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Structure–Luminescence Correlations in the Thiobarbiturates

L. A. King[1] and L. A. Gifford

Department of Clinical Pharmacology, St. Bartholomews Hospital, London, EC1, England

The ultraviolet absorption spectra, fluorescence and phosphorescence excitation and emission spectra of seven thiobarbiturates have been examined. In 5,5-disubstituted thiobarbiturates, two fluorescent species are present in solution, one of which is a mono-anion and the other a zwitterion. Compounds containing a 5-phenyl substituent exhibited anomalous features but did not show a luminescence characteristic of an isolated phenyl group. The use of luminescence techniques as a provisional means of identifying an unknown thiobarbiturate is shown.

A previous report (*1*) described the structural determinants for luminescence of oxobarbiturates at room temperature and at 77K. A requirement for fluorescence from oxobarbiturates was the presence of a dianion. However, thiobarbiturates show features in their absorption spectra, apart from a general bathochromic shift, which suggests that species different from those found in oxobarbiturates are present in solution. Numerous reports have appeared (*2–5*) describing the fluorescence analysis of thiobarbiturates extracted from biological fluids and pharmaceutical preparations although the low temperature luminescence of thiobarbiturates has hitherto received little attention.

An examination has been made of the luminescence properties of a series of thiobarbiturates. In conjunction with other studies (*6*), these results clarify the solution chemistry of the thiobarbiturates and show how various members of the group may be discriminated.

EXPERIMENTAL

The thiobarbiturates studied are shown in Table I. The 5,5-disubstituted members were shown to be homogeneous by thin-layer chromatography (*7*): melting points were within 3 °C of literature values. Barbitone (5,5-diethylbarbituric acid) was used as described earlier (*1*): 5-phenylthiobarbituric acid, mp 264°C (dec), and 5-ethyl-5-phenylthiobarbituric acid, mp 212–214 °C, were prepared by condensation of the appropriate malonic ester with thiourea in the presence of sodium ethoxide; the success of these syntheses was verified using NMR and mass spectrometry. Thiobarbituric acid was obtained from Koch-Light Ltd; Thiopentone (5-ethyl-5-(1^1-methylbutyl)thiobarbituric acid) from Abbott Laboratories Ltd; Buthalitone (5-allyl-5-isobutyl thiobarbituric acid) from May and Baker Ltd; 1-methyl-5-ethyl-5-(1^1-methylpropyl)thiobarbituric acid from Allen and Hanburys Ltd; and 1,3-dimethyl-5,5-di-*n*-propylthiobarbituric acid from ICI Ltd. Buthalitone was recrystallized by slowly neutralizing an aqueous alkaline solution. Other compounds were used without further purification.

Deuterium oxide and ethanediol were obtained from BDH Ltd. Other reagents were AR grade.

Acetate buffers were used to obtain solutions with pH's in the range 3.6–5.4; phosphate buffers in the range 6.0–8.4; and borate buffers in the range 8.4–10.2. Solutions with pH's below 3.0 and above 10.2 were obtained using dilute hydrochloric acid and sodium hydroxide, respectively. All pH's were measured at room temperature with a Pye-Unicam pH meter. In experiments where fluorescence intensity was measured as a function of pH, thiobarbiturates were prepared at *ca.* 20 μg/ml.

Uncorrected fluorescence and phosphorescence spectra were recorded using a Perkin-Elmer MPF-2A spectrophotofluorimeter. The arrangement for making measurements at liquid nitrogen temperature was similar to that described earlier (*8*). At room temperature, measurements were made in 0.5-ml silica cells with a circular cross-section (i.d., 4 mm). Fluorescence intensities were corrected for the weak emission (largely Raman scatter) from the solvent.

Ultraviolet absorption spectra were measured at room temperature with a Pye-Unicam SP8000 spectrophotometer using 10-mm path-length silica cells.

Relative quantum yields of fluorescence for the 5,5-disubstituted thiobarbituric acids were determined in aqueous alkali at 20 °C. Since these compounds showed similar excitation and fluorescence characteristics, the quantum yield of fluorescence is directly proportional to I_f/A where I_f is the fluorescence intensity at 510 nm and A is the absorbance at the excitation wavelength (310 nm). 5-Ethyl-5-phenylthiobarbituric acid was chosen as a reference, the relative quantum yield of which was arbitrarily assigned as unity.

RESULTS

Fluorescence Spectra at Room Temperature. Only 5,5-disubstituted thiobarbituric acids showed a significant fluorescence emission at 20 °C in aqueous solution. Unlike the corresponding C_2-oxybarbiturates fluorescence could be observed over the range pH 5–13.5. The pH fluorescence relationship for these thiobarbiturates is shown in Figure 1. The *N*-methyl and di-*N*-methyl derivatives of 5,5-disubstituted thiobarbiturates as well as the parent compound thiobarbituric acid were non-fluorescent at all pH's.

The fluorescence excitation and emission spectra of 5-ethyl-5-phenylthiobarbituric acid are shown in Figure 2. Thiopentone and buthalitone showed similar spectral characteristics; the wavelengths of maximum excitation (λe = 312 nm) and emission (λf = 510 nm) were independent of pH. The fluorescence intensity of 5-ethyl-5-phenylthiobarbituric acid in strongly alkaline solution was higher than that of thiopentone and buthalitone relative to values measured at pH 9–10. The relative quantum yields of fluorescence for three 5,5-disubstituted thiobarbiturates are shown in Table II.

The fluorescence of 5,5-disubstituted compounds was particularly sensitive to the solvent used. In 90% ethanol/0.1*N* KOH or 50% (v/v) aqueous ethanediol/0.1*N* KOH, the fluorescence was totally quenched. By comparison, the fluorescence of oxobarbiturates at 20 °C is reduced but not entirely quenched by addition of ethanol to aqueous solutions (*9*). Inclusion of 90% deuterium oxide in an aqueous alkaline solution (0.1*N* KOH) of thiopentone and buthalitone caused an increase in the fluorescence intensity of 1.30 and 1.33, respectively. In the case of 5-ethyl-5-phenylthiobarbituric acid, a significantly higher deuterium isotope enhancement of 1.50 was found. Under similar conditions, barbitone showed an enhancement of 1.49. No other spectral changes were associated with the fluorescence in deuterium oxide and the isotope effect was absent at pH 7.5.

Luminescence Spectra at 77K. The wavelengths of maximum excitation and emission for the thiobarbiturates studied are set out in Table III. In 50% (v/v) aqueous ethanediol containing 0.1*N* KOH, thiopentone, buthalitone, and 5-ethyl-5-phenylthiobarbituric acid showed fluorescence properties similar to those found at room temperature except that the wavelength of maximum fluorescence (λf) was blue shifted by 45 nm. The compound containing a single *N*-methyl group (1-methyl-5-ethyl-5-(1^1-methylpro-

[1] Present address, Home Office Central Research Establishment, Aldermaston, Reading, Berks, RG7 4PN, England.

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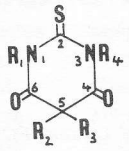
Table I. Thiobarbiturate Structure



Compound	R_1	R_2	R_3	R_4
Thiobarbituric acid	H	H	H	H
5-Phenylthiobarbituric acid	H	H	Phenyl	H
Thiopentone	H	Ethyl	1-Methylbutyl	H
Buthalitone	H	Allyl	Isobutyl	H
5-Ethyl-5-phenylthiobarbituric acid	H	Ethyl	Phenyl	H
1-Methyl-5-ethyl-5-(1¹-methylpropyl)thiobarbituric acid	H	Ethyl	1-Methylpropyl	Methyl
1,3-Dimethyl-5,5-di-*n*-propylthiobarbituric acid	Methyl	*n*-Propyl	*n*-Propyl	Methyl

Table II. Relative Quantum Yields of Three Thiobarbiturates at 20 °C in 0.1*N* Aqueous KOH.[a]

Compound	Relative quantum yield
5-Ethyl-5-phenylthiobarbituric acid	1.0 (Reference)
Buthalitone	0.41
Thiopentone	0.29

[a] The values shown are the mean of five determinations.

Table III. Luminescence Characteristics of Thiobarbiturates at 77K.[a]

	Phosphorescence			
	0.1*N* KOH		0.1*N* HCl	
Compound	λ_e(nm)	λ_p(nm)	λ_e(nm)	λ_p(nm)
5-Phenylthiobarbituric acid	304	440	304	440
	Fluorescence			
Thiobarbituric acid	n.e.	λ_f(nm)	n.e.	
5-Phenylthiobarbituric acid	304	375	n.e.	
Thiopentone	312	465	n.e.	
Buthalitone	312	465	n.e.	
5-Ethyl-5-phenylthiobarbituric acid	312	465	n.e.	
1-Methyl-5-ethyl-5-(1¹-methylpropyl)thiobarbituric acid	312	480	n.e.	
1,3-dimethyl-5,5-di-*n*-propylthiobarbituric acid	n.e.		n.e.	

[a] The solvent was 50% (v/v) aqueous ethanediol containing acid or alkali as shown (n.e. = no emission).

pyl)thiobarbituric acid) exhibited maximum fluorescence at 480 nm. None of these compounds was fluorescent in solvent glasses containing 0.1*N* HCl. Addition of ethanol did not decrease the fluorescence intensity at 77K. Again, thiobarbituric acid and 1,3-dimethyl-5,5-di-*n*-propylthiobarbituric acid were non-luminescent at any pH.

5-Phenylthiobarbituric acid exhibited phosphorescence (λ_p = 440 nm) over the range pH 0–13 but was fluorescent only in alkaline solution (λ_f = 375 nm). The excitation spectra of both fluorescence and phosphorescence were similar and independent of pH (λ_e = 304 nm) (Figure 3).

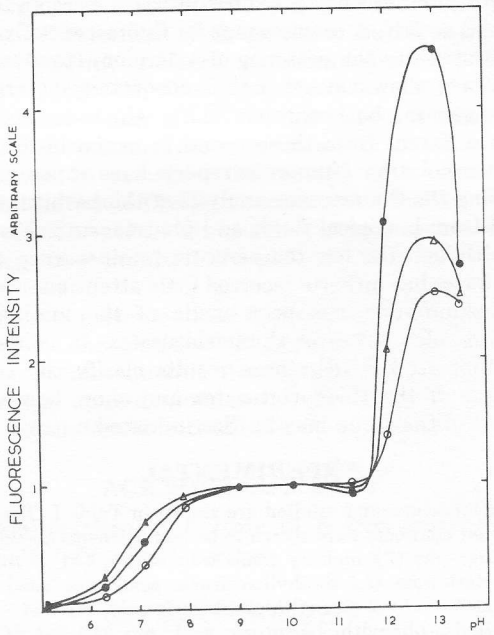


Figure 1. Fluorescence intensities of three thiobarbiturates at 20 °C as a function of pH.

Thiopentone (O); buthalitone (Δ); 5-ethyl-5-phenylthiobarbituric acid (●). Excitation wavelength 312 nm, emission wavelength 510 nm. Curves have been arbitrarily normalized at pH 10.2

The mean lifetime of the phosphorescence could not be accurately measured using only a rotating can phosphoroscope or a chart recorder with a fast chart speed but was between 0.02 and 0.5 second.

Neither of the two 5-phenyl substituted compounds showed a phosphorescence emission characteristic of an isolated phenyl group as is found in the corresponding oxobarbiturates (*1, 5*).

Absorption Spectra. The absorption spectra of 5-allyl-5-isobutylthiobarbituric acid (buthalitone) at various pH values are shown in Figure 4. A bathochromic shift was observed in changing from pH 5.1 to pH 8.1. Further increases in pH up to pH 10 produced a hyperchromic effect. Between pH 5.1 and 10.2, all curves passed through an isosbestos at 295 nm. Above pH 11, a further hyperchromic shift occurred with no change in the wavelength of maximum absorption. Similar spectra were obtained from 5-

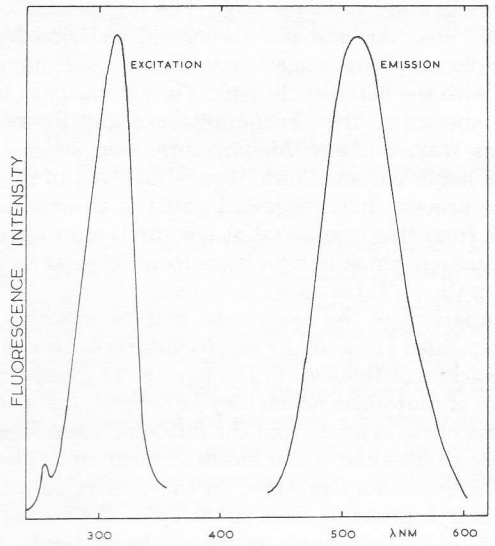


Figure 2. Fluorescence excitation and emission spectra of 5-ethyl-5-phenylthiobarbituric acid at 20 °C in 0.1*N* KOH

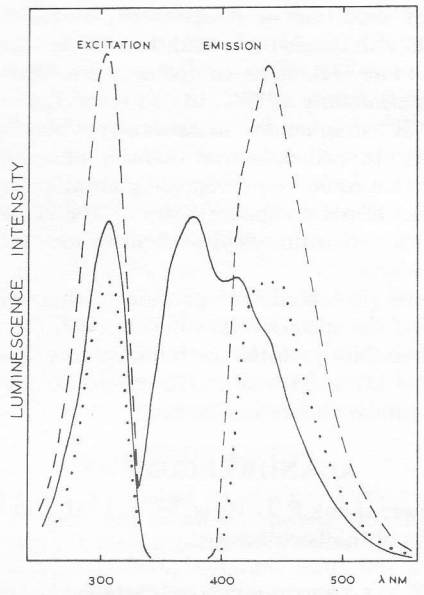


Figure 3. 77K fluorescence and phosphorescence spectra of 5-phenylthiobarbituric acid in 50% (v/v) aqueous ethanediol.

Total emission and excitation spectra in solvent containing 0.1*N* KOH ——; total emission and excitation spectra in solvent containing 0.1*N* HCl. . . ; resolved phosphorescence in 0.1*N* KOH– – – (not to same scale)

ethyl-5-(1[1]-methylbutyl)thiobarbituric acid (thiopentone) and 5-ethyl-5-phenylthiobarbituric acid. In each case, at pH 13.5, the wavelength of maximum absorption was 304–309 nm and the molar absorptivity was 27,000–28,000.

Thiobarbituric acid (Figure 5) showed wavelength maxima at shorter wavelengths than the 5,5-disubstituted acids. On changing from pH 2 to pH 5.6, a hypochromic effect occurred. At more alkaline pH's, the absorbance decreased still further. Substitution of the 5-position in thiobarbituric acid by a phenyl group produced only small changes in the absorption spectra.

These results together with those for the two *N*-methylated compounds were in good agreement with the spectra reported by Smyth *et al.* (*6*).

In none of the compounds examined was there evidence from the absorption spectra of contamination by the analogous oxobarbiturate. The effect of alkali on the absorption of thiobarbiturates was reversed by addition of acid. Solutions were stable during the period of measurement.

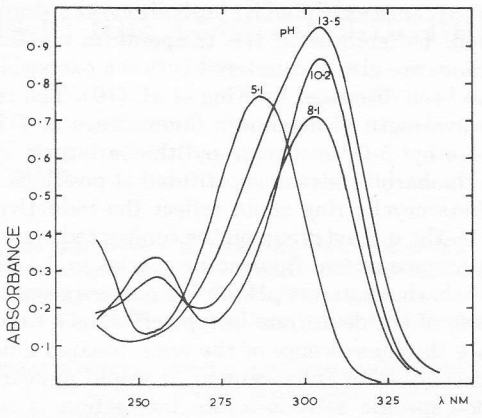


Figure 4. Absorption spectra of buthalitone ($3.30 \times 10^{-5}M$) as a function of pH

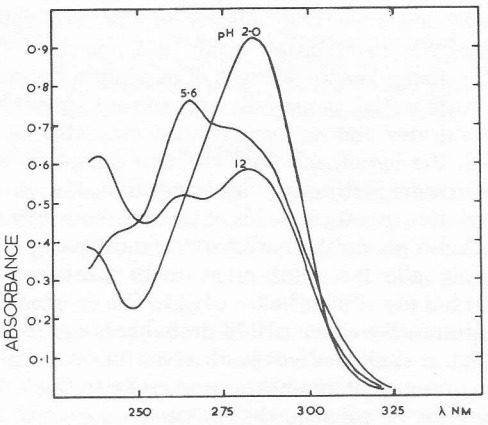
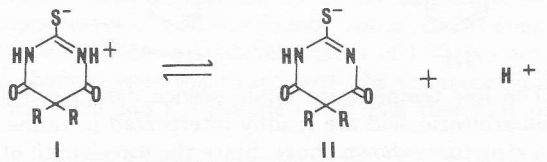


Figure 5. Absorption spectra of thiobarbituric acid ($4.65 \times 10^{-5}M$) as a function of pH

DISCUSSION

In the compounds studied here, the major changes in fluorescence intensity occurred at a similar pH to that where the absorption spectra also changed. For this reason, it was believed that the pK_a values for each substance in the excited state was similar to those of the corresponding ground state. This conclusion was further substantiated by the fact that the wavelength of maximum fluorescence of substituted thiobarbituric acids was independent of the hydrogen ion concentration.

The ultraviolet absorption spectra of a series of thiobarbiturates were recently described by Smyth *et al.* (*6*). In conjunction with polarographic studies, these authors concluded that 5,5-disubstituted thiobarbiturates exist in two predominant forms in alkaline solution. The *N*-protonated zwitterion (I) dissociates above pH 11 to form the monoanion (II). The latter authors suggested that the diketo forms were the most likely tautomers present.



I II

The results presented here are fully consistent with this hypothesis. The absence of luminescence from the di-*N*-methylated compound can be understood in terms of its inability to form an anion. The non-fluorescence of the mono-*N*-methylated compound at room temperature probably reflects a high coefficient of thermal deactivation of the excited state. At 77K, 1-methyl-5-ethyl-5-(1[1]-methylpropyl)thiobarbituric acid showed only 0.25 of the intensi-

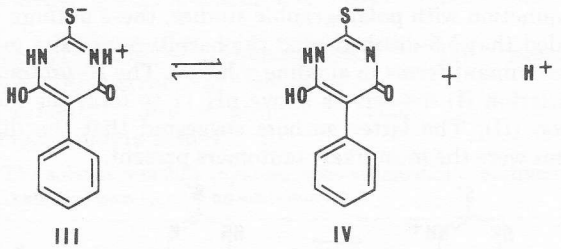
ty of fluorescence exhibited by buthalitone at a similar concentration. Differences in the temperature coefficient of fluorescence are also encountered between oxobarbiturates and have been discussed by King *et al.* (*10*). The red shift in the wavelength of maximum fluorescence at 77K of 1-methyl-5-ethyl-5-(1[1]-methylpropyl)thiobarbituric acid relative to thiobarbiturates unsubstituted at positions 1 and 3 in the heterocyclic ring could reflect the inductive effect exerted by the methyl group on the conjugated system.

The presence of two fluorescent species in 5,5-disubstituted thiobarbiturates at pH's >5 is also suggested by the magnitude of the deuterium isotope effect as a function of pH. Since the fluorescence of the unprotonated anion II is more susceptible to enhancement, it would appear that a fairly non-specific solvent–solute interaction is involved rather than specific deuteration of facile hydrogen atoms. The lower intensity of fluorescence at pH 9 relative to pH 13 may be associated with *N*-protonation and the correspondingly lower electron density in the conjugated system. However, protonation would not necessarily be expected to change the wavelength of maximum fluorescence.

The differential sensitivity to solvent quenching of thiobarbiturates and oxobarbiturates may also be associated with the hypothesis that the two classes of barbiturates form quite distinct species in solution.

The relative quantum yields of thiopentone, buthalitone, and 5-ethyl-5-phenylthiobarbituric acid differ significantly in strongly alkaline solution at room temperature (see Table II) but are similar below pH 11. The decrease in fluorescence intensity above pH 13 probably arises from alkali quenching; a similar effect is observed in oxobarbiturates (*1*). The presence of an unsaturated group in the 5-position would appear to enhance the fluorescence quantum yield. This is the reverse of the situation encountered with oxobarbiturates (*1*). The anomalous position of 5-ethyl-5-phenylthiobarbituric acid is also demonstrated by the deuterium isotope effect.

Since thiobarbituric acid is non-fluorescent, it presumably does not form species of the type shown in I and II. Furthermore, the absorption spectra of thiobarbituric acid at various pH's are quite distinct from those of the 5,5-disubstituted acids. On the basis of further studies, Smyth *et al.* (*11*) believed that thiobarbituric acid and 5-substituted acids existed predominantly in the form of enols. By analogy with the 5,5-disubstituted thiobarbituric acids, the protonated species (III) can be assumed to be in equilibrium with the corresponding mono-anion (IV). In each case, only one of the possible tautomers is shown.



III IV

The low temperature luminescence data for 5-phenylthiobarbituric acid are readily interpreted in terms of the two structures shown above. Since the wavelength of maximum excitation is constant over the pH range 0–13, it is likely that there is no change in the degree of conjugation with hydrogen ion concentration. The luminescence characteristics observed and the absence of emission from an isolated phenyl group suggest that the phenyl group is conjugated with the heterocyclic ring. The similarity in the excitation spectra of the phosphorescence and fluorescence emissions from 5-phenylthiobarbituric acid suggests that only one major chromophoric type is involved in the luminescence process. It is suggested that the absence of fluorescence from this compound at low pH is associated with *N*-protonation which in turn leads to an increase in the intersystem ($S_1 \rightarrow T_1$) crossing rate constant.

A comparison of the results obtained previously for the oxobarbiturates (*1*) and the thiobarbiturates studied here shows marked differences in the pattern of ionization and the types of tautomers which may be formed. It is probable that these changes arise from the different electronegativities of the oxygen and sulfur atoms at position 2. The presence of a quaternary nitrogen in the zwitterion causes a change in hybridization from sp^2 to sp^3 and a concomitant change in the steric configuration of the molecule. It was shown previously (*1*) that the steric configuration of the oxobarbiturates influenced their luminescence properties.

Although low temperature techniques did not improve the limit of detection of thiopentone, buthalitone, or 5-ethyl-5-phenylthiobarbituric acid, this being *ca.* 0.1 μg/ml at 20 °C and at 77K, some compounds are clearly capable of determination only at 77K. In this respect, analytical results at 77K complement measurements made at room temperature. In principle, the identity of a luminescent thiobarbiturate could be provisionally obtained by examining the effect of pH on the intensity of luminescence or by measuring the quantum yield of fluorescence relative to a known standard.

The results presented here provide further evidence for the nature of the species of thiobarbiturates present in solution and enable quantitative luminescence analysis and physiological investigations at the molecular level to proceed on a sounder theoretical basis.

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