groups to the double bounds, it is not possible to obtain on one chromatogram the whole spectrum of all the substances present in the material being analysed.

The two-dimensional system is very simple and, combined with a sensitive detection method, it constitutes a relatively quick and convenient method suitable for the separation of many critical pairs of cholesteryl esters.

This technique was used for the qualitative characterization of cholesteryl esters in various biological materials and for the identification of the spectrum of higher fatty acids in oils and fats after enzymic esterification with cholesterol.

These results will be discussed in another paper.

Laboratory of Protein Metabolism and Proteosynthesis,
Charles University,
Prague (Czechoslovakia)

Č. MICHALEC
Z. KOLMAN
M. ŠULC
J. MEŠŤAN

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Received April 26th, 1962

J. Chromatog., 9 (1962) 237–241

Notes

The decomposition of nine amino acids during chromatography on paper

It has recently been shown[1] that some decomposition of glycine takes place during chromatography on Whatman No. 4 paper using phenol–water and *n*-butanol–propionic acid–water as developing solvents. Losses of up to 38% were obtained in this system; these losses could be minimized by distillation of the phenol before use, and by pre-treatment of the paper with oxalic acid. Filter paper pre-treated in this way had a pH of 4.9, compared with 6.7 for untreated paper. This study has now been extended to include eight other amino acids.

Before use, the following amino acids were purified chromatographically on oxalic acid-treated Whatman No. 4 paper, using distilled phenol[2]–water followed by *n*-butanol–propionic acid–water[3]: DL-[2-^{14}C]alanine (8.2 μC/mg); DL-[4-^{14}C]aspartic acid (10.7 μC/mg); DL-[1-^{14}C]glutamic acid (9.0 μC/mg); [2-^{14}C]glycine (8.9 μC/mg); L-[^{14}C]leucine (8.0 μC/mg); DL-[3-^{14}C]phenylalanine (21.1 μC/mg); DL-[3-^{14}C]serine (1.7 μC/mg); DL-[2-^{14}C]tyrosine (2.3 μC/mg); and D-[4,4'-^{14}C]valine (3.1 μC/mg).

Each amino acid was eluted with water, evaporated to dryness under reduced

TABLE I

BREAKDOWN OF NINE AMINO ACIDS DURING CHROMATOGRAPHY

Pretreatment of paper	*Dimensions*	*1st solvent**	*2nd solvent**	*Extent of breakdown of amino acid (%)*									
				Alanine	*Aspartic acid*	*Glutamic acid*	*Glycine*	*Leucine*	*Phenyl-alanine*	*Serine*	*Tyrosine*	*Valine*	*Average for all amino acids*
None	1	Ph.	—	2.0	3.4	4.8	10.4	5.7	3.0	2.4	0.6	1.6	3.8
None	1	Redist. Ph.	—	4.8	4.4	5.9	10.6	6.4	3.7	2.0	1.3	1.8	4.6
None	1	But.–Prop.	—	3.5	3.2	3.7	9.2	10.7	7.7	1.0	2.5	3.3	5.0
None	2	Ph.	But.–Prop.	3.0	1.9	3.8	11.1	14.2	9.0	2.7	4.0	6.8	6.3
None	2	Redist. Ph.	But.–Prop.	3.4	2.3	4.9	12.6	12.9	11.2	2.2	4.6	6.3	6.7
None	2	But.–Prop.	Ph.	7.1	3.8	3.6	6.4	10.0	7.1	1.9	0.0	0.4	4.5
None	2	But.–Prop.	Redist. Ph.	3.9	3.7	4.3	8.9	9.8	5.5	0.8	0.5	1.6	4.3
Oxalic acid	1	Ph.	—	2.4	3.7	5.7	8.7	4.0	1.5	2.1	0.5	1.0	3.3
Oxalic acid	1	Redist. Ph.	—	1.9	3.2	5.2	8.0	3.2	2.8	1.3	0.4	0.7	3.0
Oxalic acid	1	But.–Prop.	—	2.8	2.3	3.7	6.7	8.4	7.6	1.2	3.3	2.2	4.3
Oxalic acid	2	Ph.	But.–Prop.	2.5	3.5	4.8	6.7	11.2	10.5	1.3	1.7	3.0	5.0
Oxalic acid	2	Redist. Ph.	But.–Prop.	1.5	2.2	2.4	6.6	8.4	10.4	1.4	3.3	2.8	4.3
Oxalic acid	2	But.–Prop.	Ph.	0.6	0.7	5.2	8.1	5.4	2.8	0.9	0.6	0.2	2.7
Oxalic acid	2	But.–Prop.	Redist. Ph.	0.9	7.3	1.9	9.0	2.8	2.3	1.1	0.0	0.8	2.9

* Abbreviations: Ph. = phenol–water; Redist. Ph. = redistilled phenol–water; But.–Prop. = *n*-butanol–propionic acid–water.

pressure at 40°, and redissolved in water (0.1 *N* HCl in the case of tyrosine). Aliquots of each amino acid solution containing 0.1 μC of ^{14}C were spotted onto oxalic-acid treated, or untreated, Whatman No. 4 paper, and developed either in one or in two dimensions as shown in Table I. The positions of radioactive substances were determined by radioautography (Kodak Single-Coated Blue-Sensitive Medical X-Ray Film, exposed for 36 days). The radioactive areas were excised from the chromatograms and the radioactivity in each spot was counted automatically in the apparatus described by MOSES AND LONBERG-HOLM[4].

The values reported in Table I are percentages of the total ^{14}C on each chromatogram present in substances other than the corresponding amino acid being investigated; the chemical nature of none of these other substances has been determined. Each of the amino acids investigated showed some breakdown during chromatography; in some cases as many as twenty decomposition products were detected. The extent of decomposition varied from zero with tyrosine in two instances, to over 14% with leucine in one system. Generally, glycine, leucine and phenylalanine showed the greatest degree of breakdown, and tyrosine the least; the other five substances were intermediate between these extremes.

Unlike the earlier findings with glycine[1], distillation of the phenol before use had no detectable effect on amino acid breakdown. Even glycine, the breakdown of which was earlier found to be much less extensive after distillation of the phenol, was unaffected. Presumably this is related to the quality of the undistilled phenol used on the two occasions: HUGGINS AND MOSES[1] employed British Drug Houses (Poole, England) Phenol Detached Crystals B.P.C., while in the present study the source was Mallinckrodt (St. Louis, Mo., U.S.A.) Phenol Liquefied Analytical Reagent.

Pretreatment of the chromatography paper with oxalic acid reduced the amount of decomposition by an average of 30% compared with untreated paper. The greater acidity of the oxalic acid-treated paper was probably responsible for much of this effect. However, oxalic acid specifically might also be of importance, by virtue of its binding of calcium and magnesium ions in the paper; there was some reduction in the extent of breakdown with oxalic acid-treated paper even when butanol–propionic acid–water (pH 2.5) alone was used as a solvent. The beneficial effect of oxalic acid treatment agrees with the earlier studies on the chromatographic breakdown of glycine[1].

The work described in this paper was sponsored by the United States Atomic Energy Commission.

Bio-Organic Chemistry Group,
Lawrence Radiation Laboratory, University of California, V. MOSES
Berkeley, Calif. (U.S.A.)

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Received April 12th, 1962