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Note

Detection of N-acetyl amino acids on paper and sugars on thin-layer chromatograms by a thermal–ultraviolet method

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The detection of N-acetyl amino acids is not easily accomplished by the ninhydrin procedure[1] due to the presence of a blocked amino group. Also, other techniques specific for certain acetyl amino acids such as bromophenol in ethanol and potassium permanganate proved to be unsatisfactory[2]. However, more elaborate procedures such as synthesis "*in vitro*" of radiolabelled N-acetyl amino acids[3] have been used as standards in paper chromatography[4].

A thermal-UV procedure for the detection of a large variety of organic compounds after paper chromatography has recently been reported[5]. The application of this method to the detection of N-acetyl amino acids is described in this paper. We also report the application of the thermal–UV procedure after thin-layer chromatography (TLC) with high recovery of the sample.

MATERIALS AND METHODS

N-Acetyl amino acids were detected either after ascending paper chromatography or high-voltage electrophoresis. The former method was performed with Whatman No. 1 paper using the following solvent systems: (A) pyridine–1-butanol–acetic acid–water (15:10:3:12)[6]; (B) 1-propanol–12% ammonium hydroxide (3:1); (C) 1-propanol–methyl ethyl ketone–25% formic acid (15:3:2)[7].

High-voltage electrophoresis was carried out in pyridine–acetic acid–water (1:10:189), pH 3.5, at 35 V/cm for 90 and 20 min for N-acetyl amino acids and phospho amino acids respectively.

TLC was carried out using the following solvent systems: (D) acetone–

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benzene–35% ammonium hydroxide–water (200:50:1.35:1) and (E) 1-butanol–pyridine–water (6:4:3).

After each run the paper or the plate was dried and developed according to Alperin *et al.*[5]; in the latter case an oven was used instead of a domestic iron.

[U-^{14}C]Glucose (250 Ci/mol) was purchased from New England Nuclear (Boston, MA, U.S.A.). All N-acetyl amino acids, phospho amino acids and amino acids were purchased from Sigma (St. Louis, MO, U.S.A.). Silica gel glass plates were Kieselgel 60 from E. Merck (Darmstadt, G.F.R.) and silica gel coated sheet from Eastman-Kodak (Rochester, NY, U.S.A.). Glass microfibre filters were Whatman GF/C. Toluene–PPO (2,5′-diphenyloxazole) was used for radioactivity measurements in a Beckman Model 8100 liquid scintillation spectrometer. All other procedures were as previously described[5].

RESULTS AND DISCUSSION

Detection of N-acetyl amino acids on paper chromatograms

About 1 μmol of N-acetyl derivatives of lysine, valine, glutamic acid, alanine and methionine was spotted on paper and chromatographed using solvent system A during 30 h as described in Materials and methods. After the run, the paper was dried and developed by the thermal–UV method. All the N-acetyl amino acids gave a detectable fluorescent spot as shown in Fig. 1. A sensitivity test was performed using N-acetylserine. It was found that up to 0.25 μmol/cm^2 could be detected after chro-

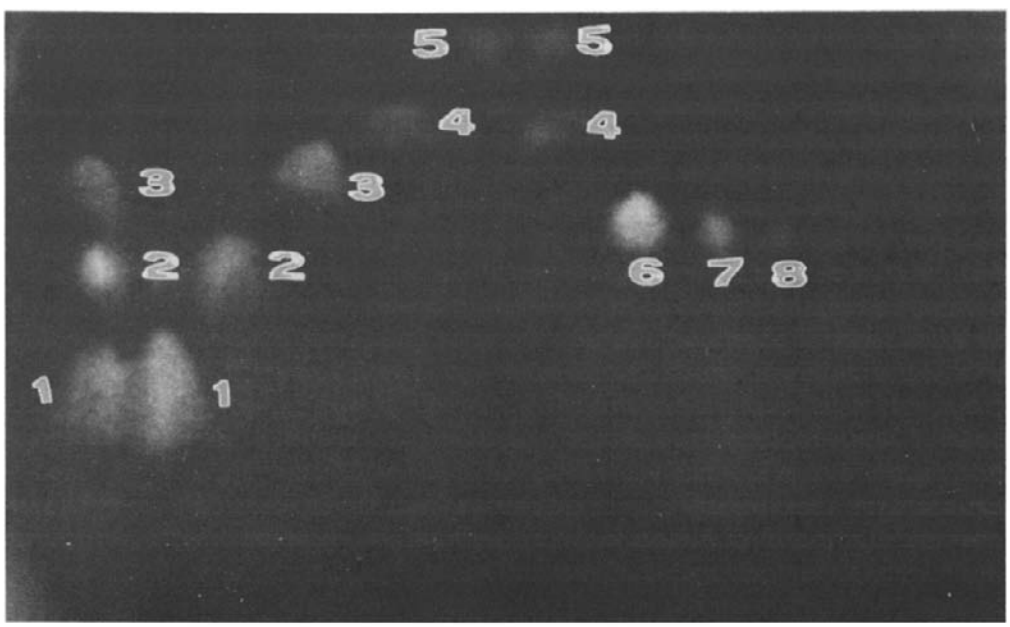

Fig. 1. Detection by the thermal–UV method of N-acetyl amino acids after paper chromatography using solvent system A. The spots correspond to about 1 μmol/cm^2 of the following compounds: 1 = N-acetylvaline; 2 = N-acetyllysine; 3 = N-acetylglutamic acid; 4 = N-acetylalanine; 5 = N-acetylmethionine. 6, 7 and 8 represent 1, 0.5 and 0.25 μmol/cm^2 of N-acetylserine respectively. Spots 1 and 5 migrated 11 and 22 cm from the origin respectively.

TABLE I

R_F AND R_{NAS} VALUES OF N-ACETYL AMINO ACIDS, PHOSPHO AMINO ACIDS AND AMINO ACIDS IN PAPER CHROMATOGRAPHY AND ELECTROPHORESIS

All the compounds were detected by the thermal–UV method. R_{NAS} = mobility relative to N-acetylserine.

Compound	R_F*	R_{NAS}**	*Compound*	R_F*	R_{NAS}**
N-Acetylserine	0.63	1.0	Serine	0.39	–
N-Acetylalanine	0.92	–	Alanine	0.47	–
N-Acetylglutamic acid	0.76	–	Glutamic acid	0.37	0.39
N-Acetylaspartic acid	0.65	–	Aspartic acid	0.26	0.72
N-Acetyllysine	0.61	0.21	Lysine	0.33	–
N-Acetylmethionine	0.83	0.86	Methionine	0.68	–
N-Acetylglycine	0.72	0.95	N-Acetylhistidine	–	0.03
N-Acetylleucine	–	0.61	N-Acetylphenylalanine	–	0.73
N-Acetylproline	0.82	1.13	N-Acetyltyrosine	–	0.69
N-Acetylvaline	0.47	0.73	Phosphotyrosine	–	1.37
O-Phosphothreonine	–	1.68	Threonine	0.48	–
O-Phosphoserine	0.18	1.80	Serine	0.39	–

* Paper chromatography in solvent system A.

** Paper electrophoresis as described in Materials and Methods. About 0.5 μmol/cm² of each substance has been used.

matography (Fig. 1). In addition, other N-substituted amino acids and phospho amino acids were detected by this method (Table I). Detection was also accomplished after high-voltage paper electrophoresis as described[8].

As mentioned before, the presence of blocked amino groups does not allow the ninhydrin reaction. As far as we know, at present, no other satisfactory chemical methods are available to detect N-acetyl amino acids on paper chromatograms. The use of radiolabelled acetyl amino acids as standards in paper chromatography is an elaborate and expensive procedure[4]. The thermal–UV method overcomes these difficulties, being an extremely simple technique.

To evaluate the recovery of the compound after heating, the following experiment was performed. About 1 μmol of N-acetylserine was spotted and chromatographed using solvent system B. After the run, the paper was dried and heated until the fluorescent spot had developed. Subsequently, the paper was run in the second dimension using solvent system C. In this case N-acetylserine and serine were used as standards. After chromatography the paper was developed again by heating and it was observed that the sample migrated as authentic N-acetylserine whereas no spot was visualized migrating at the position of serine. In addition the fluorescent spot detected in the first dimension remained at its original position (not shown).

Detection of sugars on thin-layer chromatograms

The present method was also applied for the detection of compounds after TLC. About 20,000 cpm of [^{14}C]glucose were spotted together with 1 μmol of the same unlabelled compound on a silica gel coated sheet (Eastman-Kodak). Chromatography was carried out using solvent system E. After the run, the chromatogram was heated for 3 min at 135°C in an oven until a yellow fluorescent spot was observed under UV light. Subsequently, the layer was run in the second dimension using the

same solvent system. After the run, the plate was dried and heated as above. A new yellow spot was observed which was cut out and processed for liquid scintillation counting. It is noteworthy that the R_F of the sample was the same in both runs and the recovery of the original radioactivity was about 87%.

This method has recently been applied for the detection of methyl glucosides after TLC on silica gel glass coated plates[9] (Kieselgel 60, Merck). Two methyl glucosides (2, 3, 4, 6-tetramethylglucose and 2, 3, 4-trimethylglucose) were chromatographed using solvent system D. After the run, the plate was heated for 10 min at 140°C in an oven and two fluorescent yellow spots were observed. The eluted samples revealed similar chromatographic behaviour to the authentic standard compounds when run under the same conditions as before. Thus, in this case the advantages of TLC together with the thermal–UV detection method allow the possibility of further analysis of the recovered compounds.

The fluorescence phenomena

It has been observed that after heating the paper at constant temperature (150°C) for 1–3 min only UV fluorescent spots appeared. If the heating was continued for 3–6 min the fluorescence decreased and dark visible spots could be seen. After further heating the fluorescence disappeared and maximal contrast in the visible spots was achieved. At present, we are not able to determine whether cancellation or quenching occurred during heating. Similar results were obtained using higher temperatures. As mentioned before, fluorescent spots cannot be removed from the paper matrix using water, organic solvents or several chromatographic solvent systems[5].

With several compounds initial heating to develop fluorescence revealed a light blue colour under UV light (366 nm). This phenomenon appeared to be independent of the nature of the compound tested. However, the appearance of fluorescence takes place near the decomposition temperature of any given substance. The same light blue fluorescence occurred when the paper matrix without sample was heated at approximately its decomposition temperature (260–270°C). In contrast, when the substance was spotted on silica gel (Kieselgel 60) or glass microfibre filters and heated, the colours of the fluorescende generated depended only on the nature of the substance used. These results may suggest an interaction between the substance and the paper matrix, possibly due to a differential absorption of heat. Thus, a higher temperature is produced on the area where the compound is present when the paper is heated, and results in the appearance of the characteristic light blue fluorescence of the paper.

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REFERENCES

1 E. M. Gal and D. M. Greenberg, *Proc. Soc. Exp. Biol. Med.*, 71 (1948) 88.

2 G. Zweig and J. Sherma (Editors), *Handbook of Chromatography*, Vol. 1, CRC Press, Cleveland, OH, 1972.
3 J. P. Greenstein, *Methods Enzymol.*, 3 (1957) 554.
4 A. Pestana and H. C. Pirot, *Biochemistry*, 14 (1975) 1397.
5 D. M. Alperin, V. Idoyaga-Vargas and H. Carminatti, *J. Chromatogr.*, 242 (1982) 299.
6 I. Sures and D. Gollwitz, *Biochemistry*, 19 (1980) 943.
7 A. E. Ramirez de Gluglielmone and C. I. Gomez, *J. Neurochem.*, 13 (1966) 1017.
8 D. J. Shealy and R. L. Erikson, *Nature (London)*, 293 (1981) 666.
9 R. Couso, personal communication.