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A Study of Substituted Benzimidazoles and Xanthine by Plasma Desorption Mass Spectrometry

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Plasma desorption mass spectra of biologically active substituted benzimidazoles and xanthine have been recorded and are discussed.

The present study focuses on the use of Plasma Desorption Mass Spectrometry (PDMS)† for the structural characterization of substituted benzimidazoles and xanthine with antiaggregatory activity. It is known that substituted imidazole, [2-(4-carboxyphenoxy)ethyl]imidazole, for example, is a potent and specific inhibitor of thromboxane synthetase and platelet aggregation.[2] The compounds **1–3** have been synthesized for a study of their structure-biological activity relationship.

PDMS has been widely developed during the last decade[3] as a method of obtaining a measurement of the molecular weight of non-volatile and thermolabile natural organic compounds.‡ The technique is also used for structural characterization by fragment ions.[4–6] PDMS yields interpretable spectra of cell and membrane components without such supplementary procedures as separation and chemical derivatization.[7]

The compounds investigated were obtained by chemical synthesis[8,9] and they contain aliphatic and aromatic substituents. The compounds **1f** and **2b,c** were firstly synthesized in a similar way.§

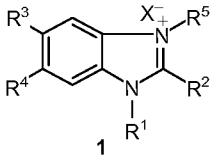
† The plasma desorption mass spectra were recorded on a commercial time-of-flight biochemical mass spectrometer BC MS (SELMI, Sumy, Ukraine).[1] The ionization was induced by ^{252}Cf spontaneous fission fragments and the samples were applied onto a gilded disk.

‡ See, *e.g.*, J. Chapman, *Practical Organic Mass Spectrometry*, J. Wiley & Sons, Chichester, 2nd edn., 1993.

§ For **1f**: m.p. 94–95 °C (hexane), IR ν/cm^{-1} 1668 (C=O), ^{1}H NMR ($CDCl_3$, δ, *J* (H,H)/Hz) 1.68–2.05 (m, 4H, 2′-CH_2-3′-CH_2), 2.92 (t, *J* 7, 2H, CH_2C=O), 3.85 (s, 3H, O–Me), 4.19 (t, *J* 7, 2H, N–CH_2), 6.84–6.93 (m, 2H, *m*-H to C=O), 7.20–7.45 (m, 3H, 2-H, 6-H, 7-H), 7.63–7.90 (m, 4H, 5-H, 8-H, *o*-H to C=O). Found: C 74.06; H 6.49; N 8.97. Calc. for $C_{19}H_{20}N_2O_2$: C 74.00; H 6.53; N 9.07%.

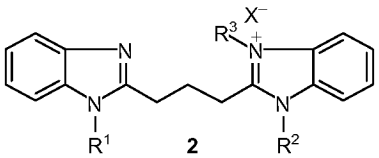
For **2b**: m.p. 54–56 °C, ^{1}H NMR ($CDCl_3$, δ, *J* (H,H)/Hz) 0.90 (t, *J* 7, 6H, 2×Me), 1.09–1.5 [m, 24H, $(CH_2)_6$ and $(CH_2)_6$], 1.6–1.9 (m, 4H, β-CH_2 and β′-CH_2), 2.5 (t, *J* 7, 2H, 2″-CH_2), 3.10 (t, *J* 7, 4H, 2-CH_2 and 2′-CH_2), 4.10 (t, *J* 7.5, 4H, N–CH_2 and N′–CH_2), 7.13–7.38 (m, 4H, 4-H, 4′-H, 7-H, 7′-H), 7.58–7.82 (m, 4H, 5-H, 5′-H, 6-H, 6′-H). Found: C 79.00; H 9.81; N 10.35. Calc. for: $C_{35}H_{52}N_4$. C 79.49; H 9.92; N 10.60%.

For **2c**: m.p. 151–153 °C, ^{1}H NMR ($CDCl_3$, δ, *J* (H,H)/Hz): 0.75–0.91 (t, *J* 7, 9H, 3 × Me), 1.07–1.58 [m, 36H, 3×$(CH_2)_6$], 1.62–2.01 (m, 6H, 3×β-CH_2), 2.40–2.61 (m, 2H, 2″-CH_2), 3.44 (t, *J* 7.5, 2H, 2-CH_2 or 2′-CH_2), 3.95 (t, *J* 7.5, 2H, 2-CH_2 or 2′-CH_2), 4.23 (t, *J* 7.5, 2H, N–CH_2 or N′–CH_2 or N″–CH_2), 4.82 (t, *J* 7.5, 4H, other N–CH_2 or N′–CH_2 or N″–CH_2), 7.10–7.39 (m, 4H, 4-H, 7-H, 4′-H, 7′-H), 7.48–7.70 (m, 4H, 5-H, 5′-H, 6-H, 6′-H). Found: C 71.36; H 9.47; N 7.39; Br 10.80. Calc. for $C_{44}H_{71}N_4Br$: C 71.80; H 9.72; N 7.61; Br 10.86%.



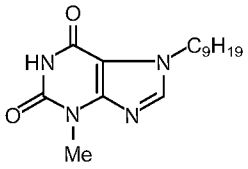
1

	M.W.	R^1	R^2	R^3	R^4	R^5	X
a	294	C_9H_{19}	Me	H	H	H	Cl
b	493	C_9H_{19}	Me	Me	Me	C_9H_{19}	Br
c	370	C_9H_{19}	CH_2Ph	H	H	H	Cl
d	324	*p*-(HOOC)$C_6H_4OC_2H_4$	Me	Me	Me	—	—
e	372	*p*-(HOOC)$C_6H_4OC_2H_4$	CH_2Ph	H	H	—	—
f	308	*p*-(MeO)$C_6H_4OC_4H_8$	H	H	H	—	—



2

	M.W.	R^1	R^2	R^3	X
a	276	H	H	—	—
b	528	C_9H_{19}	C_9H_{19}	—	—
c	735	C_9H_{19}	C_9H_{19}	C_9H_{19}	Br



3
M.W. 292

Interesting results have been obtained in the positive and negative ion mass spectral range above *m/z* 90. There are peaks corresponding to ions of various origins in the mass spectra of benzimidazoles **1**,**2** and xanthine **3** (Table 1).

We observed a set of peaks in the molecular mass region in the positive ion mass spectra. Determination of the molecular weight is realized with the most abundant molecular ion species: $M^{+\cdot}$ for **1d**, **2a**,**b** and MH^+ for **1e**,**f** and **3**. Benzimidazoles **1a–c** and **2c**, which are salts, formed an ion $[M-X]^+$ corresponding to the cation part of the molecule.

The positive fragment ions of the substituents have been identified for compounds **1–3**. They are subdivided into a number of groups (Table 1).

The closest molecular mass region peaks are $[MH-CO_2]^+$ (*m/z* 280, 328, **1d,e**); $[MH_2-OMe]^+$ (*m/z* 279, **1f**) and peak $[MH-Me]^+$ (*m/z* 310, **1d**).

In the *m/z* scale there are nine equally spaced peaks corresponding to fragmentation of the nonyl substituent for compounds **1a,c**, **2b,c** and **3**.

The group of peaks *m/z* 207 $[C_{14}H_{11}N_2]^+$, 221 $[C_{15}H_{13}N_2]^+$ and 235 $[C_{16}H_{15}N_2]^+$ was recorded for compounds **1c,e** substituted with a benzyl substituent R^2. This group is assigned to fragment ions originating from cleavage of R^1 and the unbroken structure of benzylbenzimidazole. Peaks at *m/z* 398 and 272 of **2b,c** correspond to cleavage of the propylene bridge.

In the 119–187 *m/z* region peaks due to characteristic "fingerprint" ions are observed. They have the general formula $[C_nH_nN_2]^+$, $n = 7-9$, and $[C_nH_{n+2}N_2]^+$, $n = 10-12$. The relative intensity of the peaks depends on the number of substituents and their position in the heterocyclic ring. The most intense "fingerprint" ions of **1a,c,e** and **2a–c** containing 1–3 substitutents in the imidazole ring correspond to the methyl-, ethyl- and dimethylbenzimidazole cations at *m/z* 132, 145, $n = 8,9$ [Fig. 1(*a*)]. The ion *m/z* 119 of **1f** is the most intense in this group of ions and corresponds to the loss of substituents. This compound has only one substituent on the nitrogen atom of benzimidazole.

Benzimidazoles **1b,d** are substituted in the imidazole ring (R^1, R^2, R^5) and in the benzene ring (R^3 and R^4). Compound **1d** contains four substituents. The most intense "fingerprint" peak is at *m/z* 159 $[C_{10}H_{11}N_2]^+$ [Fig. 2(*a*)]. The presence of five substituents results in an abundant peak at *m/z* 173 $[C_{11}H_{13}N_2]^+$ **1b**.

For compounds **1c–f** containing an aromatic substituent, "fingerprint" ions $[C_8H_7N_2]^+$ (*m/z* 131, **1c,f**), $[C_9H_{10}N_2]^+$ (*m/z* 146, **1e**); $[C_9H_{11}N_2]^+$ (*m/z* 147, **1f**) and $[C_{10}H_{13}N_2]^+$ (*m/z* 160, **1d**) are observed in the mass spectra.

The most intense "fingerprint" peak of **3** at *m/z* 167 $[C_6H_7N_4O_2]^+$ corresponds to cleavage of the nonyl group and a peak at *m/z* 124 $[C_4H_4N_4O]^+$ is assigned to the loss of all substituents and partial decomposition of the pyrimidine ring.

Compounds **1c–f** contain aromatic substituents R^1 and R^2. For these compounds ions characterizing the structure of the substituents were recorded. These are the benzylic cation (*m/z* 91) of **1c,e** and the *p*-methoxybenzoyl cation (*m/z* 135) and *p*-methoxybenzoylbutyl cation (*m/z* 191) of **1f**.

In the region above the molecular mass, peaks MY^+ due to the neutral molecule forming a cation with organic ions Y^+ are observed [Fig 1(*a*), 2(*a*)], as described earlier.[10] Peaks due to ions MY^+ have a high intensity when 20–70 nmol of substance is applied. MY^+ ions were not recorded for **1b** and

Table 1 PD mass spectra of positive ions of substituted benzimidazoles **1, 2** and xanthine **3**, *m/z* (relative intensity, %).

	Molecular ions[a]	Fragment ions			
		"Fingerprint"	Substituent	Others	Molecular cations[b]
1a[b]	259	119 132 145 159 173 187[c]			385 [M + 127 − HCl]
1b	413	119 132 145 159 173 187		286[c]	
	(100)	(5) (7) (8) (17) (27) (8)		(22)	
1c[b]	335	119 131 132 145 159 173 187	91	207[c]	461 [M + 127 − HCl]
1d	324	119 132 145 159 160 173 187		280 310	489 813
	(100)	(12) (12) (22) (49) (46) (19) (15)		(8) (7)	[M + 165] [2M + 165]
1e	373	132 145 146 159	91 122	207 221 235 328	435 463
	(100)	(13) (7) (7) (6)	(45) (10)	(48) (11) (15) (8)	[M + 63] [M + 91]
1f	309	119 131 132 145 147 159 173	135 191	279	371 499 679
	(100)	(33) (20) (17) (14) (20) (10) (14)	(51) (19)	(12)	[M + 63] [M + 191] [2M + 63]
2a	276	119 132 145 159 173			421 697
	(100)	(10) (40) (76) (4) (3)			[M + 145] [2M + 145]
2b	528	119 132 145 159 173 187		272 398[c]	655
	(100)	(15) (43) (54) (24) (17) (15)		(49) (22)	[M + 127]
2c	655	119 132 145 159 173 187		272 398 527[c]	
	(100)	(6) (23) (37) (12) (9) (9)		(21) (29) (5)	
3[c]	293	96 109 124 135 153 167			355
	(100)	(9) (15) (23) (9) (8) (61)			[M + 63]

[a] For **1d, 2a,b**, $M^{+\cdot}$; for **1e,f**, **3**, MH^+; for **1a–c**, **2c**, $[M-X]^+$. [b] Relative intensity is not presented for these ions, as it depends on the method of sample preparation. [c] Eight peaks equally spaced in the *m/z* scale were also recorded for **1b** between 286 and 413 (9–10); for **1c** between 207 and 335; for **2b** between 398 and 528 (6–10), between 272 and 398 (7–14); for **2c** between 272 and 398 (14–29), between 527 and 655 (5–8); for **3** between 167 and 293 (8–13). Four low intensity peaks equally spaced in the *m/z* scale were also recorded for **1a** between 187 and 259.

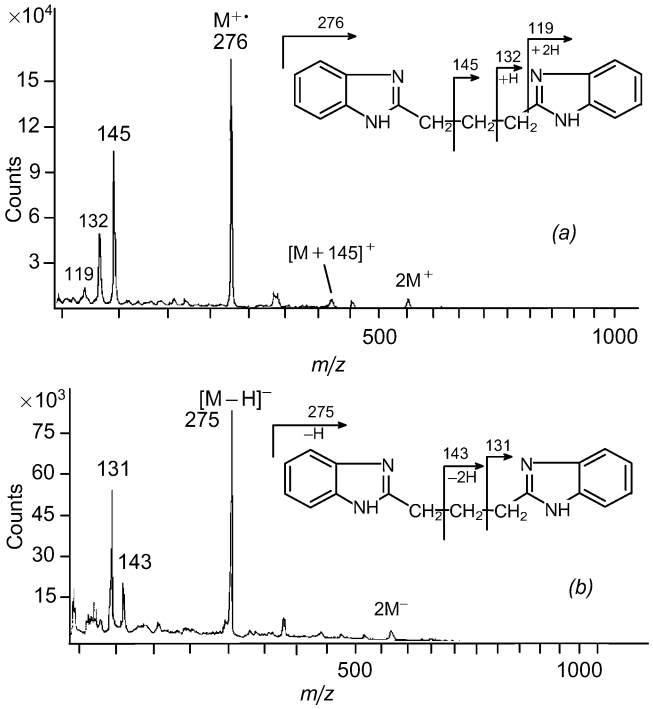


Fig. 1 Mass spectra of **2a**: (*a*) positive ions, (*b*) negative ions.

2c containing a nitrogen atom quaternized with a nonyl substituent. In the range 0.1–45 nmol we have investigated the dependence of the relative intensity of $[M+C_9H_{19}]^+$ on the amount of sample **2b**. The intensity of cation peak exceeds that of the molecular ion by 20% when 45 nmol is applied, and is 6% when 0.1 nmol is applied. Thus, about 0.1 nmol of compound should be used in order to prevent cation formation.

The low intensity peaks of oligomer ions $n\mathrm{M}^+$ up to a tetramer, inclusively, were recorded for **1d,f**, **2a,b** and **3** and $[n\mathrm{M}–\mathrm{Br}]^+$ for **1b** and **2c**.

In the negative ion mass spectra, $[\mathrm{M}-\mathrm{H}]^-$ ion peaks were observed for **1d–f**, **2a,b** and **3** [Fig. 1(*b*), 2(*b*)]. The mass spectra of **1b** and **2c** show MBr^- peaks resulting from attachment of Br^- to the neutral molecule.

Negative fragment ion peaks are of lower intensity than the positive peaks; however, they can be used for some structural elucidation. Thus, fragment ions $[\mathrm{M}-\mathrm{Me}]^-$ of **1f** are observed in the closest molecular ion mass region, and are more intense than the molecular ions. The characteristic negative "fingerprint" ions of **2a,b** are abundant *m/z* 131 and less abundant *m/z* 143 [Fig. 1(*b*)]. The peak for **1b,d** is *m/z* 159 and the peak at *m/z* 183 was also recorded for **1b**. The negative "fingerprint" ions of **3** are *m/z* 122 and less intense *m/z* 150.

Negative low intensity oligomer ions $n\mathrm{M}^-$ were recorded for **2a** [Fig. 1(*b*)] and **3** and $[n\mathrm{M}+\mathrm{Br}]^-$ for **1b**.

The data obtained may be used for the detection of substituted benzimidazoles, xanthines and their metabolites in biological systems.

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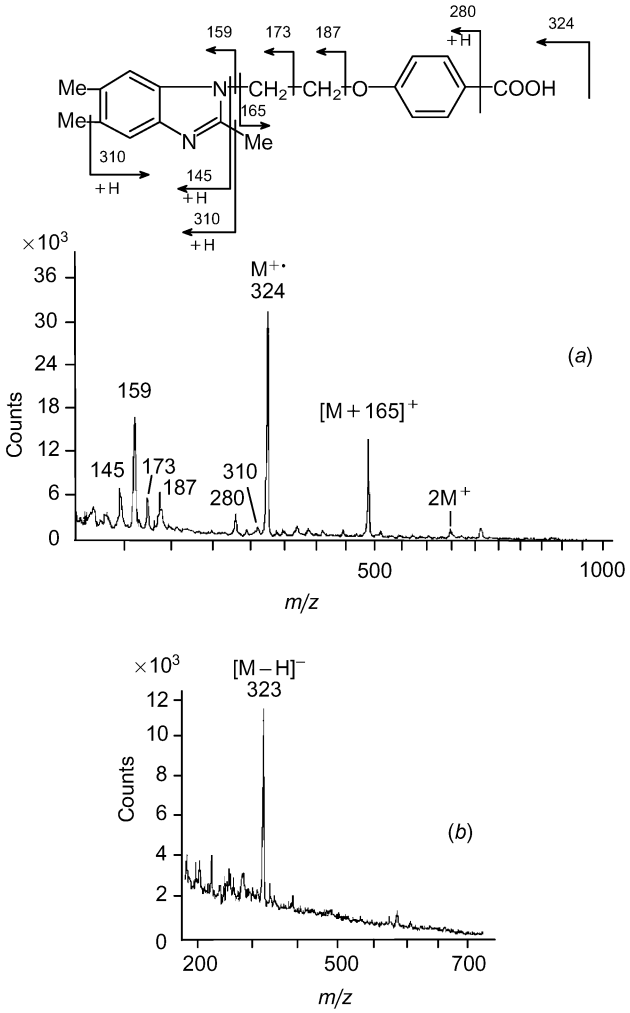

Fig. 2 Mass spectra of **1d**: (*a*) positive ions, (*b*) negative ions.

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