Photochemistry of Nitracrine and its Analogues: Acridine Derivatives as Electron Donors

J. Biedrzycki, J. Błażejowski, and J. Szychliński

Institute of Chemistry, University of Gdansk, Sobieskiego 18, 80-952 Gdansk, Poland

A. Ledóchowski

Department of Drugs Technology and Biochemistry, Institute of Organic and Food Chemistry and Technology, Polytechnical University, 80-952 Gdansk, Poland

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Studies on the electron transfer in the excited state from electron donors: acridine, 9-aminoacridine and its derivatives, including nitracrine and its isomers, to CCl_4 as a suitable electron acceptor, were undertaken. UV radiation ($\lambda = 366$ nm), absorbed only by acridine bases, generates Cl^- ions. The fluorescence quenching measurements indicate that no interaction appears between molecules of acridine bases in the excited singlet state and CCl_4 molecule. A scheme of photochemical processes is proposed which assumes that the triplet state of the base molecule is the reactive one.

Introduction

The biological activity of many acridine derivatives has been ascertained in the past, and several of these compounds are widely used as drugs [1, 2]. In the last years growing interest in this group of compounds is observed. This results from two reasons. Firstly, it has been found that some acridine derivatives show antitumor activity, e.g.: 1-nitro-9(3′-dimethylaminopropylamino)-acridine dihydrochloride monohydrate (nitracrine) [3]. Secondly, it has been established that several acridine dyes can interact with nucleic acids [2, 4–6]. This phenomenon can be described in terms of electron-donor-acceptor (EDA) interaction. It is possible that a similar interaction plays a very important role in the biological actions of acridine derivatives used as drugs. Since any acridine base may be considered as potential electron donor it seemed to be necessary to check their properties toward some simple electron acceptors. We selected CCl_4 for this purpose, which is known to show fairly strong acceptor properties toward several organic electron donors [7–10].

Preliminary investigations revealed only weak interaction between several acridine bases and CCl_4 in the ground electronic state [11]. The main aim of this work was to examine the behaviour of the excited acridine derivatives in the presence of CCl_4. This was attained both, performing fluorescence quenching measurements and analyzing photochemical processes proceeding during irradiation of appropriate solutions. The above mentioned methods are widely used in investigations of interactions of drugs with biological systems [12]. Of course, the main compound investigated was nitracrine. A complex nature of the compound requires, however, a stepwise approach to the solution of the problem. Thus, acridine, 9-aminoacridine and its derivatives, including nitracrine and its isomers, were employed successively as electron donors. The use of simpler model compounds allows one to determine the influence of particular functional groups on the properties of the acridine molecule. As solvents, methanol or ethanol were used, as well as 1,4-dioxane as an auxiliary solvent. The reason for the selection of these solvents was that the free bases were satisfactorily soluble in them, and appearing chlorides did not precipitate in the course of irradiation.

Experimental

Analytical reagent grade carbon tetrachloride was purified as reported elsewhere [11]. Spectroscopic grade solvents: methanol (Austranal Praeparate, Loba-Chemie), 99.8 and 96% ethanol (P.O.Ch., Poland) and 1,4-dioxane (Merck) were used as supplied. Acridine and 9-aminoacridine (Fluka)

Reprint requests to Dr. Jerzy Biedrzycki, Institute of Chemistry, University of Gdansk, Sobieskiego 18, 80-952 Gdansk, Poland.

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were purified by repeated crystallisation from ethanol. Other acridine derivatives were synthesized and purified according to the following reports: 1-, 2-, and 3-nitro-9(3′-dimethylaminopropylamino)-acridine [13], 1-nitroacridine [14], 9(2′-dimethylaminoethylamino)-acridine [15], 3-nitro-9-aminoacridine [16], 1-, 3-, and 4-methoxy-9-aminoacridine [17], 2-methoxy-9-aminoacridine [18].

Spectral measurements in UV and visible region were carried out on a VSU-2P (VEB Carl Zeiss, Jena) and Perkin-Elmer model 402 spectrophotometers. The fluorescence spectra were recorded on a Perkin-Elmer-Hitachi MPF-3 spectrofluorometer provided with an automatical spectral correction. The fluorescence intensities were graphically integrated over the whole emission bands. The mean intensity values were evaluated from 3 replicate measurements. The excitation wavelengths were set up at the absorption maximum of any acridine base.

The irradiations were performed in a cylindrical quartz cell that had an optical length of 6 cm and a volume of 65 cm^3. One wall of the cell was equipped with a flat window, through which the radiation penetrated to the solution, whereas the remaining walls were coated with a jacket through which water of constant temperature (293 K) circulated. The solutions were agitated with a magnetic stirrer. The source of UV radiation was a medium-pressure mercury lamp (Q-400, Hanau). The radiation passed through a combined glass filter (Schott and Gen., Jena) transmitting the 366 nm line (half width: 354 – 376 nm). The mean intensity of the incident radiation was 3.4×10^{-8} Einst s^{-1}. The amount of absorbed photons was measured using the Parker actinometer [19].

Chloride ions were assayed mercurometrically.

Results and Discussion

The irradiation of methanolic or ethanolic solutions of acridine and its derivatives in the presence of CCl_4, with a 366 nm line, produced Cl^- ions. Since neither CCl_4 nor the alcohols absorb the incident radiation, and Cl^- ions could not be detected in the irradiated alcoholic solutions of CCl_4, the formation of chloride ions must be due to the presence of acridine bases. Indeed, Fig. 1 shows that the shoulders of the long wavelength absorption

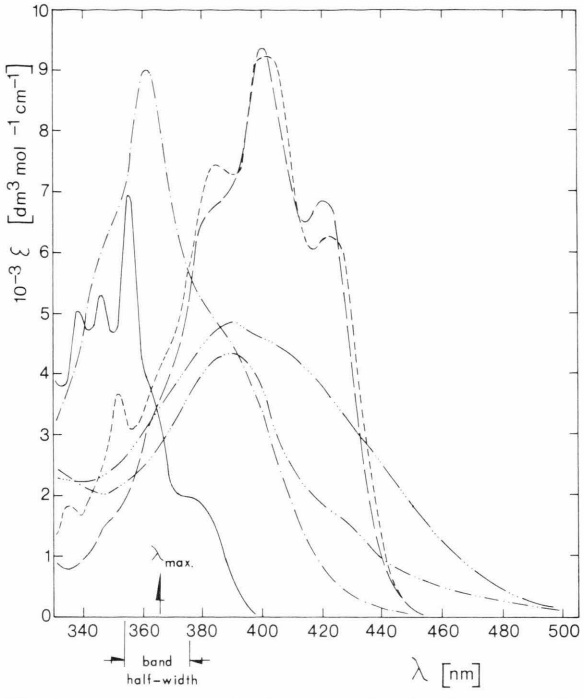

Fig. 1. Long wavelength absorption bands of some acridine bases in ethanolic solutions: acridine (——), 9-aminoacridine (– –), 4-methoxy-9-aminoacridine (- - - - - - -), 1-nitroacridine (–·–·–), nitracrine (–··–··–) and 1-nitro-9-aminoacridine (–····–····–).

bands of acridines fall within the range of transmittance of the radiation filter used.

Quantum yields of chloride ions formation (Φ) decrease with an increase of the irradiation time for all compounds investigated. Figure 2 shows this dependency in the case of 4-methoxy-9-aminoacridine. The adequate magnitude describing this phenomenon may be the ratio (X) of the number of moles of Cl^- ions ($n_{(Cl^-)}$) to the initial number of moles of acridine base in solution ($n_{0(A)}$). It may be noticed that for $X < 0.1$ Φ remains nearly constant, whereas when $X > 0.1$ Φ drops markedly in time. In a discussion which follows, only values obtained at $X < 0.1$ are considered.

Table 1 presents values of quantum yields obtained under comparable conditions for several acridine bases. Taking the value of Φ for acridine as a reference, 9-aminoacridine appears to be more photoreactive. Introduction of the methoxyl substituent to position 2 or 4 enhances the reactivity, whilst the nitro group reduces it considerably in each case. All these effects can be explained in terms of variation in electronegativity of the mole-

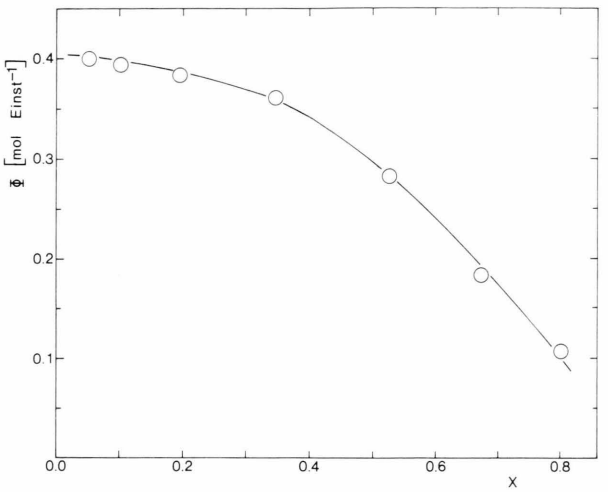

Fig. 2. Quantum yield of Cl^- ion formation as a function of X ($X = n_{(Cl^-)}/n_{0(A)}$). 4-methoxy-9-aminoacridine (c = 0.0005 mol dm^{-3}) in methanol + CCl_4 (c = 0.1 mol dm^{-3}).

Table 1. Quantum yields of formation of chloride ions upon irradiation of acridines ($c = 5 \times 10^{-4}$ mol dm^{-3}) in the mixture of methanol and CCl_4 (c = 0.1 mol dm^{-3}).

Compound	Quantum yield [mol $Einst^{-1}$]
Acridine	0.039
9-aminoacridine	0.15
1-nitroacridine	0.00074
9(2′-dimethylaminoethylamino)-acridine	0.022
3-nitro-9-aminoacridine	0.00014
1-methoxy-9-aminoacridine	0.17
2-methoxy-9-aminoacridine	0.40
3-methoxy-9-aminoacridine	0.12
4-methoxy-9-aminoacridine	0.40
1-nitro-9(3′-dimethylaminopropylamino)-acridine	0.0015
2-nitro-9(3′-dimethylaminopropylamino)-acridine	0.00083
3-nitro-9(3′-dimethylaminopropylamino)-acridine	0.00030

cules, and consequently in their electron-donor capability. Introduction of an alkylamino substituent to the 9-NH_2 group reduces markedly the reactivity. This behaviour may be interpreted as being due either to a steric hindrance, assuming C(9)-N as a reactive centre [20], or to an interaction with CCl_4 molecule already in the ground electronic state. The latter effect would appear through the aliphatic amine function as the most potent electron donor, onto which, however, the electronic excitation of the aromatic ring is not transfered. Thus, this centre can not be reactive. As a result, both excited nitracrine and its isomers exhibit a rather poor reactivity toward CCl_4. The reactivity of the latter compounds is, however, clearly differentiated. The quantum yields amounting to several hundredths of per cent for nitracrine fall to several thousandths of per cent for the two remaining isomers. Consequently, one can conclude that nitracrine, even in the electronically excited state, is a very weak electron donor. Replacement of methanol by ethanol does not lead to the appreciable changes of Φ values (Table 2).

The relation of Φ vs. X (Fig. 2) shows that the contribution of the acridine base to the photochemical processes is not limited to simple sensitization. If sensitization would occur Φ should not depend on X. A reasonable explanation of the experimental facts may be that the acridine base (A) is converted to the appropriate cation. Then the course of photochemical processes may be presented by the following general scheme:

$$A + h\nu \rightarrow A^* , \quad (1)$$

$$A^* + CCl_4 \rightarrow [A^+ \ldots CCl_4^-]^* , \quad (2)$$

$$[A^+ \ldots CCl_4^-]^* \rightarrow [A^+ \ldots CCl_3] + Cl^- , \quad (3)$$

$$[A^+ \ldots CCl_3] + \ldots \rightarrow \text{other products} . \quad (4)$$

Table 2. Quantum yields of formation of chloride ions in irradiated solutions of acridines (c = 0.0005 mol dm^{-3}) in mixtures comprising organic solvent and CCl_4 (c = 0.1 mol dm^{-3}).

Compound	Quantum yields of formation of Cl^- ions under various conditions [mol $Einst^{-1}$]					
	Ethanol 96%	Ethanol 99.8%	Methanol (solution saturated with oxygen for 1 h)	Methanol (solution de-aerated with argon 2 h)	1,4-dioxane	1,4-dioxane + water (c_{H_2O} = 0.1 mol dm^{-3})
Acridine	0.040	0.033	–	0.069	–	–
9-aminoacridine	0.17	0.12	0.16	0.26	0.042	0.042
4-methoxy-9-aminoacridine	0.49	–	–	–	–	–

The structure of a corresponding cation was proposed by Ivanoff and Walch [21] and later by Kellmann [22]. Owing to the positive charge the cation is a very weak electron donor, being rather an electron acceptor [23], and it does not participate in reactions analogous to (2) and (3). However, it absorbs active radiation, because its absorption in the long wavelength range should not differ substantially from that of the molecule of appropriate acridine base (Figure 3). Therefore, such cations should act as internal filters causing the decrease of Φ with the time of exposure.

The above mentioned facts may explain the main difference between the photochemical processes occuring in the systems investigated and those proceeding during irradiation of solutions of acridines in pure alcohols. In the latter case the central acridine ring loses its aromacity [24] and, in consequence, characteristic changes in absorption spectra, within the region 270 – 400 nm, are observed.

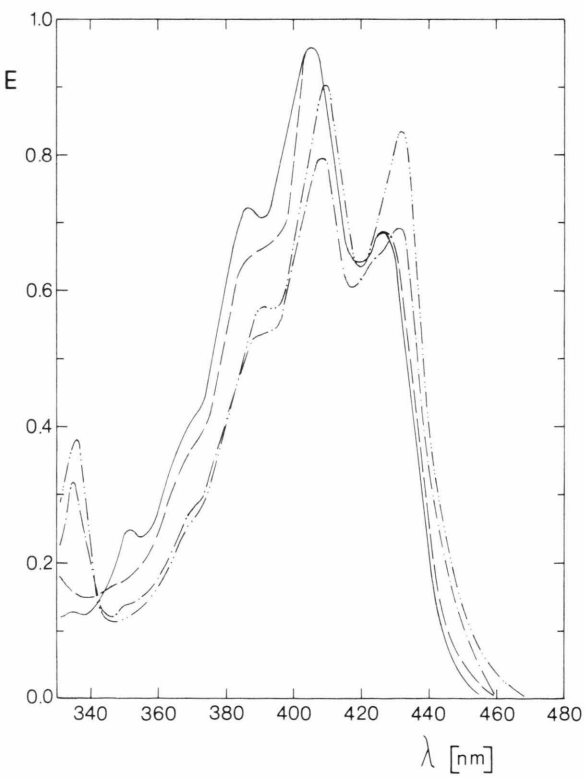

Fig. 3. Changes in long wavelength absorption bands as a result of irradiation or protonation. Optical path length = 0.2 cm. 4-methoxy-9-aminoacridine ($c = 0.0005$ mol dm^{-3}) in methanol + CCl_4 ($c = 0.1$ mol dm^{-3}): before irradiation (——), after exposure for 2 h (– – –). 2-methoxy-9-aminoacridine ($c = 0.0005$ mol dm^{-3}) in methanol: free base (–·–·–), hydrochloride (–··–··–).

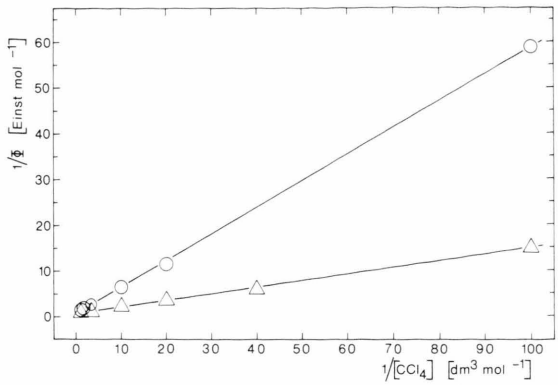

Fig. 4. Quantum yields of chloride ion formation vs. concentration of CCl_4 in methanolic solutions of acridines ($c = 0.0005$ mol dm^{-3}): 9-aminoacridine (○), 4-methoxy-9-aminoacridine (△).

Table 3. The integral fluorescence intensity ratio (I_0/I) of acridines in the presence of CCl_4[a].

Compound	Concentration of CCl_4 [mol dm^{-3}]				
	0	0.0001	0.01	0.1	1
Acridine	1	1.02	0.92	0.96	0.93
9-aminoacridine	1	1.00	0.97	1.01	0.97
3-nitro-9-aminoacridine	1	1.11	1.11	1.03	1.10
3-methoxy-9-aminoacridine	1	–	0.96	1.01	1.09

[a] I_0 represents the integral fluorescence intensity of a 0.0001 mol dm^{-3} methanolic solution of acridine base, and I the same quantitiy in the presence of CCl_4 in the solution.

For a particular acridine base the quantum yield of the chloride ion formation is a function of CCl_4 concentration. The relations presented on Fig. 4 are characteristic for all compounds investigated. These will be discussed further.

To check whether any interaction between excited acridine molecules and CCl_4 appear we conducted fluorescence measurements whose results are compiled in Table 3. Examining these data one may conclude that no quenching of the fluorescence of acridine bases is observed at varying concentrations of CCl_4. The observed differences in the I_0/I ratio show random deviations and they are comprised within experimental uncertainties. Since the fluorescence of acridines is not affected by the presence of CCl_4 it might indicate that the photochemical reac-

tion does not compete with the former process. It also might suggest that both these processes arise from different electronic states of the acridine base.

On the basis of the foregoing considerations we propose below a reaction scheme which seems to describe most satisfactorily the chloride ion formation:

$$^1A + h\nu \rightarrow {}^1A^* , \tag{5}$$

$$^1A^* \rightarrow {}^1A + h\nu' , \tag{6}$$

$$^1A^* \rightarrow {}^1A + \text{heat} , \tag{7}$$

$$^1A^* \rightarrow {}^3A^{**} , \tag{8}$$

$$^3A^{**} \rightarrow {}^1A + h\nu'' , \tag{9}$$

$$^3A^{**} \rightarrow {}^1A + \text{heat} , \tag{10}$$

$$^3A^{**} + CCl_4 \rightarrow {}^3[A^{\ddagger} \ldots CCl_4^{-}]^{**} , \tag{11}$$

$$^3[A^{\ddagger} \ldots CCl_4^{-}]^{**} \rightarrow [A^{\ddagger} \ldots CCl_3] + Cl^- . \tag{12}$$

The above scheme subsequent to reactions (1)–(4) assumes that a molecule of acridine base in the triplet state participates in the process of electron transfer. This involves that a rate of the intersystem crossing (8) is relatively high compared to the rates of the competing processes (6) and (7). Indeed, a recent estimation [25] indicates that among processes causing electronic relaxation of excited acridines the intersystem crossing is a very efficient one.

The reaction scheme proposed does not differentiate individual energy levels and it does not show which electronic state is the reactive one. A crude estimation based on the equation of Weller [26] and the acridine [27] and CCl_4 [28] redox data indicate that an electron transfer complex lies ca. 2.0 eV above the ground state. Since this is almost equal to the 1.96 eV energy of the first acridine triplet [25], the electron transfer process seems to be very likely. On the other hand, the energy of T_1 is too low to cause homolysis of the C–Cl bond. It is worth mentioning that similar processes, to those given by our reaction scheme, have been proposed for other donor – acceptor pairs [20, 29–33].

The proposed reaction scheme may help to explain qualitatively some other experimental facts. It is seen in Table 2 that de-aeration of solutions of acridine and 9-aminoacridine in methanol causes a noticeable increase in the Φ values. On the other hand saturation of the starting solutions with oxygen did not affect the Φ values. The equilibrium concentration of oxygen in solutions containing air amounts to about 0.003 mol dm^{-3}. Therefore, this amount of O_2 is sufficient to quench triplet states of acridines [34, 35]. The formation of weak complexes between oxygen dissolved in solution and acridine bases can also not be excluded [36, 37].

It is well known that the electronic relaxation of acridine derivatives depends on the nature of the solvent used [38, 39]. Generally, the increase of the polarity of the solvent favoures the intersystem crossing (8) and thus reaction (11). This is qualitatively supported by the data listed in Table 2. It is unexpected, however, that the introduction of H_2O into 1,4-dioxane did not affect the Φ values.

The system of the energy levels in acridine [25, 39] and in appropriate electron transfer complex [40] can be affected by the substituents. Thus, the behaviour of the excited molecule, i.e. its lifetime (e.g. short for nitrocompounds [41, 42]) and reactivity, should also depend on the nature of the substituent. Therefore, the above mentioned factors may account for these differences in the behaviour of individual acridine bases (Table 1) which are not due to variations in electronegativity.

A standard steady state kinetic treatment of the mechanism described by (5)–(12) leads to the equation for the quantum yield of Cl^- ion formation

$$\Phi = [k_8/(k_6 + k_7 + k_8)]/ \{(k_9 + k_{10} + k_{11}[CCl_4])/(k_{11}[CCl_4])\} . \tag{13}$$

In the absence of pertinent values of the rate constants it is convenient to present (13) in the simpler forms,

$$\Phi = A/(1 + B/[CCl_4]) \tag{14}$$

or

$$1/\Phi = 1/A + (B/A) \times (1/[CCl_4]) , \tag{15}$$

where: $A = k_8/(k_6 + k_7 + k_8)$ and $B = (k_9 + k_{10})/k_{11}$. Equations (13)–(15) should, therefore, describe the dependence of Φ on the concentration of CCl_4 in the solution. Indeed, a plot of the left-hand side of (15) vs. the reprocical CCl_4 concentration, shown in Fig. 4, yields a very satisfactorily straight line indicating the general correctness of the assumed reaction model. The derived values of the constants in (15) are $A = 1.72$ mol Einst^{-1}, $B = 1.08$ mol dm^{-3} for 9-aminoacridine and $A = 1.77$ mol Einst^{-1}, $B = 0.25$ mol dm^{-3} for 4-methoxy-9-aminoacridine.

The assumed reaction scheme predicts no dependence of the yield of fluorescence on the concentration of CCl_4, as fully supported by the experimental evidence. It is also worth noting that the intercept of an equation analogous to (15), describing $1/\Phi$, if reactions (11) and (12) would occur in the singlet state, should be equal to 1. The values of the intercept derived by us differ markedly from 1, and thus this fact also confirms the assumed reaction model.

Undoubtedly, the reaction scheme presented by (5)–(12) is oversimplified. To obtain a clearer picture of the photochemical processes proceeding it seems to be necessary to identify products comprising acridine bases. Such products could be analogous to those reported in the literature [43, 44]. Also, flash spectroscopic measurements of triplet quenching would strengthen the assumed reaction model. Nonetheless, the results obtained in this work augment our knowledge about the reactivity of excited acridine bases. They also may appear valuable in the explanation of the mechanism of biological actions of acridine drugs.

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[1] A. Albert, The Acridines, Edward Arnold, London 1966, 2nd Ed., Part IV.
[2] G. Loeber, Z. Chem. **11,** 92, 135 (1971).
[3] A. Ledochowski, Mater. Med. Pol. **8,** 237 (1976).
[4] S. Yamabe, Arch. Biochem. Biophys. **130,** 148 (1969).
[5] Y. Kubota, K. Hirano, and Y. Motoda, Chem. Lett. 123 (1978).
[6] Y. Kubota and Y. Motoda, J. Phys. Chem. **84,** 2855 (1980).
[7] J. Blazejowski, Wiad. Chem. **33,** 607 (1979).
[8] T. Takahashi, K. Kikuchi, and H. Kokubun, J. Photochem. **14,** 67 (1980).
[9] M. V. Encinas, M. A. Rubio, and E. A. Lissi, J. Photochem. **18,** 137 (1982).
[10] I. M. Brinn, Z. Naturforsch. **35a,** 418 (1980).
[11] J. Biedrzycki, J. Blazejowski, A. Ledochowski, and J. Szychlinski, Rocz. Chem. **51,** 379 (1977).
[12] C. F. Chignell, Adv. Drug Res. **5,** 55 (1970).
[13] A. Ledochowski and B. Stefanska, Rocz. Chem. **40,** 301 (1966).
[14] B. Stefanska and A. Ledochowski, Rocz. Chem. **42,** 1535 (1968).
[15] A. Ledochowski, Z., Ledochowski, J. Peryt, and H. Wojciechowska, Rocz. Chem. **38,** 1111 (1964).
[16] A. Albert and A. Gledhill, J. Soc. Chem. Ind. **61,** 159 (1942).
[17] A. Albert and A. Gledhill, J. Soc. Chem. Ind. **64,** 169 (1945).
[18] N. Drozdov and O. Tcherntsov, Zh. Obshch. Khim. **5,** 1576 (1935).
[19] C. G. Hatchard and C. A. Parker, Proc. Roy. Soc. London **A235,** 518 (1956).
[20] Yu. V. Morozov and A. K. Savenko, Mol. Photochem. **8,** 1 (1977).
[21] N. Ivanoff and F. Walch, J. Chim. Phys. **54,** 473 (1957).
[22] A. Kellmann, J. Chim. Phys. **63,** 949 (1966).
[23] V. L. Ivanoff, Al-Ainen Sami Abu, and M. G. Kuzmin, Dokl. Acad. Nauk SSSR **237,** 1400 (1977).
[24] O. L. Chapman, Organic Photochemistry, Marcel Dekker, New York 1969, pp. 117–121.
[25] K. Kasama, K. Kikuchi, K. Uji-Ie, S.-A. Yamamoto, and H. Kokubun, J. Phys. Chem. **86,** 4733 (1982) and references cited therein.
[26] H. Knibbe, D. Rehm, and A. Weller, Ber. Bunsenges. Phys. Chem. **72,** 257 (1968).
[27] K. Yasukouchi, I. Taniguchi, H. Yamaguchi, and K. Arakawa, J. Electroanal. Chem. Interfacial Electrochem. **121,** 231 (1981).
[28] C. K. Mann and K. K. Barnes, Electrochemical Reactions in Non-Aqueous Systems, Marcel Dekker, New York 1970.
[29] F. A. Carroll and D. G. Whitten, J. Phys. Chem. **80,** 2046 (1976).
[30] V. L. Pugachev, T. D. Slavnova, and A. K. Chibisov, Khimiya Vys. Energ. **11 (4),** 355 (1977).
[31] W. Muharaj, I. G. Czizmadia, and M. A. Winnik, J. Am. Chem. Soc. **99,** 946 (1977).
[32] H. Kobashi, H. Gyoda, and T. Morita, Bull. Chem. Soc. Jpn **50,** 1731 (1977).
[33] E. I. Kapinus, M. M. Aleksankina, and I. I. Dilung, J. Photochem. **21,** 125 (1983).
[34] S. Niizuma, Y. Ikeda, and M. Koizumi, Bull. Chem. Soc. Jpn **40,** 2249 (1967).
[35] A. Kira and M. Koizumi, Bull. Chem. Soc. Japan **40,** 2486 (1967).
[36] H. Tsubomura and R. S. Mulliken, J. Amer. Chem. Soc. **82,** 5966 (1960).
[37] J. C. W. Chien, J. Phys. Chem. **69,** 4317 (1965).
[38] K. Kasama, K. Kikuchi, S.-A. Yamamoto, K. Uji-Ie, Y. Nishida, and H. Kokubun, J. Phys. Chem. **85,** 1291 (1981).
[39] K. Kasama, K. Kikuchi, Y. Nishida, and H. Kokubun, J. Phys. Chem. **85,** 4148 (1981).
[40] Yu. Beilis, O. N. Chupakhin, V. L. Rusinov, and V. D. Bezuglyi, Zh. Obshch. Khim. **45,** 606 (1975).
[41] R. Hurley and A. C. Testa, J. Amer. Chem. Soc. **90,** 1949 (1968).
[42] C. Capellos and K. Suryanarayanan, Int. J. Chem. Kinet. **9,** 399 (1977).
[43] E. A. Fitzgerald, D. Wuelfing, and H. H. Richtol, J. Phys. Chem. **75,** 2737 (1971).
[44] W. Schwarz, P. Hesse, and F. Doerr, Ber. Bunsenges. Phys. Chem. **81,** 1231 (1977).