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THE (5-PHENYL-2-OXAZOLYL)PYRIDINES AS
FLUORESCENT pH INDICATORS
An Application to Chemical Radiation Dosimetry

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ABSTRACT

The three isomeric (5-phenyl-2-oxazolyl)pyridines have been shown to be sensitive fluorescent pH indicators which show a pronounced change to increased visible fluorescence as the pH is lowered. Absorption and fluorescence spectral data and pK_a values are given. Selective excitation of fluorescence from the conjugate acid in the presence of the free base was found possible. The sensitivity of the 4-isomer was demonstrated to be adequate for determining the small amounts of acid produced in certain chemical dosimeter systems.

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CHAPTER 1

INTRODUCTION

After excitation to higher electronic energy levels by the absorption of light, molecules often return to the ground state with the emission of fluorescent light. The wave lengths of the light involved are characteristic of the molecular structures; the fluorescence maxima appear at longer wave lengths than the corresponding absorption maxima. The phenomenon of fluorescence is quite common, and visible fluorescence is generally expected from organic compounds whose structures involve extended conjugation.

Many compounds are known whose fluorescence intensities and wave lengths of emission depend (among other things) on the hydrogen-ion concentration of the solutions (1,2). They may be used for the determination of pH in much the same manner as the more common colorimetric pH indicators. Most of these compounds which have been investigated as fluorescent pH indicators exhibit their more intense fluorescence at the

higher pH values; only a few fluoresce more strongly in acid solution than in basic solution, and the usable range of these is several pH units below neutrality.

During an extensive investigation of organic compounds as solutes for liquid solution scintillators (3,4), it was observed that 4-(5-phenyl-2-oxazolyl)pyridine exhibited intense blue-green fluorescence in dilute, acid solution. It was further noted that this fluorescence was visible in solutions which were only slightly acidic -- around pH 6. In basic solution, weak violet fluorescence could be observed. This suggested the possibility that compounds of this type might be valuable additions to the already long and growing list of fluorescent indicators. A further supposition was confirmed that selective excitation of fluorescence from the acid form was possible by using exciting radiation of energy insufficient to excite the conjugate base.

This report presents the data obtained in an investigation of the three isomeric (5-phenyl-2-oxazolyl)pyridines as

fluorescent pH indicators. Further application in simple chemical dosimeter systems for high-energy radiation is also described.

CHAPTER 2

EXPERIMENTAL PART

2.1 Apparatus

Absorption spectra were determined in 1-cm silica cells using a Beckman DK-1 recording spectrophotometer.

Fluorescence spectra were determined as previously described (3) in 1-cm silica cells using a modified Beckman DK-1; the excitation wave lengths were isolated from a Hanovia type-SH mercury lamp by means of a Beckman DU monochromator.

Fluorophotometer measurements were made with a sensitive instrument described by Milligan and Watts (5). The primary filter for isolating the exciting radiation was a Corning 1457 having maximum transmission in the 405-m μ region. The secondary filter for transmitting the fluorescent light to the phototube was a combination of Corning filters 3385 and 9780 which transmits between 460 and 620 m μ . Peak transmittance of the secondary filter occurs in the 520-m μ region, which does not appear to

be an efficient matching with the emission spectra of the solutions measured; however, sensitivity was adequate.

Measurements of pH were made using a Beckman Model H-2 pH Meter, which was standardized against Beckman pH 7.00 and pH 4.00 buffer solutions.

2.2 Determination of pK_a

Four milliliters of 95 per cent ethanol containing 0.666 mg of the (5-phenyl-2-oxazolyl)pyridine (4) was diluted to 100 ml with 0.1 M citric acid solution. A similar solution was prepared using 0.2 M disodium phosphate solution. Buffered solutions of desired hydrogen-ion concentration (containing the pyridine compound at 3×10^{-5} M) were prepared by mixing these citrate and phosphate solutions in the required proportions as indicated by the pH meter. Absorption spectra were determined over a pH range which was extended until the absorbances at the wave-length maxima involved became constant (Table I). From these absorbances at the low and high ends of the range were calculated the molar absorption coefficients of the conjugate acid and the free base, respectively. The relative concentrations of the basic and acidic forms of the compound were then readily calculated at intermediate pH values.

TABLE I

ABSORPTION MAXIMA OF THE (5-PHENYL-2-OXAZOLYL)PYRIDINES

Isomer	$\lambda_{\text{max}}^{\text{abs}}, \text{m}\mu \quad (\log \epsilon_{\text{max}})$	
	Base	Acid
2-	317 (4.46)	361 (4.34)
3-	310 (4.43)	328 (4.26)
4-	320 (4.37)	367 (4.34)

2.3 Fluorescence Spectra

The emission spectra were determined for the compounds at 0.01 M in acidic (0.4 M in hydrochloric acid) and basic (0.2 N in sodium hydroxide) 95 per cent ethanol solution. Wave lengths of maximum fluorescence ($\lambda_{\text{max}}^{\text{fl}}$) and relative intensity at $\lambda_{\text{max}}^{\text{fl}}$ for three excitation wave lengths (λ_{excit}) are recorded in Table II.

2.4 Fluorophotometer Measurements

Measurements were made in 15-mm-diameter Pyrex test tubes (4 ml of solution) and in 1-dram Kimble (14-mm-diameter) vials (3 ml of solution). The response from the vials was slightly

TABLE II

FLUORESCENCE MAXIMA OF THE (5-PHENYL-2-OXAZOLYL)PYRIDINES

Isomer	$\lambda_{\text{max}}^{\text{fl}}, \text{ m}\mu$	Relative Intensity $\lambda_{\text{excit}}, \text{ m}\mu$		
		313	365	405
2-, Base	389	48	17	--
2-, Acid	478	28	78	25
3-, Base	387	39	7	--
3-, Acid	510	1	3	1
4-, Base	396	53	55	--
4-, Acid	475	39	100	35

less than from the test tubes. Readings were reproducible to within a few tenths of a per cent; day-to-day variations were not noticeable. Buffered solutions were prepared as above; solutions of the compounds in triply distilled water were made with the aid of 1 per cent of 95 per cent ethanol.

2.5 Two-Phase Dosimeters

Into 1-dram vials were placed 1 ml of triply distilled water and 2 ml of tetrachloroethylene containing 4.4 mg of

4-(5-phenyl-2-oxazolyl)pyridine and 4 mg of 2,6-di-tert-butyl-p-cresol. The tetrachloroethylene had been purified by washing successively with concentrated sulfuric acid, fuming sulfuric acid, sulfuric acid, and finally with triply distilled water. Hydrochloric acid (10^{-3} N or 10^{-2} N) was added by means of lambda pipettes to a series of the dosimeters. The vials were closed with polyethylene caps, vigorously shaken, and read in the fluorophotometer using 405-m μ excitation.

Another series of the dosimeters was exposed to gamma radiation from a 130-curie Co⁶⁰ source. The vials were shaken several times over a period of 2 hours after exposure and then read in the fluorophotometer; controls, which received no radiation, were treated identically.

2.6 One-Phase Dosimeters

The solution was prepared by saturating water with trichloroethylene and incorporating 4-(5-phenyl-2-oxazolyl)pyridine at 6×10^{-5} M with the aid of 2 per cent of ethanol. Containers for the solution were 2-ml sealed ampuls. The dosimeters were exposed to 250-KVP X rays and then read in the fluorophotometer using 405-m μ excitation.

CHAPTER 3

DISCUSSION

From the absorption spectra of the three compounds in solutions buffered at various pH values can be calculated the concentrations of base and conjugate acid. Such data are plotted in Fig. 1 and from these can be obtained the pK_a values. The pK_a is the pH at which the concentration of the base and the conjugate acid are equal, according to the following equation (6):

$$pK_a = pH - \log \frac{[B]}{[BH^+]}$$

These values (2.31, 3.27, and 4.18 for the 2-, 3-, and 4-isomers, respectively) are consistent with known values for other substituted pyridines (7). Equivalent results are obtained by using only one of the two absorption maxima and taking as pK_a the pH at which the absorbance is halfway between its minimum and maximum values (8).

Fig. 1. Determination of pK_a of the (5-phenyl-2-oxazolyl) pyridines.

Both the absorption and fluorescence spectra (Tables I and II) have maxima to longer wave lengths in acid solution than in basic solution. The fluorescence maximum of the 3-isomer, in contrast to absorption, exhibits the greatest shift. The three columns in Table II show the relative fluorescent intensities with different wave lengths of exciting radiation. With 313-m μ excitation the fluorescence of the conjugate acid is less than the base; with 365-m μ excitation the reverse is found, except for the 3-isomer; and with 405-m μ excitation the basic forms of the compounds are not excited at all. The relative intensities cannot be compared horizontally in the table, i.e., the intensities of the three excitation wave lengths are not equivalent. It is evident that fluorescence of the acid forms can be selectively excited. Thus, in a solution in which both forms of a compound are present, fluorescence of the conjugate acid can be observed without interference from fluorescence originating with the free base. It is almost always the case that a solution containing two fluorescent species, when excited with radiation sufficiently energetic to cause fluorescence of both compounds, emits fluorescent light which is characteristic of only the compound emitting at the longer wave length. Energy transfer occurs -- energy from the species emitting at the shorter wave length which would normally appear

as fluorescence is transferred to and re-emitted by the other compound having the longer emission wave length. This phenomenon precludes methods of analysis for the two forms which are based on separating the fluorescent wave lengths by filters or spectrophotometry, such as the use of a fluorophotometer with a secondary filter chosen to pass only one of the two wave lengths.

Both the 2- and the 4-isomer conjugate acids have high fluorescence intensities; the latter was chosen for more detailed study because of its greater pK_a , which would provide higher sensitivity to changes in acid concentration.

Figure 2 shows the effect of pH on relative fluorescence intensity. These measurements were made utilizing 405-m μ excitation. The 3-isomer again appears considerably different from the 2- and 4-isomers, which eventually reach essentially the same intensity. The 4-isomer, however, owing to its greater fluorescence at pH values closer to neutrality, is the more sensitive to addition of acid under optimized conditions of initial pH. The effect of substrate concentration is illustrated for the 4-isomer in Fig. 3.

Several sensitive and stable chemical systems have recently been devised as radiation dosimeters (9,10). Systems which at present seem to offer the most practical advantages depend on the measurement of acid produced by ionizing radiation via a

Fig. 2. Effect of pH and concentration on fluorescence.

Fig. 3. Fluorescence of 4-(5-phenyl-2-oxazolyl)pyridine.

chemical chain reaction occurring in a halogenated hydrocarbon. The acids produced can be titrated or, more commonly, determined by color changes detected visually or spectrophotometrically in an aqueous solution of a colorimetric indicator. It was considered possible that a fluorescent indicator for determining the amount of acid produced might possess some unique capabilities.

One type of chemical dosimeter consists of tetrachloroethylene to which has been added an optimum amount of chain-reaction retarder to provide light and thermal stability and dose-rate independence. This solution can be overlaid with an indicator such as chlorphenol red in aqueous solution into which the radiation-produced acids are extracted. In the present instance, 4-(5-phenyl-2-oxazolyl)pyridine was dissolved in the tetrachloroethylene solution which was overlaid with water. Figure 4 shows the response of such a system to added hydrochloric acid. The fluorescence intensity was measured using 405-m μ excitation by which only the acid form of the indicator is excited to fluorescence. A similar series of vials was exposed to various doses of Co⁶⁰ gamma radiation. In Fig. 5 fluorescence intensity appears as a linear function of dose. By comparison with data from Fig. 4, the acid yield for these dosimeters is found to be about 5×10^{-11} equivalents of acid per roentgen per milliliter of

Fig. 4. Response of two-phase fluorescent dosimeters to added hydrochloric acid.

Fig. 5. Response of two-phase fluorescent dosimeters.

tetrachloroethylene. This is essentially the same acid yield observed with colorimetric indicator systems, and it can be assumed that the presence of the fluorescent indicator, dissolved in the tetrachloroethylene, has not influenced radiation sensitivity. The sensitivity, i.e., the response per roentgen, can be varied by changing the volume of tetrachloroethylene or the volume of the aqueous phase, by varying the starting pH or concentration of indicator, or by buffering the aqueous solution. The relative merits of such procedures would depend on the optical system of the fluorimeter, which dictates the optimum dimensions of the aqueous layer, and by other requirements on the physical dimensions of the dosimeter.

This two-phase system just described is relatively insensitive to fast neutrons. Another system is available which does respond to fast neutrons; it consists of water saturated with trichloroethylene. The response of such a system in which the 4-isomer was incorporated as the indicator is shown in Fig. 6. At the high dose values (above about 6000 r), the sensitivity starts to fall off, and the dosimeter approaches the end of its useful range. The concentration of the phenyloxazolylpyridine was 6×10^{-8} mole per milliliter, and the acid yield in such a system is about 10^{-11} mole per roentgen per milliliter. Thus, it is reasonable to assume that this

Fig. 6. Response of one-phase fluorescent dosimeters.

falling off is due to exhaustion of the supply of the pyridine base with which the acid produced can react.

These experiments indicate that fluorescent indicators can be used to measure the small amounts of acid produced in such chemical dosimeter systems. It is felt, however, that these investigations did not reveal any striking advantages to be gained over colorimetric indicators. Perhaps one advantage is that a larger dose range can be covered with the fluorescent indicator. For example, the system shown in Fig. 6 is useful to about 1200 r, when chlorphenol red is employed as a colorimetric indicator. The tetrachloroethylene two-phase fluorescent system mentioned previously remains linear to doses greater than 5000 r, whereas the same system using chlorphenol red is useful to only about 1000 r. The ranges of the colorimetric systems are commonly extended by the addition of buffer. It may be that fluorescent systems might hold peculiar advantages for other investigators.

CHAPTER 4

CONCLUSION

This investigation has provided a new series of fluorescent pH indicators which exhibit their visibly most intense fluorescence in acid solution. One of these compounds, the 4-isomer, appears to be the most useful of the three because its fluorescence changes appear at pH values nearer neutrality. It was found that fluorescence of the conjugate acids could be conveniently excited without exciting fluorescence from the free base present. The sensitivity of one of these compounds was demonstrated to be adequate for measuring small amounts of acid by utilizing it in established chemical dosimeter systems.

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