Reactions of thioamides with the ions of copper and antimycobacterial activity of the formed complexes

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Some complexes of thioamides and their oxidation products with Cu^+ were prepared. A qualitative correlation between stability of the formed complexes and the σ^* constants of the Taft equation for substituents on the nitrogen atom of the functional group was found. The antimycobacterial activities of thioamides and the formed complexes were compared.

Получены комплексы тиоамидов и продуктов их окисления с Cu^+. Обнаружена качественная корреляция между устойчивостью образованных комплексов и константой σ^* уравнения Тафта для заместителей на атоме азота функциональной группы. Проведено сравнительное изучение антимикобактериального действия тиоамидов и образованных комплексов.

Owing to their physiological activity, thioamides are used in pharmacy [1], but no simple relation between the rate of their hydrolysis and microbial activity has been found [2]. Dithiocarbamates and their copper complexes which are chemically related to these substances also exhibit antimycobacterial activity [3]. Unfortunately, only small attention has been hitherto paid to the complexes of thioamides with metal ions from both microbial and synthetic point of view. Besides some data stated in reviews [1], the complexes of dimethylthioformamide and thiobenzamide with metal ions have been described [4, 5] and the structure of the complexes of metal ions with thioacetamide has been solved [6—9]. Because of the differences consequent upon the formulation of copper complexes [5, 9] as well as the known antimycobacterial activity of dithiocarbamates and their complexes with copper ions, it seemed useful to prepare the complexes of thioamides with copper ions and to compare their antimycobacterial activity with the known activity of ligands [2, 10].

Experimental

Chemicals

N-Phenyl-*N*-(*p*-chlorophenyl)- and *N*-benzylthiobenzamide were prepared by common method [11]. The properties, melting points, and analyses of the prepared substances were consistent with literature data [12—14]. We prepared *N*-(*m*-chlorophenyl)-*m*-bromothiobenzamide as a new substance by universal procedure [11]. Thus we obtained yellow crystals which were recrystallized from ethanol. Their melting point was 117—118 °C. For $C_{13}H_9BrClNS$ (M_r=326.63) w_i(calc.): 47.65 % C, 2.78 % H, 4.29 % N, 9.28 % S; w_i(found): 47.52 % C, 2.82 % H, 4.15 % N, 9.65 % S. This thioamide was oxidized by current method [15] to give disulfide. The saturated ethereal solution of I_2 (1.27 g; 0.005 mol) was added under cooling with ice to the saturated ethereal solution of *N*-(*m*-chlorophenyl)-*m*-bromothiobenzamide (3.2 g; 0.01 mol) containing 1 g (0.01 mol) of triethylamine. The formed bis[*N*-(*m*-chlorophenyl)-*m*-bromobenzimidoyl] disulfide was isolated in usual way [15]. Thus the yellowish crystals were obtained, m. p. = 96—98 °C. For $C_{26}H_{16}Br_2Cl_2N_2S_2$ (M_r = 651.26) w_i(calc.): 47.95 % C, 2.48 % H, 4.30 % N, 9.85 % S; w_i(found): 47.51 % C, 2.30 % H, 4.25 % N, 9.99 % S. Other thioamides were substances prepared and described in the preceding paper [2]. Thioacetamide, copper(II) chloride dihydrate, hydrochloric acid and hypophosphorous acid were commercial anal. grade chemicals (Lachema, Brno).

Three methods were used for preparing the complexes.

A. A solution of hypophosphorous acid was dropwise added into the saturated alcoholic solution of copper(II) chloride (0.01 mol) up to decolorization. Before copper(I) chloride started to separate, this solution was mixed with the concentrated alcoholic solution of thioamide (0.011 mol). After a few minutes a precipitate was formed. This precipitate was washed with alcohol, dried, and analyzed.

B. The saturated alcoholic solution of copper(II) chloride (0.01 mol) was acidified by a few drops of concentrated hydrochloric acid and the concentrated alcoholic solution of thioamide (0.011 mol) was added into this solution. The separated precipitate was sucked, washed with alcohol, and analyzed.

C. The saturated alcoholic solution of bis[*N*-(*m*-chlorophenyl)-*m*-bromobenzimidoyl] disulfide (1.63 g; 0.0025 mol) was mixed with the concentrated solution of copper(II) chloride (0.42 g; 0.0025 mol) which was reduced by hypophosphorous acid beforehand. The tenfold volume of water was added into the formed solution and the complex was thus precipitated. This complex was sucked and analyzed after drying in air.

The univalence of the copper ions in the complexes was determined by measuring diamagnetism in the prepared complexes. The measurements were carried out with an apparatus made in the development laboratories and workshops of the Palacký University using the Gouy method. The equipment was calibrated in usual way [16]. $Co[Hg(SCN)_4]$ was used as a standard. All measurements were performed at the temperature of 293 K.

Microbial tests

The Šula medium was used for microbial tests *in vitro*. The minimum inhibitory concentration was read after 14 days' incubation (for *Mycobacterium fortuitum* after 7 days) at 37 °C. The complexes were dissolved in DMSO and put into the solution of the Šula medium so that DMSO represented 1 % of the volume of mixture. The resulting concentration of complexes was altered from the maximum value of 10×10^{-4} mol dm^{-3} by regular dilution to half its value down to the lowest value of 0.15×10^{-4} mol dm^{-3}. The results of testing as well as the test of Ethionamide which was used as checking sample are given in Table 2.

Results and discussion

The results of analyses of the obtained complexes are given in Table 1. It is evident that the above procedures can give rise to two kinds of complexes the first of which is characterized by the ratio of substance amounts of copper to nitrogen $n(\mathrm{Cu}) : n(\mathrm{N}) = 1:1$ while the second one exhibits $n(\mathrm{Cu}) : n(\mathrm{N}) = 1:2$. Method *A* always affords only complexes of the first type. Their formation may be simply explained by a two-step mechanism. In the first step Cu^{2+} is reduced by hypophosphorous acid to Cu^{+} and in the second one copper(I) ions are precipitated by thioamide while one chloride ion and one molecule of thioamide comes to one Cu^{+} ion. This idea is also consistent with the results of elemental analysis as well as with univalence of copper ascertained by the measured diamagnetism.

The interpretation of the results obtained by method *B* is somewhat more complicated. It is very likely that a redox reaction takes place again in the first step and thioamide acting as reduction agent is oxidized to give rise to a disulfidic bond whereas the Cu^{2+} ions are simultaneously reduced to Cu^{+} ions according to eqn (*A*)

$$2Cu^{2+} + 2R{-}C(S)NH_2 \rightarrow 2Cu^{+} + R{-}C(=NH)S{-}S(HN=)C{-}R + 2H^{+} \qquad (A)$$

The formed disulfides were a few times described in literature [15, 17, 18]. In the subsequent step a Cu^{+} complex with that ligand which is able to form a less soluble complex comes into existence. The univalence of the copper ions was confirmed again by the measured diamagnetism while the composition of the complex was verified analytically. On the basis of this mechanism, we may explain that the reaction of thiobenzamide and some of its derivatives produced by method *B* gives rise to the complex with the ratio $n(\mathrm{Cu}) : n(\mathrm{N}) = 1:2$ while the complex with the ratio $n(\mathrm{Cu}) : n(\mathrm{N}) = 1:1$ may be obtained for these ligands only by method *A*. For thioacetamide, *N*-phenyl-, and *N*-benzylthiobenzamide only the complexes with the ratio $n(\mathrm{Cu}) : n(\mathrm{N}) = 1:1$ were prepared by both methods. The properties of the

Table 1

Analyses of the prepared complexes

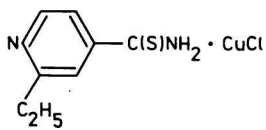
Experiment	Method	Composition of complex	M_r	w_i(calc.)/% w_i(found)/%				
				Cu	C	H	Cl	N
I	*A*	C_6H_5—C(S)NH_2 · CuCl	236.19	26.90 27.05	35.59 35.44	2.99 2.58	15.01 14.92	5.93 5.63
II	*A*		262.18	24.24 25.05	36.65 36.43	4.23 3.97	13.52 13.55	10.68 10.41
III	*A*	*p*-CH_3O—C_6H_4—C(S)NH_2 · CuCl	266.22	23.87 23.65	36.09 36.32	3.41 3.25	13.32 13.45	5.26 5.05
IV	*A*	*p*-CH_3—C_6H_4—C(S)NH_2 · CuCl	250.20	25.40 24.80	38.40 38.65	3.62 3.58	14.17 14.25	5.60 5.72
V	*A*	*m*-Cl—C_6H_4—C(S)NH_2 · CuCl	270.63	23.47 23.52	31.06 30.84	2.23 2.29	26.19 26.30	5.18 4.85
VI	*A*	C_6H_5—C(S)NH—C_6H_4—Cl-*p* · CuCl	346.73	18.79 18.63	44.76 45.35	2.89 2.85	20.33 20.12	4.02 4.05
VII	*A*	*p*-Cl—C_6H_4—C(S)N(C_6H_5)$_2$ · CuCl	422.81	15.03 15.30	53.97 53.66	3.34 2.98	16.77 16.82	3.31 3.34
VIII	*A*	*p*-Cl—C_6H_4—C(S)NH—C_6H_4—Cl-*p* · CuCl	381.17	16.66 16.95	40.96 40.81	2.38 2.21	27.90 27.52	3.68 3.41
IX	*B*	CH_3—C(S)NH_2 · CuCl	174.12	36.49 36.51	13.80 14.15	2.89 2.89	20.36 20.18	8.05 7.72

Table 1 (Continued)

Experiment	Method	Composition of complex	M_r	w_i(calc.)/% w_i(found)/%				
				Cu	C	H	Cl	N
X	*B*	C_6H_5—C(S)NH—$C_6H_5 \cdot CuCl$	312.35	20.35 20.37	50.00 49.42	3.53 3.55	11.35 11.35	4.49 4.47
XI	*B*	C_6H_5—C(S)NH—CH_2—$C_6H_5 \cdot CuCl$	326.30	19.47 19.80	51.53 52.48	4.01 4.10	10.86 10.90	4.29 3.96
XII	*B*	C_6H_5—C(=NH)S—S(NH=)C—$C_6H_5 \cdot CuCl$	371.39	17.11 17.35	45.27 45.68	3.26 3.66	9.54 9.35	7.54 7.44
XIII	*B*	*p*-CH_3—C_6H_4—C(=NH)S—S(NH=)C—C_6H_4—CH_3-*p* $\cdot$ CuCl	399.41	15.91 16.15	48.11 47.75	4.04 4.09	8.88 9.01	7.02 7.02
XIV	*B*	*p*-Cl—C_6H_4—C(=NH)S—S(NH=)C—C_6H_4—Cl-*p* $\cdot$ CuCl	440.27	14.43 14.65	38.19 37.94	2.29 2.55	24.15 23.92	6.36 6.29
XV	*B*	C_6H_5—C(=N—C_6H_4—Cl-*p*)S—S(*p*-Cl—C_6H_4— —N=)C—$C_6H_5 \cdot CuCl$	592.47	11.02 11.37	52.53 52.22	3.05 3.28	11.93 12.05	4.71 4.59
XVI	*B*	*m*-Br—C_6H_4—C(=N—C_6H_4—Cl-*m*)S— —S(*m*-Cl—C_6H_4—N=)C—C_6H_4—Br-*m* $\cdot$ CuCl	752.29	8.48 8.50	41.51 41.03	2.41 2.17	— —	3.72 3.83

complexes prepared by both methods were equal and the elemental analyses were different only in the range of observation errors. For this reason, only the results obtained by method *B* are given in Table 1.

This idea is corroborated by method *C* in which disulfide was prepared by oxidation of thioamide by iodine according to eqn (*B*)

$$I_2 + 2\,m\text{-}BrC_6H_4\text{—}C(S)\text{—}NHC_6H_4Cl\text{-}m \rightarrow 2HI +$$

$$+ m\text{-}BrC_6H_4\text{—}C(=NC_6H_4Cl\text{-}m)S\text{—}S(m\text{-}ClC_6H_4N=)C\text{—}C_6H_4Br\text{-}m \qquad (B)$$

The formed compound reacted with the Cu^+ ion to give a complex of equal properties. The results of analysis of this complex are in the range of experimental errors equal to the results obtained for substance *XVI* prepared by method *B*. Therefore the results of analysis of the complex prepared by method *C* are not quoted. The results of the mentioned experiments uphold the reduction of the copper(II) ions to the copper(I) ions as a reaction preceding the precipitation reaction and simultaneously make possible to comprehend the different composition of the complexes of thioamides with the Cu^+ ions stated in literature [5, 9].

Besides the complexes listed in Table 1, we tried to prepare the complexes containing *N*-methyl- or *N*-dimethylthioamide of *p*-chlorobenzoic acid. Method *B* gave no results and we did not succeed in preparing analytically pure substances by method *A*. The obtained complexes changed their colour as soon as in the course of drying, which might be due to oxidation, and they decomposed. A comparison of the stability of complexes thus qualitatively found with the σ^* constants of the Taft equation [19] for substituents on the nitrogen atom of the functional group showed that we succeeded in preparing the complexes with those ligands in which the substituent on the nitrogen atom had positive value of the σ^* constant (C_6H_5—, H—, C_7H_7—) while the attempts to prepare the complexes with *N*-methyl- and *N*-dimethyl derivative of *p*-chlorobenzoic acid ($\sigma^*(CH_3) = 0.00$) failed. Thus we may conclude that the stability of complex is more influenced by the polar effect of substituent than by its steric effect.

The described preparation of the complexes enabled us to investigate their antimycobacterial properties and compare them with the activity of the ligands themselves which was known from literature [2, 10]. The results of these experiments expressed in terms of the minimum inhibitory concentration of the complexes are presented in Table 2. It results from the comparison with literature data [2, 10] that the minimum inhibitory concentration (MIC) of the complexes is smaller than that of ligands and the differences in MIC of individual ligands with respect to *Mycobacterium tuberculosis* $H_{37}Rv$ and *Mycobacterium kansassii* vanish with origination of the complexes. These complexes are more efficacious for the remaining two strains than the commonly used Ethionamide. Unfortunately, this fact cannot be discussed in more detail because the set of the known experimental data is too small. For this reason, only the preparation of the complexes and

Table 2

Antimycobacterial efficacy of the complexes

No.[a]	MIC · 10^4/(mol dm^{-3}) against *Mycobacterium*			
	tuberculosis $H_{37}Rv$	*kansasii PKG 8*	*avium 80/72*	*fortuitum 1021*
I	0.6	1.25	1.25	1.25
II	0.6	1.25	1.25	1.25
III	0.3	1.25	0.6	0.6
IV	0.6	1.25	1.25	1.25
VIII	0.3	1.25	2.5	2.5
IX	0.6	2.5	2.5	1.25
XIII	0.3	1.25	1.25	5.0
XIV	0.3	1.25	1.25	5.0
XVI	0.3	1.25	2.5	2.5
Ethionamide	0.6	1.25	5.0	10.0

a) The number of complex is consistent with the number in Table 1.

determination of their antimycobacterial activity on the above level is adequate to the present state of development and knowledge of the problem.

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