The second spot of lower R_F revealed by ninhydrin staining is due to the more slowly migrating amino acid from the incubation mixture, as illustrated by carbamoylation of ornithine. According to observations of KORITZ AND COHEN[5], who tried the less sensitive diacetyl monoxime *in vitro*, semicarbazide, barbituric acid, carbamoyl phosphate and uric acid did not react to a measurable extent. Arginine, creatinine and tolbutamide were negative.

Attempts to extend the chromatographic separation to the quantitative determination of urea (extraction of the silica by methanol and photometry at 436 nm) failed, because of the faint color produced on plates. Ninhydrin spots did not disappear after subsequent antipyrine–DMG spraying, thus allowing double staining. The reagent cannot be used in paper chromatography because of its corrosive power.

*I. Medizinische Klinik der Medizinischen Akademie Lübeck (Germany)**

K. LORENTZ
A. HARTMANN***

1 K. LORENTZ, *Biochim. Biophys. Acta*, in press.
2 A. R. FAHMY, A. NIEDERWIESER, G. PATAKI AND M. BRENNER, *Helv. Chim. Acta*, 44 (1961) 2022.
3 V. KULHÁNEK AND V. VOJTIŠKOVÁ, *Clin. Chim. Acta*, 9 (1964) 95.
4 R. M. ARCHIBALD, *J. Biol. Chem.*, 157 (1945) 507.
5 S. B. KORITZ AND P. P. COHEN, *J. Biol. Chem.*, 209 (1954) 145.

Received April 28th, 1967

* Director: Prof. Dr. U. RITTER.
** Supported by Deutsche Forschungsgemeinschaft.

Rapid resolution of methylmethionine sulfonium salts and homoserine by thin-layer chromatography

Currently there is considerable interest in the natural occurrence of methylmethionine sulfonium salts (MMS). These salts, which decompose on heating to yield homoserine and dimethyl sulfide, are important in flavor development of various foods. KIRIBUCHI AND YAMANISHI[1] reported the recovery of an MMS salt from extracts of green tea and identified it as the precursor of dimethyl sulfide in green tea. These salts have also been isolated from asparagus[2], cabbage[3], and tomatoes[4].

Paper chromatography has been used for resolution and identification of MMS and homoserine, but as yet thin-layer chromatography (TLC) has not been employed. This communication reports results obtained in evaluation of a number of solvent systems for resolution of these compounds by TLC.

For evaluation of solvent systems, Eastman Chromagram Sheets were employed. Where compounds were to be recovered, glass plates (20 × 20 cm) were coated with a 250 μ layer of Silica Gel G using the Brinkman apparatus. After allowing about 15 min for the adsorbent to set, plates were heated at 110° for at least 1 h. Plates were cooled to room temperature prior to sample application.

Amino acids were detected by spraying with 0.5 % ninhydrin in 95% ethanol, drying, and heating at 70° for 5 min.

Homoserine and MMS chloride were obtained from Calbiochem. All solvents used were reagent grade or equivalent.

Solvent systems evaluated and the R_F values obtained are listed in Table I. Of the systems tested, *n*-propanol–30 % ammonia in ratios of 70:30 and 60:40 and dimethyl sulfoxide–dimethyl formamide–30 % ammonia in ratios of 20:20:15 and

TABLE I

R_F VALUES FOR METHYLMETHIONINE SULFONIUM CHLORIDE AND HOMOSERINE IN VARIOUS SOLVENT SYSTEMS

Solvent	*Ratio*	*R_F values*	
		Methylmethionine sulfonium chloride	*Homoserine*
Chloroform Isopropanol Formic acid	70 30 2	0	0
Pyridine Water Glacial acetic acid	65 35 5	0.41	0.44
95% Ethanol 30% Ammonia	70 30	0.06	0.61
Butanone Pyridine Water Glacial acetic acid	70 15 15 2	0.01	0.24
n-Propanol 30% Ammonia	70 30	0.2	0.45
n-Propanol 30% Ammonia	60 40	0.37	0.6
n-Propanol 30% Ammonia	50 50	0.47	0.63
Dimethyl sulfoxide Dimethyl formamide 30% Ammonia	20 20 10	0.12	0.61
Dimethyl sulfoxide Dimethyl formamide 30% Ammonia	20 20 15	0.19	0.73
Dimethyl sulfoxide Dimethyl formamide 30% Ammonia	20 10 10	0.17	0.71
Dimethyl sulfoxide Dimethyl formamide 30% Ammonia	10 10 20	0.48	0.81

10:10:20 gave the best resolution. For recovery of the heat-labile MMS, propanol–30% ammonia systems are more desirable, since these solvents are readily removed under vacuum at room temperatures. The use of TLC for resolution of these compounds offers the advantages of speed and sensitivity over paper chromatography. Development of plates was usually completed within 4 h and as little as 1 μg of MMS or homoserine was detectable.

These solvent systems have recently been utilized in the identification of an MMS salt occurring in milk and for following its conversion to homoserine on heating[5].

This investigation was supported by a grant from the Oregon State University General Research Fund and by a National Aeronautics and Space Administration Fellowship to the senior author.

Department of Food Science and Technology, T. W. KEENAN
Oregon State University, Corvallis, Ore. (U.S.A.) R. C. LINDSAY

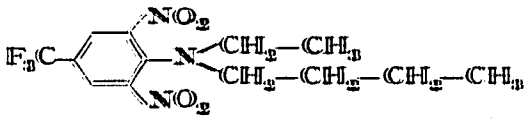
1 T. KIRIBUCHI AND T. YAMANISHI, *Agr. Biol. Chem.* (*Tokyo*), 27 (1963) 56.
2 F. CHALLENGER AND B. J. HAYWOOD, *Chem. Ind.* (*London*), (1954) p. 729.
3 R. A. MCRORIE, G. L. SUTHERLAND, M. S. LEWIS, A. D. BARTON, M. R. GLAZENER AND W. SHIVE, *J. Am. Chem. Soc.*, 76 (1954) 115.
4 F. F. WONG AND J. F. CARSON, *J. Agr. Food Chem.*, 14 (1966) 247.
5 T. W. KEENAN AND R. C. LINDSAY, in preparation.

Received April 6th, 1967

J. Chromatog., 30 (1967) 251–253

Thin-layer chromatographic separation of benefin and related substances

Benefin (I) (Balan®, N-(*n*-butyl)-N-ethyl-2,6-dinitro-α,α,α-trifluoro-*p*-toluidine) is a selective pre-emergent, soil incorporated herbicide for many agronomic and horticultural crops which controls a wide variety of annual grasses and broadleaf weeds[1]. In its herbicidal activities benefin complements another similar compound, trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-di-(*n*-propyl)-*p*-toluidine). Chromatographic separation of the latter substance and its related compounds was described previously[2].



(II)

Metabolic studies in plants and soils required knowledge of the chromatographic behavior of benefin and ten related compounds. Two-dimensional thin-layer chromatography on Silica Gel GF permitted useful separations. The best separation was obtained with benzene–methylcellosolve (96:4) and cyclohexane–ethyl acetate (95:5).

The compounds were detected by their natural colors (yellow, orange, brown),