SYNTHESIS OF IMIDAZOLE AND BENZIMIDAZOLE DERIVATIVES AND THEIR ABILITY TO INDUCE RECESSIVE LETHAL MUTATIONS IN Drosophila (FRUIT FLY)

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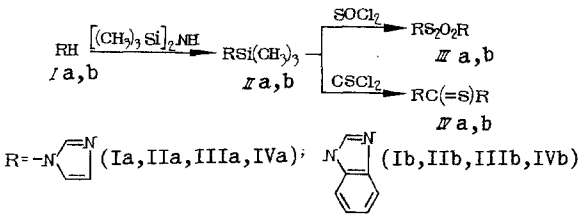
Many derivatives of imidazole and benzimidazole are widely used in industry, agriculture, photography, as well as in medical practice [1-4, 6, 9-11, 13]. Most of them have been studied for toxicity. The mutagenic activity of these compounds still has been little investigated. It was shown that benzimidazole does not show mutagenic activity on Drosophila melanogaster [2] but for Salmonella typhimurium it is a mutagen [12]. It is not known whether this activity is characteristic for benzimidazole derivatives. it is possible that imidazole and benzimidazole do not induce mutation in eucaryotes. Considering the widespread use of imidazolides and benzimidazolides in the national economy, the clarification of their mutagenic action in eucaryotes and in humans is of great interest.

In the present work, we studied the ability of a series of imidazolides and benzimidazolides, obtained by us previously and used to study their influence on the activity of acid phosphatase [5], to induce recessive lethal mutations in drosophila.

The compounds used in the investigation were obtained by the following scheme:



EXPERIMENTAL (CHEMICAL)

The IR spectra were run on a Spectromom-2000 spectrophotometer (Hungary) in mineral oil. The UV spectra were obtained on a Specord UV-VIS (Germany) and SF-26 spectrophotometers. The melting points were determined on a PTP apparatus produced at the Khimlaborpribor factory (USSR). The evaporation of all the solutions was carried out in vacuo at a temperature not higher than 40°C. The synthesis of N-trimethylsilylazoles IIa, b was carried out according to a method described in [7]. The elemental analysis data correspond to the calculated values.

N,N'-Sulfonylbisimidazole (IIIa). A 0.015 mole portion (2.02 g) of sulfuryl chloride was added dropwise, with stirring, to a solution of 0.03 mole (2.17 g) of N-trimethylsilylimidazole IIa in 5 ml of dry toluene cooled to −10°C. Stirring was continued for another 4 h at a temperature of −10°C. The light-yellow colored precipitate of IIIa that separated out was filtered off and dried over P_2O_5.

N,N'-Sulfonylbisibenzimidazole (IIIb) was obtained in a similar way as IIIa.

N,N'-Thiocarbonylbisimidazole (IVa). A solution of 0.015 mole (1.725 g) of thiophosgene in 5 ml of dry carbon tetrachloride was added dropwise with continuous stirring in the course of 90 min to a solution of 0.03 mole (2.17 g) of N-trimethylsilylimidazole IIa in 20 ml of dry carbon tetrachloride or benzene. The yellow precipitate of IVa that separated out was filtered off and dried in a vacuum desiccator over P_2O_5.

N,N'-Thiocarbonylbisibenzimidazole (IVb) was obtained in a similar way as IVa.

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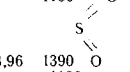
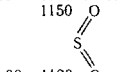
TABLE 1. Yields and Physicochemical Characteristics of IIIa, b, IVa, b

Compound	Yield, %	mp, °C	Empirical formula	(UV, λ_{max}, nm)	(IR, ν, cm^{-1})
IIIa	98	143—144	$C_6H_6N_4O_2S$	230	1500 C=N 1150 
				lg ε=3,96	1390 O 1180
IIIb	97	193—196	$C_{14}H_{10}N_4O_2S$	246,268 274	1550 C=N 1150 
				lg ε=4,38 lg ε=4,22 lg ε=4,27	1120 O 1390
IVa	94	105—106	$C_7H_6N_4S$	245,291 lg ε=3,72 lg ε=4,37	1550 C=N 1020 1300 C=S
IV b	93	138—139	$C_{15}H_{10}N_4S$	247,263 275,333 lg ε=4,28 lg ε=3,73 lg ε=3,75 lg ε=3,96	1300 C=S 1020

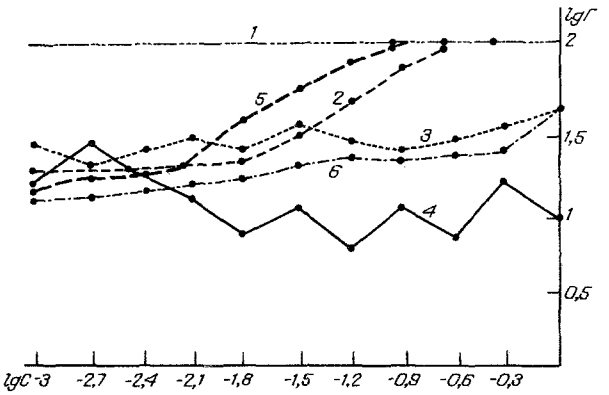


Fig. 1. Study of toxicity of compounds of the imidazole and benzimidazole series on drosophila 1) Ia, 2) Ib, 3) IIIa, 4) IIIb, 5) IVa, 6) IVb. On abscissa - log C, on ordinate - log Γ.

The yields and physicochemical characteristics of compounds IIIa, b, IVa, b are given in Table 1.

EXPERIMENTAL (BIOLOGICAL)

Larvae and imago of Drosophila melanogaster of wild lines Canton-S and Oregon-R served as the object of the investigation. The genetic activity was studied not only of compounds [IIa, b and IVa, b, but also of the starting compounds: imidazole(Ia) and benzimidazole (Ib) in the following dilutions: 1, 1/2, 1/4, 1/8. 1/16, 1/32, 1/64, 1/128, 1/512 of saturated alcoholic solution. The compounds were dissolved in a standard culture medium for Drosophila, using 1 ml of the solution of the compound tested per 20 ml of the feed solution.

The larva was placed on the feed together with the compound tested, immediately after hatching from the egg, 300 larvae for each dilution. As the control, larvae were used (300 specimens) put on a pure feed (passive control) and 300 larvae put on a feed, 20 ml of which contained 1 ml of ethanol, serving as a solvent for all the compounds tested (active control). All the variants of the experiments were repeated three times.

The statistical treatment was carried out by means of a complete one-factor dispersion analysis using, the Fischer and Student's criteria.

At the first stage, we studied the toxicity of the compounds used. The experimental results are summarized in Fig. 1.

TABLE 2. Study of Genetic Activity of IVa in a Test of Taking into Account the Recessive Lethal Mutations in Drosophila

Concentration of IVa in ethanol	Number of analyzed chromosomes	% of recessive lethal mutations, ±m
1/32	88	1,136±1,18
1/64	187	2,139±1,06
1/128	273	1,099±0,63
1/256	314	3,185±0,99
1/512	218	1,376±0,76
1/1024	164	1,22±0,86
Active control	201	0±0
Passive control	217	0±0

TABLE 3. Analysis of Genetic Activity of IVb and IIIb in the Test of Taking into Account the Recessive Lethal Mutations in Drosophila

Concentration of IVa andIIIb in a diluted alcoholic solution	IV6		III6	
	Number of analyzed chromosomes	% of recessive lethal mutations, ±m	Number of analyzed chromosomes	% of recessive lethal mutations, ±m
1	41	—	256	1,56±0,78
1/2	234	3,85±1,26	205	0,49±0,48
1/4	327	3,06±0,95	204	0,49±0,48
1/8	246	1,63±0,81	170	0,59±0,59
1/16	169	4,73±1,63	193	1,04±0,73
1/32	285	1,75±0,78	238	0,84±0,59
1/64	129	2,33±1,33	247	—
1/128	251	3,98±1,23	317	—
1/256	304	3,45±1,05	233	—
1/512	232	2,16±0,95	263	—
1/1024	224	2,23±0,99	363	—
Active control	270	—	244	—
Passive control	310	—	380	—

The analysis of the results obtained showed that the most toxic compound is Ia — 100% of larvae destroyed on diluting a saturated solution 1024 times. Other compounds were less toxic. It was found that with respect to toxicity the compounds studied can be divided into the following series for all the dilutions: Ia > Ib > IVa > IVb > IIIb > IIIa (with reliability for $p > 0.95$). The dependence of toxicity on the degree of dilution decreases in this series, although in all cases, the dilution reliably decreases the toxicity. The strength of the influence of the dilution factor decreases from Ia ($F = 323$, $\eta^2 = 49.57\%$) to IIIa ($F = 2.55$, $\eta^2 = 0.77\%$) at F 2.3 for $p > 0.95$. These data show that the thiocarbonyl group increases the toxicity to a greater extent than the sulfonyl group.

The study of the same compounds for the ability to induce recessive lethal mutations in drosophila showed the following: compound IIIa did not induce the recessive lethal mutations (number of analyzed chromosomes 2644). The genetic activity of Ia could not be investigated because of its high toxicity (183 chromosomes were analyzed, which is not sufficient for drawing a conclusion on the presence or absence of activity). As far as IIIb, IVa, and IVb are concerned, all these compounds were found to be capable of inducing recessive lethal mutations in the X chromosome of Drosophila. The experimental results are presented in Tables 2-4. The investigation of the ability of IVa to induce recessive lethal mutations was carried out at concentrations from 1/32 to 1/1024, because all the preceding dilutions caused a 100% destruction of the larvae.

The complete one-factor dispersion analysis that has been carried out showed that the dilution does not influence the number of the recessive lethal mutations (for IVa $F_a = 1.05$, $\eta^2 = 0.004$; for IVb $F_a = 0.797$, $\eta^2 = 0.003$; for IIIb $F_a = 0.55$, $\eta^2 = 0.002$ at $F_s = 1.8$ for $p < 0.95$, $F_{actual} < F_{stand.}$).

All this made it possible to sum up the analyzed chromosomes and to present the results in Table 4.

TABLE 4. Ability of IVa, IVb, and IIIb to Induce Recessive Lethal Mutations in Drosophila

Compound	Number of analyzed chromosomes	% of recessive lethal mutations, ±m
IVa	1244	1,692±0,382
IVb	2442	2,864±0,388
IIIb	2689	0,643±0,163

The data obtained by us do not contradict the known fact that for chemical mutagens, the dose–effect relationship is not existent in several cases [14].

All the compounds studied differ reliably in their ability to induce recessive lethal mutations. In comparing these values we have: IVa-IIIb: $F_a = 2.96$; IVa-IVb: $F_a = 2.24$ at $F_s = 1.96$ and $p > 0.95$.

The maximum number of mutations is induced by IVb, and the minimum by IIIb.

The results obtained indicate a high biological activity of the compounds studied and can be used both in setting sanitary-hygienic norms and in pharmacology.

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