Faculty of Chemistry[1], Adam Mickiewicz University; Department of Physical Pharmacy and Pharmacokinetics[2], Poznań University of Medical Sciences, Poznań, Poland; Department of Pharmaceutical Chemistry[3], Christian-Albrechts-University, Kiel, Germany

Electron ionisation mass spectral study of 2-(2-carboxy-4, 5-dimethoxyphenyl)-6,7-dimethoxyisoquinolinium inner salt

E. Wyrzykiewicz[1], A. Czyrski[2], T. Hermann[2], U. Girreser[3]

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Andrzej Czyrski, Department of Physical Pharmacy and Pharmacokinetics, Poznań University of Medical Sciences, Święcickiego Street 6, 60-781 Poznań, Poland
aczyrski@ump.edu.pl

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The electron ionization mass spectrum of 2-(2-carboxy-4,5-dimethoxyphenyl)-6,7-dimethoxyisoquinolinium inner salt **1** is discussed and general fragmentation routes of its molecular ion are proposed. Comparison of the data obtained for **1** with the EI-MS data of metameric protopine alkaloids (allocryptopine, argemexicaine A, argemexicaine B) and the pseudobenzyloisoquinoline alkaloid taxilamine allows a differentiation between these metamers. The data will be useful for the identification of metabolites of alkaloids of these types in biological matrices.

1. Introduction

Papaverine (4-(3′,4′-dimethoxybenzyl)-6,7-dimethoxyisoquinoline) as one of the benzyl isoquinoline alkaloids was used clinically as bronchodilator to relax various smooth muscles, smooth musculature of the larger blood vessels, especially coronary systemic peripheral and pulmonary arteries to increase cerebral blood flow (Abdel-Ghani et al. 2002).

Despite its important therapeutic values, its metabolism both *in vivo* and *in vitro*, as well as the processes of oxidation, photooxidation and degradation of its injection solutions have not been fully explained yet but have been extensively investigated (Girreser et al. 2003; Hermann et al. 2002).

Mass spectrometry is a modern powerful tool for identification and quantitative determination of papaverine metabolites and products of its oxidation and degradation which contaminate authentic samples and are also found in its injection solutions (Peng et al. 2007; Pfeiffer et al. 1972). The formation of papaverinol and papaveraldine in aqueous solutions of papaverine has been known for a long time (Colautti et al. 1987; Piotrowska et al. 2002). The structure of the final degradation product formed in papaverine solutions was found to be a 2,3,9,10-tetramethoxy-12-oxo-12-*H*-indolo[2,1-*a*]-isoquinolinium salt (Girreser et al. 2003). Lately the formation of compound **1** (Fig. 1) from aqueous solutions of papaverine has been also announced (Girreser et al. 2009). However, the EI mass fragmentation of this compound has not been reported and is clarified in this study. This investigation was also undertaken to establish whether it is possible to differentiate this compound from the metameric protopine type alkaloids allocryptopine (**2**), argemexicaine A (**3**), and argemexicaine B (**4**) as well as taxilamine (**5**, as pseudoisoquinoline alkaloid) (Fig. 2).

The protopines are characterized by the presence of a ten-membered ring hexahydrodibenzo[c,g]azeine with a methylated tertiary nitrogen atom and a keto group fused to two aryl moieties. The pseudobenzyloisoquinolines alkaloid **5** is a benzylisoquinoline which incorporates three oxygenated substituents in the bottom aromatic ring.

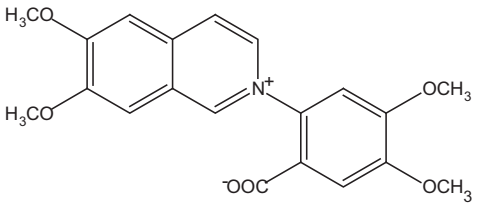


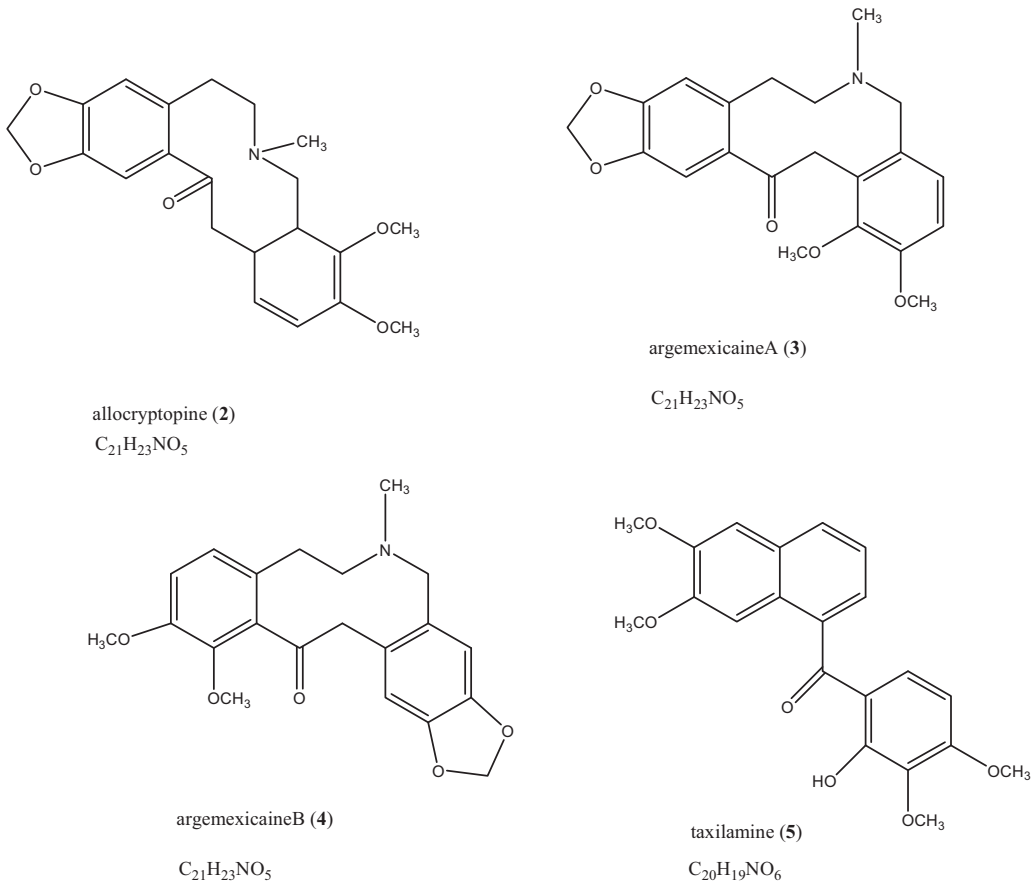
Fig. 1: 2-(2-Carboxy-4,5-dimethoxyphenyl)-6,7-dimethoxyisoquinolinium inner salt (**1**)

The aim of our study was the differentiation of isoquinoline alkaloids, which we attempted in view of the fact that the EI mass spectrometric method had been proved to be useful for identification of metabolites of alkaloids of these types in biological matrices.

2. Investigations, results and discussion

On the basis of low resolution EI mass spectra as well as B/E linked-scan spectra and exact mass determinations (Table 1) the principal mass fragmentation routes of compound **1** were interpreted as shown in the Scheme. It should be pointed out that only the fragmentation of the molecular ion **a** of **1** was confirmed by B/E linked-scan spectra and the metastable transitions observed in these experiments are labelled with asterisks in the Scheme.

As it can be seen from Scheme and Table 1, the molecular ion **a** of **1** is the base peak in the mass spectrum of this compound. The main features of the EI mass spectral fragmentation of the molecular ion **a** of **1** are the cleavages of the C_{sp2}-C_{sp2} and C_{sp2}-O bonds of the carboxy substituent of the skeleton of N-(dimethoxycarboxyphenyl)dimethoxyisoquinoline(Scheme).



allocryptopine (**2**)
$C_{21}H_{23}NO_5$

argemexicaineA (**3**)
$C_{21}H_{23}NO_5$

argemexicaineB (**4**)
$C_{21}H_{23}NO_5$

taxilamine (**5**)
$C_{20}H_{19}NO_6$

Fig. 2: Protopine alkaloids **2** (Reuffer and Zenk 1982), **3** (Change 2003), **4** (Change 2003) and pseudobenzyloisoquinoline alkaloid **5** (Blasko 1982)

The simultaneous cleavages of C_{sp2}-H and C_{sp2}-C_{sp2} bonds in the molecular ion **a** of **1** accompanied with the hydrogen migration and elimination of $^{\bullet}$COOH radical lead to the tetracyclic even-electron fragment ion **e** with the positive charge on the annular nitrogen atom of the isoquinoline ring. In the EI fragmentation of the molecular ion of **1** the cleavages of the C_{sp2}-O bond of the carboxy substituent and the C_{sp2}-H bond of the isoquinoline skeleton also follow a rearrangement involving the migration of the hydrogen atom to oxygen atom and elimination of the $^{\bullet}$OH radical. Even-electron fragment ion **c** was obtained in this way of mass decomposition. The ejection of the neutral molecule of carbon monoxide from the even-electron fragment ion **c** leads also to the even-electron fragment ion **e**. It should be pointed out that the simple cleavage of C_{sp2}-H bond with the ejection of $^{\bullet}$H radical from the molecular ion of **1** leads to the tetracyclic even-electron fragment ion **b**. The ejection of the neutral molecule of carbon monoxide from the even-electron fragment ion **b** leads to the even-electron fragment ion **d**. According to the data obtained from the metastable transitions, direct simultaneous eliminations from the molecular ion of **1** of hydrogen radical and carbon monoxide molecule have also taken place ($H^{+\bullet}$a → c → d) (Scheme).

The next steps of the EI mass fragmentation of the molecular ions of **1** involve the cleavages of the C_{sp3}-O bonds of the methoxy substituents with eliminations of methyl radicals, leading to the even-electron fragment ions (f, g and i) and odd-electron fragment ion h. (Scheme).

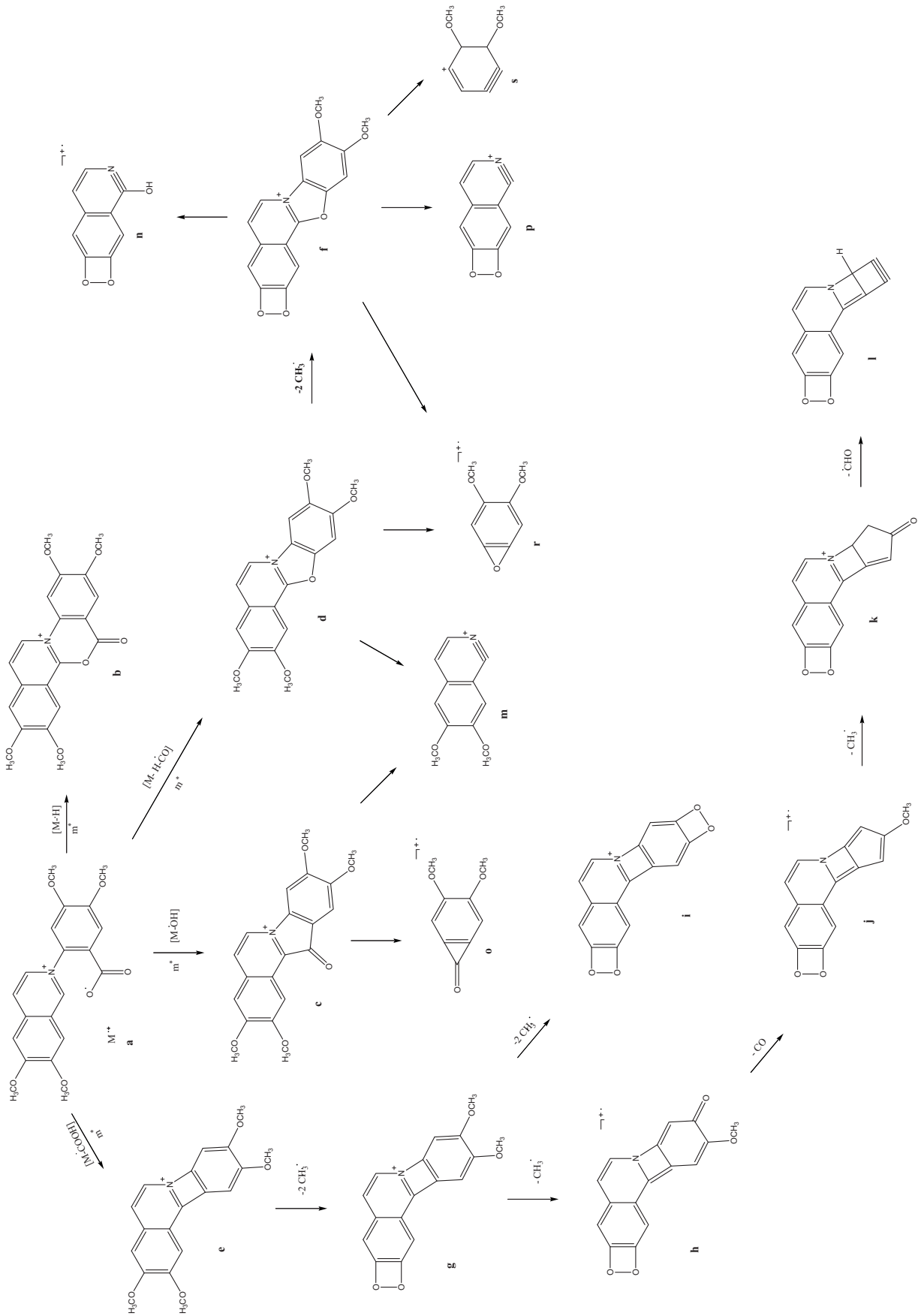
It ought to be pointed out that the cleavages of the bonds of the phenyl rings of compound **1** have been seen first in the fifth or subsequent steps of the EI mass fragmentation. The even-electron fragment ion **k** is obtained in this way as well as odd-electron fragment ions j and l.

The mass spectrum of **1** reveals some characteristic features of the EI mass fragmentation of the even-electron tetracyclic fragment ions **c**, **d** and **f**. These are the simple cleavages of the C_{sp2}-N, C_{sp2}-O and C_{sp2}-CO bonds in the 1,3-oxazole ring (ion **d**) and the pirolidone ring (ions **c** and **f**) accompanied be migration or retention of charge and also migration of hydrogen. By these inductive cleavages of two bonds the complementary even-electron and odd-electron fragment ions are obtained according to the following sequences of the EI mass

Table 1: Relative abundances of ions in the EI mass spectrum of 1

Ion	m/z	Elemental composition	% Relative abundance
a	369	$C_{20}H_{19}NO_6$	100
b	368	$C_{20}H_{18}NO_6$	86
c	352	$C_{20}H_{18}NO_5$	71
d	340	$C_{19}H_{18}NO_5$	86
e	324	$C_{19}H_{18}NO_4$	81
f	310	$C_{17}H_{12}NO_5$	68
g	294	$C_{17}H_{12}NO_4$	53
h	279	$C_{16}H_9NO_4$	72
i	264	$C_{15}H_6NO_4$	20
j	250	$C_{15}H_9NO_3$	20
k	236	$C_{14}H_6NO_3$	17
l	207	$C_{13}H_5NO_2$	20
m	188	$C_{11}H_{10}NO_2$	14
n	175	$C_9H_5NO_3$	17
o	164	$C_9H_8NO_3$	13
p	158	$C_9H_4NO_2$	5
r	152	$C_8H_8NO_3$	14
s	135	$C_8H_7O_2$	7

The elemental composition listed have masses within ± 2 ppm of those measured by peak matching of high resolution



Scheme: Pathways of the EI mass fragmentation of the molecular ion of **1**

Table 2: Relative abundances of ions in the EI mass spectra of 2, 3, 4, 5

Elemental compound **2** (Rueffer and Zenk 1982) $C_{21}H_{23}NO_5$		Elemental compound **3** (Chang 2003) $C_{21}H_{23}NO_5$		Elemental compound **4** (Chang 2003) $C_{21}H_{23}NO_5$		Elemental compound **5** (Blasko 1982) $C_{20}H_{19}NO_6$	
Ion m/z	% RA	Ion m/z	% RA	Ion m/z	% RA	Ion m/z	% RA
369	5	369	3	369	5	369	32
341	5	206	24	179	23	354	14
297	5	165	23	150	23	352	12
268	16	164	100	149	52	326	14
206	28	163	32	148	100	311	16
164	100	149	41	92	13	310	68
163	21	134	33	81	18	296	33
149	16	121	19	89	12	188	57
		104	21			181	13
		91	17			149	75
		77	18			86	50
						84	74
						49	100

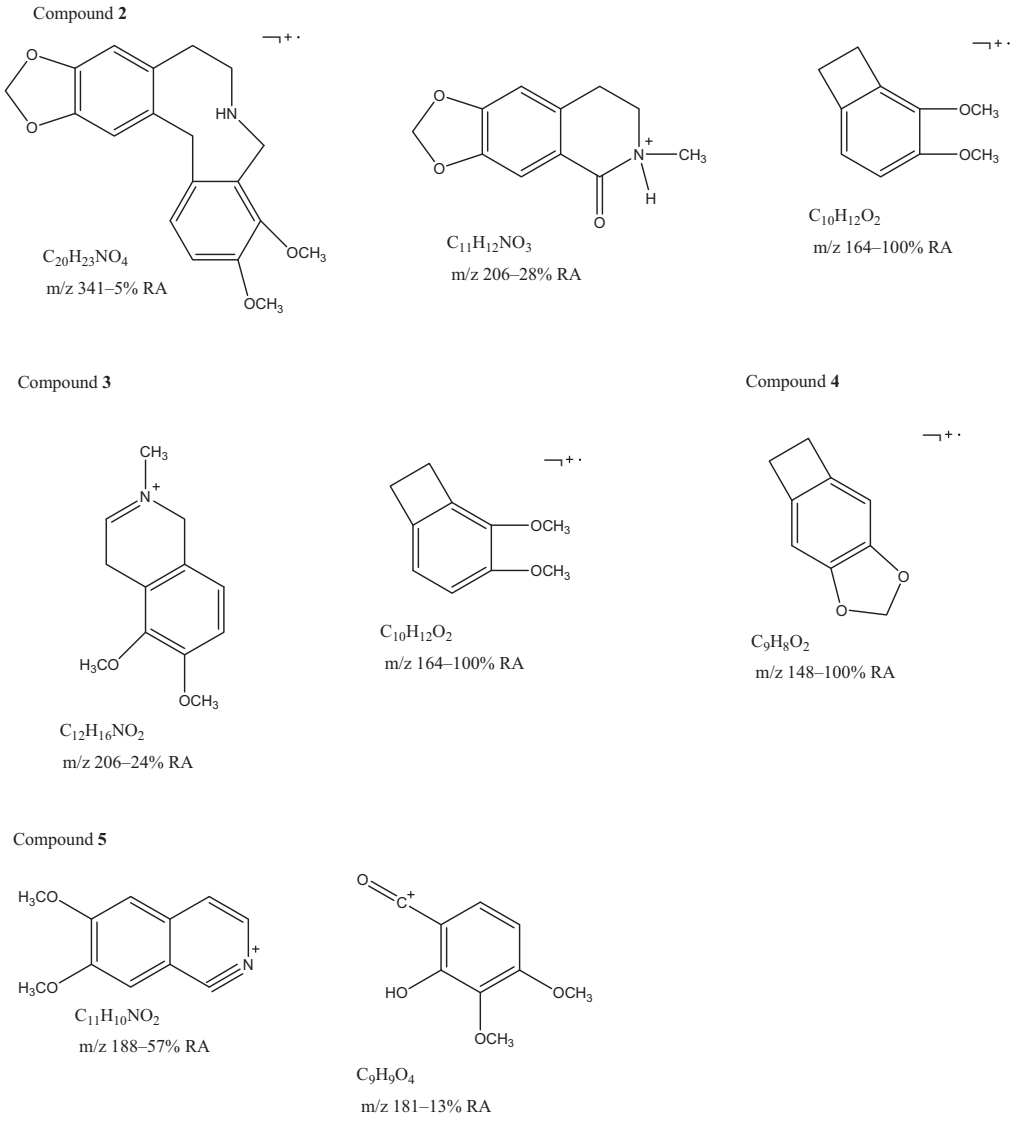


Fig. 3: Proposed structures of the analytical ions in the EI mass spectra of compounds **2**, **3**, **4**, **5**

fragmentations (Scheme).

$$c^{\neg +} \rightarrow o^{\neg +\bullet} + m^{\neg +}$$
$$d^{\neg +} \rightarrow r^{\neg +\bullet} + m^{\neg +}$$
$$f^{\neg +} \rightarrow r^{\neg +\bullet} + p^{\neg +}$$
$$f^{\neg +} \rightarrow n^{\neg +\bullet} + s^{\neg +}$$

In conclusion, the results reported here show that the presence of the carboxy substituent in the phenyl ring of **1**, together with the 'zwitterionc' structure of this compound influences the stability of the molecular ion in the EI conditions. The molecular ion **a** of **1** is the basic peak in the mass spectrum of **1**. On the

other hand, the tetracyclic structure of [M-•H] **b**, [M•H-CO] **d**, [M-•OH] **c** even electron ions is much less stable than the tetracyclic structure of [M-•COOH] **e** even-electron fragment ion. The presence of 1,3-oxazole (ions **d** and **f**) and pirolidone ring (ion **c**) in the structure of **c**, **d**, and **f** influence the facility of cleavages of the C_{sp2}-N, C_{sp2}-O and C_{sp2}-CO bonds and formation of the complementary fragment ions (**o** + **m**; **r** + **m**; **r** + **p**; **n** + **s**).

It should be pointed out that two of these ions (i.e., **o** and **m**) are suitable as the analytical ions for the differentiation of **1** from among the metameric protopines alkaloids **2**, **3**, **4** (Chang et al. 2003; Rueffer and Zenk 1982) and pseudobenzylisoquinoline alkaloid **5** (Blasko et al. 1982) (Fig. 2) on the basis of analysis of their EI mass spectra.

Analysis of the EI mass spectra of **1** and **2**, **3**, **4** and **5** (Blasko et al. 1982; Chang et al. 2003; Rueffer and Zenk 1982) implies that the analytical ions useful for selected ion monitoring of these compounds are $M^{+\bullet}$ - m/z 369 (**1**-**5**), m/z 206 (**2**, **3**), m/z 164 (**1**, **2**, **3**), m/z 148 (**4**) and m/z 188 (**1**, **5**).

The low-resolution EI mass spectra of **2**, **3**, **4** and **5** are presented in Table 2. Fig. 3 presents the proposed structures of the analytical ions. These ions are obtained by cleavages of the bonds of the ten-membered ring of **2**, **3**, **4** as well as by the cleavage of the C_{sp2}-CO bond of the skeleton of **5**. When the base peak in the EI mass spectrum of **1**-**5** is at m/z 369 the species can be recognized as **1**. If the base peak is at m/z 148 this compound can be identified as **4**. The base peak attributed to the odd-electron fragment ion situated at m/z 164 indicates that the compound of interest is **2** or **3**. Differentiation of these compounds is possible on the basis of the values of coefficients μ (ratios of the abundances of selected analytical fragment ions to that of the odd-electron ions). The comparison of the values of μ in the differentiation of isomers and metamers of organic compounds has been previously used in our laboratory (Wyrzykiewicz and Prukała 1999; Wyrzykiewicz et al. 2005; Jasiewicz and Wyrzykiewicz 2008). As shown in Table 3, the difference between the calculated values of the coefficient μ i.e. the abundance of the analytical ion at m/z 164 relative to that of the molecular ion at m/z 369 can be sufficient to differentiate between **2** and **3**. It is also seen from the data presented in Table 3 that the values of μ_2 (i.e. the abundance of the molecular ion at m/z 188 relative to that of the molecular ion at m/z 369) can be sufficient to differentiate between **1** and **5**.

The EI mass fragmentation of the molecular ion of **1** proceeds in several steps: it begins with the elimination of carboxy radical, then involves cleavages of the bonds in the 1,3-oxazole and pirolidine rings of fragment ions obtained in the subsequent steps of mass decomposition.

The result obtained have proved that it is possible to differentiate between compound **1** and its metameric protopine type alkaloids **2**, **3**, **4** as well as pseudobenzylisoquinoline alkaloid **5** on the basis of the EI mass spectra of these compounds.

This differentiation is possible on the basis of the presence of the base peak of the molecular ion at m/z 369 (**1**), the base peaks of the odd-electron fragment ions at m/z 148 (**4**) and at m/z 164 (**2,3**) as well as the even electron fragment ion at m/z 188 (5-57% RA, 1-14% RA). Detailed examination of μ coefficients, referring relative abundances of the selected fragment ions to the abundance the molecular ions, makes it possible to differentiate between isomeric **2** and **3** (Table 3 – μ_1) metameric **1** and **5** (Table 3–μ_2).

The obtained data will be useful for the selected ion monitoring in EI-MS analysis and helpful for quantitative determination of the derivatives of protopine, papaverine and pseudobenzyloisoquinolinealkaloids and their metabolites in biological matrices on the basis of mass fragmentography.

Table 3: Values of μ_1 and μ_2 determined for the EI mass spectra of 2, 3, 1 and 5

Compound	μ_1	μ_2
2	20.0	—
3	33.3	—
1	—	0.14
5	—	1.78

$$\mu_1 = \frac{\%\,RA\; m/z\;169}{\%\,RA\; M^{+\bullet}\cdot(m/z\;369)}$$

$$\mu_1 = \frac{\%\,RA\; m/z\;188}{\%\,RA\; M^{+\bullet}\cdot(m/z\;369)}$$

3. Experimental

Compound **1** was obtained by dissolving 2,3,9,10-tetramethoxy-12-oxo-12-*H*-indolo[2,1-*a*]-isoquinolinium chloride in ca 0.4% NaOH aqueous solution according to the literature (Girreser et al. 2009).

Low- and high resolution mass spectra were recorded using a model 402 two-sector-mass spectrometer (AMD- Intectra 6 MBH, Haupstadt, Germany; ionizing voltage 70 eV, accelerating voltage 8 kV, mass resolution 10000 at 10% valley). Samples were introduced by a direct injection probe at a source temperature of ~150 °C. The elemental compositions of the ions were determined by a peak matching relative to perfluorokerosene. All masses measured agreed with the elemental composition listed in column 3 of Table 1 to within ±2 ppm. The spectra from the first-field free region were recorded using linked scan at a constant B/E, with helium as the collision gas at an indicated pressure of 1.75×10^{-5} Pa with the ion source temperature of 180 °C, ionizing energy of 70 eV, and accelerating voltage of 8 kV.

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