Studies on Some New Heterocyclic Quinone Monomethine Cyanine Dyes

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New asymmetrical naphtho/quinolinoquinone[2,3-*d*]thiazole/oxazole-4,9-dione 2[4(1)]-monomethine cyanines and naphthoquinone[2,3-*e*]oxadiazine-5,10-dione 2[4(1)]-monomethine cyanine dyes were synthesized to study their spectral behaviour, solvatochromism, acid-base properties, and antimicrobial activities. This study discloses the activity of former quinoid ring in cyanine dyes when applied as photosensitizer. These dyes are characterized by elemental analysis, IR, ^{1}H NMR, and electronic absorption spectra.

Monomethine cyanines are used as photosensitizers in the blue-green light [1—3] and they are also useful as analytical reagents over a wide pH range [4, 5]. They are also used as inhibitors of the cell growth and division [6] and as anticancer agents [7].

We report here on the synthesis and studies of some new asymmetrical monomethine cyanines (*Va—Ve* and *VIa—VIc*, Scheme 1) which incorporate naphtho/quinolinoquinone[2,3-*d*]thiazole/oxazole-4,9-dione and [2,3-*e*]oxadiazine-5,10-dione moieties since such dyes might exhibit photosensitization effects. Their biological activity towards bacteria and fungi is discussed.

EXPERIMENTAL

All melting points are uncorrected. Elemental analysis was carried out at the microanalytical centre (Cairo University). The IR spectra were determined with Perkin—Elmer 127B spectrophotometer (Cairo University). The visible spectra, solvatochromism, and pH-sensitivity were recorded within the wavelength 300—750 nm on a SHIMADZU UV VIS 240 spectrophotometer using 1 cm cells (Faculty of Science, Aswan). The ^{1}H NMR spectra were recorded with EM-390 (90 MHz) spectrometer (Cairo University).

Synthesis of 2-methylnaphtho/quinolinoquinone-[2,3-*d*]thiazole/oxazole-4,9-dione *Ia—Ic* was performed in a way similar to that described in Ref. [8].

At the investigation of solvatochromism and acid-base properties the organic solvents used were of spectroscopic grade or purified according to the recommended method [9]. An accurate volume of the stock solution (10^{-3} mol dm^{-3}) of the dyes was diluted to the appropriate volume in order to obtain the required concentrations.

A series of buffer solutions with pH values ranging from 1.35—12.5 was prepared as recommended by *Britton* [10]. An accurate volume of the stock solutions (10^{-3} mol dm^{-3}) was added to 0.5 cm^3 buffer solution in a 5 cm^3 measuring flask, then completed to the mark with redistilled water. The pH of buffer solution was checked before spectral measurements. The spectra were recorded either in pure solvents or in aqueous universal buffer solutions.

Antimicrobial activity of selected newly synthesized cyanine dyes was tested in three repeated experiments using the filter paper disc method [11] according to which all the compounds used were dissolved in ethylene glycol (the bacteria used in these experiments were previously found among several soil microorganisms tested to be susceptible) and bacterial suspension. The latter was prepared by adding 10 cm^3 of sterile distilled water to a ten days old culture of the test bacteria grown on Nutrient Agar or N.A. [12]. (ρ_i/(g dm^{-3}): Beef extract 10, peptone 5, sodium chloride 5, Agar 17; pH $\cong$ 7.4.) One cm^3 aliquots of the bacteria suspension were added to N.A. Petri dishes. The excess liquid was removed and two filter paper discs (6 mm diameter) containing the test compound were placed on each plate. The plates were then incubated at 37 °C and the inhibition zones diameter was measured after 24 h.

2-Methylnaphthoquinone[2,3-*e*]oxadiazine-5,10-dione (*II*)

A mixture of ethylene glycol solution of 2,3-dichloro-1,4-naphthoquinone (0.01 mol) and monoacetyl hydrazine (0.01 mol) was refluxed for 1 h and then a reddish brown solution was attained. An amount of NaHCO$_3$ (5 cm^3 of 20 % aqueous solution) [13] was added, followed by the refluxing again for 2 h.

The reaction mixture was cooled, diluted with aqueous ethanol and the product was collected and crystallized from acetic acid.

For $C_{12}H_8N_2O_3$ ($M_r = 228$) w_i(calc.): 63.16 % C, 3.51 % H, 12.28 % N; w_i(found): 63.38 % C, 3.02 % H, 12.00 % N. M.p. = 170 °C, yield 63 %, brown crystals.

IR spectrum, $\tilde{\nu}$(KBr)/cm^{-1}: 3300—3500 (NH group), 1680 (C=O for quinone), 1600 (C=N), 1070—1170 (C—O—C cyclic), and 670 (benzene disubstituted). ^{1}H NMR spectrum (CDCl$_3$), δ: 7.4—8.3 (m, 4H, H$_{arom}$ (het), H), 2.0—2.9 (4H, for NH and CH$_3$ groups).

2-Methylnaphtho/quinolinoquinone[2,3-d]thiazole/oxazole-4,9-dione-3-methiodide *IIIa*—*IIIc* and [2,3-e]Oxadiazine-5,10-dione-3-methiodide *IV*

A pure sample of compounds *Ia*—*Ic* or *II* was suspended in excess of methyl iodide and heated in a sealed tube at 140 °C for 3 h. The sealed tube was cooled, opened and the products *IIIa*—*IIIc* and *IV*, respectively, were collected, washed with ether and then crystallized from absolute ethanol (Table 1).

IR spectrum, $\tilde{\nu}$(KBr)/cm^{-1} for *IIIa*, *IIIb*: 2820—3040 (ν(methiodide)),
1720—1835 (ν(C=O)$_{naphthoquinone}$),
1615—1640 (ν(C=N)$_{thiazole(oxazole)}$),
1020—1130 (ν(C—S—C)$_{thiazole}$ or ν(C—O—C)$_{oxazole}$).

IR spectrum, $\tilde{\nu}$(KBr)/cm^{-1} for *IV*: 3200—3700 (ν(NH)), 2940 (ν(methiodide)), 1675 (ν(C=O)), 1600 (ν(C=N)), 1020—1080 (C—O—C cyclic), 710—760 (ν(benzene disubstituted)).
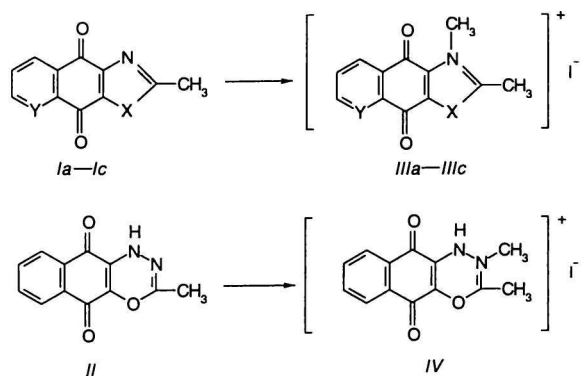
^{1}H NMR spectrum (CDCl$_3$), δ for *IIIa*, *IIIb*: 7.2—8.2 (m, 4H, H$_{arom}$), 3.5—4.2 (s, 3H, CH$_3$ joined to immonium centre), 2.0—3.1 (s, 3H, CH$_3$).

Asymmetrical Naphtho/quinolinoquinone[2,3-d]thiazole/oxazole-4,9-dione 2[4(1)]-Monomethine Cyanines *Va*—*Ve* and Naphthoquinone[2,3-e]oxadiazine-5,10-dione 2[4(1)]-Monomethine Cyanine Dyes
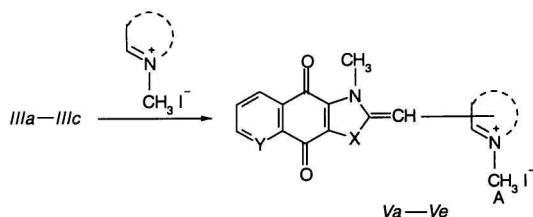
The quaternary salts *IIIa*—*IIIc* and *IV* (0.01 mol) were refluxed with *N*-methylpyridinium, -quinolinium, and -isoquinolinium iodide (0.01 mol) in the presence of ethanol (30 cm^3) and few drops of piperidine. The products *Va*—*Ve* and *VIa*—*VIc* were collected, washed with aqueous ethanol and then crystallized from absolute ethanol (*cf.* Table 1).

IR spectrum, $\tilde{\nu}$(KBr)/cm^{-1} for *Vb, Vd, Ve,* and *VIb*: 2900—3060 (ν(methiodide)), 1620—1710 (ν(C=O)) for naphthoquinone or quinolinoquinone, 1050—1110 (ν(C—S—C)$_{thiazole}$ or (C—O—C)$_{oxazole(oxadiazine)}$), 1585 (ν(C=CH)), 3200—3700 (ν(NH) for oxadiazine ring, *VIb*), 700—750 (ν(benzene disubstituted)).
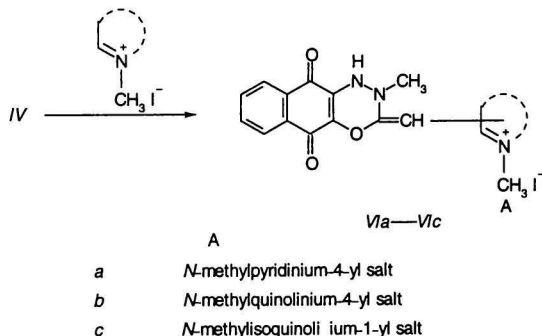
^{1}H NMR spectrum (CDCl$_3$), δ for *Vd*: 7.2—8.4

Scheme 1

(m, 10H, H$_{arom}$, =CH), 3.6—4.2 (s, 3H, CH$_3$ joined to immonium centre), 2.5 (s, 3H, CH$_3$—N).

^{1}H NMR spectrum (CDCl$_3$), δ for *VIa*: 7.0—8.2 (m, 9H, H$_{arom}$, =CH), 4.5 (s, 1H, NH), 2.5 (s, 3H, CH$_3$ joined to immonium centre), 1.2—1.8 (s, 3H, CH$_3$—N).

RESULTS AND DISCUSSION

To the synthesis of asymmetrical monomethine cyanine dyes *Va*—*Ve* and *VIa*—*VIc*, quaternization

Table 1. Characterization Data for Quaternary Starting Compounds (*IIIa—IIIc* and *IV*) and Asymmetrical Monomethine Cyani Dyes (*Va—Ve* and *VIa—VIc*).

Compound	Formula M_r	w_i(calc.)/% w_i(found)%			Yield %	M.p. °C	Colour	Absorption spectra in 95 % ethanol	
		C	H	N				λ_{max} nm	ε_{max} cm² mol⁻¹
IIIa	$C_{13}H_{10}NO_2SI$ 371	42.05 42.03	2.69 2.59	3.77 3.66	45	175	Brown		
IIIb	$C_{13}H_{10}NO_3I$ 355	43.94 43.82	2.82 2.72	3.94 3.82	60	160	Brown		
IIIc	$C_{13}H_{12}N_2O_2SI_2$ 514	30.35 30.29	2.33 2.29	5.45 5.22	20	149	Deep brown		
IV	$C_{13}H_{11}N_2O_3I$ 370	42.16 42.01	2.97 2.56	7.57 7.06	45	220	Deep brown		
Va	$C_{19}H_{15}N_2O_2SI$ 462	49.35 49.30	3.24 3.09	6.06 6.09	50	65	Shiny red	490	2200
Vb	$C_{23}H_{17}N_2O_2SI$ 512	53.96 53.70	3.32 3.12	5.47 5.37	36	110	Red	495	15000
Vc	$C_{23}H_{17}N_2O_2SI$ 512	53.91 53.80	3.32 3.31	5.47 5.35	50	97	Brownish red	490	4000
Vd	$C_{23}H_{17}N_2O_3I$ 496	55.64 55.44	3.43 3.32	5.64 5.52	35	185	Brownish red	462 510 550	3200 2000 1800
Ve	$C_{23}H_{19}N_3O_2SI_2$ 655	42.14 42.04	2.90 2.80	6.41 6.30	22	205	Deep red	445	6000
VIa	$C_{19}H_{16}N_3O_3I$ 461	49.46 49.44	3.47 3.37	9.11 9.01	35	205	Reddish brown	435	2233
VIb	$C_{23}H_{18}N_3O_3I$ 511	54.01 54.19	3.52 3.42	8.22 8.02	50	170	Reddish brown	490	1800
VIc	$C_{23}H_{18}N_3O_3I$ 511	54.01 54.07	3.52 3.62	8.22 8.16	44	120	Reddish brown	480	1966

of 2-methylnaphtho/quinolinoquinone[2,3-*d*]thiazole/oxazole-4,9-dione *Ia—Ic* [8] and [2,3-*e*]oxadiazine-5,10-dione *II* [13] using MeI in a sealed tube [14] at 140 °C afforded 2-methylnaphtho/quinolinoquinone-[2,3-*d*]thiazole/oxazole-4,9-dione methiodide *IIIa—IIIc* and [2,3-*e*]oxadiazine-5,10-dione methiodide *IV*, respectively. Interaction of equimolar amounts of *IIIa—IIIc* and *IV* with 1-methylquinolinium/isoquinolinium salts in the presence of piperidine as basic catalyst and ethanol as solvent gave the asymmetrical naphtho/quinolinoquinone[2,3-*d*]thiazole/oxazole-4,9-dione 2[4(1)]-monomethine cyanines *Va—Ve* and [2,3-*e*]oxadiazine-5,10-dione 2[4(1)]-monomethine cyanine dyes *VIa—VIc*, respectively.

The structure of asymmetrical monomethine cyanine dyes *Va—Ve* and *VIa—VIc* was established by elemental analysis, IR [15], and ¹H NMR [16] spectral data. The asymmetrical monomethine cyanines *Va—Ve* and *VIa—VIc* are fairly soluble in polar organic solvents and in concentrated H_2SO_4 liberating iodine vapour on heating. Their ethanolic solutions give red/brown colour in alkaline medium which discharges on acidification and restores their permanent colour on basification.

The electronic absorption spectra of asymmetrical monomethine cyanines *Va—Ve* and *VIa—VIc* in 95 % ethanol depend upon the nature of quaternary heterocyclic residue (A). Thus, the absorption spectra of monomethine dyes *Va* and *VIa* involving pyridinium salt moiety have absorption bands hypsochromically shifted by 5.55 nm, respectively if compared with those containing quinolinium analogues *Vb* and *VIb* (*cf.* Table 1). This may be attributed to the more extensive π-delocalization within quaternary heterocyclic moiety.

Additionally, changing the linkage position of heterocyclic quaternary residue from 4-yl salt to 1-yl salt resulted in a blue shift. Thus, the comparison of the absorption spectra of *Va—Ve* and *VIa—VIc* discloses that 4-yl linkage results in a bathochromic shift of 5—10 nm, respectively (*cf.* Table 1). This is due to the more extended conjugation in 4-yl linkage resulting in an increase of the delocalization of π-electrons in the cyanine molecule.

On the other hand, the absorption spectra of such monomethine cyanines are also influenced by the nature of five-membered ring moiety (either oxazole or thiazole) attached to naphtho or quinolinoquinone systems. Thus, the absorption spectra of the dye *Vd* incorporating the naphthoquinone[2,3-*d*]oxazole system disclose a bathochromic shift of 55 nm as the analogous dye *Vb* involving naphthoquinone[2,3-*d*]thiazole system (*cf.* Table 1). This is due to the more easier charge transfer from oxazole oxygen atom in comparison with the thiazole sulfur atom to the quinolinium-4-yl cation causing a bathochromic shift. Monomethine

Table 2. Electronic Absorption Spectra Characteristic of the Monomethine Cyanines (Vb, Vc) in Pure Solvents

Compound	λ_{max}/nm (ε_{max}/(m^{-1} cm^2))											
	Water		DMF		Ethanol		CHCl$_3$		CCl$_4$		Dioxane	
Vb	475	(11000)	497	(17800)	495	(15000)	499	(13800)	499	(11600)	498	(10400)
Vc	485	(3400)	498	(5200)	490	(4000)	498	(4000)	500	(8200)	505	(2200)
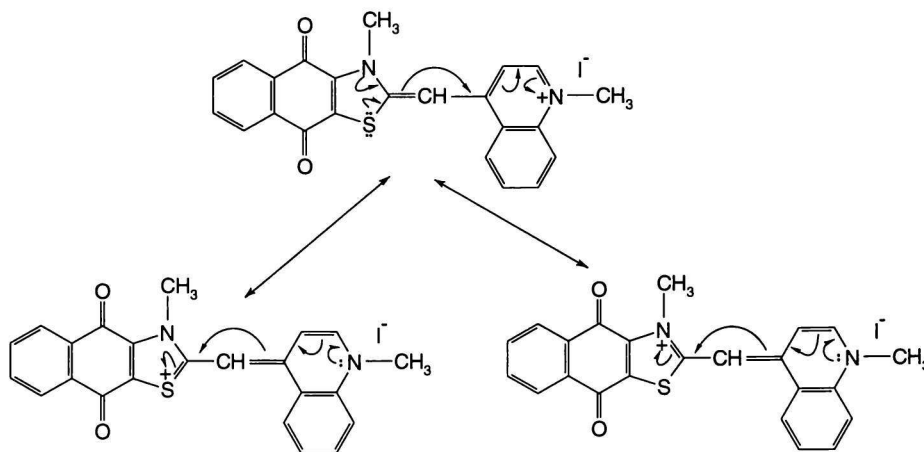
Scheme 2

cyanine incorporating quinolinoquinone ring (Ve; Y = N$^+$CH$_3$) undergoes a hypsochromic shift of 50 nm in comparison with the analogous dye (Vb; Y = CH). This is due to the fact that the positive charge on nitrogen atom decreases the electron delocalization at the cyanine conjugated system (*cf.* Table 1).

Comparison of the absorption spectra of monomethine dye Vd incorporating naphthoquinone[2,3-d]-oxazole system and of VIb involving naphthoquinone-[2,3-e]oxadiazine system discloses that the dye of oxazole moiety Vd exhibits a more bathochromic shift (20 nm) if compared to those of oxadiazine analogue VIb. This may be attributed to the increase of the electron-withdrawing ability caused by oxadiazine ring exerting an antagonistic effect (*cf.* Table 1).

The changes of colours and electronic absorption spectra of some selected monomethine cyanine dyes in some organic solvents were examined in the visible region in order to shed some light on their solvatochromic behaviour. Thus, the electronic absorption spectra of monomethine dyes Vb, Vc in pure organic solvents of different electric relative permittivity ε_r, *viz.* water (78.54), DMF (36.70), EtOH (24.3), CHCl$_3$ (4.806), CCl$_4$ (2.238), and dioxane (2.209) [17], respectively, gave different values (λ_{max}, ε_{max}) of the absorption bands due to different electronic transitions within the solute molecule in those solvents (Table 2, Fig. 1).

It is clear that the spectra of compounds Vb, Vc

in ethanol medium are characterized by one band in the visible region (above 340 nm). This band can be attributed to the intramolecular charge-transfer interaction [18], as well as $n \rightarrow \pi^*$ transitions [19]. The intramolecular CT transition can be represented by Scheme 2 (for dye Vb as an example).

From data given in Table 2 it is clear that the band corresponding to $n \rightarrow \pi^*$ or CT transitions shows a slight red shift on changing the solvent from ethanol to DMF, CHCl$_3$, CCl$_4$, and dioxane, which may be attributed to the increase in solvent polarity of DMF, and to the solute—solvent interaction through intermolecular hydrogen bond formation in case of CHCl$_3$, CCl$_4$, and dioxane.

The small blue shift observed in ethanol medium may be explained as a result of the H-bond formation between ethanol and the lone electron pair of the thiazolo sulfur atom. This decreases slightly the electron density on sulfur atom and consequently decreases to some extent the mobility of the π-electrons attached to the conjugated pathway. It is worth mentioning that the slight blue shift observed in λ_{max} in water medium relative to ethanol can be mainly ascribed to the interaction of water molecule with the lone electron pair of the thiazolo sulfur and naphthoquinone oxygen atoms through H-bonding which consequently inhibits the mobility of the π-electrons attached to the conjugated pathway (*cf.* Table 2).
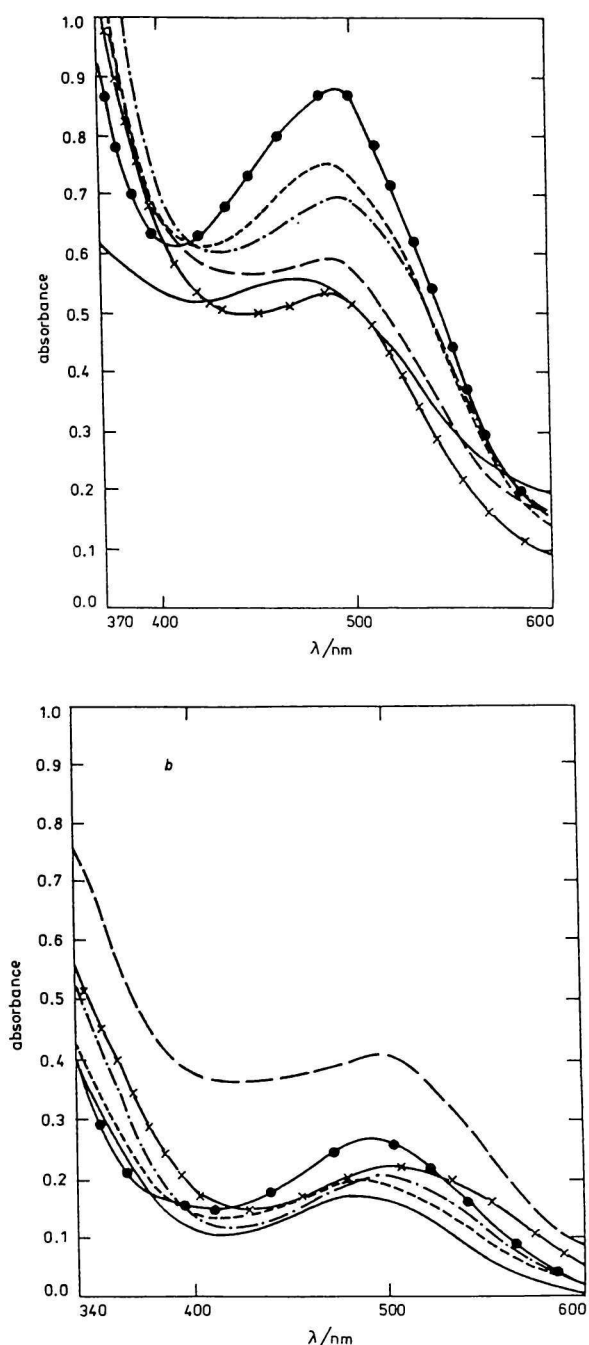
The ethanolic solutions of newly synthesized mono-

Fig. 1. Electronic absorption spectra of dyes *Vb* and *Vc* in H$_2$O (—), DMF (●), EtOH (- - - - -), CHCl$_3$ (— —), CCl$_4$ (— —), and dioxane (×). *a*) *Vb*; *b*) *Vc*.
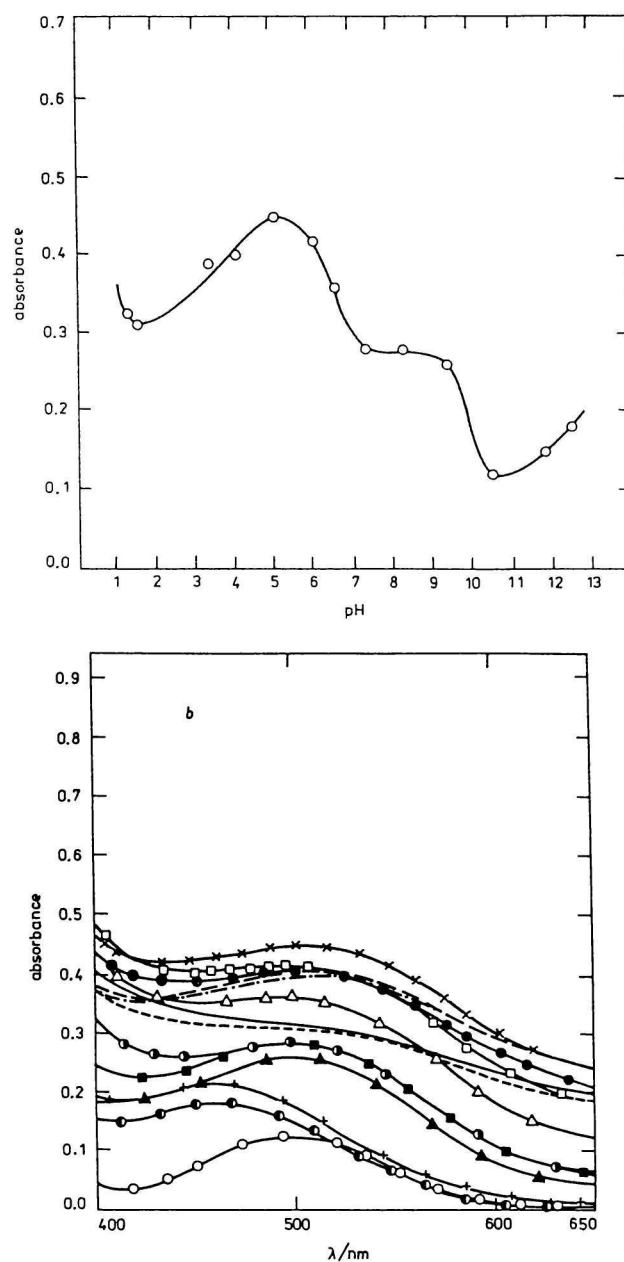
Fig. 2. *a*) $C = 1 \times 10^{-4}$ mol dm^{-3} for *Vc* at $\lambda_{max} = 495$ nm, p$K_a = 3.5$, 6.3, and 10.0. *b*) Electronic absorption spectra of dye *Vc* in aqueous universal buffer solutions at pH = 1.35 (—), 1.50 (- - - -), 3.12 (— —), 3.95 (— —), 4.98 (×), 5.48 (●), 5.90 (□), 6.50 (△), 7.36 (◐), 8.37 (■), 9.41 (▲), 10.45 (○), 11.85 (◑), and 12.50 (+).

methine cyanine dyes *Va—Ve* and *VIa—VIc* show a permanent colour in basic medium which discharges on acidification. This promoted us to study their spectral behaviour in different buffer solutions in order to ensure suitable pH medium when they are applied as photosensitizers.

The electronic absorption spectra of monomethine cyanine *Vc*, for example in universal buffer of varying pH (1.35—12.5) undergo a bathochromic shift in the

absorption band in acid medium (low pH) and a hypsochromic shift in an alkaline one (high pH). Thus, the monomethine dye *Vc* with an increased electronegativity of the oxygen of the carbonyl group in the naphthoquinone ring (increased by Sp^2 hybridization) forms hydrogen bonds at a low pH (acid medium). This leads to a criterion of positive oxonium ion on carbonyl group causing a new CT band in absorption spectra due to the charge transfer from thiazolo nitro-

gen atom to a positive oxonium ion. On increasing the pH of the media, the absorption band is hypsochromically shifted due to the inhibition of the new CT band

formed in acidic media (Fig. 2). The variation of absorbance in λ_{max} typical for monomethine cyanine dye Vc in different universal buffer solutions is as follows

pH	1.35	1.50	3.12	3.95	4.98	5.48	5.90	6.50	7.36	8.37	9.41	10.45	11.85	12.50
A_{495}	0.32	0.31	0.39	0.40	0.45	0.40	0.42	0.36	0.28	0.28	0.26	0.12	0.15	0.18

The dissociation or protonation constants of compound Vc have been determined in order to ensure the optimal pH in the application of the dye as photosensitizer. Such determination was carried out by plotting the variation of absorbance with pH using the spectrophotometric half-height limiting absorbance and *Collete* methods [20]. The effectiveness of the compound as photosensitizer increases when it is present in the ionic form which has a higher planarity [18].

The spectra at pH > 3.95 represent the absorption of the ionic (nonprotonated) forms of the dye Vc, whereas at lower pH of the medium they are due to the nonionic (protonated) species. On decreasing the pH of the medium the absorbance of the band due to the ionic form decreases in its intensity, whereas that of the nonionic form increases.

The pK_a values of compound Vc are 3.5, 6.3, 10.0. It is clear that this dye has more than one centre interacting with the acid and base of universal buffer solution. Such dye might be suggested to be a more sensitive photosensitizer in both acidic and basic media (Fig. 2).

Samples of selected newly synthesized monomethine cyanine dyes Vb, Vc were chosen to study the biological activity and the relation between the chemical structure of such dyes as bactericides and fungicides. The antibacterial activity was determined against *Bacillus stearothermophillus*, *Serratia* species, and *Pseudomonas* species. The antifungal activity was determined against *Penicillium* species and *Alternaria* species.

The monomethine cyanine dyes Vb, Vc possess the same biological activity. Thus, they show bactericidal activity (100 ppm solutions) against the bacteria under investigation (the highest effect against *Bacillus*), but they are biologically inactive against the fungi species.

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