EXAMINATION OF THE CONDITIONS OF EXTRACTION OF TOXIC ORGANIC BASES AND OF METHODS OF THEIR DETERMINATION. III. EXTRACTION OF ION-ASSOCIATES OF DIBENZ[*b,f*]-1,4-OXAZEPIN

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The protonation and distribution constants of dibenz[*b,f*]-1,4-oxazepin (CR) were measured. This substance forms stable ion-associates with Acid Red 88, extractable into chloroform. Reextraction with an aqueous phase containing basic fuchsine or safranine T brings about exchange of the CR cation for the basic dye cation. The associates so formed exhibit higher conditional extraction constants and higher molar absorptivities than the initial associates.

Dibenz[*b,f*]-1,4-oxazepin (CR) is an efficient lacrimation agent, which affects eyes, skin and partly also the respiratory organs. The compound has been identified and quantitated by ultraviolet and infrared spectrophotometry[1], gas chromatography[2], mass spectrometry[3], and nuclear magnetic resonance[4]. Direct extraction photometry has also been applied[5] because the substance protonates in aqueous solutions and forms ion-associates extractable with chloroform. The sensitivity of the analytical process can be increased by using an indirect extraction photometry method in which the CR cation in the ion-associate extracted is replaced with a suitable basic dye cation. The present paper is concerned with the examination of ion-associates of CR with the acid azo dye Acid Red 88 (AR) and with thr possibility of replacing the cation of the protonated CR with cations of Basic Violet 14 (basic fuchsine, FB) or Basic Red 2 (safranine T, ST).

EXPERIMENTAL

Chemicals and Apparatus

Dibenz[*b,f*]-1,4-oxazepin was synthesized by Military Repair Plant 072 in Zemianské Kostolany (The Slovak Republic). The active component content of CR, determined by titration with sodium tetraphenylborate using a Crytur potassium ion selective electrode[6], was 99.8%.

Acid Red 88 (sodium 4-(2-hydroxy-1-naphthyl)azo-1-naphthalenesulfate, C. I. 15 620) was obtained from BASF (Germany). Safranine T (3,7-diamino-2,8-dimethyl-5-phenylphenazinium chloride,

C. I. 50 240) and basic fuchsine (4,4′-diaminofuchoniminium chloride, C. I. 42 510) were commercial chemicals of Lachema, Brno, The Czech Republic.

The basic dyes were checked by thin layer chromatography[7] on Silufol® plates (Kavalier, Votice, The Czech Republic). Amounts of 100 μg of sample were applied to the start and a mobile phase containing butanol, butyl acetate, glacial acetic acid and water in the volume ratio of 6 : 1 : 1 : 2 was used. For ST, the component with R_F 0.29, whose content in the original chemical was 63.31%, was separated for the experiment. For FB, the component used had R_F 0.44 and its content in the dye was 76.32%. AR gave a major spot at R_F 0.66. After triple recrystallization of the commercial dye from methanol, the active content was 99.24%.

Spectrophotometric measurements were accomplished on a Specord M 40 double-beam recording spectrophotometer and on a Spekol 11 single beam spectrophotocolorimeter (both Zeiss, Jena, Germany). The pH was measured with an MW 870 pH-meter (Pracitronic, Dresden, Germany) equipped with an 01-29 combined Crytur electrode (Turnov, The Czech Republic); aqueous solutions with adjusted ionic strength after extraction were measured. The instrument was calibrated using standard (NBS) buffers obtained from the Forschungsinstitut Meinsberg (Germany); their pH was 1.68, 4.01 and 9.18 at 25 °C. The aqueous and chloroform solutions of CR and the dyes were pipetted with an MD 30 micropipette (Dioptra, Turnov, The Czech Republic), a Minilab micropipette (Plastomed, Warsaw, Poland), or with calibrated glass pipettes (Kavalier, Sazava, The Czech Republic).

Procedures

To determine the protonation constants, volumes of 200 μl of aqueous solution of CR (c = 0.55 mmol l^{-1}) were pipetted, the pH was adjusted between 0.5 and 7.0 with HCl or NaOH (1 mol l^{-1}), and the whole was diluted to 3 ml. Ionic strength was adjusted to 0.01, 0.2 or 0.3 with a solution of NaCl (c = 2 mol l^{-1}). The pH values were checked and the absorption spectra of the solutions were measured. The protonation constants of CR were calculated using absorbance values at 328 nm (ref.[8]).

For the investigation of the distribution of CR between water and chloroform, the aqueous phase contained CR in a concentration of 62 μmol l^{-1} and the pH, adjusted crudely with 10 to 100 μl of HCl or NaOH, was between 1 and 7.5. The ionic strength was adjusted to a value between 0.01 and 0.3 mol l^{-1} with a solution of NaCl (c = 2 mol l^{-1}). The whole was diluted with water to 3 ml. The same volume of chloroform was added, and the system was extracted at 20 °C for 30 min. The distribution ratios and distribution constants of CR were determined spectrophotometrically for the various pH values, which were invariably checked after the extraction.

The EXLET program[9,10] was used to evaluate the $\log D = f(\mathrm{pH})$ dependence.

For extraction of the ion-associate of CR with AR, 0.5 ml of CR (c = 0.55 mmol l^{-1}) and 1.5 ml of AR (c_{AR} = 2.67 mmol l^{-1}) were pipetted, pH was adjusted to 1.0 with HCl (1 mol l^{-1}), and the whole was diluted to 3.0 ml and extracted with 3.0 ml of chloroform for 30 min. The pH was checked after the extraction. The phases were allowed to separate, and the absorbance of the organic phase was measured at 515 nm. The excess of AR necessary to achieve the highest recovery in the extraction of the ion-associate was determined using constant additions of the dye at increasing concentrations. Pipetted were 0.1 to 2.0 ml of aqueous AR, c_{AR} = 8 mmol l^{-1}.

Associates of AR with ST or FB were obtained by reextraction of the organic phase containing the (CRH^+,AR^-) associate. To 2 ml of a chloroform solution of that associate were added 2 ml of aqueous ST or FB ($c_{ST} = c_{FB}$ = 1.5 mmol l^{-1}) at pH 5.0. The absorbance of the chloroform phase was measured after the phases separated off. The distribution ratios D, distribution constants K_D, extraction constants K_{ex} and other parameters including the molar absorptivities and limits of detection and

determination were calculated as in our previous examination of ion-associates of strychnine[9], 3-quinuclidinyl benzilate, and diethyllysergamide[10].

The conditional extraction constants were determined for equimolar solutions at $c_{CR} = c_{AR} = 0.55$ mol l^{-1}. The aqueous solution was adjusted to pH 1.0 with HCl (1 mol l^{-1}) and extracted with chloroform. The final volume of each phase was 4 ml. The absorbances of the two phases were used to calculate the distribution ratio D of the dye forming the (CRH^+,AR^-) ion-associate. After the chloroform extract separated off, the same volume of an aqueous solution at pH 1.0 or pH 5.0 (adjusted with HCl) was added, and the extraction was repeated. The absorbances of the two phases were employed to obtain the concentration of AR and the conditional extraction constants[11] K'_{ex} for the ion-associates (CRH^+,AR^-), (ST^+,AR^-) and (FB^+,AR^-). The concentrations of the dyes were as above, i.e. $c_{AR} = c_{ST} = c_{FB} = 0.55$ mmol l^{-1}.

To quantitate CR, its solutions in chloroform were prepared at seven concentrations covering the region of 10.2 to 44.5 μg ml^{-1}. To 1 ml of the solution was added 1 ml of aqueous solution of AR (c = 5 mmol l^{-1}, pH 1) and the system was agitated for 3 min. The organic phase was separated off, 1 ml of solution of ST or FB, c = 5 mmol l^{-1}, was added, and the whole was extracted. The absorbance of the organic layer was then measured at 515 and 555 nm for ST and FB, respectively.

RESULTS AND DISCUSSION

CR in aqueous solutions at pH 0 to 3 occurs predominantly in the protonated form (CRH^+), which absorbs electromagnetic radiation at 323 nm. As the proton activity is decreased, the fraction of the free CR base, which absorbs at 253 nm, increases. Typical absorption spectra at various pH values are shown in Fig. 1.

The protonation constants at ionic strength I = 0.01, 0.1 and 0.3 mol l^{-1} (adjusted with NaCl) were calculated by logarithmic analysis of the $A = f(\mathrm{pH})$ plot at pH 0.52 – 7.03 according to the formula[10]

$$\log [(A_{CRH^+} - A)/(A - A_{CR})^{-1}] \quad = \quad \log K_{H1} \quad + \quad \mathrm{pH}\,, \qquad (1)$$

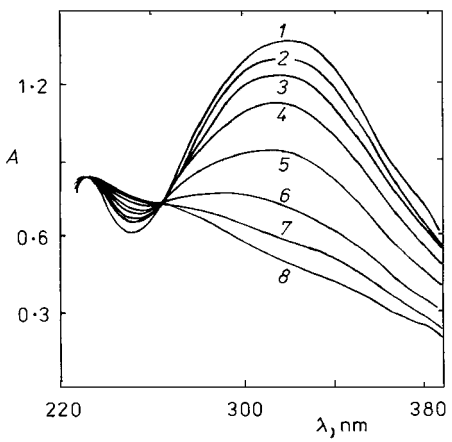

FIG. 1
Absorption spectra of CR (c = 0.55 mmol l^{-1}); pH: *1* 0.52, *2* 1.92, *3* 2.29, *4* 2.65, *5* 3.01, *6* 3.49, *7* 3.91, *8* 7.03

where A is absorbance and the subscripts CR and CRH^+ refer to the free base and the protonated form, respectively. Linear regression was applied to 20 experimental values. The resulting protonation constants K_{H1} do not differ appreciably (Table I). CR is extracted with chloroform even from acid aqueous solutions starting from pH 1. The distribution constants K_D (Table I) do not vary markedly with changing ionic strength: the value is apparently highest even from $I = 0.01$ and its additional increase does not exhibit any dependence on I, in contrast to strychnine[9]. In addition to the protonated form and the free base, the presence of the associate $(CRH^+,Cl^-)_o$ was proved (as in the cases studied previously[9,10]) after reextraction with silver nitrate and retitration of the latter with ammonium thiocyanate. The fractions of the various CR species in dependence on the pH of the aqueous phase are shown in Fig. 2.

Since the protonated form (CRH^+) is only present at pH 1 – 3, the possibility of formation and extraction of the ion-associates is limited.

From among a number of azo dyes and sulfonephthaleins, Acid Red 88 (AR) appeared to be the most suitable with respect to the recovery of the associates involving CR. Red ion-associates (CRH^+,AR^-) were extracted with chloroform from aqueous solutions of CR and AR at pH 1 to 3.57.

Figure 3 (curve *4*) and the EXLET calculations give evidence that the chloroform extract contains no free protonated CRH^+ species. Apparently, this species is transformed into ion-associates with chloride ions, $(CRH^+,Cl^-)_o$, (curve *1*) and with AR

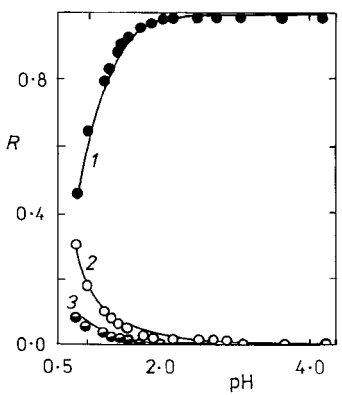

FIG. 2
Distribution diagram of CR in dependence on pH in the water (subscript w)–chloroform (subscript o) system: *1* CR_o, *2* $(CRH^+)_w$, *3* $(CRH^+,Cl^-)_o$; c_{CR} = 62 μmol l^{-1}, I = 0.1 mol l^{-1}

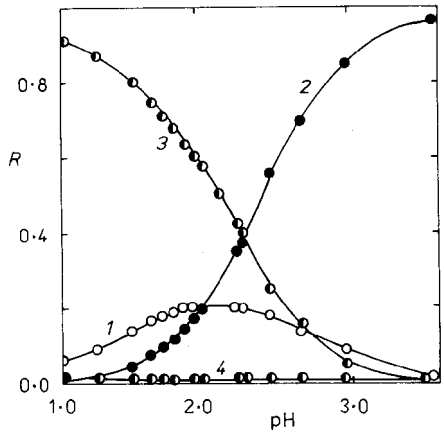

FIG. 3
Dependence of the extraction recovery p of the various CR species on pH in the water (subscript w)–chloroform (subscript o) system. *1* $(CRH^+,Cl^-)_o$, *2* (CR_o), *3* $(CRH^+,AR^-)_o$, *4* $(CRH^+)_w$

anions, $(CRH^+,AR^-)_o$ (curve *3*). The extraction recovery of the free base CR increases with increasing pH (curve *2*). The replacement of the CRH^+ cation with the basic dye cation gives rise to a new associate, and this is the underlying principle of the indirect extraction photometric analytical procedure, for which ST and FB were tested.

The absorption spectra of the ion-associates $(CRH^+,AR^-)_o$, $(ST^+,AR^-)_o$ and $(FB^+,AR^-)_o$ differ appreciably (Fig. 4). The first of them (curve *1*) is stable at pH 1 and only contains the AR^- anion absorbing in the visible part of the spectrum, whereas the two latter

TABLE I
Logarithms of protonation constants K_{H1} of dibenz[*b,f*]-1,4-oxazepin and distribution constants K_D for the extraction of the substance into chloroform, in dependence on ionic strength I

I, mol l^{-1}	log K_{H1}	log K_D
0.01	2.97 ± 0.03	2.16 ± 0.09
0.10	2.99 ± 0.02	2.15 ± 0.08
0.30	3.02 ± 0.02	2.15 ± 0.09

TABLE II
Characteristics of ion-associates of CR with AR and after reextraction of associates of AR with ST or FB

Value[a]	Associate		
	(CRH^+,AR^-)	(ST^+,AR^-)	(FB^+,AR^-)
ε	16 475	37 050	44 250
log K_{ex}	11.260	13.130	14.120
λ	515	515	555
x_L	16	5	5
n	3	2	2
pH	1	5	5
log D	0.49	1.20	1.30
R	0.85	0.94	0.95
log K_{ex}'	4.91	7.20	7.02
L_D	$5.4 \cdot 10^{-6}$	$3 \cdot 10^{-6}$	$1.6 \cdot 10^{-6}$
L_Q	$3 \cdot 10^{-5}$	$9 \cdot 10^{-6}$	$5 \cdot 10^{-6}$

[a] ε, l mol^{-1} cm^{-1} molar absorptivity; K_{ex} extraction constant; λ, nm wavelength; $x_L = c_{AR}/c_{CR}$ mole fraction of dye in the aqueous phase before extraction; n number of extractions necessary to achieve a 99% recovery; D distribution ratio; R extraction recovery; K_{ex}' conditional extraction constant; L_D, mol l^{-1} limit of detection; L_Q, mol l^{-1} limit of determination.

associates include the ST^+ and FB^+ cations whose absorbances contribute additively at close wavelengths (Table II). The optimum acidity for the ion exchange with the basic dyes is pH 5 (Table II). The extraction recovery is affected by the excess of the reagent. For the (CRH^+,AR^-) associate, the highest absorbance in the organic phase was found for a 16-fold excess of the dye and at pH 1. For the exchange of CRH^+, a fivefold excess of ST or FB in the aqueous phase is sufficient for the maximum absorbance of the associate in the chloroform extract to be achieved. The absorbance was found to change during the first three minutes of shaking. The extraction time was extended to

TABLE III
Characteristics of a determination of CR

Added	Found[a], μg		
	$L_{1,2}$	s	s_{rel}, %
10.2	10.3 ± 0.2	0.18	1.7
15.6	15.5 ± 0.3	0.40	2.6
22.0	22.1 ± 0.3	0.53	2.4
26.0	26.1 ± 0.4	0.52	2.0
29.0	29.5 ± 0.4	0.53	1.8
34.0	33.0 ± 0.3	0.56	1.7
44.5	45.0 ± 0.4	1.04	2.3

[a] $L_{1,2}$ confidence interval, s standard deviation estimate, s_{rel} relative standard deviation.

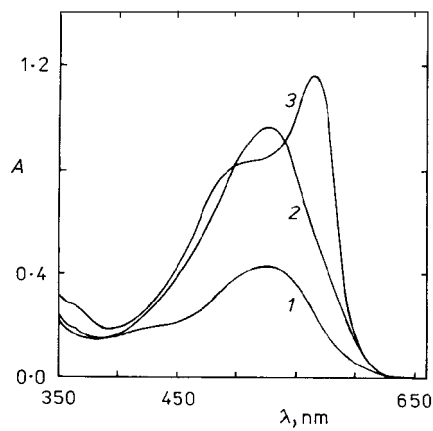

FIG. 4
Absorption spectra of the ion-associates. *1* $(CRH^+,AR^-)_o$, *2* $(ST^+,AR^-)_o$, *3* $(FB^+,AR^-)_o$

30 min to ensure equilibrium at 20 °C. The extraction characteristics of the associates of CR and AR are given in Table II.

The associates of AR and ST or FB exhibit higher molar absorptivities as well as extraction recoveries than the ion-associate of CR with AR, owing to which lower limits of detection L_D and limits of determination L_Q can be attained. The detection limits for ST and FB are 0.9 and 0.3 μg ml^{-1}, respectively. The calibration curves are linear over the regions of c_{CR}= 0.3 – 50 and 0.9 – 50 μg ml^{-1} if CRH^+ is replaced with ST and FB, respectively.

The results of analysis of model samples, including their evaluation by interval estimates[12], are given in Table III. Since the threshold concentration of CR in air is 0.2 μg l^{-1} and its unbearable concentration is 3 μg l^{-1} (ref.[13]), the procedure proposed can find use in the development of a method for the determination of CR in air.

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