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Spectral and photophysical properties of the lowest excited triplet state of 4-thiouridine and its 5-halogeno derivatives

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Abstract

The properties of the lowest excited triplet state (T_1) of 2′,3′,5′-tri-*O*-acetyl-4-thiouridine (TU) and its 5-halogeno substituted derivatives (XTU, X = F, Cl, Br and I) in CCl_4 solutions are presented. Steady-state and pulsed techniques were used to characterize the T_1 state of TU and XTU in terms of the phosphorescence quantum yields (ϕ_p^0), intrinsic lifetimes ($\tau_{T_1}^0$) and the self-quenching rate constants (k_{sq}). The quantum yields of photochemical decay (φ_{pch}) of TU and XTU were also determined. From the values of quantum yields and under assumption that the formation of the T_1 state can be approximated by $\varphi_{T_1} = 1$, the rate constants of radiative (k_p) and non-radiative (k_{nr}) decay processes from the T_1 state were calculated. The results of the studies show that the T_1 states of TU and XTU have a $\pi\pi^*$ nature. Comparison of the absorption and phosphorescence spectral properties of TU and XTU molecules with those of 1,3-dimethyl-4-thiouracil (DMTU) confirms that TU and XTU exist in CCl_4 solution as (C-2)-keto-(C-4)-thione tautomers, exclusively, and that the phosphorescence arises from the excited T_1 state of these single tautomeric forms. The effect of the halogen substituent on the T_1 state properties of XTU molecules is discussed.

Keywords: 4-Thiouridine; 5-Halogeno-4-thiouridine; Excited triplet state; Phosphorescence

1. Introduction

The spectroscopic and photophysical properties of the 4-thiouracil chromophore have been the subject of considerable interest [1–4] mostly because of the widespread use of 4-thiouridine and its analogs as biological photoprobes [5–7]. Inter-system crossing (ISC) has been shown to be the major deactivation channel for the singlet states of 4-thiouridine ($\varphi_T = 0.9 \pm 0.1$ in aqueous solution [1,5]), and the lowest excited triplet state of the chromophore was found to be responsible for its high photochemical reactivity with nucleobases and amino acids [1,3,5,8]. So far, the spectral and photophysical studies of the T_1 state of 4-thiouridine in solution have been performed in water and several polar organic solvents such as CH_3CN [1,4], C_2H_5OH [1], DMSO [1], DMF [1] and THF [1] at a single solute concentration within the range of (2–5) × 10^{-5} M, with the assumption that under these conditions self-quenching can be neglected. The quantum yield and the lifetime of the room temperature phosphorescence of 4-thiouridine in water ($\lambda_{max} = 550$ nm) were determined to be $\varphi_p = 3 \times 10^{-4}$ [3,8] and $\tau_{T_1} = 320$ ns [1], 250 ns [8], respectively. The measured lifetimes in organic solvents were in the range of 15–1060 ns [1,4] with the longest lived emission obtained in acetonitrile [4]. The emissive state of the nucleoside has been suggested to have the π, π^* configuration by analogy with 1-methyl-4-thiouracil [1,5,6,9,10].

In our recent study concerning photophysical properties of 1,3-dimethyl-4-thiouracil (DMTU) we have demonstrated that, as is the case for other aromatic thioketones, self-quenching and quenching by solvent molecules are both important and characteristic decay processes of the excited T_1 state of the 4-thiouracil chromophore [9]. Self-quenching makes an important decay channel of the DMTU T_1 state in less polar solvents even at low concentration ($c < 2 \times 10^{-5}$ M), whereas solvent quenching dominates the T_1 state decay in polar environment. These bimolecular processes disturb the measurements of the intra-molecular channels of T_1 state decay, and in order to eliminate them the spectral

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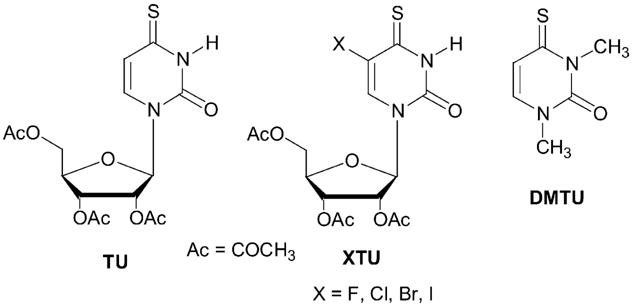

Scheme 1.

and photophysical studies should be performed in non-polar and chemically inert solvents. The most suitable solvents meeting these conditions are perfluoroalkanes. As follows from vast experimental evidence collected for aromatic and aliphatic thiocarbonyl compounds, their participation in the quenching of excited states is negligible [11]. The effect of self-quenching on the measured phosphorescence lifetime and quantum yield can be eliminated by extrapolation of the experimental values to infinite dilution of the solutions [12]. Taking account of above and using perfluoro-1,3-dimethylcyclohexane as a solvent we were able to measure a lifetime of the triplet-state of DMTU, $\tau_{T_1} = 5.3\,\mu s$, the longest obtained so far for this molecule in fluid solution [9].

As a continuation of our interest in the photophysics of the 4-thiouracil derivatives, we have undertaken detailed studies of the lowest excited triplet state properties of 4-thiouridine (TU) and its 5-halogeno derivatives (XTU, where X = F, Cl, Br, I) in non-polar solvents. The halogen-containing nucleosides XTU have been synthesized recently to evaluate their potential as novel agents in photocrosslinking technique [13]. Because of the extremely low solubility of the parent ribonucleosides in non-polar solvents their *O*-acetylated analogs (see Scheme 1) were used. They were not, however, sufficiently soluble in perfluoroalkanes, and therefore a non-polar, no hydrogen bonds forming CCl_4 was chosen as a solvent in these studies.

2. Experimental

Carbon tetrachloride (Merck) was of spectral purity and was used as received. The purity of the solvent was checked by measuring its absorption and emission spectra.

The synthesis of 2′,3′,5′-tri-*O*-acetyl-5-halogeno-4-thiouridines: FTU, ClTU and BrTU was reported previously [13]. The nucleosides TU and ITU were synthesized according to the same procedure. Selected spectral data for these compounds are given below:

TU: ^1H NMR ($CDCl_3$) δ_H (ppm): 10.08 (1, s, N–H), 7.25 (1, d, 6-H), 6.44 (1, d, 5-H), 5.99 (1, d, ribose 1′-H), 5.37 - 5.34 (2, m, ribose 2′,3′-H), 4.39 - 4.36 (3, m, ribose 4′,5′-H), 2.14 (3, s, $COCH_3$), 2.13 (3, s, $COCH_3$), 2.12 (3, s, $COCH_3$); LSIMS (*p*-nitrobenzyl alcohol) *m/z* 387.09 [$M + H^+$], $C_{15}H_{18}N_2O_8S$ requires 386.08.

ITU: ^1H NMR ($CDCl_3$) δ_H (ppm): 10.05 (1, s, N–H), 7.99 (1, s, 6-H), 6.04 (1, d, ribose 1′-H), 5.38–5.33 (2, m, ribose 2′, 3′-H), 4.43–4.33 (3, m, ribose 4′,5′-H), 2.25 (3, s, $COCH_3$), 2.13 (3, s, $COCH_3$), 2.12 (3, s, $COCH_3$); LSIMS (*p*-nitrobenzyl alcohol) *m/z* 512.97 [$M + H^+$], $C_{15}H_{17}{\cdot}IN_2O_8S$ requires 511.98.

To remove any emitting impurities the samples of nucleosides were further purified, directly prior to the measurements, by reversed phase HPLC on a Waters XTerra RP18 3.5 mm (4.6 mm × 150 mm) column eluted with 18% aqueous acetonitrile (v:v). The HPLC system (Waters 600E) was equipped with a Waters 991 photodiode array absorption and a Waters 470 scanning fluorescence detectors.

The UV absorption spectra were taken on a Cary 300 Bio (Varian) or JASCO V-550 spectrophotometers. The stationary emission measurements were made at room temperature on an upgraded Perkin-Elmer (Hitachi) MPF-3 spectrofluorometer using deoxygenated (by purging helium) solutions of nucleosides at a concentration of $c \approx 2 \times 10^{-5}$ M. Phosphorescence quantum yields (φ_p) were determined using quinine sulphate in 0.05 M H_2SO_4 as a reference ($\varphi = 0.52$) [14]. The samples were excited at 330 nm and the phosphorescence bands integrated over 470–700 nm range. The contribution of the emission from solvent impurities to the total emission of the samples was found to be negligibly small, nevertheless, for quantum yields determination it was eliminated according to the procedure described in [15] before the integration of the phosphorescence bands. Phosphorescence lifetime measurements were made on a modified laser spectrometer of nanosecond time-resolution described in details in [16]. The samples were excited at 355 nm.

The quantum yields of photochemical decay (φ_{pch}) of TU and XTU in CCl_4were determined using benzophenone–benzhydrol actinometry [17]. Deoxygenated solutions of the nucleosides ($c = 1 \times 10^{-4}$ M) were irradiated on an optical bench with a 200 W Hg arc lamp. An interference filter was used to isolate 334 nm light. Conversions were kept below 15% in all measurements. In the semi-preparative irradiation of ITU ($c = 5 \times 10^{-4}$ M) in CCl_4 the photoreaction was monitored by HPLC and UV spectroscopy.

3. Results and discussion

The UV absorption spectra of the nucleosides TU, FTU, BrTU and ITU in CCl_4 are shown in Fig. 1. The absorption properties of ClTU are almost identical with those of BrTU and therefore its spectrum is not included in the figure. The relevant absorption parameters for all the compounds are collected in Table 1.

Both TU and XTU show two absorption bands in the near UV range above 280 nm. By analogy with DMTU and other 4-thiouracil derivatives [1,9], the higher energy intense band with a maximum at $\lambda \approx 330$ nm is assigned

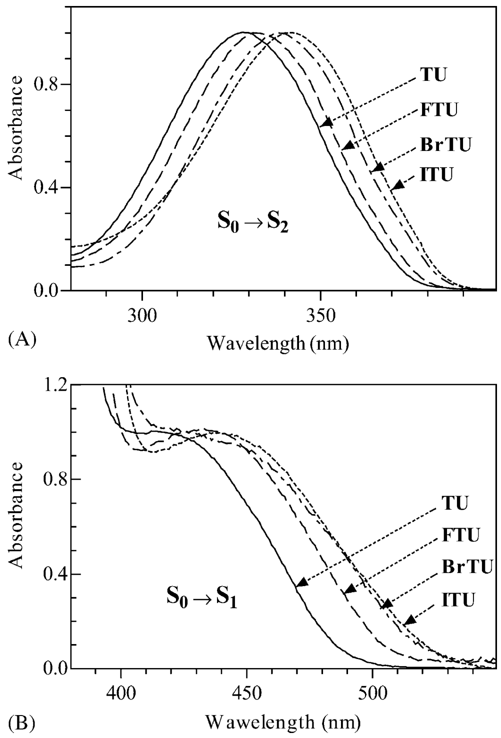

Fig. 1. The normalized: (A) $S_0 \rightarrow S_2$ and (B) $S_0 \rightarrow S_1$ absorption bands of TU and XTU in CCl_4 solution. The spectra were measured using a solution at (A) $c = 1 \times 10^{-4}$ M in a 1 cm path length cell and (B) $c = 2 \times 10^{-3}$ M in a 2 cm path length cell (B).

to the $S_0 \rightarrow S_2$ electronic transition of a π, π* character, while the very weak absorption band centered around 420–440 nm, is assigned to the $S_0 \rightarrow S_1$ transition of n, π* nature. The location of both absorption maxima, the spectral width and the profile as well as the intensity of the absorption bands of TU are identical to those of DMTU [9], whose (C-2)-keto-(C-4)-thione tautomeric structure is fixed by methyl groups. This is consistent with the results of previous experimental [18] and theoretical studies which showed that 4-thiouracil derivatives exist exclusively in this tautomeric structure in vapor, solid state as well as in aqueous, DMSO and $CHCl_3$ solutions [1,19,20]. The introduction of a halogen substituent at position 5 of the 4-thiouracil chromophore, in the case of XTU, results in a bathochromic shift of the absorption maxima, however, it has little effect on the shape, spectral width ($\Delta\nu_{1/2}$) and intensity (ε_{max}) of the $S_0 \rightarrow S_2$ and $S_0 \rightarrow S_1$ absorption bands (Fig. 1, Table 1).

Table 1
UV–vis absorption (λ_{max} (nm); ε (M^{-1} cm^{-1})) and phosphorescence (λ_{max} (nm); $\Delta\nu_{1/2}$ (cm^{-1})) spectral parameters of TU and XTU in CCl_4 at room temperature

Compound	Absorption				Phosphorescence	
	$S_0 \rightarrow S_2$		$S_0 \rightarrow S_1$			
	λ_{max}	ε	λ_{max}	ε	λ_{max}	$\Delta\nu_{1/2}$
TU	330	20600	420	40	540	3460
FTU	332	23300	432	42	570	3700
ClTU	338	17800	440	38	580	3500
BrTU	338	17200	440	40	585	3500
ITU	342	19500	440	52	595	3600

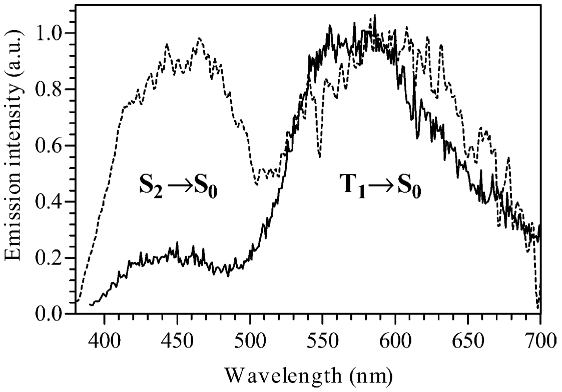

Fig. 2. The normalized emission spectra of FTU (solid line) and ITU (broken line) in deoxygenated CCl_4 solution ($\lambda_{exc} = 330$ nm; [FTU] = 1×10^{-5} M, [ITU] = 2×10^{-5} M) recorded at room temperature over 380–700 nm range.

Such a similarity of the UV–vis spectral features of XTU and TU allow one to conclude that (C-2)-keto-(C-4)-thione is also the preferred tautomeric form of the halogen substituted nucleosides in CCl_4solution.

When excited within the $S_0 \rightarrow S_2$ absorption band, TU and XTU exhibit two emission bands, a weak one in the range of 360–480 nm and a more intense one in the range of 480–700 nm. Fig. 2 shows the emission spectra of TU and ITU recorded in deoxygenated CCl_4 solution at ambient temperature. For ITU both emission bands are very weak and have similar intensity. For each compound the excitation spectra (not shown) recorded both at $\lambda_{em} = 430$ and 540 nm coincide with the absorption spectrum. The spectral range of the short-wavelength emission of TU, ITU (Fig. 2) and the remaining nucleosides studied in this work is identical with that of $S_2 \rightarrow S_0$ fluorescence band of DMTU [9].

Therefore, by an analogy, this emission of TU and XTU has been assigned to the radiative $S_2 \rightarrow S_0$ transition. The low-polarity of the solvent CCl_4 and the lack of specific solute-solvent interactions excludes the presence of emitting complexes in the systems studied. The properties of the short-wavelength emission of TU, XTU and deactivation of the excited S_2 state will be discussed in a separate paper. The long-wavelength band appearing in the TU and XTU emission spectra in the range of 480–700 nm (Fig. 2) has been assigned to the phosphorescence from the lowest excited triplet state T_1. The phosphorescence of 4-thiouridine in aqueous solution [3] and DMTU in $CCl_4$and other solvents [9] has been found to fall in a very similar spectral range.

Fig. 3 presents the normalized phosphorescence spectra of TU, FTU, BrTU and ITU.

As is the case for absorption, the phosphorescence spectrum of ClTU (not shown) almost exactly coincides with that of BrTU. As can be seen, the phosphorescence bands

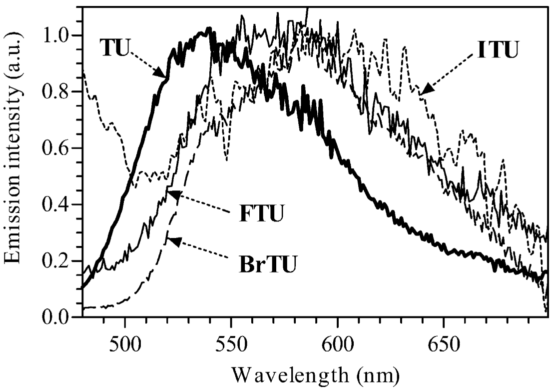

Fig. 3. Normalized phosphorescence bands of TU, FTU, BrTU and ITU in CCl_4; $\lambda_{exc} = 330$ nm; $c = 2 \times 10^{-5}$ M, deoxygenated solutions; ambient temperature.

of TU and XTU have similar profiles and width at half intensity ($\Delta\nu_{1/2}$, Table 1) and they are characterized by the lack of the vibrational structure. These spectral features and band parameters resemble closely those of the DMTU phosphorescence in CCl_4, whose emitting T_1 state is of π, π^* nature [9]. This, in turn, implies the same nature of the T_1 state of TU and XTU. Such a conclusion is supported by the published results of steady state phosphorescence study of aromatic and aliphatic thiocarbonyl compounds, showing that the phosphorescence bands resulting from $T_1(n, \pi^*) \rightarrow S_0$ and $T_1(\pi, \pi^*) \rightarrow S_0$ transitions have different shapes and the emission band from the state of a n, π^* configuration has a well marked vibrational structure [12]. Because of the lack of the vibrational structure the exact values of T_1 state energy and the energy gap ΔE [$S_1(n, \pi^*)$, $T_1(\pi, \pi^*)$] for TU and XTU could not be determined. The approximate ΔE value for TU, calculated from the positions of the $S_0 \rightarrow S_1$ absorption and $T_1 \rightarrow S_0$ emission maxima, amounts to 5290 cm^{-1} and it is by 710 cm^{-1} higher than that for DMTU [9]. The halogen substituent in XTU nucleosides causes a slightly more pronounced red shift of the phosphorescence band than that of the $S_0 \rightarrow S_1$ absorption. Therefore, the value of ΔE [$S_1(n, \pi^*)$, $T_1(\pi, \pi^*)$] in these compounds increases by 200–640 cm^{-1} as compared to the energy gap in TU.

To characterize the most important processes of deactivation of the excited T_1 state of TU and XTU in CCl_4, the phosphorescence quantum yields (φ_p) and lifetimes as well as quantum yields of the photochemical decay (φ_{pch}) were measured. For each nucleoside, kinetics of the phosphorescence decay was measured for a series of solutions of different concentrations in a range from 2.9×10^{-6} to 5×10^{-4} M. Irrespective of the sample concentration and the emission wavelength chosen from the range of 480–700 nm, single exponential decays were observed. Moreover, the kinetics of the emission decay did not depend on the energy of the exciting pulse changed in the range 0.1–10 mJ. Fig. 4 presents the experimental curves of the TU and XTU phosphorescence decays in solutions whose concentrations have been adjusted to assure a similar contribution (15–20%) of the self-quenching process (% sq, calculated from Eq. (6), see below) to the total quenching of the T_1 state.

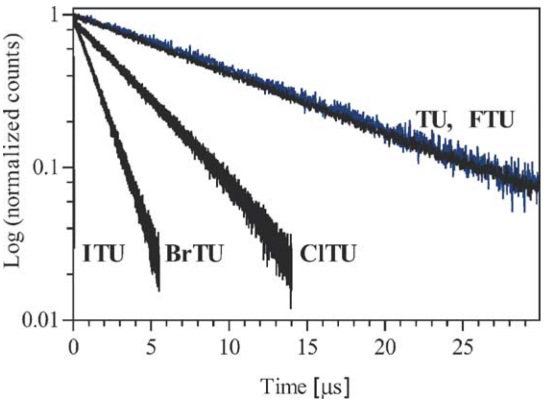

Fig. 4. The phosphorescence decays traces of TU ($c = 3.2 \times 10^{-6}$ M; 19.5% sq), FTU ($c = 2.9 \times 10^{-6}$ M; 15.2% sq), ClTU ($c = 8.2 \times 10^{-6}$ M; 12.0% sq), BrTU ($c = 2.6 \times 10^{-5}$ M; 17.8% sq) and ITU ($c = 3 \times 10^{-5}$ M) in CCl_4solution; room temperature; $\lambda_{exc} = 355$ nm, $\lambda_{em} = 580$ nm, pulse energy: 0.1 mJ.

Applying Stern–Volmer equation (Eq. (1)) [9]:

$$\frac{1}{\tau_{T_1}} = \frac{1}{\tau^0_{T_1}} + k_{sq} \times [S_0] \qquad (1)$$

and plotting a reciprocal of the experimentally measured lifetimes τ_{T_1} versus concentration of TU or XTU in the ground state ($[S_0]$), the intrinsic lifetimes $\tau^0_{T_1}$, i.e. the lifetimes of the T_1excited state in the absence of self-quenching process, and self-quenching rate constants k_{sq} were obtained from the intercepts and the slopes, respectively, of the linear Stern–Volmer plots. Because of a very low intensity of the exciting pulse (0.1 mJ) the values of $[S_0]$ in (Eq. (1)) can be well approximated by the values of the concentrations of TU or XTU solutions used in the experiments. The Stern–Volmer plots for TU and XTU are presented in Fig. 5 and the values of $\tau^0_{T_1}$ and k_{sq} determined from them are given in Table 2.

In the case of ITU, whose T_1 state is very short-lived the measured phosphorescence lifetime did not depend on the

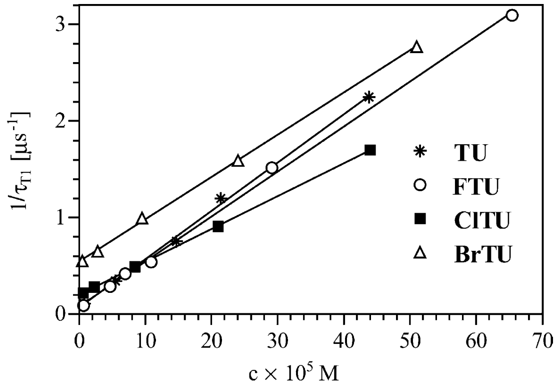

Fig. 5. Stern–Volmer plots of reciprocal of triplet lifetime $1/\tau_{T_1}$ vs. concentration of the nucleoside for TU and XTU in CCl_4.

Table 2
Photophysical properties of TU and XTU in the T_1 state in deoxygenated CCl_4 at ambient temperature

	TU	FTU	ClTU	BrTU	ITU
ϕ_p^0[a]	0.0123	0.0034	0.0026	0.0039	0.0003
$\tau_{T_1}^0$ (μs)	15.2	13.2	4.9	1.9	0.024[b]
φ_{pch}	0.008	0.010	0.001	0.005	0.021
φ_{nr}	0.99	0.99	0.99	0.99	0.98
k_{sq} ($/10^9\,M^{-1}\,s^{-1}$)	5.0	4.7	3.4	4.4	–
k_{nr} ($/10^4\,s^{-1}$)	6	7	20	52	4083
k_p ($/10^2\,s^{-1}$)	8.1	2.6	5.3	20.5	125.0
$A = k_{nr}(XTU)/k_{nr}(FTU)$	–	1	2.8	7.4	583.3
$B = k_p(XTU)/k_p(FTU)$	–	1	2.0	7.9	48.1
A/B	–	–	1.4	0.9	12.1

[a] The error of ϕ_p^0 determination is ±10%.

[b] The phosphorescence lifetime measured does not depend on the solution concentration in the concentration range used.

sample concentration in the concentration range studied ($c \approx 3 \times 10^{-6}$ to 5×10^{-4} M). For all the compounds studied, the values of self-quenching rate constant k_{sq} (Table 2) are similar and high, close to the diffusion rate constant ($k_{dif} = 10^{10}\,M^{-1}\,s^{-1}$ in CCl_4). As follows from the study of the self-quenching mechanism of the T_1 (n, π^*) excited states of several thioketones by Rajee and Ramamurthy [21] and Ramesh et al. [22] this process involves interaction of a singly occupied n-orbital of excited molecule and a lone pair of electrons on a sulfur atom of a ground state molecule. Steric crowding, e.g. the presence of a neighbouring methyl group, which diminishes the availability of n electrons, significantly reduces the k_{sq} value. Moreover, the electronic interactions leading to self-quenching are of the donor–acceptor type and the thione molecule in the S_0 state acts as a donor species. Therefore, the k_{sq} values determined for several thiones were significantly reduced when the thione molecule contained an electron withdrawing substituent [21,22]. Thus, the observed insensitivity of k_{sq} on the type of substituent in the 5-position (H, F, Cl, Br, I) in the case of TU and XTU, would indicate that the steric and electronic factors governing the self-quenching process of the π, π^* triplets are different from those responsible for the quenching of the n, π^* thione triplets.

Under the conditions where self-quenching is eliminated, the parent nucleoside TU, having no halogen substituent is characterized by the longest-lived T_1 state. The value of $\tau_{T_1}^0$ of TU in CCl_4 jest about an order of magnitude higher than the longest lifetime determined hitherto for 4-thiouracil chromophore in more polar organic solvent, which suggests that, like in the case of DMTU [9], the interactions with polar solvent make an effective channel of deactivation of the T_1 state. It is also worth noting that $\tau_{T_1}^0$ of TU in CCl_4 is about four times longer than the intrinsic lifetime found for DMTU ($\tau_{T_1}^0 = 3.6$ μs) [9] in the same solvent, which supports our previous suggestion that for the latter compound an important channel of the T_1 state deactivation is the reversible intra-molecular hydrogen abstraction involving the C=S group and the neighboring methyl group.

The T_1 state lifetime of FTU is similar to that of TU, but the lifetime gradually decreases for the nucleosides containing heavier halogen substituent (X = Br, I). As the intrinsic T_1 state lifetimes of TU and FTU are long compared to those characterizing the XTU derivatives, where (X = Cl, Br, I), in solutions of the same concentrations the contribution of self-quenching (sq(%)) to the overall deactivation of the T_1 excited state is much higher for the former two compounds. The values of sq(%), calculated using Eq. (2) for dilute solutions ($c = 1 \times 10^{-5}$ M) of TU, FTU, ClTU and BrTU in CCl_4 amount to 43, 38, 14 and 8%, respectively.

$$\mathrm{sq}\,(\%) = \frac{k_{sq}c}{k_{sq}c + 1/\tau_{T_1}^0} \times 100 \tag{2}$$

Assuming that the self-quenching process occurs at the same rate in BrTU and ITU it appears that it is not an important decay channel of the T_1 state of iodo substituted nucleoside ITU even in more concentrated solution ($c = 10^{-3}$ M, ∼10%). Using the quantum yields of phosphorescence (φ_p) of TU and XTU determined at the concentration of solutions $c \approx 2 \times 10^{-5}$ M, and applying Eq. (3) [9] we have calculated the limiting phosphorescence quantum yields ϕ_p^0 at the infinite dilution.

$$\phi_p^0 = \phi_p(1 + k_{sq}\tau_{T_1}^0 c) \tag{3}$$

As follows from the values of ϕ_p^0 given in Table 2, phosphorescence plays only a minor role in deactivation of the excited T_1 state of the investigated compounds. The value of ϕ_p^0 is the greatest for the parent nucleoside TU. The quantum yields of the F-, Cl- and Br-substituted derivatives of TU are by a factor of 3 lower than that of TU and by approximately one order of magnitude higher than the quantum yield of ITU phosphorescence.

Photochemical processes contribute only to a small extent to the T_1 state decay of TU and XTU. The quantum yields of substrate consumption φ_{pch} upon excitation into $S_0 \rightarrow S_2$ absorption band were found to be concentration dependent and the values determined for 1×10^{-4} M solutions of TU and XTU are presented in Table 2. The very low values of ϕ_p^0 and φ_{pch} indicate that the T_1 state decays mainly in radiationless processes (nr). Assuming that the quantum yield of T_1 formation for the thiouridine derivatives described in this work in CCl_4 $\varphi_{T_1} = 1$, the quantum yield of radiationless processes, φ_{nr}, was calculated from (Eq. (4)).

$$\phi_{T_1} = \phi_p + \phi_{pch} + \phi_{nr} \tag{4}$$

This assumption seems to be justified because the value of ϕ_{T_1} determined experimentally for 4-thiouridine is 0.9 ± 0.1 [1] and it should not be lower in halogen containing molecules due to the internal heavy atom effect. Finally, from Eqs. (5) and (6) the non-radiative, k_{nr}, and radiative, k_p, rate constants were calculated and their values are included in Table 2.

$$k_{nr} = \frac{\phi_{nr}}{\tau_{T_1}^0 \phi_{T_1}} \tag{5}$$

$$k_p = \frac{\phi_p^0}{\tau_{T_1}^0 \phi_{T_1}} \tag{6}$$

The value of k_p for TU is almost three times higher than for FTU, which is most probably a result of a smaller energy gap ΔE [T_2 (n, π^*), T_1(π, π^*)], and thus a greater vibronic coupling in TU [9,24]. Greater spin–orbit coupling in the TU molecule can be excluded as the origin of the difference in k_p because the estimated values of the energy gap ΔE (S_1, T_1) are almost the same for both compounds ($5290\,cm^{-1}$ for TU and $5580\,cm^{-1}$ for FTU). The values of k_p determined for TU and FTU characterizing the process of phosphorescence from the excited T_1 (π, π^*) state are about one order of magnitude smaller than those characterizing the T_1 (n, π^*) emission of aromatic and heteroaromatic thioketones in non-polar solvents. In fact, among the family of thiocarbonyl compounds the radiative decay of T_1 is slower only in thiocoumarins ($k_p = 0.1 \times 10^2\,s^{-1}$ in perfluoroalkanes) [23]. The small value of k_p for thiocoumarin was explained by the π, π^* electronic configuration of the T_1 excited state, which is not typical for thiones in non-polar solvents.

The rate constants determined for a series of nucleosides XTU with X = Cl, Br, I substituents systematically increase compared to the values of k_p and k_{nr} found for FTU. This increase expressed by the ratio $A = k_{nr}(XTU)/k_{nr}(FTU)$ and $B = k_p(XTU)/k_p(FTU)$ (Table 2), which are both the greater the higher the atomic number of the halogen substituent and can be explained by the internal effect of the heavy atom. The presence of a heavy atom in a molecule is known to increase the rate of radiative and radiationless processes of the T_1 excited state decay [25]. The presence of a heavy atom also increases the rate of the $S_1 \rightarrow T_1$ transition. However, in the substituted thiocarbonyl compounds, the heavy atom effect on the latter process is expected to be less important, because in the unsubstituted thiones the $S_1 \rightarrow T_1$ transition is of $n\pi^* \rightarrow \pi\pi^*$ type and it is already very efficient according to the El-Sayed rules [26]. The values of the *A*/*B* ratio calculated for the molecules XTU (X = Cl, Br, I) and presented in Table 2, permit comparison of the effect of the substituent X on the radiative and radiationless processes of their T_1 states. The values of *A*/*B* for ClTU and BrTU are close to 1, which means that the presence of a heavy atom increases, to a similar extent, both the radiative k_p and radiationless k_{nr} rate constants in these molecules. For ITU the *A*/*B* ratio is higher by an order of magnitude than in the other compounds, which means that the iodine substituent increases the rate of radiationless processes much more (12 times) than that of the phosphorescence. The radiationless channels of the T_1 state decay of thiouracil derivatives include the intra-molecular $T_1 \rightarrow S_0$ process and solvent quenching [9]. Neglecting the latter process, which is relatively less important in CCl_4 compared to more polar and reactive organic solvents and water, one can assume that $k_{nr} \approx k_{T_1} \rightarrow S_0$. The large value of *A*/*B* calculated for ITU indicates that either $k_{T_1} \rightarrow S_0$ is more sensitive to the heavy atom effect than k_p or that the presence of iodine has effectuated an additional fast deactivation pathway of the T_1 state, which does not lead to a stable chemical change.

Photochemistry can be initiated in ITU by photodissociation of the low-energy C–I bond leading to formation of radicals, which after a fast spin inversion recombine to generate ground state ITU molecules. Therefore, for ITU the radiationless rate constant is a sum of two processes, $k_{nr} = k_{T_1} \rightarrow s_0 + k_{diss\,C\text{–}I}$. The process of C–I photodissociation should be reversible to a large extent as the quantum yield of photochemical decay of ITU, though the highest among the whole XTU series, is still rather low (Table 2). Nevertheless, the stationary photochemistry provides evidence for such a process. Upon irradiation of ITU in CCl_4 ($c = 5 \times 10^{-4}\,M$) into $S_0 \rightarrow S_2$ band, which ultimately leads to the T_1 state ($\varphi_T \approx 1$), the solution gradually turns purple. The appearance of the purple color is consistent with the presence of free I_2. The formation of molecular iodine was confirmed spectrophotometrically by the observation of the appearance of its visible absorption (I_2 in aliphatic hydrocarbons: $\lambda_{max} = 515\,nm$, $\varepsilon = 600\,M^{-1}\,cm^{-1}$) [27] in the UV–vis spectrum of the irradiated solution of ITU (Fig. 6).

Furthermore, in support of the C–I bond photodissociation a dehalogenated nucleoside TU was identified among the several photoproducts of ITU. No C–Br bond cleavage and formation of TU was observed in the case of BrTU under the same conditions of irradiation.

4. Conclusions

The properties of the T_1 excited state of 2′,3′,5′-tri-*O*-acetylo-4-thiouridine, TU, are similar to those of the T_1 state of thiocarbonyl compounds. In the non-polar solvent CCl_4 the intrinsic lifetime $\tau_{T_1}^0$ of TU is significantly longer (>15×) than the lifetimes determined previously for

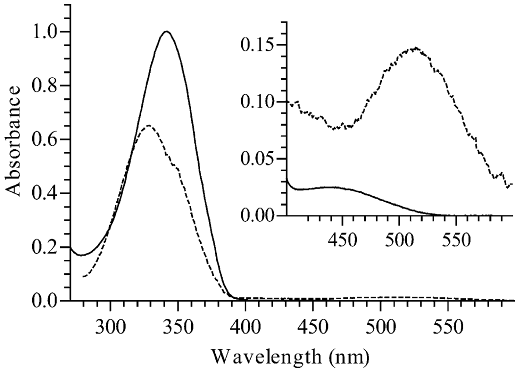

Fig. 6. The UV absorption spectra of deoxygenated solution of ITU ($c = 5 \times 10^{-4}$ M) in CCl_4 recorded in 0.1 cm cell before and after irradiation at $\lambda \geq 334$ nm up to 60% conversion of substrate; inset: the spectra recorded in 1 cm pathlength cells.

4-thiouridine. The high value of $\tau^0_{T_1}$ found for TU in CCl_4 indicates that in this chromophore, like in thiocarbonyl compounds, the solvent plays an active role in the excited T_1 state decay. Due to the long intrinsic lifetime and high self-quenching rate constant, k_{sq} about 40% of TU triplets decay by the self-quenching process even in a dilute (1×10^{-5} M) solution in CCl_4. In the absence of the bimolecular process the dominant route of T_1 state decay is the intra-molecular radiationless $T_1 \rightarrow S_0$ transition. The structureless phosphorescence band of TU in CCl_4 and the value of the radiative rate k_p constant, which is lower than that of thiocarbonyl compounds with the emitting $n\pi^*$ triplets, indicate that the T_1 state of TU has a π, π^* character. The similarity of the absorption and phosphorescence spectral data of TU and DMTU as well as the fact that the decay kinetics are independent of the emission wavelength, proves that the phosphorescence of TU occurs from the excited tautomer (C-2)-keto-(C-4) thione as the sole emitting tautomeric form.

In the series of XTU compounds the values of radiative k_p and non-radiative k_{nr} rate constants increase with increasing atomic number of the halogen substituent. The observed decrease in the phosphorescence lifetimes of XTU on going from X = F to X = Br, I can be explained in terms of the intramolecular heavy atom effect. In the case of ITU molecule an additional fast, non-radiative decay channel ascribed to photodissociation of the weak C–I bond operates.

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