

Charge-Transfer Complexes of Hydroxystyrylcyanine Dyes & Their Analogues with Nicotine: Structure—Activity Relationship Studies

MAHER M GIRGIS* & ZARIF H KHALIL

Chemistry Department, Faculty of Science, Assiut University Assiut, Egypt.

Received 20 July 1987; revised and accepted 3 November 1987

UV-visible spectrophotometric studies on the interaction of nicotine with hydroxystyrylcyanine dyes and their analogues [dyes (I-IX)] in 10% (v/v) aq ethanol at 29°C demonstrate the formation of charge-transfer complexes of dyes (I, III-VI) only. Addition of increasing amounts of nicotine to a solution containing fixed [dye] causes a decrease in the dye absorptivity with a gradual development of a new band at considerably longer wavelength characteristic of the complex formed. The spectra exhibit a clear unique isosbestic point. The stability constants and the molar absorptivities of the complexes formed have been calculated assuming the formation of 1:1 complexes. The experimental results have been interpreted in terms of molecular structure of the interacting molecules.

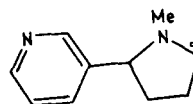
Nicotine finds growing interest for its biochemical¹ and pharmacological^{2,3} effects. The biological activity of cyanine dyes is attributed to the adsorption of the dye molecules on active sites of the living cells⁴. Structure-activity relationship has been conducted on styryl and aza cyanine dye analogues to study their effect on breaking period of dormancy in garlic⁵, essential oil production in sweet marjoram (*Origanum majorana* Linn)⁶ and chromosomal behaviour in *Vicia faba* seeds⁷. To shed some light on the cause of bio-effect of aza cyanine dyes, formation of charge-transfer (CT) complexes between one of the nucleic acid bases (adenine) and azacyanine dyes has been investigated⁸, and the results obtained were in accord with the biological activity of aza cyanine dyes when applied as bioregulator for *V. faba* seeds in the field.

The interactions between nicotine and drugs have been the subject of many investigations^{3,9}. The interactions between nicotine and cyanine dyes have not been investigated. In this work we aim to study the CT interaction of nicotine with hydroxystyrylcyanine dyes and their analogues and the effect of geometry¹⁰ on the complexation with a view to selecting an appropriate configuration for the development of radio immunoassay for tobacco alkaloids¹¹.

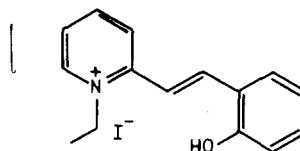
Materials and Methods

Nicotine (BDH) was used as such. Styrylcyanine dyes (I-IX) were prepared by the method described before¹² and recrystallized from ethanol. Their purity was checked by elemental analyses and spectral data.

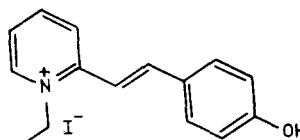
All experiments were carried out in 10% (v/v) ethanol-water mixture. The calculated volumes of nicotine (0.05 mol dm⁻³), dye (0.001 mol dm⁻³) and ethanol were mixed together and the volume made up to 10 ml in a dark-coloured bottle by adding doubly dis-



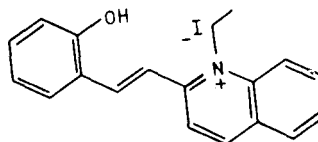
3 [1-Methyl-2-pyrrolidinyl]-pyridine
(Nicotine)



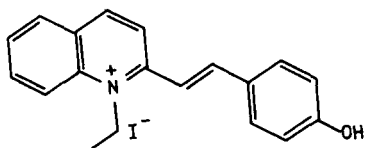
2 [2-Hydroxystyryl] pyridinium-1-ethyl
iodide. (Dye I)



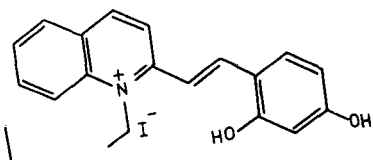
2 [4-Hydroxystyryl] pyridinium-1-ethyl
iodide. (Dye II)



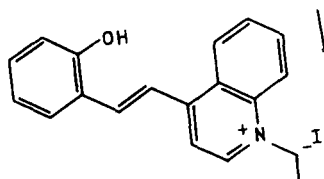
2 [2-Hydroxystyryl] quinolinium-1-ethyl
iodide. (Dye III)



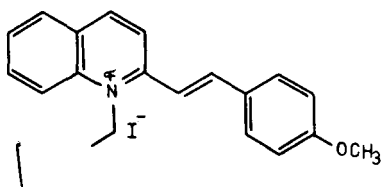
2 [4-Hydroxystyryl] quinolinium-1-ethyl iodide. (Dye IV)



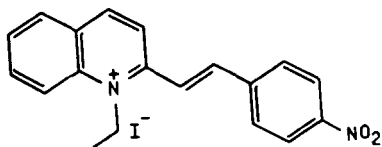
2 [2,4-Dihydroxystyryl] quinolinium-1-ethyl iodide. (Dye V)



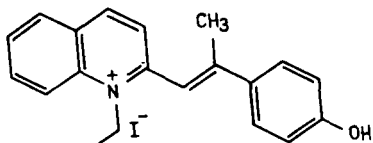
4 [2-Hydroxystyryl] quinolinium-1-ethyl iodide. (Dye VI)



2 [4-Methoxystyryl] quinolinium-1-ethyl iodide. (Dye VII)



2 [4-Nitrostyryl] quinolinium-1-ethyl iodide. (Dye VIII)



2 [2-Methyl-2(4-hydroxyphenyl)ethenyl] quinolinium-1-ethyliodide. (Dye IX)

tilled water. The solutions were freshly prepared each day, and their absorbances were measured within 5 hr. All the operations were performed at 22°C in subdued light.

Spectrophotometric measurements were made on a Shimadzu UV-200S double beam spectrophotometer, using a rectangular cell of 1 cm pathlength against solvent as the blank. The absorbances were measured at fixed wavelengths to minimize errors arising from the steepness of absorption spectra. The absorption band of the complex formed between nicotine and the dye was detected at wavelength longer than those of the individual components at concentrations identical to that in the mixture. The absorbances were measured within ~ 10 min after mixing the solutions and placing the cell in a thermostated cell holder for equilibrium. The accuracy of the measurement was ± 0.01 absorbance unit.

Results and Discussion

The absorption spectra of the dyes I, III, IV and VI are presented in Fig. 1. For some dye-nicotine mixtures, the visible absorption band of the dye diminishes gradually with increase in nicotine content in the mixture and a new band gradually appears at longer wavelength. The spectra of the mixture show a clear unique isosbestic point (Fig. 2). This behaviour is noticed only for nicotine-dye (I, III-VI) combinations, but not with other nicotine-dye (II, VII-IX) combinations. The appearance of this new longer wavelength band is attributed to the formation of a charge-transfer (CT) complex between nicotine and the dyes I and III-VI.

The stoichiometry of the CT complex ($N_n D_d$) of nicotine (N) and dye (D),

$$nN + dD \rightleftharpoons N_n D_d \quad \dots (1)$$

was determined applying Eq. (2)¹³

$$A_{\lambda}^{CT} = K_c^{CT} \cdot \epsilon_{\lambda}^{CT} [N]^n [D]^d \quad \dots (2)$$

where A_{λ}^{CT} and ϵ_{λ}^{CT} are the absorbance and the molar absorptivity, respectively, of the complex at wavelength λ , and K_c^{CT} is the thermodynamic equilibrium constant. A plot of A_{λ}^{CT} versus concentration of one component, that of other being kept constant, on log paper, proved that the complex has 1:1 composition. Thus, for the equilibrium

$$N + D \rightleftharpoons ND \quad \dots (3)$$

the equilibrium constant (K_c^{ND}) is given by Eq. (4)

$$K_c^{ND} = \frac{[ND]}{[N][D]} = \frac{[ND]}{[N]_0 - [ND] \{ [D]_0 - [ND] \}} \quad \dots (4)$$

where ND is the complex formed, and $[N]_0$ and $[D]_0$

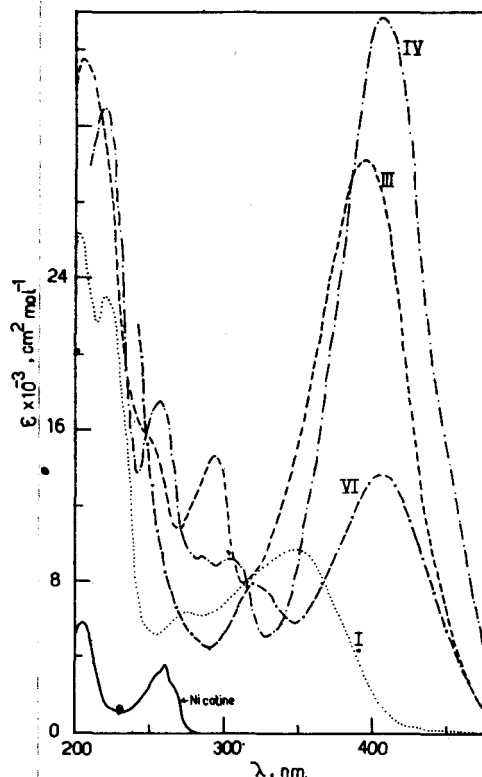


Fig. 1—Electronic absorption spectra of dyes (I, III, IV and VI) and nicotine in 10% (v/v) aq ethanol at 29°C.

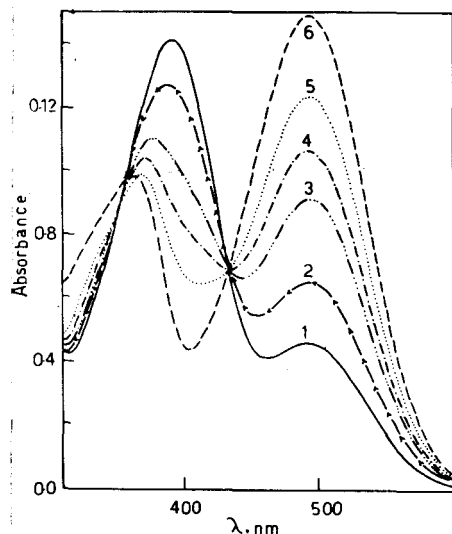


Fig. 2—Absorption spectra of $5.56 \times 10^{-5} \text{ mol dm}^{-3}$ dye (III) in 10% (v/v) aq ethanol at 29°C to which the following amounts of nicotine ($\text{mol dm}^{-3} \times 10^4$) were added: (1) 0.50; (2) 0.86; (3) 1.72; (4) 2.50; (5) 5.00; (6) 50.00.

are the initial concentrations of nicotine and the dye respectively. Eq. (4) may be rearranged to obtain Eq. (5)

$$\frac{[D]_0}{[ND]} = \frac{1}{K_c^{ND}} \cdot \frac{1}{[N]_0} + \frac{[D]_0}{[N]_0} + 1 - \frac{[ND]}{[N]_0} \quad \dots (5)$$

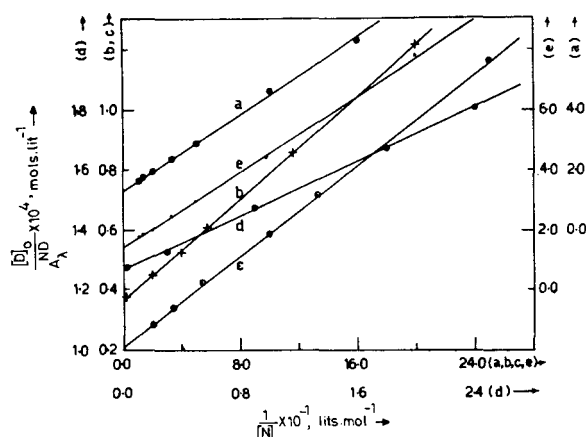


Fig. 3—Plots of $[D]_0/A_\lambda^{ND}$ versus $1/[N]_0$ for 10% (v/v) aq ethanol containing fixed [dye] and different [nicotine] at 29°C. [The combinations are: (a) nicotine-dye (I) ($[dye I] = 7.40 \times 10^{-5} \text{ mol dm}^{-3}$); (b) nicotine-dye (III) ($[dye III] = 5.58 \times 10^{-5} \text{ mol dm}^{-3}$); (c) nicotine-dye (IV) ($[dye IV] = 3.87 \times 10^{-5} \text{ mol dm}^{-3}$); (d) nicotine-dye (V) ($[dye V] = 9.27 \times 10^{-5} \text{ mol dm}^{-3}$); (e) nicotine-dye (VI) ($[dye VI] = 9.30 \times 10^{-5} \text{ mol dm}^{-3}$)].

Since the absorbance in the region of the new band is due to the complex ND (Fig. 2), and the solution is of 1 cm pathlength, for two molecules forming complex in accordance with Eq. (3), the molar absorptivity of the complex at wavelength λ is given by Eq. (6)

$$\epsilon_\lambda^{ND} = \frac{A_\lambda^{ND}}{[ND]} \quad \dots (6)$$

where A_λ^{ND} is the absorbance of the complex (ND) at the wavelength λ .

Combining Eqs (5) and (6), we get

$$\frac{[D]_0}{A_\lambda^{ND}} = \frac{1}{K_c^{ND} \cdot \epsilon_\lambda^{ND}} \cdot \frac{1}{[N]_0} + \frac{[D]_0}{[N]_0 \epsilon_\lambda^{ND}} + \frac{1}{\epsilon_\lambda^{ND}} - \frac{[ND]}{[N]_0 \epsilon_\lambda^{ND}} \quad \dots (7)$$

Since $[N]_0 \gg [ND]$ it is possible to neglect the last term in the right hand side of Eq. (7)^{14a}, so that

$$\frac{[D]_0}{A_\lambda^{ND}} = \frac{1}{[N]_0} \left(\frac{1}{K_c^{ND} \epsilon_\lambda^{ND}} + \frac{[D]_0}{\epsilon_\lambda^{ND}} \right) + \frac{1}{\epsilon_\lambda^{ND}} \quad \dots (8)$$

For a series of solutions of constant $[D]_0$ and varying $[N]_0$ a plot of $[D]_0/A_\lambda^{ND}$ against $1/[N]_0$ should be linear. The intercept of the line with ordinate gives $(\epsilon_\lambda^{ND})^{-1}$ and the gradient is equal to $[(K_c^{ND} \cdot \epsilon_\lambda^{ND})^{-1} + [D]_0(\epsilon_\lambda^{ND})^{-1}]$, so that K_c^{ND} and ϵ_λ^{ND} can be evaluated. Plots of Eq. (8) for nicotine-dye (I, III-VI) mixtures at 29°C are represented in Fig. 3 while K_c^{ND} and ϵ_λ^{ND} values are given in Table 1.

Various pyridinium salts are utilized as coenzyme models. Pyridine and its derivatives, e.g. nicotinic acid, act as electron donors on mixing with electron acceptors such as riboflavin¹⁵ and iodine¹⁶ forming CT

Table 1— $\lambda_{\max}(\epsilon_{\max})$ Values of CT Bands of Styrylcyanine Dyes (I-IX) and Nicotine-Dye (ND) Combinations, Isosbestic Points and Equilibrium Constants (K_c^{ND})^{a-c}

Dye	CT-band		$\lambda_{\max}^{ND} (\epsilon_{\max}^{ND} \cdot 10^{-3})$	Isosbestic point (nm)	$K_c^{ND} \cdot 10^{-3}$ (dm ³ mol ⁻¹)
	$\lambda_{\max}^D (\epsilon_{\max}^D \cdot 10^{-3})$	Combination			
I	348(9.7)	Nicotine-dye(I)	424(7.7)	375	5.99
II	330 ^{sh} (8.3)	Nicotine-dye(II)	—	—	—
III	394(30.2)	Nicotine-dye(III)	506(27.4)	434	16.40
IV	406(37.7)	Nicotine-dye(IV)	496(47.6)	436	7.08
V	511(4.0)	Nicotine-dye(V)	549(7.9)	473	11.66
VI	404(13.7)	Nicotine-dye(VI)	505(7.1)	444	7.61
VII	400(29.0)	Nicotine-dye(VII)	—	—	—
VIII	364(29.5)	Nicotine-dye(VIII)	—	—	—
IX	585(6.3)	Nicotine-dye(IX)	—	—	—

(a) $\lambda_{\max}$ in nm; $\epsilon_{\max}$ in cm²mol⁻¹.

(b) All measurements were carried out in 10% (v/v) aq ethanol at 29°C.

(c) Nicotine has a characteristic band: $\lambda_{\max} = 261$ nm ($\epsilon_{\max} = 3.5 \times 10^3$ cm²mol⁻¹).

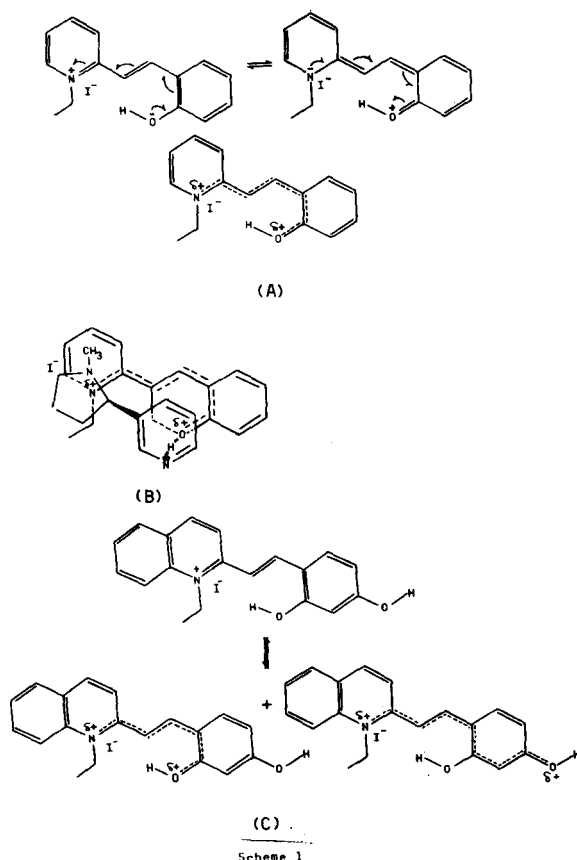
complexes. Kosower and coworkers¹⁷ have shown that pyridinium ion acts as an electron acceptor during formation of a CT complex with iodide ion as the electron donor. Such complexes are essentially ion-pairs in the ground state^{14b}. Absorption of energy within the CT-band causes excitation to a covalent excited state. The coloured nature of the quinolinium iodide¹⁸ as compared to the lack of absorption in the visible region by most of other quinolinium salts has been explained in terms of CT interaction between the two ions. Bispyridinium cation derivatives form 1:1 CT complexes with neutral donor species¹⁹.

Experimental results show that the absorption spectrum of nicotine is not changed by the addition of iodide ions, indicating that there is no CT interaction between nicotine and iodide ions.

The spectra of nicotine-dye (I, III-VI) mixtures exhibit (Fig. 2) isosbestic point which may be taken as an evidence for 1:1 complex formation²⁰. Application of Eq. (8) gives linear slopes (Fig. 3) supporting 1:1 complex formation. The results show wavelength-independence of the equilibrium constant indicating the formation of only one type of complex²¹.

Exact geometry of the complex cannot be determined from the present absorption measurements, but parallel stacking interactions between nicotine and dye can be invoked as have been for similar systems²².

In the present investigation, it is found that the ability of nicotine to form CT complexes with styrylcyanine dyes depends mainly on their molecular structures. Thus, 2-(2-hydroxystyryl)-pyridinium-1-ethyl iodide (dye I) forms CT complex on mixing with nicotine. This CT complex is assumed to be formed by 1:1 parallel stacking interaction between nicotine as an



Scheme 1

electron donor and dye(I) as an electron acceptor. The acceptor character of the dye results from the two partially positive centres located at the quaternary nitrogen and the oxygen atom of the hydroxystyryl moiety (Scheme 1A). The hydrogen atom of the hydroxyl group may act as a bridge between the O-phenyl of dye (I) and the unshared electron pair located at

the N-pyridine of nicotine molecule. The two partially positive centres of dye(I) are located at a distance equal to the distance between the two N-atoms of pyridine and pyrrolidinyll moieties of nicotine, since the distance geometry¹⁰ is approximately the same. The suggested 1:1 parallel stacking interaction is presented in Scheme 1B²³. In support of this suggestion, 2-(4-hydroxystyryl)-pyridinium-1-ethyl iodide (dye II) does not form CT complex with nicotine as the two positive centres of dye(II) do not fit the distance geometry¹⁰ and the ring-ring²⁴ size formation with nicotine.

On the other hand 2-(2-hydroxystyryl)-quinolinium-1-ethyl iodide (dye III) and 2-(4-hydroxystyryl)quinolinium-1-ethyl iodide (dye IV) form CT complexes. This behaviour of dye(IV) in contrast to that of dye(II) may be attributed to greater delocalisation of the positive charge at the N-quaternary atom of quinolinium moiety, due to the extra-fused benzene ring which enables the formation of a stable CT complex with nicotine. The stability constant (K_c^{ND}) of nicotine-dye(IV) complex ($7.08 \times 10^3 \text{ dm}^3 \text{ mol}^{-1}$) is much less than that of nicotine-dye (III) complex ($16.40 \times 10^3 \text{ dm}^3 \text{ mol}^{-1}$). The dye 2-(2,4-dihydroxystyryl)-quinolinium-1-ethyl iodide (dye V) forms a CT complex with $K_c^{ND} = 11.66 \times 10^3 \text{ dm}^3 \text{ mol}^{-1}$, a value which is half of the sum of stability constants of CT complexes of nicotine with dyes III and IV (Table 1). This may be attributed to the fact that dye(V) has two resonance hybrid structures giving two types of complexes (of Scheme 1C). 4-(2-Hydroxystyryl)-quinolinium-1-ethyl iodide (dye VI) forms a CT-complex with nicotine having $K_c^{ND} = 7.61 \times 10^3 \text{ dm}^3 \text{ mol}^{-1}$. This value is much less than that of nicotine-dye(III) complex, but is approximately the same as that of nicotine-dye (IV) complex. This is due to the fact that dyes(IV) and (VI) have approximately the same distance geometry¹⁰ between two partially positive centres.

Substitution of the hydroxyl group in 4-hydroxystyryl moiety by a methoxyl group (electron releasing) as in dye (VII), or a nitro group (electron withdrawing) as in dye(VIII) does not help in the formation of CT complexes. This provides further new evidence for the formation of H-bridge between the hydroxystyryl and the N-pyridine moieties, since this H-bridge is absent in the dyes (VII) and (VIII), and consequently no CT complexes are formed.

Substitution at the ethenyl moiety ($-\text{CH}=\text{CH}-$) of the 4-hydroxystyryl group (dye IX) also does not lead to CT complex formation with nicotine. This may be due to steric congestion between the interact-

ing molecules and the inability to adopt ring-ring conformations needed²⁴.

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