Thin-layer chromatography of carbamoyl compounds

This paper describes a technique suitable for the detection of carbamoyl derivatives, which can be easily separated on thin-layer plates.

Experimental

Urea (Merck AG, Darmstadt), allantoin (Fluka, Buchs), and amino acids (Serva, Heidelberg), analytically pure grade, were dissolved in water to yield 0.1–1 *M* solutions. Equal volumes of these solutions (except urea, allantoin, and citrulline) and 0.02 *M* carbamoyl phosphate (Boehringer, Mannheim) in 0.02 % urease–EDTA (0.02 *M*, pH 7.0) were mixed and incubated for 4 h at 37° to form the carbamoyl compounds[1]. The presence of urease is essential to destroy urea-contaminating carbamoyl phosphate.

2–20 μl of these mixtures were placed 1.5 cm from the edge of a silica gel plate (Kieselgel H, Merck AG activated for 45 min at 125°) and allowed to run in a system of *n*-butanol–glacial acetic acid–water (4:2:2) with prior equilibration. The run was stopped, when the solvent had moved 10 cm.

The spots appeared after treatment with ninhydrin reagent according to FAHMY *et al.*[2], and the carbamoyl derivatives were stained with antipyrine–DMG reagent in a modified diacetyl reaction[3,4].

Antipyrine reagent. 1.6 g phenyldimethylpyrazolone (Antipyrine from Bayer AG, Leverkusen), 30 ml concentrated sulfuric acid and 5 ml 85 % orthophosphoric acid were diluted with distilled water to 100 ml.

DMG-reagent. 0.1 g dimethylglyoxime (Merck AG, Darmstadt) was dissolved in 10 ml 96 % ethanol.

1 volume DMG and 10 volumes antipyrine reagent were mixed and yielded the indefinitely stable spray reagent. The dry plates must be sprayed twice and heated at 125° for 20 min.

Small amounts of carbamoyl compounds showed a stable yellow color, high quantities stained brown. By means of this technique, 0.5 μmole urea, allantoin, citrulline, carbamoyl glycine or carbamoyl glycylglycine could be detected, whereas other carbamoyl amino acids stained but only for amounts above 10 μmoles (carbamoyl aspartate, carbamoyl lysine, carbamoyl alanine). We found the following R_F values by the technique described above (Table I).

TABLE I

R_F VALUES OF SOME CARBAMOYL COMPOUNDS

Compound	*Ninhydrin*	*Antipyrine–DMG*
Urea	No stain	0.63
Allantoin	No stain	0.48
Carbamoyl glycine	0.35 and 0.50	0.50
Carbamoyl glycylglycine	0.33 and 0.42	0.42
Carbamoyl lysine	0.25 and 0.37	0.37
Carbamoyl aspartate	0.32 and 0.45	0.45
Carbamoyl alanine	0.39 and 0.54	0.54
Citrulline	0.33	0.33
Ornithine + carbamoyl phosphate	0.26 and 0.33	0.33

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.