UV-Induced Generation of Rare Tautomers of 2-Thiouracils: A Matrix Isolation Study

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Unimolecular photoisomerization reactions were studied for 2-thiouracil, 6-aza-2-thiothymine, 1-methyl-2-thiouracil, and 3-methyl-2-thiouracil isolated in low-temperature Ar matrixes. The IR spectra have revealed that before UV irradiation all the matrix-isolated compounds adopted exclusively the oxo−thione tautomeric form. Upon UV (λ > 320 nm) irradiation of the matrixes, two oxo−thiol photoproducts were generated for monomeric 2-thiouracil as well as for monomeric 6-aza-2-thiothymine. Generation of these products corresponds to transfer of a proton from either the N(1)−H or N(3)−H group to the sulfur atom of the C(2)═S thiocarbonyl moiety. The first of the above reactions was photoreversible. As a consequence, after prolonged UV irradiation most of the material was transformed into the oxo−thiol−N(1)H form. The hydroxy−thiol tautomers of 2-thiouracil and 6-aza-2-thiothymine were also photogenerated as minor products. For 1-methyl-2-thiouracil and 3-methyl-2-thiouracil, thione → thiol phototautomeric reactions yielded the oxo−thiol isomers of the compounds. Since these reactions were photoreversible, the final stages of the photoinduced processes corresponded, for both methylated 2-thiouracils, to photostationary states. All the products of the investigated photoreactions were identified by comparison of their IR spectra with the spectra calculated at the DFT-(B3LYP)/6-311++G(2d,p) level.

Introduction

2-Thiouracil is a modified base, closely related to uracil, a canonical nucleic acid base. Nucleobase derivatives substituted with sulfur atom(s) in the positions of the exocyclic oxygen atom(s) have been extensively studied.[1] The thiated nucleobases appear naturally in various biological materials and are important agents for numerous metabolic processes.[2−4] 2-Thiouracil has been identified as a minor component of natural tRNA's.[5−8]

2-Thiouracil and its derivatives, such as 6-*n*-propyl-2-thiouracil and fluorinated 2-thiouracils, were recognized as potent antithyroid and antitumor drugs.[9−12] The chemotherapeutic activity of these compounds is due to their ready incorporation into biological cells, direct interaction with proteins, and partial replacement of uracil in mRNA.[13−17] Recently, 2-thiouracil derivatives have been used as constituents of pseudocomplementary peptide nucleic acids, a prospective biomolecular tool for highly selective manipulation with nucleic acids.[18] Thanks to easy introduction into biological tissues and long-wavelength absorption of 2-thiouracil, the compound serves as an efficient sensitizer that enhances DNA damage caused by UVA[19−22] and could be used as a therapeutic seeker of melanomas, nonresponding to conventional treatment.[23−26]

Moreover, 2-thiouracil and its derivatives form complexes with various metal ions.[27−29] 6-Aza-2-thiothymine has recently been used as a matrix system in matrix assisted laser desorption/ionization (MALDI) mass spectrometry. The MALDI technique allowed effective studies of DNA complexes, glycoconjugates and oligosaccharides, noncovalent protein complexes, and peptide nucleic acids (PNAs).[30,31] Despite these applications, investigations of 6-aza-2-thiouracils (including 6-aza-2-thiothymine) themselves have received only very little interest hitherto.[32]

An essential prerequisite for understanding interactions of 2-thiouracil in various chemical and biological environments is the knowledge of its physicochemical properties. The most basic information concerns the molecular structure of the compound. In principle, the molecule of 2-thiouracil may adopt 13 different isomeric forms: one oxo−thione tautomeric form, four rotameric forms of the oxo−thiol tautomers, four rotameric forms of the hydroxy−thione tautomers, and four rotamers of the hydroxy−thiol tautomer. Recently, relative energies of these tautomers were theoretically predicted (at the MP2 level) by four research groups.[33−36] According to all the calculations, the oxo−thione tautomer should be the most stable form of the compound. The hydroxy−thiol, oxo−thiol, and hydroxy−thione tautomers were predicted (at the MP2/6-31+G(d,p) level[36]) to be significantly higher in energy: by 21, 30, and 49 kJ mol^{-1}, respectively. The calculations with other basis sets resulted with similar values of relative energies of 2-thiouracil tautomers.[33−35] Given such big energy gap between the oxo−thione form and the second most stable, hydroxy−thiol tautomer, only the oxo−thione isomer should be populated in the gas phase and, as a consequence, trapped in low-temperature matrixes. And indeed, in the gas phase and in low-temperature Ar and N_2 matrixes, molecules of 2-thiouracil were found only in the oxo−thione tautomeric form.[37−41] As anticipated, also for 2-thiouracil methylated at N(1) or at N(3) nitrogen atoms, no traces of forms other than the oxo−thione tautomer were observed either in vapor phase or in inert environment of a low-temperature matrix.[38,40,42]

In the present work unimolecular photoprocesses induced by UV excitation of monomeric 2-thiouracils were investigated. Photoisomerization occurrences were studied for 2-thiouracil, 6-aza-2-thiothymine, 1-methyl-2-thiouracil, and 3-methyl-2-thiouracil (Chart 1) isolated in low-temperature argon matrixes.

Experimental Section

2-Thiouracil and 6-aza-2-thiothymine used in the present study were commercial products supplied by Aldrich. Samples of 1-methyl-2-thiouracil and 3-methyl-2-thiouracil were kindly

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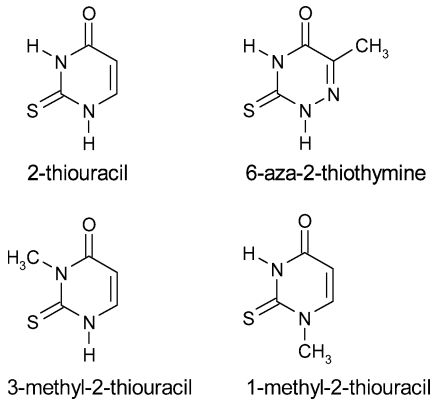
CHART 1: Canonical Oxo−Thione Tautomers of 2-Thiouracils



made available by Professor D. Shugar (Warsaw). To prepare a low-temperature matrix, the solid sample of a studied compound was electrically heated in a glass microoven placed in the vacuum chamber of a continuous-flow helium cryostat. The vapors of the compound were deposited, together with a large excess of inert gas (argon), on a CsI window cooled to 10 K. The argon matrix gas of spectral purity 6.0 was supplied by Linde AG. The IR spectra were recorded with 0.5 cm^{-1} resolution using a Thermo Nicolet Nexus 670 FTIR spectrometer equipped with a KBr beam splitter and a DTGS detector. Integral intensities of the IR absorption bands were measured by numerical integration. Matrixes were irradiated with the light from a high-pressure HBO200 mercury lamp fitted with a water filter and a suitable cutoff filter (WG320 or WG295) or with a nitrogen laser source emitting pulsed light with $\lambda = 337$ nm. UV absorption spectra of 2-thiouracils were recorded for gaseous compounds at 550 K in a quartz cell (evacuated before sealing).

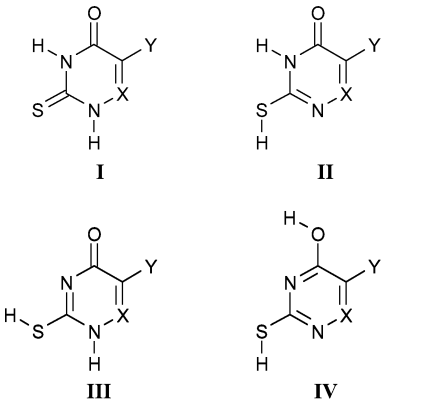
Computational Details. The geometries of isomers considered in this work were optimized using the hybrid Hartree−Fock and density functional theory method DFT(B3LYP) with the Becke's three-parameter exchange functional[43] and gradient-corrected functional of Lee, Yang, and Parr.[44] At the optimized geometries, the DFT(B3LYP) harmonic vibrational frequencies and IR intensities were calculated. All quantum-mechanical calculations were performed with the GAUSSIAN 98 program[45] using the 6-311++G(2d,p) basis set. To correct for the systematic shortcomings of the applied methodology (mainly for anharmonicity), the predicted vibrational wavenumbers were scaled down by a single factor of 0.98.

Results and Discussion

2-Thiouracil. Tautomerism and infrared spectra of matrix isolated 2-thiouracil were studied in the previous works.[38,39,41] Those studies revealed that the compound adopts exclusively the canonical oxo−thione tautomeric form **I** (Chart 2). The IR spectrum of 2-thiouracil, recorded in the current work for the compound isolated in an Ar matrix, was the same as the spectra reported earlier. This spectrum was well-reproduced by the theoretical spectra calculated, for the oxo−thione tautomeric form **I**, within and beyond[46] the harmonic approximation (Figure S1 in the Supporting Information).

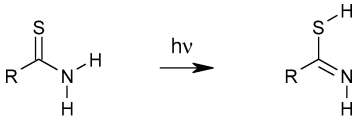
Unimolecular UV-induced processes transforming thione tautomers into the corresponding thiol forms were previously observed for simple thioamides (such as thioacetamide[47] and thiourea;[48] Scheme 1) as well as for heterocyclic thione compounds (such as 2(*1H*)-pyridinethione[49] and 4(*3H*)-pyrimidinethione;[50] Scheme 2). In all cases proton was transferred

CHART 2: Tautomeric Forms of 2-Thiouracil and 6-Aza-2-thiothymine



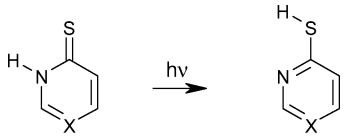
X = CH, Y = H for 2-thiouracil
X = N, Y = CH_3 for 6-aza-2-thiothymine

SCHEME 1: Unimolecular Thione → Thiol Photoreaction in Simple Thioamides



R = CH_3 (thioacetamide)
R = NH_2 (thiourea)

SCHEME 2: Unimolecular Thione → Thiol Photoreaction in Heterocyclic Thioamides



X = CH - 2(*1H*)-pyridinethione
X = N - 4(*3H*)-pyrimidinethione

from the N−H group to the sulfur atom in the α position. These reactions do not belong to any of the known types of photoprocesses. The mechanism of the thione → thiol phototautomeric reaction of the type described above remains still not elucidated, even for the simplest thioamides.

The thiocarbonyl moiety in the oxo−thione form **I** of 2-thiouracil is located between two N−H groups (Chart 2). Hence, a proton can be transferred to the sulfur atom either from the N(1)−H or from the N(3)−H group. During the experimental studies on unimolecular photochemistry of 2-thiouracil, carried out in the present work, the matrix isolated monomers were irradiated using UV light with $\lambda > 320$ nm, that is at the long-wavelength edge of the UV absorption spectrum of the compound (see Figure 1). Under such conditions the monomers of 2-thiouracil were not subjected to photodecomposition, but the energy of the absorbed photons was high enough to induce photoisomerization processes in the molecule. Irradiation of 2-thiouracil isolated in an Ar matrix with UV light of such wavelength resulted in continuous decrease of the IR spectrum of the initially observed oxo−thione tautomer **I** (e.g. bands at 3456 and 3414 cm^{-1}, presented in Figure 2). At initial stages of the phototransformation new bands, ascribable to NH stretching vibrations, appeared at 3467 and 3405 cm^{-1} (Figure 2B). Prolonged UV irradiation demonstrated that these bands belong to the spectra of two different photoproducts. After the

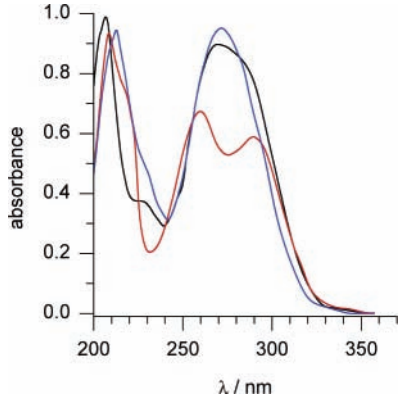


Figure 1. UV absorption spectra of gaseous 2-thiouracil (black), 1-methyl-2-thiouracil (red), and 3-methyl-2-thiouracil (blue).

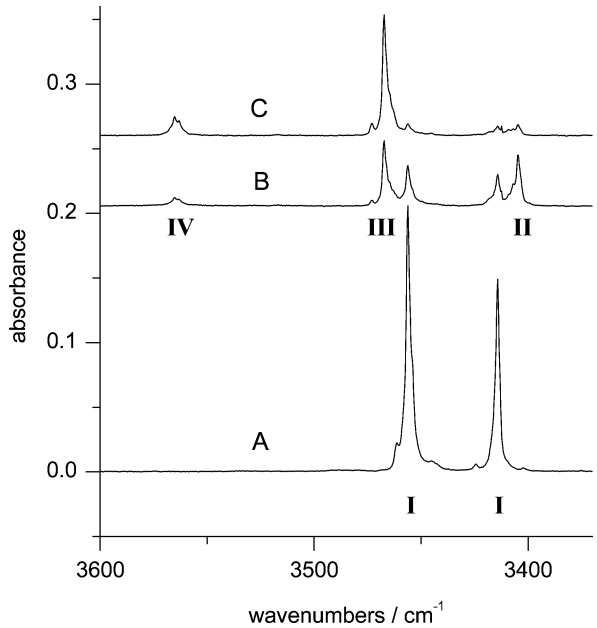


Figure 2. High-frequency region of the IR spectrum of 2-thiouracil isolated in an Ar matrix: (A) after deposition of the matrix; (B) after 2 h of irradiation with UV (λ > 320 nm) light; (C) after 8 h of irradiation with UV (λ > 320 nm) light. **I**−**IV** as in Chart 2.

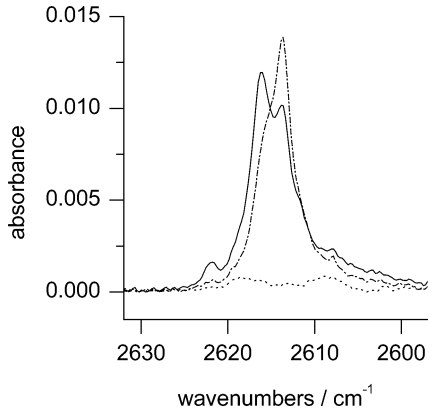


Figure 3. SH stretching frequency region (2600−2630 cm^{-1}) of the IR spectrum of 2-thiouracil isolated in an Ar matrix: (dotted line) after deposition of the matrix; (solid line) after 2 h of irradiation with UV (λ > 320 nm) light; (dashed line) after 8 h of irradiation with UV (λ > 320 nm) light.

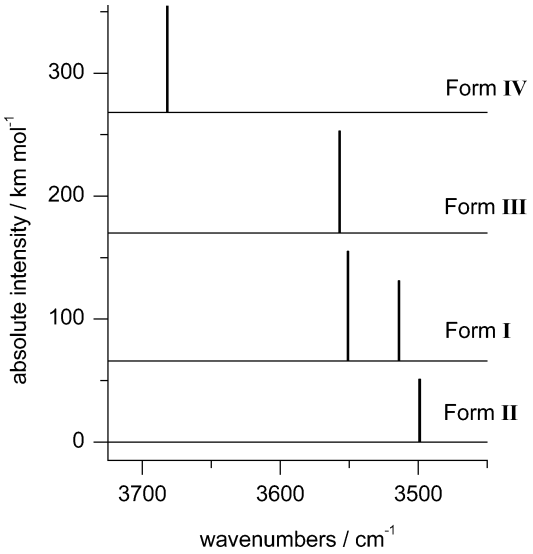


Figure 4. High-frequency region of the IR spectra of 2-thiouracil tautomers **I**−**IV** (see Chart 2) theoretically simulated at the DFT-(B3LYP)/6-311++G(2d,p) level. The calculated wavenumbers were scaled by the single factor of 0.98.

majority of the initial oxo−thione tautomer **I** had been consumed, the intensity of the band at 3405 cm^{-1} started to diminish (Figure 2, traces B and C). On the contrary, the band at 3467 cm^{-1} grew continuously, until the oxo−thione substrate was totally consumed (Figure 2, trace C). Apparently, the photoproduct represented in the IR spectrum by the band at 3405 cm^{-1} is photogenerated from the initial oxo−thione form, but there must occur also a concomitant photoreaction that consumes this species. The second photoproduct, represented by the band at 3467 cm^{-1}, can be considered as the major, ultimate product of the observed photoisomerizations (see Figure S2 in the Supporting Information).

New bands of the photoproducts appeared also in the frequency range 2620−2610 cm^{-1}, that is in the spectral region where the bands due to S−H stretching vibrations are expected (Figure 3). Two bands representing two thiol photoproducts were observed at 2616 and 2614 cm^{-1}. Although these bands partially overlap, it is clear that during the UV irradiation one of them (at 2616 cm^{-1}) first grows, but then decreases, whereas the other one (at 2614 cm^{-1}) continues to grow until the end of the photoreaction.

The observations described above strongly suggest that there are two major products of the photoreaction, each of them having one N−H and one S−H bond in a molecule. The obvious candidates for the structures of these two photoproducts are the oxo−thiol forms **II** and **III** presented in Chart 2. Form **II** can be generated by a shift of the N(1)−H proton to the sulfur atom, and in the case of product **III** the proton should be shifted from the N(3)−H group. The comparison of the high-frequency region of the experimental spectrum (Figure 2) with the corresponding fragment of the spectra of forms **I**−**III** theoretically predicted at the DFT(B3LYP)/6-311++G(2d,p) level (Figure 4) suggests the assignment of the ultimate oxo−thiol product of the photoisomerizations to form **III** and of the other oxo−thiol photoproduct to form **II**. Interestingly, the position of the ν(N(1)H) band (3467 cm^{-1} (Ar)) in the spectrum of photoproduct **III** is quite similar to the position of the ν(N(1)H) band (3456 cm^{-1} (Ar)) in the spectrum of the initial form **I**. The same is true for the bands due to the ν(N(3)H) vibrations observed in the experimental spectra of isomers **II** and **I** at 3405 and 3414 cm^{-1}, respectively (Figure 2).

The identification of the product first growing but then being consumed during the photoreaction is confirmed by a good agreement between the whole mid-infrared experimental spectrum of this photoproduct and the spectrum theoretically

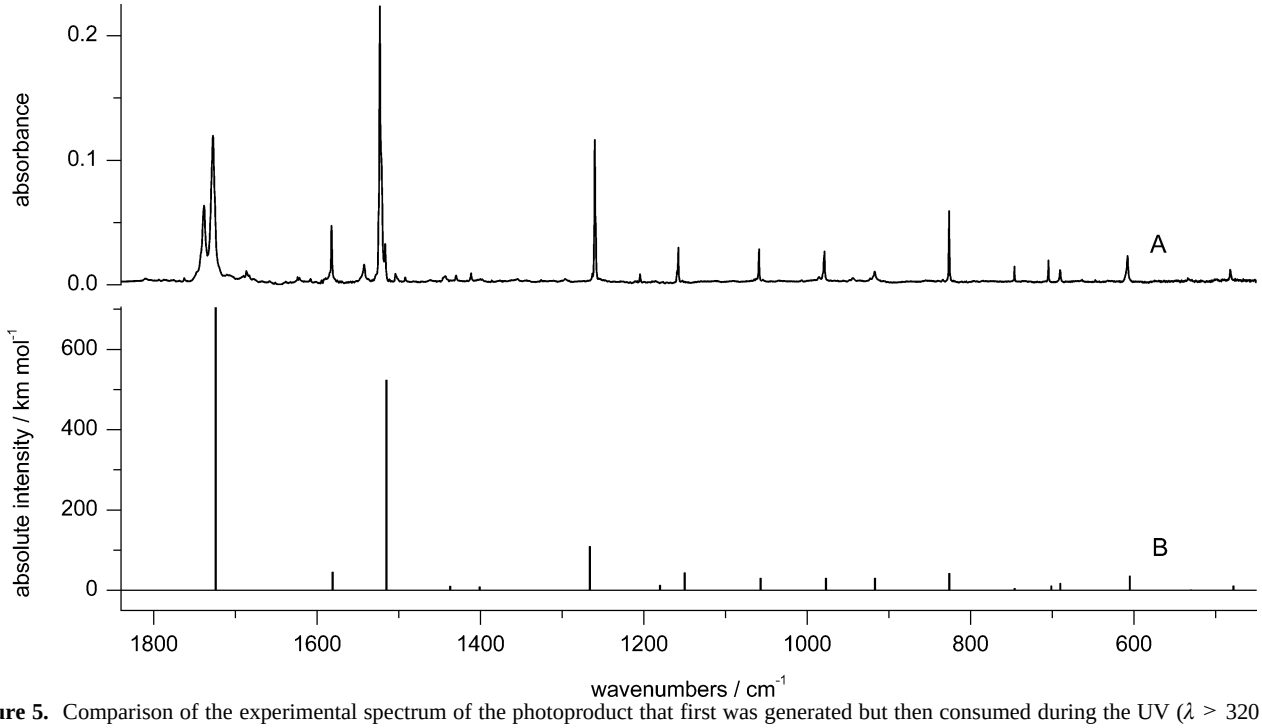


Figure 5. Comparison of the experimental spectrum of the photoproduct that first was generated but then consumed during the UV (λ > 320 nm) irradiation of 2-thiouracil isolated in an Ar matrix with the spectrum of tautomer **II** theoretically simulated at the DFT(B3LYP)/6-311++G(2d,p) level. The calculated wavenumbers were scaled by the single factor of 0.98.

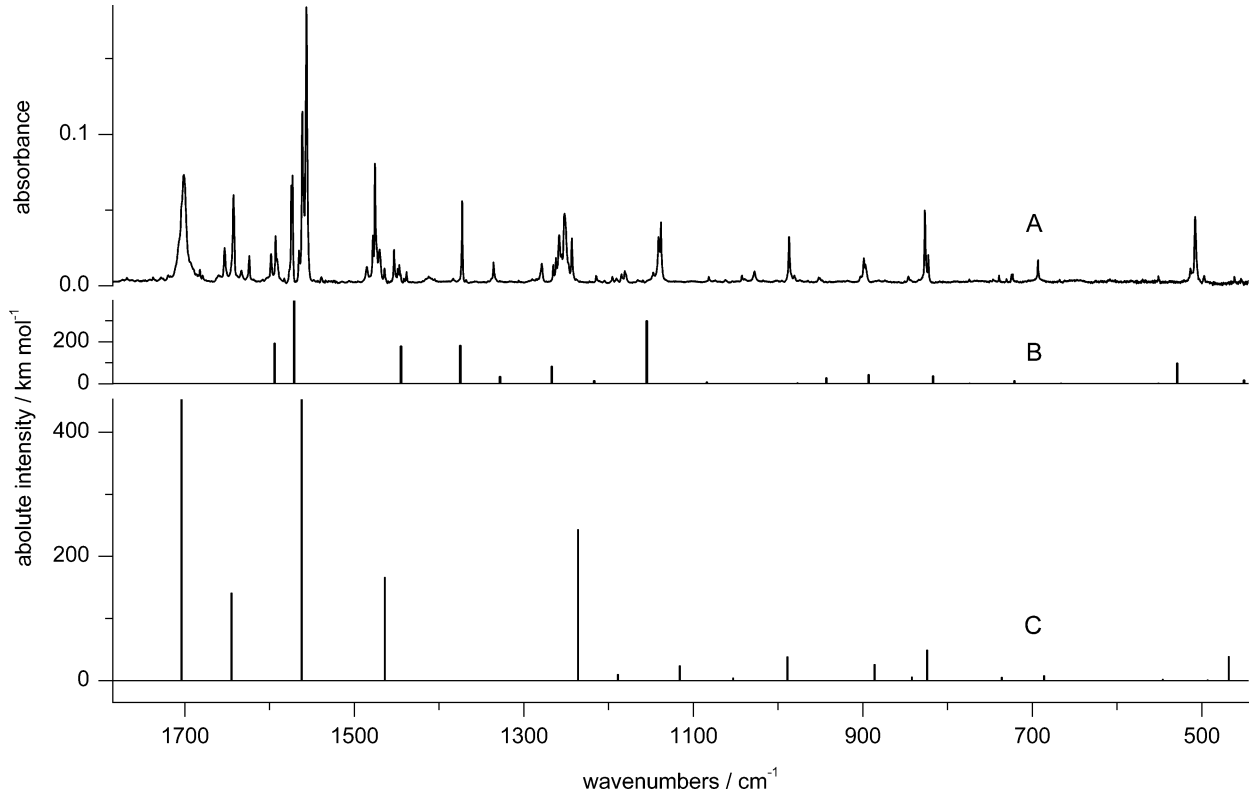
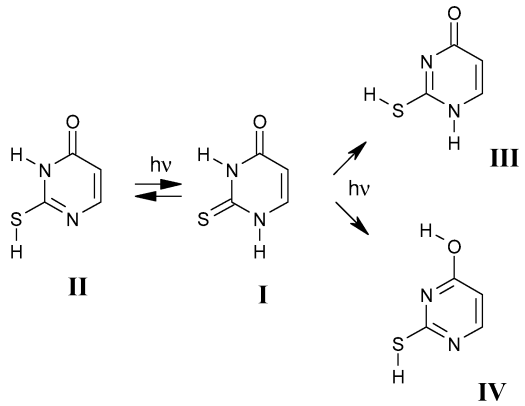


Figure 6. Comparison of the experimental spectrum of the ultimate photoproducts generated upon the UV (λ > 320 nm) irradiation of 2-thiouracil isolated an Ar matrix (A) with the spectra of tautomers **III** (C) and **IV** (B) theoretically simulated at the DFT(B3LYP)/6-311++G(2d,p) level. The calculated wavenumbers were scaled by the single factor of 0.98.

predicted for the oxo−thiol tautomer **II** (Figure 5). The experimental IR spectrum of this photoproduct consists of the bands behaving during the progress of UV irradiation in the same way as the absorptions at 3405 and 2616 cm^{-1}. This spectrum was separated from the bands due to other species present in the matrix by removing from the spectrum obtained after 2 h of irradiation the bands of the initial form **I** (by subtracting the initial spectrum) and then removing the bands of the ultimate photoproduct(s) (by subtracting the final spectrum recorded after 8 h of UV irradiation).

The spectrum of the ultimate photoproduct(s) of the photoreaction can be obtained by numerical subtraction of the last traces of the spectra of forms **I** and **II** from the spectrum collected after 8 h of UV (λ > 320 nm) irradiation. All the

SCHEME 3: Photoisomerization Reactions Observed for 2-Thiouracil[a]



[a] Analogous scheme is postulated for 6-aza-2-thiothymine.

strong IR absorptions found in this spectrum (Figure 6) can be assigned to the bands present in the theoretical spectrum of isomer **III**. But, in the spectrum shown in Figure 6A one can easily find the bands that have no counterparts in the spectrum calculated for form **III.** Among all other tautomeric forms of 2-thiouracil, only the hydroxy−thiol form **IV** (Chart 2) has a theoretical spectrum that reproduces well the pattern of the IR bands, which were not assigned to form **III** (Figure 6). The IR spectrum of the photogenerated hydroxy−thiol form **IV** should involve a high-frequency band due to O−H stretching vibration, which could be easily identified in the spectrum recorded after UV irradiation. And indeed, a low-intensity band appeared in the spectrum of photoproducts at 3565 cm^{-1} (Figure 2). This band was growing monotonically during UV irradiation in the same manner as other IR bands assigned to the form **IV** (see Figure S2 in the Supporting Information). The theoretically calculated intensity (87 km mol^{-1}) of the ν(OH) band in the spectrum of tautomer **IV** is close to the absolute intensity of the ν(NH) band predicted in the spectrum of form **III** (83 km mol^{-1}). From the ratio of intensities of the bands at 3565 and 3467 cm^{-1} in the spectrum measured after final irradiation the amount of the photogenerated hydroxy−thiol tautomer with respect to the oxo−thiol form **III** was assessed at 20%.

This was not the first observation of an UV-induced oxo → hydroxy phototautomerism. Unimolecular photoreactions converting the oxo tautomers of formamide,[51] 2(*1H*)-pyridinone,[52] and 4(*3H*)-pyrimidinone[53] into the corresponding hydroxy forms were observed in the past. However, the photochemical reaction generating the hydroxy−thiol form **IV** of 2-thiouracil is a first example of the combined oxo → hydroxy and thione → thiol phototautomerization.

The intramolecular photoisomerizations observed for matrix isolated 2-thiouracil can be explained by the photoreactions shown in Scheme 3. According to this scheme the **I** → **II** phototautomerization is photoreversible. Up to now, no example of photoreversibility of a thione → thiol phototautomerization has been reported. To prove the photoreversibility of the reaction **I** → **II**, the following experiment was performed. The matrix was first exposed to the radiation from a nitrogen laser of λ = 337 nm and then to filtered radiation from a mercury lamp (λ > 320 nm). During the first irradiation forms **II** and **III** of the compound were produced, whereas, during the second irradiation, alongside the continuous growth of the bands due to form **III**, partial recovery of the population of initial form **I** accompanied by a decrease of population of form **II** was observed (see Figure 7). The above observation is a direct proof of the occurrence of the process **II** → **I**. Indeed, if all the photoreactions observed for 2-thiouracil were irreversible, then there would be no possibility of UV-induced partial recovery of the population of **I**. Such experimental evidence of the photoreversibility of the **I** → **II** photoreaction, and the formation of tautomers **III** and **IV** as final products of irradiation, strongly support the correctness of the interpretation of the observed photoprocesses in terms of phototautomeric reactions presented in Scheme 3.

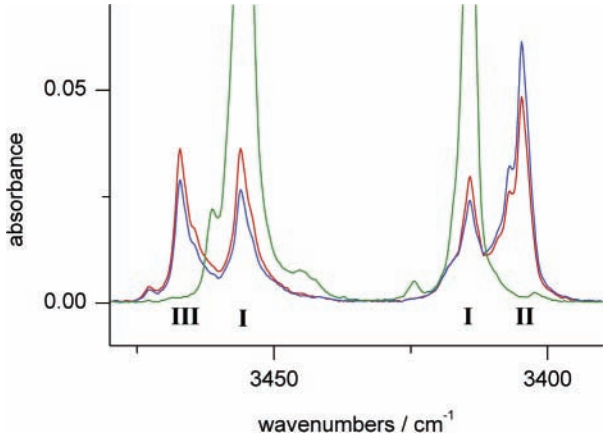


Figure 7. Portion of the IR spectrum of 2-thiouracil isolated in an Ar matrix: (green line) after deposition of the matrix; (blue line) after 5 h of UV irradiation with a nitrogen laser (λ = 337 nm); (red line) after a subsequent 30 min of irradiation with a high-pressure mercury lamp fitted with a WG 320 filter.

6-Aza-2-thiothymine. Substitution of the C(6)−H group by a nitrogen atom does not significantly change the tautomerism of a 2-thiouracil. The previous matrix isolation study on 6-aza-2-thiouracil[32] revealed that the compound adopts exclusively the oxo−thione tautomeric form. Introduction of the methyl group at the C(5) atom was not expected to change that picture. 6-Aza-2-thiothymine, studied in the present work, was found in an Ar matrix only in the oxo−thione tautomeric form **I** (Chart 2). Two bands, due to the N(1)H and N(3)H stretching modes, observed in the high-frequency region of the spectrum of 6-aza-2-thiothymine at 3454 and 3407 cm^{-1}, are placed nearly at the same positions as the corresponding bands (at 3453 and 3406 cm^{-1}) observed previously[32] in the spectrum of 6-aza-2-thiouracil isolated in an Ar matrix.

The effects of UV (λ > 320 nm) irradiation of matrix isolated 6-aza-2-thiothymine (Figure 8) demonstrate that the unimolecular photoinduced processes in this compound are very similar to the photoisomerizations observed for 2-thiouracil and can be summarized as a set of photoprocesses presented in Scheme 3. Photoproduct **II** (represented in Figure 8 by the bands at 3401 and 768 cm^{-1}) is generated at the initial stages of irradiation of the matrix, but upon prolonged irradiation its population diminishes. Most probably the photoreaction transforming form **I** into form **II** is photoreversible, in similarity to the case of 2-thiouracil. The band (at 3457 cm^{-1}) due to the stretching vibration of the N(1)−H group in photoproduct **III** partially overlaps with the ν(N(1)H) band (at 3454 cm^{-1}) from the spectrum of the initial form **I**. Nevertheless, it is clear that the band at 3457 cm^{-1} grows monotonically during the progress of UV-induced photoisomerizations (Figure 8). Such behavior was characteristic of a whole set of IR bands ascribable to form **III**, e.g. of the band at 783 cm^{-1} (Figure 8).

In comparison to the photoisomerization observed for 2-thiouracil, the photogeneration of the hydroxy−thiol isomer **IV** was less effective in the case of 6-aza-2-thiothymine. Only a very low intensity band due to the stretching vibration of the OH

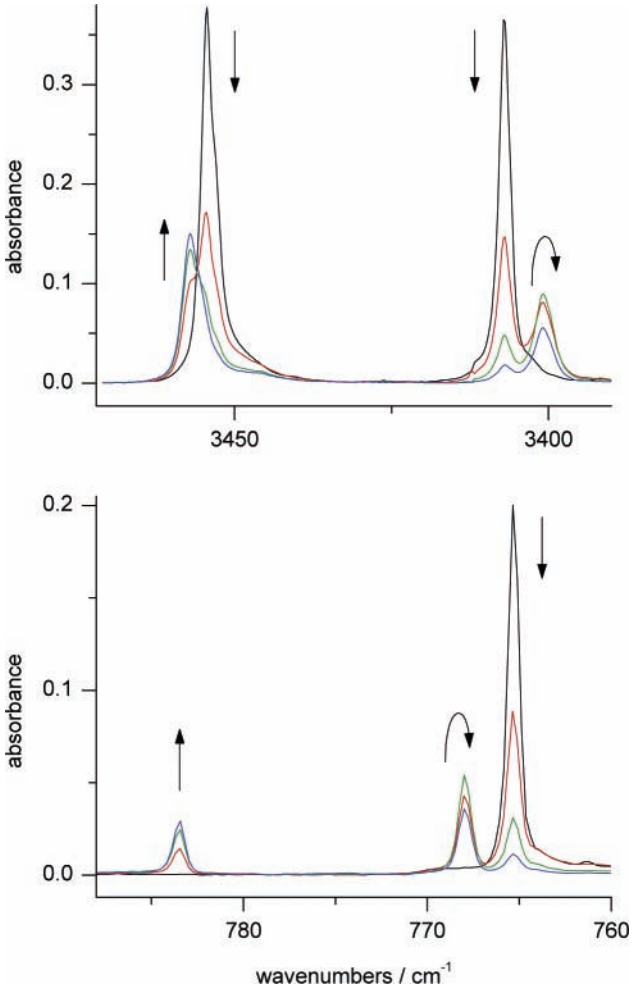


Figure 8. Fragments of the IR spectrum of 6-aza-2-thiothymine isolated in an Ar matrix: (black) after deposition of the matrix; (red) after 45 min of irradiation with UV (λ > 320 nm) light; (green) after 2 h of irradiation with UV (λ > 320 nm) light; (blue) after 7.5 h of irradiation with UV (λ > 320 nm) light. Arrows indicate the direction of changes of the band intensities with the time of irradiation.

group appeared at 3552/3550 cm^{-1} in the spectra of UV-irradiated 6-aza-2-thiothymine isolated in an Ar matrix. In a matrix irradiated for 7.5 h with UV (λ > 320 nm) light, the estimated amount of form **IV** was ca. 3%, with respect to the amount of form **III**. Hence, although the general scheme of photoreactions found for 6-aza-2-thiothymine was the same as that described above for 2-thiouracil, some differences of quantitative nature concerning relative effectiveness of photogeneration of products **II**−**IV** were observed.

1-Methyl-2-thiouracil and 3-Methyl-2-thiouracil. Methylation at N(1) or at N(3) nitrogen atom of 2-thiouracil limits the possibilities of transferring protons to the C═S group and simplifies the schemes of reactions. Therefore, the photochemical behavior was investigated for two methylated derivatives: 1-methyl-2-thiouracil and 3-methyl-2-thiouracil isolated in Ar matrixes. In low-temperature matrixes, both methylated compounds adopt only oxo−thione tautomeric forms.[38,40] Upon exposure of monomeric 3-methyl-2-thiouracil to UV (λ > 295 nm) light, transformation of the initial oxo−thione form **V** into the oxo−thiol tautomer **VI** was observed (Scheme 4). Taking into account the structure of **V**, no phototautomerization processes competing with the transfer of the N(1)−H proton to the C═S group in the α-position should be expected.

However, even upon prolonged UV irradiation the photoreaction did not result in total consumption of the substrate **V**.

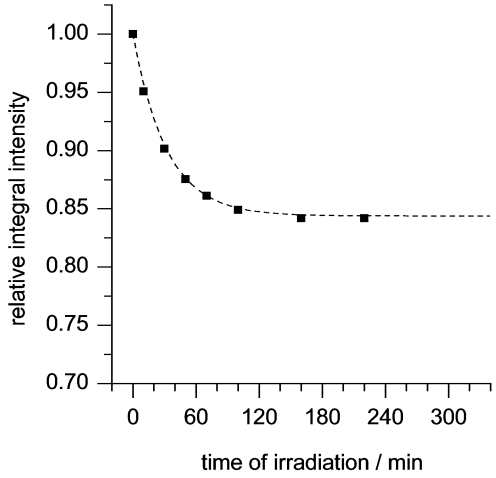
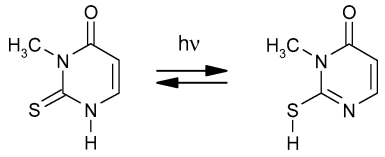


Figure 9. Progress of the phototautomeric reaction in 3-methyl-2-thiouracil isolated in an Ar matrix. The progress was monitored as a decrease of the intensity of the ν(N(1)H) band (at 3452 cm^{-1}), with respect to the intensity of this band recorded before UV irradiation. Dashed line represents the approximation of the experimental time dependency by the function given in eq 2.

SCHEME 4: Photoisomerization Reaction Observed for 3-Methyl-2-thiouracil



V **VI**

The progress of the photoreaction as a function of irradiation time is shown in Figure 9. Evidently, the reaction led to a photostationary state, which corresponded to transformation of only 16% of the initial material into the photoproduct. A photostationary state as a final stage of a photoprocess indicates that the reaction (Scheme 4) is photoreversible. For a reversible photoreaction:[54]

$$A \underset{k_1}{\overset{k_2}{\rightleftharpoons}} B \qquad (1)$$

the dependence of the substrate concentration [A] on time of irradiation, t, is governed by the following equation:

$$\frac{[A]}{[A_0]} = \left(1 - \frac{[A_{eq}]}{[A_0]}\right) \cdot e^{-(k_1+k_2)t} + \frac{[A_{eq}]}{[A_0]} \qquad (2)$$

where $[A_0]$ is the initial concentration of the substrate before irradiation and $[A_{eq}]$ is the concentration when photoequilibrium is achieved. This function fits very well the experimentally determined dependence of the amount of the oxo−thione form of matrix isolated 3-methyl-2-thiouracil on the time of UV irradiation (see Figure 9).

The photogenerated oxo−thiol form **VI** was identified by comparison of the IR spectrum emerging upon UV irradiation with the theoretical spectrum of **VI** calculated at the DFT-(B3LYP)/6-311++G(2d,p) level. The good agreement between the experimental IR spectrum of the photoproduct and the theoretical spectrum of the oxo−thiol form **VI** is illustrated by the fragment presented in Figure 10.

In similarity to the phototautomerism observed for 3-methyl-2-thiouracil, the photoreaction occurring (upon irradiation with UV (λ > 295 nm) light) for matrix isolated 1-methyl-2-thiouracil

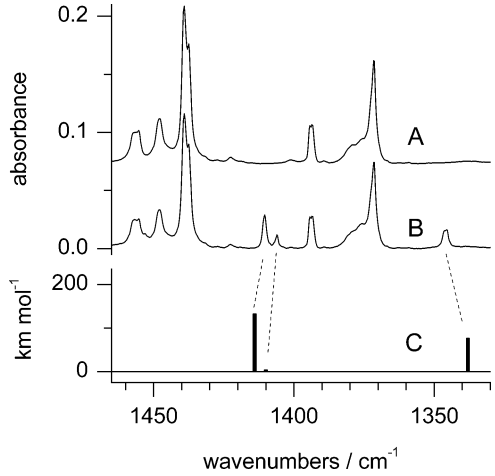
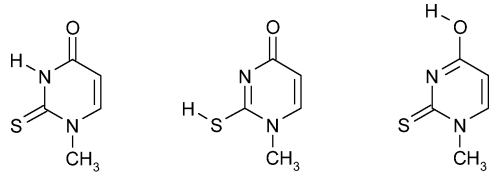


Figure 10. Fragment of the IR spectrum of 3-methyl-2-thiouracil isolated in an Ar matrix: (A) after deposition of the matrix; (B) after 220 min of UV (λ > 295 nm) irradiation; compared with a corresponding fragment (C) of the spectrum calculated for the isomer **VI** at the DFT(B3LYP)/6-311++G(2d,p) level. The calculated wavenumbers were scaled by the single factor of 0.98.

CHART 3: Tautomeric Forms of 1-Methyl-2-thiouracil



VII **VIII** **IX**

did not result in total conversion of the initial oxo−thione form **VII** into the oxo−thiol photoproduct **VIII** (Chart 3). Decrease of the population of the substrate **VII** as a function of the time of the UV illumination of the matrix is presented in Figure 11. This time dependency follows well the kinetics of reversible reactions (eq 2). In comparison to the case of 3-methyl-2-thiouracil, the amount of the initial form of 1-methyl-2-thiouracil transformed into the photoproduct was quite significant. Nevertheless, after 58% of the oxo−thione tautomer **VII** had been consumed, no further progress of the phototransformation was observed. This shows that also for 1-methyl-2-thiouracil the phototautomeric reaction is reversible and leads to a photostationary state.

The oxo−thiol photoproduct **VIII** was identified on the basis of a good agreement between the experimental IR spectrum appearing upon UV irradiation of the matrix and the theoretical spectrum of **VIII**. The comparison of fragments of the experimental spectra of 1-methyl-2-thiouracil, recorded before and after UV irradiation, with the spectrum predicted for tautomer **VIII** is presented in Figure 12.

In the molecule of 1-methyl-2-thiouracil, not only the thiocarbonyl C(2)═S group is situated in the direct vicinity of the N(3)−H fragment, but also the carbonyl C(4)═O moiety. Hence, photogeneration of the hydroxy−thione tautomer **IX** cannot be excluded for this compound. The matrix isolation experiments, carried out within the present work, suggest that some very small amount of this form (**IX**) is actually photoproduced. There are only very few spectral features found in the IR spectrum of 1-methyl-2-thiouracil, recorded after UV irradiation, that could be ascribed to **IX**. One of them is the weak band at 1560 cm^{-1} (see Figure 12). This band can be readily assigned to the spectrum of **IX** but not to the spectrum of **VIII**. The hypothesis of photogeneration of a minor amount

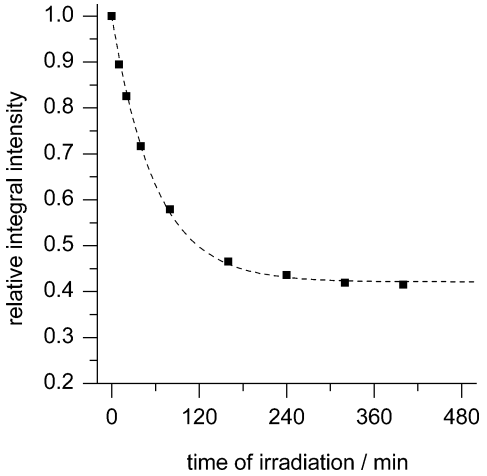


Figure 11. Progress of the phototautomeric reaction in 1-methyl-2-thiouracil isolated in an Ar matrix. The progress was monitored as a decrease of the intensity of the ν(N(3)H) band (at 3412 cm^{-1}), with respect to the intensity of this band recorded before UV irradiation. Dashed line represents the approximation of the experimental time dependency by the function given in eq 2.

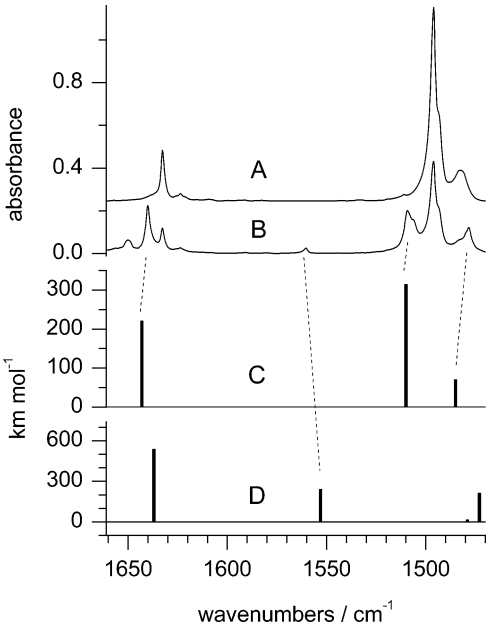


Figure 12. Fragment of the IR spectrum of 1-methyl-2-thiouracil isolated in an Ar matrix: (A) after deposition of the matrix; (B) after 400 min of UV (λ > 295 nm) irradiation; compared with corresponding fragments (C and D) of the spectra calculated at the DFT(B3LYP)/6-311++G(2d,p) level for isomers **VIII** and **IX**, respectively. The calculated wavenumbers were scaled by the single factor of 0.98.

of the hydroxy−thione tautomer **IX** is further supported by appearance upon UV irradiation of a very weak band at 3543 cm^{-1}, that is at a spectral position characteristic of the bands due to stretching vibrations of O−H groups. The efficiency of production of form **IX** was so small that it did not significantly affect the time dependency shown in Figure 11.

Concluding Remarks

The photoprocess dominating for UV-excited monomers of 2-thiouracil, 6-aza-2-thiothymine, 1-methyl-2-thiouracil, and 3-methyl-2-thiouracil is the transfer of either N(1)−H or N(3)−H proton to the sulfur atom of the thiocarbonyl group. Photoproducts generated by the transfer of the N(1)−H proton were observed for 2-thiouracil, 6-aza-2-thiothymine, and 3-methyl-

2-thiouracil, whereas products obtained by the transfer of the N(3)−H proton were detected for 2-thiouracil, 6-aza-2-thiothymine, and 1-methyl-2-thiouracil.

Although intramolecular proton-transfer photoreactions converting thione forms of compounds into the thiol tautomers were previously observed for a number of thione heterocycles, the results of the current work provide for the first time convincing proof of the occurrence of a back thiol → thione photoreaction. The experimental evidence of the thiol → thione photoprocesses consists of two types of observation:

(i) For 1-methyl-2-thiouracil and for 3-methyl-2-thiouracil the final stage of the UV-induced phototransformations was a photostationary state—this is possible only if the thione → thiol photoreaction is accompanied by a concomitant thiol → thione photoprocess.

(ii) For 2-thiouracil partial recovery of the oxo−thione initial tautomer was directly observed—this is possible only if there occurs a photoprocess transforming one of the oxo−thiol photoproducts back into the oxo−thione form.

Alongside the dominating oxo−thione → oxo−thiol photoreaction, a minor oxo−thione → hydroxy−thiol photoprocess generating the less populated form **IV** (with a fully aromatic pyrimidine ring) was also observed for 2-thiouracil and 6-aza-2-thiothymine. This photoreaction could involve simultaneous double-proton-transfer or a sequence of two single-proton-transfer processes. Analogous phototautomerization converting dithione tautomer into the corresponding dithiol form (with a fully aromatic pyrimidine ring) was observed previously for 2,4-dithiouracil isolated in an Ar matrix.[55]

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Supporting Information Available: Figure S1 providing the comparison of the experimental spectrum of 2-thiouracil isolated in an Ar matrix with the spectrum of the oxo−thione tautomeric form **I** theoretically simulated within and beyond the harmonic approximation and Figure S2 showing the evolution of intensities of the IR bands representing photoproducts **II**−**IV** with the time of irradiation of matrix-isolated 2-thiouracil. This material is available free of charge via the Internet at http://pubs.acs.org.

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