CHROM. 3617

THE USE OF SOLUBILIZING AGENTS IN CHROMATOGRAPHY AND ELECTROPHORESIS

IV. STUDY OF SOLUBILIZATION OF ISOQUINOLINE ALKALOIDS*,**

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SUMMARY

The solubilizing effects of 7-(2-hydroxyethyl)-theophylline, tetramethyluric acid and theophylline-7-acetic acid on papaverine, papaverinol, papaveraldine, 3,4-dihydropapaverine, 3,4-dihydropapaveraldine and narcotine were studied by paper chromatography and electrophoresis. The formation of molecular complexes of the purine derivatives with the isoquinoline alkaloids, with the exception of narcotine, has been anticipated. Systems containing Britton–Robinson's buffer pH 3.5–4 in the mobile phase and *o*-xylene in the stationary phase were suitable for the separation of isoquinoline alkaloids.

The formation of molecular association compounds of papaverine with barbiturates and purines has been reported by MOSSINI AND RECORDATI[1], and WINDER AND KAISER[2]. Analogous complexes with quinine alkaloids were later studied by FRENCH AND MORRISON[3]. Preparation of these compounds was undertaken, the objective being the study of the modified pharmacological properties of the individual components. The formation of molecular complexes in solutions can be demonstrated by changes in solubility of the components of the complex.

These changes of solubility can be studied on a micro scale by chromatographic methods[4]. The formation of the molecular complexes can also be studied by changes in electrophoretic mobility of isoquinoline alkaloids in buffer solutions containing solubilizing agents.

* For Part III see ref. 4.
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MATERIALS AND METHODS

Chemicals

Papaverine, papaverinol, papaveraldine and 3,4-dihydropapaverine were products of Farmakon; narcotine was a product of Slovakofarma, purified by crystallization from ethanol; 7-(2-hydroxyethyl)-theophylline (HET), Slovakofarma, was purified from admixture of theophylline[5]. Tetramethyluric acid (TMUA)[6], theophylline-7-acetic acid (T-COOH)[7] and 3,4-dihydropapaveraldine[8] were synthesized in the laboratory of the above institute.

CHROMATOGRAPHY

A descending technique with reversed phases was used. The chromatographic paper Schleicher & Schüll 2043b Mgl was impregnated with *o*-xylene, previously shaken with the mobile phase. The impregnation treatment was performed by dipping the chromatogram in *o*-xylene. Excess of *o*-xylene was removed by absorbing the same between two sheets of filter paper. Alkaloids (10 μg) were spotted on the impregnated paper. Chromatograms were suspended in a chamber saturated with stationary phase and after 2 h a mobile phase was admitted. The mobile phase consisted of buffer solutions of HET and TMUA previously shaken with *o*-xylene. The universal Britton–Robinson's (B–R) buffer was used. Chromatograms were developed during 12 h and alkaloids were detected by Dragendorff's reagent. The R_F values shown in Figs. 1 and 2

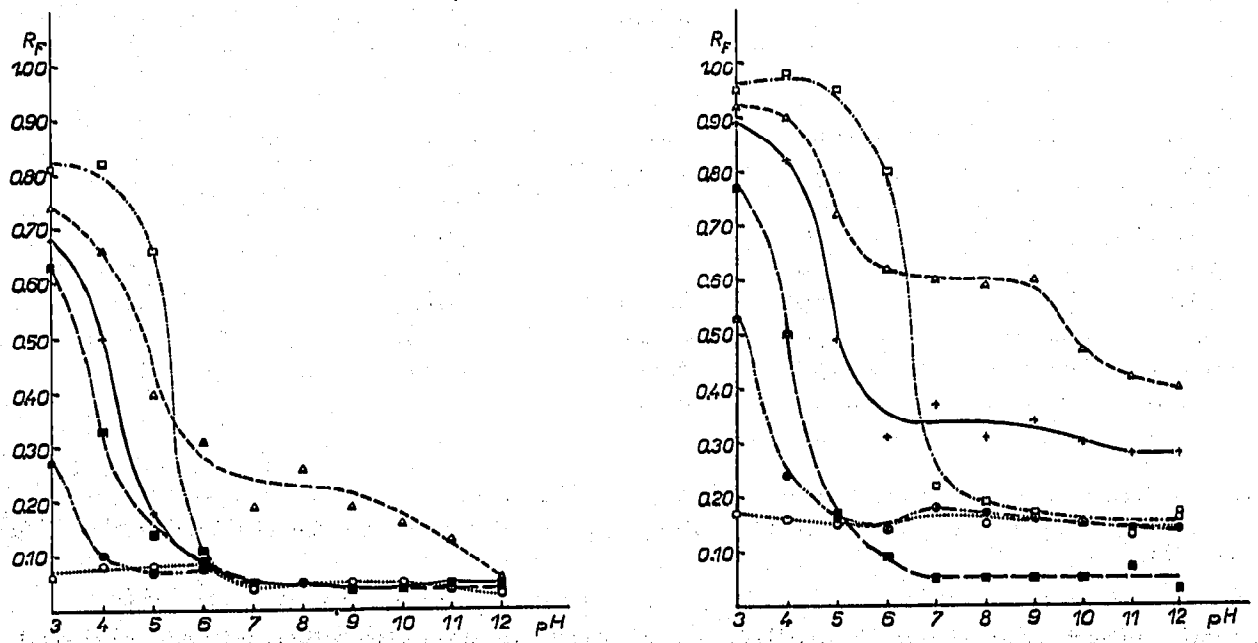

Fig. 1. Dependence of R_F values of isoquinoline alkaloids on the pH of the mobile phase. Mobile phase: B–R buffer; stationary phase: *o*-xylene. Papaverine (——+——+——); papaverinol (– – – –△– – – –△– – – –); papaveraldine (· · · ·○· · · ·○· · · ·); 3,4-dihydropapaverine (—·—□—·—□—·—); 3,4-dihydropapaveraldine (—··—●—··—●—··—); narcotine (— — ▨ — — ■ — —).

Fig. 2. Dependence of R_F values of isoquinoline alkaloids on the pH of the mobile phase. Mobile phase: $1.0 \cdot 10^{-1}$ *M* HET in B–R buffer; stationary phase: *o*-xylene. Papaverine (——+——+——); papaverinol (– – –△– – –△– – –); papaveraldine (· · · ○ · · · ○ · · ·); 3,4-dihydropapaverine (—·—□—·—□—·—); 3,4-dihydropapaveraldine (—··—●—··—●—··—); narcotine (— — ■ — — ■ — —).

are average values from different chambers. The above values are measured with reference to the second front (closer to the starting line) visible under U.V.-light. The chloroform solutions of papaveraldine and 3,4-dihydropapaverine used for spotting are not very stable. Their decomposition is indicated by appearance of new spots visible under U.V.-light which do not give any reaction with Dragendorff's reagent.

ELECTROPHORESIS

The apparatus used in this study has already been described[5]. The electrolytes used, consisted of universal B–R buffer and a 5.10^{-2} *M* solution of T-COONa in B–R buffer. The ionic strength of both electrolytes was adjusted to $\mu = 0.2$, using NaCl.

The composition of the electrolytes for electrophoresis is listed in Table I. Electropherograms S&S 2043b Mgl 110 × 420 mm were impregnated with electrolyte and placed in the apparatus. Alkaloids in 10 μg amounts were spotted on the wet electropherograms. The apparatus was assembled, and the current was switched on

TABLE I

COMPOSITION OF THE ELECTROLYTES USED FOR ELECTROPHORESIS

pH	μ*	*a ml 0.2 M NaOH*	*B–R buffer*	*B–R buffer with 0.05 M T-COONa*	
			g NaCl 100 + a ml	*g NaCl 100 + a ml*	*g T-COONa 100 + a ml*
2.87	0.031	17.5	1.1605	0.8171	1.5345
3.29	0.034	20.0	1.1643	0.8135	1.5672
4.10	0.040	25.0	1.1687	0.8035	1.6325
5.02	0.052	35.0	1.1676	0.7731	1.7631
6.09	0.063	42.5	1.1409	0.7245	1.8611
7.00	0.085	52.5	1.0249	0.5793	1.9917
7.96	0.098	60.0	0.9538	0.4862	2.0869
8.95	0.104	67.5	0.9398	0.4503	2.1879
9.91	0.110	77.5	0.9336	0.4149	2.3182
11.20	0.120	85.0	0.8541	0.3243	2.4161

* μ = ionic strength of 100 ml (0.04 *M* H_3BO_3, 0.04 *M* H_3PO_4 and 0.04 *M* CH_3COOH) + *a* ml 0.2 *M* NaOH.

after 30 min when a uniform humidity of the paper was expected to be reached. Electrophoresis was performed for 2 h at a potential gradient of 15 V/cm and temperature 20° ± 0.1°. Mobilities were corrected for electro-osmosis. For detection of alkaloids, Dragendorff's reagent was used. The T-COONa was detected using 1% aqueous solution of sodium 2-hydroxy-3,6-naphthalenedisulphonate[9]. In the alkaline region of pH it is necessary to spray the electropherogram with B–R buffer pH 2 prior to the detection. The ability of purines to quench fluorescence of the reagent under U.V. light is utilized here for detection.

RESULTS AND DISCUSSION

The bases of isoquinoline alkaloids are sparingly soluble in water, hence in the case of alkaline buffers as mobile phase, they remain at the start with the exception of papaverinol, which due to its hydroxyl group in place of the linking methylene bridge has acquired a more hydrophilic character. In the acid region of pH where dissociation of alkaloids takes place, their R_F values increase and reach an optimum at pH 3.5–4.0 (Fig. 1).

The use of buffered aqueous solutions of HET and TMUA as mobile phases causes an increase of R_F value of isoquinoline alkaloids, which is evident mainly in the alkaline region (Fig. 2). The course of R_F dependence upon pH is similar in both solubilizers studied here.

The ΔR_F value representing the difference $R_{F\ \text{solubilizer}} - R_{F\ \text{buffer}}$ is a measure of the solubilizing effect (Table II). A greater solubilizing ability of TMUA, in comparison to HET, was observed. It is evident from Table II that in the alkaline region narcotine is not solubilized. For the other alkaloids, the solubilizing effect decreases in the following order: papaverinol > papaverine > 3,4-dihydropapaverine. In the case of papaveraldine and 3,4-dihydropapaveraldine, the solubilizing effect is small and practically the same.

TABLE II

THE SOLUBILIZING EFFECT OF PURINE DERIVATIVES ON SOME ISOQUINOLINE ALKALOIDS IN ALKALINE REGION

*Compound***	$\Delta R_F \cdot 100$*									
	pH 7.0		*pH 8.0*		*pH 9.0*		*pH 10.0*		*pH 11.0*	
	TMUA 0.05 M	*HET 0.05 M*	*TMUA 0.05 M*	*HET 0.05 M*	*TMUA 0.05 M*	*HET 0.05 M*	*TMUA 0.05 M*	*HET 0.05 M*	*TMUA 0.05 M*	*HET 0.05 M*
1	31	20	31	16	32	20	24	16	24	15
2	18	16	24	13	23	15	17	13	22	12
3	16	9	9	7	9	6	7	6	6	5
4	9	7	9	5	9	6	6	6	6	4
5	7	7	7	6	9	6	6	5	6	5
6	2	0	2	0	2	0	1	0	0	1

* $\Delta R_F = R_F$ solubilizer $- R_F$ buffer.

** 1 = Papaverinol; 2 = papaverine; 3 = 3,4-dihydropapaverine; 4 = papaveraldine; 5 = 3,4-dihydropapaveraldine; 6 = narcotine.

The use of solubilizing agents in electolytes makes it possible to study the electrophoretic mobility of isoquinoline alkaloids in the alkaline region. In the absence of solubilizing agents, only papaverinol and 3,4-dihydropapaverinol shows some cathodic movement, while the others remain at the start (Fig. 3). As in electron donor–acceptor interactions of aromatic compounds with purines the occurrence of an extreme case of electron transfer from donor to acceptor resulting in the formation of ions, is not possible, it is only a case of mutual polarization of molecules without exchange of an electron, where N-methylated xanthines appear as acceptors due to polarization of the carbonyl groups[10].

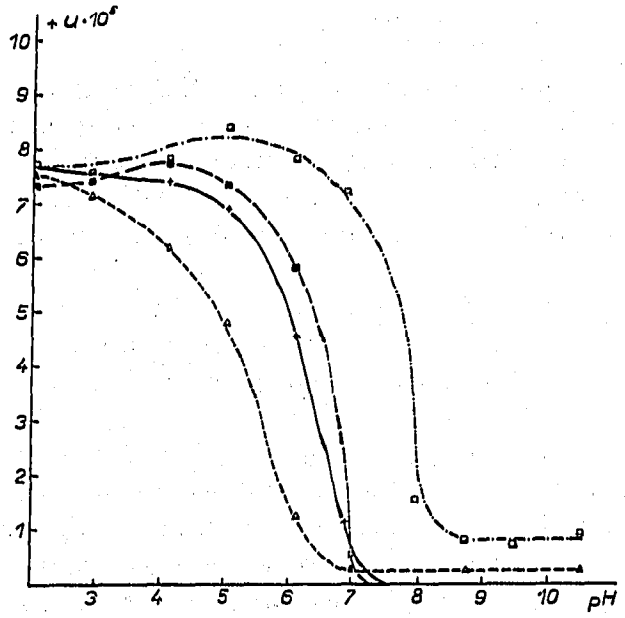

Fig. 3. Electrophoretic mobility of some isoquinoline alkaloids. Electrolyte: B–R buffer, μ 0.2 (NaCl); $+u$ = mobility towards cathode [$cm^2 \cdot V^{-1} \cdot sec^{-1}$]. Papaverine (——+——+——); papaverinol (– – –△– – –△– – –); 3,4-dihydropapaverine (—·—□—·—□—·—); narcotine (— — ■ — — ■ — —).

Isoquinoline alkaloids are electron donor compounds. When a molecule of the solubilizer is one moiety of the resulting electron donor–acceptor complex, the electrophoretic mobility of isoquinoline alkaloids is always situated between those of the pure components, when processed separately in B–R buffer.

The curve representing electrophoretic mobility as a function of pH expresses also its dependence upon the concentration of [T-COO$^-$] according to the following relation:

$$[\text{T–COO}^-] = c \cdot \frac{K}{[\text{H}^+] + K} \; ; \; K_{\text{T–COOH}} = 1.02.10^{-3}$$

The region of coexistence of comparable concentrations of alkaloids and their complexes is located on descending sections of the mobility curve, whereas the plateau region always shows a definite stoichiometric composition. This helps in studying the approximate comparative stability constants of the complexes formed, similarly as for complexes of the chelate type[11]. The considerable difference in stability of individual types of complexes can be observed from the breaks in the mobility curve (see the curve of papaverinol, Fig. 4). The breaks in the curves of electrophoretic mobility as well as those of R_F as a function of pH are located in identical regions (compare Fig. 2 with Fig. 4). No complex formation was observed with the base of narcotine.

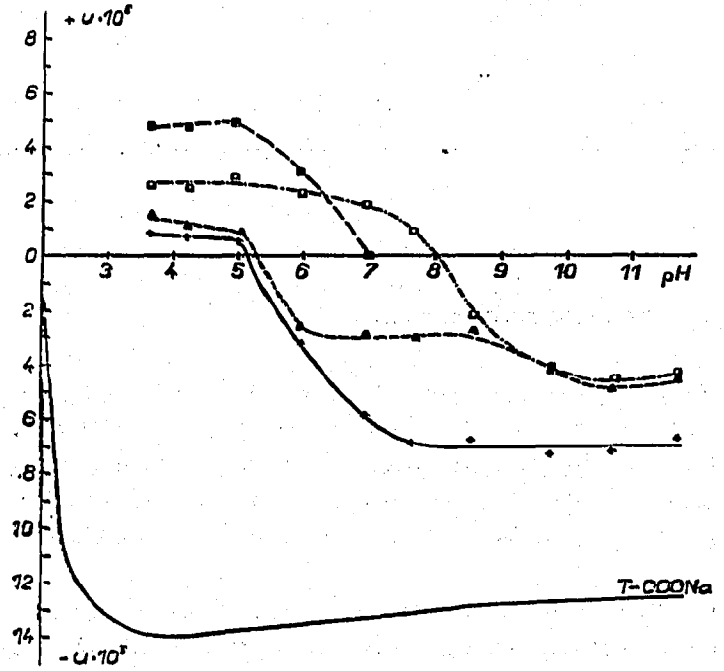

Fig. 4. Electrophoretic mobility of some isoquinoline alkaloids. Electrolyte: 5.10^{-2} *M* T-COONa in B–R buffer, μ 0.2 (NaCl). T-COONa curve = mobility in B–R buffer; $+u$ = mobility towards cathode [$cm^2 \cdot V^{-1} \cdot sec^{-1}$]. Papaverine (——+——+——); papaverinol (---△---△---); 3,4-dihydropapaverine (—·—□—·—□—·—); narcotine (——■——■——).

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