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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF PRODUCTS OBTAINED VIA ANIONARYLATION OF ACRYLIC AND METAHCRYLIC ACIDS AMIDES AND NITRILES BY 5-CARBOXYPHENYLENE-1,3-BISDIAZONIUM SALTS

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Abstract. 3-[3-amino(cyano)-2-chloro(bromo, thiocyanato)-(2-methyl)-3-oxopropyl]-5-chloro(bromo, thiocyanato)benzoic acids have been obtained *via* copper catalytic anionarylation of acrylic and methacrylic acids amides and nitriles. Their antimicrobial activity has been examined.

Keywords: anionarylation, amides and nitriles of unsaturated acids, 5-carboxyphenylene-1,3-bisdiazonium tetrafluoroborate, antimicrobial activity.

1. Introduction

Aromatic bisdiazonium salts are suitable arylation agents in the Meerwein and anionarylation reactions [1, 2]. The presence of two diazo groups in their structures allows to carry out the regioselective synthesis of unsaturated compounds arylalkyl derivatives which are hard-to-reach and have effective antimicrobial properties [3-5].

Thus, we obtained the products of halogen- and thiocyanatoarylation of acrylic and methacrylic acids amides *via* interaction between aryldiazonium tetrafluoroborates based on benzidine, diaminodiphenylmethane and diaminodiphenylsulfone with the participation of both diazogroups [6]. *m*-Phenylenebisdiazonium chlorides form monochlorarylation adducts in the presence of acrylonitrile, acrylates and methacrylates due to electronic and steric effects [7]. The same regularities are observed when unsaturated amides are thiocyanatoarylated by *p*- and *m*-phenylenebisdiazonium tetrafluoroborates [8].

Previously synthesized products [9, 10] have sufficiently effective antibacterial and antimycotic activity [11, 12]. 2-Thiocyanato-(2-methyl)-3-arylpropanamides are characterized by pronounced anticandidiasis activity [12].

It is well-known that 5-thiocyanatobenzoic acid derivatives are biologically active compounds capable to destruct protein molecules. 2-Nitro-5-thiocyanatobenzoic acid (NTCB) is used for the break of polypeptide bonds formed by cysteine residues [13]. NTCB cyanates the cysteine thiol group and transforms it into *b*-thiocyanatoaniline residue which is cyclized into 2-iminothiazolidine carbonic acid with the break of peptide bond under soft conditions [14, 15].

Therefore, we investigated the interaction between 5-carboxyphenylene-1,3-bisdiazonium salts and unsaturated carbonic acids derivatives under the conditions of chloro-, bromo- and thiocyanatoarylation in order to synthesize new biologically active compounds.

2. Experimental

2.1. Analysis

IR-spectra of the compounds **I–V** were recorded in vaseline oil using Specord M80 within the range of 4000–400 cm^{-1}. 1H NMR spectra were obtained in DMSO-*d*6 and $CDCl_3$ using Varian Mercury device (400 MHz) and Bruker Avance DRX-500 (500 MHz). Tetramethylsilane was used as an internal standard. The elemental analysis was carried out according to the standard methods. The elemental analysis data correspond to the gross-formulas. The individuality of the synthesized compounds was established by TLC on Silufol UV-254 plates eluting with benzene : methanol (4:1) and methanol benzene : acetone (1:3:1) mixtures.

2.2. Synthesis

3-(3-Amino-2-chloro-3-oxopropyl)-5-chlorobenzoic acid (Ia).

7.6 g (0.022 mol) of 5-carboxyphenylene-1,3-bisdiazonium tetrafluoroborate are added to 1.7 g (0.024 mol) of acrylamide, 0.8 g (0.0023 mol) of copper(II) tetrafluoroborate hexahydrate and 2.8 g (0.048 mol) of sodium chloride in 150 ml of water-acetone (1:2.5) mixture during 30 min. The nitrogen release was observed at 278–283 K for 1 h. Then 30 ml of water is added 50 ml of diethyl ether is extracted. The extracts are washed by water and dried by anhydrous calcium chloride. After ether evaporation the residue is sustained at 253 K for 24 h of crystallization. The obtained solid phase is recrystallized from methanol and 2.5 g (40 %) of the compound **Ia** is obtained as light-yellow crystals with the melting point of 438 K. Found, %: N 5.17, Cl 27.21. $C_{10}H_9Cl_2NO_3$. Calc., %: N 5.34, Cl 27.06.

The compounds **Ib** and **IVa, b** are obtained according to the similar procedure.

3-(3-Amino-2-bromo-3-oxopropyl)-5-bromobenzoic acid (IIa).

7.6 g (0.022 mol) of 5-carboxyphenylene-1,3-bisdiazonium tetrafluoroborate are added to 1.9 g (0.026 mol) of acrylamide, 0.9 g (0.0025 mol) of copper(II) tetrafluoroborate hexahydrate and 6.3 g (0.053 mol) ofpotassium bromide in 150 ml of water-acetone (1:2.5) mixture during 1 h. The nitrogen release was observed at 258–263 K for 1 h. The target product is extracted similar to the compound **Ia.** 4.9 g (58 %) of **IIa** is obtained as light-brown crystals with the melting point of 447 K. Found, %: N 4.07, Br 45.42. $C_{10}H_9Br_2NO_3$. Calc., %: N 3.99, Br 45.53.

The compounds **IIb** and **Va, b** are obtained according to the similar procedure.

3-(3-Amino-3-oxo-2-thiocyanatopropyl)-5-thiocyanatobenzoic acid (IIIa).

6.4 g (0.018 mol) of 5-carboxyphenylene-1,3-bisdiazonium tetrafluoroborate are added to 1.4 g (0.02 mol) of acrylamide, 0.7 g (0.002 mol) of copper(II) tetrafluoroborate hexahydrate and 3.9 g (0.04 mol) of potassium thiocyanate in 120 ml of water-acetone (1:2.5) mixture during 1 h. The nitrogen release was observed at 248–253 K for 1.5 h. The target product is extracted similar to the compound **Ia.** 3.5 g (63 %) of **IIIa** is obtained as light-brown crystals with the melting point of 470 K. Found, %: N 13.81, S 20.79. $C_{12}H_9N_3O_3S_2$. Calc., %: N 13.67, S 20.87.

The compound **IIIb** is obtained in the similar way.

The yields, melting points and ^{1}H NMR spectra of 3-[3-amino(cyano)-2-chloro(bromo, thiocyanato)-(2-methyl)-3-oxopropyl]-5-chloro(bromo, thiocyanato)benzoic acids **I–V** are represented in Table 1.

Table 1

Yields, melting points and ^{1}H NMR spectra of 3-[3-amino(cyano)-2-chloro(bromo, thiocyanato)-(2-methyl)-3-oxopropyl]-5-chloro(bromo, thiocyanato)benzoic acids I–V

Compound	An	X	R	Yield, %	mp, K*	^{1}H NMR spectrum (*d*), ppm
Ia	Cl	$C(O)NH_2$	H	40	438	13.27 br.s (1H, HOOC), 7.72 s, 7.51 s (2H, NH_2), 7.83 s, 7.80 s, 7.62 s (3H, C_6H_3), 4.57 t (1H, CH(Cl)), 3.52 d.d, 3.33 d.d (2H, –CH_2–C_6H_3)
Ib	Cl	$C(O)NH_2$	CH_3	45	412	13.19 br.s (1H, HOOC), 7.70 s, 7.52 s (2H, NH_2), 7.91 s, 7.89 s, 7.70 s (3H, C_6H_3), 3.55 d, 3.49 d (2H, –CH_2–C_6H_3), 1.83 s (3H, CH_3)
IIa	Br	$C(O)NH_2$	H	58	447	13.41 br.s (1H, HOOC), 7.66 s, 7.59 s (2H, NH_2), 7.89 s, 7.82 s, 7.69 s (3H, C_6H_3), 4.49 t (1H, CH(Br)), 3.46 d.d, 3.27 d.d (2H, –CH_2–C_6H_3)
IIb	Br	$C(O)NH_2$	CH_3	51	435	13.34 br.s (1H, HOOC), 7.63 s, 7.55 s (2H, NH_2), 7.93 s, 7.88 s, 7.73 s (3H, C_6H_3), 3.52 д, 3.45 д (2H, –CH_2–C_6H_3), 1.76 s (3H, CH_3)
IIIa	SCN	$C(O)NH_2$	H	63	470	13.45 br.s (1H, HOOC), 7.77 s, 7.52 s (2H, NH_2), 8.04 s, 7.96 s, 7.80 s (3H, C_6H_3), 4.30 t (1H, CH(SCN)), 3.42 d.d, 3.21 d.d (2H, –CH_2–C_6H_3).
IIIb	SCN	$C(O)NH_2$	CH_3	71	464	13.27 br.s (1H, HOOC), 8.19 s, 8.00 s (2H, NH_2), 8.07 s, 7.92 s, 7.70 s (3H, C_6H_3), 3.55 d, 3.23 d (2H, –CH_2–C_6H_3), 1.87 s (3H, CH_3)
IVa	Cl	CN	H	33	394	13.30 br.s (1H, HOOC), 8.03 s, 7.99 s, 7.74 s (3H, C_6H_3), 5.52 t (1H, CH(Cl)), 3.50 d.d, 3.41 d.d (2H, –CH_2–C_6H_3)
IVb	Cl	CN	CH_3	38	421	13.18 br.s (1H, HOOC), 7.99 s, 7.96 s, 7.72 s (3H, C_6H_3), 3.60 d, 3.47 d (2H, –CH_2–C_6H_3), 1.97 s (3H, CH_3)
Va	Br	CN	H	50	422	13.42 br.s (1H, HOOC), 7.98 s, 7.96 s, 7.87 s (3H, C_6H_3), 5.43 t (1H, CH(Br)), 3.56 d.d, 3.47 d.d (2H, –CH_2–C_6H_3)
Vb	Br	CN	CH_3	53	428	13.38 br.s (1H, HOOC), 8.02 s, 7.99 s, 7.91 s (3H, C_6H_3), 3.53 d, 3.44 d (2H, –CH_2–C_6H_3), 1.82 s (3H, CH_3)

* The compounds are recrystallized from methanol

2.3. Microbiological Investigations

The antimicrobial activity of the synthesized compounds was determined using the method of serial dilutions in a liquid nutrient medium – meat peptone broth (MPB). At first 1% mother waters of the compounds in dimethylformamide (DMF) were prepared. Directly before the experiments they were dissolved in MPB from 1:10 to 1:320. The investigated bacterial suspension in the amount of 0.2 ml was brought in the test-tube with microbes concentration of 10^5 in 1 ml according to McFarland. Germs were incubated at 310 K for 18–24 h and then the presence or absence of microorganisms growth was observed. The least quantity of the compounds in the presence of which the culture growth was depressed we accepted as a minimum inhibitory concentration (MIC). We put non-growing germs on meat peptone agar in Petri dishes and thus determined the minimum bactericidal concentration (MBC). For the control we used test tubes with the equivalent amount of DMF.

Each experiment was repeated ten times. The results were statistically analyzed using Microsoft Excel computer programs.

3. Results and Discussion

3.1. Synthesis of 3-[3-Amino(cyano)-2-chloro(bromo, thiocyanato)-(2-methyl)-3-oxopropyl]-5-chloro(bromo, thiocyanato)benzoic Acids

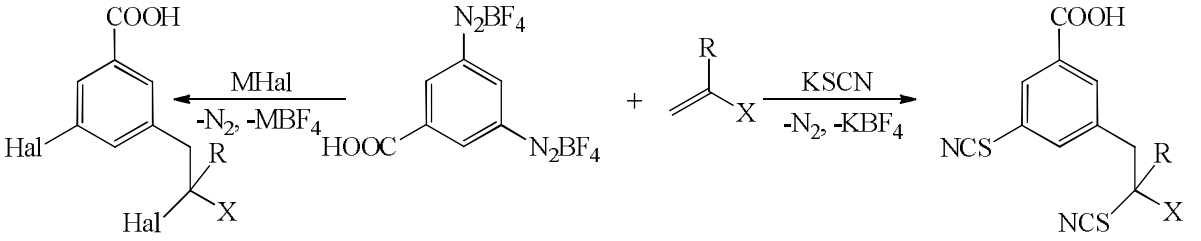
Anionarylation products are obtained *via* interaction between 5-carboxyphenylene-1,3-bisdiazonium tetrafluoroborate and acrylic and methacrylic acids amides and nitriles in the presence of chloride, bromide and rodanide anions. One group participates in the process with the parallel substitution of other diazo group for halogen atoms or thiocyanate group – 3-[3-amino(cyano)-2-chloro(bromo, thiocyanato)-(2-methyl)-3-oxopropyl]-5-chloro(bromo, thiocyanato)benzoic acids **I–V** (Scheme).

The reactions proceed in water-acetone (1:2.5) medium at 248–283 K in the presence of a catalyst – copper(II) tetrafluoroborate. The yields of halogen(thiocyanato)arylation products are 33–71 %. The reactions are accompanied by the formation of 3,5-dichloro(bromo, thiocyanato)benzoic acids in the amount of 10–30 % to calculate for diazonium salt and resinoid compounds of undetermined structure.

Our attempts to implement the reaction of thiocyanatoarylation of acrylic and methacrylic acids nitriles using 5-carboxyphenylene-1,3-bisdiazonium salts failed. Under the reaction conditions 3,5-dithiocyanatobenzoic acid is the main product. The possible reason is that usual Sandmeyer reaction proceeds at higher temperature compared with that of anionarylation but in case of sufficiently strong nucleophile, such as rodanide-anion, and active arylation reagents the temperatures of these competitive processes are almost the same.

3.2. Structure of Obtained Halogen(thiocyanato)amides(nitriles)

The structure of the anionarylation products **I–V** is in agreement with the data of IR- and ^{1}H NMR spectroscopy. In IR-spectra of the compounds **I–III** we observe the absorption bands of carbonyl and amide groups at 1660–1684 and 3386–3400 cm^{-1}, respectively. The absorption bands of the nitrile group of the compounds **IV** and **V** are observed at 2232–2244 cm^{-1}. Thiocyanatoamides **III** are additionally characterized by absorption bands of thiocyanate groups at 2152–2164 cm^{-1}.



I a, b, IIa, b, IVa, b, Va, b

IIIa, b

X = C(O)NH_2 (**I-III**), CN (**IV, V**); Hal = Cl (**I**, **IV**), Br (**II**, **V**); R = H (a), CH_3 (b); M = Na, K

Scheme

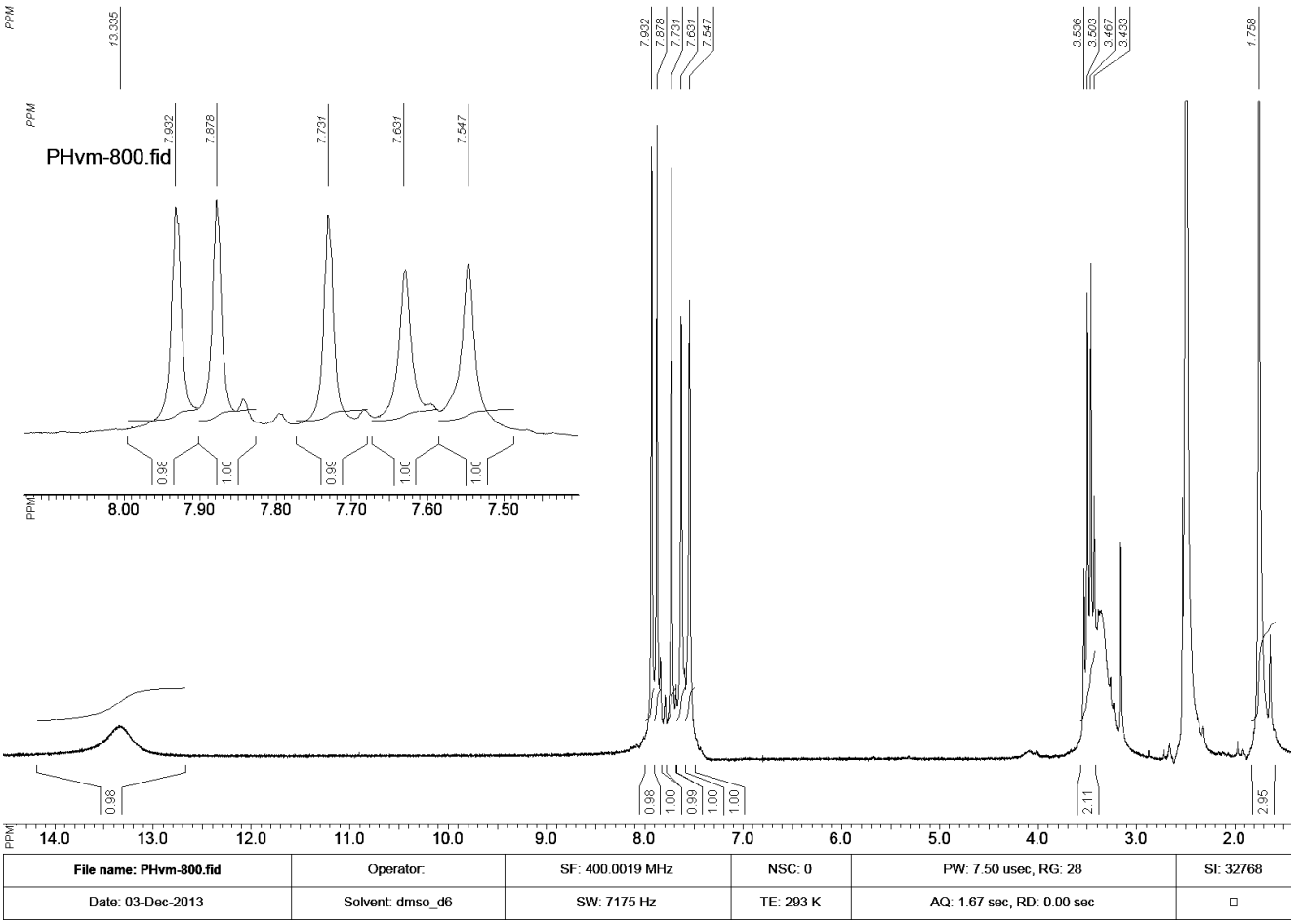


Fig. ^{1}H NMR spectrum of 3-(3-amino-2-bromo 2-methyl-3-oxopropyl]-5-bromobenzoic acid (IIb)

Table 2

Antibacterial and antimycotic activity of 3-[3-amino(cyano)-2-chloro(bromo, thiocyanato)-(2-methyl)-3-oxopropyl]-5-chloro(bromo, thiocyanato)benzoic acids I–V

Compound	An	X	R	Investigated microorganisms				
				S.aureus	*E.coli*	*C.albicans*	*B.subtilis*	*P.aeruginosa*
				MIC, μg/ml				
Ia	Cl	$C(O)NH_2$	H	15.6	3.9	3.9	N/A	15.6
Ib	Cl	$C(O)NH_2$	CH_3	15.6	3.9	3.9	N/A	15.6
IIa	Br	$C(O)NH_2$	H	7.8	3.9	3.9	N/A	7.8
IIb	Br	$C(O)NH_2$	CH_3	7.8	3.9	7.8	N/A	7.8
IIIa	SCN	$C(O)NH_2$	H	3.9	1.9	1.9	125.0	3.9
IIIb	SCN	$C(O)NH_2$	CH_3	3.9	1.9	1.9	125.0	3.9
IVa	Cl	CN	H	15.6	7.8	15.6	N/A	31.2
IVb	Cl	CN	CH_3	15.6	7.8	15.6	N/A	31.2
Va	Br	CN	H	31.2	15.6	7.8	N/A	31.2
Vb	Br	CN	CH_3	31.2	15.6	7.8	N/A	31.2

^{1}H NMR-spectra of halogen(thiocyanato)amides (nitriles) **I–V** (Table 1, Fig.) contain signals of aromatic protons (three singlets in the region of 8.07–7.62 ppm) and carboxyl group protons (wide singlets in the region of low field ~13.4–13.2 ppm). Methylene group protons bounded with aromatic nuclei (compounds **Ia–Va**) form two doublets at 3.56–3.21 ppm and for the compounds **Ib–Vb** – two doublets at 3.60–3.23 ppm. Protons of amide fragment NH_2-groups (compounds **I–III**) are characterized by two singlets at 8.19–7.52 ppm, protons of methenyl group (compounds **Ia–Va**) – by triplets at 5.52–4.30 ppm, and protons of methyl groups (compounds **Ib–Vb**) – by singlets at 1.97–1.76 ppm. The ratio between integral intensities of signals indicates the presence of only one propanamide fragment in the structure of compounds **I–V**. This fact reveals the formation of anionarylation products with the participation of one diazo group.

3.3. Antimicrobial Activity of 3-[3-Amino(cyano)-2-chloro(bromo, thiocyanato)-(2-methyl)-3-oxopropyl]-5-chloro(bromo, thiocyanato)benzoic Acids

We investigated the antimicrobial properties of 3-[3-amino(cyano)-2-chloro(bromo, thiocyanato)-(2-methyl)-3-oxopropyl]-5-chloro(bromo, thiocyanato)benzoic acids **I–V** relative to bacteria *S.aureus* ATCC 6538, *B.subtilis* ATCC 6633, *E.coli* ATCC 25922, *P.aeruginosa* ATCC 9027 and fungus *C.albicans* ATCC 885-653.

The results show that the compounds **I–V** have pronounced antimicrobial activity relative to the investigated test-microorganisms (Table 2).

The most sensitive to the investigated compounds are gram-negative bacteria *E.coli*. The amides derivatives **I–III** have the bactericidal activity at minimum concentrations of 1.9–3.9 μg/ml. The culture of spore-forming gram-positive bacillus *B.subtilis* is found to be the most stable one. Only thiocyanatoamides **IIIa** and **IIIb** have a slight antimicrobial effect on it.

The culture *S.aureus* is the most sensitive toward the compounds **IIIa** and **IIIb** (MIC is 3.9 μg/ml). The similar activity of the mentioned compounds is observed relative to gram-negative bacillus *P.aeruginosa*.

All investigated compounds have an essential antimycotic activity with the concentrations of 1.9–15.6 μg/ml.

4. Conclusions

Bisdiazonium salts based on 1-carboxy-3,5-phenylenediamine are suitable and highly reactive arylation agents for the reactions of dediazonation in the presence of unsaturated compounds and nucleophiles. This fact allows to combine in one molecule anionarylation processes and nucleophilic substitution of diazo group for anion.

The analysis of antimicrobial activity of the synthesized 3-[3-amino(cyano)-2-chloro(bromo, thiocyanato)-(2-methyl)-3-oxopropyl]-5-chloro(bromo, thiocyanato) benzoic acids **I–V** shows the essential increase in their antibacterial and antomycotic activity compared with 2-halogen(thiocyanato)-(2-methyl)-3-phenylpropanamides [11, 12]. This regularity is caused most of all by the modification of aromatic nucleus *via* introducing chlorine atom, bromine atom or thiocyanate group together with carboxyl group which considerably increases the molecule hydrophilicity.

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СИНТЕЗ ТА ПРОТИМІКРОБНА АКТИВНІСТЬ ПРОДУКТІВ АНІОНАРИЛЮВАННЯ АМІДІВ ТА НІТРИЛІВ АКРИЛОВОЇ І МЕТАКРИЛОВОЇ КИСЛОТ СОЛЯМИ 5-КАРБОКСИФЕНІЛЕН-1,3-БІСДІАЗОНІЮ

Анотація. *Купрокаталітичним аніонарилюванням амідів та нітрилів акрилової і метакрилової кислот одержані 3-[3-аміно(ціано)-2-хлоро(бромо, тіоціанато)-(2-метил)-3-оксопропіл]-5-хлоро(бромо, тіоціанато)бензойні кислоти та вивчена їх протимікробна активність.*

Ключові слова: *аніонарилювання, аміди та нітрили ненасичених кислот, тетрафлуороборат 5-карбоксифенілен-1,3-бісдіазонію, протимікробна активність.*