

CHROM. 3628

# THE PAPER ELECTROPHORETIC STUDY OF ION PAIR FORMATION

## III. ION PAIR FORMATION WITH QUATERNARY AMMONIUM IONS

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**SUMMARY**

The ion pair formation between quaternary ammonium ions and various anions was studied by high voltage paper electrophoresis.

There seems to be little ion pair formation in 0.1 *N* solutions but quite considerable interaction in 1 *N* solutions.

**INTRODUCTION**

In the two previous papers of this series we have shown that ion pair formation can be used to improve the separation of metal complexes by paper electrophoresis.

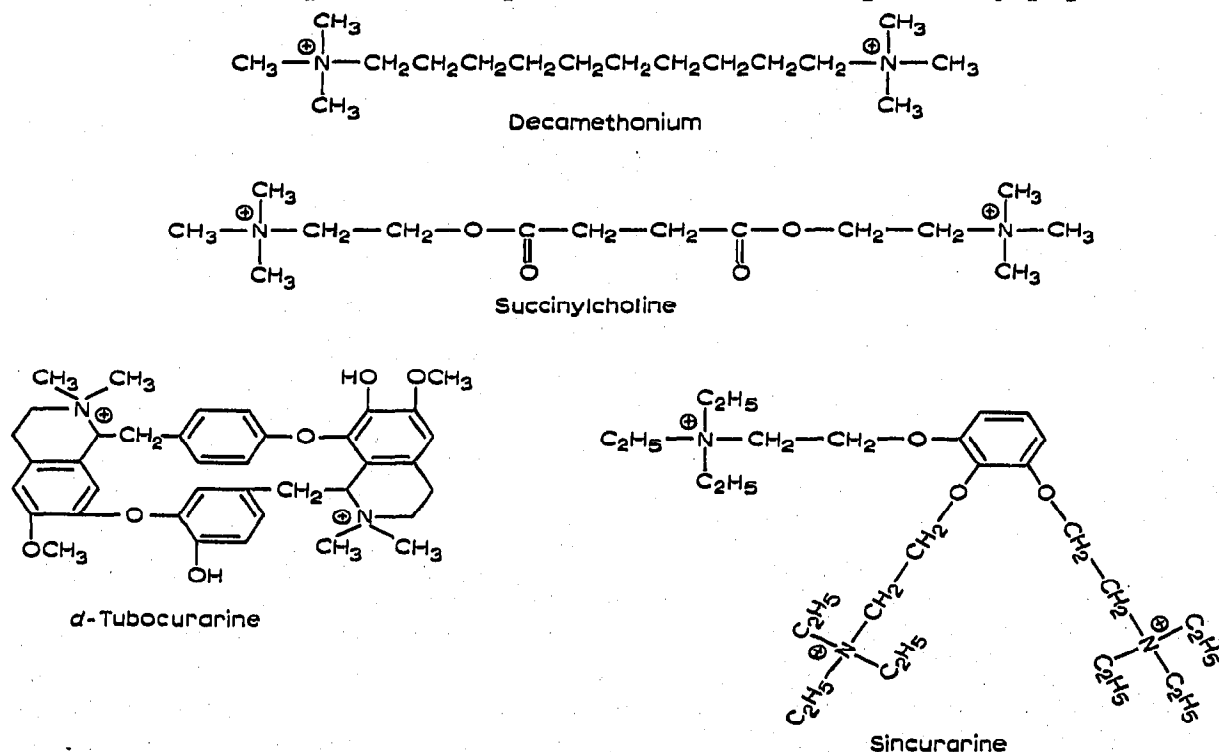


Fig. 1. Structural formulae of the quaternary ammonium compounds studied.

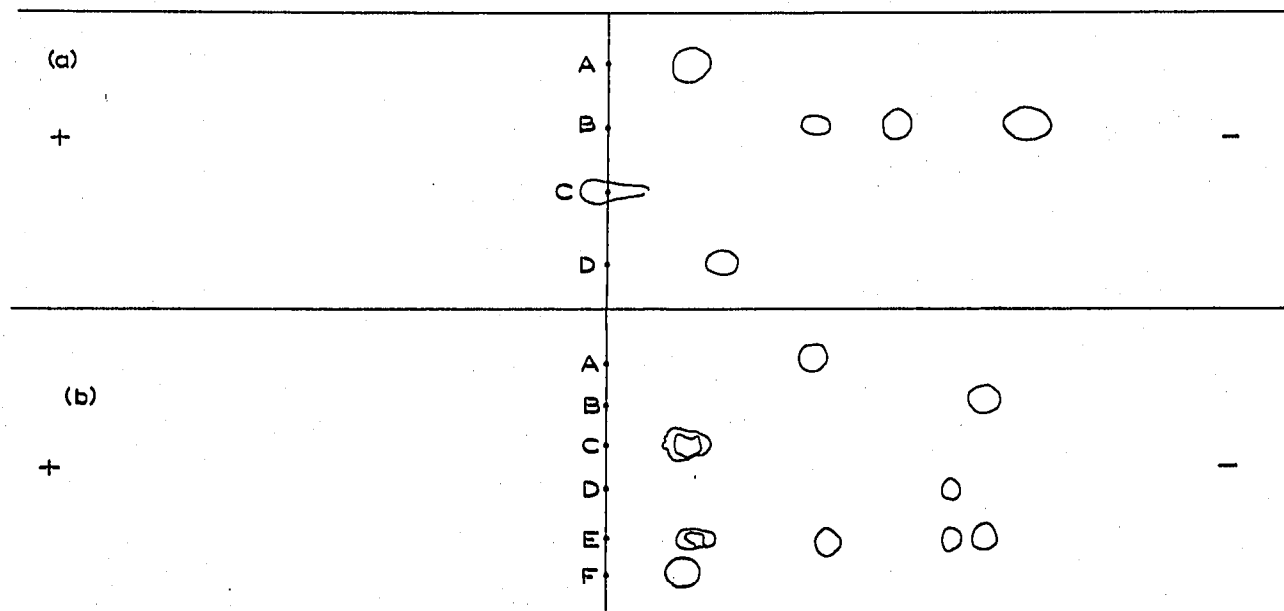


Fig. 2. Separation of some curarising compounds ( $1/3 \times$  actual size). (a) Electrolyte: sodium trichloroacetate 1 *N* at pH  $\sim$  4.5. 1500 V for 1 h on Whatman No. 1 paper. A = Sincurarine; B = succinylcholine (centre spot is succinylcholine, the other two spots are hydrolysis products); C = *d*-tubocurarine; D =  $H_2O_2$  (for measurement of electroosmotic flow). (b) Electrolyte: sodium dichloroacetate 1 *N* at pH 4.5. 1500 V for 1 h. A = Sincurarine; B = succinylcholine (solid, freshly dissolved in water, and does not show spots of hydrolysis products); C = *d*-tubocurarine; D = decamethonium; E = mixture of A-D; F =  $H_2O_2$ .

Quaternary ammonium salts are a group of compounds which usually yield multispots in paper chromatography due to ion pair formation<sup>1</sup> and we thus decided to continue our studies by examining several quaternary ammonium compounds, especially those of pharmaceutical interest.

#### EXPERIMENTAL AND RESULTS

High-voltage paper electrophoresis with a Camag apparatus was employed as described before<sup>2</sup> (Whatman No. 1 paper at *ca.* 15°C with 1500 V for 30 min and  $H_2O_2$  run for the measurement of electro-osmotic flow). All compounds were detected with Dragendorff's reagent.

Table I shows the distances moved by  $Co(NH_3)_6^{3+}$  (so as to have some comparison with metal complexes studied previously) tetramethylammonium ion, tetraethylammonium ion and sincurarine (Fig. 1) a trivalent cation. The last two columns show the movement of tetraethylammonium relative to tetramethylammonium, and sincurarine relative to tetramethylammonium.

Table II shows the movement of several quaternary ammonium compounds used in pharmacy for their curare-like action.

Succinylcholine usually yields two to three spots owing to rapid hydrolysis in solution (see Fig. 2). Only choline and succinylmonocholine were detected here. In Table II the distance moved by the original compound is indicated.

Fig. 3 shows the movement of the four compounds of Table II in a graphical form. The different separation effects which can be obtained in various anions become evident.

TABLE I  
ELECTROPHORETIC MOVEMENT OF QUATERNARY AMMONIUM IONS

Electrolyte	Concentration (N)	Distance moved in mm in 30 min with 1500 V			Ratio of movement of $NEt_3^+/NMe_3^+$	Ratio of movement of sincurarine/ $NMe_4^+$	
		$Co(NH_3)_6^{3+}$	Sincurarine	$NMe_3^+$			
LiCl	0.1		57	86	59	0.69	0.66
LiCl	0.5		50	91	62	0.68	0.55
LiCl*	1.0		26	51	34	0.67	0.51
NaBr	0.1	110	49	87	56	0.65	0.56
NaBr	0.5	127	41	93	57	0.62	0.44
NaBr**	1.0	50	12	40	23	0.58	0.30
NaClO <sub>4</sub>	0.1	101	27	76	52	0.69	0.35
NaClO <sub>4</sub> *	0.5	104	18	72	43	0.60	0.25
NaClO <sub>4</sub> *	1.0		0	34	18	0.52	0.00
Li <sub>2</sub> SO <sub>4</sub>	0.1	21	51	77	58	0.75	0.66
Li <sub>2</sub> SO <sub>4</sub>	0.5	11	58	82	64	0.78	0.71
Li <sub>2</sub> SO <sub>4</sub>	1.0	13	61	84	66	0.79	0.73
AcH-AcNa	0.1	134	73	105	70	0.67	0.69
AcH-AcNa (pH ~ 4.5)	0.5	94	44	70	49	0.70	0.63
AcH-AcNa	1.0	94	48	84	53	0.63	0.57
CH <sub>2</sub> ClCOOH	0.1	105	53	82	55	0.67	0.65
CH <sub>2</sub> ClCOOH (pH ~ 4.5)	0.5	103	48	86	57	0.66	0.56
CH <sub>2</sub> ClCOOH	1.0	97	43	91	55	0.61	0.47
CHCl <sub>2</sub> COOH	0.1	101	45	78	55	0.70	0.58
CHCl <sub>2</sub> COOH (pH ~ 4.5)	0.5	108	33	84	50	0.60	0.39
CHCl <sub>2</sub> COOH	1.0	64	14	52	27	0.52	0.27
CCl <sub>3</sub> COOH	0.1	111	41	83	55	0.66	0.49
CCl <sub>3</sub> COOH (pH ~ 4.5)	0.5	94	16	74	39	0.53	0.22
CCl <sub>3</sub> COOH	1.0	65	— 8	51	16	0.31	

\* With 1000 V for 30 min.

\*\* With 1000 V for 25 min.

## DISCUSSION

Several interesting points emerged from the results in Tables I and II. The ratio of the movement of tetraethylammonium to tetramethylammonium is more or less constant for 0.1 *N* electrolyte concentrations. This would indicate that there is similar or no ion pair formation at this concentration. The ratio increases considerably at 1 *N* electrolyte concentration for some anions, *e.g.*, for perchlorate, dichloroacetate and trichloroacetate.

The ratio of the movement of sincurarine to tetramethylammonium ion is around 0.66 (actually 0.66, 0.66, 0.69 and 0.65) for 0.1 *N* chloride, sulphate, acetate, monochloroacetate and below that for bromide, perchlorate, dichloroacetate and trichloroacetate indicating that some ion pair formation seems to take place even in 0.1 *N* solutions. Zero movement is obtained in 1 *N* perchlorate and anionic movement in 1 *N* trichloroacetate and these are also the anions which showed most interaction with cobalt complexes such as the tris-*o*-phenanthroline Co(III)<sup>3+</sup>.

Somewhat unexpected results were obtained with sulphate. Here the difference in movement decreases with the increase in the concentration of the anion. As the distance moved by the tetramethylammonium ion is of the same order as in the other electrolytes this does not seem to be caused by unduly strong ion pair formation by this ion. We have no explanation for this phenomenon.

TABLE II  
ELECTROPHORETIC MOVEMENT OF CURARE AGENTS

Electrolyte	Concentration ( <i>N</i> )	Distances moved in mm in 30 min with 1500 V			
		Deca- methonium	Succinyl- choline	Sin- curarine	<i>d</i> -Tubo- curarine
LiCl	1		29 (34*)	17	7
NaClO <sub>4</sub>	1		6 (24*)	— 4	— 4 T
Li <sub>2</sub> SO <sub>4</sub>	1	62	60	51	17
AcH-AcNa (pH ~ 4.5)	1	54	58	42	14
CH <sub>2</sub> ClCOOH (pH ~ 4.5)	1	54	58	36	11
CHCl <sub>2</sub> COOH (pH ~ 4.5)	1	37	42	20	3
CCl <sub>3</sub> COOH (pH ~ 4.5)	1	9	24	— 5	— 15

\* Hydrolysis product in brackets.  
T = comet.

Table II and Fig. 3 illustrate the main aim of the investigation, *i.e.*, that ion pair formation can be profitably employed to change and improve separations by paper electrophoresis.

Incidentally, ion pair formation need not increase with the number of charges in the molecule. *d*-Tubocurarine has two quaternary ammonium groups and is more anionic than sincurarine with three. The polarity and size, etc., of the molecule are equally important.

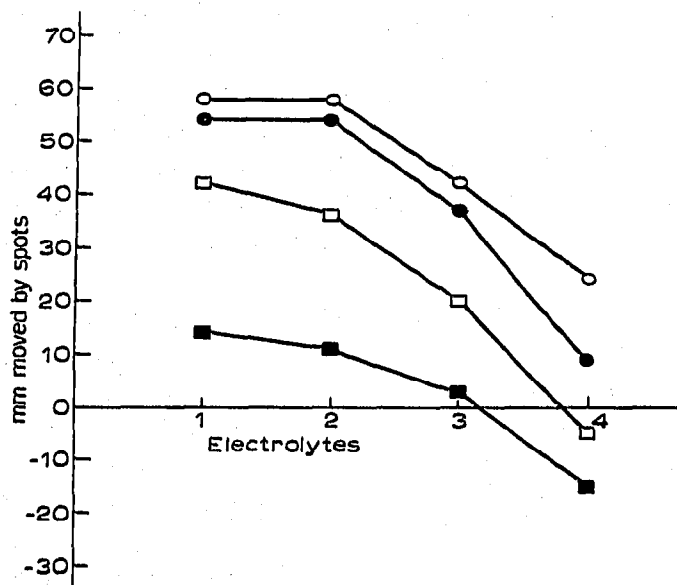


Fig. 3. Graphical representation of the movement of curare-like compounds in 1 *N* acetate and mono-, di- and trichloroacetate. ● = Decamethonium; ○ = succinylcholine; □ = sincurarine; ■ = *d*-tubocurarine. 1 = CH<sub>3</sub>COOH-CH<sub>3</sub>COONa 1 *N*; 2 = CH<sub>2</sub>ClCOOH 1 *N*; 3 = CHCl<sub>2</sub>COOH 1 *N*; 4 = CCl<sub>3</sub>COOH 1 *N*; 2, 3 and 4 adjusted to pH  $\approx$  4.5 with NaOH.

Most theoretical treatments of paper electrophoresis (for example ref. 3) ignore ion pair formation, and perhaps justly so, because it plays only a negligible role in 0.1 *N* or less concentrated electrolytes. In an apparatus with adequate cooling (*e.g.* the Camag apparatus) there is no reason why 1 *N* electrolytes should not be used and at this concentration not only the size, shape, charge and degree of ionisation of an ion decides its movement but, as shown here, interactions with the surrounding anions play an essential role. Quaternary ammonium compounds were chosen for this study because they are strong bases and hence their movement is not influenced by the pH of the electrolyte and because their separation by chromatographic methods is less satisfactory than for many other classes of compounds. In principle ion pair formation should also be useful in altering separations of other classes of compounds which can be made to migrate in an electric field.

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