(4) D. L. Trepanier, K. L. Shriver, and J. N. Eble, *J. Med. Chem.*, **12**, 257(1969).
(5) T. Taguchi (Sankyo Co., Ltd.), Japanese pat. 6346 (Cl. 16E4) (1967); through *Chem. Abstr.*, **67**, 21947n(1967).
(6) O. Straub and P. Zeller (Hoffmann-La Roche, Inc.), U. S. pat. 3,073,819 (Cl. 260-240) (1963); Swiss Appl. (1959).
(7) D. H. R. Barton, R. E. O'Brien, and S. Sternhell, *J. Chem. Soc.*, **1962**, 470.
(8) J. B. Miller, *J. Org. Chem.*, **24**, 560(1959).
(9) P. A. S. Smith, "The Chemistry of Open-Chain Organic Nitrogen Compounds," vol. II, W. A. Benjamin, New York, N. Y., 1966, p. 119.
(10) G. H. Coleman, H. Gilman, C. E. Adams, and P. E. Pratt, *J. Org. Chem.*, **3**, 99(1938).
(11) S. Garattini, A. Giachetti, A. Jori, L. Pieri, and L. Valzelli, *J. Pharm. Pharmacol.*, **14**, 509(1962).
(12) T. Fujita and D. H. Tedeschi, *Pharmacologist*, **7**, 155(1965).
(13) J. Barsky, W. L. Pacha, S. Sarkar, and E. A. Zeller, *J. Biol. Chem.*, **234**, 389(1959).
(14) A. M. El Masri, J. N. Smith, and R. T. Williams, *Biochem. J.*, **68**, 587(1958).

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Application of Fluorescence Spectroscopy to Study of Intramolecular Hydrogen Bonding in Pamaquine

STEPHEN G. SCHULMAN▲ and KENNETH ABATE

Abstract □ The fluorescences of the antimalarial pamaquine and of its precursor 8-amino-6-methoxyquinoline were studied in sulfuric acid and *n*-heptane and throughout the pH range in water. Quenching of the fluorescences of both molecules in concentrated acid solutions upon addition of water was found to be the result of dissociation in the excited state from the protonated 8-amino groups. The anomalously long wavelengths of fluorescence of singly protonated pamaquine in the biological pH range in water and in *n*-heptane are shown to be due to intramolecular proton transfer, in the excited state, from the diethylamino group of the side chain to the heterocyclic nitrogen atom of the quinoline ring. This phenomenon appears to be mediated by the presence of an intramolecular hydrogen bond between the protonated diethylamino group and the ring nitrogen atom in the ground state, a result that may be of significance in the interpretation of the pharmacodynamics of pamaquine.

Keyphrases □ Pamaquine—fluorescence, intramolecular hydrogen bonding, acidity dependence □ Fluorescence, pamaquine—effect of intramolecular hydrogen bonding, acidity dependence □ Spectrophotofluorometry—pamaquine fluorescence, acidity dependence

The fluorescence of pamaquine has been a subject of some controversy. Brodie *et al.* (1) found that the naphthoate salt of pamaquine emitted a blue fluorescence in alkaline solutions. Irvin and Irvin (2) subsequently studied the free base dissolved in concentrated sulfuric acid solutions, and they observed fluorescence in solutions greater than 7 *M* in sulfuric acid. No fluorescence was observed at lower acidities, and the emission in concentrated sulfuric acid was attributed to a solvent effect and protonation of the methoxy group. Later work by Udenfriend *et al.* (3) showed the fluorescence of pamaquine naphthoate to be due to the naphthoate anion. Recently, Schulman and Sanders (4) showed that the failure of 8-aminoquinoline to fluoresce in fluid dilute acid solutions was a result of hydrogen bonding of the monocation with the solvent, water, in the lowest excited singlet state. The reason for the failure of the dication of the aminoquinoline to fluoresce in fluid concentrated sulfuric acid solutions was less certain and was attributed either to hydrogen-bonding-assisted internal conversion, as for the monocation, or to the acidity of the dication in the excited state which may have been so great that the latter species could not form even in the most concentrated sulfuric acid solutions available.

To evaluate the nature of the fluorescence of pamaquine in concentrated acid solutions in light of modern fluorescence theory, the present study of the acidity dependence of the fluorescence of pamaquine and of its simple aromatic precursor, 8-amino-6-methoxyquinoline, was undertaken.

EXPERIMENTAL

Instrumentation—Absorption spectra were obtained using a spectrophotometer[1]. Fluorescence measurements were performed on a fluorescence spectrophotometer[2] whose monochromators were calibrated against the xenon line emission spectrum and whose output was corrected for instrumental response by means of a rhodamine-B quantum counter. The pH measurements were made using a digital pH meter[3] with a silver–silver chloride–glass combination electrode[4].

Reagents—8-Amino-6-methoxyquinoline[5] and pamaquine phosphate[6] were used without further purification.

Analytical reagent grade sulfuric acid was purchased[7] and used without further purification. Solutions of varying acidity for fluoro-

[1] Beckman DG-BT.
[2] Perkin-Elmer MPF-2A.
[3] Orion model 801.
[4] Beckman.
[5] K & K Laboratories, Inc., Plainview, N. Y.
[6] Sterling-Winthrop Research Institute, Rensselaer, N. Y.
[7] Mallinckrodt Chemical Works, Inc., St. Louis, Mo.

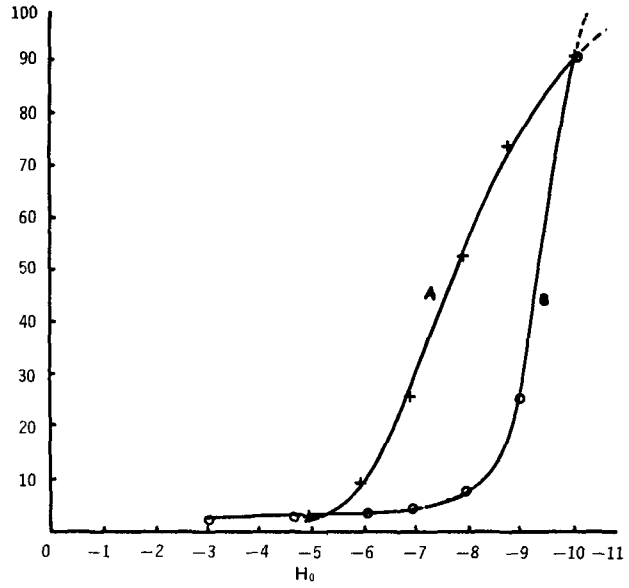


Figure 1—*Fluorometric titrations of (A) pamaquine and (B) 8-amino-6-methoxyquinoline in concentrated sulfuric acid. The abscissa (H_0) is the Hammett acidity scale for sulfuric acid solutions. The ordinate (I_f) is fluorescence intensity in arbitrary units. The emission maxima, at which I_f values were measured, were at 433 nm. for both compounds.*

metric and absorptiometric titrations were prepared by dilution of the sulfuric acid with distilled deionized water.

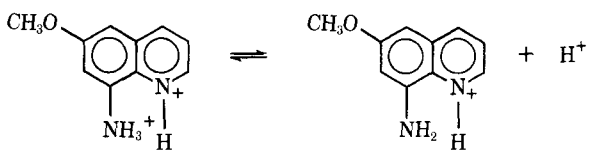
Spectroscopic grade *n*-heptane was purchased[8] and used without further purification.

For fluorescence studies in *n*-heptane, the doubly protonated form of pamaquine was prepared by dissolving pamaquine diphosphate directly in *n*-heptane to saturation. The singly protonated species was prepared by making the latter solution 1×10^{-2} *M* in morpholine. The neutral species was prepared by dissolving the free base pamaquine directly in *n*-heptane.

RESULTS AND DISCUSSION

Both pamaquine and 8-amino-6-methoxyquinoline demonstrated intense blue fluorescence at 433 nm. in the most concentrated acid solutions. The fluorometric titration curves of these compounds are shown in Fig. 1. Neither titration curve was complete at the upper end of the acidity scale employed ($H_0 = -10.0$ in 96% H_2SO_4) (5). The long wavelength absorption maxima of both compounds demonstrated substantial shifts to shorter wavelengths on going from dilute (0.1 *M*) to concentrated sulfuric acid (Tables I and II). The pKa for dissociation of the triply protonated cation of pamaquine to the doubly protonated cation is −1.3 (6). Spectrophotometric determination of the pKa of doubly protonated 8-amino-6-methoxyquinoline, in this work, yielded a value of −0.4. These dissociations occur from the protonated 8-amino groups (4); the bathochromic absorption shift, upon dissociation, indicates that both compounds become stronger acids in the lowest excited singlet state, a result consistent with the normal excited state behavior of arylamines (7, 8). It is, therefore, concluded that the fluorometric titrations of both compounds in the region near H_0 −10 are the result of ionization in the lowest excited singlet states of the fluorescent arylammonium ions to form nonfluorescent 8-amino-6-methoxyquinolinium ion and its analog in pamaquine.

Although the fluorometric titration curves of neither pamaquine nor 8-amino-6-methoxyquinoline were complete in the most acidic solutions employed, the slope of the fluorometric titration curve of 8-amino-6-methoxyquinoline indicated that the Henderson–Hasselbach equation was obeyed for the distribution of excited acid and conjugate base species derived from this compound. Therefore, it could be concluded that prototropic equilibrium was achieved within the lifetime of the lowest excited singlet state according to Scheme I. It was thus possible to extrapolate the fluorometric titration curve for the equilibrium in Scheme I to obtain the approximate excited state dissociation constant pKa* = −10.

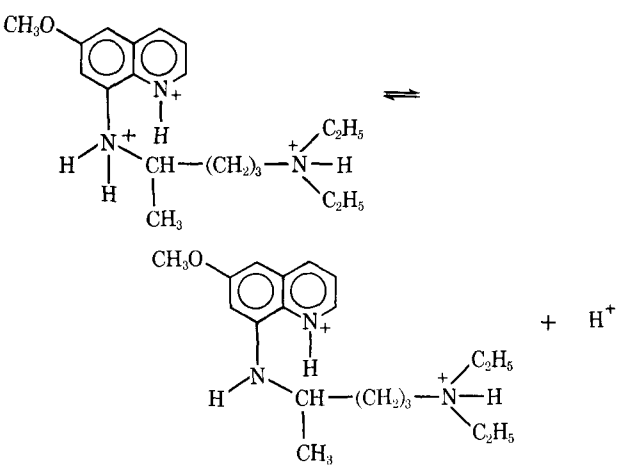


Scheme I

The fluorometric titration of pamaquine in concentrated sulfuric acid, although not complete at H_0 −10, originated at lower Hammett acidities than that for 8-amino-6-methoxyquinoline. Moreover, at the more acidic end of the titration curve, it was obvious that the inflection point (H_0 = pKa*) was well passed. The pKa* estimated for dissociation of triply protonated pamaquine from the amino group (Scheme II) is −8.0. The lower acidity of pamaquine relative to 8-amino-6-methoxyquinoline may be due to the inductive effect of the alkyl side chain of the 8-amino function of pamaquine. However, the slope of the fluorometric titration curve of pamaquine in concentrated acid (Fig. 1) is not quite as steep as that of 8-amino-6-methoxyquinoline. This suggests that prototropic equilibrium may not be complete during the lifetime of the lowest excited singlet state, so the apparently lower pKa* of pamaquine may be due to kinetic rather than thermodynamic factors (8).

No fluorescence was observed for 8-amino-6-methoxyquinoline below H_0 −5 in aqueous solutions. This appears to be typical of 8-aminoquinolines and has been shown, for unsubstituted 8-aminoquinoline, to be the result of quenching of the fluorescences of the neutral and singly protonated species by hydrogen-bonding interactions with the solvent in the lowest excited singlet state (4). That this is also the case for 8-amino-6-methoxyquinoline is demonstrated by the observation of fluorescence from both the neutral species and hydrochloride at 438 and 543 nm., respectively, in the nonhydrogen-bonding solvent *n*-heptane (Table I). However, the doubly protonated species derived from 8-aminoquinolines are apparently capable of fluorescing in fluid concentrated acid solutions provided that the excited doubly protonated species is not too acidic to be generated in available acid solutions. The pKa* of excited, doubly protonated, 8-aminoquinoline has been estimated to be −14 (4) and is thus too acidic for the latter to exist in concentrated sulfuric acid. The 6-methoxy derivative, however, is apparently not as strong an acid as the unsubstituted 8-aminoquinoline, probably as a result of charge transfer in the excited state from the methoxy group to the amino group, and this accounts for the observation of fluorescence from the former doubly protonated cation in fluid sulfuric acid solutions.

Although the blue fluorescence of pamaquine in sulfuric acid is quenched by dilution of the acid, in very dilute acid solutions there appears a weak-green fluorescence (λ_{max} = 525 nm.), persisting through the pH 3–14 range. Quenching of the green fluorescence in dilute acid solutions (pH ~ 3) is half complete at pH 3.5. Since the latter pH corresponds to the ground state pKa for dissociation from



Scheme II

[8] Matheson, Coleman and Bell Inc., Rutherford, N. J.

Table I—Low Frequency Absorption Maxima ($\bar{\nu}_a$) and Fluorescence Maxima ($\bar{\nu}_f$) of 1×10^{-4} *M* 8-Amino-6-methoxyquinoline in Media of Different Acidities[a]

Solvent	$\bar{\nu}_a$	$\bar{\nu}_f$
18 *M* H_2SO_4 (H_0 −10.0)	2.88	2.31
0.1 *M* Aqueous H_2SO_4	2.63	—
0.1 *M* CF_3COOH in *n*-heptane	—[b]	1.88
Aqueous phosphate buffer (pH 7)	2.96	—
n-Heptane	—[b]	2.28
7 *M* Aqueous NaOH (H_- 15.6)	2.96	—

[a] Maxima are reported in $\mu m.^{-1}$ ($cm.^{-1} \times 10^{-4}$). [b] Solubility in *n*-heptane was too low to obtain accurate absorption spectra.

Table II—Low Frequency Absorption Maxima ($\bar{\nu}_a$) and Fluorescence Maxima ($\bar{\nu}_f$) of Pamaquine Diphosphate in Media of Different Acidities[a]

Solvent	$\bar{\nu}_a$	$\bar{\nu}_f$
18 *M* H_2SO_4(H_0 −10.0)	2.84	2.31
0.1 *M* Aqueous H_2SO_4	2.39	—
n-Heptane	—[b]	1.88
Aqueous phosphate buffer (pH 7)	2.76	1.91
n-Heptane + morpholine	—[b]	1.92
7 *M* Aqueous NaOH(H_- 15.6)	2.71	2.05
Pamaquine (free base in *n*-heptane)	—[b]	2.26

[a] Maxima are reported in $\mu m.^{-1}$ ($cm.^{-1} \times 10^{-4}$). [b] Solubility in *n*-heptane was too low to obtain accurate absorption spectra.

the heterocyclic nitrogen atom of pamaquine (6) (Scheme III), it can be concluded that the green fluorescence originates from a species formed as a result of the excitation of pamaquine, protonated only at the diethylamino function. At high pH, the green fluorescence at 525 nm. diminishes with increasing pH in the region near pH 14 and a blue-green fluorescence (λ_{max} = 485 nm.) concurrently appears. The midpoint of this conversion occurs at pH 14.0. In this pH region, the only species present in the ground state is the neutral species or free base pamaquine [the pKa for dissociation of the protonated diethylamino group is 10.2 (6)] so that the absorption spectrum does not vary appreciably with pH. Consequently, the shifting of the fluorescence of pamaquine in the pH 14 region must be due to a reaction taking place in the excited state.

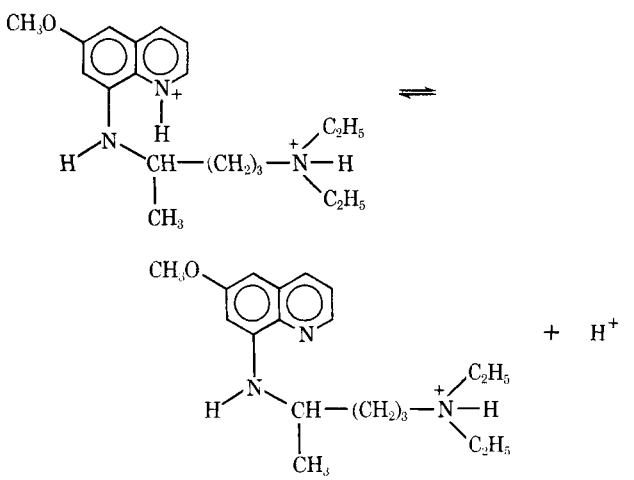
To characterize the green fluorescence and the blue-green fluorescence arising from pamaquine, the fluorescence spectra of pamaquine in various states of protonation were taken in *n*-heptane. Heptane is a low dielectric, nonhydrogen-bonding solvent in which excited state processes involving intermolecular proton transfer between solvent and solute are not possible (10). Thus, excepting intramolecular proton transfer processes, fluorescence in heptane must originate from the species that is excited. The fluorescences of the neutral, singly protonated, and doubly protonated species arising from pamaquine in *n*-heptane as well as the emissions of pamaquine in aqueous media are listed in Table II.

The data of Table II show that the fluorescences of the singly protonated pamaquine in heptane and in water are almost identical and are much closer in wavelength to the fluorescences of the doubly protonated species in heptane than to those of the free base in heptane or in water. This is rather unusual because the singly protonated species is protonated at the diethylamino group, which is aliphatic; and if isolated from the aromatic system as depicted in Scheme III, it should not affect the electronic structure of the aromatic system. Thus, it is to be expected that the singly protonated species should show fluorescence very similar to that of the free base. The lack of direct effect of the alkyl side chain on the electronic structure of the aromatic group is illustrated by the similarities of the luminescence frequencies in *n*-heptane of the various species derived from 8-amino-6-methoxyquinoline (Table I) with those of the corresponding species derived from pamaquine. Moreover, the fluorometric titration of pamaquine in the region near pH 14, which is due to dissociation in the excited state, does not appear to be consistent with titration of the protonated diethylamino group since the aliphatic side chain would be expected to show ground state titration behavior even when the aromatic part of the molecule is electronically excited.

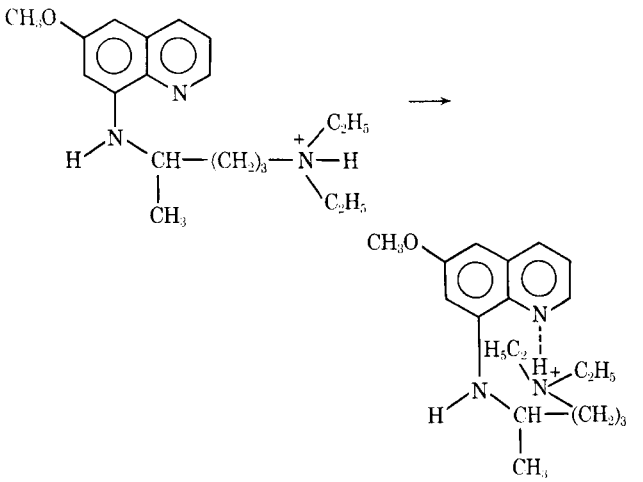
These results strongly suggest that the protonated diethylamino group of the aliphatic side chain of pamaquine is intimately associated with the aromatic ring in the excited state; for this reason, the fluorescence of singly protonated pamaquine is sensitive to protolytic dissociation. The fluorescence properties and titration behavior of singly protonated pamaquine suggest the following explanation for the observed results.

The failure of pamaquine to fluoresce in the region from H_0 −5 to pH 3 while doubly protonated pamaquine shows green fluorescence in *n*-heptane indicates that the excited state of the doubly protonated species is quenched by hydrogen bonding with the aqueous solvent. In the region near pH 3.5, doubly protonated pamaquine is converted in the ground state to the singly protonated species (Scheme II). Although the diethylamino group of the side chain is more basic than the heterocyclic nitrogen atom in the ground state and, therefore, is covalently bonded to the remaining proton, there is additional stabilization energy to be gained by the formation of an intramolecular hydrogen bond between the proton of the diethylammonium group and the ring nitrogen (Scheme IV).

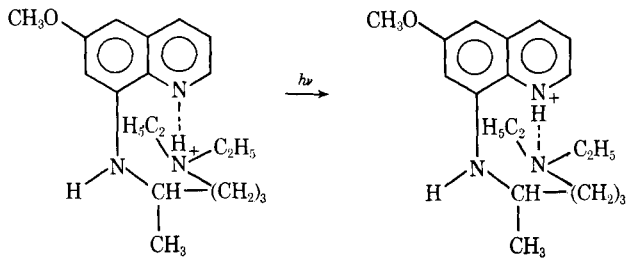
Thus, the intramolecularly hydrogen-bonded, singly protonated species derived from pamaquine is the stable, thermally equilibrated, singly protonated species in the ground state, a result which may be of importance in the interpretation of the biological action of pamaquine since this species is predominant at biological pH. The shifts to shorter wavelengths of both absorption and fluorescence spectra of 8-amino-6-methoxyquinoline and pamaquine upon protolytic dissociation from the heterocyclic ring indicate that the ring nitrogen atom becomes considerably more basic in the lowest excited singlet state (7–9). If the ring nitrogen atom in the excited state of pamaquine is more basic than the diethylamino



Scheme III



Scheme IV



Scheme V

group, excitation of the singly protonated species would result in transfer of the proton, across the intramolecular hydrogen bridge, from the diethylamino group to the ring nitrogen. Presumably, in the thermally equilibrated excited state, the proton would be covalently bonded to the ring nitrogen and electrostatically hydrogen bonded to the diethylamino group (Scheme V).

Thus the excited singly protonated species would have an electronic distribution similar to that of the doubly protonated species in the aromatic ring, and this would account for the similarity of emission frequency between the singly and doubly protonated species. That the emission frequency of the singly protonated species is slightly higher than that of the doubly protonated species in *n*-heptane can be explained by the intramolecular hydrogen bond with the diethylamino group which partially withdraws the proton from the ring nitrogen atoms, thus diminishing its polarizing effect on the π-electron distribution of the aromatic system. The intramolecular hydrogen bond with the diethylamino group in the excited state also serves to shield the proton of the excited singly protonated species from the solvent. This accounts for the observation of fluorescence from the singly protonated species of pamaquine but not from the doubly protonated pamaquine or singly protonated 8-amino-6-methoxyquinoline, both of which have similar electronic structures to that of the singly protonated pamaquine.

The fluorometric titration of pamaquine near pH 14 can be attributed to protolytic dissociation, in the excited state, from the heterocyclic nitrogen atom of singly protonated pamaquine. The pKa* corresponding to this dissociation is 14.0. This dissociation occurs at such high pH because of the increase in basicity of the ring nitrogen upon excitation and supports the hypothesis of protonation of the ring nitrogen in the excited state of singly protonated pamaquine. That the fluorescence of the free base pamaquine occurs at substantially lower frequencies than in *n*-heptane is probably the result of electrostatic stabilization of the excited state of the free base by dipole–dipole interactions with the highly polar aqueous solvent. That pamaquine fluoresces as the free base in aqueous solutions while 8-amino-6-methoxyquinoline does not appears to be the result of protection of the ring nitrogen atom of pamaquine from hydrogen-bonding interactions with the solvent. Although dissociation removes the hydrogen bond between the alkyl side chain and the ring nitrogen atom, the time required for the chain to become disoriented from the ring nitrogen atom may be slow compared with the lifetime of the excited state of pamaquine, so the chain may provide a steric blockage to approach of solvent molecules which would quench the fluorescence of the excited free base.

REFERENCES

(1) B. B. Brodie, S. Udenfriend, and J. V. Taggart, *J. Biol. Chem.*, **168,** 327(1947).

(2) J. L. Irvin and E. M. Irvin, *ibid.*, **174,** 577(1948).

(3) S. Udenfriend, D. E. Duggan, B. M. Vasta, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **120,** 26(1957).

(4) S. G. Schulman and L. B. Sanders, *Anal. Chim. Acta*, **56,** 91(1971).

(5) M. J. Jorgenson and D. R. Hartter, *J. Amer. Chem. Soc.*, **85,** 878(1963).

(6) S. R. Christophers and J. D. Fulton, *Ann. Trop. Med. Parasitol.*, **34,** 1(1940).

(7) A. Weller, *Progr. React. Kinet.*, **1,** 85(1971).

(8) S. G. Schulman, *J. Pharm. Sci.*, **60,** 371(1971).

(9) S. G. Schulman, *Rev. Anal. Chem.*, **1,** 85(1971).

(10) S. G. Schulman and A. C. Capomacchia, *Anal. Chim. Acta*, **58,** 91(1972).

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Kinetics of Solvolysis of Intrazole

V. K. PRASAD, R. A. RICCI, and A. P. GRANATEK▲

Abstract □ The kinetics of degradation of intrazole [1-(*p*-chlorobenzoyl)-3-(1*H*-tetrazol-5-ylmethyl)indole] in solution was investigated at 65 ± 0.1° at constant ionic strength of 0.5 over a wide pH range. The observed rates, followed by measuring intact intrazole, obeyed first-order kinetics. The catalytic effect of a phosphate buffer was found to be greater than the acetic acid catalysis. The apparent rate of hydrolysis of intrazole increased with the increasing concentration of hydrochloric acid. Primary salt effects were observed in both acidic and basic solutions. The rate of hydrolysis decreased in acidic and alkaline solutions, with increasing concentrations of ethanol in the solvent system. The apparent heats of activation for intrazole degradation in solution were determined to be 19.87 kcal./mole in 0.1 *N* HCl, 21.40 kcal./mole in pH 4.10 acetate buffer, 20.30 kcal./mole in pH 8.0 phosphate buffer, and 7.25 kcal./mole in pH 9.10 and 10.10 borate buffers. From the rate–pH profile, the pH of minimum degradation or maximum stability of the compound under buffer-free conditions was found to be 3.20. The products of hydrolysis formed in acid- and alkali-catalyzed degradation of intrazole were identified by TLC. A mechanism consistent with the above observations is proposed.

Keyphrases □ Intrazole—solvolysis, pH–rate profile □ pH–solvolysis rate profile—intrazole

The anti-inflammatory, antipyretic, and analgesic properties of intrazole (I), the most effective member of a series of 1-substituted 3-(5-tetrazolylmethyl)indoles, were described by Fleming *et al.* (1). It was shown to be equally active in intact and adrenalectomized rats as an anti-inflammatory agent (carrageenin foot edema assay).