ION-ASSOCIATES OF *N,N*-DIETHYLLYSERGAMIDE WITH SOME SULFOPHTHALEINS AND AZO DYES

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Ion-associates of *N,N*-diethyllysergamide with the sulfophthaleins: Bromoxylenol Blue, Eriochrome Cyanine R, Xylenol Blue, and Cresol Red, and the azo dyes: Acid Black I and Orange-I were studied by extraction spectrophotometry. The extraction recoveries, distribution ratios, conditional extraction constants and limits of detection and determination were calculated.

D-Lysergic acid and its amides are serotonin derivatives which contain in their molecules substituted indole rings inducing hallucination effects. *N,N*-Diethyllysergamide (LSD) is a prominent compound of this kind; it is one of the biologically most efficacious substances, causing psychic debilitation of people even in very low doses[1] (1 to 2 μg kg^{-1}).

Lysergic acid derivatives give with the Marquis reagent colours that are usable for their photometric determination[2]. Similar colours emerge in the presence of a mixture of acetaldehyde and sulfuric acid[3]. In addition to the dehydrating effect of sulfuric acid, the catalytic effect of some salts also appears. Copper(II) chloride and iron(III) chloride have been employed in the modified Winkler reaction[4,5] and in the Keller reaction[6], respectively. LSD can be detected through its reaction with *p*-dimethylaminobenzaldehyde in hydrochloric acid, giving rise to violet colour[7]. Lysergic amide has been determined fluorimetrically at 408 nm using excitation radiation of 314 nm wavelength[8]. Lysergic acid derivatives have been quantitated by direct spectrophotometry[9] at 312 nm. Infrared spectrometry[10] and mass spectrometry[11] have also been applied to the analysis of lysergamides. The ability of LSD to form ion-associates extractable into nonpolar solvents has been made use of in the extraction spectrophotometric determination with azobenzenenaphthalenesulfonates[12], nitrophenols[13] and sulfophthaleins[14].

Spectrophotometric investigation of the formation of ion-associates on *N,N*-diethyllysergamide with some anionic dyes from the azo and sulfophthalein groups, after extraction into chloroform, is the subject of this paper.

EXPERIMENTAL

Chemicals and Apparatus

LSD (99.50) was a product of SPOFA, Prague. Its purity was checked by titration with perchloric acid in glacial acetic acid[15]. Acid Black I (C.I. 20 470) (*I*), Bromoxylenol Blue (3,3′-dibromo-*p*-xylenolsulfophthalein) (*II*), Xylenol Blue (*p*-xylenolsulfophthalein) (*III*), Eriochrome Cyanine R (C.I. 43 820) (*IV*), and Cresol Red (*o*-cresolsulfophthalein) (*V*) were obtained from Merck (Germany), Orange-I (Tropaeolin 000 No. 1, C.I. 14 600) (*VI*) was a chemical of Fluka (Switzerland).

Chloroform was destabilized by triple shaking with distilled water, followed by distillation at 60 °C. The other chemicals used were commercial products of reagent grade purity (Lachema, Brno).

Citrate buffers at pH 1.0 to 6.5 and phosphate buffers at pH 7.0 to 8.0, in steps of 0.5, were prepared following ref.[16] and checked with an OP-208/1 pH-meter equipped with an OP-0808P combined electrode (both Radelkis, Hungary). The spectrophotometric measurements were performed on a Spekol 11 single-beam spectrophotometer (Zeiss Jena, Germany) using a glass cell 1 cm optical pathlength.

Procedures

The wavelengths of the absorption maxima of the ion-associate extracts in the visible spectral region (λ_{max}) were determined after extraction of the aqueous solutions with 2 ml of chloroform for 2 min. The solutions were obtained by mixing 0.1 ml of an aqueous solution of LSD (c_{LSD} = 1 mmol l^{-1}), 0.1 ml of a methanolic solution of the dye (c_d in mmol l^{-1}): *I* (3.0), *II* (5.0), *III* (2.7), *IV* (5.7), *V* (10.0), or *VI* (18.0), and 1.8 ml of citrate buffer at pH 2.5 (*I*, *III*, *V*), 3.0 (*II*) or 2.0 (*IV*, *VI*).

The effect of pH on the ion-associate formation was studied by measuring the $A = f(\text{pH})$ dependence (*A* is absorbance). Mixtures were prepared as above, the pH of the citrate buffer, however, was from 1.0 to 8.0 in steps of 0.5. The pH values of the aqueous mixtures were measured. The mixtures in test tubes were extracted with 2 ml of chloroform for 2 min at room temperature, and the absorbances of the extracts were measured at λ_{max}; the pH at which the absorbance was highest (pH_{opt}) was recorded.

The $A = f(c_d)$ dependence of the ion-associate was also examined (c_d is the concentration of the dye). Mixtures containing 0.1 ml of LSD solution (c_{LSD} = 0.1 mmol l^{-1}) in buffer at pH_{opt} and 0.01 to 0.1 ml of methanolic solution of the dye (c_d in mmol l^{-1}): *I* (4.5), *II* (8.0), *III* (2.7), *IV* (5.7), *V* (10.0) or *VI* (1.70), in 0.01 ml steps were prepared in test tubes and diluted to 0.2 ml with methanol, and 1.8 ml of buffer at pH_{opt} was added. The ion-associate was extracted with 2 ml of chloroform for 2 min. The concentration of the dye at which the extraction recovery was highest while low blank values were maintained were considered as optimum (c_{opt}).

To investigate the time dependence of the extraction recovery, 0.1 ml of LSD solution (c_{LSD} = 0.1 mmol l^{-1}) in buffer at pH_{opt} and 0.1 ml of methanolic solution of the dye (c_d in mmol l^{-1}): *I* (4.5), *II* (8.0), *III* (2.7), *IV* (5.7), *V* (1.7) or *VI* (10.0), were pipetted and diluted to 2 ml with buffer at pH_{opt}. The absorbance at λ_{max} was measured after shaking for 0.5, 1, 2, 5, 10, 15 and 30 min.

The ion-associate composition was elucidated from the $A = f(x_d)$ plot ,where x_d is the mole fraction of the dye. Volumes of 0.1 to 1 ml of dye solution at c_d = 0.4 mmol l^{-1} (*I*, *II*, *VI*) or 0.2 mmol l^{-1} (*III*, *IV*, *V*) were pipetted and diluted to 1 ml with LSD solution in buffer at pH_{opt}.

Molar absorptivities of the dyes (ε_d) were determined so that volumes of 0.1 to 1 ml of aqueous solutions of the dyes in 0.1 ml steps (c_d = 0.1 mmol l^{-1}) were pipetted into test tubes and diluted to 4 ml with NaOH (c = 0.1 mol l^{-1}), and their absorbances were measured at $\lambda_{max,d}$.

Molar absorptivities of the ion-associates (ε) in conditions of quantitative extraction of the dye were determined so that 1 ml of LSD solution in buffer at pH_{opt} (c_{LSD} = 10 mmol l^{-1}) and 0.1 to 1 ml

of the dye (c_d in mmol l^{-1}): *I* (0.05), *II* (0.1), *III* (0.09), *IV* (0.2), *V* (0.1) or *VI* (0.04), in 0.1 ml steps were pipetted, and the $A = f(c_d)$ dependence was measured. The concentration of the dye in the aqueous phase was checked after pH adjustment with NaOH (0.1 mmol l^{-1}), by measuring the absorbance at $\lambda_{max,d}$.

The calibration plot of extract absorbance vs LSD concentration ($A = f(c_{LSD})$) was obtained for a set of samples at various concentrations. The samples were prepared by pipetting 0.1 to 1 ml of LSD solution in buffer at pH_{opt} in 0.1 ml steps, adding 0.1 ml of methanolic solution of dye at $c_{d,opt}$ and diluting with the buffer to 2 ml.

To determine the distribution ratio of the ion-associate (D_a) and the conditional extraction constant (K'_{ex}), 50 ml of LSD solution in buffer at pH_{opt} (c_{LSD} = 0.1 mmol l^{-1}), 5 ml of dye at c_{opt}, and 45 ml of buffer at pH_{opt} were pipetted into a flask, 100 ml of chloroform were added, the whole was shaken, and the absorbance of the ion-associate (A_0) was measured at λ_{max}. A 15 ml aliquot of the extract was taken, 15 ml of buffer at pH_{opt} were added, and the system was reextracted for 60 min. The absorbance of the ion-associate (A_1) was measured again after phase separation, and the dye concentration in the aqueous phase was determined after pH adjustment with NaOH (0.1 mol l^{-1}).

RESULTS AND DISCUSSION

The molar absorptivities of the basic species of the dyes at their absorption maxima are given in Table I. The absorption spectra of the ion-associate extracts are shown in Fig. 1. The extracts of the ion-associates of LSD with *I*, *II*, *IV*, *V* and *VI* exhibited pronounced maxima, wheras the solution containing the associate with *III* did not exhibit any maximum in the visible region: the absorbance decreased with increasing wavelength. The measurement was performed at λ_{max} = 370 nm.

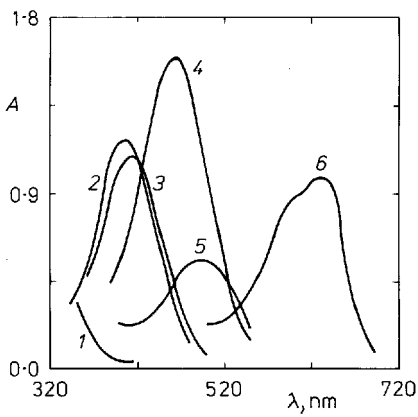


FIG. 1
Absorption spectra of chloroform extracts of ion-associates of LSD with some dyes: *1 III*, *2 V*, *3 II*, *4 IV*, *5 VI*, *6 I*; pH 2.0 (*IV*, *VI*), 2.5 (*I*, *III*, *V*), 3.0 (*II*)

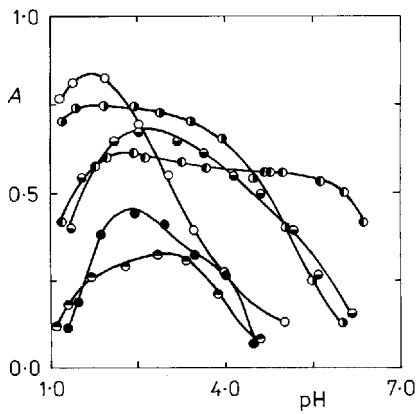


FIG. 2
pH-Dependences of absorbances of extracts of LSD ion-associates with dyes; c_{LSD} = 1 mmol l^{-1}; dye, c_d (mmol l^{-1}), λ_{max} (nm): ● *I*, 3.0, 625; ❍ *II*, 5.0, 412; ❍ *III*, 2.7, 370; ❍ *IV*, 5.7, 462; ❍ *V*, 20.0, 406; ❍ *VI*, 18.0, 495

TABLE I
Parameters of dyes and ion-associates of LSD

Parameter[a]	Dye					
	I	*II*	*III*	*IV*	*V*	*VI*
$\lambda_{max,d}$, nm	619	610	594	584	571	515
ε_d, l mol^{-1} cm^{-1}	41 300	36 800	39 100	19 900	49 328	32 400
λ_{max}, nm	625	412	370	462	406	495
pH_{opt}	2.4	3.0	2.7	1.8	2.5	2.0
c_{opt}, mmol l^{-1}	4.5	8.0	2.7	17.0	20.0	15.7
t, min	5	2	2	2	2	3
x_d/x_{LSD}	1 : 2	1 : 1	1 : 1	1 : 1	1 : 1	1 : 1
r	0.964	0.999	0.998	0.999	0.999	0.997
$S_{x,y}$	0.015	0.013	0.011	0.016	0.016	0.014
q	−0.132	0.006	0.002	0.025	0.025	0.023
RSD, %	6.02	0.39	1.13	0.88	0.73	1.23
ε, l mol^{-1} cm^{-1}	43 200	23 800	15 500	28 300	26 300	18 300
ε', l mol^{-1} cm^{-1}	11 960	22 290	9 410	24 800	24 100	17 000
E, %	27.7	93.7	60.7	87.6	91.6	92.9
D	0.33	5.23	11.43	1.49	46.80	4.57
log K_{ex}'	4.94	4.70	6.66	3.69	6.89	5.63
L_D, μg ml^{-1}	11.63	0.48	1.69	1.25	0.94	1.74
L_Q, μg ml^{-1}	31.52	1.08	4.08	2.75	2.01	3.96

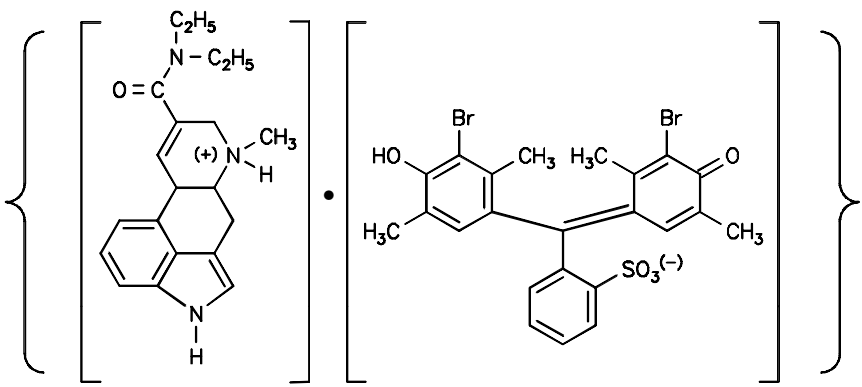
[a] $\lambda_{max,d}$ absorption maximum wavelength of the dye (basic species), ε_d molar absorptivity of the dye (basic species), λ_{max} absorption maximum of the ion-associate extract, pH_{opt} optimum pH for the formation of the ion-associate, c_{opt} reagent concentration, t extraction time, x_d/x_{LSD} ion-associate stoichiometry, q calibration straight line intercept, r correlation coefficient, $S_{x,y}$ standard deviation estimate for the calibration straight line points, RSD relative standard deviation of the straight line slope, ε molar absorptivity of the ion-associate in conditions of complete transfer of the dye into the associate form, ε' molar absorptivity of the ion-associate calculated from the $A = f(c_d)$ calibration straight line, E extraction recovery, D ion-associate distribution ratio, K_{ex}' conditional ion-associate extraction constant, L_D limit of detection, L_Q limit of determination.

The effect of pH on the formation of the ion-associates is illustrated by Fig. 2. The effect of pH was appreciable for Eriochrome Cyanine R, Orange-I and Acid Black I, for which the absorbance was highest at citrate buffer pH 1.8, 2.0 and 2.4, respectively. The ion-associate with Bromoxylenol Blue is formed across the entire pH region, the absorbance of the ion-associate extract being little affected by acidity within the range of pH 2.5 to 5.5. The pH values at which the ion-associate extracts gave the highest absorbances are given in Table I.

The effect of time of extraction on the extraction recovery was different for the different dyes. A time of 2 min was sufficient for the dyes *II* through *V*. The extraction periods used in the subsequent measurements are given in Table I. The blank absorbances were unaffected by extended extraction.

Attempts to promote the ion-associate formation by increasing the excess of dye resulted in an increase in the blank absorbances for *IV*, *V* and *VI*, as the dyes passed into the organic phase. The optimum dye concentrations giving the highest extraction recoveries while maintaining low detection limits are given in Table I. The lowest blank absorbances were observed for Acid Black I (0.003), Xylenol Blue (0.005) and Bromoxylenol Blue (0.008).

The ion-associate stoichiometry was determined by the continuous variations method[17]. The absorbances of the ion-associate extracts were highest at $x_d = 0.32$ for *I* and at $x_d = 0.49 - 0.52$ for the remaining dyes, which suggests that the LSD-to-dye ratio in the ion-associate is 2 : 1 for Acid Black I and 1 : 1 for the other dyes. The assumed formula of the ion-associate of LSD with *II* is shown in formula (*A*).



(*A*)

The $A = f(c_{LSD})$ linear calibration plot was calculated by the least squares method[18]. The calibration parameters for the LSD concentration regions (μmol l^{-1}): 26 – 86 (*I*), 4.8 – 48 (*II*), 5.5 – 55 (*III*), 5 – 50 (*IV*), 5.3 – 42 (*V*), and 5.2 – 42 (*VI*), are given in Table I. The results with *II* were most precise. The relative standard deviation of the straight line was 0.39%.

The molar absorptivities of the ion-associates under the conditions of quantitative extraction of the dye into chloroform are also given in Table I.

The extraction recoveries *E* were calculated as[19]

$$E\ (\%) \quad = \quad (\varepsilon'/\varepsilon)\ 100\ . \qquad (1)$$

The values were highest for *II*, *V* and *VI* (Table I).

The ion-associate distribution ratios D_a and the conditional extraction constant of the ion-associate K_{ex}' were calculated as

$$D_a \quad = \quad A_1/(A_0 - A_1) \qquad (2)$$

$$K_{ex}' \quad = \quad D_a/c_{re}\ , \qquad (3)$$

where A_0 is the initial absorbance, A_1 is the ion-associate absorbance after reextraction in the buffer at pH_{opt}, and c_{re} is the concentration of the dye in the aqueous phase after reextraction. The values are also included in Table I.

The results obtained can serve as underlying data in the development of methods for the spectrophotometric determination of LSD. Bromoxylenol Blue (*II*) emerges as the most suitable of the dyes with respect to the extraction recovery and limits of detection and determination. As compared to 3,3′,5,5′-tetrabromocresolsulfophthalein, employed in ref.[14], the use of Xylenol Blue enables the limit of determination of LSD in aqueous solutions to be reduced from 600 to 480 ng ml^{-1}.

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