THE INTERACTIONS OF TENSIDES WITH CALCONALIDE I

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A study was made of the effect of cationogenic, anionogenic and nonionogenic tensides on the absorption spectra and dissociation constant of calconalide I. It was found that the changes in the absorption spectra are a result of influences on the dissociation of the dye and a shift in the equilibrium between the tautomeric forms. The mechanism of the effect of the tenside on dye dissociation was also discussed.

The azo dye calconalide I (calcon *m*-nitroanilide, the disodium salt of 1-(2-hydroxy-3-(*m*-nitrophenylcarbamoyl)-1-naphthylazo)-2-naphthol-4-sulphonic acid, summary formula $C_{27}H_{16}N_4Na_2O_8S$, structure depicted in Scheme 1) has been used for only a relatively short time in analytical chemistry. At the end of the nineteen sixties, Ditz, Neumann and Suk[1-3] described a method for preparing members of the group of arylides of calconcarbonic acid and studied the acid-base properties of calconalide I (ref.[4]). As a result of its favourable properties, this dye has been proposed as a reagent for the complexometric determination of calcium and the stability constants of its complex with calcium have been determined[5]. The spectrophotometric utilization of this complex has also been studied[3].

As it has been shown that the properties of organic dyes used as spectrophotometric reagents are strongly affected by surface-active substances, termed tensides, this effect was utilized in a number of spectrophotometric determinations (primarily to improve the sensitivity, to affect the optimum pH for complex formation and to improve the stability of the reaction products); consequently, we decided to study this effect for calconalide I. The effects of the cationogenic tenside Septonex, the anionogenic tenside sodium dodecyl sulphate and the nonionogenic tenside Triton X-100 on the absorption spectra and conditional dissociation constant of this dye were examined.

EXPERIMENTAL

Reagents

Calconalide I (Spolana, Neratovice) was purified prior to use[6]: 5 g of the substance were transferred to a glass chromatographic column and gradually dissolved by rinsing with methanol (about one third of the substance remained undissolved). The concentrated methanolic extract obtained was precipitated with a ten-fold excess of diethyl ether. The precipitae was centrifuged off and rinsed with diethyl ether until a colourless liquid phase was obtained. The precipitated calconalide was dried in a vacuum drying box at a temperature of 40°C and dried and stored in a dessicator over silica gel and then over P_2O_5. The efficiency of the purification was controlled chromatographically by the thin-layer technique (Silufol, mobile phase: methanol–chloroform); the substance yielded only a single spot. The following results were obtained for elemental organic analysis: for $C_{27}H_{16}N_4Na_2O_8S$ calculated 53·82% C, 2·67% H, 9·30% N; found 54·93%C, 3·02% H, 10·15% N.

The stock solution of $1 \,.\, 10^{-3}$ mol l^{-1} calconalide I in 50% v/v methanol was prepared by grinding 0·15062 g of purified substance in 100 ml of methanol and transferring to a 250 ml volumetric flask. A further 25 ml of methanol was added and the flask was filled to the mark with distilled water. This solution was stored in the cold and dark and was stable for at least several weeks.

The stock solutions of Septonex (carbethoxypentadecyl trimethyl ammonium bromide, Slovakofarma, $M_r = 422·48$), sodium dodecyl sulphate (Serva $M_r = 288·38$) and Triton (Serva, $M_r = 624·9$) with concentrations of $1 \,.\, 10^{-1}$ mol l^{-1} were prepared by dissolving the substances in water. Prior to weighing, Septonex was dried for 24 h in a dessicator over silica gel.

The pH was adjusted using solutions of HCl and NaOH, as the salts present in buffers produce polymerization of the dye[7].

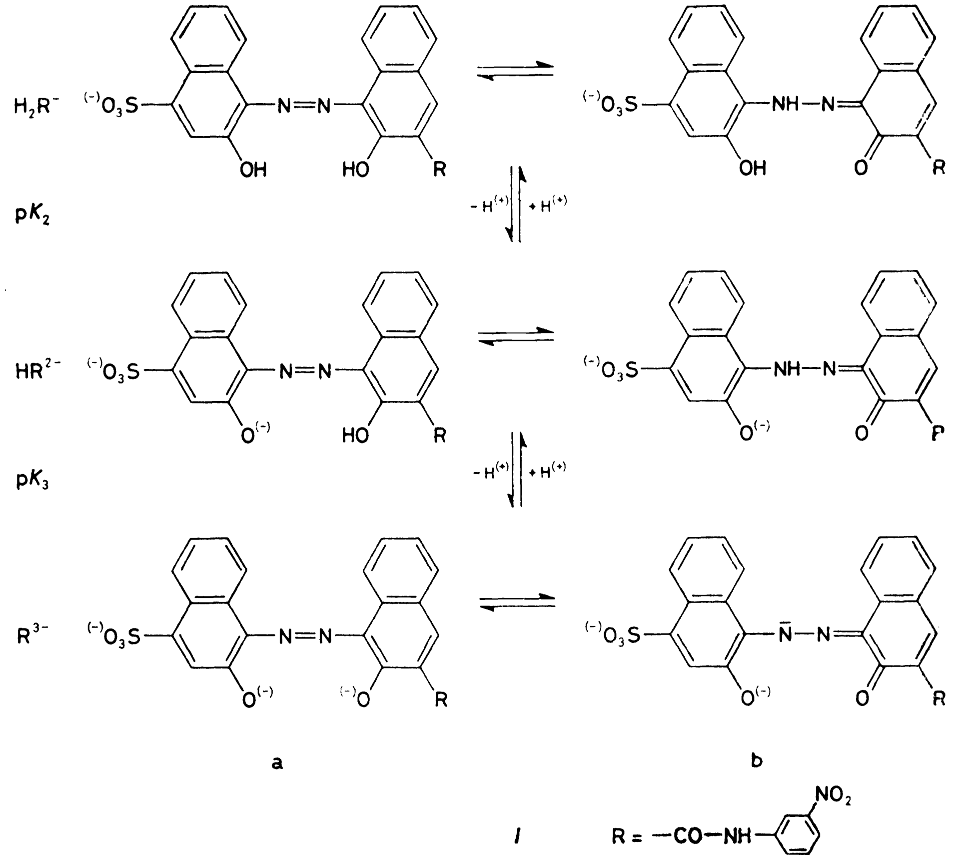
Instruments

The UV/VIS PU 8800 recording spectrophotometer (Philips/Pye Unicam, Cambridge) was employed with quartz cuvettes with an internal pathlength of 1·00 cm. The PHM 26 pH-meter Radiometer, Copenhagen) was employed with a GK 2401 C combined glass electrode.

RESULTS AND DISCUSSION

The Effect of the Tensides on the Absorption Spectra

The absorption spectra of *o*- and *p*-hydroxyazo dyes[8] in the visible region consist of two absorption bands corresponding to the hydroxyazo form (for calconalide, this is form a in Scheme 1), absorbing shorter wavelength radiation, and to the keto-hydrazo form (form b), absorbing at longer wavelengths. The ratio of the contents of the azo and hydrazo forms of the azo dye in solution depends on the dye structure[8] and on the solvent properties[9]. Depending on the pH, calconalide I exists in several dissociated forms[4]; the H_2R^- and HR^{2-} forms are most important for analytical applications, as the H_3R form exists only in very acidic solutions and the R^{3-} form only in very alkaline solutions (pK_1 was not found; $pK_2 = 7·62 \pm 0·01$, $pK_3 = 13·03 \pm 0·04$)[4].



SCHEME 1
Dissociation scheme of calconalide I

It was found in a study of the effect of Septonex on the absorption spectrum of calconalide I that, as the tenside concentration increased, a new long wavelength maximum was gradually formed on the absorption curves of the H_2R^- form (Fig. 1). It follows from comparison with the absorption curves for calconalide at higher pH values (Fig. 2) that this maximum corresponds to the HR^{2-} form. Thus, the cationogenic tenside increases the dissociation of the H_2R^- form with production of the HR^{2-} form, and shifts the region of existence of these two forms to lower pH values. Consequently, the absorption curves for the solutions with increasing Septonex concentrations were measured again at pH values corresponding to the existence of a single dye form (Fig. 3). Curves *1* in Figs 3*a*, 3*b* correspond to forms H_2R^- and HR^{2-} of the dye in the absence of Septonex. As the Septonex concentration is increased, the heights of both absorption maxima increase (the long wavelength maximum of the H_2R^- form simultaneously exhibits a hypsochromic shift from 620 nm to 600 nm, while that of the HR^{2-} form exhibits a bathochromic shift from 660 to 675 nm). However, the absorbance of the ketohydrazo form increases far more, so that the absorbance of this form exceeds that of the hydroxyazo form. Consequently, the presence of Septonex affects the equilibrium between the two tautomeric forms of the dye and simultaneously causes a shift in the individual absorption bands.

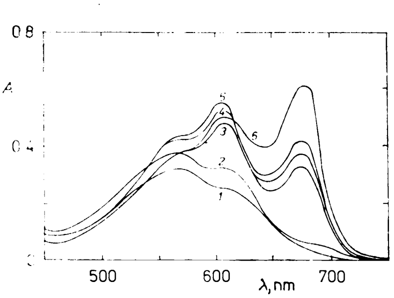


FIG. 1

The effect of Septonex on the absorption spectrum of calconalide I. $c_{calc} = 2\cdot5 \,.\, 10^{-5}$ mol l^{-1}, pH 3·40–3·60. c_{Sept}(mol . l^{-1}): *1* 0, *2* 1 . 10^{-4}, *3* 5 . 10^{-4}, *4* 1 . 10^{-3}, *5* 5 . 10^{-3}, *6* 1 . 10^{-2}

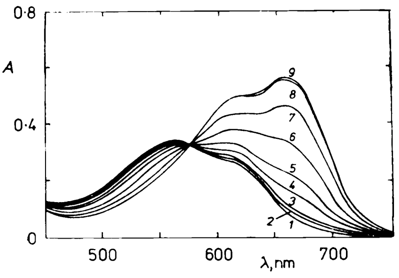


FIG. 2

The effect of the pH on the absorption spectrum of calconalide I. $c_{calc} = 2\cdot5 \,.\, 10^{-5}$ mol. . l^{-1}, pH: *1* 5·27, *2* 6·25, *3* 6·53, *4* 6·90, *5* 7·20, *6* 7·70, *7* 8·30, *8* 9·60, *9* 10·00

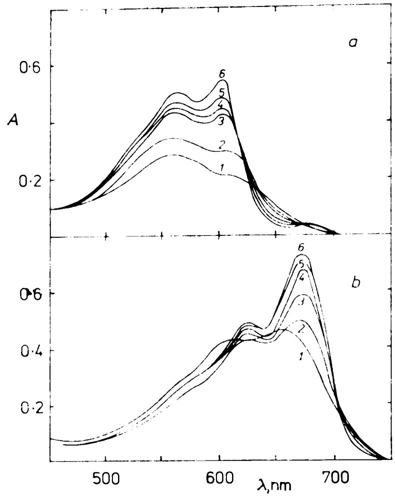


FIG. 3

The effect of Septonex on the absorption spectrum of the H_2R^- form (*a*) and the HR^{2-} form (*b*) of calconalide I. $c_{calc} =$ = $2\cdot5 \,.\, 10^{-5}$ mol l^{-1}, c_{Sept}(mol l^{-1}): *1* 0, *2* 1 . 10^{-4}, *3* 5 . 10^{-4}, *4* 1 . 10^{-3}, *5* 5 . 10^{-3}, *6* 1 . 10^{-2}. *a*: pH 1·70–1·75, *b*: *1* pH 9·70, *2*–*6* pH 7·10–7·20

The effect of the cationogenic tenside on the tautomeric equilibrium has been described for *p*-hydroxyazonaphthol sulphonate[10], where, however, the absorption maximum of the hydrazo form is suppressed and that of the azoform is increased. It follows from the spectral changes for the dye pyridinazoresorcinol[11] that interaction of the H_3R^+ form with the anionogenic tenside sodium dodecyl sulphate leads to changes analogous to those during interaction of calconalide I with Septonex, i.e. to an increase in the amount of hydrazo form. The effect of the tenside on the tautomeric equilibrium is apparently connected with the structure of the test dye and of the tenside micelles; this effect will be the subject of a detailed study.

The absorption spectra of the calconalide I–Septonex system were recorded from a Septonex concentration of $1 . 10^{-4}$ mol l^{-1} as turbidity appeared in the solution at lower concentrations. This cloudiness is usually attributed to the formation of insoluble ion pairs of the dye and tenside monomer. The above-described changes in the absorption spectra occur already from a Septonex concentration of $1 . 10^{-4}$ mol l^{-1}, i.e. a concentration below its critical micelle concentration[12] (no strong electrolytes were present in the solution[12] so that cmc = $7 . 10^{-4}$ mol l^{-1}). As such substantial changes can occur only as a result of considerable changes in the dye environment, this finding confirms the conclusions[10] that tenside micellization occurs at $c_{\text{tens}} <$ cmc to produce micelles induced by the presence of the dye. Initially, the dye molecules predominate in these micelles and the tenside molecules become to predominate as cmc is approached.

In the presence of the nonionogenic tenside Triton X-100, changes occur in the absorption spectra that are analogous to those observed in the presence of Septonex (hyperchromic shifts of the long wavelength bands are somewhat smaller). These changes once again occur at Triton concentrations of less than cmc (cmc = $8 . 10^{-4}$ mol l^{-1} ref.[13]), thus confirming the above conclusions.

The aniogenic tenside sodium dodecyl sulphate does not produce changes in the height or position of the absorption bands of any of the forms of calconalide I. The absorbance of all the bands first decreases slightly and then once again attains the initial values. Consequently, sodium dodecyl sulphate does not affect the tautomeric equilibrium of the dye.

We could not study the effect of the ionic strength on the interaction between calconalide I and the tensides as the presence of salts led to the polymerization or salting out of the dye, reflected in the appearance of turbidity.

The Effect of the Tensides on the Dissociation Constants

The conditional dissociation constants K_2 were found from the curves of the dependence of the absorbance on the pH. Dissociation constant K_2 is defined by the relationship

$$K_2 = \frac{[H^+][HR^{2-}]}{[H_2R^-]}\,. \tag{1}$$

It holds in the pH interval 3·0 – 10·0 where the measurements were carried out (at $l = 1$ cm) that

$$A = \varepsilon_1[H_2R^-] + \varepsilon_2[HR^{2-}]\,, \tag{2}$$

$$c_{calc} = [H_2R^-] + [HR^{2-}]\,. \tag{3}$$

Substitution of Eqs (*1*) and (*3*) into Eq. (*2*) yields the expression

$$A = c_{calc}\frac{\varepsilon_1[H^+]^2 + \varepsilon_2K_2[H^+]}{[H^+]^2 + K_2[H^+]}\,. \tag{4}$$

The Marquardt method of nonlinear regression was employed[14] to estimate the values of parameters K_2, ε_1 and ε_2.

Figure 4 depictst he dependence of pK_2 on the negative logarithm of the concentration for the three studied tensides. The relative standard deviations of the pK_2 values ranged from 0·4 to 1·2% (for 4 measurements). The cationogenic and nonionogenic tensides decrease the pK_2 value (curves *1* and *2*), while the anionogenic tenside slightly increases the pK_2 value (curve *3*). Compared with the literature data for the changes in the dissociation constants of other dyes (e.g. refs[11,15–17]), the decrease in the pK_2 value for calconalide I in the presence of Septonex is unusually large (about 4 units). The decrease in pK_2 produced by Triton X-100 is somewhat smaller.

The decrease in the pK_2 value in the presence of a cationogenic tenside (or an increase in the presence of an anionogenic tenside) is in agreement with the theory of the pseudophase ion exchange model[15]. If the dye is solubilized in the micelle pseudophase (solubilization is a result primarily of hydrophobic interactions) then OH^- ions from the solution are concentrated on the surface of the cationogenic

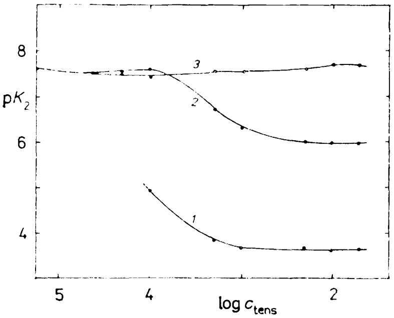


FIG. 4

Effect of the tenside concentration on the pK_2 value of calconalide I. *1* Septonex, *2* Triton X-100, *3* sodium dodecyl sulphate tenside micelle and facilitate dye dissociation (pK decreases). The OH^- ions are electrostatically repulsed from the surface of the aniogenic tenside micelle, and dissociation is thus suppressed (pK increases). The effect of the nonionogenic tenside cannot be unambiguously explained on this basis; the pK values are sometimes decreased, sometimes increased.

As the pK_2 changes in the presence of cationogenic or nonionogenic tenside again occur at tenside concentrations below cmc, they can once again be explained on the basis of incorporation of the dye and tenside in mixed micelles, with a size that increases with increasing tenside concentration. These changes terminate when $c_{\text{tens}} \approx$ cmc, when classical micelles are formed in the solution, with an aggregation number given by the type of tenside, in which the concentration of solubilized dye ions is negligible compared with the tenside concentration.

The increase in pK_2 produced by the presence of sodium dodecyl sulphate is very small and occurs at $c_{\text{SDS}} >$ cmc (cmc $= 7 \,.\, 10^{-3}$ mol l^{-1})[13], where hydrophobic interactions can occur between the dye anion and the tenside micelles, however, they are limited by electrostatic repulsion between their identical charges.

The changes in the dissociation constants of organic dyes in the presence of submicelle concentrations of tensides have also been explained on the basis of the formation of associates between dye ions and tenside monomers[18].

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ERRATA:

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Collect. Czech. Chem. Commun. *56*, 2100 (1991), p. 2105, Fig. 4:
For log c_{tens} read $-\log c_{\text{tens}}$.