Confirmation of Iminoacridan Structure of Singly Protonated 9-Aminoacridine

ANTHONY C. CAPOMACCHIA and STEPHEN G. SCHULMAN ˣ

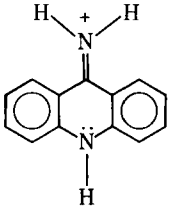
Abstract □ The occurrence of the second absorption band of singly protonated 9-aminoacridine, which arises from an acridinium ring-localized transition, at anomalously short wavelength indicates the disruption of the aromaticity of the central ring of the singly charged cation. The shifting of the two longest wavelength absorption bands and the fluorescence band of 10-methyl-9-aminoacridine monocation, a model of singly protonated 9-aminoacridine which is constrained to dissociate from the exocyclic nitrogen atom, to shorter wavelengths, upon dissociation, indicates the imine-like nature of the 10-methyl derivative. These observations support the conclusion that the predominant site of positive charge in singly protonated 9-aminoacridine is the exocyclic nitrogen atom, even though the heterocyclic nitrogen atom is the site of protonation of the neutral molecule.

Keyphrases □ 9-Aminoacridine—singly protonated, confirmation of iminoacridan structure □ Iminoacridan—confirmed as structure of singly protonated 9-aminoacridine □ Acridine derivatives—confirmation of iminoacridan structure of singly protonated 9-aminoacridine

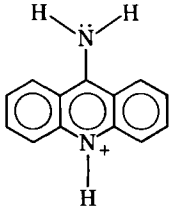
In a recent paper (1), it was shown that singly protonated 9-aminoacridine, the predominant prototropic species derived from 9-aminoacridine at physiological pH, existed in aqueous solution as the protonated iminoacridan (I) form rather than as the protonated aminoacridine (II). This finding is of pharmacological significance because of the ionic interaction of 9-aminoacridine and presumably other antimicrobials derived from 9-aminoacridine with DNA, an interaction whose geometrical characteristics are determined by the site of positive charge in the acridine derivative.

Previously (1), the anomalous shifting of the long wavelength absorption and fluorescence bands of singly protonated 9-aminoacridine with protonation, dissociation, and solvent polarity relative to the corresponding shifts of the singly protonated cation of 2-aminoacridine, a "typical" *N*-heterocyclic amine, was suggested to indicate that I was closest to the actual electronic structure of the 9-aminoacridine monocation.

Recently, in this laboratory, the *N*-methyl monocation of 9-aminoacridine (10-methyl-9-aminoacridinium iodide) was prepared and the pH dependences of its absorption and fluorescence spectra were studied. Moreover, the second absorption band of 9-aminoacridine, whose position in the electromagnetic spectrum is indicative of the extension of aromaticity in the aromatic ring (acridine), was examined as a function of pH. The results of these experiments confirm the assignment of Structure I as most representative of the electronic structure of singly protonated 9-aminoacridine.



I



II

EXPERIMENTAL

The pH measurements were made on a pH meter[1] with a combination silver–silver chloride glass electrode. Electronic absorption spectra were taken in 1-cm silica cells on a grating-type spectrophotometer[2]. Fluorescence measurements were taken on a fluorescence spectrophotometer[3] whose monochromators were calibrated against the line emission spectrum of xenon. Emission spectra were corrected for the wavelength response of the monochromator and phototube by means of a corrected spectra accessory employing a rhodamine-B quantum counter.

10-Methyl-9-aminoacridinium iodide was prepared by methylating 9-aminoacridine[4] according to the literature method (2). Spectra were taken of solutions that were 1×10^{-5} *M* in 9-aminoacridine or 10-methyl-9-aminoacridine. The spectrophotometric titration procedure employed was described previously (1).

RESULTS AND DISCUSSION

The longest wavelength absorption bands of acridines may be considered to be derived from the short axis polarized transition [$^1L_a \leftarrow {}^1A$ in Platts' nomenclature system (3)] of the parent hydrocarbon, anthracene. This band generally is most sensitive to substituents in what corresponds to α-positions of anthracene (4) (positions 1, 4, 5, 8, 9, and 10 in acridine) and, therefore, to protonation of the heterocyclic nitrogen atom of acridine. The second absorption bands of acridines are derived from the long axis polarized transition of anthracene ($^1L_b \leftarrow {}^1A$), are generally more structured and narrower than the ^1L_a bands, are sensitive in position to β-type substituents (4) (positions 2, 3, 6, and 7 in acridine), and are relatively insensitive to α-substituents (*e.g.*, a 9-amino group or protonation of the heterocyclic nitrogen atom) in acridine.

Thus, while the ^1L_a bands of α-substituted acridines appear in the absorption spectrum anywhere from 380 to >500 nm, the ^1L_b bands occur from 350 to 370 nm (5). Even in 2-aminoacridine, which has a β-substituent and no α-functional group other than the heterocyclic nitrogen atom, the O–O feature of the ^1L_b band always occurs within the latter interval, at 354, 370, and 356 nm in the dication, monocation, and neutral molecule, respectively. Hence, while the ^1L_a band in acridines is an "intramolecular charge-transfer band" whose position is determined by the interactions of the functional groups with the aromatic ring as well as by the size of the aromatic ring itself, the ^1L_b band in acridines is essentially a "ring-localized $\pi \rightarrow \pi^*$ transition" whose position is largely "fixed" by the size of the aromatic ring system. In anthracenes and the corresponding heterocyclic molecules, the ^1L_b band occurs between 350 and 370 nm; in naphthalenes, it occurs between 315 and 340 nm; and in benzenes, it occurs between 255 and 300 nm.

The ^1L_b bands of the various prototropic species derived from 9-aminoacridine indicate that the dication and anion are "well-behaved" acridine derivatives. The neutral molecule also is on the

[1] Model 801, Orion Research, Cambridge, Mass.
[2] Model DB-GT, Beckman Instruments, Fullerton, Calif.
[3] Model MPF-2A, Perkin-Elmer Corp., Norwalk, Conn.
[4] Pfaltz and Bauer, Flushing, N.Y.

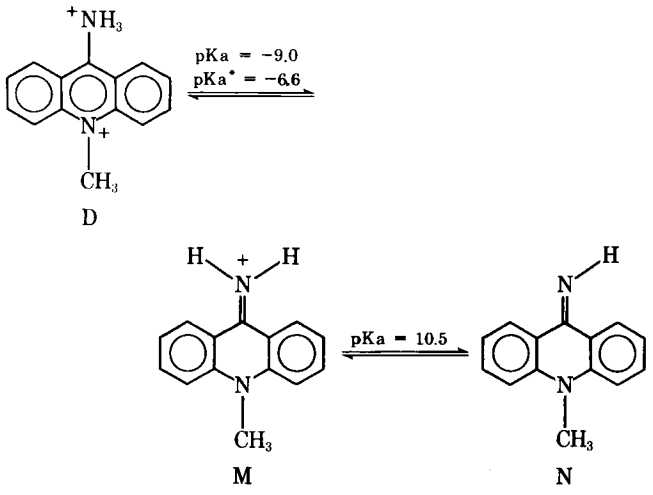
Table I—The O–O Vibronic Maxima of the Two Longest Wavelength Absorption Bands (λ^1L_a and λ^1L_b) and the Fluorescence Bands (λ) of the Various Prototropic Species Derived from 9-Aminoacridine and 10-Methyl-9-aminoacridinium Iodide

	λ^1L_b, nm	λ^1L_a, nm	λf, nm
9-Aminoacridine			
Dication ($H_0 - 10.0$)	366	440	475
Monocation (pH 1.0)	324	423	430
Neutral molecule (pH 12.0)	348	426	445
Anion (H_17.5)	353	443	470
10-Methyl-9-amino-acridinium iodide			
Dication ($H_0 - 10.0$)	368	445	480
Monocation (pH 1.0)	327	431	433
Neutral molecule (pH 12.5)	312	408	431

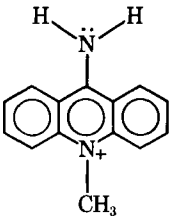
borderline of being a "typical" acridine derivative. However, the ^1L_b band of the monocation is at a much shorter wavelength than that indicative of an acridine ring system and suggests disruption of the aromaticity of the tricyclic system. Actually, the ^1L_b maximum at 324 nm is reasonable for an extended benzenoid system as represented by Structure I.

The 10-methyl-9-aminoacridinium ion has an electronic structure very much like that of the 9-aminoacridinium cation (Table I). The methylated cation can be protonated or dissociated according to Scheme I. An excited-state equilibrium occurs for the dication (D)–monocation (M) pair but not for the monocation–neutral (N) pair. The pKa* and pKa, as well as the spectral shifts occurring upon going from the dication to the monocation of the methylated derivative, are similar to the corresponding quantities measured for the dication–monocation interconversion of 9-aminoacridine (1) and therefore support the validity of using the *N*-methyl derivative as a model of the 9-aminoacridinium ion; which cannot dissociate from the heterocyclic function.

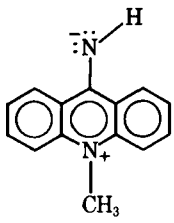
The dissociation of the monocation to form the neutral *N*-methyl derivative results in shifts of the long wavelength absorption (^1L_a) and fluorescence bands to shorter wavelengths. This result is expected for dissociation from a structure such as M to form one such as N because it indicates the electron-withdrawing nature of the exocyclic imino group (similar to but weaker than that of a carbonyl group).



Scheme I



M′



N′

If the alternative amino structures of M′ and N′ are taken to represent M and N, a shift of the ^1L_a and fluorescence bands to longer wavelengths would be expected upon dissociation, because of the increased lone-pair repulsion on the optical electron (stabilization of the charge-transfer excited state of N′ relative to that of M′) produced by dissociation of the electron donor amino group. These conclusions are supported by the positions of the ^1L_b bands of the 10-methyl monocation and neutral species at 327 and 312 nm, respectively. Both are at anomalously short wavelengths for acridinium ions (*i.e.*, M′ and N′) and also rule out the possibilities that the 10-methyl monocation is in the form M′ and that the neutral species is in the form N, a circumstance that might also be expected to yield a shift of the ^1L_a and fluorescence bands to shorter wavelengths upon dissociation.

The loss of acridine-like aromaticity in the monocation derived from 9-aminoacridine, as reflected in the short wavelength of the ^1L_b band and the imine-like dissociation and spectral behavior of the *N*-methylated monocation, supports the assignment of Structure I as that of singly protonated 9-aminoacridine. This conclusion is also supported by a recent X-ray diffraction study of 9-aminoacridine hydrochloride monohydrate (6), in which it was shown that the length of the bond joining the amino group to the aromatic ring is indicative of a double bond as represented in Structure I.

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