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Structure-Luminescence Correlations in the Oxybarbiturates

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The ultraviolet absorption spectra, fluorescence and phosphorescence excitation and emission spectra of sixteen barbiturates and certain model compounds have been examined and correlated. The structure of the fluorescent dianion in the 5,5-disubstituted barbiturates was shown. Phosphorescence was observed at a lower wavelength than that of the prompt fluorescence at room temperature for those barbiturates containing a 5-phenyl substituent. The fluorescence derived from the dianionic form of the heterocyclic ring, whereas the phosphorescence derived from the aromatic nucleus. The effects of steric interactions and pH on the phosphorescence of the phenyl substituted barbiturates are discussed. Detection limits at room temperature and at 77 K for the barbiturates in common use have been determined.

The luminescence properties of the oxybarbiturates have been studied by several groups of workers (*1–5*) usually with the intention of developing analytical methods at microgram or submicrogram levels. In some cases, only room-temperature fluorescence characteristics have been described, but more recently the development of low-temperature (77 K) techniques has permitted observations of phosphorescence also (*5–7*).

Many oxybarbiturates exhibit an analytically-useful fluorescence in alkaline solutions at room temperature. Those containing *N*-methyl groups, however, are virtually nonfluorescent. Barbiturates containing a phenyl ring at the 5-position may exhibit a fairly strong phosphorescence, with lifetimes of several seconds, even when *N*-methyl groups are also incorporated. The present workers, and more recently Miles and Schenk (*7*) have noted that this phosphorescence occurs at a shorter wavelength than the room-temperature fluorescence.

In an attempt to explain these phenomena, the pH-dependence of the absorption and emission spectra of a number of oxybarbiturates have been studied. The results presented here may enable the luminescence analysis of these molecules to proceed on a sounder theoretical foundation, and provide further information on structure–luminescence relationships in heterocyclic species.

EXPERIMENTAL

The barbiturates studied are shown in Table I: the melting points were within 2 °C of literature values, and they were shown to be homogeneous using the thin-layer chromatography technique of Bogan, Rentoul, and Smith (*8*). 5-Ethylbarbituric acid, mp 190–191 °C; 5-phenylbarbituric acid, mp 265–266 °C; and 5-benzylbarbituric acid, mp 209–210 °C were prepared by condensation of the appropriate malonic ester with urea in the presence of sodium ethoxide; the success of these syntheses was verified using NMR spectroscopy and mass spectrometry. Other barbiturates were gifts from the companies named in Table I.

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 8.4–10.0. Solutions with pH's below 2.0 and above 10.0 were obtained using dilute hydrochloric acid and sodium hydroxide, respectively. All pH's were measured at room temperature with a Pye-Unicam 290 pH-meter.

Uncorrected excitation, fluorescence and phosphorescence spectra, phosphorescence lifetimes, and detection limits were determined using a Baird-Atomic SF100E spectrofluorimeter, as previously described (*5*). Relative quantum yields were determined using excitation and emission wavelengths at 260 and 405 nm, respectively. Solutions containing approximately 30 μg of barbiturate per ml of 0.1*M* ethanolic NaOH were used. All solutions had optical densities of $\leq$0.02. The solvents used for low temperature work were obtained by adding equal volumes of ethanediol to the appropriate aqueous solutions. Water was distilled three times from an all-glass apparatus and ethanol was purified as previously described (*5*).

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

RESULTS

Absorption Spectra. Table II shows the principal absorption maxima and molar extinction coefficients of several important oxybarbiturates at various pH values. In the spectra of barbituric acid (Figure 1), the curves obtained in the pH range 1.0–7.0 apparently passed through a single isosbestic point. There were no changes in the spectrum between pH 7.0 and pH 10.0, but above pH 10 there was a bathochromic shift in the absorption maximum and a decrease in the extinction coefficient. All the curves in this alkaline pH region passed through a new isosbestic point. Sodium hydroxide concentrations greater than 1*M* produced no further changes in the spectrum. All the changes described could be reversed by addition of acid to the solution.

Substitution of a single alkyl or aryl group at the 5-position had no marked effect on these phenomena: thus, 5-ethyl-, 5-phenyl-, and 5-benzylbarbituric acids all had similar properties to those of barbituric acid itself.

The absorption spectra of 5-phenyl 5-ethylbarbituric acid (phenobarbitone) at various pH values are shown in Figure 2. Two isosbestic points were again found but these were distinct from those of barbituric acid. Again a bathochromic shift was observed at high pH's, with an accompanying slight fall in extinction coefficient. The properties of 5-phenyl 5-methylbarbituric acid (rutonal) were very similar.

Methylphenobarbitone spectra (Figure 3) showed only a single isosbestic point, which occurred at a similar wavelength to that of the low-pH isosbestic point in phenobarbitone. 1,3-Dimethylphenobarbitone, however, had an ab-

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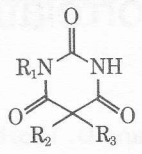
Table I. Oxybarbiturate Structure



Compound[a]	No.	R_1	R_2	R_3
Barbituric acid	1	H	H	H
5-Substituted acids				
Ethylbarbituric acid	2	H	H	Ethyl
Phenylbarbituric acid	3	H	H	Phenyl
Benzylbarbituric acid	4	H	H	Benzyl
5,5-Substituted acids				
*Rutonal	5	H	Methyl	Phenyl
*Phenobarbitone	6	H	Ethyl	Phenyl
*Barbitone	7	H	Ethyl	Ethyl
*Butobarbitone	8	H	Ethyl	*n*-Butyl
*Butabarbitone	9	H	Ethyl	sec-Butyl
*Pentobarbitone	10	H	Ethyl	1-Methylbutyl
*Amylobarbitone	11	H	Ethyl	Isoamyl
*Nealbarbitone	12	H	Neo-pentyl	Allyl
*Seconal	13	H	1-Methylbutyl	Allyl
*Cyclobarbitone[b]	14	H	Ethyl	Δ′-Cyclohexenyl
Heptabarbitone	15	H	Ethyl	Δ′-Cycloheptenyl
Diphenyl-barbituric acid	16	H	Phenyl	Phenyl
1,5,5-Substituted acids				
*Hexobarbitone	17	Methyl	Methyl	Δ′-Cyclohexenyl
Mebaral	18	Methyl	Ethyl	Phenyl
Metharbital	19	Methyl	Ethyl	Ethyl
Methohexitone[c]	20	Methyl	Allyl	1-Methylpent-2-ynyl
1,3,5,5-Substituted acids				
1,3-Dimethylphenobarbitone	21	*N,N′*-dimethyl	Ethyl	Phenyl

[a] Methylphenobarbitone was obtained from Winthrop Laboratories, Methohexitone from Eli Lilly, Diphenyl barbituric acid from ICI and Heptabarbitone from Geigy. Other commercial barbiturates* were obtained from May and Baker Ltd. [b] Ca salt. [c] Na salt.

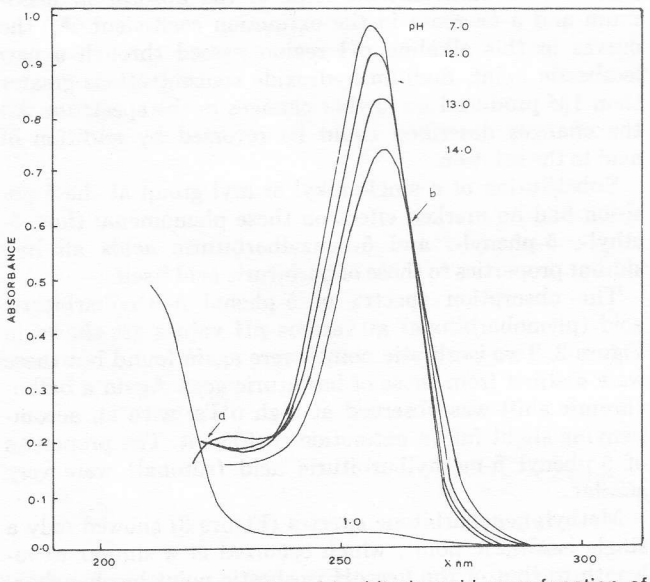


Figure 1. Absorption spectra of barbituric acid as a function of pH showing two isosbestic points *a* and *b*

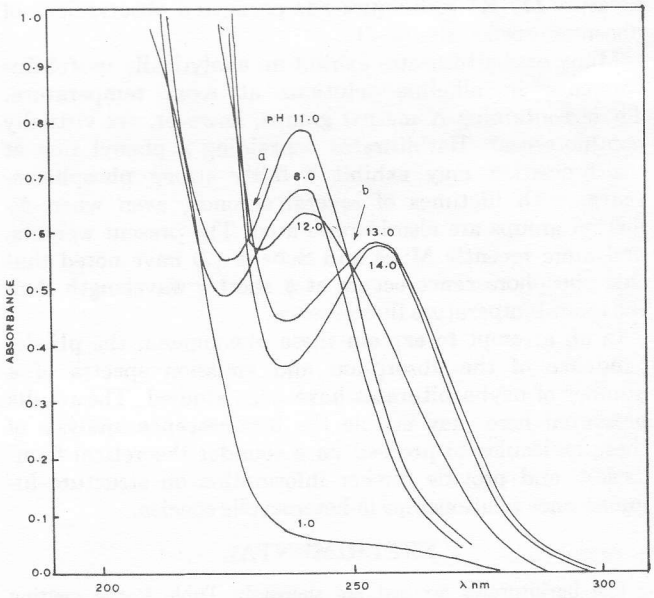


Figure 2. Absorption spectra of phenobarbitone as a function of pH showing two isosbestic points *a* and *b*

sorption spectrum which was independent of pH, with a strong absorption below 235 nm.

Fluorescence Spectra at Room Temperature. At 20 °C, the fluorescence of barbituric acid, 5-ethylbarbituric acid, and 5-benzylbarbituric acid in 0.1*M* aqueous NaOH showed a very weak emission at approximately 400 nm. The intensity in each case was 50% less than the solvent Raman scatter signal. 5-Phenylbarbituric acid showed a more intense fluorescence (λ_e = 300 nm, λ_f = 410 nm) which, however, remained less intense than that of a typical 5,5-disubstituted barbiturate (Figure 4). In neutral ethanol, 5-phenylbarbituric acid was nonfluorescent, but

Table II. Principal Absorption Maxima and Extinction Coefficients of Oxybarbiturates

Compound	pH	λ_{max}	$\epsilon \times 10^{-3}$
Barbituric acid	1	257	0.6
	7–10	257	19.8
	12	258	18.6
	13	259	16.9
	14	259.5	15.1
5-Phenylbarbituric acid	1	262	8.0
	7–11	267	21.6
	12	267	19.7
	13	268	18.8
	14	269	17.9
Benzylbarbituric acid	1	250	5.6
	10	263	17.1
	13	264	13.5
	14	265	13.3
5-Ethyl-5-phenyl-barbituric acid	1	240	0.7
	8	240	8.0
	11	240	9.3
	12	242	7.5
	13	255	6.9
	14	256	6.8
1-Methyl-5-ethyl-5-phenylbarbituric acid	1	246	1.3
	7	246	2.6
	8	246	5.6
	10–14	246	8.5

Table III. Fluorescence Characteristics of 5,5-Disubstituted Oxybarbiturates at Room Temperature in 0.1*M* Aqueous NaOH

Compound	λ_e, nm	λ_f, nm	Detection limit, μg/ml
Rutonal	278	420	1.5
Phenobarbitone	278	420	1.5
Barbitone	277	420	0.1
Butobarbitone	276	420	0.1
Butabarbitone	276	420	0.1
Pentobarbitone	276	420	0.05
Amylobarbitone	276	420	0.1
Nealbarbitone	277	420	1.0
Seconal	277	420	1.0
Cyclobarbitone	277	420	3.0
Heptabarbitone	277	420	3.0

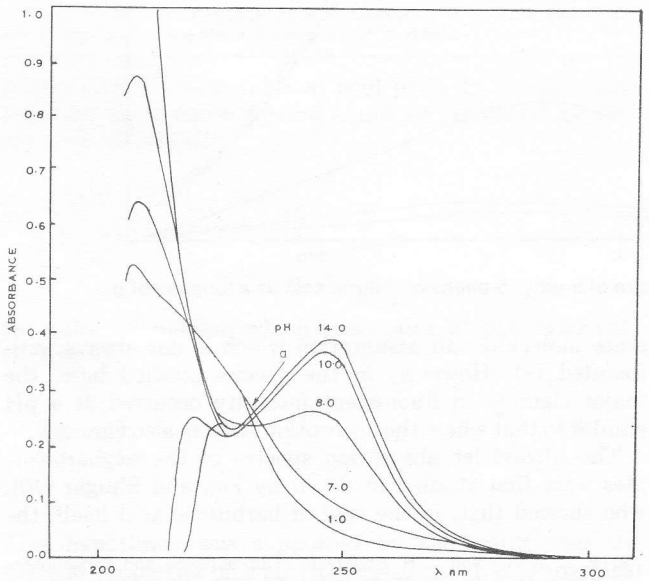


Figure 3. Absorption spectra of *N*-methyl phenobarbitone as a function of pH showing a single isosbestic point *a*

Table IV. Relative Quantum Yields of Some Oxybarbiturates at Room Temperature in 0.1*M* Ethanolic NaOH

Compound	Relative quantum yield
Phenobarbitone	0.05
Barbitone	0.55
Butabarbitone	1.00
	Reference
Pentobarbitone	0.96
Amylobarbitone	0.61
Seconal	0.13

Table V. Luminescence Characteristics of Oxybarbiturates at 77 K. The Solvent Was 0.1*M* NaOH in Ethanediol: Water, 1:1 v/v

Compound	λ_e, nm	λ_p, nm	Detection limit, μg/ml
Phosphorescence			
Rutonal	266	395	0.5
Phenobarbitone	266	395	0.3
Mebaral	260	395	0.5
Fluorescence (total luminescence)			
	λ_e, nm	λ_f, nm	
Rutonal	266	395	0.3
Phenobarbitone	266	395	0.2
Barbitone	265	410	0.3
Butobarbitone	265	410	0.4
Butabarbitone	266	410	0.2
Pentobarbitone	266	410	0.2
Amylobarbitone	265	410	0.1
Nealbarbitone, Seconal, Cyclobarbitone, Heptabarbitone	Weak fluorescence of comparable intensity to that of Raman solvent emission.		

the fluorescence reappeared on the addition of NaOH to a final concentration of 0.1*M*.

Most of the 5,5-disubstituted barbiturates were fluorescent in alkaline solution, the excitation and emission spectra of phenobarbitone (Figure 5) being typical. This fluorescence had a maximum intensity at pH 13.0–13.5, and was absent at pH's below 10.0. Emission and fluorescence wavelengths and detection limits for these compounds are listed in Table III. Species with aromatic or unsaturated aliphatic substituents (compounds 5, 6, 12–15) had a less intense fluorescence than the others. Absolute quantum yields were not determined, but the relative quantum yields of some of the barbiturates are given in Table IV. *N*-Methyl derivatives of 5,5-disubstituted barbiturates (*i.e.*, compounds 17–21) were nonfluorescent at all pH's.

Luminescence Spectra at 77 K. In a 1:1 v/v water: ethanediol solvent containing 0.1*M* NaOH, the barbiturates listed in Table V had fluorescence properties similar to those exhibited at room temperature, although a slight blue shift was observed in the wavelength maxima of the absorption and emission spectra. Again the fluorescence of these compounds was only observed in alkaline solution, but in the same conditions barbiturates with allyl and other non-aromatic unsaturated substituents (compounds 12–15) were only weakly luminescent.

All barbiturates with a 5-phenyl substituent showed a delayed luminescence at 77 K, with a lifetime of several seconds. This emission had a maximum intensity at *ca.* 395 nm and showed a considerable degree of fine structure, similar to that found in the phosphorescence spec-

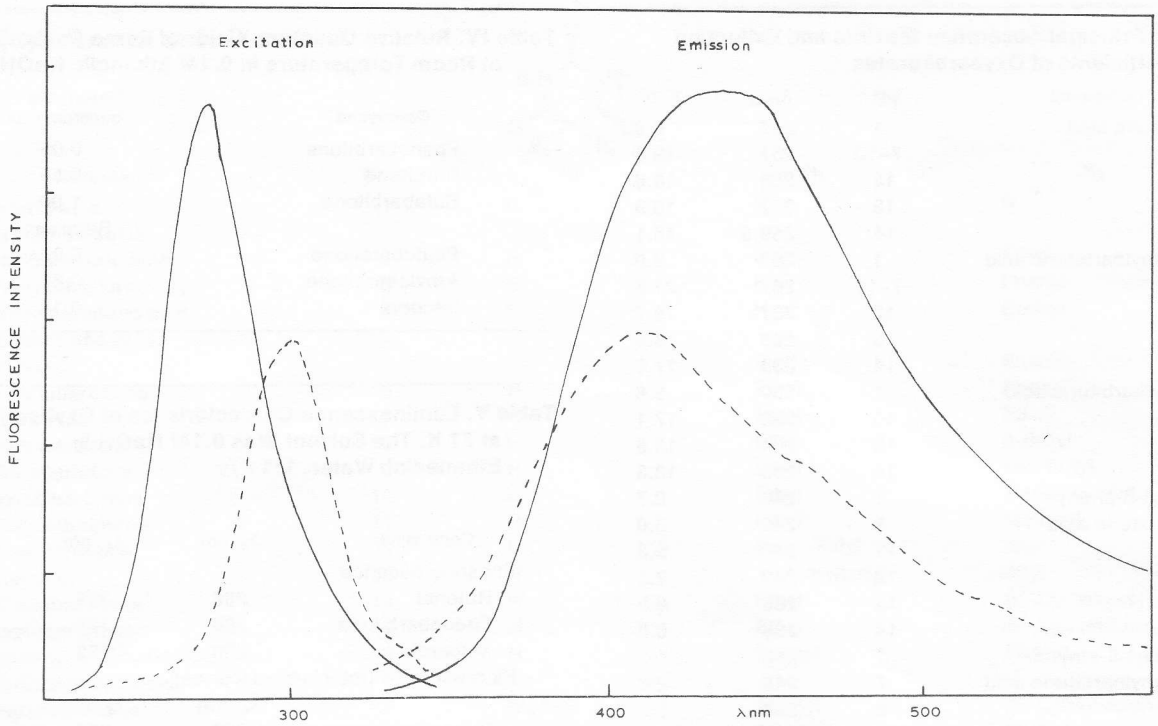


Figure 4. Room temperature fluorescence excitation and emission spectra of 5,5-diethylbarbituric acid (——) and 5-phenylbarbituric acid (- - - -)

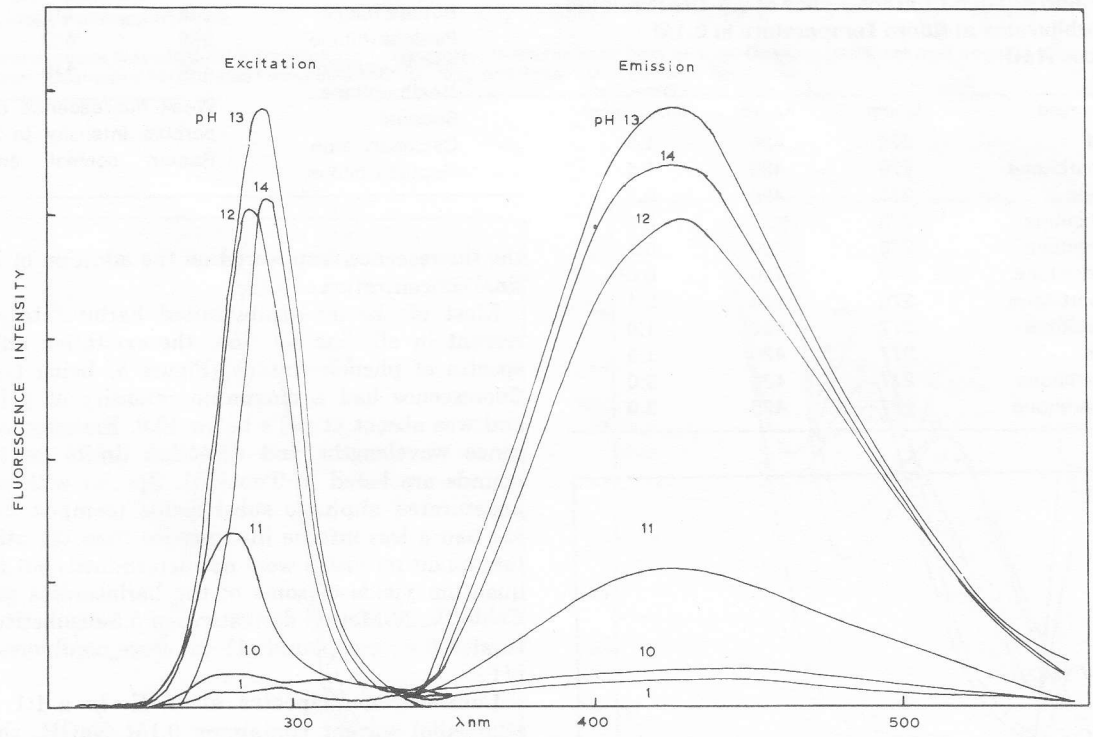


Figure 5. Room temperature fluorescence excitation and emission spectra of 5-ethyl-5-phenylbarbituric acid as a function of pH

trum of toluene (Figure 6). Furthermore, except in the case of 5-phenylbarbituric acid, it was maintained over the entire pH range 0–14, although some changes in lifetime did occur (Table VI). Benzylbarbituric was also phosphorescent, but only in alkaline solution.

DISCUSSION

Any attempt to interpret luminescence spectra on the basis of absorption spectroscopy rests upon the assumption that the pK_a values for each substance in the excited state are similar to those of the corresponding ground state molecules, an assumption which is not always well-founded (*9*). However, in the species studied here, the major changes in fluorescent intensity occurred at a pH similar to that where the absorption spectra also changed.

The ultraviolet absorption spectra of the oxybarbiturates were first studied in detail by Fox and Shugar (*10*), who showed that, in the case of barbituric acid itself, the

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(10) J. J. Fox and D. Shugar, *Bull. Soc. Chim. Belg.*, **61**, 44 (1952).

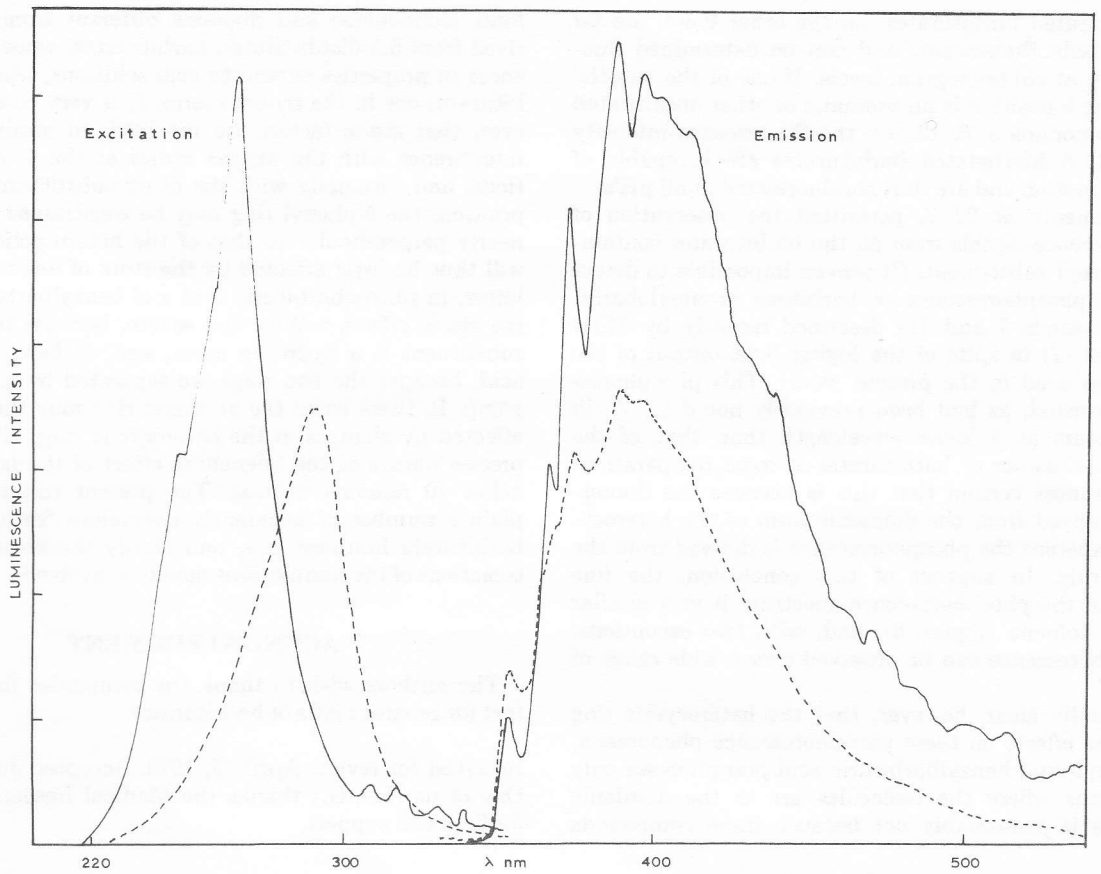


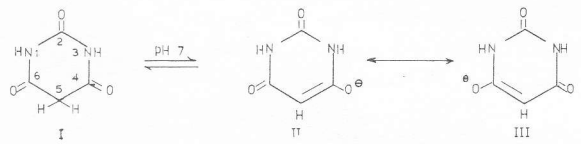
Figure 6. Luminescence excitation and emission spectra of toluene (——) and 5-ethyl-5-phenylbarbituric acid (----) in 0.1*M* ethanolic NaOH at 77 K

Table VI. Luminescence Characteristics of Oxybarbiturates at 77 K, in Various Conditions in Ethanediol: Water Solvent

	Conditions[a]		
Compound	0.1*M* H_2SO_4	Neutral	0.1*M* NaOH
5-Phenylbarbituric acid	N.L.	N.L.	P, τ = 2.0
Benzylbarbituric acid	F (W)	F (W)	P, τ = 4.6
Rutonal	P, τ = 3.0	P, τ = 3.0	P, τ = 4.1
Phenobarbitone	P, τ = 2.9	P, τ = 4.1	P, τ = 5.8
Diphenylbarbituric acid	P, τ = 2.2	P, τ = 2.4	P, τ = 2.4
Mebaral	P, τ = 3.5	P, τ = 3.6	P, τ = 3.7

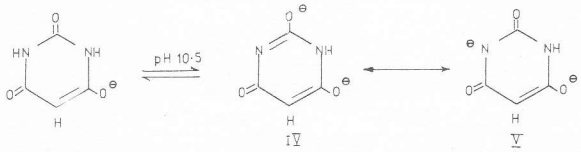
[a] N.L. = Non-luminescent, F = Fluorescent, P = Phosphorescent, τ = Phosphorescence lifetime, seconds, W = Weak.

triketo form (I) was stable at acid pH's. In the pH range 7–10 the mono-anion formed could be stabilized by tautomerism (II and III)



I II III

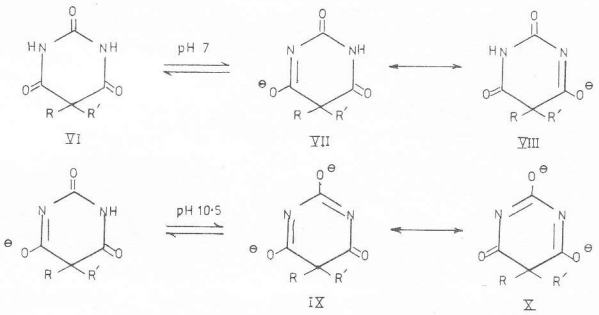
as could the dianion which was stable at pH's above 11 (IV and V):



IV V

This hypothesis was supported by the result that the spectral properties of methylbarbituric acid were identical to those of the parent compound. In the present work the spectra obtained for barbituric acid are similar to those of Fox and Shugar, and their hypothesis is further supported by the finding that replacement of a single hydrogen atom at the 5-position (compounds 2–4) has little effect on the spectra.

5,5-Disubstituted barbituric acids (compounds 5–15) are also capable of forming mono- and dianions, but as their absorption spectra are distinct from those of barbituric acid, the anions are presumably formed by a different mechanism:



VI VII VIII IX X

On this basis, compounds 17–20 (containing an *N*-methyl group) would be expected to show only the first ionization, and the spectra of *N*-methyl phenobarbitone indicate that this is indeed so. Dimethylphenobarbitone (compound 21) is incapable of ionization.

The pH dependence of the fluorescence of the barbiturates at room temperature shows clearly that the dianions are the fluorescent species. The dianions produced in barbituric acid itself and in other compounds with at least one hydrogen atom at the 5-position exhibit feeble fluorescence, except in the case of 5-phenylbarbituric acid: the enhanced fluorescence of this compound may be due to the ability of the phenyl ring to form a conjugated π-bonded system with the heterocyclic ring when the latter is in the dianionic form (IV and V). The dianions derived from

5,5-disubstituted barbiturates, on the other hand, are far more intensely fluorescent, and can be determined fluorimetrically at submicrogram levels. If one of the substituents at the 5-position is an aromatic or other unsaturated group (compounds 5, 6, 12–16), the fluorescence intensity is reduced. *N*-Methylated barbiturates are incapable of dianion formation and are thus nonfluorescent at all pH's.

Measurements at 77 K permitted the observation of phosphorescence signals from all the barbiturates containing a 5-phenyl substituent. (It proved impossible to detect the feeble phosphorescence of barbitone or amylobarbitone (compounds 7 and 11) described recently by Miles and Schenk (*7*) in spite of the higher light output of the xenon lamp used in the present work). This phosphorescence is unusual, as had been previously noted (*5, 7*), in that it occurs at a lower wavelength than that of the prompt fluorescence of barbiturates at room temperature. It seems almost certain that this is because the fluorescence is derived from the dianionic form of the heterocyclic ring, whereas the phosphorescence is derived from the aromatic ring. In support of this conclusion, the fine structure of the phosphorescence spectrum is very similar to that of toluene (Figure 6), and, with two exceptions, the phosphorescence can be observed over a wide range of pH's.

It is equally clear, however, that the heterocyclic ring exerts some effects on these phosphorescence phenomena, since phenyl- and benzylbarbituric acid phosphoresce only in conditions where the molecules are in the dianionic form. This is presumably not because these compounds form monoanions and dianions different from those derived from 5,5-disubstituted barbiturates, since the differences in properties extend to acid solutions, where all barbiturates are in the tri-keto form. It is very possible, however, that steric factors are involved: to minimize steric interference with the oxygen atoms at the 4- and 6-positions, and especially with the other substituent at the 5-position, the 5-phenyl ring may be constrained in a plane nearly perpendicular to that of the heterocyclic ring, and will thus be little affected by the state of ionization of the latter. In phenylbarbituric acid and benzylbarbituric acid, the steric effects will be less severe, because the other 5-substituent is a hydrogen atom, and, in benzylbarbituric acid, because the two rings are separated by a methylene group. In these cases the aromatic ring may thus be more affected by changes in the heterocyclic ring, although the precise nature of the quenching effect of the latter at pH below 10 remains unclear. The present results thus explain a number of apparently anomalous features of oxybarbiturate luminescence, and clarify the nature and interactions of the luminescent moieties involved.

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