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Mechanism of 4-methyl-1,2,4-triazol-3-thione reaction with formaldehyde

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We have recently described theoretically the mechanism of the reaction between 4-methyl-1,2,4-triazol-3-thiol and formaldehyde that leads to the *N*-substituted product, *N*^{1}-hydroxymethyl-4-methyl-1,2,4-triazol-3-thione. New experimental findings indicate that the thione tautomeric form in reaction with formaldehyde also yields this product. This observation could not be explained on the basis of previous calculations, which predicted that the thione tautomer undergoes nucleophilic substitution at the sulfur atom, leading to the *S*-substituted product. We present theoretical explanation of the observed reactivity. We show that under experimental conditions this reaction proceeds with the intervention of the anionic form of the triazole with the Gibbs free energy of activation of only 1.8 kcal/mol. Copyright © 2008 John Wiley & Sons, Ltd.

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INTRODUCTION

Mechanisms of reactions of 1,2,4-triazole-3-thio-derivatives remain unclear in spite of the importance of this class of organic compounds that exhibit diverse biological activities. Apart from the documented anticonvulsant, antidepressant, anti-inflammatory, antitumor, analgesic, antiviral, and antibacterial activities[1–7] that led to approved drugs, such as alprazolam,[8] etizolam,[9] or vibunazole,[10] 1,2,4-triazoles are also potent inhibitors of enzymes such as monoamine oxidase,[11] methionine aminopeptidase-2[12] and farnesyltransferase.[13] They are also used as intermediates in the synthesis of antifungal agents such as fluconazole, voriconazole, and itraconazole.[14,15] *N*^{1}-hydroxymethyl derivatives that are formed in reactions of 1,2,4-triazole-3-thio-derivatives with formaldehyde show significant antibacterial and antifungal activity.[16] In an attempt to shed some light on the mechanisms of these reactions we have shown[17] that 4-methyl-1,2,4-triazol-3-thiol (**S$_{SH}$** in Scheme 1) in the reaction with formalin yields *N*^{1}-hydroxymethyl-4-methyl-1,2,4-triazol-3-thione (**P$_{NC}$**) rather than the expected 3-hydroxymethylsulfanyl-4-methyl-1,2,4-triazole (**P$_{SC}$**). However, recently we have observed that the thiono tautomer, **S$_{NH}$**, also leads to the same product. This observation cannot be explained on the basis of the previously studied potential energy surface (PES).

Herein we present results that explain the newly found reactivity of 4-methyl-1,2,4-triazol-5-thione and provide details of the mechanism that leads to the *N*-substituted derivative. It is found that this reaction occurs via the 4-methyl-1,2,4-triazol-3-thion anion that reacts to form **P$_{NC}$** with the Gibbs free energy barrier of only 1.8 kcal/mol.

RESULTS AND DISCUSSION

All calculations have been carried out at the DFT level using B3LYP functional[18–20] with aqueous solution approximated by the PCM implicit solvent model.[21] It has been shown that the dielectric properties of the formalin solution are close to that of pure water.[22] Experimentally, the reaction was initiated by mixing aqueous solution of the triazol **S$_{NH}$** with formalin at 25°C. The **S$_{NH}$** substrate was initially crystallized and its tautomeric form was confirmed by the X-ray measurements. Formalin is an aqueous solution in which the predominant form is monohydrate, $CH_2(OH)_2$[23] that is, however, unreactive and it is free formaldehyde, present in smaller quantity, that is the reactive species. Thus we consider that the reaction under consideration involves formaldehyde molecules, the abundance of which is maintained in solution via rapid equilibrium with formol. We have determined experimentally the pK_a of the triazole substrate; it is equal to 7.79 at 25°C in water.

From the PES reported previously[17] the reaction of **S$_{NH}$** with formaldehyde should yield the product containing the sulfur–carbon bond (**P$_{SC}$**) since the Gibbs free energy of activation of this reaction is equal to 10.3 kcal/mol, while the corresponding value for the S$_N$2 reaction leading to the observed product (**P$_{NC}$**) is equal to 25.0 kcal/mol. Similarly, the alternative route involving tautomerization to the **S$_{SH}$** form, followed by the conversion to **P$_{SC}$**, also exhibits Gibbs free energy of activation of nearly 25 kcal/mol. It is thus evident that another mechanism, not yet explored theoretically, is responsible for the experimentally observed reactivity.

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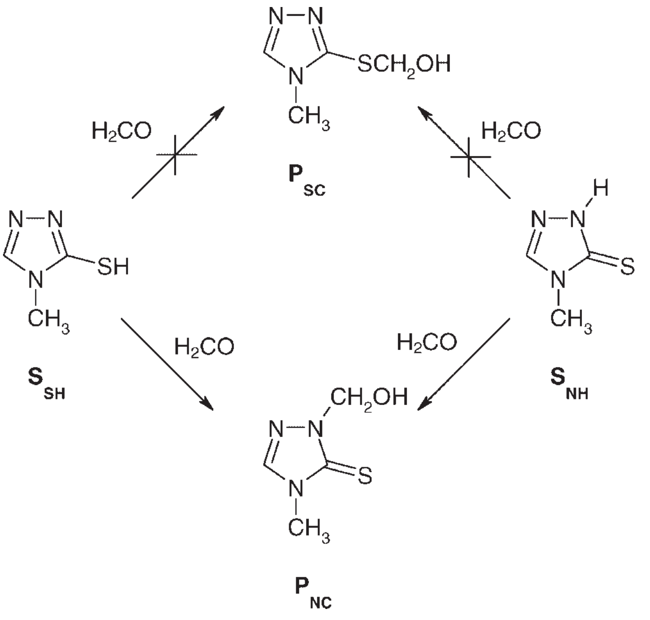


Scheme 1.

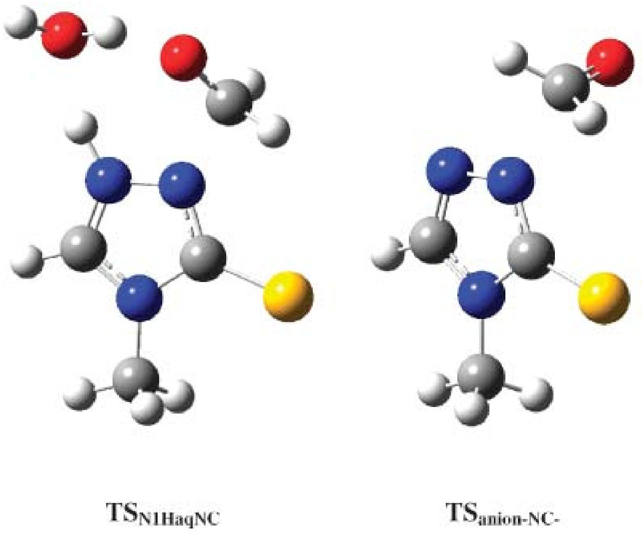


Figure 1. Transition state structures for the reaction of $\mathbf{S_{N1H}}$ ($\mathbf{TS_{N1HaqNC}}$) and the anion $\mathbf{S_{NS\text{-}}}$ ($\mathbf{TS_{anion\text{-}NC-}}$).

As the first option we have considered a mechanism that proceeds via a tautomer not considered thus far, which, although less stable, may react much faster in the experimentally observed direction. Tautomeric forms of **S**, illustrated in Scheme 2, leave two candidates, labeled $\mathbf{S_{N1H}}$ and $\mathbf{S_{N4H}}$. (For the sake of compatibility with the symbols used previously[17] we label the N^2 atom as N, while N^1 and N^4 atoms are identified by N1 and N4, respectively.) The relative stability of the latter is lower than that of $\mathbf{S_{SH}}$ (by over 27 kcal/mol) and therefore only 4-methyl-1,2,4-triazol-1-ium-3-thiolate ($\mathbf{S_{N1H}}$) has been considered. This tautomer is less stable than $\mathbf{S_{NH}}$ by 6.6 kcal/mol, and thus with the activation barrier lower than 3.7 kcal/mol it could play the role of an intermediate on the pathway from $\mathbf{S_{NH}}$ to $\mathbf{P_{NC}}$.

We have found a transition-state structure (given in the Supporting Information) for the reaction of this tautomer with formaldehyde using the model that comprises both substrates and one explicit water molecule ($\mathbf{TS_{N1HaqNC}}$). This is illustrated in Fig. 1 while the major geometric data are collected in the second column of Table 1. This transition state is characterized by one imaginary frequency of 215.0i cm^{-1} that corresponds predominantly to the motion of the triazole N^2 atom and formaldehyde carbon atom that leads to formation of the new bond. The other main contributions to this vibration come from the transfers of two protons: between oxygen atoms of formaldehyde and water molecule, and between trialoze N^1 nitrogen and water oxygen atoms. The Gibbs free energy of activation of this reaction (from complex of reactants to the transition state, both with one explicit water molecule) is 8.1 kcal/mol. When the Gibbs free energy difference between $\mathbf{S_{NH}}$ and $\mathbf{S_{N1H}}$ is accounted for, the overall barrier of the conversion of $\mathbf{S_{NH}}$ to $\mathbf{P_{NC}}$ by this route (14.7 kcal/mol) is higher than the barrier corresponding to the S_N2 reaction leading to $\mathbf{P_{SC}}$ (10.3 kcal/mol). It is thus evident that another pathway is responsible for the observed reaction direction.

Since neither of the mechanistic scenarios that involve different tautomeric forms of **S** seem to be able to describe correctly the observed experimentally reactivity of $\mathbf{S_{NH}}$ we have considered a possibility that the reactive species is either dimeric or deprotonated form of the triazole. Calculations of all possible dimeric forms (structures of dimers are given in the Supporting Information) did not provide any reasonable reactive

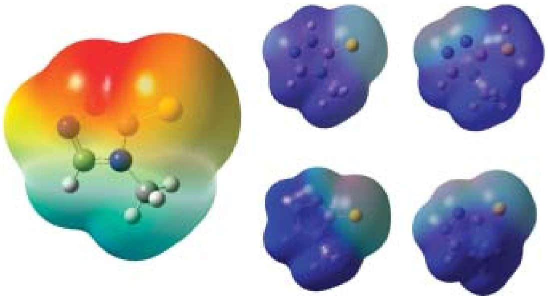
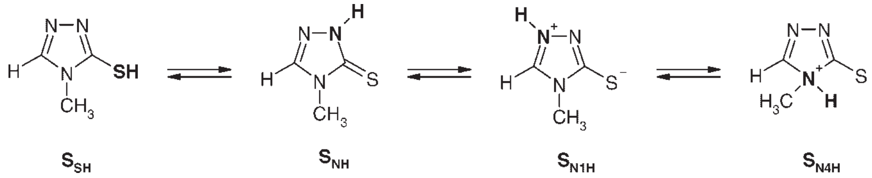


Figure 2. Electrostatic potential mapped on the electron density of the triazole anion (left). Electrostatics around different tautomeric forms (right), from top left clockwise: $\mathbf{S_{NH}}$, $\mathbf{S_{SH}}$, $\mathbf{S_{N4H}}$, and $\mathbf{S_{N1H}}$ in the same scale −0.25 (red) to 0 (blue) e.u.



Scheme 2.

Table 1. Bond distances (in Å), valence and dihedral angles (in degree) of transition state structures

Coordinate/property				$\mathbf{TS}_{\mathbf{N1HaqNC}}$	$\mathbf{TS}_{\mathbf{anion\text{-}NC\text{-}}}$
S	C	—	—	—	1.726
O^{a}	H	—	—	1.014	—
N	C	—	—	1.760	1.962
O	C	N	—	110.7	111.5
N	H	O	—	162.1	—
O	H	O	—	160.1	—
N	N	C	O	36.2	76.4
N	C	O	H	−57.7	—
C	O	H	O	41.9	—
H	O	H	N	−11.4	—
O	H	N	N	2.8	—
S	C	N	N	179.5	179.9

^{a} Proton being transferred between water and formaldehyde molecules.

complex, which could lead in experimentally observed direction with energetics competitive to the barrier of the reaction leading to $\mathbf{P}_{\mathbf{SC}}$. The anion of the triazole, on the other hand, was found to react with formaline to yield $\mathbf{P}_{\mathbf{NC}}$ with the Gibbs free activation barrier of only 1.8 kcal/mol. The corresponding transition state structure $\mathbf{TS}_{\mathbf{NS\text{-}NC\text{-}}}$ is illustrated in Fig. 1 and major geometric parameters are given in the third column of Table 1. This transition state is characterized by one imaginary frequency of $191.7i\,\mathrm{cm}^{-1}$ that corresponds exclusively to the motion of the triazole N^2 atom and formaldehyde carbon atom that leads to formation of the new N—C bond. This N—C distance in the transition state indicates that it is a very early transition state in good agreement with the low barrier. The possibility of the reaction of the anion with formaldehyde in the direction leading to $\mathbf{P}_{\mathbf{SC\text{-}}}$ has been explored by the PES scan along the S—C bond. It has been established that the energy increases monotonically indicating that the anion of 3-hydroxymethylsulfanyl-4-methyl-1,2,4-triazole ($\mathbf{P}_{\mathbf{SC\text{-}}}$) is not a competitive product compared to $\mathbf{P}_{\mathbf{NC\text{-}}}$.

The preference of the nucleophilic attack by the nitrogen center rather than the sulfur center is not surprising when the charge distribution in the anion is considered. Figure 2 illustrates charge distribution obtained from the Merz–Kollman fitting procedure mapped on the electron density surface. It can be clearly seen that the highest density is located around the N^2 atom. For comparison, the same electrostatic properties of **S** tautomers are also illustrated in Fig. 2.

In order to establish if the reaction of the anion with formaldehyde leading to $\mathbf{P}_{\mathbf{NC\text{-}}}$ is kinetically competitive we have compared reaction rates:

$$v_{\mathrm{NC-}} = \frac{\mathrm{d}[\mathbf{P}_{\mathbf{NC-}}]}{\mathrm{d}t} = k_{\mathrm{anion-NC}}[\mathrm{S}_{\mathrm{anion}}][\mathrm{H_2CO}] \tag{1}$$

$$v_{\mathrm{SC}} = \frac{\mathrm{d}[\mathbf{P}_{\mathbf{SC}}]}{\mathrm{d}t} = k_{\mathrm{NHaqSC}}[\mathbf{S}_{\mathbf{NH}}][\mathrm{H_2CO}] \tag{2}$$

where ν are reaction rates, k are rate constants, brackets denote concentrations, and subscripts refer to different reactants as defined earlier. Division of Eqn (1) by (2) and inclusion of the equilibrium constant for the deprotonation of the neutral triazole, K_a, leads to the expression for the ratio of competitive products:

$$\frac{[\mathbf{P}_{\mathbf{NC-}}]}{[\mathbf{P}_{\mathbf{SC}}]} = \frac{k_{\mathrm{anion-NC}}}{k_{\mathrm{NHaqSC}}}\cdot\frac{[\mathrm{S}_{\mathrm{anion}}]}{[\mathbf{S}_{\mathbf{NH}}]} = \frac{k_{\mathrm{anion-NC}}}{k_{\mathrm{NHaqSC}}}\cdot\frac{K_a}{[\mathrm{H}^+]} \tag{3}$$

By taking logarithm of both sides of Eqn (3), introducing experimentally determined value of pK_a, and taking into account that rate constants are proportional to exponents of the corresponding activation Gibbs free energies we obtain Eqn (4) relating relative yield of products as a function of the pH of the reacting mixture:

$$\log\left(\frac{[\mathbf{P}_{\mathbf{NC-}}]}{[\mathbf{P}_{\mathbf{SC}}]}\right) = (\Delta\Delta G^{\neq})/2.303\mathrm{RT} - \mathrm{p}K_a + \mathrm{pH} = \mathrm{pH} - 1.6 \tag{4}$$

where $\Delta\Delta G^{\neq}$ is the difference in Gibbs free activation energies of the competitive processes with the transition states $\mathbf{TS}_{\mathbf{NHaqSC}}$[17] and $\mathbf{TS}_{\mathbf{anion\text{-}NC\text{-}}}$, respectively.

As expected the relative fluxes of the formation of the competitive products ($\mathbf{P}_{\mathbf{NC\text{-}}}$ and $\mathbf{P}_{\mathbf{SC}}$) depend on the relative concentrations (difference between pH and pK_a) and relative activation barriers ($\Delta\Delta G^{\neq}$). We have found experimentally the pK_a of the triazole to be equal to 7.79_2. Calculated difference in the Gibbs free activation barriers is equal to 8.5 kcal/mol. Introduction of these numeric values to the lefthand-side part of Eqn (4) yields the expression given on its right side. As can be seen, the relative concentrations are dependent on the pH of the reacting solution. According to these calculations, under experimental conditions reported here (pH = 3.2), nearly 98% of the products should be a result of the reaction occurring through the anion form, which agrees very well with the experimental observation.

CONCLUSIONS

In summary, we have examined computationally the reaction between 4-methyl-1,2,4-triazol-3-thione and formaldehyde leading to N^1-hydroxymethyl-4-methyl-1,2,4-triazol-5-thione. It is found that the reaction proceeds with the intervention of the anionic form of the triazole with the Gibbs free energy of activation of only 1.8 kcal/mol rather than the unionized form of the substrate.

EXPERIMENTAL METHODS

Materials

4-methyl-1,2,4-triazol-3-thione was obtained by the crystallization of 4-methyl-1,2,4-triazol-3-thiol from water with traces of acetic acid. The tautomeric form was confirmed by the X-ray spectroscopy. Both 4-methyl-1,2,4-triazol-3-thiol and formaldehyde (37%) were obtained from commercial suppliers. Previously reported analytical procedures were used.[17] The value of pK_a was determined by fitting results of pH-metric titration to the Henderson equation.

Reaction conditions

4-methyl-1,2,4-triazol-3-thione (0.01 mol) and 0.01 mol of formaldehyde solution (37%) were mixed (final pH 3.21) and left at room temperature (25°C) for ½ h. The obtained product was filtered, dried, and crystallized from ethanol. Yield 86%. M.p.

125–126°C. $C_4H_7N_3OS = 145.184$. ^{1}H NMR (DMSO) δ 3.47 (s, 3H, CH_3); 5.36 (d, 2H, $J = 7.5$ Hz, CH_2); 6.79 (t, 1H, $J = 7.5$ Hz, OH); 8.48 (s, 1H, CH). ^{13}C NMR (DMSO) δ 32.1 (CH_3), 70.5 (CH_2), 141.5 (CH), 166.2 (C). ^{15}N NMR (DMSO) δ 169.9 (d, $J = 8.1$ Hz, *N*-CH3), 206.3 (d, J = 5.6 Hz, N=C—SH), 279.5 (d, J = 13.1Hz, N=CH).

Computational methods

Density functional theory (DFT) calculations were performed. Geometry optimization of reactants, transition states, and products was carried out using the B3LYP functional[18,19] and the standard 6-31+G(d,p) basis set[24,25] as implemented in the Gaussian03 package[26] with PCM implicit solvent model.[27] Standard parameters for aqueous solution were used. One-dimensional PES scans along forming bonds (N—C in the case of $\mathbf{P_{NC\text{-}}}$ and S—C in the case of $\mathbf{P_{SC\text{-}}}$) have been performed with the appropriate distance changing (from that for the complex of reactants to the bond length in the corresponding product) in ten equal steps. The energy increased monotonically with the decrease of distance in the case of S—C bond. The point of largest energy on the N—C PES scan was reoptimized to the transition state. Once the transition state structures were optimized the IRC protocol[28] was used to identify reactants and products of the corresponding reaction. Structures obtained from these calculations were subsequently optimized to the nearest stationary points. For all stationary points energies were then calculated at the B3LYP/6-311++G(d,p) level.[29] All calculations were carried out using default convergence criteria. Vibrational analysis was performed for the optimized structures to confirm that they represent stationary points on the PESs (3n-6 real normal modes of vibration for the reactant and one imaginary frequency for the transition state). Gibbs free energy values correspond to temperature of 298 K.

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