## Ion Exchange Paper Chromatography

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Separation or organic acids by paper chromatography usually requires the presence of an acid such as acetic or formic acid in the developing solvent to prevent streaking.[1] In a previous communication[2] the use of papers impregnated with alginic acid was described whereby the so-called swamping acids could be dispensed with and any desirable solvent utilized. This paper deals with the separation of organic acids using papers containing ion exchange resins[3] and *O*-(carboxymethyl)cellulose[4] in conjunction with non-acidic solvents.

The resin containing papers were prepared by adding the powdered ion exchange resin to the cellulose pulp before the sheets were made.[3] Four types of ion exchange resin papers were tested, two containing acid resins and two containing basic resins. Before use the papers containing the cation exchange resins were irrigated for 6 hr. with *N* hydrochloric acid, and those containing the anion exchange resins were treated with *N* sodium hydroxide. Thereafter all the papers were washed thoroughly with water to remove the excess of reagent and dried in the air.

The *O*-(carboxymethyl)cellulose papers were made by dipping Whatman No. 1 filter paper in a 1.5% aqueous solution of *O*-(carboxymethyl)cellulose (Type 130, medium)[4] and, after draining off the excess of the solution, the papers were dipped into 2*N* hydrochloric acid to precipitate the *O*-(carboxymethyl)cellulose onto the cellulose fibers. The papers were then washed with water to remove excess acid and dried in the air.[cf. 2]

Glyconic and glycuronic acids can be separated on the strongly acidic type resin paper using 1-butanol:ethanol:water (4:1:5) as the developing solvent. All the acids except D-glucosaccharo-6,3-lactone gave compact spots on the chromatograms as revealed by ammoniacal silver nitrate (Tollen's reagent). Malic and citric acids gave white spots on a light brown background whereas the rest (see Table I) appeared as dark spots.

Mono- and disaccharides showed about the same $R_f$ values on paper impregnated with resins as on untreated cellulose papers. The resins do not interfere with the detection of the components.

The weakly acidic type of resin paper was most useful for separating amino acids[5] (see Table II) although it was noted that overloading had to be avoided or else streaking occurred. The weakly basic type of resin papers proved to be of no value for separating the amino acids; streaking was common and aspartic acid and glutamic acid displayed no movement.

TABLE I

SEPARATION OF ORGANIC ACIDS

| Acid | Ion Exchange Paper[b] | *O*-(Carboxymethyl)-cellulose Paper |
|---|---|---|
| $R_f$ values using 1-butanol:ethanol:water (4:1:5)[a] | | |
| L-Ascorbic acid | 0.48 | 0.39 |
| L-Arabono-γ-lactone | 0.43 | 0.39 |
| Citric acid | 0.62 | 0.47 |
| D-Galactono-γ-lactone | 0.34 | 0.30 |
| D-Galacturonic acid | 0.26 | 0.15 |
| D-Glucoheptono-γ-lactone | 0.22 | 0.16 |
| D-Glucosaccharo-6,3-lactone | 0.35 | 0.20 |
| D-Glucurone | 0.38 | 0.29 |
| D-Glucuronic acid | 0.31 | 0.17 |
| L-Gulonic acid | 0.25 | 0.20, 0.16[c] |
| 5-Keto-D-gluconic acid | 0.43 | 0.27 |
| 2-Keto-L-gulonic acid | 0.33 | 0.17 |
| Malic acid | 0.63 | 0.52 |
| D-Mannurone | 0.44 | 0.33 |
| D-Mannuronic acid | 0.31 | 0.22 |
| L-Tartaric acid | 0.50 | 0.31 |

[a] Components detected with ammoniacal silver nitrate. [b] Impregnated with Amberlite IR-120 cation exchange resin ($H^+$ form). [c] The spot with the lower $R_f$ value is due to acid and the other to the lactone.

TABLE II

SEPARATION OF AMINO ACIDS

| Amino Acid | Ion Exchange Paper[b] | *O*-(Carboxymethyl)-cellulose Paper |
|---|---|---|
| $R_f$ values using 1-butanol:ethanol:water (4:1:5)[a] | | |
| Alanine | 0.20 | 0.20 |
| Aspartic acid | 0.09[d] | 0.13 |
| Cysteine[c] | 0.08[d] | 0.22 |
| Glycine | 0.14 | 0.10 |
| Glutamic acid | 0.17 | 0.19 |
| Histidine | 0.06[d] | 0.04 |
| Leucine | 0.38 | 0.57 |
| Methionine | 0.35 | 0.36 |
| Phenylalanine | 0.38 | 0.62 |
| Proline | 0.22 | 0.17 |
| Threonine | 0.18 | 0.16 |
| Tryptophan | 0.39 | 0.44 |
| Valine | 0.29 | 0.44 |

[a] Amino acids detected with ninhydrin. [b] Impregnated with Amberlite IRC-50 cation exchange resin ($H^+$ form). [c] Applied as hydrochloride. [d] Evaluated from leading edge of spot which extends back to origin.

Filter paper impregnated with *O*-(carboxymethyl)-cellulose[6] gave a good separation of the uronic acids

(1) J. W. H. Lugg and B. T. Overell, *Australian J. Sci. Research, Ser. A*, **1**, 98 (1948).

(2) F. Smith and D. Spriesterbach, *Nature*, **174**, 466 (1954).

(3) The authors thank The Rohm & Haas Co., Philadelphia, Pa., for a generous supply of these papers.

(4) The authors thank The Hercules Powder Co., Wilmington, Del., for the *O*-(carboxymethyl)cellulose.

(5) *Cf.* M. M. Tuckerman, *Anal. Chem.*, **30**, 231 (1958).

(6) *Cf.* T. Willard and A. Berg, *Angew. Chem.*, **64**, 418 (1952); E. Peterson and H. Sober, *J. Am. Chem. Soc.*, **78**, 751 (1956).

and the lactones, D-glucurone and D-mannurone (see Table I). All the acids and lactones tested appeared as compact spots on the chromatograms.

The $R_f$ values of the amino acids tested on the *O*-(carboxymethyl)cellulose paper were of the same order of magnitude as previously observed[7] and lower than those observed with phenol as the solvent.[8] In the case of histidine and proline the $R_f$ values 0.04 and 0.17, respectively, were so much lower than those found on chromatograms developed with phenol:water[8] that the difference might be used to characterize these two amino acids.

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(7) J. A. Cifonelli and F. Smith, *Anal. Chem.*, **27**, 1501 (1955).

(8) R. Consden, A. H. Gordon and A. J. P. Martin, *Biochem. J.*, **38**, 224 (1944).

## Synthesis and Reduction of Nitrosotrimethylhydrazine[1]

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As a logical extension to an investigation in this laboratory of the lower alkylhydrazines, an attempt was made to prepare an alkyl derivative of the unknown triazane, $H_2NNHNH_2$. A survey of the literature did not disclose any attempts to prepare compounds of this type, although a few aromatic triazanes have been prepared and characterized.[2] One route to the synthesis of such a compound appeared to lie in the reduction of nitrosotrimethylhydrazine: This compound was selected as a pos-

$$(CH_3)_2NNCH_3NO + 4\ [H] \longrightarrow (CH_3)_2NN(CH_3)NH_2 + 2H_2O$$

sible precursor to *N,N,N'*-trimethyltriazane because (*1*) nitrosamines can be reduced to hydrazines with little or no reductive cleavage of the N—N bond,[3] (*2*) *N*-nitroso-*N*-phenyl-*N'*-formylhydrazine has been successfully reduced to *N*-formyl-*N'*-phenyltriazane, which was characterized through its benzaldehyde derivative,[4] and (*3*) trimethyltriazane may be considerably more stable than a triazane containing fewer alkyl groups. This is suggested by the high order of stability of tetramethylmethylenediamine, $[(CH_3)_2N]_2CH_2$,[5] compared to that of the nonisolable parent compound, methylenediamine.

The unknown nitrosotrimethylhydrazine was synthesized from trimethylhydrazine and nitrous acid. It gives a positive Lieberman test for the nitroso group, and is miscible with water to give a neutral solution. The compound is stable for long periods of time at Dry Ice temperatures, but at room temperature it slowly decomposes. A sample stored at 23° became orange-yellow in about three days, and orange-brown in a week. An elemental analysis gave somewhat high values for nitrogen, but the results are considered satisfactory in view of the instability of the compound. The reduction of nitrosotrimethylhydrazine was characterized by cleavage of at least one of the N—N bonds in attempts to prepare the triazane by three different methods. In the direct hydrogenation of the nitroso compound under mild conditions with a catalyst of 10% palladium-on-charcoal, trimethylhydrazine was isolated in addition to unreacted nitroso compound. With lithium aluminum hydride in ether dimethylamine was obtained, while with sodium amalgam in ethanol, hydrazine and dimethylamine were obtained. The course of the reduction to produce hydrazine is not clear, although other nitroso compounds have also been found to yield hydrazine when reduced with sodium amalgam under similar conditions.[6]

### EXPERIMENTAL[7]

*Starting materials.* Trimethylhydrazine was prepared by the method of Class.[8] A value of 100.5% was obtained for the purity of the product, b.p. 59–60° 749 mm., by titration with standard potassium iodate (four-electron change).[9] Lithium aluminum hydride was obtained from Metal Hydrides, Inc., Beverly, Mass. Sodium amalgam was prepared by the method given by Fieser.[10]

*Preparation of nitrosotrimethylhydrazine.* A solution of 25.9 g. (0.35 mole) of trimethylhydrazine in 100 ml. of water was neutralized to a *p*H of 7.00 with 1:1 hydrochloric acid, diluted to 280 ml. with water, and cooled to 0°. A solution of 72.5 g. (1.05 moles) of sodium nitrite in 200 ml. of water was then added dropwise to the well stirred trimethylhydrazine hydrochloride solution at such a rate that the temperature did not rise above 5°. After the addition, the *p*H was adjusted to 5.18 by the dropwise addition of 1:1 acetic acid. When the resulting solution, which degassed continuously, was stored overnight at 0°, the *p*H increased to 5.83 and the solution deepened in color (yellow). The *p*H was adjusted to 8.00 by the addition of solid potassium carbonate, and the solution was extracted continuously with 150 ml. of ether until nearly all of the yellow color had passed

(1) This investigation was carried out under a contract with the Office of Naval Research.

(2) T. C. Chaudhuri, *J. Chem. Soc.*, **117**, 1081 (1920).

(3) E. Fischer, *Ann.*, **199**, 283 (1879).

(4) A. Wohl, *Ber.*, **33**, 2759 (1900).

(5) F. Klages, G. Nober, F. Kircher, and M. Bock, *Ann.*, **547**, 24 (1941).

(6) L. F. Audrieth and B. A. Ogg, *The Chemistry of Hydrazine*, John Wiley & Sons, Inc., New York, N. Y., 1951, p. 16.

(7) The analysis of nitrosotrimethylhydrazine was performed by Elek Microanalytical Laboratories, Los Angeles.

(8) J. B. Class, J. G. Aston, and T. S. Oakwood, *J. Am. Chem. Soc.*, **75**, 2938 (1953).

(9) G. S. Jamieson, *Am. J. Sci.*, **33**, 352 (1912).

(10) L. F. Fieser, *Experiments in Organic Chemistry*, 2nd ed., D. C. Heath & Co., New York, N. Y., 1941, p. 418.