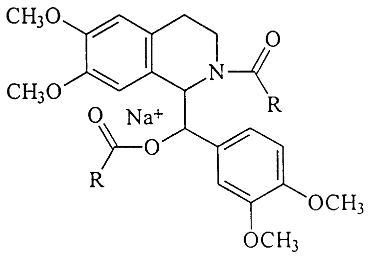
2. Experimental

Both ESI and atmospheric pressure chemical ionization (APCI) experiments were performed on an Agilent 1100 Series LC/MSD Trap VL&SL instrument. The 10^{-5} M solutions of the different compounds in H_2O/CH_3CN (1/1 v/v) was directly infused into the ion sources by a syringe pump at a flow rate of 8 μL/min. In ESI conditions, the spray capillary voltage was set at 4 kV and the entrance capillary temperature was set at 270 °C. The nebulizing gas was N_2. MS/MS experiments were obtained by resonance activation of pre-selected species. The APCI measurements were performed by a vaporizer temperature of 430 °C and the corona discharge was operating at 3.5 kV.

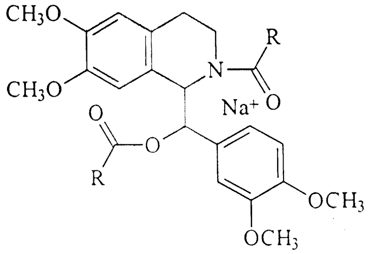
Accurate mass measurements were performed on a Jeol AccuTof, operating in the same ESI conditions described above, at a resolving power of 10,000.

3. Results and discussion

In a previous paper on ESI mass spectrometry of stereoisomeric isoquinoline alkaloids, namely the precursors of compounds **3** and **4**, some specific behaviours were observed [8]. First of all, the *RR* and *RS* isomers were differentiated in terms of the energy required for the primary fragmentation process implicating the two chiral carbon atoms, lower in the case of the *RS* isomers. Furthermore, for the compounds containing the ester groups (and hence very similar to **3** and **4**), the ESI spectra showed the facile formation of $[M+Na]^+$ and $[M+K]^+$ ions, and this behaviour was justified by invoking a donor effect of the oxygen atoms of the carboxyl group (structure **k**) or to the presence of nitrogen and oxygen atoms (as well as to the electron-donating power of the methoxy group) leading consequently to a stable **j** structure.



k



j

In the present case, this last behaviour was not observed. As can be seen in Figs. 1 and 2, showing the spectra of compounds **4** and **3**, respectively, in the molecular ion region, the peak of the protonated molecules is always the most abundant one. The $[M+Na]^+$ and $[M+K]^+$ species are also present, but in quantity much lower than that observed in the case of isoquinoline alkaloids.

These results suggest that for the cationized ions of the latter compounds, structure **j** is the most reasonable one: once the cyclization reaction has taken place and both N atom and carbonyl oxygen are no more

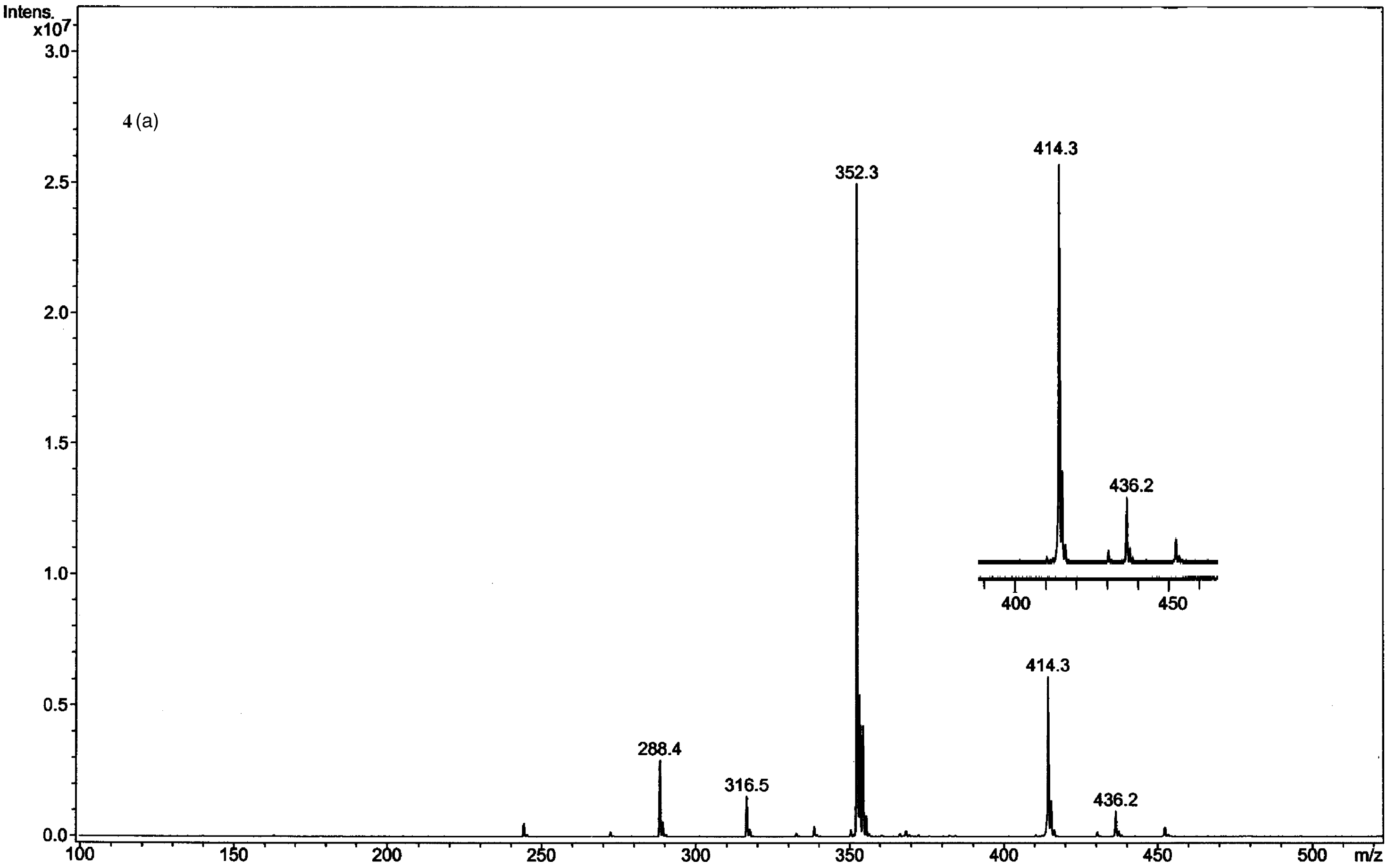


Fig. 1. ESI spectra of compounds **4a** and **4b**.

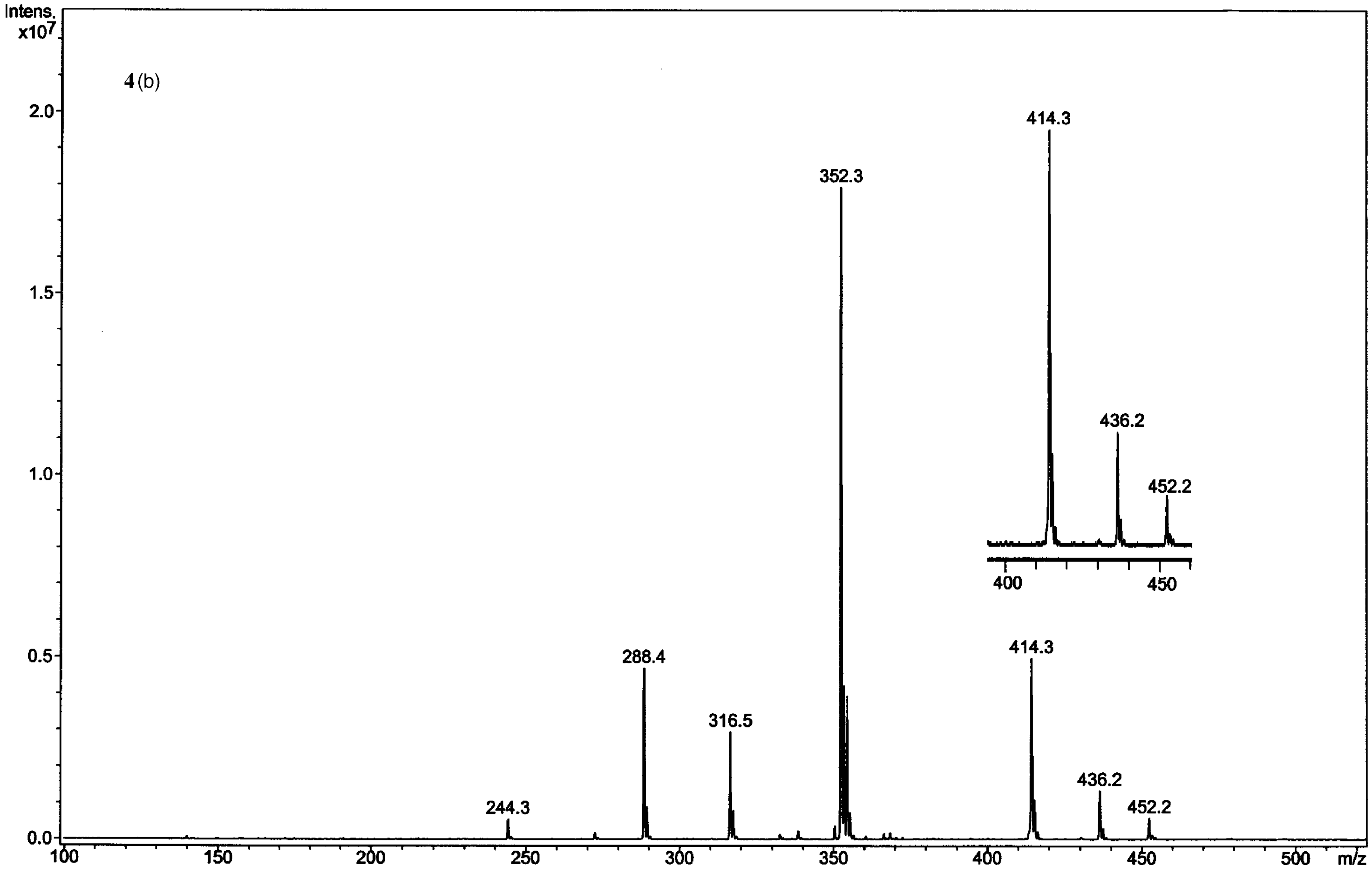


Fig. 1. (*Continued*).

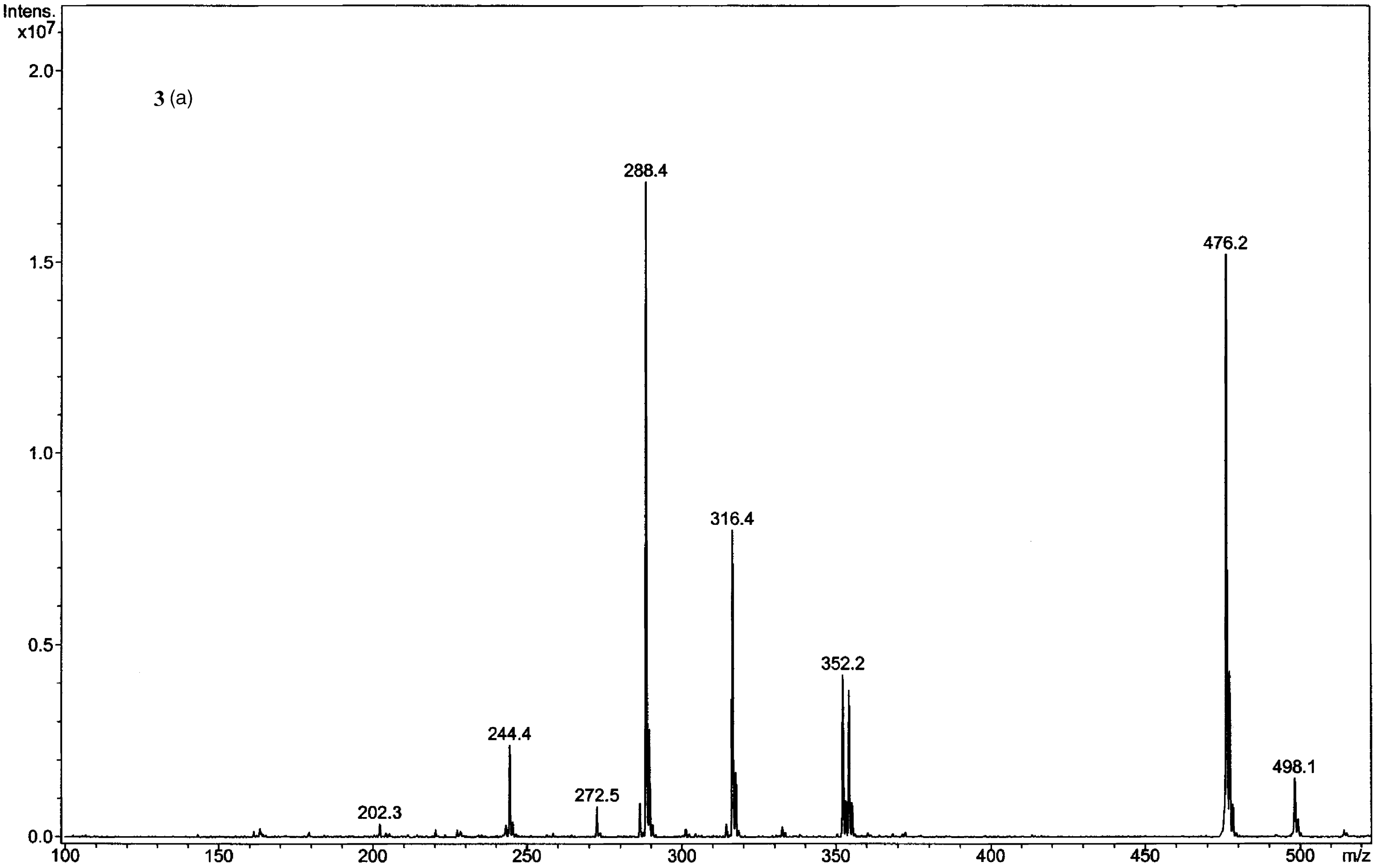


Fig. 2. ESI spectra of compounds **3a** and **3b**.

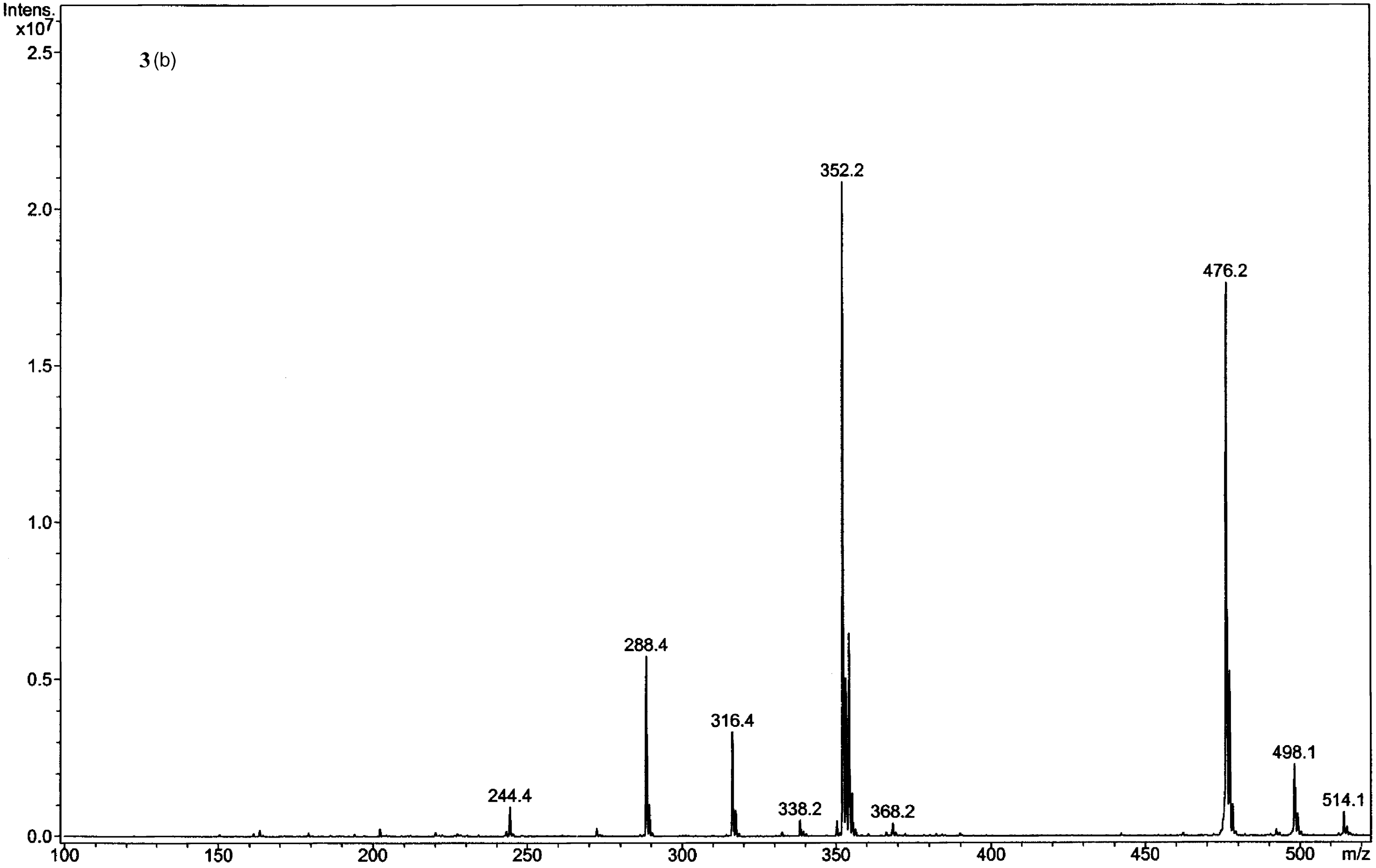


Fig. 2. (*Continued*).

available to coordinate with Na^+ and K^+ ions, the abundance of the related cationized molecules show a dramatic decrease.

The ESI spectra of stereoisomeric compounds **4a** and **4b** (Fig. 1) show only minor differences. A higher abundance of $[M+Na]^+$ and $[M+K]^+$ ions is present for **4b**. This difference has been confirmed by a series of different measurements which show that, even in a low extent, the formation of $[M + Na]^+$ and $[M + K]^+$ ions is more favoured for the *RR* isomer (compound **4b**).

To further investigate this phenomenon, different amounts of sodium chloride were added to solutions of **4a** and **4b**, so that molar ratio compound/salt of 10:1, 2:1, 1:1 were obtained. The spectra of the samples so obtained show an increasing amount of $[M + Na]^+$ ions with the complete depression of the signal due to $[M + K]^+$ species. However, the $[M + Na]^+$ ions obtained with the 1:1 molar ratio solution became about the 80% of the $[M + H]^+$ species in the case of **4b**. The difference between **4a** and **4b** is still present for **4a**: in these conditions, the abundance of $[M + Na]^+$ ions is 63% of that due to the protonated molecule.

The ESI spectra of both **4a** and **4b** show the base peak at *m*/*z* 352, corresponding to the loss of a neutral species of 62 u from the protonated molecule. It is to emphasize that, in the case of ESI mass spectrometry of the analogues isoquinoline alkaloids, the loss of acetic acid (60 u) was detected. This fragmentation process is also present for **4a** and **4b** (see the peak at *m*/*z* 354 in the spectra of Fig. 1) but the formation of the ionic species at *m*/*z* 352 predominates. This behaviour could be explained by considering that this ionic species originates by sequential losses of CH_3COOH and H_2, consequently leading to the cation **a** shown in Scheme 2, highly stabilized by resonance phenomena. In the case of isoquinoline analogues, the further H_2 loss was not observed, due to the lower thermodynamic stability of the product ion so formed. Furthermore, it must be stressed that the H_2 loss, subsequent to the CH_3COOH one, could be activated by two different processes, i.e., the intrinsic instability of the $[MH–CH_3COOH]^+$ cation and/or the oxidative conditions present in the ESI source. This point will be discussed below, with the aid of collisional data and further measurements in APCI conditions.

In order to be confident on the hypothesis given above, the use of accurate mass measurements was retained essential and a series of experiments were performed on a time of flight instrument, operating in ESI conditions. The spectra obtained were practically superimposable with those shown in Fig. 1 and the accurate mass data (available on request) fully confirm the neutral losses above discussed.

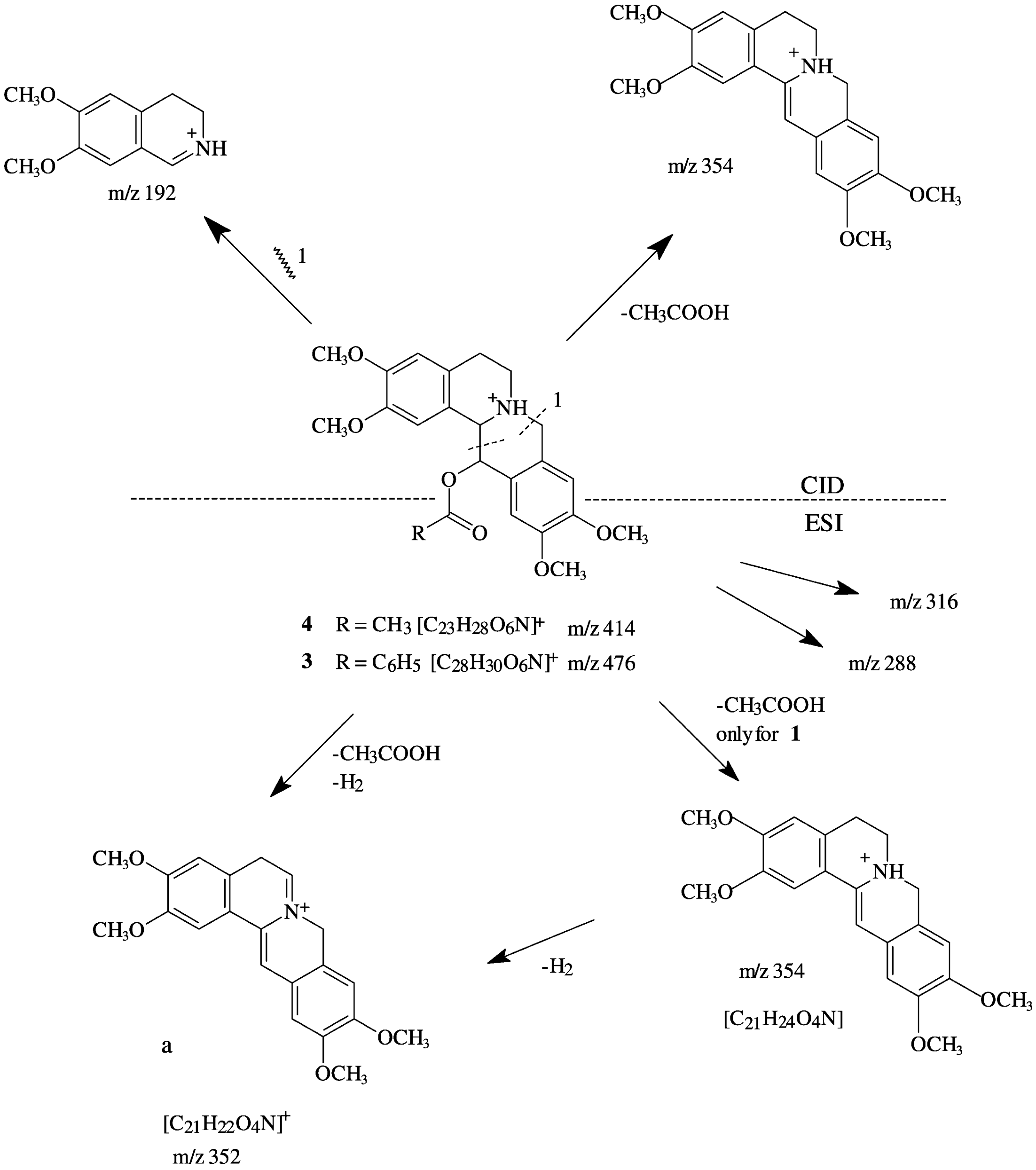
First of all, it is to be emphasized that the APCI spectra of **4a** and **4b** (data available on request) show the presence of $[M + H]^+$ and $[MH–CH_3COOH]^+$ ions only. Any trace of $[MH–CH_3COOH–H_2]^+$ ions is completely absent, proving that the H_2 loss is an artefact of ESI conditions.

The spectra obtained by selecting the ESI-generated, protonated molecules of **4a** and **4b** and colliding them at the same collision energy and buffer gas pressure show the presence of only two fragment ions at *m*/*z* 354 ($[MH–CH_3COOH]^+$) and 192 (due to the cleavage 1 reported in the upper part of Scheme 2). The ions at *m*/*z* 352, base peak of the ESI spectrum, are undetectable.

This discrepancy between ESI vs. APCI and MS/MS data could be explained by two different reasons:

(i) The H_2 loss from the $[M–CH_3COOH]^+$ ions is a decomposition process requiring an activation energy available in ESI conditions and not in APCI or collisional experiments.
(ii) The H_2 loss is an oxidation process occurring in the ESI source.

Considering the low internal energy deposition typical of ESI experiments, even taking into account that collision experiments by ion trap privilege the decomposition channels at lowest activation energy, we retain mechanism (ii) as the most reasonable one. The occurrence of this oxidation process can be justified considering the high stability of the product ion so obtained (see Scheme 2). For the ion **a**, in which a high conjugation level is present and the positive charge is



Scheme 2.

highly stabilized by the nitrogen atom, many different resonance formulae can be proposed, accounting for this high stability. A further point to emphasise is that its formation is not due to intrinsic instability of $[M + H]^+$ and $[M–CH_3COOH]^+$ ions being them completely absent either in APCI or in collision spectra. The "electrochemical" role of the ESI source must necessarily be invoked for their formation.

The product ion spectra put in evidence a different behaviour of the two stereoisomeric compounds. The primary CH_3COOH loss seems more favoured for compound **4a** (the *RS* isomer). Considering that the collision conditions were rigorously the same, the abundance ratio $[M + H]^+$/ $[MH–CH_3COOH]^+$ is 0.148 for **4a** and 0.469 for **4b**. In other words, at the same collision energy, the *RS* isomer show an easier occurrence of the CH_3COOH loss, implying the chiral centres of the molecule.

In the ESI spectra of compounds **4**, further fragment ions are detectable at *m*/*z* 316 and 288 (see Fig. 1). Accurate mass measurements gave for them the elemental formulae $C_{19}H_{24}O_4$ and $C_{18}H_{20}O_7$, respectively, which cannot be justified by simple decomposition pathways either of the protonated (or cationized) molecules or of the most abundant artefact ion at *m*/*z* 352. Furthermore, they do not appear among the product ions of the collision spectra of $[M + H]^+$, $[MH–CH_3COOH]^+$ and $[MH–CH_3COOH–H_2]^+$ ions (see, for example, Fig. 3). Considering that the samples injected in the ESI source were analytically pure, it follows that the ions at *m*/*z* 316 and 288 may originate by complex oxidation–reduction processes occurring in the ESI environment (or, alternatively, by the possible production of undetectable complexes which decompose very fast).

Compounds **3** show a strong different behaviour in ESI conditions (see Fig. 2). The protonated molecules, leading to the signal at *m*/*z* 476, are present in an

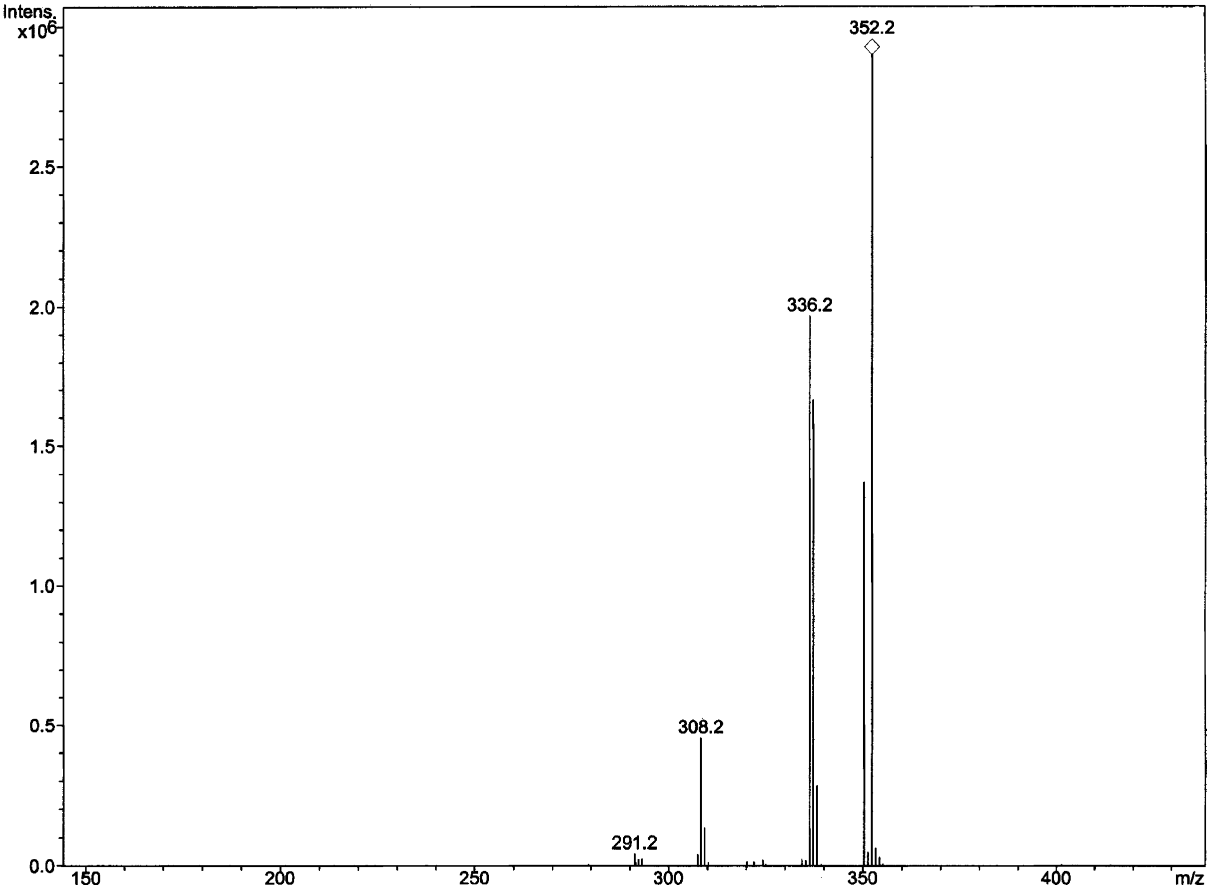


Fig. 3. MS/MS spectrum of the ions at *m*/*z* 352 observed in the ESI spectra of compound **4a**.

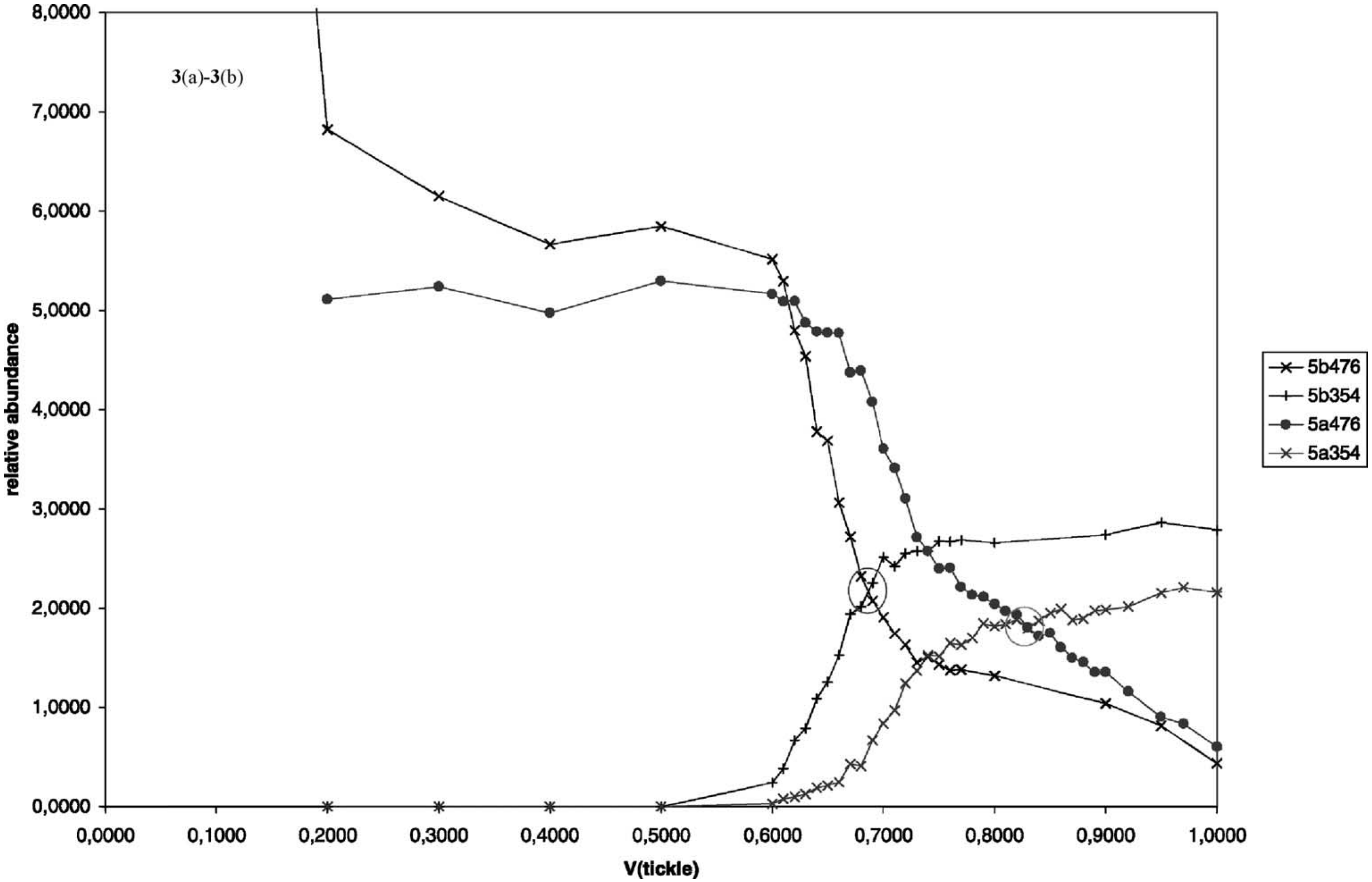


Fig. 4. Breakdown curves of $[M+H]^+$ ions of the diastereisomers **3a** and **3b**.

abundance higher than that observed for compounds **4**. While compound **3b** shows a series of fragments at *m*/*z* 354, 352, 316 and 288 of abundance comparable to that present for compounds **4**, compound **3a** shows an abundance decrease of the ion at *m*/*z* 352 and a clear abundance increase of the species at *m*/*z* 316 and 288. This result could be justified by two different mechanisms:

(i) The ESI-induced decompositions leading to the ions at *m*/*z* 316 and 288 are primary processes more favoured for **3a**.
(ii) These fragment originate from the $[MH–C_6H_5\text{-}COOH–H_2]^+$ ions.

The low abundance of this last species in the case of **3a** could indicate its lowest stability reflecting, according to point (ii), to the easiest formation of the two fragments. However, it is to be emphasised that the related losses are not observed in the product ion spectra of either $[M + H]^+$, or $[MH–C_6H_5COOH–H_2]^+$ and $[MH–C_6H_5COOH]^+$ ions.

Furthermore, collisional spectra put in evidence an unexpected behaviour, i.e., the CH_3COOH loss is slightly more favoured in the case of *RR* isomer (**3b**), on the contrary of what was observed in the case of compounds **4** and for isoquinoline alkaloids [8]. To be more confident on the results shown by the collision spectra, the breakdown curves of $[M + H]^+$ of **3a** and **3b** were determined and compared (see Fig. 4). They unequivocally prove that the primary CH_3COOH loss is energetically more favoured for compound **3b** (the *RR* isomer); in fact, the crossing point between the decreasing abundance of $[M + H]^+$ and the increasing abundance of $[MH–C_6H_5COOH]^+$ ions is at 0.685 V for **3b** and at 0.835 V for **3a**.

This discrepancy between the behaviour of **4** and **3** might be rationalised by considering the steric hindrance of the benzoyl group present in compounds **3**, which would favour the C_6H_5COOH loss for the *RR* isomer.

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