ATLANTIS PRESS

Advances in Biological Sciences Research, volume 7

1st International Symposium Innovations in Life Sciences (ISILS 2019)

Reactions of thiourea oxides with amines, aminoacids and proteins

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***Abstract*—The publication discusses new data about chemistry and application of thiourea dioxide and its sulfur-containing relatives.**

Keywords—thiourea dioxide, guanidine-based pharmaceuticals

I. INTRODUCTION

Thiourea dioxide $(NH_2)_2CSO_2$ (TDO, aminoiminomethanesulfinic acid) is widely used in the different fields of chemistry and chemical technology. In the recent years a few reviews on the chemistry and application of TDO and its sulfur-containing relatives – sodium dithionite and sodium hydroxymethanesulfinate have been published [1-4]. In alkaline solutions, TDO manifests strong reductive properties due to the formation of sulfoxylate ion SO_2H^- (SO_2^{2-}). An important aspect in using thiourea dioxide and the product of its oxidation - thiourea trioxide $(NH_2)_2CSO_3$ (TTO, aminoiminomethanesulfonic acid) is the synthesis of guanidines and guanidine acids [1, 2, 5-8]. Here we discuss new data appeared after publication of mentioned reviews and compare them with earlier results.

Guanidines are considered as fundamental entities in medicinal chemistry [9]. The guanidine moiety is an essential substructure in many molecules of biological importance, such as arginine and creatine phosphates [9]. Many natural guanidines have been isolated and tested for biological activity. The isolation, structural identification and synthesis of naturally occurring guanidines have been reviewed by Berlinck and coworkers [10, 11].

Guanidine organocatalysts have emerged as a powerful group of organic Brønsted bases over the last years [12].

For the synthesis of guanidines, the reaction of thiourea oxides with amines or aminoacids in alkaline solutions is performed. The mechanism of reaction between thiourea oxides (or thioureas plus peroxides) and amines and aminoacids has been considered in many papers [6-8, 13]. Two likely intermediates of this reaction are a carbodiimide (resulting from elimination of the oxidized sulfur) which can undergo addition of amine or izomerize to a cyanamide or a tetracoordinate adduct which can undergo elimination of the oxidized sulfur function. Maryanoff and coworkers [7] have proved that the favorable mechanism includes the addition/elimination steps Fig. 1.

Recently a mechanistic study on tert-butyl hydroperoxide-promoted guanidinylation of amines with benzoylthioureas by monitoring short lifetime intermediates using electrospray ionization/time-of-flight high resolution mass spectrometry (ESI-Q-TOF HRMS) as well as ^{1}H NMR has been performed [13]. The results suggested an addition/elimination mechanism involving the benzoylthiourea dioxide and the nucleophilic amine as the main pathway to yield the guanidine [13].

Guanidine derivatives with antimicrobial and antifungal activity have been investigated as medical and crop protection agents and antiseptics for industry products, food and daily necessities [14-17]. Guanidinylation of chitosan can not only enhance the water solubility of the chitosan, but also improve the stability of low molecular weight guanidine salt; guanidinylated chitosan (GCS) treated wool fabrics show significantly improved antimicrobial characteristics.

In view of the analogous transmembrane function to cell penetrating peptides, guanidine group was incorporated into chitosan by chemical modification to enhance the transfection performance of chitosan vectors. The results of flow cytometry indicated that guanidinylation promoted an eightfold increase in the cell uptake. The study revealed that guanidinylated chitosan is a promising candidate as an effective nonviral vector for in vivo gene delivery [18]. It was confirmed later that guanidinylation of chitosan could

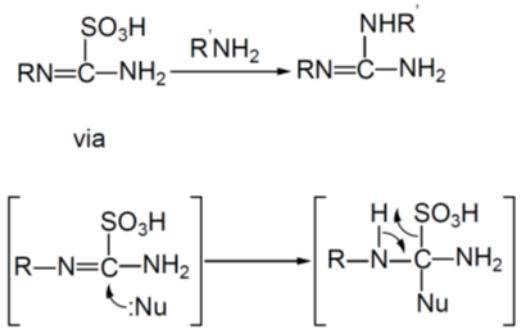

Fig.1. Schematic illustration of the addition/elimination steps of reaction between thiourea oxides (or thioureas plus peroxides) and amines and aminoacids proved by Maryanoff and coworkers [7].

provide novel additives for protein/peptide delivery systems [19].

Guanidinylated chitosan derivatives with different molecular weights have been synthesized by the guanidinylation reaction of chitosan with TTO [16] (Fig.2).

The structure of guanidinylated chitosan was characterized by UV, FT-IR, fluorescence and 13C NMR [16]. The substitution degrees of guanidinylated chitosan were confirmed by elemental analysis. In vitro antibacterial activity of guanidinium derivatives was evaluated against Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa. Compared with chitosan, guanidinylated chitosan had much better antibacterial activity, whose minimum inhibitory concentrations in aqueous hydrochloric acid (pH 5.4) were 4 times lower than those of chitosan. Interestingly, guanidinylated chitosan inhibited the growth of S. aureus and B. subtilis at pH 6.6. The antibacterial activity of guanidinylated chitosan enhanced with decreasing pH.

Guanidinylated chitosan as a heterogeneous alkaline catalyst for the transesterification of soybean oil with methanol, was synthesized by grafting TTO onto chitosan [20]. The guanidinylated chitosan catalyst exhibited very high catalytic activity and stability. Thus, the soybean oil conversion reached 98.8% at the first run and 96.6% at the fifth run. Results show that the transesterification mainly took place on the surface of the catalytic membrane. The kinetics study of the transesterification was undertaken in terms of pseudo-homogeneous model. A kinetic model of the transesterification by the catalyst was established.

In acidic media, the reaction between thiourea oxides and amino acids, amines, and other amino-containing compounds proceeds in another manner: the sulfur-containing moiety is not eliminated (thiourea oxides are much more stable in acidic than alkaline solutions), and the substitution proceeds at one of NH_2 groups. The reaction of thiourea dioxide with amines and aminoacids in the presence of sodium acetate was shown to give the products of general formula $NH_2NHRCSO_2$ (R being aliphatic or aromatic radical) and $O_2SCNH_2NH(CH_2)_nCOONa$ [21] (in the case of glycine, $O_2SCNH_2NHCH_2COONa$).

In acidic media, the reaction with aminoacids gives rise to the formation of products possessing much higher solubility than that of thiourea dioxide (poor solubility in water, ~2.5 g/100 g water at room temperature, is a strong drawback limiting its application [22]; besides, the presence

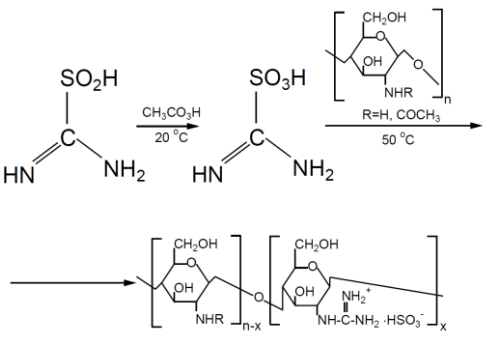

Fig.2 The guanidinylation reaction of chitosan with TTO [16].

of network of hydrogen bonds in solid thiourea dioxide leads to the fact that it is not only poorly soluble in water but its dissolution is a slow process). The results of the work [21] allow making also the following important conclusion. Earlier, it was found that thiourea dioxide is capable to deactivate glutamine synthetase [23]. Inactivation also occurred in the absence of oxygen and in the presence of catalase, thereby ruling out mixed-function oxidation-reduction as the mode of amino acid modification. Fluorescence and circular dichroism measurements indicated that modification by thiourea dioxide causes detectable changes in the structure of the protein [24]. The effect was assumed to be caused by the reaction of thiourea dioxide with lysine and histidine resulting in the formation of guanidine derivatives of aminoacids (thiourea trioxide reacts with the same lysine residues of glutamine synthetase as thiourea dioxide [25]). However, data mentioned above suggest that in neutral and weakly acidic media, close to physiological conditions, more probable explanation of inactivating effect of thiourea dioxide or thiourea trioxide is the formation of derivatives of aminoacids containing SO_2 or SO_3 fragment in the molecule. Their formation due to activation of the amino group upon oxidation of thiourea can be a reason of toxicity of many thioureas (the toxicity of thioureas is known to be related with the processes of their oxidation in the organism [6]). It should be noted also that reactions of thiourea oxides with aminoacids, peptides and proteins are accompanied by formation of harmful compound – ammonia [21].

II. CONCLUSION

It should be noted in conclusion, that chemists continue to use thiourea oxides in the synthesis of guanidine-based pharmaceuticals. Thus, in 2019 Cho and coworkers have received guanidine-based compound which suppresses angiogenesis in human umbilical vein ebdothelial cells [26]. In the intermediate steps of the synthesis of this compound they used thiourea trioxide.

ACKNOWLEDGMENT

This work was supported by Russian Foundation for Basic Research (project 19-33-90019).

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