Scientific paper

Mass Spectrometry of Bis-quinolizidine Alkaloids: Unexpected Dimerization in Electron Ionization Mass Spectrometry

Beata Jasiewicz* and Tomasz Pospieszny

Faculty of Chemistry, Adam Mickiewicz University, Umultowska 98b, 61-614 Poznañ, Poland

** Corresponding author: E-mail: beatakoz@amu.edu.pl;*
Tel.: +48—61-829-1354; fax: +48-61-829-1505

Received: 01-07-2013

Abstract

The electron-ionization mass spectra of dimers of bis-quinolizidine alkaloids: α-isosparteine, 2-methylsparteine, 17-hydroxylupanine, lupanine *N*-oxide and 2,17-dimethylsparteine are discussed and general fragmentation routes of their molecular cations are proposed. Dimers structures are also studied using PM5 methods. The recognition of the fragmentation pathways and relative abundances of the ions obtained will provide important information, useful for the identification of similar dimeric compounds.

Keywords: Alkaloids dimerization, bis-quinolizidine alkaloids, EI-MS spectra, semiempirical calculations

1. Introduction

The quinolizidine alkaloids have interested chemists for several decades. Interesting examples of stereochemical effects on EI mass spectra of this important group of alkaloids can be found in the literature.[1–4]

Sparteine, the main representative of bis-quinolizidine alkaloids, is a chiral diamine and has a widespread

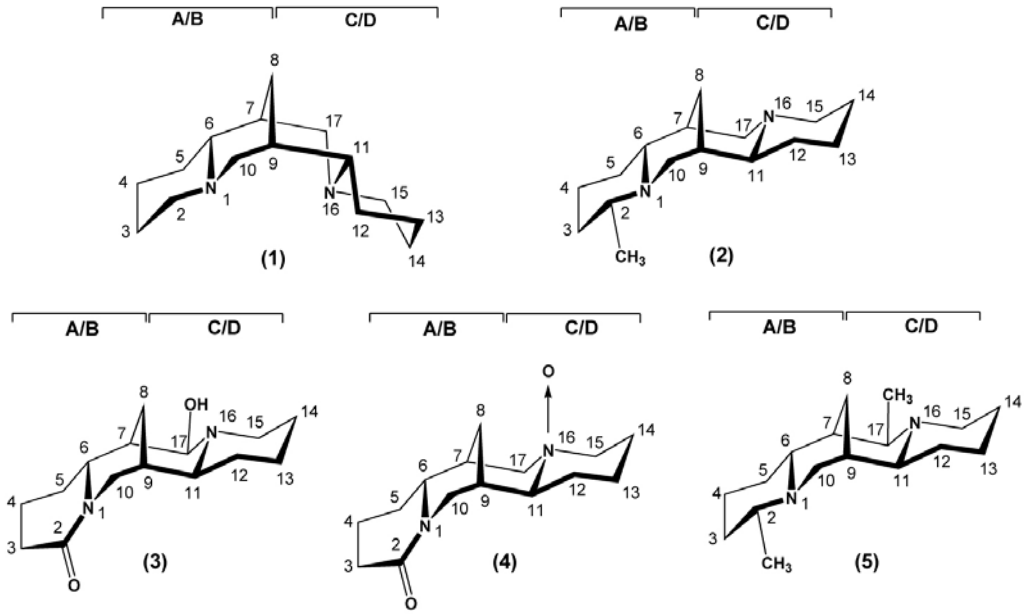


Figure 1. Structure of bis-quinolizidine alkaloids **1**–**5**.

use as a chiral ligand in asymmetric synthesis.[5,6] It appears to offer protection to plants from *Leguminosae* family from insects and grazing mammals.[7,8] Various pharmacological and toxicological properties have been attributed by lupine alkaloids such as antiinflammatory, antiarrhythmic, diuretic, hypotensive, antidiabetic, respiratory, depressant and stimulant.[9]

As a continuation of our study of the mass fragmentation of quinolizidine alkaloids,[10–12] we focused our attention on unexpected dimerization of α-isosparteine [$C_{15}H_{26}N_2$, M = 234] (**1**), 2-methylsparteine [$C_{16}H_{28}N_2$, M = 248] (**2**), 17-hydroxylupanine [$C_{15}H_{24}N_2O_2$, M = 264] (**3**), lupanine *N*-oxide [$C_{15}H_{24}N_2O$, M = 234] (**4**) and 2,17-dimethylsparteine [$C_{17}H_{30}N_2$, M = 262] (**5**) (Fig. 1) in the electron-impact ionization chamber of the mass spectrometer at inlet probe temperature 200–280 °C. Simultaneously, we found that other alkaloids such as: 2-cyano-2-methylsparteine, lupanine (2-oxosparteine), 17-oxosparteine, 17-oxolupanine and 2-thiosparteine under the same conditions did not form dimers.

There are a few reports found in the literature describing formation of bis-quinolizidine alkaloid dimers.[13–15] Dimeric sparteines described in the literature have been obtained synthetically and have bonds between the carbon atom C(17) of the first molecule and the carbon atoms (C5'), C(12'), C(14') or C(17') of the second molecule. They are characterized as anti-arrhythmic agents having the basic sparteine ring structure, but with considerably improved pharmacological properties.[13] The application of these alkaloids in biology requires a detailed knowledge of their structure after drug metabolism. We hope that the recognition of the fragmentation pathways and relative abundances of the ions obtained will provide important information, useful for the identification and analysis of similar dimeric compounds.

2. Experimental

Low- and high-resolution electron ionization (EI) mass spectra were recorded on AMD-Intectra (Harpstedt, Germany) D-27243 model 402 double-focusing mass spectrometer of BE geometry (ionizing voltage 70 eV, accelerating voltage 8 kV, resolving power 10 000). Samples were introduced by a direct insertion probe at a source temperature of ~200 and 280 °C. The elemental compositions of the ions were determined using the same instrument by peak matching method relative to perfluorokerosene. All masses measured agreed within ±2 ppm of those of the composition listed in Table 1.

The spectra from the first field-free region were recorded using linked scans at constant B/E, with helium as collision gas at an indicated pressure of 1.75×10^{-5} Pa with an ion source temperature of 200–280 °C, ionizing energy of 70 eV, and an accelerating voltage of 8 kV.

The values of μ_1–μ_3 were calculated as averages of three measurements. The normal measured variation of ion intensities was 1–2% of the relative intensity. PM5 semiempirical calculations were performed using the WinMopac 2003 program.[16–18]

3. Results and Discussion

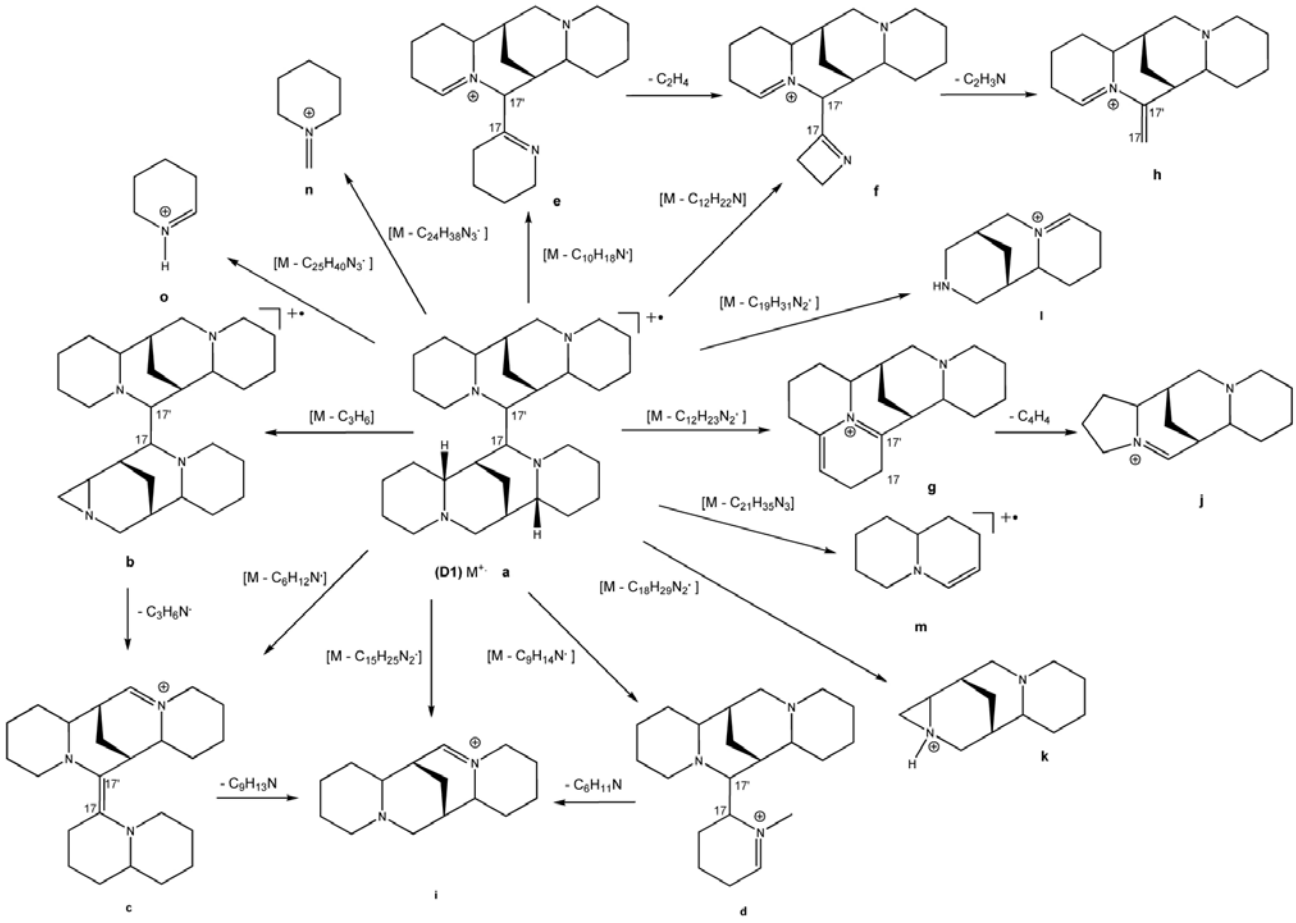
On the basis of the low-resolution EI mass spectra, exact mass determination (Table 1) and B/E linked scan spectra (Figures 2), the principal mass spectra fragmentation routes of dimers **D1**–**D5** are interpreted as shown in Schemes 1–4. It can be concluded that the formation of C(17)–C(17') bonds for **D1**, **D2** (elimination of H_2 molecule), C(17) = C(17') bonds for **D3, D4** (elimination of H_2O molecule) and C(12)–C(12') for **D5** (elimination of H_2 molecule again) is preferred.

It should be pointed out that all the fragmentation pathways have been confirmed by B/E linked scan spectra and that many of the cyclic ion structures shown in Schemes 1–4 are conjectural, similarly to those discussed previously in the literature.[2–4,10–12,19] The common feature of the mass spectral fragmentation of molecular ions of dimers **D1**–**D5** is the cleavage of N(1)–C(10), C(6)–C(7), C(9)–C(10) (ring B); C(7)–C(17), C(9)–C(11) (ring C); C(15)–C(14), C(13)–C(12) (ring D) as well as of the C(17)–C(17') or C(12)–C(12') bonds of the skeleton of bis-quinolizidine alkaloid dimers. All suggested structures of fragment ions are consistent with the Bredt's rule, that states that a double bond cannot be placed at the bridgehead of a bridged ring system, unless the rings are large enough.[20]

In the mass fragmentation of dimers **D1**–**D5** fragment ions formed from two molecules of bis-quinolizidine alkaloids can be observed. The even-electron fragment ions (EE^+) **c, d, e, f** as well as cyclic ion **g** are characteristic of **D1**, whereas in the mass fragmentation of **D2**, **D3** and **D4** the even-electron fragment ions **b, c, d** and **e** are present. It should be pointed out that for **D2** the even-electron fragment ion **c** has been obtained from the even-electron fragment ion **b** by elimination of the C_3H_2 molecule and the even-electron fragment ion **d** has been obtained from the even-electron fragment ion **c** by elimination of the C_4H_7N molecule in the second and third step of the mass fragmentation of $\mathbf{M^{+\bullet}a}$, respectively. The N(1)–C(2) bond cleavage of ring A of the molecular ion of **D1** with elimination of the C_3H_6 molecule leads to the odd-electron ion ($OE^{+\bullet}$) **b**. In the case of molecular ions **D1**,**D2** and **D5** the charge is situated on an annular nitrogen atom N(1) or N(16) whereas in the case of molecular ions **D3** and **D4** the charge is probably situated on an annular nitrogen atom N(1). This fact suggests a better stabilization of the charge at N(1) of these even-electron or odd-electron fragment ions at N(16). It is probably connected with the presence of the carbonyl functional group at C(2) in the skeleton of **3** (or **4**).

Table 1. Elemental compositions and relative intensities of the ion peaks in the EI mass spectra of dimers **D1–D5**.

Ion	*m/z*	Elemental composition	Relative abundance [*RA*, %]			
			D1	D2	D3/D4	D5
$M^{+\cdot}$ **a**	522	$C_{34}H_{58}N_4$	–	–	–	20
	494	$C_{32}H_{54}N_4$	–	10	–	–
	492	$C_{30}H_{44}N_4O_2$	–	–	15/10	–
	466	$C_{30}H_{50}N_4$	30	–	–	–
b	424	$C_{27}H_{44}N_4$	2	–	–	–
	410	$C_{27}H_{44}N_3$	–	–	–	8
	396	$C_{26}H_{42}N_3$	–	5	–	–
	393	$C_{25}H_{37}N_4$	–	–	1/0	–
c	382	$C_{24}H_{36}N_3O$	–	–	25/4	–
	368	$C_{24}H_{38}N_3$	14	–	–	–
	358	$C_{23}H_{40}N_3$	–	3	–	–
	291	$C_{19}H_{35}N_2$	–	–	–	3–
d	340	$C_{21}H_{30}N_3O$	–	–	7/4	–
	330	$C_{21}H_{36}N_3$	5	–	–	–
	289	$C_{19}H_{33}N_2$	–	4	–	–
	275	$C_{18}H_{31}N_2$	–	–	–	8
e	314	$C_{20}H_{32}N_3$	6	–	–	–
	286	$C_{17}H_{24}N_3O$	–	–	4/2	–
	274	$C_{17}H_{28}N_3$	–	5	–	–
	262	$C_{17}H_{30}N_2$	–	–	–	75
f	286	$C_{18}H_{28}N_3$	11	–	–	–
	261	$C_{17}H_{29}N_2$	–	18	–	–
	259	$C_{16}H_{23}N_2O$	–	–	20/8	–
	247	$C_{16}H_{27}N_2$	–	–	–	46
g	271	$C_{18}H_{27}N_2$	37	–	–	–
	247	$C_{16}H_{27}N_2$	–	65	–	–
	246	$C_{15}H_{22}N_2O$	–	–	100/100	–
	205	$C_{13}H_{21}N_2$	–	–	–	28
h	245	$C_{16}H_{25}N_2$	19	–	–	–
	233	$C_{15}H_{25}N_2$	–	22	–	–
	231	$C_{15}H_{23}N_2$	–	–	31/6	–
	178	$C_{11}H_{18}N_2$	–	–	–	24
i	233	$C_{15}H_{25}N_2$	40	–	–	–
	207	$C_{13}H_{23}N_2$	–	18	–	–
	189	$C_{11}H_{13}N_2O$	–	–	4/3	–
	164	$C_{11}H_{18}N$	–	–	–	30
j	219	$C_{14}H_{23}N_2$	54	–	–	–
	193	$C_{12}H_{21}N_2$	–	8	–	–
	163	$C_{10}H_{15}N_2$	–	–	6/5	–
	151	$C_{10}H_{17}N$	–	–	–	97
k	193	$C_{12}H_{21}N_2$	18	–	–	–
	151	$C_{10}H_{17}N$	–	49	–	–
	148	$C_9H_{10}NO$	–	–	48/50	–
	136	$C_9H_{14}N$	–	–	–	50
l	179	$C_{11}H_{19}N_2$	9	–	–	–
	136	$C_9H_{14}N$	–	57	–	–
	134	$C_9H_{12}N$	–	–	63/16	–
	124	$C_8H_{14}N$	–	–	–	38
m	137	$C_9H_{15}N$	27	–	–	–
	112	$C_7H_{14}N$	–	69	14/8	100
n	98	$C_6H_{12}N$	100	100	7/8	33
o	84	$C_5H_{10}N$	25	30	10/3	30



Scheme 1. Proposed fragmentation pathway observed in the EI MS spectrum of **D1**.

As can be seen from Schemes 2 and 4 and Table 1, the principal fragmentation pathways of **D2** and **D5** are similar, but show differences in the abundances of important fragment ions. In the mass spectra of **2** and **5** there are fragment ions which have been obtained by the elimination of methyl radicals from the A or/and C rings of the molecules investigated. The elimination of the methyl radical from the molecular ions of **2** and **5** can be explained by the cleavage of the C(2)–CH_3 or C(17)–CH_3 bond.[12] Such an elimination of the methyl radical can be seen only in the mass fragmentation of the dimer **D5**. The even-electron fragment ions **d** and **f** of **D5** have been obtained by the simple δ cleavage of C(2)–CH_3 and C(17)–CH_3 bond, respectively.

The common characteristic features of the EI MS fragmentation of **D1**–**D5** are the presence of ions corresponding to the monomer part of the bis-quinolizidine alkaloids. The even-electron fragment ions **i** (for **D1**) and **g** (for **D2**) have been obtained by the cleavage of C(17)sp^3–C(17')sp^3 bonds. Ion **i** ($C_{15}H_{25}N_2$, *m/z* 233) has only 40% of the relative intensity whereas ion **g** ($C_{16}H_{27}N_2$ *m/z* 247) has 65% of the relative intensity. In both presented ions the positive charge is probably located on the nitrogen atom N(16). The odd-electron fragment ions **g** (for **D3** or **D4**) and **e** (for **D5**) are obtained by the cleavage of C(17)sp^2 = C(17')sp^2 and C(12)sp^3–C(12')sp^3 bonds, respectively. The relative abundance of this ions is 100% of **g** ($C_{15}H_{22}N_2O$ *m/z* 246) and 75% of **e** ($C_{17}H_{30}N_2$ *m/z* 262), respectively.

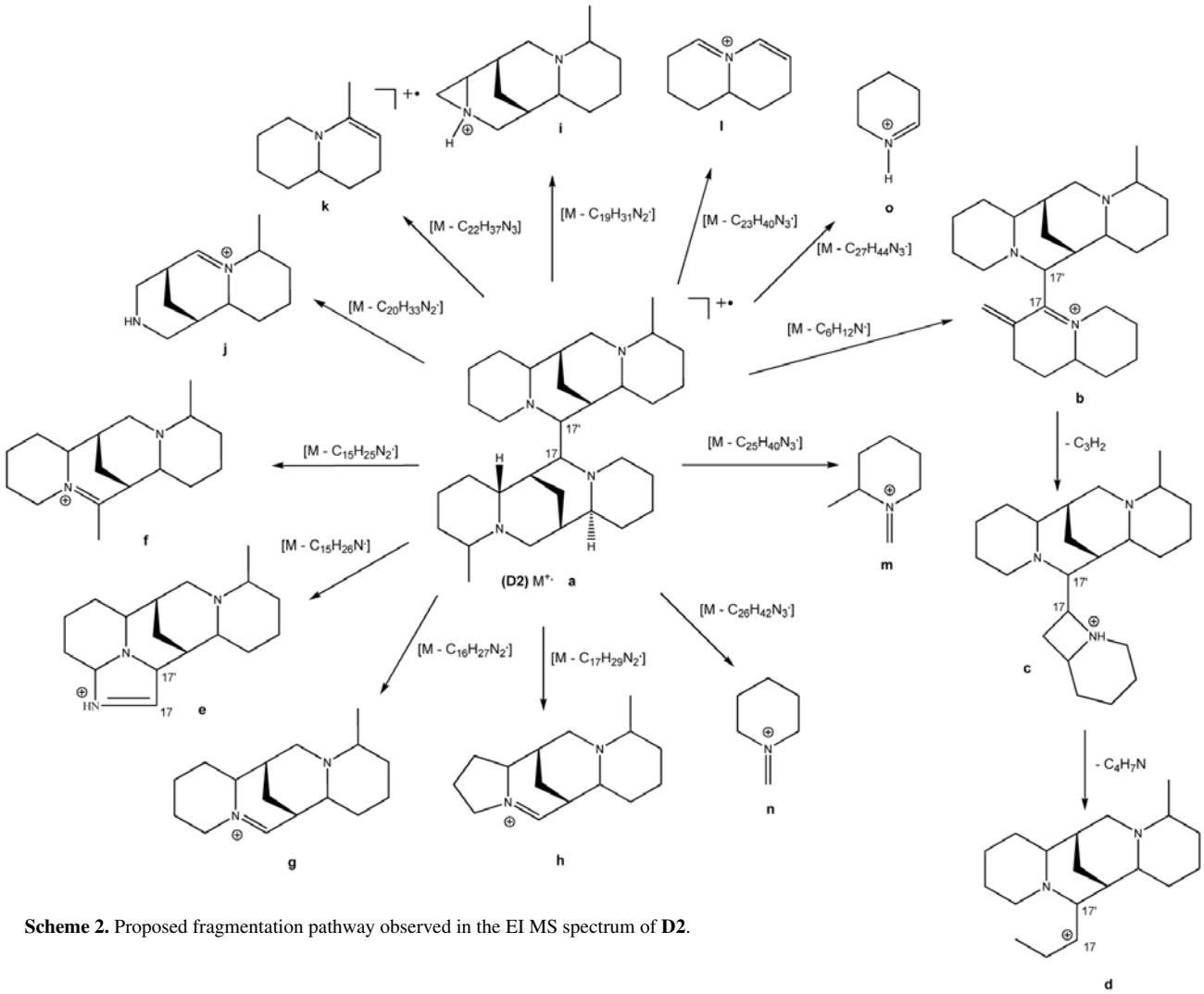
For **D2**–**D5** ions **m** formed by cleavage of ring B are located at *m/z* 112, whereas for compound **D1** the odd-electron ion **m** is located at *m/z* 137. Although dimers **D3** and **D4** have the same structure and show the same fragmentation patterns, they can be distinguished from each other based on different abundances of ions: **a**, **c**, **f**, **h**, **l**, **o**.

The differences in the fragmentation of **D3**–**D4** (molecular ions of dimers with the same elemental composition) have been expressed quantitatively by comparing the calculated values of the μ coefficients, i.e. the abundances of selected fragment ions relative to the abundances of the odd-electron ions **a**.

Because the values of μ are highly dependent on the intensities of the ions, it is necessary to average three scans to obtain adequate statistical results. Table 2 presents the values of μ_1, μ_2 and μ_3 coefficients for dimers as:

μ_1 = % *RA* **g** / % *RA* **a** ; μ_2 = % *RA* **m** / % *RA* **a** ;

μ_3 = % *RA* **o** / % *RA* **a**



Scheme 2. Proposed fragmentation pathway observed in the EI MS spectrum of **D2**.

The analytical ions for the calculation of the coefficients μ_1, μ_2 and μ_3 were chosen similarly as previously for the differentiation of the stereoisomers and substituted sparteines.[10–12]

As can be seen from the data in Table 2, dimer **D4** may be distinguished from **D3** on the basis of its lower values of μ_2 and μ_3 and higher value of μ_1:

μ_2, μ_3 **D3** > μ_2, μ_3 **D4** μ_1 **D4** > μ_1 **D3**.

Table 2. Values of fragment ions peak intensities relative to those of the corresponding molecular ions (μ, see text for definitions) determined from the EI mass spectra of the dimers **D3** and **D4**. [Intra- and inter-day repeatabilities were of the order of 1–3%.]

Dimer	μ_1	μ_2	μ_3
D3	6.66	0.93	0.66
D4	10.0	0.80	0.30

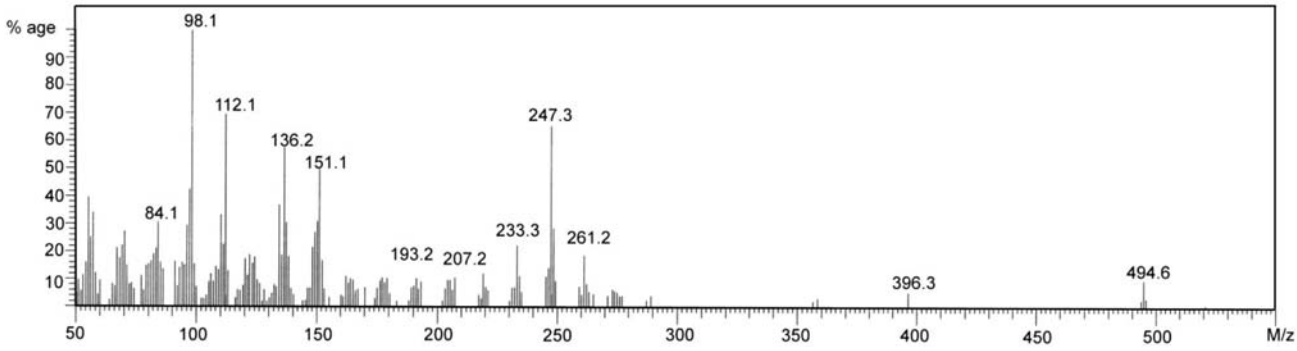
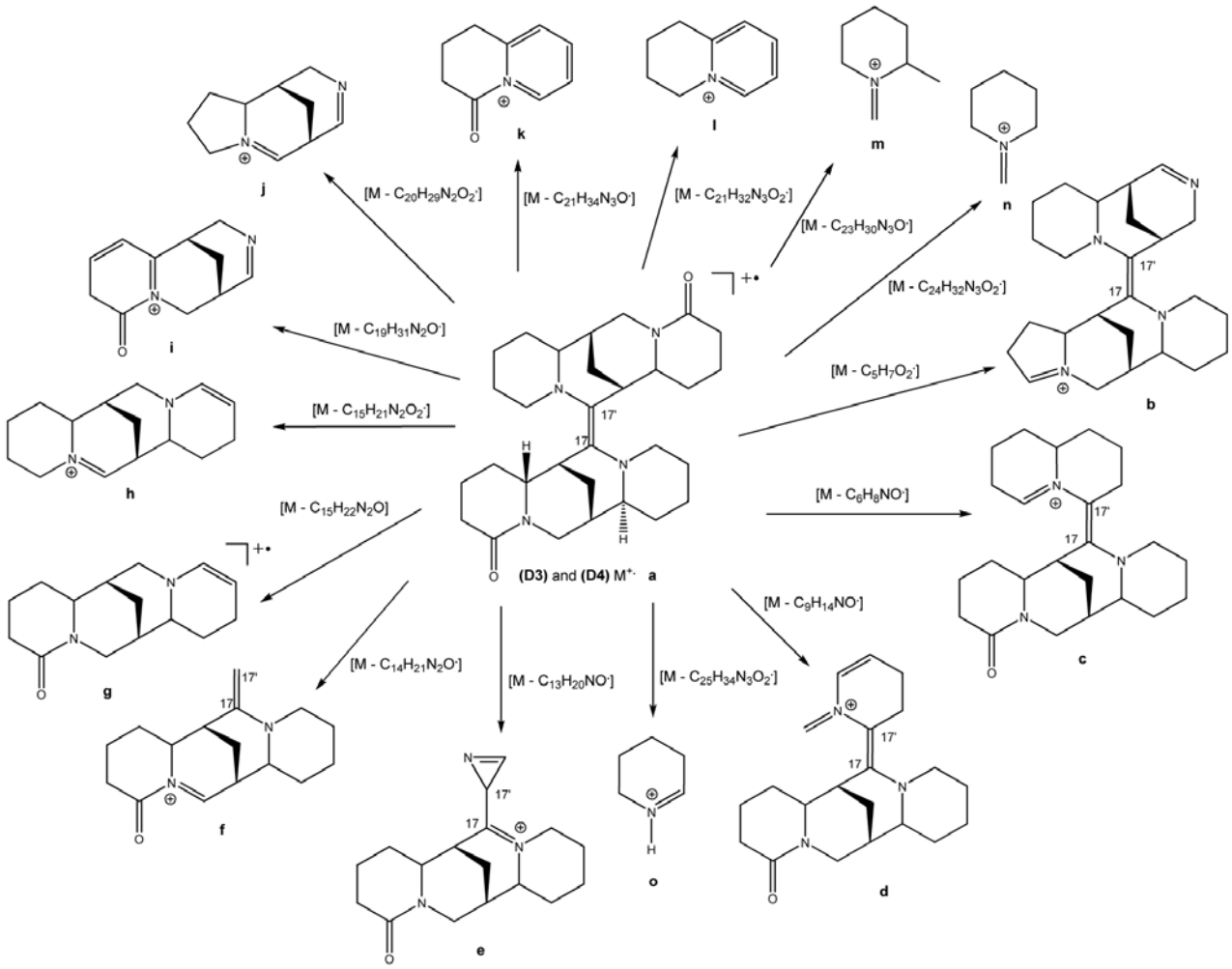


Figure 2A. EI MS spectrum of the dimer **D2**.



Scheme 3. Proposed fragmentation pathway observed in the EI MS spectra of **D3** and **D4**.

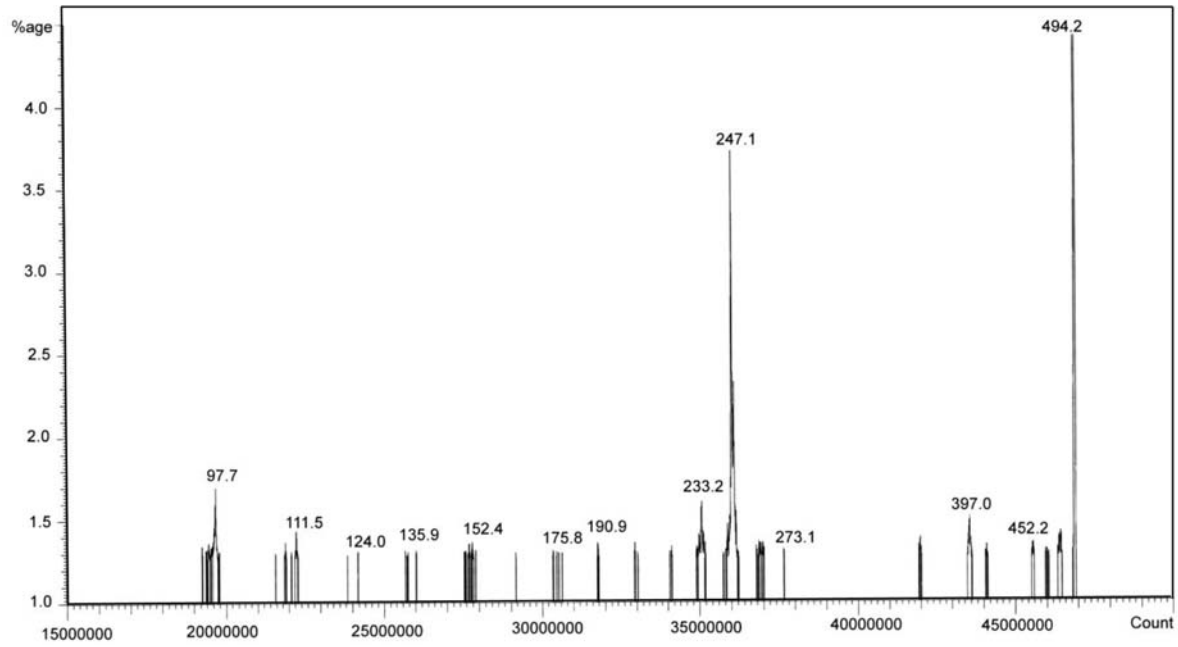
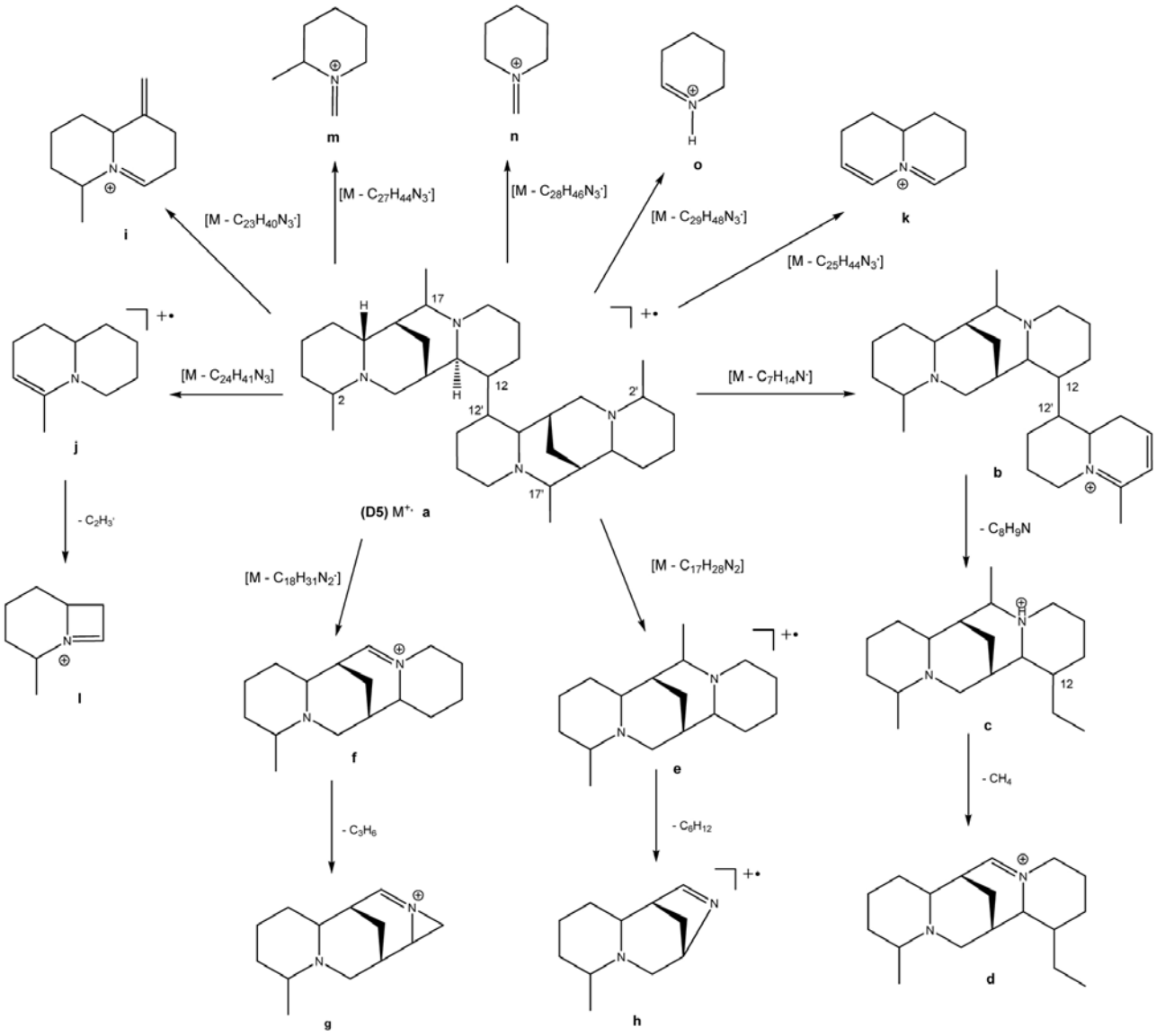


Figure 2B. B/E mass spectrum of the **[M+· a]** ion at *m/z* 494 of **D2**.



Scheme 4. Proposed fragmentation pathway observed in the EI MS spectrum of **D5**.

Table 3. Heats of formation of alkaloids and its dimers **D1–D5** calculated by PM5 semiempirical method.

Compound	Heat of formation [kcal/mol]
1	–26.3335
2	–31.4112
3	–107.4384
4	–69.3802
5	–36.3871
D1	–35.9377
D2	–45.3540
D3/D4	–86.9464
D5	–63.8692

Thus, PM5 semiempirical method is reliable method for visualization of the structures in the gas phase. Calculated structure of dimers **D1**–**D5** are presented in Figure 3. Calculation shows that heat of formation (HOF) of α-isosparteine, 2-methylsparteine, 2,17-dimethylsparteine and lupanine *N*-oxide is higher than that of corresponding dimers, whereas the HOF of 17-hydroxylupanine is lower than that of **D3** (Table 3). The latter may be explained by the fact that the water loss is much more favorable in the monomer **3** than **4**.[21] The highest heat of formation observed for α-isosparteine and its dimer is probably related to the *trans-trans* all chair conformation of rigid molecule.

4. Conclusion

In the mass fragmentation of dimers **D1**–**D5** fragment ions formed from two molecules of bis-quinolizidi-

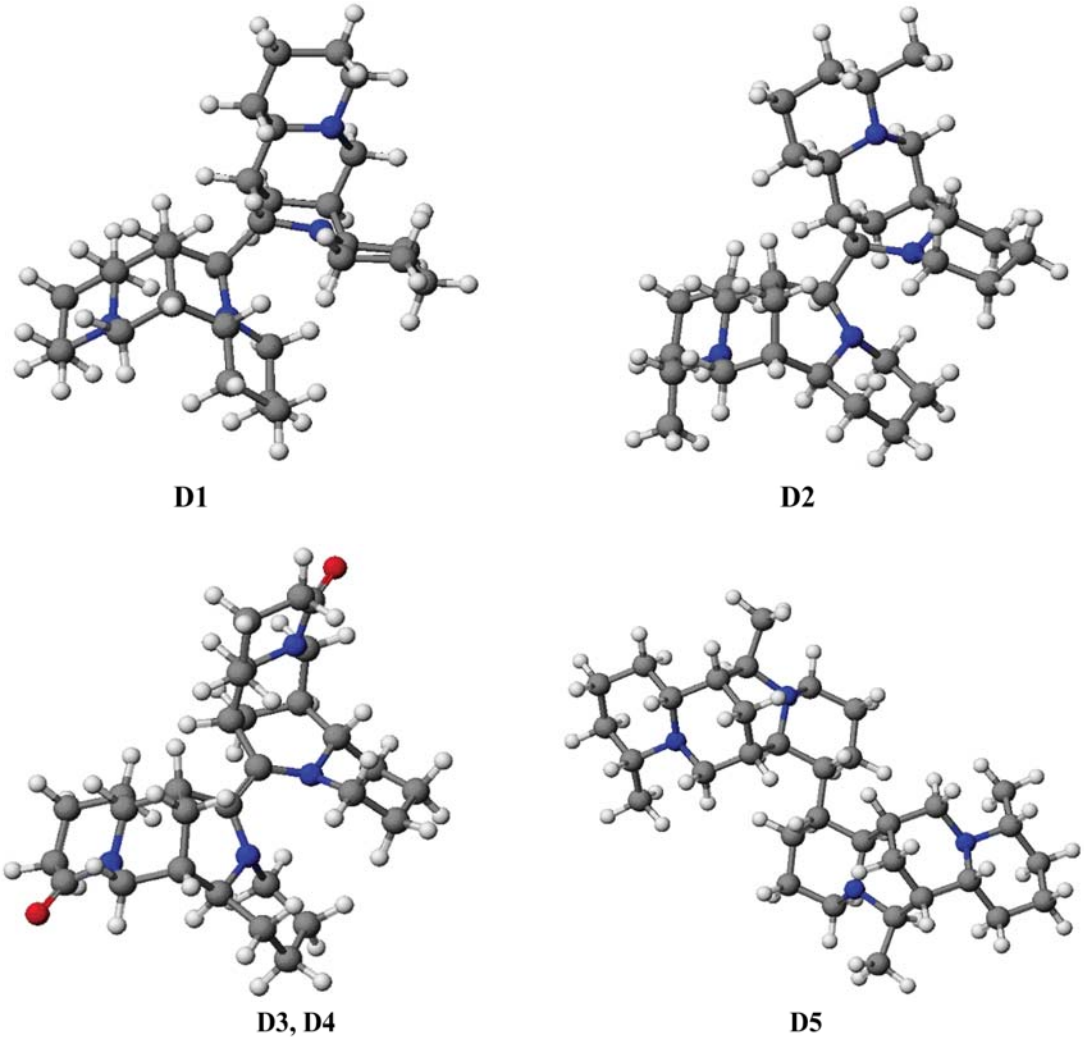


Figure 3. The optimized structures of the bis-quinolizidine dimers **D1-D5.**

ne alkaloids as well as the presence of ions corresponding to the monomer part of the alkaloids can be observed. The results obtained have proved that it is possible to differentiate between dimers obtained. On the basis of the EI mass spectra, differentiation of the dimers **D3** and **D4** can be achieved using the values of coefficients μ.

4. 1. Dedication

This paper is dedicated to the memory of Prof. E. Wyrzykiewicz.

5. References

1. S. P. Sastry, in: G. R. Waller (Ed.): Biochemical Application of Mass Spectrometry, Wiley-Interscience, New York, **1972**, p.655.
2. E. Wyrzykiewicz, W. Wysocka, *Org. Mass Spectrom.* **1990**, *25,* 453–456.
3. E. Wyrzykiewicz, W. Wysocka, M. Wiewiórowski, *Org. Mass Spectrom.* **1988**, *23,* 700–704.
4. U. Majchrzak-Kuczyñska, M. Wiewiórowski, E. Wyrzykiewicz, *Org. Mass Spectrom.* **1984**, *19*, 600–604.
5. J. L. Stymiest, G. Dutheuil, A. Mahmood, V. K. Aggarwal, *Angew. Chem. Int. Ed.* **2007**, *46,* 7491–7494.
6. I. Coldham, J. J. Patel, S. Raimbault, D. T. E. Whittaker, H. Adams, G. Y. Fang, V. K. Aggarwal, *Org. Lett.* **2008**, *10*, 141–143.
7. M. Wink, in: E. A. Bernays (Ed.): Insect-plant interaction, CRC-Press, Boca Raton, **1992**, p.136.
8. M. Wink, in: G. A. Cordell (Ed.): The Alkaloids: Chemistry and Biology, San Diego, **1993**.
9. T. Schmeller, M. Sauerwein, F. Sporer, M. Wink, W. E. Müller, *J. Nat. Products* **1994**, *57*, 1316–1319.
10. E. Wyrzykiewicz, W. Boczoñ, B. Kozioł, *J. Mass Spectrom.* 1**998**, *33,* 971–975.
11. B. Jasiewicz, W. Boczoñ, E. Wyrzykiewicz, *J. Mass Spectrom.* **2004**, *39,* 541–547.
12. E. Wyrzykiewicz, W. Boczoñ, B. Kozioł, *J. Mass Spectrom.* **2000**, *35*, 1271– 1277.

13. B. Hachmeister, W. Kehrbach, U. Kuehl, G. Buschmann, Bis-Sparteine Derivatives and Method of Using Same In Therapy, U.S. Pat. No. 4,415,577.
14. H. G. Bolt, Achievements in Alkaloid Chemistry Up to 1960, Akademie Verlag, Berlin, **1961**, p. 197.
15. M. Hesse, H. O. Bernhard, in H. Budzikiewicz (Ed.): Alkaloide, Verlag Chemie GmbH, Weinheim, **1975**, p. 264.
16. CAChe 5.04, UserGuide, Fujitsu. **2003**
17. J. J. P. Stewart, *J. Comput. Chem.* **1998**, *10,* 209–220.
18. J. J. P. Stewart, *J. Comput. Chem.* **1998**, *10*, 221–264.
19. J. Tamas, in J. S. Splitter, F. Turecek (Eds.): Applications of Mass Spectrometry to Organic Stereochemistry, New York, **1994**, pp. 625–626.
20. G. L. Buchanan, *Chem. Soc. Rev*. **1974**, *3,* 41–63.
21. B. Jasiewicz, E. Wyrzykiewicz, *Int. J. Anal. Chem*. **2011**, Article ID 652589.

Povzetek

Razpravljamo o masnih spektrih z elektronsko ionizacijo za dimere bis-kvinolizidinskih alkaloidov: α-izospartein, 2-metilspartein, 17-hidroksilupanin, lupanin *N*-oksid in 2,17-dimetilspartein. Predlagamo tudi splošne fragmentacijske poti njihovih molekulskih kationov. S PM5 metodami preučujemo strukture dimerov. Prepoznavanje fragmentacijskih poti in relativnih intenzitet opaženih ionov daje pomembne informacije, koristne pri identifikaciji podobnih dimernih spojin.