680. *Behaviour of Some Alkylphosphonic Acids in Paper Chromatography and Paper Electrophoresis.*

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The migration rates of several lower alkylphosphonic acids were determined by paper chromatography and paper electrophoresis. The variation of migration rate with the size of the alkyl group permits location but not separation of the acids.

Many of the methods commonly used of synthesis of alkylphosphonic acids require as final step the separation of inorganic acids of phosphorus from the phosphonic acids. In some instances this separation is tedious and often can be accomplished only by fractional distillation of some derivative of the phosphonic acid. At the same time very little is known about the behaviour of mixtures of alkylphosphonic acids with the result that in some syntheses, particularly those using aluminium chloride as catalyst, one is uncertain of the isomeric identity or purity of the final product. For control of these factors, paper-chromatographic and -electrophoretic means of identification and, possibly, purification, of alkylphosphonic acids were examined. When this work was commenced, no data on true organophosphorus acids existed, but since then Weil[1] reported his results on chromatographic migration rates, on paper, of some aromatic phosphonic acids. In view of our primary interest, at that time, in the aliphatic unsubstituted phosphonic acids, chromatography or electrophoresis necessitated finding means of detection of small amounts of these acids on paper. Since alkylphosphonic acids are stable and no coloured derivatives are known, possibilities were limited. After preliminary experimentation, we found it most convenient to use direct indicator application or formation of an insoluble salt, particularly that of lead. For alkylphosphonic acids Weil's methods did not give such clean location of the spots as ours, which permitted location of as little as 0·01 mg. on the paper.

With butan-1-ol, equilibrated with water, as the mobile phase in ascending paper strip chromatograms, the higher ($>C_{10}$) alkylphosphonic acids migrated totally with the advancing solvent front, as also did the lower dialkylphosphinic acids. Addition of up to 10% of pyridine or acetic acid to the solvent failed to change the mode of advance of these acids, as was to be expected from the very low solubility of the higher alkylphosphonic acids in the aqueous phase. The annexed Table lists R_F values for the lower alkylphosphonic acids, established by runs of seven hours at 30—31° with water-saturated butanol alone. It shows that the approximate trend toward higher R_F values in a homologous series of alkylphosphonic acids has many and large exceptions, the two most remarkable

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[1] Weil, *Helv. Chim. Acta*, 1955, **38**, 1274.

being the high R_F values of the propyl and the butyl members, indicating a higher lipophilic character of these acids than would have been expected from their position in the series. Branching tends to reduce the R_F values, owing probably to steric effects at least to some extent. The variation of the R_F cannot be directly related to the steady variation of acidic dissociation constants which were reported from this Laboratory a few years ago.[2]

Alkylphosphonic acids.

Alkyl	Me	Et	Pr^n	Bu^n	n-C_5H_{11}	n-C_6H_{13}	Pr^i	Bu^i	Bu^s	Bu^t
R_F	0·54	0·51	0·80	0·81	0·62	0·74	0·78	0·54	0·72	0·38
Migration (mm.)...	113	107	100	107	99	96	107	97	100	97

For electrophoresis of alkylphosphonic acids on paper strips, the findings may be summarized as follows. If the acids are made to migrate along a paper strip which is in flat contact with the table of the apparatus, the bands of the migrating acids show convex fronts, but a suspended paper strip in which the migrating acid is allowed to come in contact only with the paper strip yields bands which have sharp flat fronts which can be readily measured. In an acidic medium (acetate buffer, pH 5·2) on Whatman No. 1 paper (also used for the chromatographic work) strips, the Reco electrophoresis apparatus being used, the migrations in one hour with 500 v were as in the Table (the actual physical distance between the two electrolyte cell liquid surfaces was 50 cm.; hence the values of field strength in v cm.$^{-1}$ can be readily obtained). The results indicate a rather narrow total band of migration distances for the group as a whole, without any satisfactory order within it. Runs in which the time was varied from 1 to $>$7 hr. gave similar results.

A comparison of the methods of suspension of the paper strips is shown in the following Table. The duration, as above, is 1 hr., the total applied voltage being 700 v.

Distances migrated (mm.)

	Me	Et	Pr^n	Bu^n	Pr^i	Bu^t
Paper flat	121	131	99	110	98	95
Paper suspended	136	131	125	132	130	125

Attempts to obtain a better spread of migration distances by the use of lower and higher voltages were unsuccessful. Even less satisfactory were the results with basic media, borax–sodium hydroxide buffer with pH 10·8, and borax buffer with pH 9·3. Not only were the bands of the acids quite diffuse and difficult to delineate, but they were accompanied by diffuse slow-moving bands which tended to obscure the picture. Evidently these secondary bands were produced by partially and completely dissociated units, since they were not apparent in acidic media and were weaker in the pH 10·8 buffer than in the pH 9·3 buffer.

While paper chromatography and electrophoresis on paper yields locatable spots of various lower alkylphosphonic acids, the spread of the migration values is small and irregular, making these methods rather unsuitable for separation of mixtures, although they may be used for identification of individual acids. The electrophoretic method failed to cause any motion of the higher alkylphosphonic acids even with the highest potential (750 v) under all conditions tried, including both acidic and alkaline media.

EXPERIMENTAL

The phosphonic acids were prepared by conventional methods.[2]

Paper chromatography was done in a large bell jar with one-inch wide strips of Whatman No. 1 paper for ascending chromatograms, and butan-1-ol saturated with water as the fluid. The acids were deposited by a micropipette on the strips in the form of one carefully measured drop located at the same distance from the level of the butanol in the tank. The acid was deposited in 0·1M-solution and the spotted paper was allowed to equilibrate suspended in the tank overnight.

The electrophoretic work was done with Reco Electrophoretic apparatus manufactured by

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[2] Crofts and Kosolapoff, *J. Amer. Chem. Soc.*, 1953, **75**, 3379.

Research Equipment Corp., of Oakland, California, U.S.A., as Model E-800-2. The acid samples were applied stripe-wise across the paper strips precisely 14 cm. from the centre-line of the apparatus, after the strips had been moistened with the desired buffer solution and excess of electrolyte removed by blotting, again with Whatman No. 1 paper. The application site was nearer to the cathode terminal.

With either technique it is undesirable to encircle the location of the initial spot or stripe by a pencil mark. Comparative runs, especially for paper chromatography, showed that encirclement with graphite (and probably owing to the compression of the paper) slows the motion of the phosphonic acid, *i.e.*, the pencilled circle retards or " captures " the substrate for an appreciable time, giving low values for extent of migration.

The following methods were found to be satisfactory for locating the alkylphosphonic acids on paper in amounts of 0·01 mg. or greater.

Indicator Method.—After being dried under a pair of infrared heat lamps, the strips were sprayed uniformly by means of a compressed air-atomiser with bromothymol-blue solution prepared as follows. To a solution of bromothymol-blue (0·20 g.) in acetone (200 ml.) and ether (200 ml.) were added distilled water (1 ml.) and potassium hydroxide (1 g.). The mixture was shaken vigorously and kept for several hours, during which the solution changed from yellow to deep blue. After several days, the mixture deposits a viscous aqueous phase. The supernatant solution is used for charging the atomizer, yielding a very stable colour which can be read very readily, with excellent blue–yellow contrast on the chromatograms.

A somewhat less satisfactory, although still usable, indicator solution is obtained by treatment of methyl-red in acetone with very concentrated aqueous potassium hydroxide. The red–yellow contrast produced by this indicator is also readily seen on the chromatogram.

Lead Salt Method.—Salts of copper and iron failed to give good results under a variety of conditions. The lead salts gave the best results. The paper strip was dipped briefly into a saturated solution of lead acetate, rinsed thoroughly in running tap water, and then dipped into, or sprayed with, a dilute solution of sodium sulphide or ammonium sulphide. The concentration of the sulphide solution was not critical. The location of the phosphonic acid is shown by a black spot on a light tan background.

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