STUDY OF SOME REACTIONS OF ACRIDINE ORANGE

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The nitration and formylation of acridine orange were studied, and its dinitro derivatives and mono and diformyl derivatives were obtained. By measurement of the ionization constants of the conjugated cations and PMR spectroscopy it was proved that the protons attached to $C_{(4)}$ and $C_{(5)}$ are substituted.

We studied two electrophilic substitution reactions of 3,6-bis(dimethylamino)acridine (I) – the introduction of an aldehyde group and nitration – in order to obtain substances that are of interest for the synthesis of biologically active compounds, since it is known that I has biological activity [1-3] and is able to give complexes with nucleic acid [4]. The aldehyde group was introduced via the Vilsmeier–Haack reaction by the addition of a solution of I in dimethylformamide to a cooled mixture of phosphorus oxychloride and dimethylformamide. Depending on the ratio of the components, monoformyl and diformyl derivatives (II, III) could be obtained.

The literature relative to the nitration of I and the reduction of the nitro derivative consists only of patent data [5] in which there is no mention of the characteristics of the products obtained, and the site of entry of the nitro group into the I molecule is not indicated. We accomplished the nitration of I with a 10-fold excess of concentrated nitric acid in glacial acetic acid. The dinitro derivative (IV) was isolated and crystallized from dichloroethane and reduced with hydrazine hydrate in the presence of Raney nickel to the diamino derivative (V).

From the distribution of the electron density of the 3,6-diaminoacridine cation [6], the molecular diagram of which is similar to that for the cation of I, it can be assumed, with a high measure of probability, that the electrophilic substitution will primarily occur at the $C_{(4)}$ and $C_{(5)}$ atoms, which have the greatest negative charge.

It is known [7] that the basicity of 4,5-diaminoacridine (pK_a 3.55) is somewhat lower than that of unsubstituted acridine (pK_a 4.11), while the introduction of two amino groups into the 2 and 7 positions leads to a considerable increase in the basicity (the pK_a of 2,7-diaminoacridine is 5.64). We measured the ionization constants of the conjugated cations of the compounds (Table 1). We found that diamino derivative V

TABLE 1. Chemical Shifts (δ) of the Protons and Ionization Constants of Acridine Orange Derivatives

Compound	δ, ppm (No. of protons)							
	$N(CH_3)_2$	$C_{(2)}$, $C_{(7)}$	$C_{(1)}$, $C_{(8)}$	$C_{(4)}$, $C_{(5)}$	$C_{(5)}$	$C_{(9)}$	CHO	pK_a
I	3,11 (12)	7,28 (2)	7,80 (2)	7,23 (2)	—	8,53 (1)	—	9,65
II	3,23 (6)	7,29 (1)	7,68 (1)	—	8,25 (1)	8,77 (1)	9,77 (1)	8,75
	3,35 (6)	7,62 (1)	8,08 (1)					
III	3,25 (12)	7,16 (2)	7,66 (2)	—	—	8,39 (1)	9,75 (2)	8,55
IV	3,01 (12)	7,28 (2)	7,70 (2)	—	—	8,53 (1)	—	4,20

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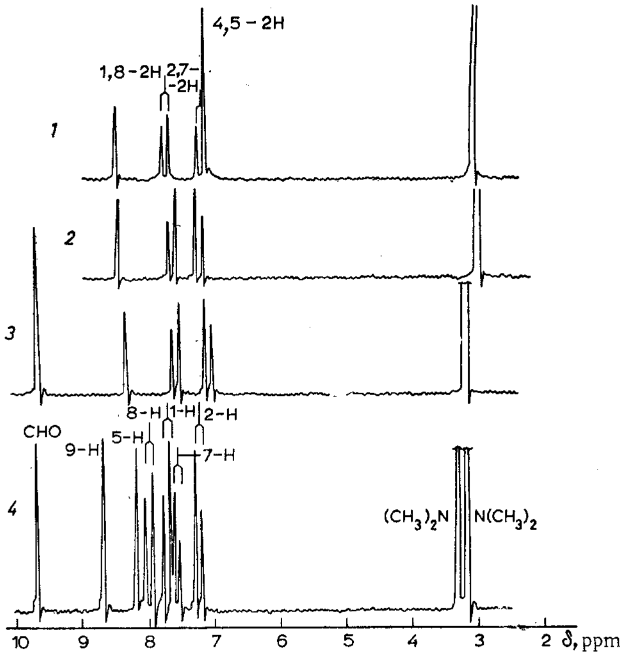
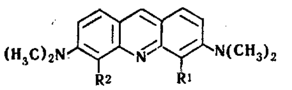


Fig. 1. PMR spectra (in trifluoroacetic acid): 1) 3,6-bis(dimethylamino)acridine (I); 2) 3,6-bis(dimethylamino)-4,5-dinitroacridine (IV); 3) 3,6-bis(dimethylamino)-4,5-diformylacridine (III); 4) 3,6-bis(dimethylamino)-4-formylacridine (II).

has two ionization constants (3.50 and 4.70) that are lower than those of starting I (pK$_a$ 9.65). These results provide a basis for assuming that, in this case, the hydrogen atoms attached to C$_{(4)}$ and C$_{(5)}$ are substituted.

The above assumption is confirmed by the PMR spectra of I-IV.



I R^1=R^2=H; II R^1=CHO, R^2=H; III R^1=R^2=CHO; IV R^1=R^2=NO$_2$; V R^1=R^2=NH$_2$

The PMR spectrum of I (Fig. 1) is characterized by absorption in the form of singlets with the appropriate intensities for two dimethylamino groups at strong field, by absorption of the protons attached to C$_{(4)}$, C$_{(5)}$, and C$_{(9)}$, and by two doublets (more accurately, an AB quartet) for the ortho protons attached to C$_{(1)}$, C$_{(8)}$, and C$_{(2)}$, C$_{(7)}$ with spin–spin coupling constant $J_{1(8),2(7)} = 10$ Hz. The assignment was made in accordance with [8]. In contrast to the spectrum presented in [8], the singlet of the protons attached to C$_{(4)}$ and C$_{(5)}$ in the PMR spectrum is shifted to weak field, which apparently can be explained by the solvent (trifluoroacetic acid) effect.

A singlet from the proton attached to C$_{(9)}$ and a quartet from the protons attached to C$_{(2)}$, C$_{(7)}$ and C$_{(1)}$, C$_{(8)}$ are observed at weak field in the PMR spectrum of dinitro derivative IV. There is no signal for protons attached to C$_{(4)}$ and C$_{(5)}$, which is evidence for substitution of these protons by a nitro group. The PMR spectrum of dialdehyde III is similar to the spectrum of dinitro derivative IV and differs from it only by the appearance at weak field of a signal from two protons of aldehyde groups. The protons attached to C$_{(4)}$ and C$_{(5)}$ are consequently substituted during formylation. An analysis of the PMR spectrum of the monoaldehyde made it possible to isolate individual singlets for the aldehyde group proton, the proton attached to C$_{(9)}$, and the protons of the two dimethylamino groups, the nonequivalence of the absorption of which, in contrast to starting I and disubstituted derivatives (III and IV), indicates they have different chemical environments. The one-proton singlet for one of the protons attached to C$_{(4)}$ or C$_{(5)}$ indicates monosubstitution in one of these positions. As a result of this, the protons attached to C$_{(1)}$, C$_{(8)}$ and C$_{(2)}$, C$_{(7)}$ become chemically and magnetically equivalent, and two two-proton AB quartets for the protons attached to C$_{(1)}$, C$_{(2)}$, C$_{(7)}$, C$_{(8)}$ can be isolated in the spectrum (Fig. 1). The assignment of the lines in the spectrum of the monoaldehyde (II) was made on the basis of a comparison of the spectra of I and of dialdehyde III. The accurate chemical shift and the ratios of the integral intensities (number of protons) are presented in Table 1.

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EXPERIMENTAL

A sample of I isolated from the zinc salt by the method in [9] and purified by chromatography according to the method in [10] was used in this study.

3,6-Bis(dimethylamino)-4-formylacridine (II). A 0.36 g (5 mmole) sample of dimethylformamide (DMF) was added dropwise to 0.76 g (5 mmole) of phosphorus oxychloride at 10°C, and a solution of 1.32 g (5 mmole) of I in 20 ml of DMF was added to this mixture in small portions after 15 min. The resulting mixture was then heated at 65° for 4 h. The bright-orange precipitate was removed by filtration, treated with 25% ammonium hydroxide, and crystallized from alcohol to give 0.4 g (27.8%) of yellow crystals with mp 168°. Found: C 73.8; H 6.6; N 14.4%. $C_{18}H_{19}N_3O$. Calculated: C 73.7; H 6.5; N 14.4%.

3,6-Bis(dimethylamino)-4,5-diformylacridine (III). This compound was similarly obtained in 62% yield with a fivefold amount of $POCl_3$–DMF complex. The yellow crystals had mp 205° (from alcohol). Found: C 71.0; H 6.0; N 13.4%. $C_{19}H_{19}N_3O_2$. Calculated: C 71.0; H 5.9; N 13.1%.

3,6-Bis(dimethylamino)-4,5-dinitroacridine (IV). A total of 3 ml (100 mmole) of nitric acid (sp. gr. 1.47) was added dropwise with vigorous stirring and cooling to a solution of 2.65 g (10 mmole) of I in 40 ml of glacial acetic acid, and the mixture was held at room temperature for 30 min. The resulting precipitate was treated with dilute ammonium hydroxide and crystallized from dichloroethane to give 2.1 g (69%) of orange crystals with mp 303-304°. Found: C 57.4; H 4.9; N 19.9%. $C_{17}H_{17}N_5O_4$. Calculated: C 57.5; H 4.8; N 19.7%.

3,6-Bis(dimethylamino)-4,5-diaminoacridine (V). A total of 15 ml of hydrazine hydrate and a catalytic amount of Raney nickel were added to a solution of 1 g (2.8 mmole) of IV in 200 ml of ethanol, and the mixture was refluxed for 1 h. The alcohol was then removed by distillation to one fourth of the initial volume, and the residue was diluted with hot water. The precipitate was removed by filtration and purified by reprecipitation from alcohol solution by the addition of water to give 0.4 g (42%) of dark-red crystals with mp 120-121°. Found: C 69.3; H 6.9; N 23.9%. $C_{17}H_{21}N_5$. Calculated: C 69.2; H 7.1; N 23.7%.

The ionization constants were measured by potentiometric titration of 0.001 N solutions of hydrochlorides I-V in 50% aqueous alcohol with a 0.1 N NaOH solution at 21 ± 1°. The PMR spectra of 8% solutions in trifluoroacetic acid were recorded with a Varian HA-100 spectrometer. The chemical shifts are given in parts per million relative to tetramethylsilane.

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