

Study of diketone derivatives as inhibitors of mycological corrosion

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Abstract. The article describes the possibility of using two derivatives of diketones as inhibitors for protection against micromycete corrosion. Compositions based on a mixture of solidol, derivatives of 4,4,4-trichloro-1- (3-chlorophenyl) butane-1,3-dione, which in preliminary studies have shown their fungicidal properties in low concentrations, have been investigated. The study was carried out on samples of steel grade St3. After 28 days under the influence of micromycetes of the genera *Aspergillus spp.*, *Penicillium spp.* and *Trichoderma spp.* the rate of growth and smear from the surface were studied (to determine the fungal resistance of the material). Based on the data obtained, it was concluded that it is impossible to use lis-24 and lis-86 as inhibitors of mycological corrosion due to the low fungal resistance of the coating and the high growth rate of the samples. Lis-89 sufficiently protects against micromycetes of the genus *Aspergillus*, however, it copes with other micromycetes worse than the control group.

Keywords: biological corrosion, micromycetes, corrosion inhibitors, diketones

Introduction

At the moment, more and more attention is paid to the processes occurring at the intersection of sciences. One of these processes is biological corrosion. Microbiological corrosion (MBC) is a form of corrosion that is caused by the metabolic activity of microbial colonies, which may include bacteria and fungi [1]. This type of corrosion is one of the most destructive; some researchers cite data that $\frac{3}{4}$ of all losses from pipeline corrosion are caused precisely by microorganisms living in the soil [2].

Depending on the belonging of corrosive organisms to different kingdoms of the living, bacterial, mycological and mixed corrosion are distinguished.

The type of destruction caused by microscopic fungi or micromycetes is called mycological. Corrosion occurs under the influence of various aggressive environments that arise due to the presence of mold fungi. One of the biggest problems of this type of corrosion is the high adaptability of micromycetes to changing environmental conditions, as well as their huge species diversity. Another problem is that the habitat of micromycetes is soil and air, which is why almost all metal structures can be exposed to mold. Compared to bacterial corrosion, a larger volume of metal structures is susceptible to mycological. Not only ferrous, but non-ferrous metals are exposed to corrosion. Typical representatives of corrosive fungi are the genus *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium* and *Trichoderma*.

If we talk about the effect of microorganisms on metals, then there are several types. The most common: direct influence of metabolites, often of various acids, such as hydrogen sulfide and various organic, or ammonia [3], on the metal surface, or coating the surface with build-ups and films, under which pitting corrosion can develop.

The effect of micromycetes on the corrosion rate can be diametrically opposite, as some researchers cite data on the inhibitory effect of *Cladosporium herbarum* on the surface of aluminum samples and a simultaneous increase in the corrosion rate under the influence of *Aspergillus niger*. Some authors [4] have shown that the inhibiting effect of fungi on steel and aluminum corrosion consists primarily in increasing the resistance to charge transfer through the inner oxide layer [5].

Many organic substances can be used to protect metals from biocorrosion. However, a problem arises as some substances may be suitable for bacterial corrosion, but not suitable for mycological. For example, N-benzyl-diethylenediammonium chloride has a high inhibitory activity against bacteria, but its effect on micromycetes has not been studied [6].

From all of the above, the relevance of the study follows. There are not so many methods of protection against micromycete corrosion, while micromycetes do great damage to metal structures.

Methodology

To study the antimicrobial activity of substances that in the future can be used as inhibitors of mycological corrosion, we used the method of two-fold serial dilutions in a liquid nutrient medium by the micro method [7]. In the wells of a sterile 96-well flat-bottomed microplate, two parallel rows of two-fold serial dilutions of chemical compounds in Sabouraud broth were prepared. Each well contained 150 µl of a certain concentration of the test substance and 150 µl of culture inoculum. The last rows contained the nutrient medium and culture in equal

volumes (control). The microplate was placed in a thermostat of an Epoch spectrophotometer and the optical density (OD) was measured at a wavelength of 540 nm. After 48 hours and 7 days, the OD of the culture fluid was again recorded. The study involved 3 substances, the structure of which is shown in Figure 1.

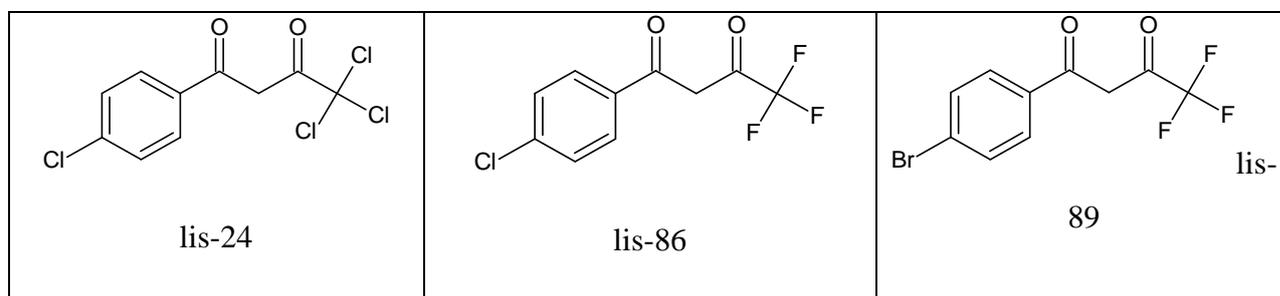


Fig. 1. Structural formulas of the most effective compounds

An aqueous suspension of fungal spores was prepared from the micromycete colonies (*Aspergillus*, *Penicillium*, and *Trichoderma*) isolated from soil and water samples from the Perm Territory, grown for 14 days at a temperature of $29 \pm 1^\circ\text{C}$ on a chapek slant nutrient medium. The spore load of each fungus in distilled water was prepared according to the McFarland turbidity standard using a densitometer OD = 1.0 equal to 109 microbial cells / μl .

Due to the fact that the selected substances are poorly soluble in water, but well in organic solvents, it was decided to use solidol as a solvent for the substances.

To study the inhibitory effect of the selected substances, the method of studying the fungal resistance of the metal was used. Samples of steel grade St3 (C 0.14-0.22%; Ni not more than 0.3%; Cu not more than 0.3%; Cr not more than 0.3%; Mn 0.4-0.65%; Si 0.05-0.17%; As not more than 0.08%; S not more than 0.05; P not more than 0.04% [8]). Samples with an area of 14 and 22 cm^2 were covered with a mixture of solidol and a representative of a number of diketones, kept for a day, after which they were weighed and placed in a Petri dish. An aqueous suspension of spores of each type of fungi was used to infect prototypes of steel with inhibitors; the steel surfaces were irrigated with a spray gun until they were completely moistened, preventing droplets from merging. The contaminated samples were placed in pre-prepared desiccators, on the bottom of which water was poured and thermostatic at a temperature of $29 \pm 1^\circ\text{C}$ and a relative humidity of more than 90% for 28 days. After testing, the samples were removed from the desiccator and examined visually, re-weighed on an analytical balance to determine the rate of rise, and then using a Micromed microscope with a ToupCam digital camera and Toup View software, at a magnification of $\times 10$, each steel variant was evaluated for the presence or absence of mushrooms according to the point system [9]. The control of the viability of mold spores was pure cultures applied in the form of drops on the surface of the slant

agar culture medium in test tubes. The results of the growth and development of micromycetes were confirmed after 5 days.

Results

Anti-fungicidal activity tests

Diketone halide derivatives were chosen as the proposed inhibitors. Earlier [11], similar compounds proved their antimicrobial activity in small doses. Within the framework of this study, the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) were determined based on the data on the change in optical density over time (Fig. 2). It was found that lis-24, lis-86 and lis-89 exhibit their antimycological activity already at concentrations of 3.9 - 7.8 $\mu\text{g} / \mu\text{l}$.

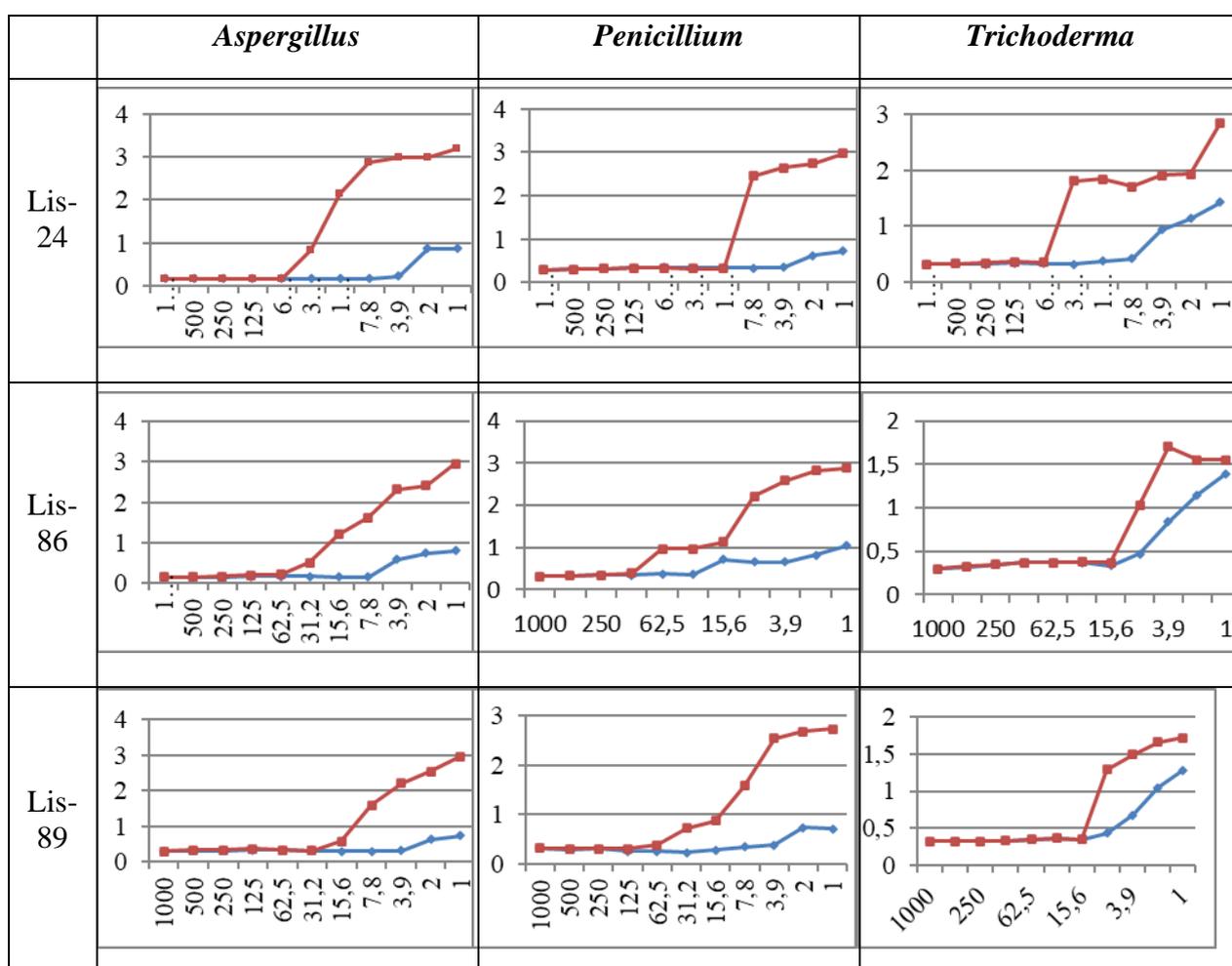


Fig 2. Change in optical density of samples on the second (blue) and tenth days (red)

Corrosion tests

After carrying out gravimetric tests for each compound, the average growth rates of the samples were determined. The results are shown in Figure 3. Samples treated with solidol were used as a control group. The results of the effect of micromycetes on the surface of steel without

protective coatings were described earlier [10]. Along with this, the protective effect of the diketone derivatives was calculated. However, none of the substances showed a positive value.

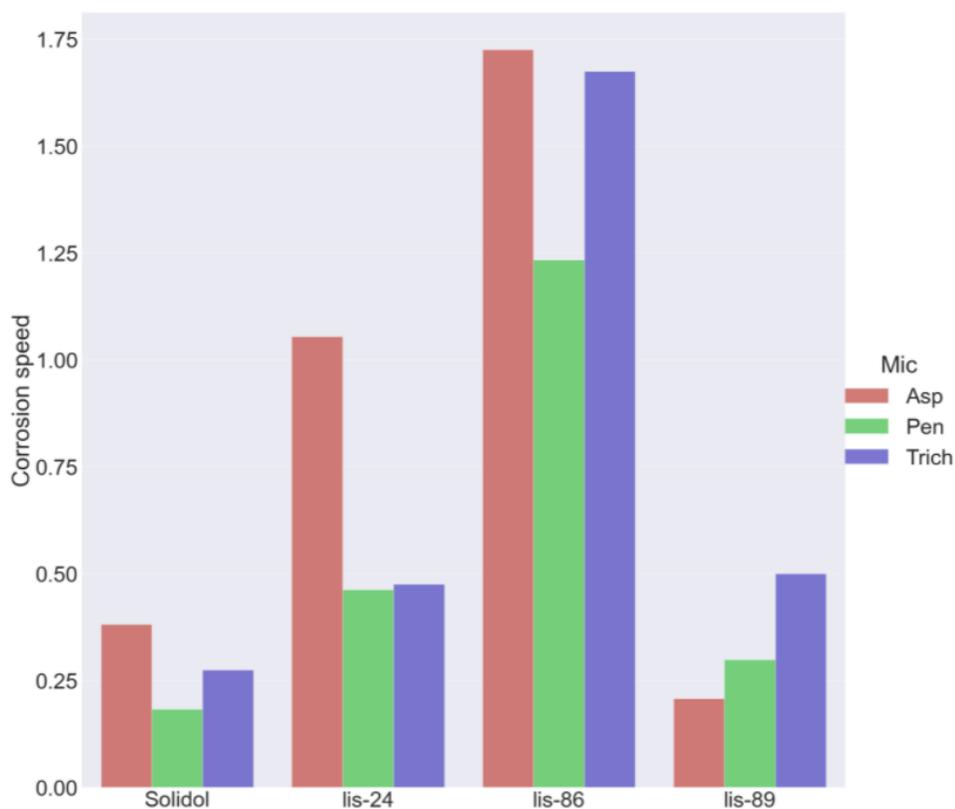


Fig. 3. Growth rate diagram of samples, g/(m²·hour)

To determine the intensity of development of micromycetes, scraping from the metal surface was studied in two ways: microscopy of scraping (Fig. 4) and determination of the presence of reductase using sodium resazurin. The transition from blue to pink indicates the presence of waste products of micromycetes.

The results obtained confirmed themselves, and a summary table was constructed from them (Table 2). The presence or absence of micromycetes was assessed using a point system [9]. As you can see, lis-86 and lis-89 cope with the task a little better, however, the indicator of fungal resistance is insufficient for the use of substances as an inhibitor of mycological corrosion.

Inhibitor	Microscopy	Grade
Lis-24	Discovered spore germination, mycelium, sporulation	3
Lis-86	Discovered spore germination, mycelium	2
Lis-89	Discovered spore germination, mycelium	2
Solidol	Discovered spore germination, mycelium	2

Table 2. The results of the study of scraping from the surface of metals

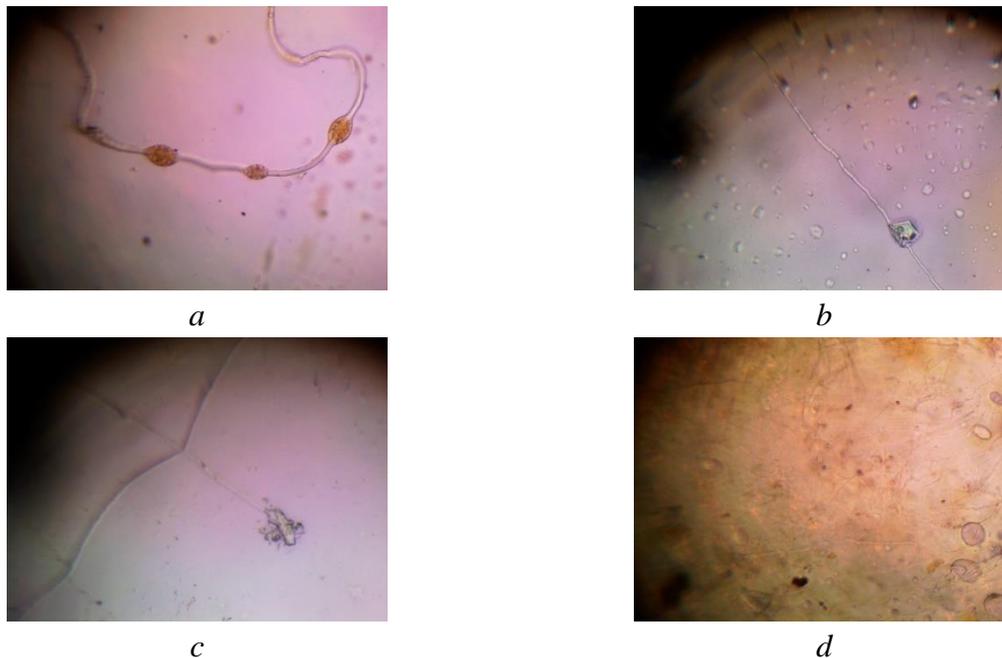


Fig. 4. Micrographs of a smear taken from a sample: *a* – lis-24, *b* – lis-86, *c* – lis-89, *d* – Solidol

Conclusion

To protect metals from micromycetes, substances of the diketone halide group were proposed, which in preliminary studies showed fungicidal properties in low concentrations. To study their possible use as inhibitors of mycological corrosion, the effect of micromycetes on metals coated with protective films of solidol with the proposed substances was studied. Based on the results of the data on the rate of growth and the study of scraping to assess fungal resistance, it was concluded that it was impossible to use the substances lis-24, and lis-86 as inhibitors. Lis-89 partially copes with the task, however, its concentration is insufficient for use as an inhibitor.

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