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Collision-induced dissociations of trimethylsilylated lysergic acid diethylamide (LSD) in ion trap multiple stage mass spectrometry

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Dedicated to Yannik Hoppilliard on the occasion of her 60th birthday. It has been a great honor to have been her students and a pleasure to continue working with Yannik.

Abstract

The first part of this work was devoted to the determination of the most sensitive GC–MS^2 method for LSD detection on the ion trap apparatus. On neat samples, it has been shown that trimethylsilylation of LSD decreases the detection threshold by a factor 10 and that chemical ionization using acetonitrile as the reagent gas provides a method seven times more sensitive than electron ionization. The decomposition pathways of protonated trimethylsilylated LSD were determined owing to isotopic labeling and MS^n experiments with n up to 4. The ion trap analyzer allowed the observation of intermediate ions that are not observed in triple quadrupole analysis. The resort to MS^3 and MS^4 experiments also permitted to show evidence of isobaric daughter ions and to attribute the corresponding structures. (Int J Mass Spectrom 219 (2002) 615–624)

Keywords: LSD; Ion trap mass spectrometry; Collision-induced dissociations

1. Introduction

Illicit use of lysergic acid diethylamide (LSD) has undergone a significant increase since the early 1990s, leading to a renewal of interest in its analysis in laboratories of toxicology. The detection of LSD in urine or blood samples is known to be particularly difficult because LSD is ingested in very small amounts (20–80 μg) and rapidly metabolized [1]. Its metabolism remains not totally known but is now assumed to lead to sub-nanogram/milliliter concentrations in body fluids, within a few hours after ingestion [2,3]. Because of cross-reactivity, immunological techniques [4–9] tend to be progressively replaced by chromatography–mass spectrometry techniques that provide better selectivity and sensitivity. In most cases, tandem mass spectrometry is performed using triple quadrupole (TQ) analyzers. In a very complete study, Nelson and Foltz showed the great efficiency of a TQ instrument in detecting very small amounts of LSD in urine and blood extracts: a detection limit about 10 pg/mL was obtained for both urine and blood extracts [10]. Specific detection of LSD in pubic hair at very low levels (0.5 pg/mg) has also been recently performed with the TQ analyzer [11]. Most of GC–MS methods use trimethylsilylation of the analyte prior to chromatographic introduction but LSD detection has also been performed without derivatization, from urine and blood extracts with

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GC-ion trap MS/MS methods [12,13]. The limit of detection obtained was 20 pg/mL for blood and urine extracts. Recently, great results were also obtained with LC–MS methods since limits of quantification of 20 and 25 pg/mL were reached, with LC-TQ instruments, for blood and urine extracts, respectively [14,15]. Cougnard et al. developed a LC-ion trap MS/MS method allowing the detection of LSD in urine down to 12 pg/mL [16].Though LC–MS studies showed the most promising results with sensitive methods requiring quite simple extraction processes, GC–MS instruments still remain rather less expensive and more widespread in analytical laboratories. Among mass spectrometers, ion traps allow performing MS/MS experiments at a cost much lower than triple quadrupole instruments. Moreover, ion trap mass spectrometers constitute very powerful tools to elucidate fragmentation pathways since they allow performing MS^n experiments with n greater than 2. Prior to a study on spectral reproducibility for toxicological extracts in ion trap analysis, the purpose of this paper is to establish MS^2 and MS^3 ion trap reference spectra of trimethylsilylated LSD and to rationalize its fragmentation mechanisms.

2. Experimental

2.1. Materials and sample preparation

Lysergic acid diethylamide (LSD) and LSD-d3 (in which the methyl group is deuterated) were purchased from Promochem (Molsheim-France) and stored at −18 °C. Toluene and acetonitrile HPLC grade were obtained from Prolabo (Fontenay-sous-Bois-France). *N*,*O*-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% of trimethylchlorosilane (TMCS) was purchased from Merck (Nogent-sur-Marne-France). TMCS is a catalyst that increases the silylating power of BSTFA [17].

LSD and LSD-d3 were purchased in acetonitrile, which is not an ideal solvent for gas chromatography. Solutions of trimethylsilylated LSD and trimethylsilylated LSD-d3 were thus prepared as follows: acetonitrile of the commercial solution was evaporated under a N_2 stream at 25 °C. Thirty microliter of BSTFA were added to the residue. The sample was then heated at 70 °C for 20 min and let to cool down to ambient temperature; 30 μL of toluene were added prior to GC–MS analysis. Trimethylsilylated LSD is referred as "TMS-LSD" in what follows. TMS-LSD and TMS-LSD-d3 were injected at 200 pg/μL in MS^2 and MS^3 experiments, at 2 ng/μL in MS^4 experiments.

2.2. Instrumentation

All analysis were performed on a VARIAN "Saturn 2000" apparatus consisting in a gas chromatograph coupled with an ion trap mass spectrometer and fitted with an autosampler. The gas chromatograph was equipped with a temperature-programmed, liquid CO_2-cooled injector.

2.2.1. Gas chromatography

Samples were introduced owing to a cold on-column injection process. Injections were performed into a 2.5 m retention gap (internal diameter: 0.53 mm) of deactivated fused silica which has been pressfit to the 30 m CP-Sil8CB-MS (VARIAN-SA) analytical column (internal diameter: 0.25 mm, film thickness: 0.25 μm). Helium was used as the carrier gas and the flow was held constant at 1.4 mL/min owing to an electronic flow controller. The amount of 5 μL of sample were automatically injected at a rate of 1 μL/s. The injector was held at 80 °C for 0.25 min and then raised to 300 °C at 200 °C/min where it was held for 18.55 min. The analytical column was held at 200 °C for 1.50 min, then ramped to 220 °C at 30 °C/min, to 280 °C at 20 °C/min, and finally to 300 °C at 2 °C/min where it was held for 4.83 min. The total duration of the method was 20 min. The transfer line was held at 300 °C.

2.2.2. Mass spectrometry

The "Saturn 2000" mass spectrometer is not fitted with an external source; molecules are thus ionized between the ion trap electrodes, just as they are eluted from the GC capillary column. In the following work, the ion trap electrodes and manifold temperatures were 250 and 80 °C, respectively. Positive ionization was performed either by electron ionization (EI) or by chemical ionization (CI) using acetonitrile (ACN) as reagent gas [16]. In all cases, ACN provided CI source spectra displaying only the MH^+ ion. Precursor ions were isolated within a 3 unit mass window for all the MS^n experiments but those devoted to dissociation studies for which a 1 unit mass window was used. Ionization was performed with an emission current of 80 μA in EI and 20 μA in CI. The number of precursor ions stored was automatically adjusted to 5000 in the EI mode and 2000 in the CI mode. CID was performed in the resonant mode, at a *qz* value of 0.25, with isolation and excitation times of 5 and 20 ms, respectively (activation voltage values are mentioned for each experiment). The radio frequency of the storage field was modulated in a range corresponding to 0.3 *m*/*z*, with a modulation rate of 10 ms/(*m*/*z*), to minimize the effect of shifts in secular frequency during the collision step. Spectra were recorded with a scan rate of 0.8 s per scan within the range 180–400 amu.

3. Results and discussion

3.1. Method development

In the first part of this work, we determined the ionization mode providing the best sensitivity for LSD detection; we also studied the improvements resulting from trimethylsilylation of the sample. Two GC–MS^2 methods were first developed for the detection of underivatized LSD. One was operated in the EI mode, the other one in the CI mode. In both cases, CID was performed in the resonant mode, at a *qz* value of 0.25, with the activation energy value that provided the best signal-to-noise ratio for the selected ion current of the five main daughter ions. Detection thresholds of 7 and 1 pg/μL were obtained in EI and CI, respectively. GC–MS^2 methods performing CID on trimethylsilylated LSD were optimized using the same conditions. They provided detection thresholds of 0.7 in EI and 0.1 pg/μL in CI. The comparison of these values with the detection thresholds given in the introduction of this paper must take into account that current extraction processes usually concentrate the samples by a factor 50–100. Because toxicological analysis on biological samples needs the highest possible selectivity, the addition of a supplemental collision step to the most sensitive MS^2 method was tested. The activation energy of the first CID step was adjusted to provide the maximum sensitivity for the major daughter ion at *m/z* 295 while the second CID step was optimized for the best sensitivity on the five major ions of the MS^3 spectrum. With "neat" samples, performing MS^3 instead of MS^2 increases the detection threshold of derivatized LSD by a factor 5. The sensitivity of the MS^3 method could yet compete with that of the MS^2 method for the analysis of some biological extracts such as putrefied blood for which the chromatographic background is particularly high. The main parameters of the optimized methods and the corresponding detection thresholds are given in Table 1. These results showed that the method involving CI on derivatized LSD is clearly the more sensitive one. Collision-induced dissociations of protonated TMS-LSD were studied; they are presented below.

3.2. Collision-induced dissociations of TMS-LSDH$^+$

Fig. 1 shows CID spectra of TMS-LSDH$^+$ in the resonant mode: (a) for MS^2 of MH^+, (b) for MS^3 with *m*/*z* 295 as the second precursor ion. With the aim of interpreting decomposition pathways, MS^3 was also performed on TMS-LSDH$^+$ with a second CID step dissociating the main daughter ions *m*/*z* 381, 365, 353, 323, 295, 280 and 269 (relative intensities of at least 5%) of the MS^2 spectrum. The ion *m*/*z* 396 was submitted to resonant excitation under 0.75 V, with $qz = 0.25$; daughter ions were submitted to CID under voltage values of 0.30, 0.50 and 0.75 V, at the same *qz* value. MS^2 was also performed on TMS-LSD-d3H$^+$ (*m*/*z* 399); 3 mass unit shifts were observed for ions at *m*/*z* 323, 295 and 269 that led to *m*/*z* 326, 298 and 272, respectively. Table 2 displays the daughter ions obtained in each MS^n experiment. Structures for the

Table 1
Main parameters and detection thresholds of the optimized GC–MS^n methods

	Underivatized LSD		Trimethylsilylated LSD		
	EI-MS^2	CI-MS^2	EI-MS^2	CI-MS^2	CI-MS^3
Precursor ion (*m/z*)	323.4	324.4	395.2	396.2	396.2
Isolation window (*m/z*)	3	3	3	3	3
CID voltage (V)	0.65	0.75	0.65	0.75	0.60
Storage threshold (*m/z*)	88.9	89.2	108.8	109.1	109.1
Precursor ion (*m/z*)[a]					295.2
CID voltage (V)[a]					0.70
Storage threshold (*m/z*)[a]					81.2
Qualifier ions (*m/z*)[b]	280, 265, **222**, 221, 207, 196	281, 251, **223**, 222, 208, 197	352, 337, 295, **294**, 268, 253	381, 365, 353, 323, **295**, 280	**280**, 264, 254, 221, 196
Detection threshold (pg/μL)	**7**	**1**	**0.7**	**0.1**	**0.5**

[a] Values are related to the second CID step of the MS^3 experiment.

[b] The major ion is given in bold.

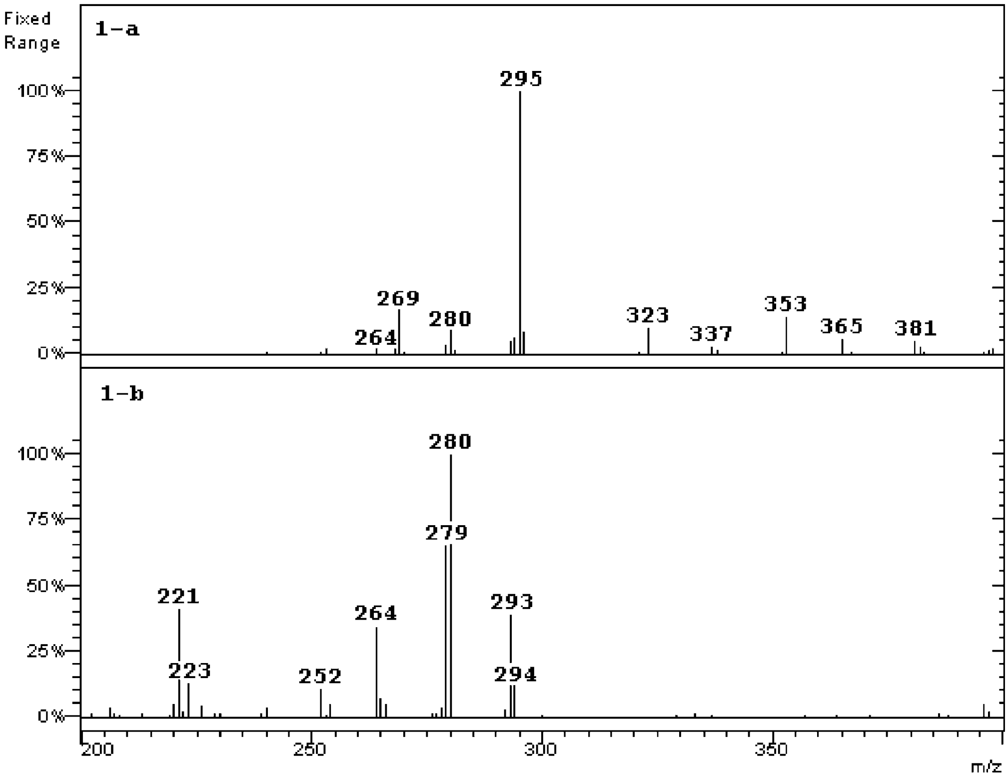

Fig. 1. CID spectra of TMS-LSDH$^+$ in the resonant mode. (a) MS^2 spectrum of MH$^+$, (b) MS^3 spectrum of *m/z* 295 as second precursor ion.

Table 2
Product ions from CID of TMS-LSDH$^+$, TMS-LSD-d3H$^+$ and of their main daughter ions[a]

Precursor ion (*m/z*)	Daughter ions (*m/z*)
396 (399)	381 (381), 365 (365), 353 (353), 323 (326), 295 (298), 269 (272)
381	380, 280, 254
365	292, 264
353	280, 252
323 (326)	322 (322), 308 (308 + 311), 295 (298), 280 (280)
295 (298)	294 (297), 293 (296), 280 (280 + 283), 279 (279 + 282), 264 (264), 252 (252), 223 (226), 221 (224), 196 (199)
269 (272)	254 (254), 197 (200)

[a] Ions in parenthesis are those observed from the deuterated compound.

daughter ions of the MS^2 spectrum are proposed in Fig. 2, in accordance with the isotopic shifts observed with TMS-LSD-d3. It is to be noticed that two structures (referred as (a) and (b)) are proposed for ions *m/z* 323 and for ions *m/z* 280. The formation of ion *m/z* 381 is assumed to result from direct bond cleavage of the N–CH$_3$ bond of TMS-LSDH$^+$. In MS^3, ion *m/z* 381 fragments into ions *m/z* 380 (loss of H$^\bullet$), *m/z* 280 (loss of HCON(Et)$_2$), and *m/z* 254. The latter probably results from the elimination of $H_2C{=}CHCON(Et)_2$ via a retro-Diels Alder like mechanism. Methylamine elimination from ion *m/z* 396 leads to ion *m/z* 365, which fragments in turn into ions *m/z* 292 and *m/z* 264. Ion *m/z* 292 has been isolated via *m/z* 365 in a MS^4 experiment and led only to ion *m/z* 264 by CO elimination. This establishes ion *m/z* 292 as an intermediate in the formation of ion *m/z* 264 and eliminates the possibility of ion *m/z* 292 resulting from a $^\bullet Si(CH_3)_3$ loss from ion *m/z* 365. CO elimination from a structure such as that proposed for ion *m/z* 292 in Fig. 3 is known to be thermodynamically easy, explaining why ion *m/z* 292 is not observed in the MS^2 spectrum of TMS-LSDH$^+$ although *m/z* 264 is observed with a relative abundance of 4%. The loss of CH_3NCH_2 through a retro-Diels Alder like mechanism leads to ion *m/z* 353, which fragments into ions *m/z* 280 (b) and *m/z* 252 by successive eliminations of diethylamine and CO. A MS^4 experiment demonstrated the loss of CO from ion *m/z* 280 (b). The combination of isotopic labeling with MS^3 experiments showed evidence of two different ions at *m/z* 323: ion *m/z* 323 (a) results from diethylamine elimination from ion *m/z* 396 while ion *m/z* 323 (b), results from a $^\bullet Si(CH_3)_3$ elimination from ion *m/z* 396. Activation of ion *m/z* 323 led to ions *m/z* 322 (–H$^\bullet$), *m/z* 308 (–$CH_3^\bullet$), *m/z* 295 (–CO) and *m/z* 280 (–CO–$CH_3^\bullet$). In experiments with TMS-LSD-d3, the ion at *m/z* 322 is not mass shifted, meaning that H$^\bullet$ elimination occurs only from ion *m/z* 323 (b) and thus demonstrating the existence of this structure. When formed from ion *m/z* 326, the ions at *m/z* 308 are only partially mass shifted to *m/z* 311. The three mass units shift occurs when a methyl radical is eliminated from structure (a) (the remaining ions at *m/z* 311 then result from the loss of a methyl radical from the trimethylsilyl group); *m/z* 308 ions that are not mass shifted result from $CD_3^\bullet$ loss from structure (b). The fact that ion *m/z* 295 is shifted to ion *m/z* 298 while ion *m/z* 280 remains unchanged is in good agreement with the proposed mechanisms. CID on ion *m/z* 323 previously showed that the formation of ion *m/z* 295 from TMS-LSDH$^+$ can occur via successive eliminations of diethylamine and CO; direct loss of HCON(Et)$_2$ via a concerted mechanism can also be envisaged, as displayed in Fig. 2. The ion *m/z* 269 is assumed to result from direct $H_2C{=}CHCON(Et)_2$ elimination from ion *m/z* 396, through a retro-Diels Alder like mechanism. CID on ion *m/z* 269 provides ions *m/z* 254 ($CH_3^\bullet$ loss by homolytic cleavage of the N–CH$_3$ bond) and *m/z* 197 –$H_2C{=}Si(CH_3)_2$; only the latter is mass shifted into *m/z* 200 when CID is performed on the deuterated ion *m/z* 272. These results are in good agreement with the structure proposed for ion *m/z* 269. The structures of ions *m/z* 280 resulting from *m/z* 381 and *m/z* 323 correspond to (a). They were attributed following MS^4 experiments that were performed isolating ions *m/z* 280 through, in turn, *m/z* 353, *m/z* 295, *m/z* 381 and *m/z* 323. Spectra of ions *m/z* 280 derived from ions *m/z* 381 and *m/z* 323 correspond to those of ions *m/z* 280 derived by CID of

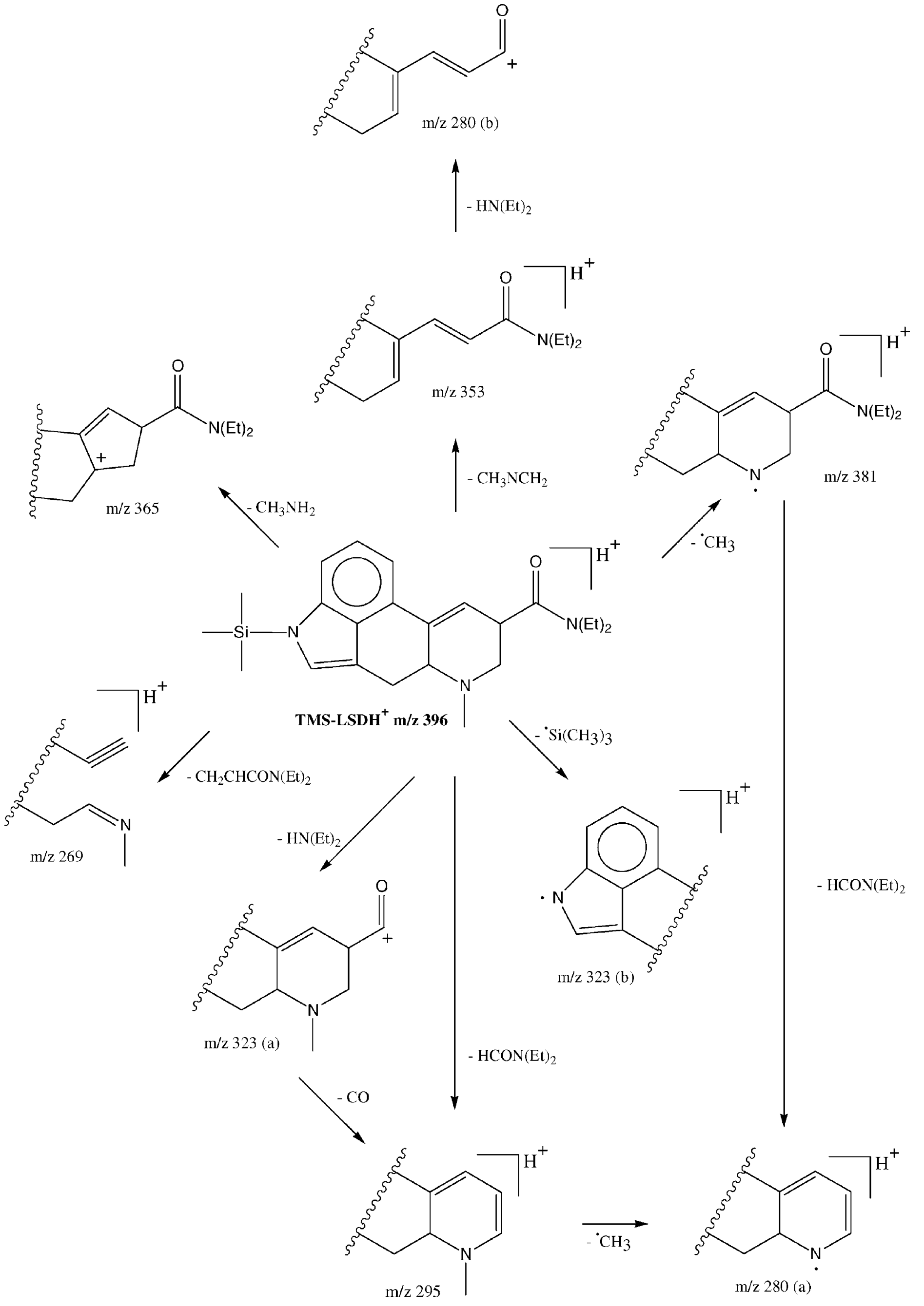

Fig. 2. Decomposition pathways from collision-induced dissociation of protonated trimethylsilylated LSD. Only the ions of which the relative abundances are greater than 5% in the MS^2 spectrum are displayed.

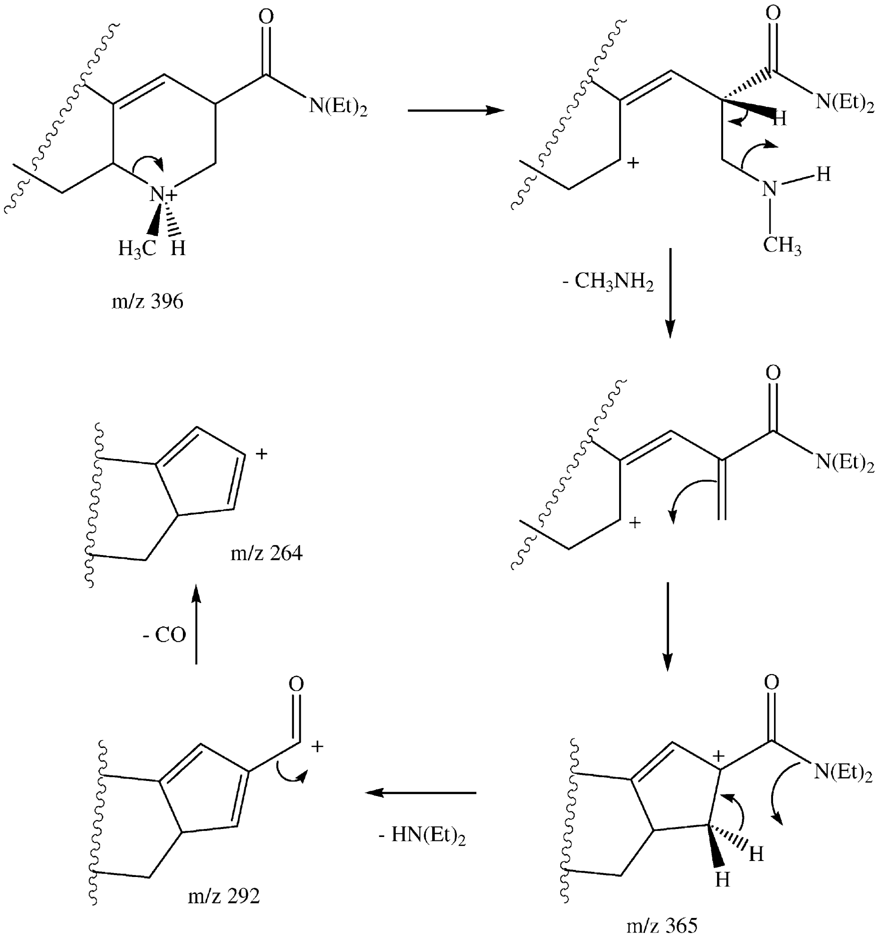

Fig. 3. Dissociation pathways from TMS-LSDH$^+$ to ion *m*/*z* 264.

ions *m*/*z* 295 and fragmenting only to ions *m*/*z* 279 (H$^\bullet$ loss), while activation of ion *m*/*z* 280 (b), isolated as a daughter ion of *m*/*z* 353, only led to ion *m*/*z* 252 by CO elimination. These results confirm the structures (a) and (b) proposed for the ion *m*/*z* 280.

Fragmentation pathways of ion *m*/*z* 295 are proposed in Fig. 4. CID was performed on the deuterated analogue at *m*/*z* 298. Ions at *m*/*z* 294, 293, 223 and 221 were shifted by three mass units and the other daughter ions remained unchanged. The structures proposed for the main daughter ions observed in the MS^3 spectrum (Fig. 4) are in good agreement with the isotopic shifts. By CID, ion *m*/*z* 295 dissociates into ions *m*/*z* 294 (loss of H$^\bullet$) and *m*/*z* 293. Performing CID on ion *m*/*z* 298 showed that ions *m*/*z* 280 and *m*/*z* 279 were partially mass shifted to ions *m*/*z* 283 and *m*/*z* 282, respectively. This demonstrates the existence of a third structure (referred as (c)) for the ion *m*/*z* 280, resulting from the loss of a methyl radical from the trimethylsilyl group of *m*/*z* 295. Two structures are thus logically proposed for ion *m*/*z* 279; they are referred as (a) and (b) in Fig. 4. MS^4 experiments where CID was performed to ion *m*/*z* 294 clearly showed that the formation of ion *m*/*z* 293 occurs via ion *m*/*z* 294. A mechanism involving direct H$_2$ elimination from ion *m*/*z* 295 can also be envisaged but not be proved. In the

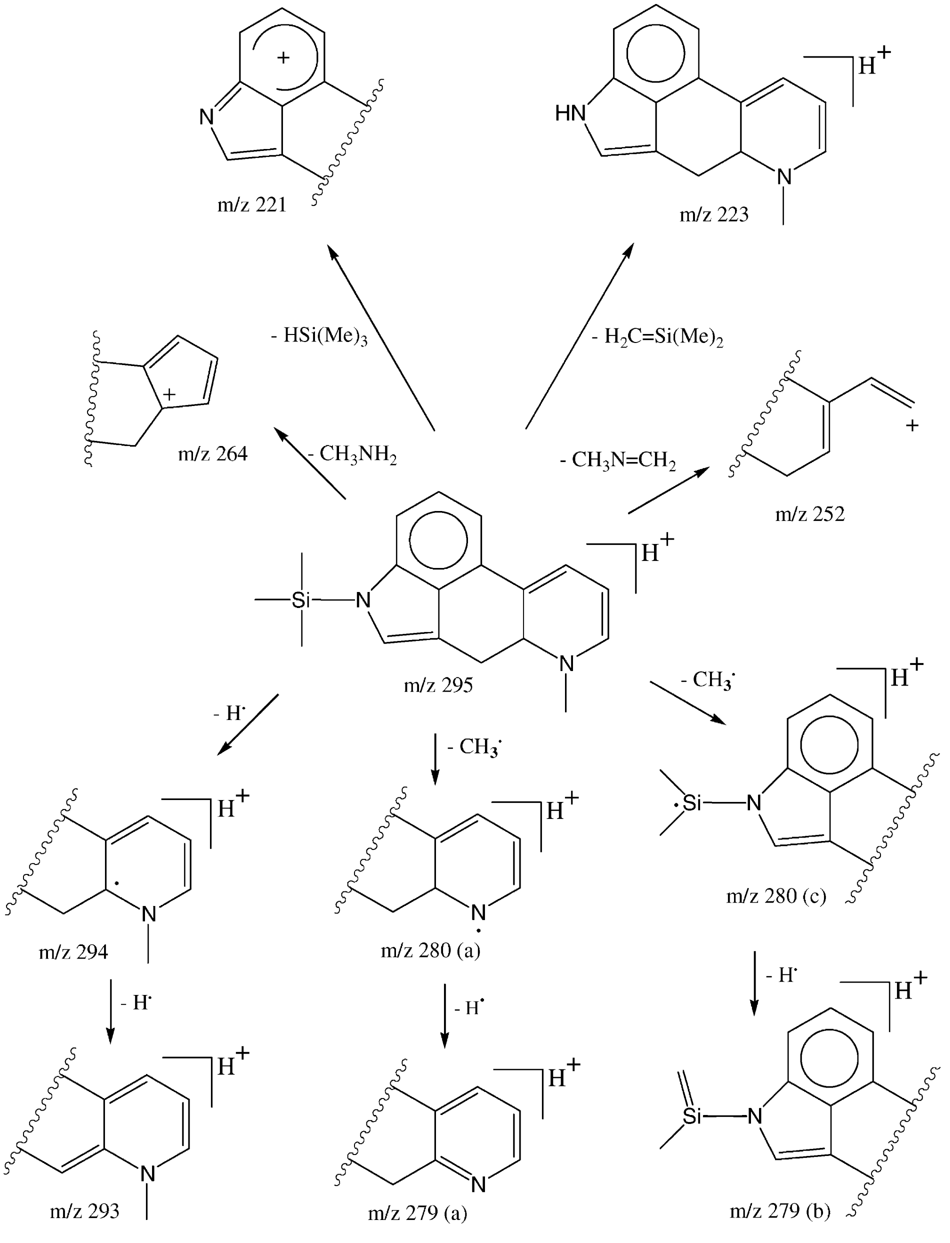

Fig. 4. Decomposition pathways from collision-induced dissociation of ion *m/z* 295 in MS^3 experiments on $TMS\text{-}LSDH^+$.

same manner, the formation of ion *m/z* 279 has been shown to occur from ion *m/z* 280 but direct methane elimination from ion *m/z* 295 cannot be discarded. By losses of methylformimine and methylamine, the ion *m/z* 295 also dissociates into ions *m/z* 252 and 264, respectively. The formation of ions *m/z* 223 and 221 involves loss of the trimethylsilyl group: $H_2C{=}Si(Me)_2$ in the first case, and $HSi(Me)_3$ in the second one.

To our knowledge, there has been only one article about CID on protonated TMS-LSD; Nelson and

Foltz performed MS^2 studies on a triple quadrupole mass spectrometer, dissociating the major ions of the source spectrum produced by methane chemical ionization [10]. Comparison of ion trap and TQ MS^2 spectra shows, as one should expect, that both display identical main daughter ions and that, considering "classical" collision energies (35 eV for the TQ, 0.70 V for the ion trap in the resonant mode), the TQ favors the formation of the ions of the lower *m*/*z* values. For instance, the *m*/*z* 252 ion, resulting from $H_3CN{=}CH_2$ elimination from *m*/*z* 295 is only displayed in the ion trap MS^3 spectrum, after activation of *m*/*z* 295, while it is straight observed in the TQ MS^2 spectrum of $TMS\text{-}LSDH^+$. This phenomenon can be explained by two factors: (i) collisions with Helium atoms are much less efficient than with argon atoms, leading to a lower increase in internal energy of parent ions; (ii) triple quadrupole analyzers favors secondary fragmentations since daughter ions may undergo energetic collisions during the CID process which is not the case with ion trap mass spectrometers where, in the resonant mode, daughter ions are thermalized by the cooling effect of Helium [18]. On a mechanistic point of view, ion trap allows the observation of intermediate ions that are not displayed in TQ MS^2 spectra, providing useful information to confirm or infirm the dissociation pathways postulated according to TQ experiments. In a general way, our experiments confirmed most of the mechanisms suggested from the TQ spectra but some noticeable differences were found. The MS^4 experiments confirmed the formation of ion *m*/*z* 264 by loss of methylamine from ion *m*/*z* 295 but they also showed evidence of a second mechanism involving the formation of ion *m*/*z* 264 through ions *m*/*z* 365 and *m*/*z* 292 intermediates. Such intermediates were not detected in TQ MS^2 experiments. In the same manner, our experiments showed evidence of two different structures for ions *m*/*z* 323. The elimination of the trimethylsilyl group leading to ion *m*/*z* 323 (b) could not be deduced without the CID spectra, at the MS^3 level, of ion *m*/*z* 323 and its deuterated analogue *m*/*z* 326. Multiple stage MS also permitted to establish that ions *m*/*z* 323 (a) and *m*/*z* 381 are precursors of ions *m*/*z* 295 and *m*/*z* 280 (b), respectively. It is interesting to note that, unlike studies performed on TQ, ion trap experiments showed evidence of eliminations involving the trimethylsilyl group of TMS-LSD. Performing additional experiments using TMS(d9) derivatives would be of great interest but deuterated BSTFA is not commercially available.

4. Conclusions

The first part of this study showed that trimethylsilylation of LSD increases its GC–MS^2 detection threshold by a factor 10. It also demonstrated that methods involving ACN CI provides sensitivities seven times greater than those involving EI. These conclusions result from studies performed on neat samples; their extensions to biological extracts are in progress. MS^2 and MS^3 spectra of protonated trimethylsilylated LSD were fully interpreted owing to MS^n experiments with *n* up to 4 and to deuterium labeling. The ion trap analyzer allowed the observation of intermediate ions that are not observed in triple quadrupole analysis. Most of the main mechanisms postulated from triple quadrupole experiments were confirmed but a few noticeable differences were reported. The resort to MS^3 and MS^4 experiments also permitted to show evidence of isobaric daughter ions and to suggest the corresponding structures. Dissociations involving elimination of the trimethylsilyl group were reported for the first time.

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