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BIOCIDAL ACTIVITY OF AMINOMETHYL DERIVATIVES OF 2-MERCAPTOBENZOXAZOLE

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Abstract: *The biocidal activity of the aminomethyl derivatives of 2-mercaptobenzoxazole synthesized by us and being of interest as potential antimicrobial additives to petroleum products and cutting fluids, was studied. The aminomethylation reaction proceeded with the participation of methylene-bis-amines. As a result, the appropriate N-substituted benzoxazolin-2-thiones were obtained. Their structure was proved by NMR ^{1}H. Due to the poor solubility of the synthesized compounds in petroleum products, their salts with petroleum naphthenic acid were obtained for microbiological testing. The results of tests in lubricating oil M-8 show that the naphthenic salts of the synthesized compounds exhibit biocidal activity and their fungicidal properties are higher than bactericidal ones. The petroleum naphthenic acid used in the test in its pure form in the composition of the M-8 lubricating oil is not biostable and is affected by microorganisms. N-piperidinomethylbenzoxazolin-2-thione in the composition of the coolant proved to be a biocide, and its bactericidal properties are higher than fungicidal ones. The results of a microbiological study of an aqueous solution of this compound showed high bactericidal properties in the absence of effectiveness against mold fungi.*

Keywords: *2-mercaptobenzoxazole, N-substituted benzoxazolin-2-thiones, aminomethylation, methylene-bis-amines, biocidal activity, antimicrobial additives, petroleum products, cutting fluid, petroleum naphthenic acid.*

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Introduction

Derivatives of 2-mercaptobenzoxazole, along with derivatives of 2-mercaptobenzothiazole and 2-mercaptobenzimidazole, are of interest as heterocyclic compounds with diverse functional properties. Unsubstituted benzoxazole has little practical application. This notwithstanding, its numerous derivatives are widely used in various fields, including industry (for example, as optical brighteners for detergents) [1, 2].

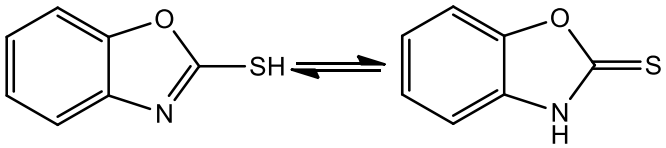
According to Osipova et al. [2], the heterocyclic nucleus of benzoxazole is the starting cyclic system for the synthesis of biologically active compounds. The reason of relative stability of benzoxazol is its aromaticity. In addition, it has active sites for functionalization [3]. Benzoxazole derivatives, both natural and synthetic, have a wide range of biological activity [4]. Namely, they exhibit antibacterial [5], anti-inflammatory [6], antiviral [7], anti-allergic [8], antifungal [9, 10], anthelmintic [11] properties and are inhibitors of cyclooxygenase-2 [12]. The benzoxazole moiety is a constituent of flunoxaprofen, a non-steroidal anti-inflammatory drug. Besides, substituted benzoxazoles and related heterocycles have low toxicity and high chemotherapeutic efficacy as evidenced by recent studies [13].

Bio-damage of fuels and lubricating oils manifests itself in the deterioration of their performance properties, results in corrosion of tanks, fuel and lubrication systems of engines. In addition, microorganism-contaminated petroleum products and cutting fluids (coolants) can cause skin, allergic, and other diseases

among persons in contact with them [14]. For many decades, the laboratory of protective additives of acad. A.M. Guliyev Institute of Chemistry of Additives (ICA) is engaged in the synthesis of chemical compounds with biocidal activity, exploring the possibilities for their use as antimicrobial additives for petroleum products and cutting fluids. In continuation of these studies, aminomethyl derivatives of 2-mercaptobenzoxazole were synthesized. Synthesis and study of their physiological properties are given in the article [15]. The results showed that as inhibitors of a number of enzymes, all tested compounds outperform the standards used in world pharmaceuticals. The purpose of this study was to evaluate the biocidal activity of aminomethyl derivatives of 2-mercaptobenzoxazole, considering them as potential antimicrobial additives to petroleum products.

Experimental part

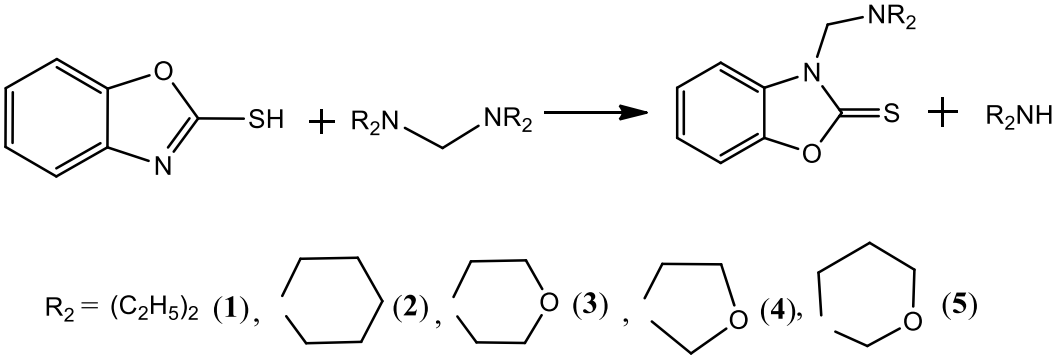
2-Mercaptobenzoxazole has tautomeric properties and, depending on the reaction conditions, can react to form compounds having a thio-keto- or thio-enol structure:



There are literature sources in which the thio-keto structure of 2-mercaptobenzoxazole is confirmed by physio-chemical methods, completely excluding the presence of the thioenol tautomer [16]. However, in substitution reactions, both S- and N-substituted derivatives of the above heterocyclic thiol are obtained [17].

To obtain aminomethyl derivatives of 2-mercaptobenzoxazole, we used methylene-bis-amines or aminals, according to Boehme. The method for obtaining methylene-bis-amines used in this study was developed by researchers from the laboratory of protective additives of the ICA and consists in the condensati of secondary amines with formaldehyde in a ratio of 2:1 or amino alcohols with formaldehyde in a ratio of 2:3. The results of the synthesis and study of the properties of these aminals are presented in the article [18],

Aminomethylation of 2-mercaptobenzoxazole was carried out by us according to the scheme [15]:



As a result of the interaction of 2-mercaptobenzoxazole with methylene-bis-amines, crystalline substances were obtained and purified by recrystallization from absolute methanol. Physio-chemical characteristics of the synthesized compounds, along with the aminomethyl derivatives of 2-mercaptobenzoxazole and 2-aminothiazole, are given in the article [15].

The results of ^{1}H NMR spectroscopy analysis of the synthesized compounds [15] prove the obtaining of N-substituted derivatives of benzoxazolin-2-thione, namely a singlet at 3.15-5.2 ppm, which is characteristic for two protons of NCH_2N group was observe in their ^{1}H NMR spectra. The spectra of some synthesized compounds are shown in Fig.1, 2.

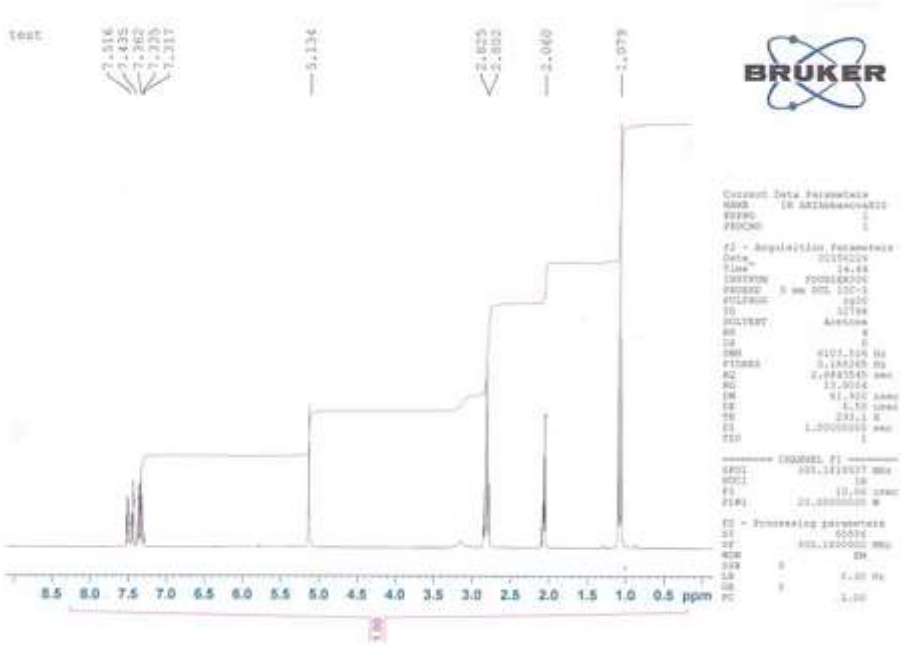


Fig. 1. ^{1}H-NMR spectra of N-diethylaminomethylbenzoxazoline-2-thione

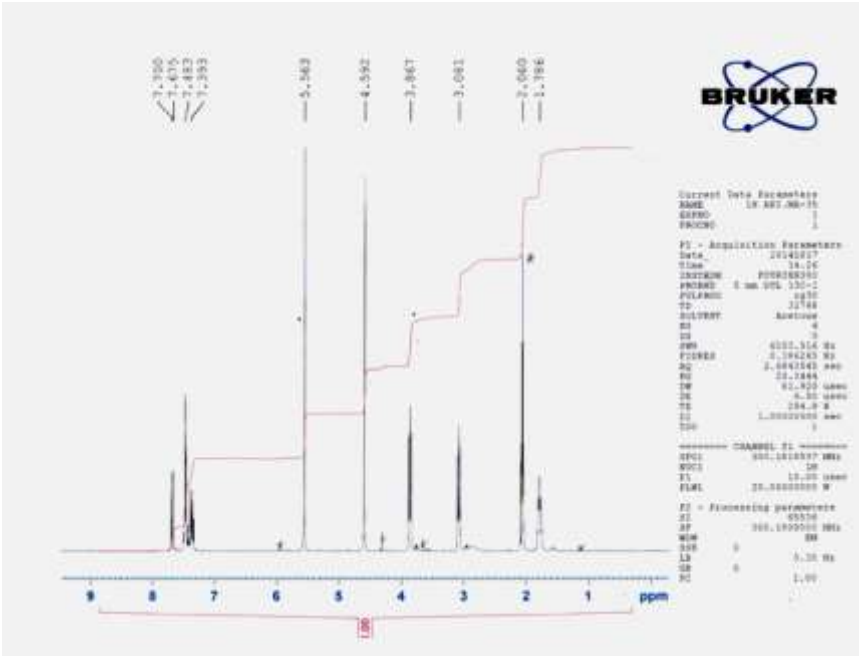


Fig. 2. ^{1}H-NMR spectra of N-oxazinomethylbenzoxazoline-2-thione

Attempts to conduct X-ray analysis of N-aminomethyl derivatives of benzoxazolin-2-thione every time came to failure due to the fact that the crystals were destroyed when X-rays hit them.

Results and discussion

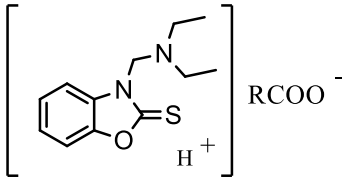
In common with N-substituted derivatives of benzothiazoline-2-thiones [15, 19], the derivatives of benzoxazolin-2-thione obtained by us are poorly soluble in fuel, lubricating oils, hexane, and readily soluble in ethanol and acetone. To study into the microbiological activity of the synthesized compounds, their salts with petroleum naphthenic acid were preliminarily obtained at an equimolar ratio of heterocyclic thione and petroleum naphthenic acid [19, 20], which were well dissolved in lubricating oil. Petroleum naphthenic acid fraction 200-250°C with an average molecular weight of 200 was taken.

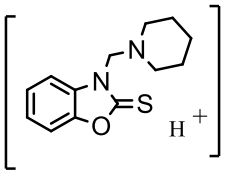
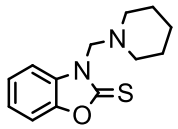
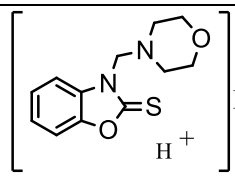
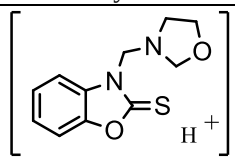
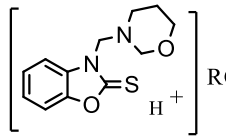
Tests for biocidal activity of 1% solutions of these salts were carried out in M-8 lubricating oil using a mixture of the following types of bacteria: *Pseudomonas aeruginoza*, *Mycobacterium lacticolum*, according to ГОСТ 9.082-77 "Unified protection corrosion and ageing system. Oils and lubricants. Methods of laboratory tests for resistance to bacteria action", as well as in relation to mold fungi *Aspergillus niger* according to ГОСТ 9.052-88 "Unified system of corrosion and ageing protection. Oils and greases. Laboratory test methods for mould resistance". In parallel, the bio-stability of a 1% solution of petroleum naphthenic acid in M-8 lubricating oil was studied. N-piperidinomethylbenzoxazolin-2-thione (2) was dissolved in water and tested in the form of an aqueous solution, as well as in the composition of coolant in line with ГОСТ 9.085-78 "Single corrosion and ageing protection system. Cooling lubricant. Bio-resistance test methods". The test results are presented in Table. 1.

As can be seen from Table 1, salts of aminomethyl derivatives of benzoxazolin-2-thione with naphthenic acid proved to be biocides and their fungicidal action is higher than bactericidal. Petroleum naphthenic acid itself is not biostable and is attacked by both bacteria and fungi during testing. The result of the study of N-piperidinomethylbenzoxazolin-2-thione (2) in the coolant composition is indicative that it exhibits both fungicidal and bactericidal activity, with the latter predominating. Evaluation of a 0.5% solution of compound (2) in distilled water showed that bactericidal properties were higher than the properties of its salt with naphthenic acid in M-8 lubricating oil, as well as in the coolant composition. At the same time, this solution turned out to be absolutely ineffective in respect of mold fungi.

The fungicidal activity of the studied salts of naphthenic acid increases from compounds with a linear structure substituents at the nitrogen atom to compounds with substituents with a cyclic structure. The maximum result is given by N-oxazolidinomethylbenzoxazolin-2-thione (4). When analyzing the bactericidal activity of aminomethyl derivatives of benzoxazolin-2-thione, no such regularity was observed. It is also impossible to speak of a clear increase in both fungicidal and bactericidal activity as the substituent becomes heavier.

Table 1. Antimicrobial properties of N-aminomethyl derivatives of benzoxazolin-2-thione

№/№	Compound	Oil product/ solvent	Concen-tration, %	Microbial growth inhibition zone diameter, cm	
				Bacteria	fungi
1	 Naphtenic salt of N-diethylaminomethyl-benzoxazolin-2-thione	Lubricating oil M-8	1	2.5 - 2.3	2.5 - 2.2

2	 Naphtenic salt of N-piperidinomethylbenzoxazoline-2-thione	-«-	- « -	2.0-1.8	3.6 – 3.5
		Coolant	0.5	2.5-2.0	1.5-1.5
		Distilled water	0.5	2.6-2.5	+
3	 Naphtenic salt of N-morfolinomethylbenzoxazoline-2-thione	Lubricating oil M-8	1	+	3.0 - 3.0
4	 Naphtenic salt of N-oxazolidinomethylbenzoxazoline-2-thione	- « -	- « -	2.3 - 2.3	4.0 - 4.0
5	 Naphtenic salt of N-oxazinomethylbenzoxazoline-2-thion	- « -	- « -	1.0 – 1.1	2,8 - 2.8
6	Petroleum nathtenic asid	- « -	- « -	+	+
7	Lubricating oil M-8			+	+
8	Coolant 5 % without biocide			++++	++++
9	Distilled water			+	+

+ - growth of microorganisms

Conclusion

By condensation of 2-mercaptobenzoxazole with methylene-bis-amines, we synthesized a number of aminomethyl derivatives of benzoxazolin-2-thione. Two compounds, namely, N-oxazolidinomethylbenzoxazolin-2-thione (4) and N-oxazinomethylbenzoxazolin-2-thione (5), were synthesized for the first time. The structure of the obtained compounds was established by ^{1}H NMR.

The synthesized compounds were tested for biocidal activity in M-8 lubricating oil. The results of the study showed that the naphthenic salts of these compounds exhibited bactericidal and fungicidal properties. Since petroleum naphthenic acid is not biostable, it can be concluded that the tested compounds exhibit antimicrobial activity against microorganisms affecting oil products, which means that these substances can be recommended as potential biocides to protect oil products from microbiological damage. In addition, N-piperidinomethylbenzoxazolin-2-thione (4) showed biocidal activity in the composition of cutting fluids, which makes it possible to use it as an antimicrobial additive in coolant. The bactericidal activity of an aqueous solution of

this compound also makes it necessary to study the possibilities of its use.

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2-MERKAPTOBENZOKSAZOLUN AMİNOMETİL TÖRƏMƏLƏRİNİN BİOSİD AKTİVLİYİ

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Xülasə: Neft məhsulları və yağlayıcı-soyuducu mayelər üçün potensial antimikrob aşqarlar kimi maraq doğuran 2-merkaptobenzoksazolun aminometil törəmələri tərəfimizdən sintez edilmiş və onların biosid aktivliyi tədqiq edilmişdir. Aminometilləşmə reaksiyası metilen-bis-aminlərin iştirakı ilə aparılmışdır. Nəticədə quruluşu ^{1}H NMR spektroskopiya üsulu ilə təsdiq edilmiş müvafiq N-əvəzedilmiş benzoksazolin-2-tionlar alınmışdır. Sintez edilmiş birləşmələr neft məhsullarında pis həll olduğuna görə mikrobioloji tədqiqatlar üçün onların neft naften turşusu ilə duzları alınmışdır. M-8 sürtkü yağında aparılan sınaqların nəticələri göstərir ki, sintez edilmiş birləşmələrin naften duzları biosid aktivlik nümayiş etdirir və onların fuhgisid xassələri bakterisid xassələrdən yüksəkdir. M-8 sürtkü yağının tərkibində təmiz halda olan neft naften turşusu biostabil olmadığına görə sınaq zamanı mikroorqanizmlərin təsirinə məruz qalır. Yağlayıcı-soyuducu mayenin tərkibində N-piperidinometilbenzoksazolin-2-tionun biosid olduğu sübut edildi və onun bakterisid xassəsi fungisid xassəsindən daha yüksəkdir. Mikrobioloji tədqiqatların nəticələrinə əsasən, demək olar ki, bu birləşmənin sulu məhlulunun kif göbələklərinə qarşı effektivliyi olmasa da, o, yüksək bakterisid xassə göstərir.
Açar sözlər: *2-merkaptobenzoksazol, N-əvəz edilmiş benzoksazolin-2-tionlar, aminometilləşmə, metilen-bis-aminlər, biosid aktivlik, antimikrob aşqarlar, neft məhsulları, yağlayıcı-soyuducu maye, neft naften turşusu.*

БИОЦИДНАЯ АКТИВНОСТЬ АМИНОМЕТИЛЬНЫХ ПРОИЗВОДНЫХ 2-МЕРКАПТОБЕНЗОКСАЗОЛА

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Аннотация: Исследована биоцидная активность синтезированных нами аминометильных производных 2-меркаптобензоксазола, представляющих интерес в качестве потенциальных антимикробных присадок к нефтепродуктам и смазочно-охлаждающим жидкостям. Реакцию

аминометилирования проводили с участием метилен-бис-аминов. В результате получены соответствующие N-замещенные бензоксазолин-2-тионы, структура которых доказана методами ЯМР ^{1}H спектроскопии. Ввиду плохой растворимости синтезированных соединений в нефтепродуктах для микробиологических испытаний получены их соли с нефтяной нафтеновой кислотой. Результаты испытаний в смазочном масле М-8 показывают, что нафтеновые соли синтезированных соединений проявляют биоцидную активность, причем их фунгицидные свойства выше бактерицидных. Использованная в испытании нефтяная нафтеновая кислота в чистом виде в составе смазочного масла М-8 не биостойка и поражается микроорганизмами. N-пиперидинометилбензоксазолин-2-тион в составе смазочно-охлаждающей жидкости проявил себя как биоцид, и его бактерицидные свойства выше фунгицидных. Результаты микробиологического исследования водного раствора этого соединения показали высокие бактерицидные свойства при отсутствии эффективности против плесневых грибов.

***Ключевые слова**: 2-меркаптобензоксазол, N-замещенные бензоксазолин-2-тионы, аминометилирование, метилен-бис-амины, биоцидная активность, антимикробные присадки, нефтепродукты, смазочно-охлаждающая жидкость, нефтяная нафтеновая кислота.*