

E. Wyrzykiewicz* and D. Prukała

Department of Mass Spectrometry of Organic Compounds, Faculty of Chemistry,
Adam Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland

Received June 16, 1998

Revised April 7, 1999

EI induced mass spectral fragmentations of twelve new hydrazones of 2-(3- and 4-)-pyridinecarboxaldehydes and hydrazides of (*E*)-stilbenyloxyacetic acid as well as *N*-(*E*)-stilbenyloxyalkylcarbonyl substituted amino acids were investigated. Fragmentations pathways are proposed on the basis of accurate mass and B/E linked scan spectra measurements. The correlation between the intensities of M^{+} and the selected fragment ions of these compounds is discussed. The data obtained create the basis for distinguishing isomers.

J. Heterocyclic Chem., **36**, 739 (1999).

Introduction.

In a previous communication [1], we described the synthesis and physicochemical properties of isomeric hydrazones of 2-(3- and 4-)-pyridinecarboxaldehydes bearing *N*-(*E*)-stilbenyloxyalkylcarbonyl- and *N*-(*E*)-stilbenyloxyalkylcarbonylaminoalkylcarbonyl substituents [1]. Additionally, the differentiation of the metameric *N*-(*E*)-stilbenyloxyalkylcarbonyl- [or (*E*)-4'-chlorostilbenyloxyalkylcarbonyl]substituted amino acids, their methyl esters and hydrazides on the basis of EIMS analysis in conjunction with the interpretation of the values of μ (*i.e.* the ratio of intensity of the selected fragment ions peak to that of the molecular ion peak) have been studied previously in our laboratory [2]. Our investigations have now been extended to mass spectral fragmentation of isomeric *N*-(*E*)-stilbenyloxymethylenecarbonyl **1-6**, *N*-(*E*)-stilbenyloxymethylenecarbonylamino-(1-phenylethyl)carbonyl **7-9**, as well as *N*-(*E*)-stilbenyloxymethylenecarbonylamino(methyl)methinecarbonyl **10-12** substituted hydrazones of 2-, 3- and 4-pyridinecarboxaldehydes, to establish whether it is possible to distinguish these isomers on the basis of the differences in the values of μ . This paper deals with the elucidation of the mass fragmentation of the hydrazones of 2-, 3- and 4-pyridinecarboxaldehydes which have been obtained in the reactions with the hydrazides of (*E*)-stilbenyloxyacetic acid [or (*E*)-4'-chlorostilbenyloxyacetic acid] **1-6**, as well as hydrazides of *N*-(*E*)-stilbenyloxymethylenecarbonyl substituted β -phenyl- β -alanine **7-9** and α -alanine **10-12** (Figure 1).

Results and Discussion.

Based on low and high resolution electron-impact as well as B/E linked scan mass spectra (Tables 1-3), the principal mass spectral fragmentation routes of **1-6** are interpreted as shown in Scheme 1, and those of **7-9** and **10-12** in Schemes 2 and 3, respectively. It should be pointed out that 9,10-dihydrophenanthrene-type structures of the molecular ions shown in Schemes 1-3 are con-

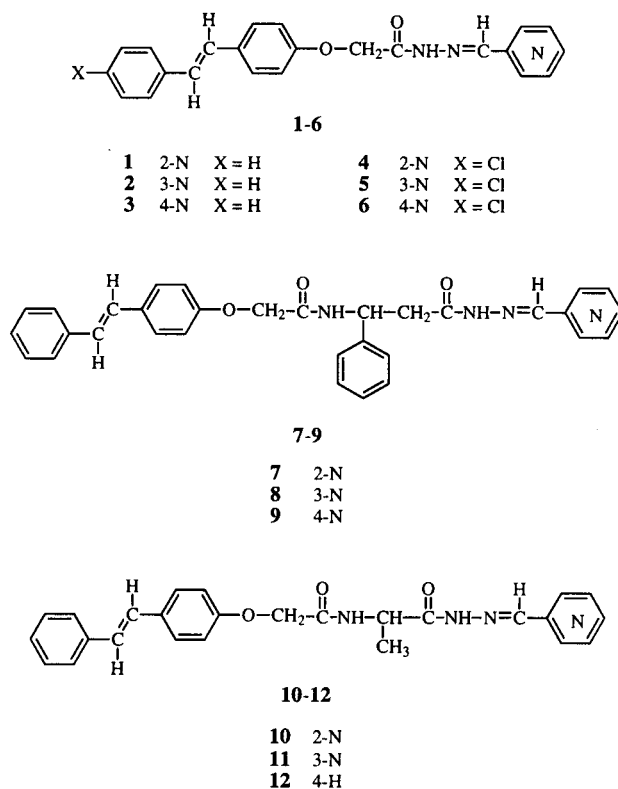


Figure 1.

tural, but consistent with previously published literature [3-7]. The principal mass fragmentation pathways of *N*-substituted hydrazones of 2-pyridinecarboxaldehydes **1,4,7,10** are similar to those of their isomeric *N*-substituted hydrazones of 3-pyridinecarboxaldehydes **2,5,8,11** as well as *N*-substituted hydrazones of 4-pyridinecarboxaldehydes **3,6,9,12** but show differences in the abundances of the important ions.

Table 1
Elemental Compositions and Relative Intensities of the Ion Peaks in the Spectra of 1-6 According to High-resolution Data

Ion	m/z	Elemental Composition	Relative Intensity (%)					
			1	2	3	4	5	6
M ⁺ a	357	C ₂₂ H ₁₉ N ₃ O ₂	100	63	100	—	—	—
	391	C ₂₂ H ₁₈ N ₃ O ₂ Cl	—	—	—	43	100	91
b	253	C ₁₆ H ₁₅ NO ₂	1	2	2	—	—	—
	287	C ₁₆ H ₁₄ NO ₂ Cl	—	—	—	1	1	3
c	209	C ₁₅ H ₁₃ O	1	2	6	—	—	—
	243	C ₁₅ H ₁₂ OCl	—	—	—	1	3	4
d	196	C ₁₄ H ₁₂ O	40	100	46	—	—	—
	230	C ₁₄ H ₁₁ OCl	—	—	—	14	71	100
e	178	C ₁₄ H ₁₀	42	43	26	53	52	13
f	165	C ₁₃ H ₉	14	5	37	5	9	20
g	162	C ₈ H ₈ N ₃ O	10	12	10	17	25	25
h	148	C ₇ H ₆ N ₃ O	13	3	7	39	1	1
i	120	C ₆ H ₆ N ₃	66	6	4	100	5	6
j	92	C ₆ H ₆ N	82	1	3	74	5	6

Table 2
Elemental Compositions and Relative Intensities of the Ion Peaks in the Spectra of 7-9 According to High-resolution Data.

Ion	m/z	Elemental Composition	Relative Intensity (%)		
			7	8	9
M ⁺ a	504	C ₃₁ H ₂₈ N ₄ O ₃	49	100	100
c	356	C ₂₄ H ₂₂ NO ₂	8	16	12
d	253	C ₁₆ H ₁₅ NO ₂	7	24	18
e	209	C ₁₅ H ₁₃ O	9	8	6
f	196	C ₁₄ H ₁₂ O	48	51	22
g	178	C ₁₄ H ₁₀	34	24	16
h	165	C ₁₃ H ₉	13	7	3
i	148	C ₇ H ₆ N ₃ O	89	30	1
j	120	C ₆ H ₆ N ₃	100	39	8
k	92	C ₆ H ₆ N	38	5	3

Table 3
Elemental Compositions and Relative Intensities of the Ion Peaks in the Spectra of 10-12 According to High-resolution Data.

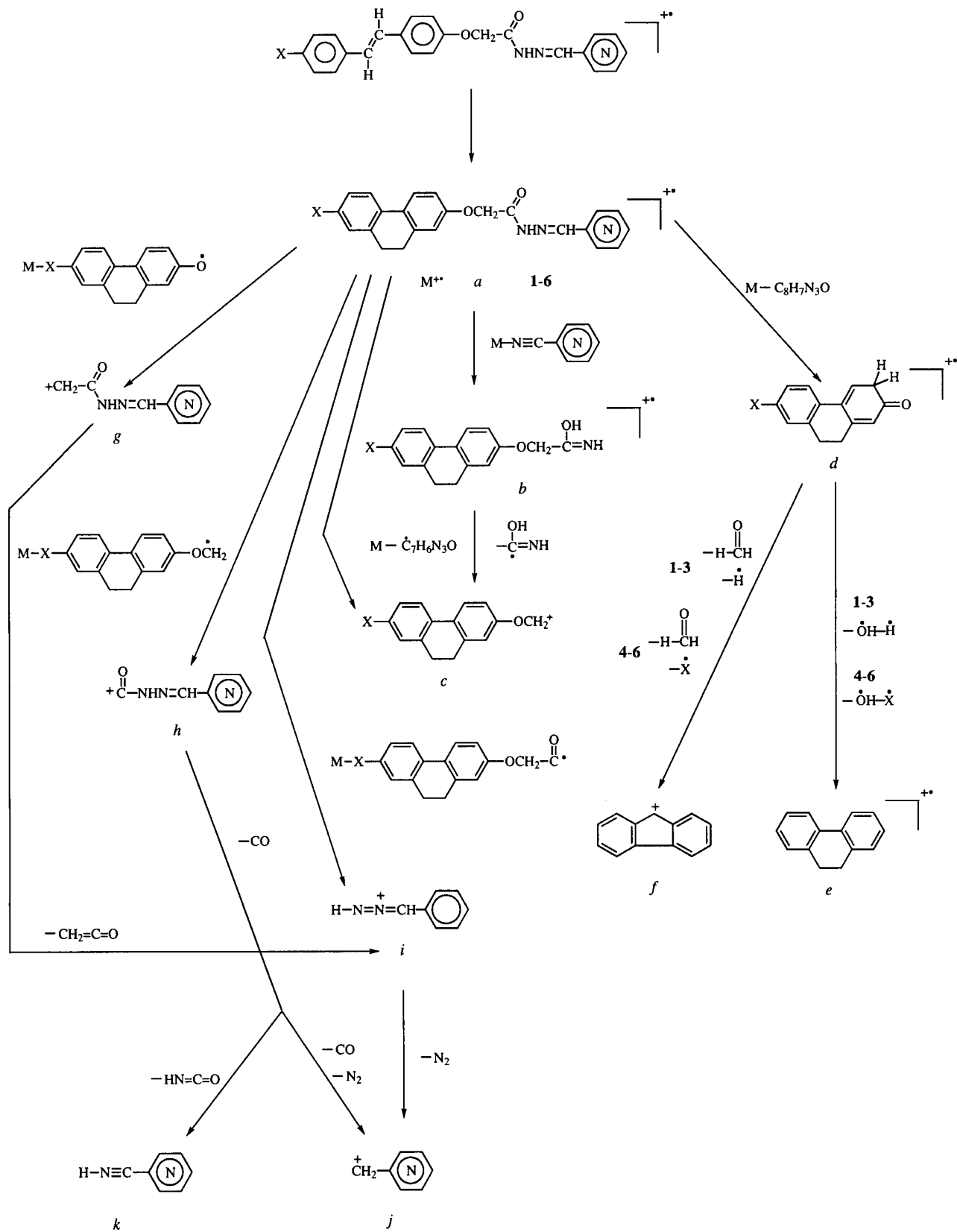
Ion	m/z	Elemental Composition	Relative Intensity (%)		
			10	11	12
M ⁺ a	428	C ₂₅ H ₂₄ N ₄ O ₃	58	100	100
b	307	C ₁₉ H ₁₈ NO ₃	7	2	2
c	280	C ₁₈ H ₁₈ NO ₂	11	7	13
d	209	C ₁₅ H ₁₃ O	11	9	15
e	196	C ₁₄ H ₁₂ O	33	45	51
f	178	C ₁₄ H ₁₀	34	19	27
g	165	C ₁₃ H ₉	7	7	7
h	148	C ₇ H ₆ N ₃ O	98	17	5
i	120	C ₆ H ₆ N ₃	100	21	11
j	92	C ₆ H ₆ N	4	3	3

The EI-induced mass fragmentation of 1-6 begins with the cleavages of methoxycarbonylhydrazonazabenzylidene chain linking (*E*)-stilbene and pyridine molecules of these compounds (Scheme 1). The charge-site initiated inductive cleavages (i) of O-Csp₃ and Csp₃-Csp₂ bonds proceed with the eliminations of C₁₄H₁₀OX⁺, C₁₅H₁₂OX⁺ and C₇H₆N₃O⁺ radicals giving *g*, *i* and *c* even-electron fragment ions. The radical-site initiated α-cleavage of Csp₃-Csp₂ bond proceed with the elimination of C₁₆H₁₂OX⁺ radical. By this fragmentation route, the *h* even-electron fragment ion is derived. On the other hand, it is found that the cleavage of the O-Csp₃ bond of the methoxy group of this chain, follows a McLafferty rearrangement involving the migration of hydrogen atom to a double bond of the phenyl ring of (*E*)-stilbene skeleton. Odd-electron fragment ions *d* were obtained by this fragmentation pathway. These were the base peaks in the mass spectra of 2 and 6.

It should be mentioned that the molecular ions M⁺ a in the mass spectra of 1, 3 and 5 were the base peaks. For structures 2, 4 and 6, M⁺ a was 43-91% of the base peak intensity. In contrast the base peak in the mass spectrum of 4 is this the even-electron fragment ion *i*. In the mass spectra of 1-6 this ion is also formed by the inductive cleavage of the Csp₂-N bond in the even-electron fragment ions *g* and *h*, with the ejection of the molecules of ketene, as well as carbon monoxide, respectively.

The even-electron fragment ions *i* decompose further by the cleavage of Csp₃-N bond, with the simultaneous hydrogen transfer, nitrogen molecule elimination, as well as the charge migration giving even-electron fragment ion *j*. This ion is also derived from the even-electron fragment ion *h*, in the analogous processes of the simultaneous eliminations of carbon monoxide and nitrogen molecules with neighboring hydrogen and charge migration. In the

Scheme 1



second step of the mass decomposition of the molecular ions of **1-6** odd-electron fragment ions *e* are derived, by the eliminations of $\cdot\text{OH}$ and H (or $\cdot\text{X}$) radicals from the odd-electron fragment ion *d*. On the other side the simultaneous ejections from the ion *d* of the neutral molecules of formaldehyde and $\cdot\text{H}$ (or $\cdot\text{X}$) radical gave even-electron fragment ions *f*. It is highly probable that this ion has a fluorenyl cation structure, as was proposed earlier for the fragmentation of the unsubstituted stilbene [7].

In the processes of the mass decomposition of the molecular ions of **1-6** is also seen the second McLafferty rearrangement involving the cleavage of N-N bond, with simultaneous transfer of hydrogen from methine to carbonyl group and elimination of neutral molecule of benzonitrile. This decomposition produces the odd-electron fragment ion *b*. In the second step of the mass fragmentation, the inductive cleavage of the $\text{Csp}_3\text{-Csp}_3$ bond in this ion eliminates $\text{OH}\cdot\text{C}=\text{NH}$ radical giving even-electron fragment ion *c*. The characteristic features of the first steps of the mass fragmentation of the molecular ions of **7-9** (Scheme 2) are the cleavages of N-N (ion *d*), N- Csp_3 (ion *b*) as well as O- Csp_3 (ion *f*) bonds of the methoxycarbonylamino(phenyl)ethylcarbonylhydrazonabenzylidene chain according to McLafferty rearrangements. The mechanism of α -cleavage of $\text{Csp}_3\text{-Csp}_2$ bond (ion *i*), as well as inductive cleavage of $\text{Csp}_2\text{-N}$ bond (ion *j*) of this chain in the molecular ions of **7-9** (Scheme 2) show key similarities with these observed in the mass decomposition processes of the molecular ions of **1-6** (Scheme 1). The same similarities are seen in the second steps of mass fragmentation of the molecular ions of **7-9** in the processes of the cleavages of $\text{Csp}_2\text{-N}$ (ion *j*) and $\text{Csp}_3\text{-N}$ (ion *k*) bonds of the even-electron fragment ion *i*, as well as $\text{Csp}_3\text{-Csp}_2$ bonds (ion *h*) and $\text{Csp}_2\text{-O}$, $\text{Csp}_2\text{-H}$ bonds (ion *g*) of odd-electron fragment ions *f*.

The characteristic features of the mass decomposition of the molecular ions of **10-12** are three inductive and two α -cleavages of $\text{Csp}_2\text{-Csp}_3$ and $\text{Csp}_2\text{-N}$ bonds of methoxycarbonylamino(methyl)carbonylhydrazonabenzylidene chain linking (*E*) stilbene and pyridine molecules of these compounds (Scheme 3). In this way even-electron fragment ions *c, d, e* (inductive cleavages) as well as *b* and *h* (α -cleavage) have been obtained.

It should be pointed out that *c* and *h*, as well as *b* and *i* even-electron fragment ions are complementary. In the fragmentation of the molecular ions of **10-12** is also seen McLafferty rearrangement. The odd-electron fragment ion *e* has been produced by this mechanism of mass decomposition. In the second step of the mass fragmentation, this ion decomposes by the simultaneous ejection of the neutral molecule of formaldehyde and $\cdot\text{H}$ radical (even-electron fragment ion *g*), or $\cdot\text{OH}$ and $\cdot\text{H}$ radicals (odd-electron fragment ion *f*).

Table 4 presents for all compounds investigated, **1-12**, the ratios of the intensities *h, c, d, 1-6, i, j, f, 7-9 and *h, i, e, 10-12* ion peaks to those of the parent ion peaks, *i.e.**

$$\mu_1 = \frac{h}{a} \quad \mathbf{1-6, 10-12}$$

$$\mu_1 = \frac{i}{a} \quad \mathbf{7-9}$$

$$\mu_2 = \frac{j}{a} \quad \mathbf{1-6, 10-12}$$

$$\mu_2 = \frac{f}{a} \quad \mathbf{7-9}$$

$$\mu_3 = \frac{d}{a} \quad \mathbf{1-9}$$

$$\mu_3 = \frac{e}{a} \quad \mathbf{10-12}$$

Table 4

The Values of $\mu_1\text{-}\mu_3$ Calculated from the EI Mass Spectra of **1-12**

Compound	μ_1	μ_2	μ_3
1	0.13	0.66	0.40
2	0.04	0.09	1.58
3	0.03	0.04	0.46
4	0.90	2.32	0.32
5	0.01	0.05	0.71
6	0.01	0.06	1.09
7	1.80	2.04	0.97
8	0.30	0.39	0.51
9	0.01	0.08	0.22
10	1.68	1.72	0.56
11	0.17	0.26	0.45
12	0.05	0.11	0.51

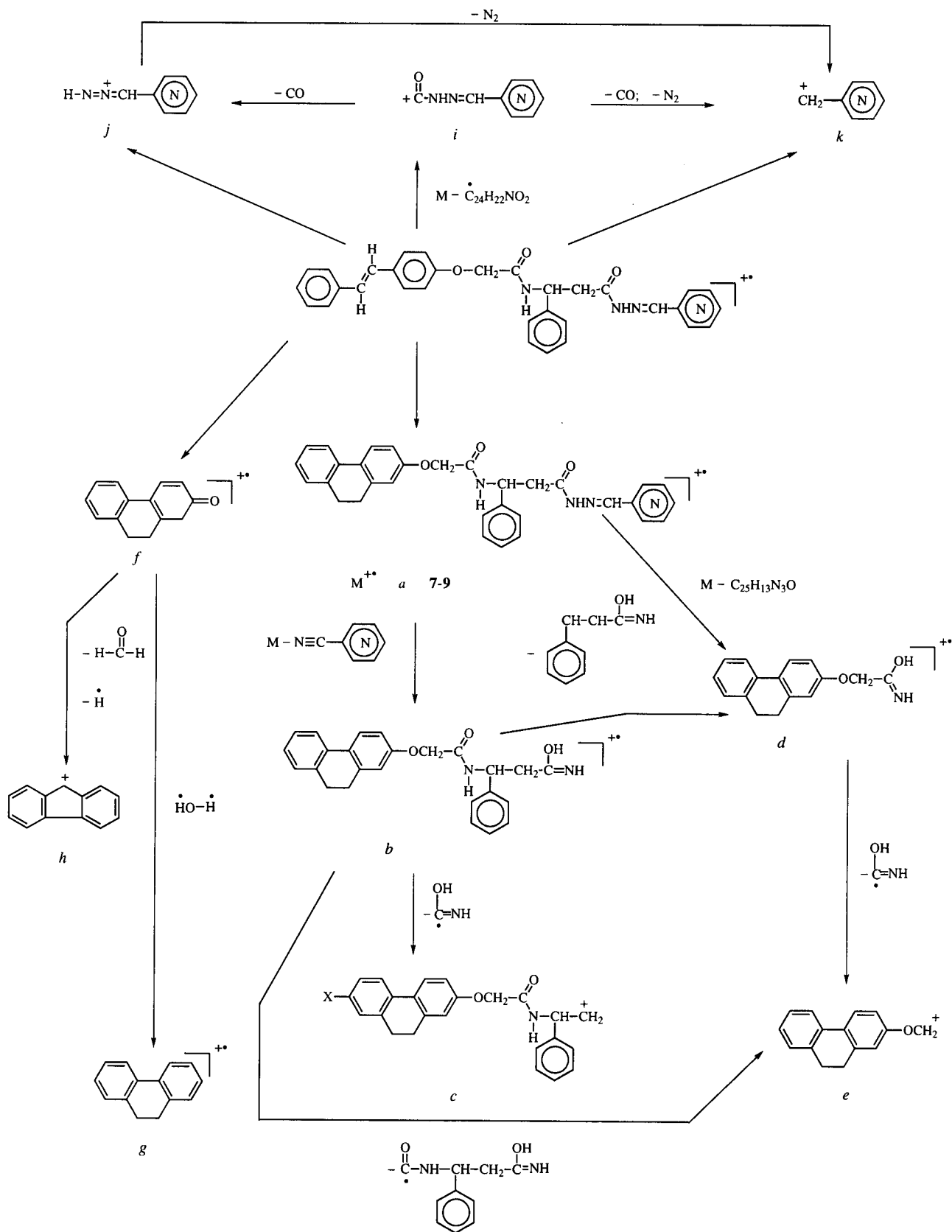
Because the values of μ are highly dependent on the relative intensities of the ions, it is necessary that three measurements be averaged, to be able to obtain adequate statistical results. This procedure guarantee that the results with the differences of the relative intensities of the ions on level of 2-3% between each particular scan are reliable. As can be seen from the data in Table 4, the differences between the relative intensities of the peaks of the selected fragment ions *d, e, h, i, j*, and $\text{M}^{+\cdot}$ *a* ions *i.e.* the values of $\mu_1\text{-}\mu_3$ for *N*-substituted hydrazones of 2-(3- and 4-)pyridinecarboxaldehydes **1-12** may be sufficient to differentiate isomers. It is possible to distinguish isomeric hydrazones with annular nitrogen atom situated at 2-, 3- or 4-position of the pyridine ring. This problem seems not to have been tackled previously.

The isomers of hydrazones with nitrogen atom in position 2- of the pyridine ring, **1,4,7,10**, may be distinguished from isomeric **3-2,5,8,11** and **4-3,6,9,12** substituted compounds on the basis of the highest values of μ_1 and μ_2 .

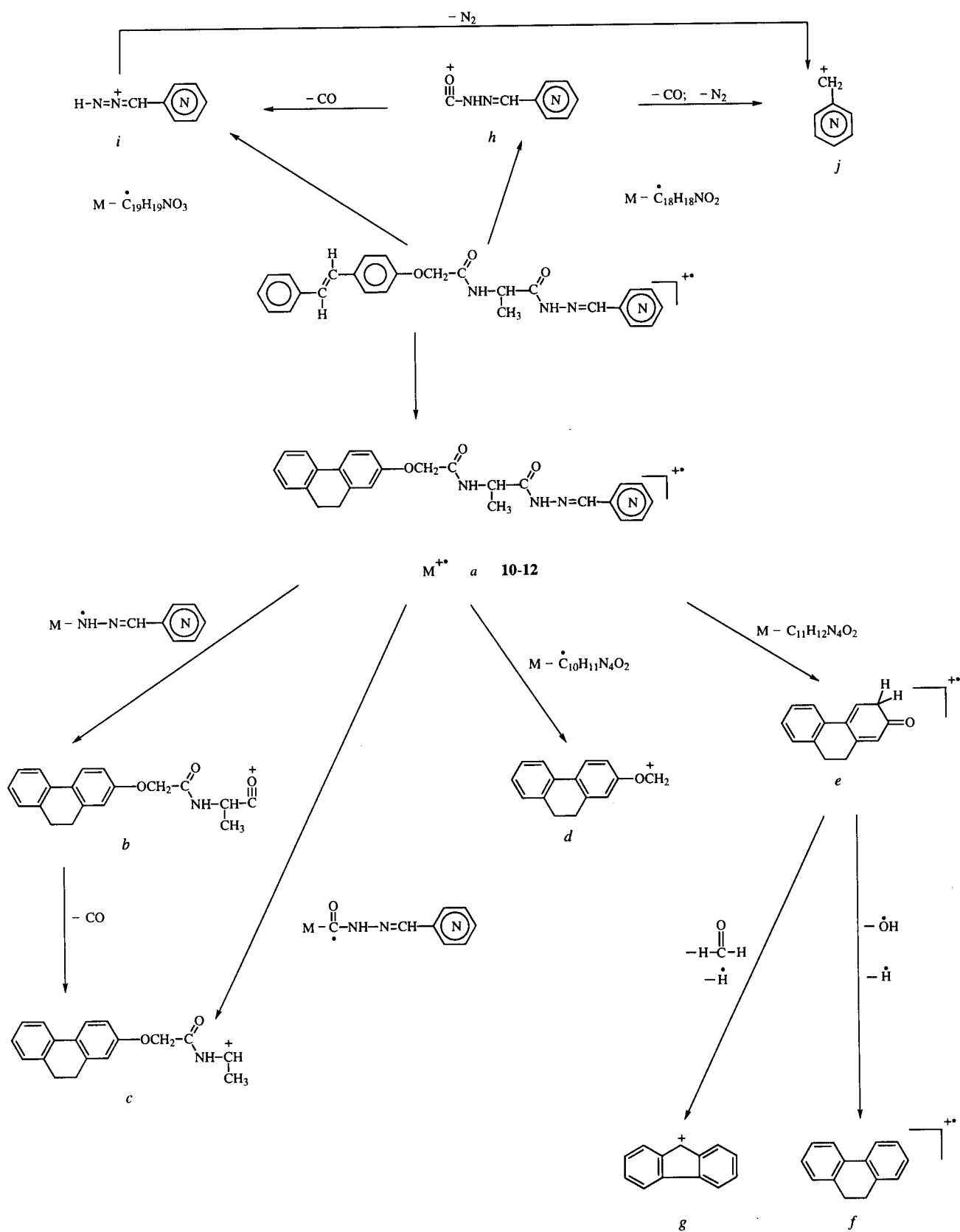
In the series of **7-12** it is also possible to use the values of μ_1 and μ_2 in the differentiation of the position of nitrogen atom in the pyridine ring according to the following sequences:

$$\mathbf{7-12} \quad \mu_1, \mu_2 \quad \mathbf{2} > \mu_1, \mu_2 \quad \mathbf{3} > \mu_1, \mu_2 \quad \mathbf{4}$$

Scheme 2



Scheme 3



The highest value of μ_3 is useful for the differentiation of 3-substituted isomer in the series of **1-3**. The values of μ_3 are also useful for the differentiation of 2-, 3- and 4-substituted isomers in the series of **4-6** and **7-9** according to the following sequences:

$$\mu_3 \mathbf{4} > \mu_3 \mathbf{3} > \mu_3 \mathbf{2} \quad \mathbf{4-6}$$

$$\mu_3 \mathbf{2} > \mu_3 \mathbf{3} > \mu_3 \mathbf{4} \quad \mathbf{7-9}$$

Conclusions.

The basic mass fragmentation of **1-12** is due to cleavages of O-Csp₃, Csp₃-Csp₃, Csp₃-N, Csp₂-N and N-N bonds of methoxycarbonylhydrazonazabenzylidene, and methoxycarbonylaminoalkylcarbonylhydrazonazabenzylidene chains linking (*E*)-stilbene and pyridine molecules of these compounds. The values of μ_1 - μ_3 (*i.e.* the ratio of the intensities of the selected ion peaks to that of the molecular ion M⁺) depend on the structure of the hydrazone and the (*E*)-stilbenyloxyalkylcarbonyl, or (*E*)-stilbenyloxyalkylcarbonylaminoalkylcarbonyl *N*-substituent. The differences in the values of μ_1 - μ_3 in the series of isomeric hydrazones **1-12** are useful for the differentiation of isomers as, follows: *N*-(*E*)-stilbenyloxymethylenecarbonyl-**1,4** and *N*-(*E*)-stilbenyloxymethylene carbonylaminoalkylcarbonyl-substituted hydrazones of 2-pyridinecarboxaldehydes **7,10** may be distinguished from isomeric derivatives of 3-pyridinecarboxaldehyde **2,5,8,11** and 4-pyridinecarboxaldehyde **3,6,9,12** on the basis of the highest values of μ_1 and μ_2 . *N*-(*E*)-stilbenyloxymethylenecarbonyl-substituted hydrazone of 3-pyridinecarboxaldehyde **2** may be distinguished from isomeric derivatives of 2-pyridinecarboxaldehyde **1** and 4-pyridinecarboxaldehyde **3** on the basis of the highest value of μ_3 . *N*-(*E*)-stilbenyloxymethylenecarbonylaminoalkylcarbonyl-substituted hydrazones derivatives of 2-(**7,10**); 3-(**8,11**) and 4-(**9,12**)-pyridinecarboxaldehydes may be differentiated on the basis of the following sequence of the values of μ_1 and μ_2 .

$$\mu_1, \mu_2 \mathbf{2} > \mu_1, \mu_2 \mathbf{3} > \mu_1, \mu_2 \mathbf{4}$$

The sequences of dwindling values of μ_3 are useful for the differentiation of isomeric *N*-(*E*)-stilbenyloxymethylenecarbonyl substituted hydrazones of pyridinecarboxaldehydes **4-6**, as well as *N*-(*E*)-stilbenyloxymethylenecarbonylamino(phenyl)ethylcarbonyl substituted hydrazones of pyridinecarboxaldehydes **7-9**.

$$\mu_3 \mathbf{2} > \mu_3 \mathbf{3} > \mu_3 \mathbf{4} \quad \mathbf{7-9}$$

$$\mu_3 \mathbf{4} > \mu_3 \mathbf{3} > \mu_3 \mathbf{2} \quad \mathbf{4-6}$$

EXPERIMENTAL

Low- and high-resolution mass spectra were recorded on an AMD-402 two-sector mass spectrometer (ionizing voltage 70 eV, accelerating voltage 8 kV, resolution 10,000). Samples were introduced by a direct insertion probe at the source temperature of ~150°. The elemental compositions of the ions were determined by a peak matching method relative to perfluorokerosene and using the same instrument. All measured masses agreed with those of the composition listed in column 3 of the Tables 1-3 to within ± 2 ppm. B/E linked scan spectra on the first field-free region were investigated using helium as the collision gas at a pressure of 1.73×10^{-5} with the ion source temperature of 180°, ionization energy of 70 eV and an accelerating voltage of 8 kV. Compounds **1-12** were obtained according to the literature [1]. The values of μ_1 - μ_3 were calculated as averages of three measurements.

REFERENCES AND NOTES

- [1] E. Wyrzykiewicz and D. Prukała, *J. Heterocyclic Chem.*, **35**, 381 (1998).
- [2] E. Wyrzykiewicz and D. Prukała, *Org. Mass Spectrom.*, **29**, 347 (1994).
- [3] P. F. Donaghue, P. Y. White, J. H. Bowie, B. D. Roney and H. J. Rodda, *Org. Mass Spectrom.*, **2**, 1061 (1969).
- [4] H. Güsten, L. Klasinc, V. Kramer and J. Marsel, *Org. Mass Spectrom.*, **8**, 323 (1974).
- [5] H. Güsten, L. Klasinc, V. Kramer and J. Marsel, *Adv. Mass Spectrom.*, **6**, 79 (1974).
- [6] M. Mintas, K. Jakopčič, L. Klasinc and H. Güsten, *Org. Mass Spectrom.*, **12**, 544 (1977).
- [7] A. R. Gregory and D. F. Williams, *J. Phys. Chem.*, **83**, 2352 (1979) and references cited therein.