MEDICINAL PLANTS

ADAPTATION OF *Staphylococcus aureus* TO SYNTHETIC TRIAZOLIDES

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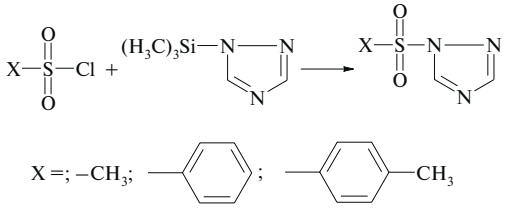
The ability of *Staphylococcus aureus* to adapt to the toxic action of 1,2,4-triazole and its sulfuryl derivates has been studied. A positive correlation is found between the lipophilicity and toxicity of substances. It is established that methylated derivates are more toxic than substances without methyl radicals. *St. aureus* species grown on a medium containing triazolides in a nontoxic dose become resistant to highly toxic doses of these substances. The possible mechanisms of *St. aureus* adaptation to triazolides are discussed.

In recent years, the appearance of highly resistant strains of *Staphylococcus aureus* has been observed in cases of chronic administration of various antibiotics [1, 2]. This situation leads to the search for new compounds that can serve as a base for highly effective antistaphylococcal preparations and to the investigation of mechanisms responsible for the ability of *St. aureus* species to adapt to new drugs. In this respect, a promising direction of research is related to the antistaphylococcal properties of 1,2,4-triazole derivatives, many of which are used in pharmacology [3] and agriculture [4 – 6]. Previously, we demonstrated that some triazole derivatives exhibited mutagenicity [7, 8] and antibacterial activity [9, 10].

Despite extensive investigation, relationships between the structure and activity of various groups of compounds are for the most part still unclear. This study was aimed at an analysis of the antibacterial activity of 1,2,4-triazole (I) and its sulfuryl derivatives (II – IV) (Fig. 1), and the evaluation of the ability of *St. aureus* species to adapt to these compounds.

EXPERIMENTAL CHEMICAL PART

Compounds II – IV were synthesized via the interaction of N-trimethylsilyl-1,2,4-triazole with chloroanhydrides of methane-, benzene-, and toluenesulfonic acids, respectively, in anhydrous tetrahydrofuran (THF) according to the following scheme:



The IR absorption spectra of the synthesized compounds were measured using a Spectromom-200 instrument (Hungary) using samples prepared as nujol mulls. The UV spectra were recorded on a Specord UV-VIS spectrophotometer (Germany). The melting points were determined on a PTP device (Khimlaborpribor, Russia). During the synthesis, solutions were evaporated in vacuum at a temperature not exceeding 40°C.

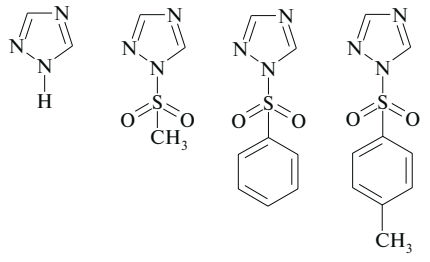


Fig. 1. Structures of 1,2,4-triazole (I), methanesulfonic acid N-triazolide (II), benzenesulfonic acid N-triazolide (III), and toluenesulfonic acid N-triazolide (IV).

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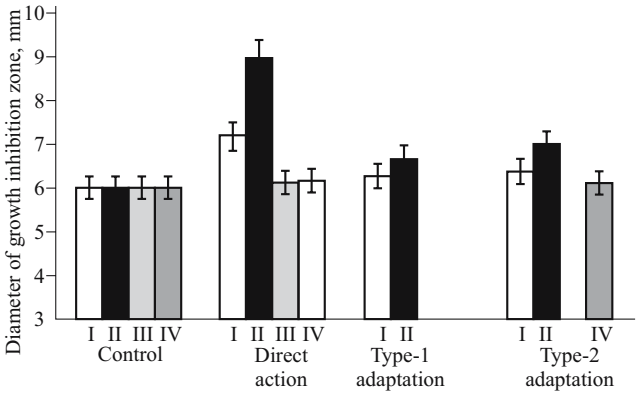

Fig. 2. A histogram of the ability of *St. aureus* to develop resistance with respect to compounds I – IV.

The lipophilicity of compounds I – IV was evaluated from data on the chromatographic mobility, which was determined by TLC in a toluene – chloroform – methanol (5 : 2 : 1) solvent system. Some characteristics are summarized in Table 1.

The initial **N-trimethylsilyl-1,2,4-triazole** was obtained using a procedure proposed by Birkofer et al. [11].

Methanesulfonic acid N-traizolide of (II) was synthesized as described in [12].

Benzenesulfonic acid N-triazolide (III). A solution of 2 g (0.014 mole) of N-trimethylsilyl-1,2,4-triazole in 10 ml of dry toluene was cooled down to –10°C. To this solution was added dropwise with stirring 2 g (0.015 mole) of benzenesulfonic acid chloroanhydride in 5 ml of toluene and the mixture was stirred for 3 h at the same temperature. Then, the reaction mixture (with precipitate) was evaporated in vacuum to dryness and the residue was recrystallized from ethanol to obtain 2.5 g (0.012 mole) of product III in the form of white crystals with a yield of 85%. The purity and identity of the product was confirmed by the data of elemental analyses, TLC, and IR spectroscopy; additional evidence was provided by the melting point determination (Table 1).

Toluenesulfonic acid N-triazolide (IV) was obtained using a procedure analogous to that described above for compound III. The target product IV was isolated in the form of white crystals with a yield of 85%. The melting point and characteristic IR absorption band positions are given in Table 1.

TABLE 1. Meting Points and Characteristic IR Absorption Bands of Compounds III and IV

Compound	M.p., °C	Characteristic optical absorption band, cm^{-1}
III	95	1372
		1185
IV	70	1372
		1185

EXPERIMENTAL BIOLOGICAL PART

The biological tests were performed with *St. aureus* strain ATCC No. 6587p. The antibacterial activity of compounds I – IV in aqueous solution with concentrations 0.001, 0.01, 0.1, 1, and 10 mg/ml was determined using a conventional method based on the determination of the zone of microbial growth inhibition as a result of the direct diffusion of drugs into agar from impregnated paper disks placed onto a bacterial lawn [13]. There were three series of experiments.

Series 1. Determination of the dependence of the antistaphylococcal activity of compounds I – IV on the concentration (five independent runs for each experimental point).

Series 2. Evaluation of the ability of small doses of compounds I – IV to favor the development of adaptation to highly toxic doses of same drugs. For this purpose, each compound was introduced into the cultural medium to a concentration of 0.001 mg/ml and then *St. aureus* species were seeded in these media. The colonies grown on these media were used to prepare suspensions for seeding on a fresh agar not containing drugs. The suspensions were diluted with isotonic NaCl solution to a final bacterial load of 5×10^9 microbial cells per milliliter (with reference to the turbidity standard obtained from the Tarasevich Culture Nursery). Then, test disks impregnated with 10 mg/ml solutions of compounds I – IV were placed onto inoculated bacterial lawn and the growth inhibition zone size was determined (seven independent runs for each experimental point).

Series 3. Evaluation of the ability of a nontoxic dose (0.001 mg/ml) of compound I to favor the development of adaptation to highly toxic (10 mg/ml) doses of compounds II – IV. For this purpose, *St. aureus* species were grown in a medium containing compound I at a concentration of 0.001 mg/ml and then reseeded on a fresh agar not containing drugs. Then, the experiment was carried out as in series 2.

RESULTS AND DISCUSSION

The results of investigation of the ability of compounds I – IV to inhibit the growth of *St. aureus* are presented in Table 2. As can be seen from these data, compounds I and II (but not III and IV) exhibit the concentration-dependent ability to inhibit the growth of staphylococcoccal species. The results of a two-factor dispersion analysis showed evidence for reliable ($p < 0.01$) dependence of the antistaphylococcal activity both on the concentration and on the structure of compounds studies.

Table 3 presents data on the physicochemical parameters of triazolides I – IV and their ability to inhibit the growth of *St. aureus*. A mathematical correlation analysis of these data confirmed the presence of a positive correlation between lipophilicity and antistaphylococcal activity ($r = 0.66$ at $p < 0.01$) and between the total energy of a molecule and the

TABLE 2. Diameter of the Zone of *St. aureus* Growth Inhibition by Compounds I – IV

Compound	Drug concentrating, mg/ml					
	0	0.001	0.01	0.1	1	10
I	6.0 ± 0.00	6.5 ± 0.03	6.5 ± 0.05	6.8 ± 0.04	6.9 ± 0.02	7.2 ± 0.06
II	6.0 ± 0.00	6.8 ± 0.02	7.0 ± 0.07	7.8 ± 0.09	8.0 ± 0.07	9.0 ± 0.11
III	6.0 ± 0.00	6.1 ± 0.04	6.1 ± 0.04	6.2 ± 0.06	6.1 ± 0.03	6.1 ± 0.05
IV	6.0 ± 0.00	6.2 ± 0.06	6.3 ± 0.07	6.4 ± 0.10	6.2 ± 0.05	6.2 ± 0.06

antistaphylococcal activity ($r = 0.66$ at $p < 0.01$). It was established that methylated derivates are more toxic than substances without methyl radicals: II > I; IV > III. It should also be noted that methylation frequently renders compounds mutagenic [14].

In order to elucidate the mechanism of the antibacterial action of compounds I – IV, we checked for the ability of these drugs to favor the development of adaptation (type-1 preadaptation) with respect to highly toxic doses of same compounds (experimental series 2). It was found that the preliminary action of compounds I – II in a concentration of 0.001 mg/ml induces the ability of *St. aureus* species to adapt to large (10 mg/ml) doses of these compounds, whereby the growth inhibition zone size reliably decreases (Fig. 2). Moreover, the preadaptation induced by compounds III and IV in a small dose even leads to the so-called "overgrowth" effect, whereby the disks impregnated with these drugs at a concentration of 10 mg/ml not only fail to inhibit the growth of *St. aureus*, but even are overgrown with the bacterial species.

In order to refine the mechanisms of adaptive response, the preadaptation in experimental series 3 was induced by the initial 1,2,4-triazole (I) in a nontoxic dose and checked on the highly toxic doses (Fig. 2) of its derivatives (II – IV). It was established that *St. aureus* pretreated with compound I at a concentration of 0.001 mg/ml becomes resistant to a high dose of its derivatives (II – IV). However, the growth inhibition zone size was reliably greater than in series 1 (the phenomenon of overgrowth was observed only for compound III).

Assuming that the adaptation to toxic doses of triazolides was related to the fact that a small dose induces the production of enzymes capable of modifying the toxicity, one would have to suggest that these enzymatic systems must specifically recognize triazole and hydrolyze the triazolides at the N–S bonds with the formation of a highly toxic sulfonic acid radical. However, this mechanism does not explain the "overgrowth" observed upon the type-2 preadaptation for compound III. In the experiments with type-1 preadaptation, one could suggest the induction of enzymes of the methyl transferase type, but this mechanism also cannot explain the "overgrowth" observed for compound III.

TABLE 3. Comparative Data on Antistaphylococcal Activity and Physicochemical Characteristics of Compounds I – III

Compound	Total dipole moment	Total energy of molecule	R_f	$R_m = \log\frac{1-R_f}{R_f}$	Diameter of growth inhibition zone, mm
I	2.73	– 760.21	0.385	0.203	7.2 ± 0.06
II	3.867	– 1277.6	0.538	– 0.066	9.0 ± 0.11
III	4.774	– 2209.82	0.785	– 0.562	6.1 ± 0.05
IV	5.399	– 2493.15	0.862	– 0.796	6.2 ± 0.06

We believe that, being capable of influencing the metabolism of purines [15], 1,2,4-triazole is a mutagen for *St. aureus*. Mutant clones possess highly active enzymatic systems, which can both modify the action of triazolides and actively use triazolides containing benzene rings (compounds III and IV) in the bacterial cell metabolism. Compounds III and IV also induce such mutations in *St. aureus*. Thus, *St. aureus* species most frequently maintain the cell homeostasis using the genetic mechanisms of cell adaptation, which account for the rapid development of resistance to various drugs.

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