Hydrolysis of some imidazole, benzimidazole, and 1,2,3-benzotriazole derivatives according to HPLC and NMR diffusimetry data

Yu. L. Polyakova,[a] A. V. Bulanova,[a]★ and R. Sh. Vartapetyan[b]

[a]*Samara State University,*
1 ul. Akad. Pavlova, 443011 Samara, Russian Federation.
Fax: +7 (846 2) 34 5417. E-mail: polyak@ssu.samara.ru
[b]*Institute of Physical Chemistry, Russian Academy of Sciences,*
31 Leninsky prosp., 119991 Moscow, Russian Federation.
Fax: +7 (095) 952 0065. E-mail: vartapet@serv1.phyche.ac.ru

Hydrolysis of 1-mesylimidazole, 1-mesylbenzotriazole, and 1-tosylbenzimidazole was studied by reversed-phase HPLC and pulsed field gradient NMR diffusimetry. The hydrolysis rate constants and half reaction times were determined. The self-diffusion coefficients of the substances in aqueous solutions were measured. The reversed-phase HPLC data agree well with those of NMR diffusimetry.

Key words: hydrolysis, hydrolysis rate constant, self-diffusion coefficients, biologically active substances, reversed-phase HPLC, NMR diffusimetry.

Chromatography becomes a more promising method of physicochemical studies of various substances and biological objects, which is due to apparatus and methodical improvement of variants of the method and development of new high-quality sorbents.[1,2]

The purpose of this work was to study the interrelation between the structure and physicochemical properties of substances and the behavior in aqueous solutions of *N*-sulfonyl-substituted derivatives of some heterocycles, 1-mesylimidazole (**1**), 1-tosylbenzimidazole (**2**), and 1-mesylbenzotriazole (**3**), by reversed-phase HPLC and NMR diffusimetry.*

These objects for studying were chosen because a series of drugs used for various diseases was synthesized from five-membered nitrogen-containing heterocycles.[5—8] As shown in Ref. 9, *N*-tosylbenzimidazole (**2**) possesses an antibacterial activity. Therefore, it is probable that other similar derivatives of five-membered nitrogen-containing heterocycles can be biologically active. The study of the capability of hydrolysis in aqueous solutions with the pH close to that in biological liquids will help to forecast the behavior of these compounds in living organisms.

Since molecular weights of the starting substances and hydrolysis products should differ, it is reasonable to assume that the hydrolysis process can conveniently be monitored using self-diffusion of molecules in solutions: the values of self-diffusion coefficients (SDC), under other equivalent conditions, are determined by the weight of diffusing particles. The study of self-diffusion, in particular, by NMR diffusimetry, can provide an additional fruitful information on the physicochemical properties of liquids.

* The NMR method with pulsed magnetic field gradient (PFG NMR) is implied by NMR diffusimetry.[3,4]

Experimental

Chromatographic studies were performed using a Milikhrom-4 liquid chromatograph (Russia) with a UV-spectrometric detector at λ = 190 and 220 nm. A column 80 mm long with an inner diameter of 2 mm was packed with silica gel (Separon SGX RPS) with grafted octadecyl groups (specific surface 320 $m^2\ g^{-1}$, particle size 5—7 μm). Bidistilled water served as an eluent. The mobile phase velocity was 100 $\mu L\ min^{-1}$. Current concentrations of the starting substances and hydrolysis products were calculated by the peak surface areas in the chromatograms obtained in experiments.

Compounds **1—3** were synthesized according to a previously described procedure.[10] Substances were identified by retention times of the hydrolysis products.

Hydrolysis of 1-mesylimidazole (**1**), 1-tosylbenzimidazole (**2**), and 1-mesylbenzotriazole (**3**) was performed as follows. A buffer solution (2 mL) (a mixture of orthophosphoric (0.1 *M*), acetic (0.1 *M*), and boric acids (0.05 *M*) with the addition of an aliquot of a 0.2 *M* solution of sodium hydroxide (all substances were analytically pure grade)) with an exact pH was added to a freshly prepared 1% solution (2 mL) of a substrate.[11] Buffer solutions with pH 4.0, 7.0, 8.0, and 9.0 were used. The time of pouring together the solutions was fixed. Samples were taken and chromatographed at certain intervals. The last chromatographic measurement was performed 24 h after the beginning of the reaction. To estimate the accuracy of quantitative measurements and reproducibility of the obtained results, substances were hydrolyzed five times at a specified pH. The final products were identified by retention times of standard substances. The relative error of a single measurement did not exceed 5%. (The experimental error was determined specifying the degree of reliability according to Student $P = 0.95$).

The reaction of a pseudo-first order was observed due to an excess of a reactant (H_2O).

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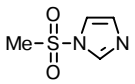
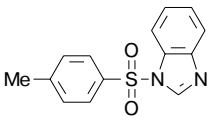
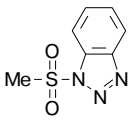
Table 1. Estimation of biological activity of substances under study by the PASS program[12—14]

Compound	Structural formula	Type of biological activity	P_a	P_i
1-Mesyl-imidazole (**1**)		Vascular (periferal) disease treatment	1.000	0.001
		Dermatologic	1.000	0.071
		Antihypertensive	0.878	0.000
		Antimigraine	0.635	0.003
1-Tosylbenz-imidazole (**2**)		Cardiovascular analeptic	0.824	0.005
		Restenosis, Agent for	0.792	0.005
		Antihypertensive	0.639	0.010
		cAMP phosphodiesterase inhibitor	0.579	0.003
		Antiishemic	0.607	0.112
1-Mesylbenzo-triazole (**3**)		Vascular (periferal) disease treatment	1.000	0.005
		Antitrypanosomal	0.900	0.008
		Dermatologic	0.875	0.166
		Antiishemic	0.656	0.035
		cAMP phosphodiesterase inhibitor	0.444	0.091

The rate constant (k) was calculated from the equation

$$k = 1/t(\ln c_0/c), \quad (1)$$

where c_0 and c are the initial and current concentrations of the substrate, respectively.

The half reaction period ($\tau_{1/2}$) was found from the formula

$$\tau_{1/2} = \ln 2/k. \quad (2)$$

The objects of the study were preliminarily tested by the PASS C&T program (Prediction of Activity Spectra for Substances: Complex and Training).[12—14] According to the PASS C&T program, the biological activity is described in a qualitative manner ("yes"/"no"). The prognosis results obtained include, along with the names of activity types, estimations of probabilities of their presence (P_a) or absence (P_i), whose values vary from 0 to 1.*

The P_a and P_i values show that the compounds under study possess a high probability of the appearance of various types of pharmacological effects (Table 1).

The behavior of compounds **1—3** in aqueous solutions with pH 7 was also studied by the PFG NMR method, which enables the measurement of self-diffusion coefficients in solutions.[3,4] With this purpose, solutions of the substances under study were prepared directly in NMR tubes. The duration of SDC measurement in each case was 2—3 min. The SDC values were determined at certain time intervals in an NMR diffusimeter (proton resonance frequency 60 MHz, pulsed gradient amplitude ≤50 T m^{-1}) from diffuse decay (DD) according to the formula $A(g^2)/A(0)$, where $A(g^2)$ is the amplitude of the spin echo signal at the gradient value g.

In pure liquids the plot of the logarithm of spin echo amplitude $A(g^2)$ *vs.* square of the PFG amplitude is linear (DD of spin echo is exponential). This plot is described by the expression for DD due to Brownian diffusion in a uniform medium[4]:

$$\ln A(g^2)/A(0) = -\gamma^2\delta^2 g^2 t_d D, \quad (3)$$

where $A(0) = A_0\exp(-\tau_1/T_2 - 2\tau_2/T_1)$ is the spin echo amplitude in the absence of a gradient pulse; τ_1 is the time interval between the first and second pulses, and τ_2 is the interval between the first and third radio frequency pulses in the "stimulated echo" procedure[3,4]; T_1 and T_2 are the times of spin-lattice and spin-spin relaxation, respectively; γ is the gyromagnetic ratio of a proton; δ is the duration of a gradient pulse with the amplitude g; t_d is the diffusion time; and D is the SDC value.

The deviation of the shape of the DD curve from exponential can indicate the presence of molecules that move with different rates. In this case, mean SDC values are used, which are calculated from the tangent slope to the initial region of DD ($g \to 0$).

A standard three-pulse sequence of stimulated echo was mainly used in measurements.[3,4] The diffusion times were 5—20 ms, and the temperature was 309 K. The observed DD curves were close to exponential. In all cases, mean SDC values were determined.

Results and Discussion

The chromatograms of hydrolysis of 1-mesylimidazole (**1**) show that hydrolysis was completed over 24 h, and the number of peaks in the chromatogram corresponds to the number of final products (Fig. 1).

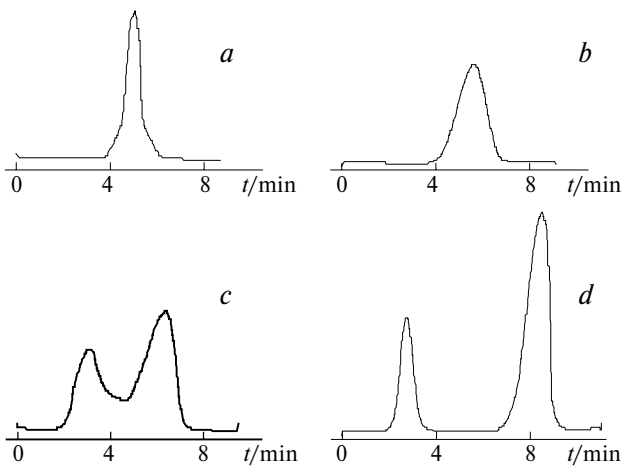


Fig. 1. Chromatograms of the reaction mixture during hydrolysis of 1-mesylimidazole (**1**) (pH 7) 7 (*a*), 20 (*b*), 150 (*c*), and 350 min (*d*) after the beginning of the reaction. Wavelength of UV spectrometric detection 190 nm, sample volume 1 μL.

* Since the probabilities P_a and P_i are calculated independently, their sum can be not equal to unity.

Table 2. Hydrolysis constants (k) and half reaction times ($\tau_{1/2}$) of sorbates under study at 293 K

Sorbate	pH	k^*/min^{-1}	$\tau_{1/2}$/min
1	7	$4.54 \cdot 10^{-3} \pm 5.12 \cdot 10^{-4}$	151.0±20.2
2	4	$5.38 \cdot 10^{-3} \pm 8.93 \cdot 10^{-4}$	128.8±15.5
	7	$4.59 \cdot 10^{-3} \pm 3.04 \cdot 10^{-4}$	151.0±10.1
	8	$3.15 \cdot 10^{-3} \pm 6.55 \cdot 10^{-4}$	220.1±35.7
3	7	$4.78 \cdot 10^{-3} \pm 1.28 \cdot 10^{-4}$	144.9±4.5
	9	$4.01 \cdot 10^{-3} \pm 1.78 \cdot 10^{-4}$	172.9±10.9

* $n = 5$, $P = 0.95$.

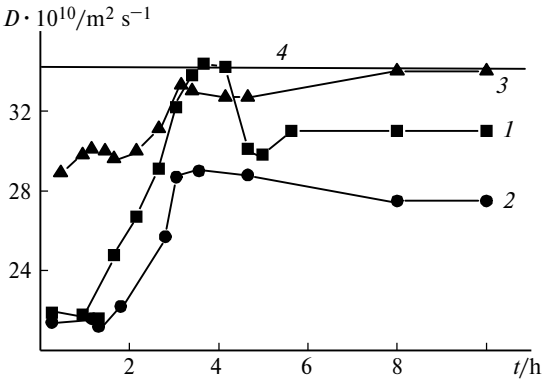


Fig. 2. Plots of SDC *vs.* time of a 1% solution of 1-tosylbenzimidazole **2** (*1*); a 3% solution of 1-mesylimidazole **1** (*2*) in D_2O, and a 1% solution of 1-mesylimidazole in H_2O (*3*) at 309 K. *4*, SDC of H_2O at 309 K.

The hydrolysis reaction in the general form can be written as follows:

$$RSO_2Het + H_2O \longrightarrow RSO_2OH + HetH,$$

R = Me, 4-MeC_6H_4; Het = imidazolyl, benzimidazolyl, benzotriazolyl.

The rate constants and half reaction periods for hydrolysis of compounds **1—3** are presented in Table 2. The hydrolysis rate constants of the studied substrates are close, and hydrolysis is completed for 5 h. It can easily be seen that the rate constants increase when pH decreases. Thus, we can assume that hydrolysis of these compounds can be catalyzed by acids.

The plots of the self-diffusion coefficients of mesyl- and tosyl-substituted derivatives of **1** and **2** *vs.* time at 309 K are presented in Fig. 2. The coefficient for pure water at the same temperature is given for comparison (curve *4*). It is seen that the plot of SDC for compound **2** (curve *1*) has a characteristic shape: for the first 1.5 h the coefficient values are lower than the SDC of H_2O by almost 35%, then they increase virtually achieving the SDC of H_2O and remain unchanged for ~1 h, then somewhat decrease, and further remain unchanged (by 10—15% lower than the SDC of H_2O). The same tendency of SDC changing is observed for compound **1** (curve *2*). A similar plot for a solution of tosyl-substituted derivative **2** in water (curve *3*) is presented for comparison. It has a resembling, although less pronounced shape than that in the case of D_2O.

The half reaction period of compounds **1** and **2** at pH 7, according to the chromatographic data, is about 2.5 h (see Table 2). This corresponds to approximately half an increase in the SDC values, and 5 h after the curve of SDC changing reaches a plateau (see Fig. 2).

Thus, the PFG NMR studies gave the results that agree well with the kinetic parameters of hydrolysis obtained in the chromatographic experiment.

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