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CR(VI) OXIDATION OF THIOBENZANILIDE IN ACETONE MEDIA MONITORED BY HPLC

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Abstract: *The conversion of Cr(VI) to stable Cr(III) species involves a three-electron change which has only a low probability to occur in a single step. Our results indicated that the Cr(VI) oxidation of thiobenzanilide in acetone media is not simple process and probably involves at least two side reactions. When treated with Jones' reagent, the oxidation of thiobenzanilide undergoes elimination of the sulfur moiety forming benzanilide in high yield; and at the same time thiobenzanilide with oxidative cyclization is converted to a heterocyclic compound, 2-phenylbenzothiazole. For monitoring the course of the oxidation of thiobenzanilide directly from the reaction mixture and to investigate the influence of Jones' reagent concentration on this oxidation in acetone media, a reversed-phase HPLC- DAD method for separation and simultaneous determination of thiobenzanilide and its possible oxidation products was developed.*

Keywords: *Cr(VI) oxidation, Jones' reagent, thiobenzanilide, acetone media, RP HPLC.*

INTRODUCTION

Compounds containing a thiocarbonyl group, e.g. thiobenzamides, thiobenzanilides, dithio-oxalamides and related thiosalicylanilides have been reported to show general antibacterial, antimycobacterial, antifungal, antiviral, anticancer, anti-inflammatory, photosynthesis–inhibiting, insecticidal, herbal and antialgal activity. Thiocarbonyl compounds are also particularly interesting as convenient simplified models for *in vitro* investigation of the interactions between pharmacologically active substances and living organisms. Therefore, in the past few years, the research in our laboratory has been focused on synthesis of compounds that exhibit biological activity [1-3], and investigation of their structural properties [4-7].

A report about toxicity of thioamides [8] gave us an additional reason to study the possible ways of metabolic degradation of thiobenzanilide. The majority of metabolic ways of drugs run by electron-transfer processes and therefore the attention in this work has been focused on investigation of the oxidation of thiobenzanilide as a model.

Oxidations of thiobenzamides, where *S*-oxides, sulfur free products, heterocycles, diimidoyl disulfides and sulfides are formed, have been reviewed [9-14]. Although the oxidations of thiobenzanilide derivatives with various oxidants have been known, relatively little is known concerning Cr(VI) oxidation. Chromium oxidation, well known and widely explored in organic chemistry, is the topic of our current interest in developing new techniques and procedures for oxidation of thiobenzanilide.

The present study was undertaken to obtain such base-line information that can help in better understanding of the course of the oxidation of thiobenzanilide with Cr(VI) so

called Jones' reagent. In this work, we present a relatively rapid and simple procedure for Cr(VI) oxidation of thiobenzanilide in acetone media and the course of the reaction has been monitored directly from the reaction mixture. The influence of Jones' reagent concentration on the oxidation of thiobenzanilide, has been studied by a reversed-phase HPLC method with UV-diode array detection developed by our group [15].

Our study on the Cr(VI) oxidation of thiobenzanilide, indicates that this reaction is not a simple process and probably involves two or more side reactions. When treated with so called Jones' reagent, the oxidation of thiobenzanilide undergoes elimination of the sulfur moiety forming benzanilide in high yield; and at the same time thiobenzanilide with oxidative cyclization is converted to a heterocyclic compound, 2-phenylbenzothiazole. The composition of the mixture depends on the molar fractions of the substrate-thiobenzanilide and the oxidant-Jones' reagent.

2. EXPERIMENTAL

2.1. Chemicals

Thiobenzanilide and its oxidation products were synthesized by known or modified methods.

(**1**) Thiobenzanilide was synthesized by thionation of benzanilide using Klingsberg - Papa's method [1].

(**2**) Benzanilide was prepared by addition of benzoyl chloride into aniline in 10 % aqueous NaOH [16].

(**3**) 2-phenylbenzothiazole was synthesized by oxidation of thiobenzanilide with potassium fericyanide [17].

(**4**) Thiobenzanilide-*S*-oxide was prepared by oxidation of thiobenzanilide with H_2O_2 [18].

(**5**) The synthesis of bis(*N*-phenylbenzimidoyl) disulfide by oxidation of thiobenzanilide with lead tetraacetate was modified [14,19].

(**6**) Bis(*N*-phenylbenzimidoyl) sulfide was obtained in high yield using thermal decomposition of non-polar organic solution from bis(*N*-phenylbenzimidoyl) disulfide [19].

The standard chromium trioxide - sulfuric acid solution was prepared using the Jones' method [20].

All crude products were purified with crystallization. The physical properties of these compounds (melting points, elemental microanalysis and infrared spectra) were in good agreement with the literature values.

2.2. Apparatus

Melting points were taken in a Melt-Temp apparatus and are uncorrected.

FT IR spectra were recorded with Perkin-Elmer System 2000 interferometer in the 4000-400 cm^{-1} frequency range. The spectra of all obtained crystal solids were recorded in KBr pellets.

Varian HPLC system equipped with ternary gradient pump Model 9012, a Rheodyne injector with 20 μL sample loop and an UV - Diode Array detector Model 9065, was used for the HPLC analysis. Separations were performed on an analytical column LiChrospher 60 RP-select B (LiChroCART, dimensions 250×4 mm and 5 μm particle diameter).

2.3. Procedure

The procedure involves addition of Jones' reagent to diluted acetone solution of thiobenzanilide in different proportions. The temperature of the reaction mixture was kept under 35 °C. Chromatography of the reaction mixture immediately after mixing the reagents, every 30 minutes during 3 hours and after 24 hours, was performed on a HPLC system Varian at ambient temperature. Optimal HPLC conditions [15] were: mobile phase

consisted of solvent A (water with phosphoric acid with pH = 3.0) and solvent B (acetonitrile, HPLC grade from Merck). The gradient elution program was 0-10 min: 60 % A; 15-25 min: 25 % A, the flow rate was 1.0 mL/min. The diode array detector was set to monitor the signals of the analyte at a wavelength of 254 nm. Data acquisition and management was performed using Star Workstation 4.5 software package.

3. RESULTS AND DISCUSSION

Initially the reaction of oxidation of thiobenzanilide with Cr(VI), as a model for oxidative biotransformation of drugs with thioamide substructural fragment like a pharmacophore, was investigated to identify the products formed. Cr(VI) compound was chosen as the oxidant because it is appropriate for visual monitoring of the progress of the reaction with dramatic color change from orange Cr^{6+} solutions to green Cr^{3+} salts. In addition, chromium(VI) derivatives, with respect to their high oxidation potential, behave as strong oxidizing reagents capable of oxidizing almost every oxidation eligible organic functional group [20], and give high yield of obtained products.

In this work, the chromium(VI) oxidation of thiobenzanilide was carried out under acidic conditions with standard chromium trioxide - sulfuric acid solution, in the presence of co-solvent acetone. The seeming contradiction between the non-aqueous medium of an *in vitro* experiment and the aqueous medium of living organisms is explainable by the localization of the metabolism of drugs in biological membranes created by lipid molecules and their environment is non-aqueous. One of the reasons for using non-aqueous acetone medium is a poor solubility in water of thiobenzanilide and its oxidation products. Also electrochemically determined electrode potential of chromium trioxide in acetone is higher than the electrode potential measured in water at the same conditions [20], so acetone increases the oxidizing power of Cr(VI) reagent. It is known that the alkaline CrO_4^- has a reduced oxidizing power, while strong acids enhance the oxidizing power of Cr(VI) [20], which is the main reason for involving a sulfuric acid solution.

Continuing our investigation on Jones' oxidation of thiobenzanilide, the influence of the concentration of Jones' reagent was studied. Because of the instantaneous reaction and strong acidic conditions, first the Jones' reagent was diluted.

The reaction of thiobenzanilide with excess Cr(VI) was monitored using HPLC method, previously developed for this purpose [15]. Under the described conditions above in the procedure, the reversed-phase HPLC shows excellent performance for investigation the oxidation reaction of thiobenzanilide. The elution order of the separation of thiobenzanilide and its possible oxidation products was the following: thiobenzanilide-*S*-oxide (t_r=10.363 min), benzanilide (t_r=11.133 min), thiobenzanilide (t_r=15.839 min), 2-phenylbenzothiazole (t_r=17.992 min), bis(*N*-phenylbenzimidoyl) sulfide (t_r=21.077 min) and bis(*N*-phenylbenzimidoyl) disulfide (t_r=22.868 min). The wavelength of 254 nm was selected for detection because all of the analytes exhibit significant absorption, which produces good sensitivity for all the investigated compounds, except for thiobenzanilide-*S*-oxide. In order to achieve a better resolution from benzanilide and better sensitivity for this oxidation product, a wavelength of 344 nm was used for its determination.

The linearity was calculated by linear regression in the range from 10^{-6} to 10^{-3} mol/L for all compounds. Calibration graphs were constructed by plotting the peak area as a function of the injected amount of the active ingredient. The equations for the calibration curves and correlation coefficients in the working concentration range (10^{-4} – 10^{-3} mol/L) for thiobenzanilide and its oxidation products obtained by oxidation of thiobenzanilide with Jones' reagent, benzanilide and 2-phenyl-benzothiazole, are listed in Table 1.

Tab. 1: Statistical data of calibration curves of investigated compounds

Compound	Regression equation	R^2
thiobenzanilide (**1**)	$A = 3.384 \cdot 10^9 c + 4.065 \cdot 10^4$	0.998
benzanilide (**2**)	$A = 2.754 \cdot 10^9 c - 1.129 \cdot 10^5$	0.998
2-phenylbenzothiazole (**3**)	$A = 1.201 \cdot 10^9 c - 4.260 \cdot 10^4$	0.999

A-peak area, *c*-concentration in mol/L

On examining the oxidation of thiobenzanilide with Jones reagent by means of HPLC, the conditions were changed using a several different molar excess of oxidant, such as 2:1, 4:1, 6:1 and 8:1. Results obtained from the chromatogram in Figure 1 show that the composition of the reaction mixture is markedly influenced by the molar fractions of the oxidant.

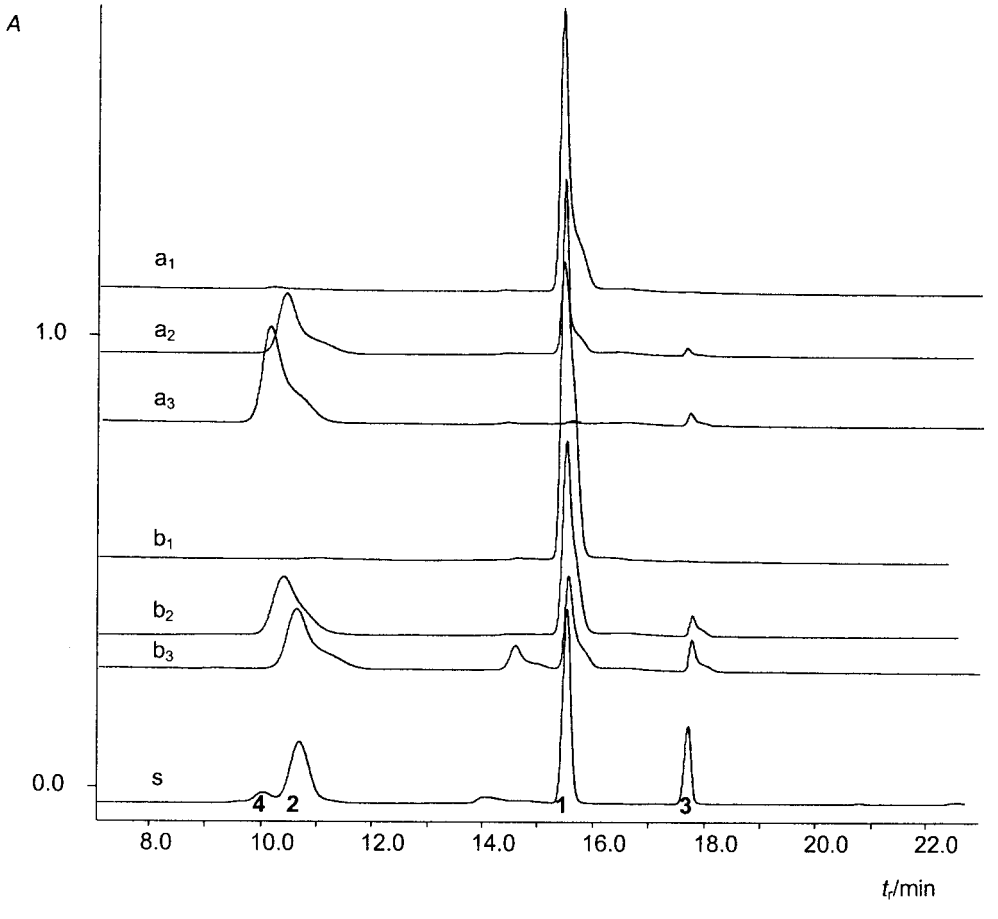

Fig. 1: Chromatograms at 254 nm of s) mixture of standards **1**) thiobenzanilide **2**) benzanilide **3**) 2-phenylbenzothiazole **4**) thiobenzanilide-*S*-oxide, and of reaction mixtures obtained for molar ratio oxidant/substrate a) 8:1 b) 4:1, the subscripts indicate: 1) injection immediately after mixing the reagents; 2) after 3 hours, and 3) after 24 hours

As can be seen from Figure 1, three peaks appear in the chromatograms of the reaction mixture. These peaks were identical by comparison of their retention times and UV-spectra with the ones obtained for pure substances. Peak with retention time t_r=15.839 min was identified as substrate-thiobenzanilide (**1**) and this peak is missing

when the substrate has been completely transformed in the high excess of Jones' reagent, after 24 hours (chromatogram a_3). Two peaks due to the presence in traces of benzanilide (**2**) and 2-phenylbenzothiazole (**3**) were identified as oxidation products at the very beginning of the reaction of oxidation of thiobenzanilide with excess of Jones reagent (chromatograms a_1 and b_1). The analyses have shown that these oxidations in dilute solutions are instantaneous and the first oxidation product thiobenzanilide-*S*-oxide (**4**) is hard to detect. It is obvious that thiobenzanilide is rapidly oxidized to *S*-oxide, which is then converted successively to benzanilide and 2-phenylbenzothiazole (chromatograms a_2 and b_2).

As can be seen from Fig.1, the concentration of the substrate-thiobenzanilide (**1**) decreased during the reaction time and this tendency is characteristic for all investigated different concentrations of Jones' reagent. The reaction mixtures stay for 24 hours and the all substrate spontaneous reverts to oxidation products only in molar excess of Jones' reagent, when the molar ratio oxidant/substrate is 6:1 and 8:1 (Fig. $1a_3$).

The concentration of benzanilide (**2**) continuously increased after mixing thiobenzanilide and Jones' reagent, on almost identical way at all different concentrations of Cr(VI). Maximum benzanilide was obtained in reaction of oxidation of thiobenzanilide in acetone media with Jones reaction, when the molar ratio between the oxidant and substrate-thiobenzanilide is 6:1 and 8:1 (Fig. $1a_3$), 24 hours after mixing the reactants.

The maximum concentration of 2-phenylbenzothiazole (**3**) was detected in reaction mixture where the molar ratio oxidant/substrate is 4:1(Fig. $1b_3$), 24 hours after their mixing.

Monitoring the course of the reaction and the composition of the reaction mixture by HPLC have shown that with increasing the concentration of Jones' reagent in the reaction of oxidation of thiobenzanilide, formation of benzanilide - oxidation product without sulfur is favoured.

The conversion of Cr(VI) to stabile Cr(III) species involves a three-electron change which has only a low probability to occur in a single step. This implies the formation of chromium in intermediate oxidation states [Cr(V) and Cr(IV)] in practically every oxidation reaction. Our results indicate that the oxidation of thiobenzanilide with Jones' reagent is not simple process and probably involves at least two side reactions. Evidence for benzanilide formation in this reaction of oxidation has been obtained, so the main reaction is desulfuration of thiobenzanilide giving amide - benzanilide (**2**) and sulfur. The proposed mechanism for oxidative sulfur elimination reaction involves formation of thiobenzanilide-*S*-oxide (**4**), by rapid S-oxidation. The absence of the peak of thiobenzanilide-*S*-oxide in chromatograms, indicates that thiobenzanilide-*S*-oxide and thiobenzanilide-*S,S*-dioxide appear as reactive intermediates with a very short life when the oxidation is performed in dilute solution.

The minor pathway is oxidative cyclization of thiobenzanilide, forming heterocyclic 2-phenylbenzothiazole (**3**). The general scheme of the reaction of Jones' oxidation of thiobenzanilide is shown in Fig.2.

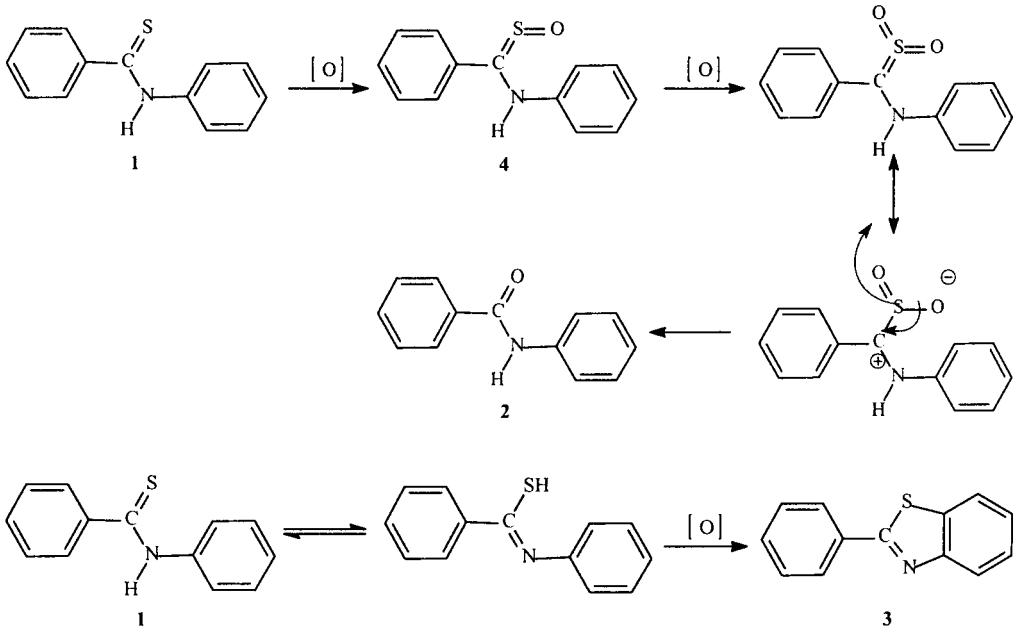

Fig. 2

4. Conclusion

Our results indicate that the Cr(VI) oxidation of thiobenzanilide in acetone media is not simple process and probably involves at least two side reactions. When treated with Jones' reagent, the oxidation of thiobenzanilide undergoes elimination of the sulfur moiety forming benzanilide in high yield; and at the same time thiobenzanilide with oxidative cyclization is converted to a heterocyclic compound, 2-phenylbenzothiazole.

All the substrate is transformed to oxidation products after 24 hours only in molar excess of Jones' reagent, when molar ratio oxidant/substrate is 6:1 and 8:1 and maximal yield of benzanilide is obtained.

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