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The Effect of Alkaloids on Fluorescence in Solutions

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The quenching of fluorescence of different substances by a series of alkaloids has been studied. The alkaloids generally exert a quenching action on the fluorescence. In evaluating these effects, in certain cases it is necessary to take into consideration the absorption of exciting light by the molecules of the quencher. The quenching effect of the anions of alkaloid salts should also be taken into account. By mathematical corrections of experimental data for the absorption of the exciting light and the quenching by anions, the constants and semi-concentrations of quenching are obtained for the cationic part of the alkaloid. It may be assumed that the quenching effect of alkaloids involves an external static mechanism, *i. e.* the formation of molecular compounds between the quencher and the fluorescent substance.

INTRODUCTION

From the general chemical point of view, alkaloids are nitrogenous bases which form salts with anorganic and organic acids. These salts are molecular compounds, because acids are bound to the free pairs of electrons of the nitrogen atoms. On the other hand the quenching of fluorescence in solutions by addition of foreign matter is, according to Förster's classification[1], generally of the external, static type. According to this mechanism the quenching is due to the formation of molecular compounds or similar aggregates between the fluorescent substance and the quencher.

With regard to the chemical structure of alkaloids and the static theory of quenching it seems very probable that alkaloids will quench the fluorescence of different substances in solutions. The quenching effect may be due to the cationic as well as the anionic part of the alkaloid, as it is known[2] that not only different anions but also cations effectively reduce the intensity of fluorescence in solution.

The action of alkaloids on fluorescence has been studied so far by Boutaric *et al.*[3], who compared the action of different alkaloids on the fluorescence of uranine with the action of the corresponding gen-alkaloids. They had found that alkaloids exert a quenching effect on the fluorescence while the corresponding gen-alkaloids, which are less toxic, and comprise an amino-oxyde group instead of the amino group of alkaloids, produce no significant effect on the fluorescence of uranine. So far the curves of quenching (intensity *vs.* alkaloid concentration) have not been determined, neither have the semi-concentrations, the quenching constants, the effect of the anionic part of the alkaloid molecule, or a possible absorption of the exciting ultraviolet light by the molecules of the quencher. It

has been of great theoretical interest to study the effect of a series of alkaloids on the fluorescence of different substances in solutions by determining the above characteristic physico-chemical parameters.

EXPERIMENTAL

The intensity of fluorescence was measured with a photoelectric fluorometer, which was obtained[4] by combining the electronic part of the Zeiss-Jena Universal spectrophotometer, Model A, with a corresponding high pressure mercury lamp and optical filters. In this fluorometer the »straight through« arrangement is used with respect to the excitation and detection components. The fluorescence was excited by 365 nm radiation and the intensity of the entire fluorescence band was measured. The results of fluorescence measurements in the presence of alkaloids (Φ) are expressed as percentage of the intensity of the fluorescent solution with the quencher absent ($\Phi_0 = 100$).

The fluorescent substances used were of the highest possible purity. The alkaloids were of Ph. Jug. II. purity, and the other substances were reagent grade or puriss. grade chemicals.

Fluorescence intensity (Φ) measurements were carried out in series of solutions with increasing concentrations of the alkaloids and compared to the intensity of fluorescence of an identical solution containing no alkaloids (Φ_0). In those cases when the alkaloids absorbed the exciting ultraviolet light, absorption curves were measured with a Perkin Elmer UV 137 spectrophotometer.

RESULTS

Fluorometric measurements were carried out in the following solutions of fluorescent substances: quinine 1×10^{-4} in 10^{-1} *M* H_2SO_4; acridine 1×10^{-4} *M* in 10^{-1} *M* H_2SO_4; riboflavine 1×10^{-5} *M* in water; sodium naphthionate 1×10^{-4} M

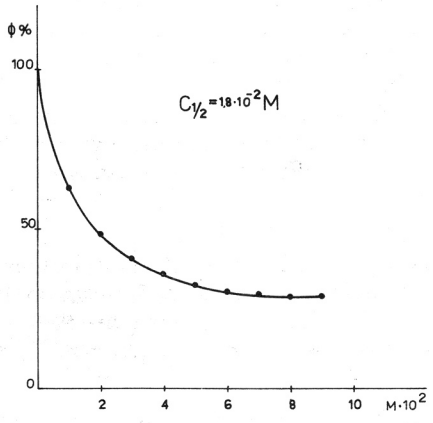

Fig. 1. Quenching of the fluorescence of acridine by cotarnine chloride

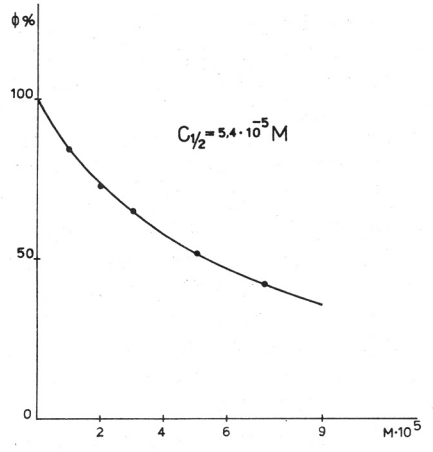

Fig. 2. Quenching of the fluorescence of sodium naphthionate by caffeine

in water and uranine (sodium fluorosceinate) 1×10^{-4} *M* in water. In the above solutions of quinine and acridine the fluorescent substance was a cation, in the solution of riboflavin a neutral molecule, while in the solutions of sodium naphthionate and uranine it was an anion. The following alkaloids were tested for quenching action: papaverine hydrochloride, narcotine hydrochloride, cotarnine chloride, narceine hydrochloride, morphine hydro-

chloride, codeine hydrochloride, codeine phosphate, homatropine hydrobromide, pilocarpine hydrochloride, strychnine nitrate and caffeine. Figs. 1 and 2 present the curves of the quenching of acridine with cotarnine chloride and of sodium naphthionate with caffeine respectively. In the first case the quenching is very efficacious, while in the other one it is rather weak. By graphic interpolations of the curves it is possible to determine the semi-concentrations of quenching $c_{½}$, *i. e.* the molarities of the quenchers necessary to reduce the intensity of fluorescence to half of its unquenched value ($\Phi = 50$). Table I gives the values of $c_{½}$ for all the systems investigated. The values range from 5.4×10^{-5} *M* to 4.3×10^{-2} *M*, and to still higher concentrations, whose numerical values cannot be determined experimentally.

The relationship between the extent of quenching and the concentration of the quencher can be expressed in most cases by the Stern-Volmer[5] hyperbolic equation

$$\Phi = \Phi_0 \frac{1}{1 + \beta c} \tag{1}$$

where Φ_0 is the intensity of fluorescence in absence of the quencher, Φ the intensity of fluorescence with a concentration c of the quencher, and β the quenching constant. This constant can be determined both mathematically (Table II) and graphically (Fig. 3). The plot of the fraction Φ_0/Φ *vs.* alkaloid concentration (c) yields a straight line, which intersects the ordinate at value 1, and the abscissa at that (negative) concentration of the alkaloid, which cor-

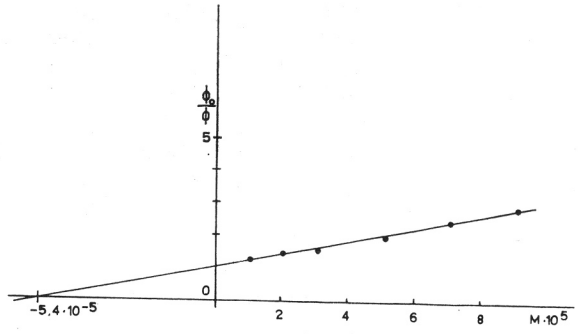

Fig. 3. Quenching of acridine fluorescence by cotarnine chloride, ϕ_0/ϕ *vs.* cotarnine chloride concentration

responds to the relation $1/\beta = -c$. The values for $1/\beta$ obtained by equation (1) are also listed in Table I. The less the purely experimental values of $c_{½}$ differ from the mathematically obtained data for $1/\beta$, the more conformed is the equation (1).

From the alkaloids investigated papaverine hydrochloride and cotarnine chloride absorb the exciting ultraviolet light ($\lambda = 365$ nm). The result is an »inner filter effect«: a decrease in fluorescence intensity caused by partial absorption of the exciting radiation. To determine the »true« quenching it is necessary to determine this effect by corresponding light absorption measurements and to take it into consideration mathematically. When two substances

TABLE I

Experimental Values of $c_{1/2} \times 10^4$ M without Corrections (a), and Values for 1/β Obtained Mathematically by Equation (1).

		Quinine	Acridine	Riboflavin	Sodium naphthionate	Uranine
Papaverine hydrochloride	a	7.8	7.6	7.6	7.9	6.1
	b	7.57	7.57	7.25	7.91	6.10
Narcotine hydrochloride	a	> 100	50.0			
	b		50.0			
Cotarnine chloride	a	0.61	0.54	1.0	> 100	0.57
	b	0.65	0.54	0.89		0.56
Narceine hydrochloride	a	23.0	32.5	59.0		1.3
	b	21.1	29.6	58.8		2.2
Morphine hydrochloride	a	32.0	49.0	> 1000	no quenching	270.0
	b	32.7	52.3			286.0
Codeine hydrochloride	a	27.0	45.0			
	b	27.7	44.0			
Codeine phosphate	a	> 100	75.0			
	b		72.4			
Homatropine hydrobromide	a	22.5	15.5	400.0	no quenching	> 1000
	b	21.4	15.7	412.0		
Pilocarpine hydrochloride	a	31.0	120.0	no quenching	no quenching	1050.0
	b	31.5	125.0			
Strychnine nitrate	a	> 100	60.0	> 100	no quenching	> 100
	b		60.2			
Caffeine	a	230.0	43.0	220.0	180.0	430.0
	b	248.0	42.7	250.0	238.0	413.0
Sodium chloride	a	29.5	180.0	no quenching	no quenching	no quenching
	b	30.3	189.0			
Sodium bromide	a	31.0	21.5	410.0	no quenching	> 1000
	b	30.6	21.9	429.0		

TABLE II

Values of the Constant β for the Quenching of Acridine Fluorescence by Cotarnine Chloride

Cotarnine chloride $c \times 10^5$ M	$\beta \cdot 10^{-2}$
1	190
2	184
3	177
5	180
7	190
9	195

$\beta = 1.86 \cdot 10^4$

in the same solution (fluorescent substance and alkaloid) absorb monochromatic light, the individual absorption of one substance (the alkaloid) in the reaction mixture is expressed by the equation[6]

$$A_2 = 100 \frac{\varepsilon_2 c_2}{\varepsilon_1 c_1 + \varepsilon_2 c_2} (1 - 10^{-\varepsilon_1 c_1 p - \varepsilon_2 c_2 p}) \quad (2)$$

where ε_1 and ε_2 are the extinction coefficients of the fluorescent substance and the quencher respectively, c_1 and c_2 the corresponding concentrations, and p the width of the layer. A_2 is expressed in percents. The individual absorption (A_1) of the fluorescent substance can be expressed by an analogous equation. The sum $A_1 + A_2 = A$ yields the light absorption of the entire system. Quenching by inner filter effect increases with the increase in A_2 value.

Substitution of the values of A_2 into the hyperbolic equation of quenching (1) for each combination of the concentration of the fluorescent substance and the quencher yields an equation with a new quenching constant (β'),

TABLE III

	ε	$c \times 10^5 / M$	A_2	A_1	β	β'	$c'_{1/2} \cdot 10^4 / M$
Quinine	3200	10.0	34.0	55.8	1320	307	32.6
Papaverine hydrochloride	250	78.0	37.0	63.0			
Acridine	3100	10.0	34.2	55.8	1320	315	31.7
Papaverine hydrochloride	250	76.0	38.0	62.0			
Riboflavin	7200	1.0	50.4	18.9	1380		
Papaverine hydrochloride	250	76.0	72.0	28.0			
Sodium naphthionate	470	10.0	54.5	19.0	1265		
Papaverine hydrochloride	250	79.0	75.0	25.0			
Uranine	1230	10.0	39.5	31.6	1638		
Papaverine hydrochloride	250	61.0	55.0	45.0			
Quinine	3200	10.0	44.2	48.9	983	653	15.3
Cotarnine chloride	4800	6.1	47.0	52.0			
Acridine	3100	10.0	41.8	50.2	1851	1798	5.6
Cotarnine chloride	4800	5.4	45.0	55.0			
Riboflavin	7200	1.0	80.0	11.9	11200		
Cotarnine chloride	4800	10.0	87.0	13.0			
Uranine	1230	10.0	57.1	26.0	17900		
Cotarnine chloride	4800	5.7	68.0	32.0			

$$\Phi = \frac{\Phi_0 - A_2}{1 + \beta' c} \tag{3}$$

which is corrected with regard to the absorption of the exciting light in the molecules of the quencher. The corrected constant is easily derived from the experimentally obtained semiconcentrations of quenching by the expression

$$\beta_i' = \frac{\frac{100 - A_2}{50} - 1}{c_{1/2}} \tag{4}$$

Table III summarizes the values obtained for the molar extinction coefficients (ε) of the substances used, the individual absorptions (A_1 and A_2), the constants from equations (1) and (4), and the corrected semi-concentrations of quenching $c_{1/2} = 1/\beta'$. The latter constants and semi-concentrations correspond to the quenching without the inner filter effect.

Of the anions of the above mentioned alkaloids nitrate ion does not quench fluorescence, while chloride and bromide ions exert a quenching effect when present in somewhat higher concentrations (Table I). For solutions which contain

TABLE IV

Values of $c_{1/2}$ $M \times 10^3$ Corrected for the Action of the Anions and for Absorption of the Primary Light

	Quinine	Acridine	Riboflavin	Sodium naphthionate	Uranine
Papaverine hydrochloride	no quenching	3.8	no quenching	no quenching	no quenching
Narcotine hydrochloride	> 10	5.0			
Cotarnine chloride	1.5	0.55	no quenching	no quenching	no quenching
Narceine hydrochloride	6.9	3.5	5.9		0.13
Morphine hydrochloride	no quenching	7.2	> 100	no quenching	27.0
Codeine hydrochloride	32.0	5.7			
Codeine phosphate	> 10	7.5			
Homatropine hydrobromide	7.0	5.5	no quenching	no quenching	> 100
Pilocarpine hydrochloride	no quenching	36.0	no quenching	no quenching	105.0
Strychnine nitrate	> 10	6.0	> 10	quenching no	> 10
Caffeine	23.0	4.3	22.0	18.0	43.0

two different substances or two ions, both of which quench fluorescence, the following quenching equation is generally valid:

$$\Phi = \Phi_0 \frac{1}{1 + \beta_1 c_1 + \beta_2 c_2}, \tag{5}$$

in which β_1 and c_1 relate to one and β_2 and c_2 to the other quenching substance. In the case of quenching with alkaloid salts which also absorb the exciting light, the above equation is modified:

$$\Phi = \frac{\Phi_0 - A_2}{1 + (\beta''_1 + \beta_2) c} \tag{6}$$

as $c_1 = c_2 = c$. The new constant β''_1 is corrected both for light absorption and for the action of anions. In case of absorption of the exciting light by the alkaloids $\beta''_1 = \beta'_1 - \beta_2$, while in the absence of such absorption the expressions $A_2 = 0$ and $\beta''_1 = \beta - \beta_2$ are valid. The reciprocal values of these constants always represent the semi-concentrations of quenching. The semi-concentrations of quenching, corrected for absorption of the primary light as well as for the quenching effect of anions, are listed in Table IV.

DISCUSSION

From the results obtained it is evident that alkaloids are efficient quenchers of fluorescence in solutions. Besides »true« quenching by alkaloids, the anions of their salts also exert a quenching effect, and in certain cases the quenching is due to the absorption of exciting light (»inner filter effect«). The quenching by alkaloids does not depend on the type of the fluorescent substance in the solution, *i. e.* whether it is a cation, an anion or a neutral molecule. The alkaloids studied quenched most effectively the fluorescence of acridine. Certain alkaloids proved equally efficient in quenching the fluorescence of riboflavine, uranine and quinine. It is evident that individual relationship exists between the fluorescent substance and the quencher and this relationship is obviously conditioned by the chemical properties of both the fluorescent substance and the quencher. It may be therefore considered that we are concerned with the formation of molecular compounds, which are not capable of emitting fluorescence.

This conclusion is interesting from the point of view of pharmacology as well. Alkaloids are known as effective drugs and in modern pharmacology it is generally assumed that the *in vivo* action of certain drugs is a result of formation of molecular compounds between the drug and a certain chemical receptor of the cell, *e. g.* an enzyme. Since the capability of alkaloids to form molecular compounds has been demonstrated by our results, it is probable that they act in the same way *in vivo* as well.

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IZVOD

Djelovanje alkaloida na fluorescenciju u otopinama

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Istraživano je gašenje fluorescencije različitih tvari utjecajem niza alkaloida. Alkaloidi redovito izrazito gase fluorescenciju. Kod prosuđivanja ustanovljenih efekata potrebno je uzimati u obzir u određenim slučajevima još i apsorpciju primarnog svjetla u molekulama gasila (alkaloida). Pored toga je važno istaknuti da i anioni alkaloidnih soli gase fluorescenciju. Računskim korigiranjem eksperimentalnih podataka u pogledu apsorpcije primarnog svjetla, kao i gašenja anionima, dobiju se konstante i polovične koncentracije gašenja za kationski dio alkaloida. Može se smatrati, da se kod gašenja fluorescencije alkaloidima radi o statičkom vanjskom mehanizmu gašenja, koji je u vezi sa stvaranjem molekularnih spojeva fluorescentne tvari i gasila.

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