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# Application of Filter Paper Partition Chromatography to Qualitative Analysis of Volatile and Non-Volatile Organic Acids.*

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In attempting to apply filter paper chromatography to organic acids one encounters streaking of many of the acids at the concentrations required for detection by color reaction, spreading or evaporation from the paper of the volatile members of the group, and too rapid migration, essentially with the solvent boundary, of the compounds with low solubility in water. Lugg and Overell[1] have reported a procedure which minimizes streaking and permits separation of many of the non-volatile organic acids. In an attempt to make filter paper chromatography more generally applicable to this class of compounds, a number of salts and derivatives were investigated. Of those tested, the potassium hydroxamate derivative appears most nearly to satisfy the requirements of the procedure. By its use it has been possible to separate most of the common organic acids with chain lengths of about 8 carbon atoms or less.

*Methods.* The potassium hydroxamates were synthesized from the methyl esters of the organic acids, essentially according to the procedure of Hauser and Renfrow[2]. Separate solutions of hydroxylamine hydrochloride (2.4 g or 0.033 mole in 15 ml methyl alcohol) and potassium hydroxide (2.8 g or 0.05 mole in 10 ml methyl alcohol) were prepared at the boiling point of methyl alcohol. Both were allowed to cool to 30-40°C, the one containing alkali was added with shaking to the hydroxylamine solution, and the mixture was placed in an ice bath for 5 minutes to permit complete precipitation of potassium chloride. To this mixture was added with thorough shaking, 0.017 mole of the methyl ester of the organic acid (or one-half that quantity of the methyl ester of a dicarboxylic acid). The mixture was filtered immediately with suction, and the precipitate rinsed with a few ml of methyl alcohol. The precipitate remaining in the funnel was discarded. Methyl alcohol was added to the filtrate to make a final volume of 30 ml if 0.017 mole of the ester was added. When 0.0085 mole was used, the hydroxamate was concentrated on the steam bath to a final volume of 15 ml, and for some of the hydroxamate derivatives, especially of the dicarboxylic acids, it was necessary to evaporate off most of the methyl alcohol and add water to obtain solution of the precipitate which formed. No determinations were made of the yields of the hydroxamates in their synthesis from the acid.

Details of the apparatus used and the technique for partition chromatography have been described.[3] For the chromatograms, 5 microliters ($3 \times 10^{-6}$ mole) of the potassium hydroxamate solution were used. A saturated solution of ferric chloride in n-butyl alcohol saturated with water was used as the color developing reagent.

*Results.* A number of solvents were found to be satisfactory for separating short-chain monocarboxylic acids, and $R_F$ values for a number of such acids are given in Table I. Acids containing more than one carboxyl group frequently streaked, or gave numerous spots, or did not move sufficiently far from the initial position to permit a good separation in many of the solvents which were tried.

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1 Lugg, J. W. H., and Overell, B. T., *Nature*, 1947, **160**, 87.

2 Hauser, C. R., and Renfrow, W. B., Jr., *Organic Syntheses*, 1939, **19**, 15.

3 Dent, C. E., *Biochem. J.*, 1948, **43**2, 169.

TABLE I.
$R_F$ Values of Hydroxamate Derivatives of Organic Acids in Various Solvents on Whatman No. 1 Filter Paper.

| | Solvent | | | | | | |
|---|---|---|---|---|---|---|---|
| Hydroxamate | n-Hexyl alcohol | n-Amyl alcohol | n-Butyl alcohol | sec-Butyl alcohol | Methyl ethyl ketone | Isobutyric acid | Phenol |
| Formic | .06 | .12 | .40 | .54 | .22 | .45 | .57 |
| Acetic | .23 | .35 | .51 | .63 | .40 | .57 | .70 |
| Propionic | .43 | .56 | .68 | .78 | .61 | .68 | .78 |
| Butyric | .63 | .71 | .79 | .87 | .75 | .74 | .80 |
| Valeric | .73 | .78 | .86 | .90 | .84 | .83 | .84 |
| Caprylic | .86 | .88 | .90 | .91 | .91 | .87 | .90 |
| Pelargonic | .84 | .85 | .91 | .90 | .91 | .88 | .90 |
| Capric | .89 | .89 | .92 | .90 | .90 | .92 | .95 |
| Benzoic | .69 | .73 | .82 | .86 | .83 | .79 | .85 |
| Phenylacetic | .73 | .76 | .83 | .83 | .84 | .75 | .86 |
| Lactic | .14 | .23 | .42 | .53 | .28 | .50 | .66 |

TABLE II.
$R_F$ Values of Hydroxamate Derivatives of Organic Acids in Phenol and in Isobutyric Acid on Whatman No. 1 Filter Paper.

| | Solvent | |
|---|---|---|
| Hydroxamate | Phenol | Isobutyric acid |
| Oxalic | .14, .40 | .23, .28, .32 |
| Malonic | .11, .23 | .19, .32 |
| Succinic | .40, .72 orange | .45, .52 orange |
| Glutaric | .47 | .37, .52 |
| Adipic | .54, .67 | .44, .60 |
| Pimelic | .60, .73 | .52, .69 |
| Azelaic | .63, .74 | .66 streaked |
| Sebacic | .89 | .74, .89 |
| Citric | .09, .23 | .20, .29 |
| Tartaric | .10 | .19 |
| Pyruvic | .59, .86 | .54, .62 orange, .73 |

The most satisfactory solvents of those tested for these acids were isobutyric acid and phenol. $R_F$ values for a number of these acids and for pyruvic acid are given in Table II, and a diagram of a two dimensional chromatogram is given in Fig. 1.

*Discussion.* Converting the organic acids to their hydroxamate derivatives increases the polarity, decreases the volatility, and makes possible the use of a convenient and reasonably sensitive color reaction for development of the chromatogram without increasing the molecular weight to such a degree that solubility differences between members of a homologous series are seriously diminished. In general, the color reaction is sufficiently sensitive to detect on the finished chromatogram a spot containing in the order of $10^{-7}$ mole of acid, assuming a quantitative conversion of the ester to the hydroxamate in the preparative procedure, while use of a mixture containing more than about a milligram of any one component is likely to lead to spreading or streaking due to the overloading of the paper. With proper controls, a rough quantitative estimation may be made of the amount of acid in a spot by judging from the size of the spot and the intensity of the color.

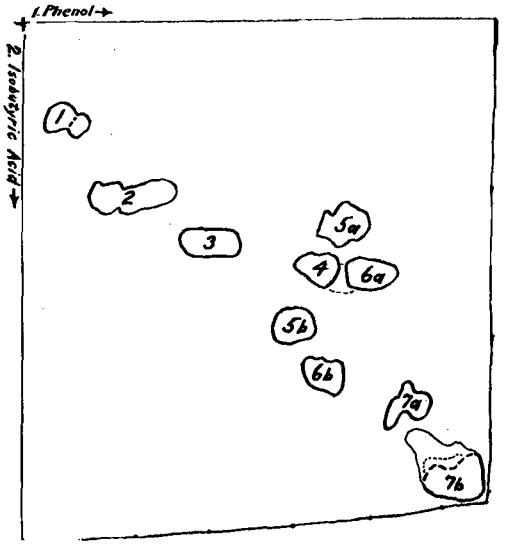


FIG. 1.
Diagram of a two dimensional chromatogram of hydroxamate derivatives of organic acids. The hydroxamates were applied at the point indicated by the cross. Phenol was used as the first solvent and isobutyric acid as the second. The numbers refer to the acids as follows:

1. tartaric
2. malonic
3. succinic
4. lactic
5. adipic
6. pimelic
7. sebacic

Disadvantages in the use of the hydroxamate derivatives include the manipulations involved in their preparation and the multiple spots obtained from the acids with multiple functional groups. The multiple spots are presumably due to failure to carry the preparative reactions to completion.

The colors in the developed chromatogram are relatively stable, although continued exposure to strong light causes a loss of contrast, principally by action on the ferric chloride background. Intense spots of the ferric hydroxamates tend to spread to other papers in contact with them, so that in filing it is well to insert blank sheets of paper between the chromatograms.

One dimensional chromatogram may be made on filter paper previously impregnated with ferric chloride, in which case the chromatogram may be examined visually at any stage of the development, but better results are usually obtained by use of non-impregnated paper.

*Summary.* A procedure for the application of filter paper partition chromatography to the separation of both volatile and non-volatile organic acids with chain lengths of about eight carbons or less is described. The potassium hydroxamate derivatives of the organic acids are prepared by reacting the methyl ester with about a two-fold excess of a mixture of potassium hydroxide and hydroxylamine in methyl alcohol. The hydroxamate derivatives obtained from about $10^{-6}$ mole of each of the esters are applied to the filter paper, the chromatogram developed with suitable solvents, and then sprayed with ferric chloride to make the derivatives visible as purple spots on a yellow background. Isobutyric acid and phenol gave the best results of the solvents tried for two-dimensional chromatograms of the dicarboxylic acids. $R_F$ values for a number of hydroxamate derivatives are given for several solvents.

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## Destructive Action of Human Cancer Extracts on Red Blood Cells *in vitro*.*

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It was recently observed in this laboratory that centrifugated or filtered extracts prepared from spontaneous mouse mammary carcinomas hemolyze mouse erythrocytes *in vitro*.[1]

Experiments reported in this study were designed with the purpose of determining whether extracts prepared from human cancer also exert a destructive action on red blood cells *in vitro*.

*Materials and methods. Tumor extracts.* Sterile specimens of tumors were obtained from the operating room.† The tumor tissue was weighed, cut with scissors into small pieces, then ground thoroughly for several minutes in a porcelain mortar, 0.85% solution of sodium chloride being added to obtain cell suspensions varying in concentration from 20 to 30%. The suspensions thus obtained were cleared from cells by 2 successive centrifugations at 5,000 t.p.m. for 10 minutes each; the final supernatant fluid was then used, and designated "tumor extract." In several cases, tumors of hard, fibrous consistency had to be ground into fine cell suspensions in a Latapie Grinding Apparatus (Thomas), and then centrifugated.

Cell suspensions prepared from human

* Published with the permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by the author.

1 Gross, L., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 292.

† Most of the tumor specimens were obtained from the operating room of our hospital (Veterans Administration Hospital, Bronx, N. Y.); 10 of the 12 specimens of human breast carcinomas were obtained from the Memorial Hospital, New York City.