

THE ELECTROPHORETIC BEHAVIOUR OF SOME STRUCTURAL ANALOGUES OF DICARBOXYLIC ACIDS OF THE KREBS CYCLE*

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A paper chromatographic procedure for the detection of epoxysuccinic, mercaptosuccinic and mercaptomalic acids was described in a previous paper¹. There remained, however, a need for a quicker procedure matching the rates at which these compounds are utilized by bacteria. Since Gross² has reported good separations of non-volatile organic acids by paper electrophoresis, the electrophoretic behaviour of epoxysuccinic, mercaptosuccinic, mercaptomalic acids, and their structural analogues involved in the Krebs cycle, was investigated. It became apparent that a paper electrophoretic procedure was suitable as a rapid means of separating and detecting these acids.

MATERIALS AND METHODS

Epoxysuccinic, mercaptosuccinic and mercaptomalic acids** were chromatographically pure. Oxaloacetic, fumaric, succinic and malic acids were all of the best grade available. Solutions containing mixtures of the acids and supernatants of reaction mixtures from microbiological experiments were investigated. The various substances were identified by comparing the migration value of the unknown spots with those of 1% standard solutions, using 50 μ l per spot.

Spots were applied on strips (4.9 \times 40 cm) of unwashed Macherey-Nagel No. 261 filter paper. In all the experiments electrophoresis was carried out with 400 V for 90 min at 27°. The composition and the pH of the electrolytes used are given in Table I. Immediately after the electrophoresis the ends of the strips which were immersed in the electrolyte were cut off and the strips dried at 50°, to constant weight. Higher temperatures must be avoided because of the instability of the mercaptoacids.

For the detection of the spots the general and specific reagents referred to in a previous paper¹ were used.

The migration distances were measured in centimeters from the centers of the spots to the point of application.

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RESULTS AND DISCUSSION

The acids investigated can be separated from mixtures by the procedure described, if an appropriate electrolyte is selected, as shown in Table I. Diffusion phenomena or electroendosmotic effects³ caused no noticeable interference.

TABLE I
ELECTROPHORETIC MIGRATION OF THE ACIDS ASSAYED
200 V, 90 min, 25°. Migration distances in cm.

Electrolytes	A	B	C	D	E	F	G
pH values	1.9	2.5	3.0	4.0	5.0	8.9	10.0
μ values	0.01	0.09	0.03	0.06	0.04	0.3	0.1
<i>Acids</i>							
1. Epoxysuccinic	6.0	0.4	3.5	1.2	2.0	5.2	6.0
2. Succinic	2.3	1.0	1.8	18.1	13.2	4.6	—
3. Mercaptosuccinic	2.3	0.4	4.2	1.5	7.0	4.6	6.0
4. Mercaptomalic	2.4	2.8	3.4	22.0	19.0	5.0	—
5. Malic	2.3	2.2	2.8	14.2	11.2	5.0	—
6. Fumaric	2.3	1.7	—	1.5	1.5	4.6	—
7. Oxaloacetic	1.6	1.5	2.8	1.0	2.0	3.2	—

Electrolytes: A = formic acid 0.75 M, pH 1.9.
 B = acetic acid 30% (w/v), pH 2.5.
 C = acetic acid 0.2 M, pH 3.0.
 D = sodium acetate 0.01 M, pH 4.0.
 E = sodium acetate 0.025 M, pH 5.0.
 F = ammonium carbonate 0.1 M, pH 8.9.
 G = sodium borate 0.02 M, pH 10.

In electrolyte A, of pH 1.9, oxaloacetic acid, being in the keto-form, migrated slowly; epoxysuccinic acid, behaving as a strong acid, was able to dissociate at such a low pH value, and migrated satisfactorily. Since the other acids gave a mixed spot between the spots corresponding to oxaloacetic and epoxysuccinic acid, the two last-mentioned compounds can be isolated in that electrolyte, as is evident from Fig. 1.

Electrolytes B and C did not allow good separations under the conditions employed.

In electrolytes D and E the mercaptoacids were well resolved from each other and from their structural analogues, succinic and malic acids, as shown in Figs. 2 and 3. These results are especially interesting because of the difficulties encountered in separating these compounds by paper chromatography¹. Electrolyte F proved unsatisfactory. In electrolyte G, the detection of the spots failed because the solution could not be removed from the strips and the spots had to be extracted, after acidification, with ethyl ether.

Attempts to correlate the pH values of the electrolytes and the electrophoretic behaviour of the spots failed, as was to be expected⁴. If the migration distances in the various electrolytes are plotted against the ionic strengths of the respective electrolytes, a definite relation can be established, as can be seen in Fig. 4.

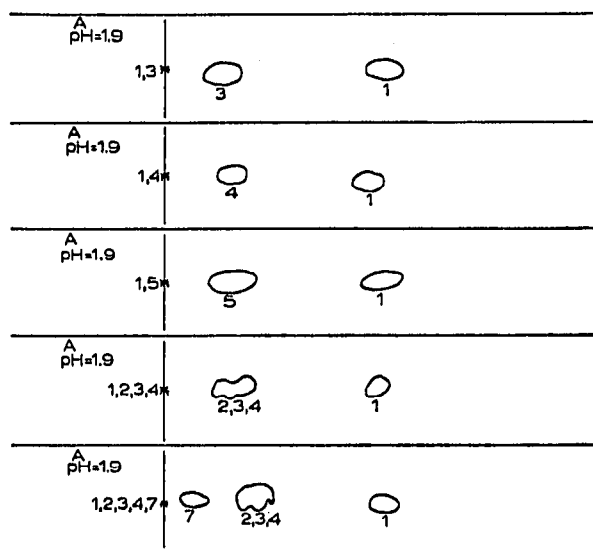


Fig. 1. Electrophoretic behaviour of the acids in 0.75 *M* formic acid of pH 1.9. Conditions: 400 V, 90 min, 27°. 1 = epoxysuccinic acid; 2 = succinic acid; 3 = mercaptosuccinic acid; 4 = mercaptomalic acid; 5 = malic acid; 6 = fumaric acid; 7 = oxaloacetic acid.

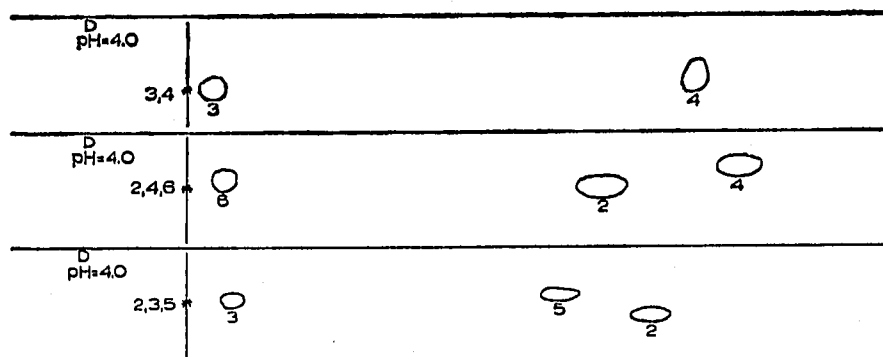


Fig. 2. Electrophoretic behaviour of the acids in 0.01 *M* sodium acetate buffer of pH 4.0. Conditions: 400 V, 90 min, 27°. 1 = epoxysuccinic acid; 2 = succinic acid; 3 = mercaptosuccinic acid; 4 = mercaptomalic acid; 5 = malic acid; 6 = fumaric acid; 7 = oxaloacetic acid.

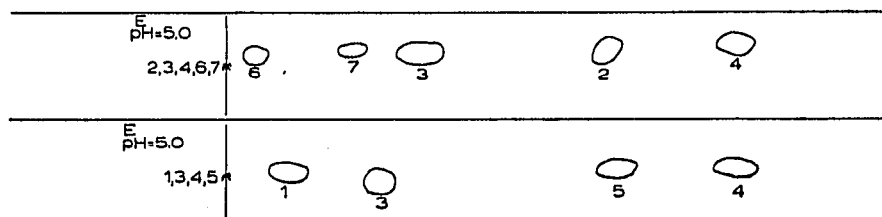


Fig. 3. Electrophoretic behaviour of the acids in 0.025 *M* sodium acetate buffer of pH 5.0. Conditions: 400 V, 90 min, 27°. 1 = epoxysuccinic acid; 2 = succinic acid; 3 = mercaptosuccinic acid; 4 = mercaptomalic acid; 5 = malic acid; 6 = fumaric acid; 7 = oxaloacetic acid.

For epoxysuccinic acid the greatest migration distance occurs in the electrolyte with the lowest ionic strength, further values decreasing proportionally with the increase of μ . The recorded data indicate that the substitution of hydrogen atoms by an epoxy group in the molecule of the C_4 dicarboxylic acid enhances the migration when the ionic strength of the electrolyte decreases. This effect can be considered as configurational, since the keto group lowers it.

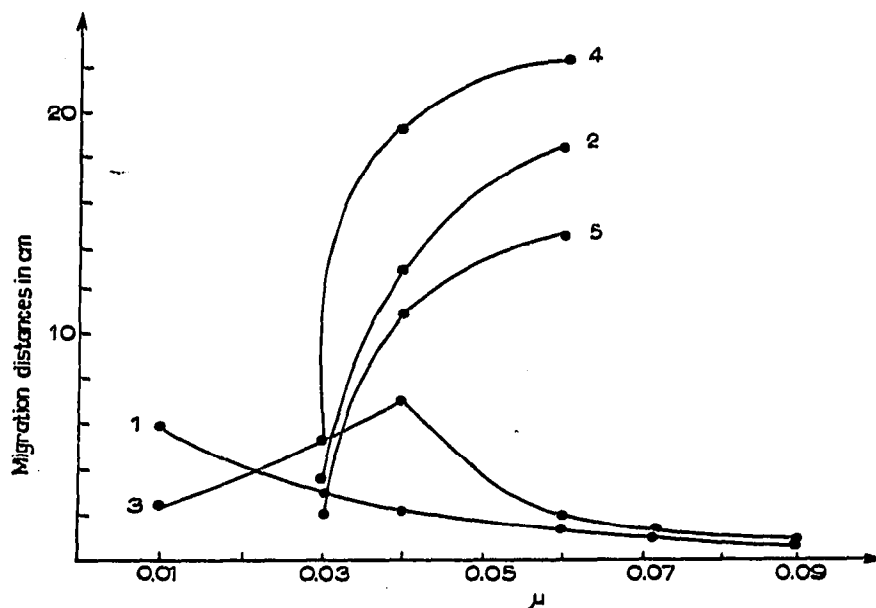


Fig. 4. Correlation between migration distances in cm and the ionic strength of the electrolytes (μ). Conditions: 400 V, 90 min, 27°. 1 = epoxysuccinic acid; 2 = succinic acid; 3 = mercaptosuccinic acid; 4 = mercaptomalic acid; 5 = malic acid.

The behaviour of the mercaptoacids is different: the migration distance values exhibit a maximum for a definite value of μ , this being 0.04 for mercaptosuccinic acid and 0.06 for mercaptomalic acid.

The slopes of the curves corresponding to succinic and mercaptosuccinic acid suggest that the introduction of the SH group in the molecule of succinic acid causes a decrease in the migration distance for the same value of μ . A similar shift in the migration value occurs when OH is the substituent, though to a lesser extent, as can be observed in the malic acid curve. On the other hand, the interaction between the adjacent OH and SH groups in mercaptomalic acid largely influences the electrophoretic behaviour of this compound.

CONCLUSIONS

1. The behaviour of the dicarboxylic acids investigated, on electrophoresis with 400 V for 90 min at 27°, provides a means for their rapid separation.
2. Epoxysuccinic acid can easily be separated in 0.75 M formic acid solution of pH 1.9.
3. Mercaptosuccinic and mercaptomalic acids are well separated in both 0.01 M and 0.025 M sodium acetate buffer of pH 4.0 and 5.0 respectively.
4. The migration distances can be related to the ionic strength of the electrolytes and to the chemical composition of the compounds.

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SUMMARY

A paper electrophoretic procedure is described for the separation of epoxysuccinic, mercaptosuccinic and mercaptomalic acids from their structural analogues involved in the Krebs cycle, namely succinic, malic, oxaloacetic and fumaric acids. Several electrolytes were tried and the migration distances recorded. The best conditions are discussed and also the influence of the substituent groups on the electrophoretic migration of the metabolic compounds.

REFERENCES

- ¹ H. L. MARTELLI AND O. PIRES, *J. Chromatog.*, 6 (1961) 159.
- ² D. GROSS, *Chem. & Ind. (London)*, (1959) 1219.
- ³ E. W. BERMES, JR. AND H. J. McDONALD, *J. Chromatog.*, 4 (1960) 34.
- ⁴ L. P. RIBEIRO, E. MITIDIERI AND O. R. AFFONSO, *Paper Electrophoresis*, Elsevier, Amsterdam, 1961.

J. Chromatog., 8 (1962) 227-231