in identifying unknown nitrogenous organic compounds.

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Electronic Spectra of 8-Mercaptoquinoline

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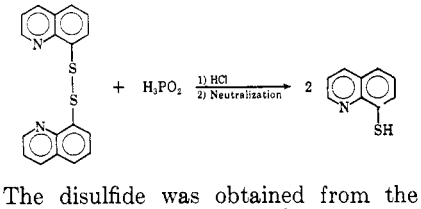
▶ **The electronic spectra of 8-mercaptoquinoline have been studied in aqueous solution and in nonaqueous solvents. The molar absorptivities of the various species have been redetermined and causes for disagreement with values of earlier investigators discussed. The blue color of 8-mercaptoquinoline has been shown to arise from a charge-transfer transition of the zwitterionic form, the analogous transition in 8-hydroxyquinoline being less evident and blue-shifted. The absorption spectra of 8-mercaptoquinoline are compared to those of 8-hydroxyquinoline and quinoline; differences between these spectra are discussed. Results of SCF-CI molecular orbital calculations support the spectral interpretations. The fluorescence of 8-mercaptoquinoline has been studied in nonaqueous solvents. Fluorescence spectra, both in wavelength and intensity, vary as a function of solvent. These and other observations are consistent with fluorescence from the second excited state of the 8-mercaptoquinoline zwitterion. Also, fluorescence spectra of the dihydrate of the mercaptan and of the cadmium(II) and zinc(II) chelates are reported.**

MANY contrasts are exhibited by 8-mercaptoquinoline (8MQ) to 8-hydroxyquinoline (8HQ), the most striking of which are the colors of the mercaptan and its chelates with metal ions. While 8HQ is a colorless solid and most of its chelates are light yellow, 8MQ is a deep blue liquid which forms a red crystalline hydrate, and its chelates show a variety of colors. Some spectral data have been reported which point up these differences (*1*, *3–5*, *14*), and some tentative assignments of the origins of the absorption bands of 8MQ have been suggested. However, to date there have been no detailed studies of the spectral characteristics of the mercaptan.

The present investigation was undertaken to provide a complete investigation of the electronic spectra of 8MQ, with a particular interest in differences of spectral behavior between 8MQ and 8HQ. The aqueous absorption spectra have been investigated as a function of pH and the molar absorptivities for 8MQ redetermined. The spectra of 8MQ and 8HQ are compared in detail and indicate that a larger extent of zwitterion formation in 8MQ is responsible for most of the spectral differences. In addition, 8MQ fluoresces and forms a fluorescent dihydrate, and some of its metal chelates show strong fluorescence.

EXPERIMENTAL

The mercaptan was prepared by the method of Corsini, Fernando, and Freiser (*11*) using the following reaction sequence:



The disulfide was obtained from the Dojin Pharmaceutical Co., Kumamotoski, Japan.

All solutions used were deaerated by bubbling with nitrogen, and the entire procedure was carried out under a nitrogen atmosphere. A portion of the freshly prepared mercaptan was dissolved immediately in deaerated distilled water to prepare an aqueous stock solution; the remaining mercaptan was dehydrated by drying overnight in a vacuum desiccator over sodium hydroxide pellets, and was then vacuum distilled (ca. 1 mm. Hg) into tubes which were sealed under vacuum. The mercaptan was stable indefinitely when stored in this manner.

The aqueous stock solution was analyzed to determine the mercaptan concentration by titration with iodine solution and an amperometric dead stop end point. The precision was better than $\pm 1\%$ of the determined concentration. Aliquots of the stock solution were then diluted to the appropriate concentrations with buffers of the desired pH. Hydrochloric acid, sodium hydroxide, acetic acid, and sodium bicarbonate were used to prepare the buffers. The pH of each mercaptan solution was measured after dilution.

Aqueous absorption spectra were obtained at pH −1, 5.2, and 13 to determine the molar absorptivities of the acidic, neutral, and basic forms, respectively, of the mercaptan. Spectra were also obtained at pH's about 2.0 and 8.4 (corresponding to the pK_a values) to determine the values of the acid-base equilibrium constants. Absorption spectra were obtained on a Cary Model 14 recording spectrophotometer. A Leeds and Northrup Model 7664 pH meter was used for pH measurement. Data processing calculations were done on the M.I.T. Laboratory for Nuclear Science IBM 7044 computer.

For nonaqueous studies, sealed tubes of anhydrous mercaptan were opened as needed, and the mercaptan was either dissolved in chloroform to prepare a stock solution or dissolved directly in the desired solvent. When chloroform stock solution was used, aliquots of the stock solution were diluted with the desired solvent before absorption spectra were obtained.

About 2% chloroform in the solutions caused no noticeable effect on the spectra. The concentration of the stock solution was determined by extracting the mercaptan from chloroform with 1*M* hydrochloric acid and measuring the absorbance of the acid solution. The extraction was quantitative.

Purification of Solvents. All solvents were deaerated by bubbling with nitrogen. For several of the solvents, elaborate steps were taken to ensure purity.

Carbon Tetrachloride. Fisher certified reagent #C-187 was degassed by the freeze-thaw technique. The solvent was then distilled onto sodium to remove water, and then was distilled into a tube which was sealed off and stored for later use. For fluorescence work, Matheson Coleman & Bell #SG2601 spectroquality reagent was used without further purification.

Acetonitrile. Eastman spectro grade #S488 was degassed by the freeze-thaw technique, distilled onto phosphorus pentoxide to remove water, and then distilled and stored for later use. For fluorescence work, Matheson Coleman & Bell #SF2726 spectroquality reagent was used without further purification.

Acetone. Harleco fluorimetric grade #92487 was treated as for carbon tetrachloride, except that Drierite was used to remove water.

Chloroform. Fisher certified reagent #C-298 was treated according to the method of Weissburger and Proskauer (*31*). For fluorescence work, Matheson Coleman & Bell #SG 5023 spectroquality reagent was used without further purification.

Ethanol. U.S.I. Absolute U.S.P.—N.F. (reagent quality) was deaerated by bubbling with nitrogen and used without further purification. (The solvent was used as soon as possible after the container was opened. If more than 1 day had elapsed, a fresh, previously unopened one was used.)

E.P.A. Solvent. (Ethyl ether, isopentane, and ethyl alcohol mixed in a 5:5:2 volume ratio). Harleco #92922 was used without further purification. (Here again the solvent was used as soon as possible after the container was opened. If more than 1 day had elapsed, a fresh, previously unopened one was used.)

Phosphorescence spectra and some fluorescent spectra were obtained on an instrument assembled from Aminco building blocks components. The fluorescence spectra obtained on the Aminco instrument used a 1P21 multiplier phototube. Both types of spectra are uncorrected. Other fluorescence spectra and all excitation spectra were obtained on a Turner Spectro 210 absolute spectrofluorometer.

The fluorescence spectrum of the solid mercaptan dihydrate was obtained by smearing a sample on a quartz plate and inserting it in the Turner spectrofluorometer at a 45° angle to the exciting radiation. Fluorescence of the zinc and cadmium chelates of 8MQ was obtained by extraction into chloroform from an aqueous solution of the metal ion and the mercaptan. Fluorometric grade reagents were used throughout.

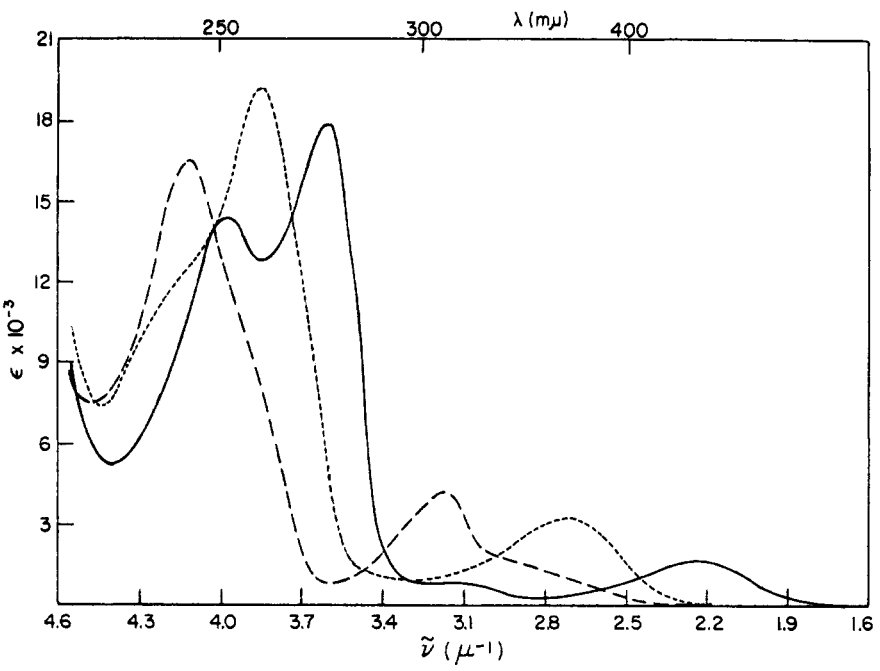


Figure 1. Aqueous absorption spectra of mercaptoquinoline

——— Neutral form, pH = 5.2
— — — — Acidic form, pH = −1
---------- Basic form, pH = 13

RESULTS

The absorption spectra of the three forms of 8MQ—cationic, neutral (zwitterionic), and anionic—responsible for absorption at various pH's are shown in Figure 1. The molar absorptivities of these species are tabulated in Table I along with the corresponding peaks for 8HQ and quinoline.

The molar absorptivities of the absorption maxima of 8MQ are tabulated in Table II, along with those reported by Albert and Barlin (*1*), Lee and Freiser (*14*), and Bankovskii, Chera, and

Table I. Aqueous Absorption Maxima of 8-Mercaptoquinoline, 8-Hydroxyquinoline, and Quinoline

Compound	pH	$\tilde{\nu}_{max}$(microns^{-1})	ϵ_{max}
8-Mercaptoquinoline			
Cation	−1	4.12	16,570
		3.16 (2.8)	4,280 (shoulder)
Neutral	5.2	3.97[a]	14,400
		3.60[b]	17,970
		3.16[a]	820
		2.24[b]	1,600
Anion	13	3.85	19,290
		2.72	3,250
8-Hydroxyquinoline (*28*)			
Cation	1	3.98	31,600
		3.25, 313	1,480 1,550
Neutral	7.6	4.18[a]	32,400
		3.71[b]	2,840
		3.28[a]	2,630
		2.32[b]	64
Anion	12	3.97	30,200
		2.99, 2.84	2,880 2,820
Quinoline (*12*)			
Cation	1.1	4.29	31,600
		3.19	6,310
Neutral	6.3	4.42	22,900
		3.64	3,240
		3.44, 3.20	2,880, 3,310

[a] Molecular form.
[b] Zwitterionic form.

Table II. Molar Absorptivities of Absorption Maxima of 8-Mercaptoquinoline

	This study		Albert and Barlin (1)		Lee and Freiser (14)		Bankovskii et al. (5)	
	$\tilde{\nu}_{max}$ (microns^{-1})	ϵ	$\tilde{\nu}_{max}$ (microns^{-1})	ϵ	$\tilde{\nu}_{max}$ (microns^{-1})	ϵ	$\tilde{\nu}_{max}$ (microns^{-1})	ϵ
Cation (pH 2 to 0)	4.12	16,570	4.13	20,000	...	...	4.17	20,870
	3.16	4,280	3.12	5,260			3.17	5,250
Neutral (pH 5.2)	3.97	14,400	3.94	14,800	...	...		
	3.60	17,970	3.57	19,100	...	...	3.61	26,400
	3.16	820	3.10	980	...	...	...	...
	2.24	1,600	2.17	1,740	2.23	2,032	2.15	2,460
Anion (pH 12 to 14)	3.85	19,290	3.80	19,100	...	...	3.85	22,300
	2.72	3,250	2.67	3,550	...	...	2.72	4,320

Table III. Solvent Dependence of $\tilde{\nu}_{max}$ of Visible Absorption Band of 8-Mercaptoquinoline

Solvent	$\tilde{\nu}_{max}$ (microns^{-1})	ϵ_{max}[a]	Mercaptan[b] in zwitterionic form, %
Water (pH 5.2)	2.24	1600	97
Ethanol	1.99	22	1.3
Acetonitrile	1.82	8	0.5
Acetone	1.77	2	0.14
Chloroform	1.78	1.5	0.09
Ethyl ether	...	$<10^{-4}$	$<10^{-5}$
Isooctane	...	$<10^{-4}$	$<10^{-5}$

[a] An apparent molar absorptivity calculated from total concentration of mercaptan in solution.
[b] Per cent zwitterion in water from Albert and Barlin (1); remaining figures are estimates based on apparent molar absorptivity of zwitterion absorption band.

Ievinsh (5). Table II shows that, in almost every case, the molar absorptivities found in the present study are lower than those reported in other studies. However, repeated purification of the mercaptan resulted in lowering of the apparent molar absorptivities until the values reported in this study were reached, after which further purification caused no change in extinction coefficients. When a solution of the mercaptan was allowed to stand in air so that some of the mercaptan was oxidized, the apparent molar absorptivities increased again. Thus, one (or more) of the mercaptan oxidation products absorbs more strongly throughout the ultraviolet and visible range than the mercaptan itself.

The major oxidation product of the mercaptan is 8,8′-diquinolyl-disulfide. A study of vacuum-sublimed samples of this material showed that although it absorbs strongly in the ultraviolet region, there is no absorption in the visible region. The pure mercaptan and the pure disulfide were both shown not to fluoresce in aqueous solution. However, when a solution of the mercaptan was allowed to oxidize in air, a green fluorescence appeared. Fluorescence excitation spectra of the solution showed definite differences from the absorption spectra of the pure mercaptan or of the pure disulfide (2). The shift in the absorption maximum of the long wavelength band reported by Albert and Barlin (1) is further evidence for the presence of an absorbing impurity in their solutions.

The molar absorptivity of the visible band reported by Bankovskii *et al.* (5) is not based on the total mercaptan in solution, but on that portion of the mercaptan calculated to be in the zwitterionic form. If the molar absorptivity is corrected, based on the total mercaptan as is the case in all the other studies being compared, the value obtained is 1950. This value is much nearer those reported in the other studies, and the difference between it and that in the present study can also be explained by the presence of an absorbing impurity.

The reported values for the acid-base equilibrium constants of the mercaptan do not show a wide range as do the molar absorptivities, indicating that the presence of a small amount of impurity has little effect on such determinations. Albert and Barlin (1) report pK_a values of 2.05 and 8.29 for the two constants; Bankovskii *et al.* (6) report values of 2.02 and 8.48; Barkovskii and Kharkover (7) report values of 2.11 and 7.9 to 8.6. The values obtained in the present study are 2.08 and 8.35 (for pK_{NH} and pK_{SH}, respectively).

Absorption spectra of 8MQ in non-

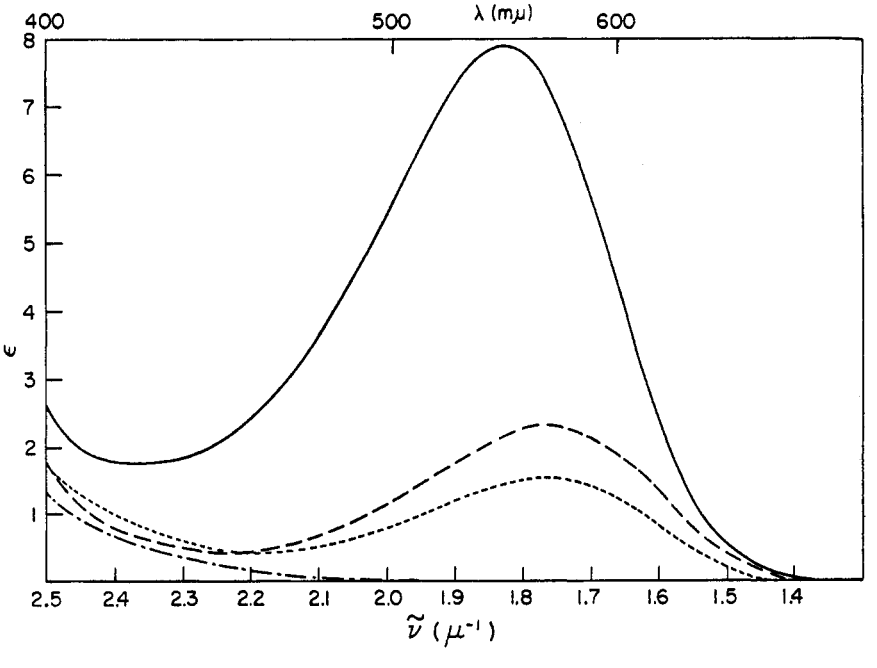


Figure 2. Visible absorption spectra of 8-mercaptoquinoline

Acetonitrile
Acetone
Chloroform
Isooctane

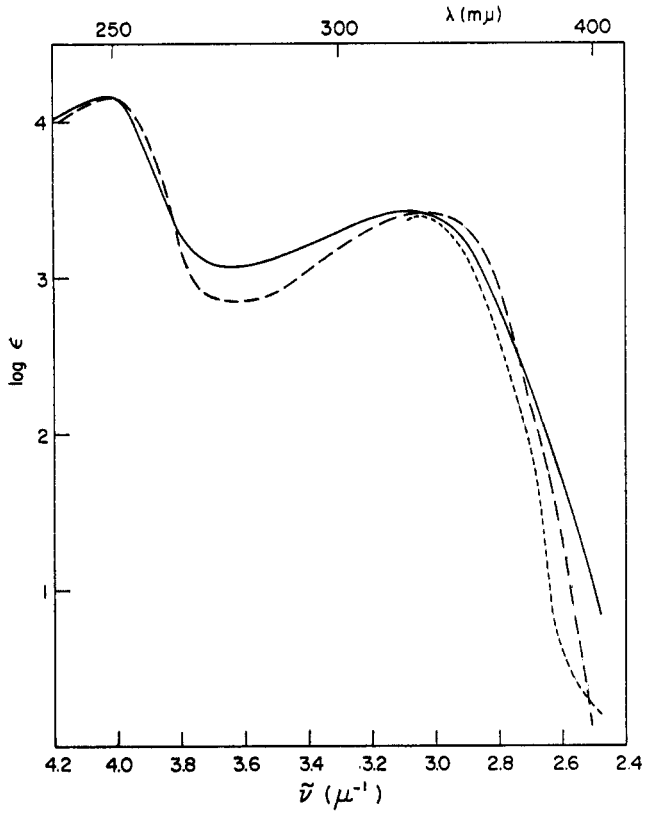


Figure 3. Ultraviolet absorption spectra of 8-mercaptoquinoline

——— Ethanol
---------- Acetone
— — — — Isooctane

aqueous solvents are shown in Figures 2 and 3. Figure 2 covers the visible region of the spectrum in which there appears one absorption band. The decrease in intensity and shift in frequency as the solvent polarity is reduced is evident. Figure 3 shows, for the same solvents, that intensities for the ultraviolet band are essentially unaffected by solvent, and the positions of the bands are shifted only slightly as is normal in changing from a polar to a nonpolar solvent. In Table III the intensities and positions of the visible absorption bands in nonaqueous solvents are compared to those in water and an estimate of the amount of zwitterionic form in solution is given for each solvent. The value for per cent of zwitterion in aqueous solution is from Albert and Barlin (*1*). The values for nonaqueous solutions were calculated from the apparent molar absorptivities in each of the solutions, assuming that the oscillator strength of the visible zwitterion absorption band is unaffected by the solvent. These are only rough estimates of the amount of zwitterion present.

In Figure 4 the absorption spectra of the mercaptan are shown in various mixtures of water and ethanol. An equilibrium between two species is responsible for the spectral changes observed. The absence of a well defined isobestic point is due to the frequency shift and slight changing of shape of the two bands as the solvent composition is varied.

Nuclear magnetic resonance spectra of the mercaptan were obtained to verify the presence of zwitterionic formation. In carbon tetrachloride a peak due to the mercaptal proton was observed at $\delta = 6$ p.p.m. (from TMS); but in acetonitrile, the peak was broadened, shifted to $\delta = 5.2$ p.p.m., and reduced in intensity. This behavior is characteristic of tautomeric equilibria, such as keto-enol tautomerism, and is consistent with increased zwitterion formation in acetonitrile.

The fluorescence spectra of 8MQ in several solvents are shown in Figure 5. Intensity is expressed in units of quantum efficiency per angstrom, the area under the curve giving the total quantum efficiency. The values of the quantum efficiencies are based on Melhuish's (*21*) value of 0.546 for dilute solutions of quinine sulfate. (A solution of 1-p.p.m. quinine sulfate in 1*N* H_2SO_4 was used as a standard.) The wavelength of maximum emission and the total quantum efficiency for the mercaptan in each solvent studied are listed in Table IV. The precision of the quantum efficiency determination is probably about ±0.003 or better, so the fluorescence observed in ethanol, which gave a small amount of fluorescence in

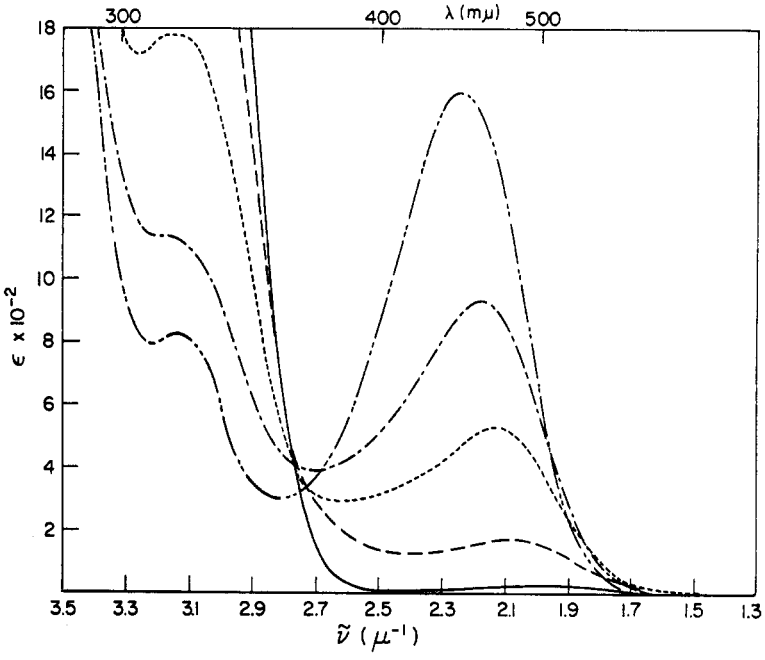


Figure 4. Absorption spectrum of 8-mercaptoquinoline at various solvent compositions: ethanol–water

——— 100% ethanol
— — — — 80% ethanol–20% water
---------- 50% ethanol–50% water
— — — — 30% ethanol–70% water
— — — — — 100% water

the blank, may be doubtful. The fluorescence in carbon tetrachloride is probably real because the blank gave no detectable fluorescence, and the purity of the mercaptan is such that impurities should contribute less than 0.001 to the quantum efficiency.

Table IV shows that the quantum efficiency of 8MQ increases as a function of solvent polarity in nonhydrogen bonding solvents. In fact, the ratio of the quantum efficiency in each solvent to the molar absorptivity of the visible absorption band is approximately constant. (Although the fluorescence efficiency in CH_3CN is largest of the solvents, the ratio is somewhat low.) This implies that the same form is responsible for both the fluorescence and the visible absorption band.

To verify that the observed fluorescence did not arise from impurities, the emission characteristics of the oxidation products of 8MQ were investigated. 8,8'-Diquinolyl disulfide (the major oxidation product) did not fluoresce but did phosphoresce weakly. Other oxidation products (the identities of which were not determined) phosphoresced with the same spectrum as that of the disulfide, but more intensely. These same oxidation products also showed fluorescence only slightly different from that of the mercaptan, but the fluorescence excitation spectra were different.

Because fluorescence was observed from the oxidation products of 8MQ, care was taken to ensure that the fluorescence was not due to impurities. The mercaptan was purified until the fluorescence remained unchanged; excitation spectra were obtained which matched exactly the absorption spectra of the mercaptan in the particular solvent; and the fluorescence efficiency at various excitation wavelengths and various concentrations was determined and found to be constant.

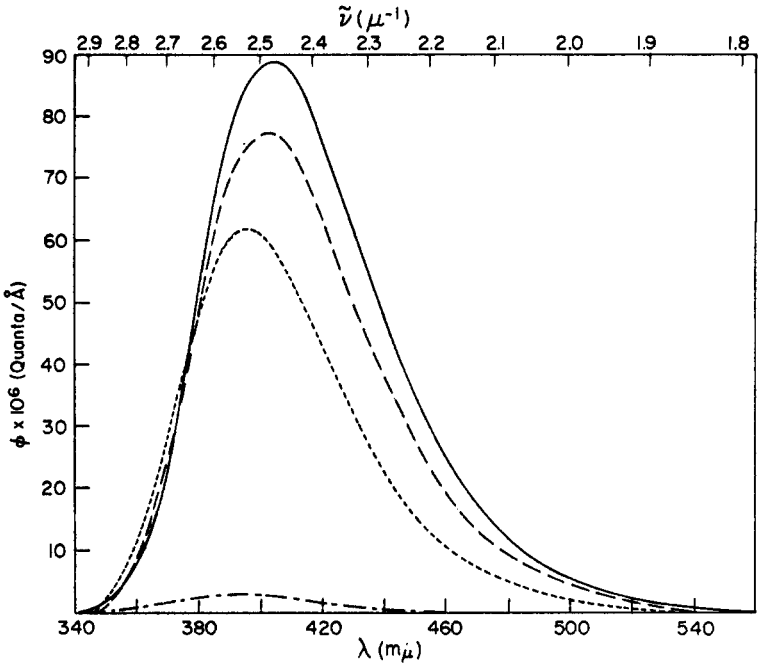


Figure 5. Fluoroescence spectra of 8-mercaptoquinoline

——— Acetonitrile
— — — — Acetone
..... Chloroform
— — — — — Carbon tetrachloride

Concentration, 1×10^{-5}M; temp. = 24° C. Spectra were obtained on Turner Spectro 210 and are absolute spectra

The mercaptan in hydrogen bonding solvents such as water and ethanol does not fluoresce. This can be attributed to the hydrogen bonding properties of the solvents. Fluorescence quenching through hydrogen bond formation has been reported for 8-hydroxyquinolates and other nitrogen heterocycles (*17–20*).

The dihydrate of 8MQ fluoresced showing a maximum at 1.48 micron^{-1}. Because the sample was solid rather than in solution, absorption and absolute fluorescence spectra could not be obtained readily. An excess or deficiency of water decreased the fluorescence intensity, indicating that it was the solid hydrate fluorescing. Furthermore, the color of the dihydrate is red and its fluorescence was red, a situation normal for Stokes' shift. The fluorescence intensity was reduced by the addition of excess water and was also reduced whenever the mercaptan was made anhydrous. No fluorescence was observed from the anhydrous liquid. Although the anhydrous mercaptan when frozen is the same red color as the dihydrate, no fluorescence was observed from frozen samples of the liquid at 77° K. The fluorescence intensity of the dihydrate was greatly enhanced at 77° K.

Zinc and cadmium chelates of 8MQ fluoresced in chloroform solution and the data on these chelates are summarized in Table V. The experimental error in determination of quantum efficiencies was about ±0.003. The absorption spectra of the chelates are not shown because a considerable amount of free mercaptan was extracted into the chloroform solvent, thus giving absorption bands for both chelate and mercaptan. From fluorescence excitation spectra, however, a good estimate could be made

Table IV. Dependence of Frequency and Quantum Efficiency of Fluorescence on Solvent

Solvent	$\tilde{\nu}_{max}$ (microns^{-1})	Quantum efficiency	Quantum efficiency/vis ϵ
Ethanol	2.44	<0.002	...
Acetonitrile	2.46	0.064	8×10^{-3}
Acetone	2.48	0.055	24×10^{-3}
Chloroform	2.53	0.041	27×10^{-3}
Carbon tetrachloride	2.56	0.002	$>20 \times 10^{-3}$
Water	No fluorescence observed		

Table V. Absorption and Fluorescence Maxima of 8-Mercaptoquinolates in Chloroform

Chelate	$\tilde{\nu}_{max}^{abs.}$ (microns^{-1})	$\tilde{\nu}_{max}^{fluor}$ (microns^{-1})	Quantum efficiency
Cadmium	2.52 3.70 shoulder[a]	1.87	0.111
Zinc	2.48 3.70	1.86	0.066

[a] The true positions of this peak should be at a considerably longer wavelength because it appears on the long wavelength side of a much larger band which causes a shift toward a shorter wavelength.

of the absorption spectra of the chelates, and these are the maxima reported in Table V. The absorption and fluorescence spectra of the chelates are almost identical to the absorption and fluorescence spectra of the mercaptan anion and the chelates of 8-hydroxyquinoline. This behavior is not unexpected because of the similarity between 8MQ and 8HQ. At 77° K., the fluorescence intensities of the mercaptan and the chelates in EDTA solution were increased; no phosphorescence was observed from either the mercaptan or its chelates.

DISCUSSION

Visible Absorption Spectra of 8MQ. In neutral solutions, 8MQ exists in two tautomeric forms—the molecular form (I) and the zwitterionic form (II).

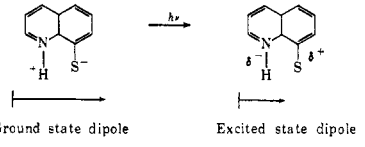
(I) (II)

The neutral molecule is expected to predominate in nonpolar solvents, while the zwitterion should become more prevalent in polar solvents. Although for 8HQ the zwitterionic form is present only to a small extent in any solvent, the greater acidity of the mercapto group (about two orders of magnitude) causes form (II) to become more prevalent for 8MQ in polar solvents. Albert and Barlin (*1*) have calculated that 97% of 8MQ in water is in the zwitterionic form, while less than 4% of 8HQ exists as the zwitterion in aqueous solution. This interpretation is supported by the behavior of the mercaptal proton peak observed in the NMR spectra.

Hydrogen bonding solvents will also have an effect on formation of the zwitterion. Because the SH group shows little tendency to form hydrogen bonds (*12*), hydrogen bonding will be expected only between proton donor solvents and the nitrogen of the mercaptan. On the other hand, both the NH^+ and the S^- groups may be strongly hydrogen bonded in both proton donor and proton acceptor solvents. Thus, hydrogen bonding solvents, especially hydroxylic solvents such as water and alcohols, will tend to stabilize the zwitterion more than the neutral molecule. Therefore, in nonpolar solvents, the neutral mercaptan will predominate, and as the polarity or the hydrogen bonding strength of the solvent increases, the equilibrium will be shifted toward the zwitterionic form.

Figure 2 and Table III show that intensity of the visible absorption band follows a pattern identical to that for zwitterion formation—in nonpolar solvents the intensity approaches zero, but as the solvent polarity and hydrogen bonding strength increase, the intensity of the band increases. Thus, the visible absorption band can be attributed to the zwitterionic form of the molecule. This conclusion is consistent with the interpretation of other workers (*11*, *12*, *14*). [The ratio of the molar absorptivity of the 2.24-micron^{-1} band in the mercaptan to that of the 2.23-micron^{-1} band in 8HQ is equal to the ratio of the percentages of zwitterion in the two compounds in aqueous solution, as determined by Albert and Barlin (*1*). This suggests that the two bands are identical in nature, and also that their intrinsic extinction coefficients are equal.]

The type of transition responsible for the visible absorption band of 8MQ has not yet been determined, although Lee and Freiser (*14*) have suggested the possibility that it arises from an $n \rightarrow \pi^*$ transition. Figures 2 and 3 and Table III show that the visible band undergoes a blue shift as solvent polarity is increased. This behavior is characteristic of Bayliss and McRae's case IV-a (*8*) where the solute dipole decreases during the transition. A transition within the ring structure could not possibly cause a large enough change in dipole to account for the magnitude of the solvent shift observed in 8MQ. The promotion of an electron from an orbital on the sulfur to a ring orbital would, on the other hand, easily give rise to a large enough change in the solute dipole:



There are two possibilities for such a transition: The first is an $n \rightarrow \pi^*$ transition from a sulfur orbital; the second is an intramolecular charge-transfer (*C-T*) transition involving a sulfur orbital. The large molar absorptivity (1600 in water), the blue shift observed when solvent polarity is increased, and the disappearance of the band in nonpolar solvents suggest that the low-energy absorption band in the mercaptan is due to a charge-transfer transition in the zwitterionic form of the molecule, rather than an $n \rightarrow \pi^*$ transition.

The rapid decrease in molar absorptivity of the *C-T* absorption band as solvent polarity and hydrogen bonding strength are decreased can be explained as follows. Because the calculated molar absorptivity is based on the total concentration of mercaptan in the solution, the apparent molar absorptivity of the *C-T* band will decrease as the concentration of the zwitterionic form is reduced. Thus, the *C-T* band becomes less intense as the equilibrium is shifted toward the neutral form of the mercaptan by less polar or less hydrogen bonding solvents.

Comparison of Spectra 8MQ with 8HQ. The absorption spectra of 8MQ and 8HQ are similar, the major differences being in the relative intensities of the bands. Generally, the shifts in positions of the bands correlate with changes in the electronegativities of the atoms perturbing the π-electron system.

A comparison of the data from Table I for all forms of the substituted quinolines shows that the intense bands at about 4.0 to 3.8 micron^{-1} are analogous to the $\pi \rightarrow \pi^*$ band of quinoline occurring at 4.42 micron^{-1}. Also, the weaker bands in the vicinity of 3.0 to 3.2 micron^{-1} appear to arise largely from the $\pi \rightarrow \pi^*$ band of quinoline at 3.6 to 3.2 micron^{-1}. This band in quinoline is interpreted as arising from two overlapping $\pi \rightarrow \pi^*$ transitions (*15*). Neither of these bands in the substituted quinolines is a pure $\pi \rightarrow \pi^*$ transition, but they have varying amounts of charge-transfer character, particularly the low-frequency band.

In the substituted quinolines there are three bands which do not have direct analogies in the quinoline spectrum. A band at about 2.8 micron^{-1}, on the low energy side of the 3.2-micron^{-1} band, appears most prominently in the spectra of the cationic and anionic species. It is proposed that this absorption band arises largely from a charge-transfer transition from the sulfur (or oxygen) atom to the quinoline ring. This interpretation is consistent with two features observed for the 2.8-micron^{-1} band. First, it occurs at lower energies in 8MQ species than in 8HQ species due to the lower electronegativity of the sulfur atom. Second, the band shows a red shift upon either protonation of the ring or ionization of the mercapto (or hydroxyl) group. This is because either process makes the ring a better acceptor relative to the substituent group and lowers the energy required for the transition. In addition, SCF-CI calculations indicate a large amount of charge-transfer character for this band.

The second band not having an analogy in quinoline is the band at 2.2 to 2.3 micron^{-1}. This band has been identified as being a charge-transfer transition in the zwitterionic form of the molecule. On the basis of the discussion above, it is reasonable to expect such a transition to occur even at lower energies because both protonation of the nitrogen and ionization of the mercapto (or hydroxyl) group have occurred in the zwitterion.

The third band, which has no analogy in quinoline, is located at 3.6 to 3.7 micron^{-1}. This band is absent from the spectra of both 8MQ and 8HQ in nonpolar solvents but occurs in polar solvents, its appearance paralleling that of the visible zwitterionic band at 2.2 to 2.3 micron^{-1}. This indicates that this band arises from a transition of the

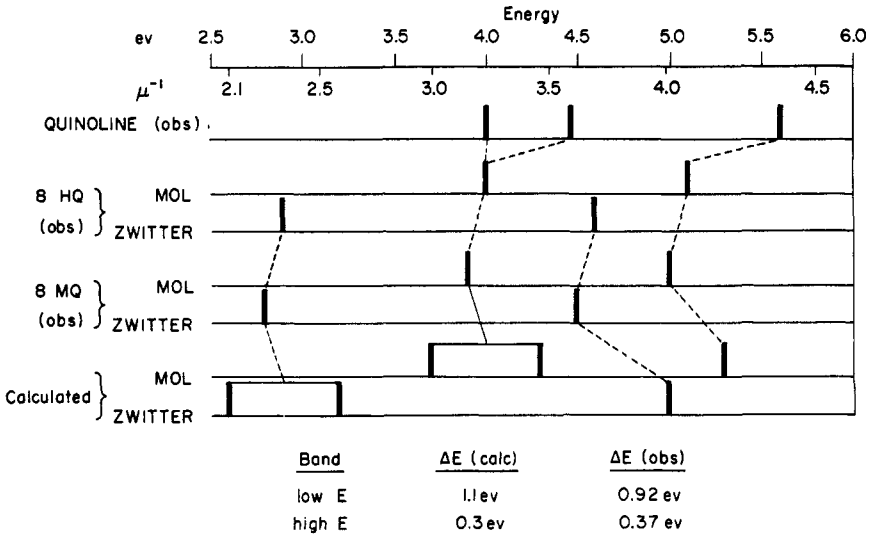


Figure 6. Comparison of calculated and observed energies for electronic transitions of 8-mercaptoquinoline

zwitterionic form of the molecule and that it is probably analogous to the 4.42 micron^{-1} band in quinoline. The relative intensities of this band in 8-hydroxyquinoline and 8-mercaptoquinoline support this assignment.

Molecular orbital calculations were performed to substantiate conclusions derived from the experimental data. First, semiempirical Pariser-Parr, self-consistent field calculations with configurational interaction were done for the molecular form of 8MQ. The parameters used were estimated from those of Pariser and Parr (*23–26*), Streitweiser (*29*), and Orloff (*22*). The results of this calculation gave a good qualitative fit to the observed transitions for the neutral molecule in solution (Figure 6). The calculated energies show two closely spaced transitions in the vicinity of 4 e.v. (3.0 to 3.5 micron^{-1}) (obs. = 3.6 micron^{-1}), and a third transition at about 5.3 e.v. (413 micron^{-1}) (obs. = 3.97 micron^{-1}). The lowest energy transition is largely y polarized, the second is largely x polarized; both of these transitions show considerable charge transfer character with the charge being transferred from the sulfur to the ring, especially to the nitrogen atom. The observed 3.16-micron^{-1} band is fairly broad and could easily be a composite of the two calculated charge-transfer transitions. In this respect, 8MQ is not unlike an alpha-substituted naphthalene where the two low-energy electronic transitions are merged into a single band. The third transition is largely x polarized and also shows some charge transfer character. Thus, the SCF–CI calculations support our interpretation that the 3.97-micron^{-1} band of the molecular form of 8MQ arises from a transition similar to the 4.42-micron^{-1} band of quinoline (with some charge-transfer admixture), while the 3.16-micron^{-1} band results from a charge-transfer transition. The present argument also is supported by observations by Bhatnagar and Forster (*10*) and Perkampus and Kortüm (*27*).

Energy level calculations were done for the zwitterion by performing first-order perturbation calculations on the SCF orbitals of the molecular form using Mason's perturbation parameters (*16*). The results gave a good qualitative fit to the zwitterion spectrum (Figure 6). The two low-energy bands are shifted to approximately 3 e.v. (2.11 to 2.57 micron^{-1}) (obs. = 2.24 micron^{-1}), while the high-energy band is shifted to about 5 e.v. (4.05 micron^{-1}) (obs. = 3.60 micron^{-1}). These predictions correlate well with the small experimental shift (0.37 micron^{-1}) observed for the high-energy band on going from molecular to zwitterionic form, and with the larger shift (0.92 micron^{-1}) observed for the low-energy band.

Fluorescence Spectra. Interpretation of the fluorescence data for 8MQ is not completely straightforward. The fluorescence seems to parallel formation of the zwitterion in 8MQ, as is evident from the relationship between fluorescence intensity and solvent polarity given in Figure 5 and Table IV. As with the low-energy absorption band, the intensity of fluorescence increases with increasing solvent polarity and fluorescence maximum shifts to lower energies. The energy of fluorescence makes it certain that the observed emission does not arise from the excited state responsible for the long wavelength charge-transfer transition of the zwitterion. (In chloroform the *C-T* absorption is at 1.78 micron^{-1} and emission is at 2.53 micron^{-1}, requiring an impossible anti-Stokes shift of 0.75 micron^{-1}.)

The following are possible explanations for the fluorescence observed for 8MQ: trace impurities; emission from the lowest excited singlet state of the molecular form of 8MQ; emission from the second excited singlet of the zwitterionic form of 8MQ.

It is likely that an impurity giving an apparent quantum efficiency of 6% would be present in sufficient quantity that it could be detected by thin-layer chromatography, or that it should absorb sufficiently to perturb the excitation spectrum. These arguments combined with fluorescence behavior on repeated purification reported above tend to indicate that the fluorescence observed for 8MQ is not due to an impurity.

One argument favoring fluorescence from the molecular species is that the fluorescence excitation spectra match the observed absorption spectra which are due to the molecular species in the solvents studied. However, this is not conclusive because excited state zwitterion formation could cause fluorescence to be observed that would be characteristic of the zwitterion rather than the molecular form.

The magnitude of the integrated absorption spectrum for the molecular form is consistent with the fluorescence efficiency of 0.06 observed for 8MQ in acetonitrile. However, the second electronic transition in the zwitterionic form also has sufficient intensity to account for the observed fluorescence efficiency. The fact that the fluorescence spectrum shows a considerable solvent shift and solvent dependence of intensity tends to argue against fluorescence by the molecular species because the absorption spectra depend little on solvent. The disulfide of 8MQ is not fluorescent in any of the solvents studied, and on this basis one would also expect a lack of fluorescence from the molecular form of 8MQ. Furthermore, there is some evidence for the presence of a long wavelength $n \rightarrow \pi^*$ transition in nonpolar solvents in 8MQ similar to that observed for 8-hydroxyquinoline (*1, 28*). All of these data taken together tend to indicate fluorescence does not come from the molecular form of 8MQ.

The relationship between fluorescence intensity and solvent polarity, the solvent dependency of band frequency, and the location of the fluorescence band all are consistent with the postulate that the 8MQ zwitterion fluoresces from the second excited singlet state. To date there has been reported only one authenticated example of fluorescence from the second excited singlet state and this is for azulene (*9, 30*) which bears some striking similarities to the 8MQ zwitterion.

Both azulene and the 8MQ zwitterion are blue in color, having widely separated first and second excited singlet states. In azulene the separation is

about 1.4 micron^{-1}, while in 8MQ it is 1.3 micron^{-1} in the pure liquid and slightly larger in nonpolar solvents. (This is a sufficiently large gap to slow internal conversion and allow fluorescence to occur.) Also, both azulene and the 8MQ zwitterion are not phosphorescent, indicating strong coupling between the lowest excited singlet state and the ground state via the lowest triplet. Therefore, we propose that the fluorescence in 8MQ arises from fluorescence of the second excited singlet state of the zwitterionic species. We recognize that this proposal must be regarded as a tentative assignment awaiting confirmation from life-time and polarization studies.

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Sensitive Spectrophotometer for Measuring Kinetics of Reversible Systems

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▶ **Details are given for construction of a sensitive spectrophotometer whose components include a double monochromator for achieving a high spectral purity in the detecting beam and a feedback circuit for stabilizing the light emitted from the detecting beam source. The instrument is capable of quantitatively measuring changes as small as 5×10^{-5} absorbance unit, has a resolution of 1 A. over much of the spectrum (2600 to 12000 A.), and can measure precisely kinetic parameters which may vary from 10^{-4} second to minutes. Typical light-induced absorbance changes for the photosynthetic bacterium *Rhodospirillum rubrum* are presented, and the importance of using a low intensity detecting beam for such systems is underscored.**

Measurement of fast photochemical reactions in solution was first achieved by use of a high-intensity flash lamp (*14–16*) for rapid (10^{-6} to 10^{-4} second) excitation followed by a second low-intensity flash for measuring absorbance changes. Extension of this method (*23–25*) has been employed for studies of photosynthetic systems. While more rapid changes (10^{-6} to 10^{-4} second) may be followed, the present limitation in sensitivity is of the order of 5×10^{-4} absorbance unit.

A more sensitive method takes advantage of the availability of commercial digital memory averagers as signal improvement and storage devices (*8*). However, the fastest reactions that can be followed are of the order of 10^{-4} second. One of the most rewarding applications (*7, 9, 10*) is the measurement of the small changes in absorbance displayed by photosynthetic material as a response to light excitation. Such light-induced absorbance changes were first discovered by Duysens (*4*). The photosynthetic systems are ideal for signal improvement because they are rapidly excited by radiation in the visible region of the spectrum, the quantum yield for excitation is near 1 (*2, 22*), there is a wide range of absorbance changes that can be observed (from 200 to 1300 mμ, some positive and some negative), the dark decay times may vary from a few milliseconds to many seconds depending on the conditions, and most changes are fully reversible (*21*).

From performance characteristics of previously reported kinetic spectrometers which utilize the signal averaging technique (*7, 10*), 10^{-3} absorbance unit of reversible change can be detected with a peak to peak $S/N = 10$ after a few minutes averaging time. Each of these instruments uses a single monochromator for its detecting beam and operates with a typical resolution of approximately 50 A. The kinetic spectrometer reported herein has a 10-fold increased sensitivity, uses a double monochromator for its detecting beam having a resolution of less than 1 A. over much of the spectrum (260 to 1200 mμ), and is capable of measuring precisely kinetic parameters which may vary from 10^{-4} second to minutes.

EXPERIMENTAL

Methods and Materials. A schematic drawing of the optical arrangement used is shown in Figure 1. The detecting beam light source is a tungsten-halogen lamp powered by a programmable supply (Hewlett-Packard 6367A) having less than 0.5-mv. ripple and 5 mv. per hour drift. The double monochromator is that of a Cary 14R recording spectrophotometer with the scattered transmission attachment No.