STUDY OF THE CONDITIONS FOR THE EXTRACTION OF TOXIC ORGANIC BASES AND METHODS FOR THEIR DETERMINATION.
II. EXTRACTION OF ION ASSOCIATES OF 3-QUINUCLIDINYL BENZILATE AND DIETHYL LYSERGAMIDE WITH AZO DYES AND SULFOPHTHALEINS

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Diethyl lysergamide (LSD) and 3-quinuclidinyl benzilate (BZ) are both extracted by chloroform in dependence on the pH value of the solution. The protonation constants were determined for both the bases and their distribution constants were calculated from their distribution constants in the water–chloroform system. Conditions were studied for the formation of the ion associates with 13 dyes and for their extraction with chloroform.

Diethyl lysergamide (LSD) and 3-quinuclidinyl benzilate (BZ), similar to strychnine[1], are organic nitrogen bases. Both compounds affect human psychical states and are considered to be incapacitating substances[2]. They are cholinesterase inhibitors. LSD is abused as a drug, while BZ blocks the activity of the central nervous system. It is mostly detoxified in the kidneys to yield benzilic acid and quinuclidinol[3]. As a poison, it is on the list of substances to be destroyed in the framework of the present disarmament program[4]. The test compounds can be determined by gas and liquid chromatography, and by ultraviolet and infrared spectrophotometry[5,6]. Because of the protonation ability of these substances in aqueous solutions, and their ability to subsequently form associates with suitable anions that can be extracted by chloroform, a study was carried out of their determination by extraction spectrophotometry; this study was described in the previous work[1].

This contribution describes the extractability of 3-quinuclidinyl benzilate and diethyllysergamide and the conditions for the formation and extraction of their ion associates with selected dyes.

EXPERIMENTAL

Instruments and Chemicals

The spectrophotometric measurements were carried out using a two-beam recording Specord UV VIS spectrometer and single-beam Spekol 11 spectrophotocolorimeter (Zeiss, Jena, Germany). All the measurements were carried out in 1 cm cuvettes. The pH values of the aqueous solutions were adjusted by the addition of 0.001 to 0.1 mol l^{-1} HCl or NaOH and controlled using a MW 870 pH meter (Präcitronic, Dresden Germany) with an SE 20 reference calomel electrode and GA 50N glass measuring electrode (Forschungsinstitut, Meinsberg, Germany). Organic and aqueous solutions were shaken on a TE III laboratory shaker (Chirana, Stará Turá, The Slovak Republic).

3-Benziloyloxyquinuclidinium chloride (BZHCl) was manufactured in VOZ 072 Zemianské Kostoľany. Diethyllysergamide chloride (LSDHCl) was supplied by Zdravotnické zásobování, Praha. The content of active components was determined by titration with sodium tetraphenyl borate using a potassium measuring electrode (Crytur, Monokrystaly, Turnov[7]). The purity of BZ was 99.2 wt.%; the content of LSD active component was 99.5%.

The following dyes were tested for the formation of the ion associates: 3',3"-dibromothymol sulfophthalein, bromothymol blue; *I*; 3',3",5',5"-tetrabromo-*m*-cresol sulfophthalein, bromocresol green *II*; 3',3",5',5"-tetrabromophenol sulfophthalein, bromophenol blue, *III*; 3',3",5',5"-tetrabromoxylenol sulfophthalein, bromoxylenol blue, *IV*; sodium 2-hydroxy-naphthalene-1-azo-4'-benzene sulfonate, orange II, C.I. 15510, *V*; sodium 4-dimethylaminoazobenzene-4-sulfonate, methyl orange, C.I. 13025, *VI*; sodium 4-anilinazobenzene-*m*-sulfonate, methanil yellow, C.I. 13065, *VII*; sodium 1-(2-hydroxynaphthylazo)-2-naphthol-4-sulfonate, calcone, C.I. 15705, *VIII*; 2,4,6-trinitrophenol, picric acid, *IX*; 5',5"-dibromopyrogallol sulfophthalein, bromopyrogallol red, *X*; disodium 2-(2,4-dinitrophenylazo)-1-naphthol-3,6-disulfonate, nitrazine yellow, C.I. 14890, *XI*; disodium 2-(5-chloro-2-hydroxy-1-phenylazo)-1,8-dihydroxynaphthalein-3,6-disulfonate, eriochrome blue SE or Plasmocorinth B, C.I. 16680, *XII*; tetrasodium 2,2'-(3,3'-dimethyl-4,4'-biphenylenebisazo)bis(8-amino-1-naphthol-5,7-disulfonate), Evans blue, C.I. 23860, *XIII*.

Procedure

For the extraction of the bases, 10 ml of an aqueous solution of BZHCl with a concentration of c_{BZHCl} = 9.3 mmol l^{-1} or LSDHCl, c_{LSDHCl} = 0.1 mmol l^{-1} was pipetted and the pH was adjusted by the addition of 0.01 to 2 ml of HCl or NaOH. The ionic strength was adjusted by 2 mol l^{-1} NaCl to a value of 0.01, 0.1 or 0.3. In the extraction, the volume of the aqueous solution was diluted to 20 ml and it was shaken for 40 min with 20 ml of freshly distilled chloroform. Then the pH of the aqueous phase was measured and the absorbances of both phases were read at a wavelength of λ = 258 nm for BZ and 314 nm for LSD. The distribution ratio D was calculated from the ratio of the absorbances of the organic and aqueous phases. Nonlinear regression of the dependence $\log D = f(\mathrm{pH})$ was employed to calculate the distribution and extraction constants using the EXLET program[8]. The base associates with chloride ions in the chloroform extracts, obtained after re-extraction with an standard $AgNO_3$ solution, were extracted using the same procedure as for strychninium chloride[1].

To determine the protonation constants, 5 ml of a base solution with a concentration of 1 mmol l^{-1} were pipetted into a titration vessel and aliquots of 50 µl of NaOH with a concentration of 10 mmol l^{-1} were added from a Bang burette until a constant potential change was attained. The protonation constants were read from the titration curves.

For extraction of the ion associates, an amount of 1 ml of buffer solution of pH 1.1 to 7.5 in intervals of 0.1 pH units was pipetted, along with 0.1 ml of dye solution (c_L = 5 mmol l^{-1}) and 0.1 ml of BZHCl or LSDHCl solution ($c_{BZHCl} = c_{LSDHCl}$ = 0.5 mmol l^{-1}) and the mixture was diluted with water to 2 ml. After shaking with chloroform, the aqueous phase was drawn off and the absorbance of the extract was measured. The value of λ_{max} was determined and the absorbances of all the chloroform solutions of the ion associates were read at this wavelength.

The stoichiometric ratios of the components yielding the ion associates were determined by the method of continuous variations. Solutions of BZHCl or LSDHCl were mixed with solutions of *I* to *XIII* of the same concentration, in buffers with a pH value best suited to the formation of the corresponding associate, ($c_{BZHCl} = c_{LSDHCL} = c_L$), where c_L is the dye concentration. An amount of 0.2 to 2.0 ml of BZHCl or LSDHCl was pipetted in 0.2 ml aliquots and the solution was diluted with the solution of *I* to *XIII* to 2 ml and shaken with the same volume of chloroform. To achieve the maximum extraction yield, an amount of 0.3 ml BZHCl or LSDHCl was pipetted, ($c_{BZHCl} = c_{LSDHCL} = 0.1$ mmol l^{-1}) and the smallest dye excess required to achieve the highest possible yield was determined by the method of constant addition of dye *I* to *XIII*. The total volume of the aqueous and chloroform phases was 2 ml. The optimum dye concentration, at which the maximum yield is achieved, was chosen for each dye.

To determine the calibration dependence $A = f(c_L)$, amounts of 0.1 to 1.0 ml of BZHCl or LSDHCl were added in aliquots of 0.1 ml, along with 1 ml of *I* to *XIII* in the buffer. The solution was diluted with water to 2 ml and extracted with the same volume of chloroform; the base concentration corresponding to the dye concentration for the given stoichiometric ratio was calculated from the absorbance of the organic phase. The conditional molar absorption coefficient ε' was calculated as the slope of the calibration straight line.

The conditional extraction constant of the ion associate K'_{ex} was determined as the pH and ionic strength dependent value. An amount of 5 ml of equimolar aqueous solution of BZHCl or LSDHCl was mixed with *I* to *XIII*, ($c_{BZHCl} = c_{LSDHCL} = c_L = 0.5$ mmol l^{-1}), the pH was adjusted, the solution was diluted with water to 15 ml and was shaken with the same volume of chloroform at a temperature of 20 °C for 40 min. An amount of 10 ml of the organic phase was separated and 10 ml of water were added, with pH adjusted for each associate according to Table II. After repeated shaking under the same conditions, the aqueous and organic phases were separated, the absorbance was measured and the distribution ratio of the dye D_L and its concentration in the aqueous solution were calculated. The conditional extraction constant of the associate formed K'_{ex} was calculated from six parallel determinations according to the relationship[9]

$$K'_{ex} = D_L [L]^{-1} . \qquad (1)$$

RESULTS AND DISCUSSION

Both compounds BZ and LSD are extracted by chloroform in dependence on the proton activity. It was confirmed experimentally, similarly as for strychnine[1], that the 3-quinuclidinyl benzilate and diethyllysergamide cations form associates with chloride ions. The $\log D = f(\mathrm{pH})$ dependence was calculated using the EXLET program[8], where the distribution ratio D is given by the relationship

$$D = \{[BH^+, Cl^-]_o + [B]_o\} \{[BH^+] + [Cl^-] + [B]\}^{-1} , \qquad (2)$$

where subscript o refers to the species in the organic phase and quantities without a subscript are present in the aqueous phase. The concentration of chloride ions was adjusted to a value of 0.1 mol l^{-1} using sodium chloride.

The contents of the individual base forms ($LSDH^+$ and BZH^+ cations, $\{LSDH^+, Cl^-\}$ and $\{BZH^+, Cl^-\}$ associates and LSD or BZ free bases in dependence on the pH are depicted in Fig. 1.

The following equilibria were considered for the individual components of base B in treatment using the EXLET computer program:

protonation of the monoacidic base B

$$B + H^+ \rightleftharpoons BH^+ \qquad K_{H1} = [BH^+]\,[H^+]^{-1}\,[B]^{-1} \tag{3}$$

extraction of the associate of the cations of the bases BH^+ with the halide ions X^-

$$BH^+ + X^- \rightleftharpoons \{BH^+, X^-\}_o \qquad K_{ex} = [BH^+, X^-]_o\,[B]^{-1}\,[H^+]^{-1}\,[X^-]^{-1} \tag{4}$$

distribution of the base B

$$B \rightleftharpoons B_o \qquad K_D = [B]_o\,[B]^{-1}, \tag{5}$$

The experimentally determined and calculated values of the equilibrium constants are given in Table I together with the molar absorption coefficients and the corresponding wavelengths at which the absorbances of both substances were measured in aqueous and chloroform solutions. The difference in the position of the absorption maximum of the free base and its associate with chloride ions in the organic phase was not found. The calculated extraction constant values K_{ex} are related to this associate (Table I), while the distribution constants K_D are given for the base.

The best conditions for the extraction of the associates of the individual dyes with 3-quinuclidinyl benzilate and diethyllysergamide were found experimentally and are summarized in Table II.

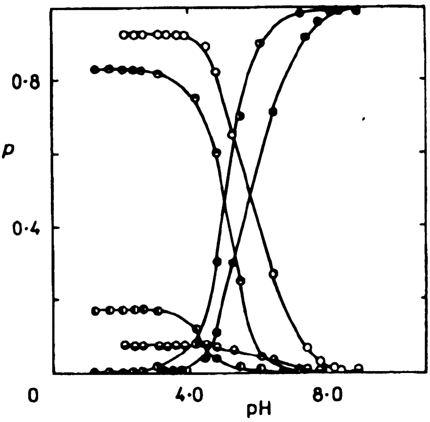

FIG. 1

Dependence of the yield *p* of the BZH^+ (○) and $LSDH^+$ (◓) forms, $\{BZH^+, Cl^-\}_o$ (◒) and $\{LSDH^+, Cl^-\}_o$ (◐) associates, and BZ (●) and LSD (◑) free bases on the pH of the aqueous phase. $c_{BZHCL} = 4.83$ mmol l^{-1}, $c_{LSDHCl} = 0.593$ mmol l^{-1}, $I = 0.1$ mol l^{-1}

The wavelength of the maximum λ_{max} and the pH of the aqueous solution after extraction for maximum absorbance were found for each chloroform solution of the ion associate. In order to achieve the maximum extraction yield, it is necessary to know the dye excess in the aqueous phase expressed as the number of moles of dye n_L per mole of base. Table II also gives the range of concentrations of BZHCl or LSDHCl within which the condition of linearity of the dependence of the absorbance on the concentration of the test substance is fulfilled. The BZH^+ and $LSDH^+$ cations form associates with the anions of selected dyes on the basis of the number of sulfo groups in their molecules, as can be seen from Table II. In the choice of a dye suitable for the determination, it is necessary to take into account a number of requirements simultaneously. The method of multicriterial analysis[10] is suitable for this purpose. Of the relatively wide range of acidic dyes, the individual reagents were selected primarily on the basis of the yield p, which is affected by the number of individual extraction steps n yielding the maximum value $p = 0.99$. Quantitative extraction can also be achieved by increasing the dye concentration in the aqueous solution. The distribution ratio of the test base D_B depends on the pH, which affects the value of the conditional extraction constant K'_{ex}, yield p and slope of the calibration curve, which can be considered to be dependent on the molar absorption coefficient ε'

$$A = \varepsilon' c \pm q , \qquad (6)$$

where A is the absorbance of the chloroform solution of the dye in the form of the associate with the required base, c is its analytical concentration and q is the intercept on the y-axis. The K'_{ex} and ε' values are dependent on the experimental conditions and thus are denoted as conditional constants. The yield p is also dependent on these conditions, and corresponds to the amount of dye transferred to the organic phase as the ion associate. The detection limit L_Q and determination limit L_D were calculated for the individual associates[11]. The method of simple multicriterial analysis[10] was employed to

TABLE I

Constants and characteristic values[a] for BZHCl and LSDHCl

Quantity	BZHCl	LSDHCl
λ, nm	258	314
ε, l mol^{-1} cm^{-1}	460	20 100
$\log K_{HI} \pm L_{1,2}$	8.53 ± 0.11	6.66 ± 0.12
$\log K_D \pm L_{1,2}$	2.18 ± 0.05	1.80 ± 0.08
$\log K_{ex} \pm L_{1,2}$	7.81 ± 0.03	6.63 ± 0.08

[a] $L_{1,2}$ reliability interval.

determine the order of the individual dyes suitable for the determination of BZ and LSD as the ion associates (Table III).

The chloroform solutions of the associates are stable. The decrease in the absorbance of most extracts over two hours is negligible. The associate of calcone (*VIII*) is an exception, as its colour decreases to 86% of the original value over this period of time. It follows from Table III that selected dyes can be considered suitable for the determination of 3-quinuclidinyl benzilate and diethyl lysergamide. The order in the group of

TABLE II
Conditions for the formation of the ion associates of 3-quinuclidinyl benzilate and diethyllysergamide with selected dyes[a]

Associate	pH	λ, nm	n_L	Δc_B, mmol l^{-1}	B : L
{*I*, LSD}	6.5	415	2	0.02 – 0.5	1 : 1
{*I*, BZ}	6.5	415	2	0.03 – 0.5	1 : 1
{*II*, LSD}	4.0	415	2	0.03 – 0.5	1 : 1
{*II*, BZ}	4.0	415	2	0.03 – 0.5	1 : 1
{*III*, LSD}	3.5	415	3	0.03 – 0.5	1 : 1
{*III*, BZ}	3.5	415	4	0.04 – 0.5	1 : 1
{*IV*, LSD}	2.0	415	9	0.05 – 0.5	1 : 1
{*IV*, BZ}	4.0	415	11	0.07 – 0.6	1 : 1
{*V*, LSD}	2.0	485	16	0.05 – 0.5	1 : 1
{*V*, BZ}	1.6	485	19	0.05 – 0.5	1 : 1
{*VI*, LSD}	3.5	420	16	0.05 – 0.5	1 : 1
{*VI*, BZ}	3.5	420	20	0.05 – 0.5	1 : 1
{*VII*, LSD}	4.0	405	19	0.04 – 0.5	1 : 1
{*VII*, BZ}	4.0	405	23	0.04 – 0.5	1 : 1
{*VIII*, LSD}	2.0	515	8	0.09 – 0.6	1 : 1
{*VIII*, BZ}	2.0	515	11	0.1 – 0.9	1 : 1
{*IX*, LSD}	3.0	400	14	0.1 – 0.9	1 : 1
{*IX*, BZ}	3.0	400	18	0.1 – 0.9	1 : 1
{*X*, LSD}	2.0	510	48	0.4 – 1.1	1 : 1
{*X*, BZ}	2.0	510	53	0.3 – 1.2	1 : 1
{*XI*, LSD}	2.0	470	55	0.5 – 1.4	2 : 1
{*XI*, BZ}	2.0	470	55	0.5 – 1.4	2 : 1
{*XII*, LSD}	3.0	525	55	0.6 – 1.5	2 : 1
{*XII*, BZ}	3.0	525	55	0.7 – 1.6	2 : 1
{*XIII*, LSD}	5.0	615	55	1.1 – 25.0	4 : 1
{*XIII*, BZ}	5.0	615	55	1.1 – 25.0	4 : 1

[a] At the most suitable pH for the extraction of the given associate; λ wavelength; n_L excess dye ensuring maximum associate yield; Δc_B range of concentrations within which the extraction can be carried out with maximum yield; B : L stoichiometric ratio of cations of base and dye forming the ion associate.

sulfophthaleins is the same as that given by Yamamoto and Motomizu[12] for a similar group of dyes. On the basis of the order in Table III, picric acid (*IX*), bromopyrogallol red (*X*), nitrazine yellow (*XI*), eriochrome blue SE (*XII*) and Evans blue (*XIII*) can be considered as unsuitable for the determination of the test compounds. It is also apparent from the multicriterial analysis that the two organic bases cannot be determined simultaneously by the extraction method. To increase the amount of information

TABLE III

Constants and characteristic values of the ion associates of 3-quinuclidinyl benzilate and diethyllysergamide with selected dyes[a]

Associate	$\varepsilon' \pm L_{1,2}$ l mol^{-1} cm^{-1}	n	p	$\log K'_{ex}$	L_Q, μg ml^{-1}
{*I*, LSD}	20 840 ± 250	1	0.9960	8.44	1.0
{*II*, LSD}	23 530 ± 300	2	0.9100	6.22	1.8
{*II*, BZ}	18 700 ± 450	1	0.9920	7.05	2.4
{*I*, BZ}	20 540 ± 500	2	0.9860	7.57	3.6
{*III*, LSD}	14 900 ± 400	2	0.9890	7.71	3.3
{*IV*, LSD}	16 300 ± 400	2	0.9880	7.63	3.8
{*V*, LSD}	19 330 ± 600	2	0.9690	7.02	3.8
{*III*, BZ}	19 900 ± 850	2	0.9440	5.05	3.6
{*II*, LSD}	18 800 ± 900	2	0.9750	7.25	3.8
{*VI*, BZ}	23 300 ± 900	2	0.9480	5.35	3.7
{*IV*, BZ}	19 230 ± 1 100	2	0.9699	6.82	5.6
{*V*, BZ}	18 800 ± 1 000	3	0.8800	5.81	6.3
{*VII*, LSD}	19 750 ± 850	3	0.8920	5.31	6.5
{*VII*, BZ}	19 100 ± 800	3	0.8010	4.76	6.6
{*VIII*, LSD}	8 800 ± 1 150	3	0.8710	5.22	7.2
{*IX*, LSD}	12 000 ± 1 000	4	0.7580	5.18	9.6
{*VIII*, BZ}	8 800 ± 1 350	3	0.7960	4.42	7.8
{*IX*, BZ}	10 700 ± 970	4	0.7580	4.80	26.0
{*X*, BZ}	9 300 ± 550	16	0.2610	3.13	31.0
{*XI*, LSD}	2 300 ± 550	11	0.3630	3.85	32.0
{*XI*, BZ}	3 150 ± 300	320	0.1830	2.35	88.0
{*X*, LSD}	9 200 ± 350	83	0.0538	2.94	91.0
{*XII*, BZ}	900 ± 120	83	0.0499	1.95	95.0
{*XII*, LSD}	1 300 ± 200	83	0.0524	3.13	96.0
{*XIII*, LSD}	20 ± 9	459	0.0101	1.00	6 250.0
{*XIII*, BZ}	25 ± 10	11 511	0.0004	−2.39	5 000.0

[a] At the most suitable pH for the extraction of the given associate; ε' conditional molar absorption coefficient; $L_{1,2}$ reliability interval; n number of extractions required to obtain the maximum yield; p extraction yield; K'_{EX} conditional extraction constant; L_Q determination limit.

obtained from the analysis of a two-component mixture of BZ and LSD, it is necessary to introduce a separation step, as described by Eckschlager[13]. After separation of the two components, the extraction spectrophotometric method can be used.

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