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Thin-layer chromatography of aldehyde hydrazones of 2-hydrazinobenzothiazole

Thin-layer chromatographic (TLC) methods for the identification of carbonyls using the separation of 2,4-dinitrophenylhydrazones do not readily distinguish between aldehydes and ketones other than by differences in R_F values. A previous paper[1] described a TLC method for the separation of aliphatic aldehydes in the presence of ketones using azine derivatives of 3-methyl-2-benzothiazolone hydrazone. Aromatic and aliphatic aldehydes can be present together in complex mixtures and a method for identifying them in the presence of ketones is desirable. Such a method is described in this work.

The reactions involved are firstly between an aldehyde and 2-hydrazinobenzothiazole to form a hydrazone. This hydrazone reacts with *p*-nitrobenzenediazonium fluoborate to form a coloured dye which reacts in turn with a base to form a blue or green anion. These reactions were described by SAWICKI AND STANLEY[2] as a test for aliphatic, aromatic and heterocyclic aldehydes. For the purposes of TLC identification the aldehyde hydrazones of 2-hydrazinobenzothiazole are chromatographed on silica gel. Detection is by successive spraying with the *p*-nitrobenzenediazonium fluoborate reagent and alcoholic KOH. The final spraying with base is necessary as a confirming test because the colours formed with the diazonium reagent are not specific for the hydrazones. Ketones form hydrazones with 2-hydrazinobenzothiazole, however the hydrazones do not form coloured compounds with the diazonium reagent or by subsequent treatment with base.

Experimental

Apparatus and reagents. E. Merck precoated Silica Gel F_{254} TLC plates with a layer thickness of 250 μ were used for the investigation. 2-Hydrazinobenzothiazole and *p*-nitrobenzenediazonium fluoborate were obtained from Distillation Products Industries, Rochester 3, N.Y., U.S.A.

Preparation of hydrazones. One mmole of the aldehyde was added to 30 ml of ethanol containing 1 mmole of 2-hydrazinobenzothiazole and a drop of glacial acetic acid. The mixture was warmed under reflux on a steam bath for 30 min to 1 h. The hydrazone was obtained from the reaction mixture by cooling in an ice bath and if necessary by the addition of water. The hydrazones of citral and isovaleraldehyde were obtained by pouring the reaction mixture into ice water. The derivatives were recrystallised from ethanol–water mixtures. Chloroform or ethyl acetate solutions of the hydrazones were stored under refrigeration.

Plate preparation. Silica Gel F_{254} plates were activated at 110° for 1 h and then stored over blue silica gel in a desiccator.

Thin-layer chromatography. Chloroform or ethyl acetate solutions of the hydrazones were applied to a start line 2 cm from the bottom of a TLC plate. After 1 h equilibration of the spotted plate in a solvent vapour saturated chamber, development was performed with a solvent system of light petroleum (b.p. 30–40°)–ethyl acetate–acetic acid (88:10:2). After the front had advanced 15 cm from the start-line, the plate was air dried.

TABLE I

R_F VALUES AND COLOURS OBTAINED WITH SPRAY REAGENTS FOR ALDEHYDE HYDRAZONES OF 2-HYDRAZINOBENZOTHIAZOLE

Aldehyde hydrazone	R_F *value*	*Colour*	
		With p-nitrobenzenediazonium fluoborate	*With p-nitrobenzenediazonium fluoborate, then alcoholic KOH or* NH_3 *vapour*
Formaldehyde	0.35	orange	blue
Acetaldehyde	0.40	orange	blue
Propionaldehyde	0.44	orange	blue
Butyraldehyde	0.50	orange	blue
Isobutyraldehyde	0.48	purple	blue
2-Methylbutyraldehyde	0.52	purple	blue
Hexyl aldehyde	0.55	orange	blue
Heptyl aldehyde	0.56	orange	blue
Octyl aldehyde	0.57	orange	blue
Nonyl aldehyde	0.58	orange	blue
Benzaldehyde	0.38	purple	blue
p-Tolualdehyde	0.36	purple	blue
Cuminyl aldehyde	0.44	purple	blue
Phenylacetaldehyde	0.40	orange	blue
Hydrotropaldehyde	0.39	orange	blue
Cinnamaldehyde	0.38	orange	green
Hydrocinnamaldehyde	0.36	orange	blue
α-amylcinnamaldehyde	0.52	orange	blue
α-Hexylcinnamaldehyde	0.51	orange	blue
Citral	0.49, 0.57	orange	blue
Citronellal	0.54	orange	blue
Hydroxycitronellal	0.09	orange	blue
Salicylaldehyde	0.25	purple	blue
Acrolein	0.35	red	blue
Isovaleraldehyde	0.45	orange	blue
Piperonal	0.23	pale green	blue
Anisaldehyde	0.26	olive	blue

Detection. Detection of the separated hydrazones was accomplished by spraying firstly with an ethanol solution of 0.1% *p*-nitrobenzenediazonium fluoborate until the coloured spots appeared, then with a solution of 10% alcoholic KOH to form the final blue or green anions. Alternatively, the final colours can be developed by exposure of the diazonium reagent detected spots to ammonia vapour.

Results and discussion

The R_F values and colours obtained with the spray reagents for the hydrazones of some aliphatic, aromatic and terpenoid aldehydes are shown in Table I.

Fig. 1 shows the separation of $C_1 - C_8$ aliphatic aldehyde hydrazones and Fig. 2 the separation of some aromatic and terpenoid hydrazones.

The colours formed with the diazonium reagent are stable under prolonged storage, however, the blue to green anions formed with the alcoholic KOH spray fade and revert to the colours initially formed with the diazonium reagent. The hydrazone solutions are stable for about a week when stored under refrigeration. All the hydra-

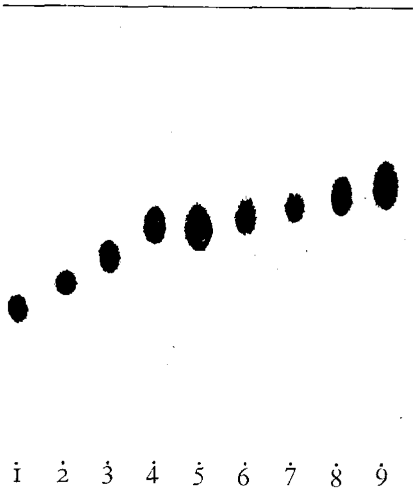

Fig. 1. Thin-layer chromatogram of C_1–C_8 aliphatic aldehyde hydrazones of 2-hydrazinobenzothiazole on Silica Gel F_{254}. Solvent: light petroleum (b.p. 30–40°)–ethyl acetate–acetic acid (88:10:2). Hydrazones of : (1) formaldehyde; (2) acetaldehyde; (3) propionaldehyde; (4) butyraldehyde; (5) isobutyraldehyde; (6) 2-methylbutyraldehyde; (7) hexyl aldehyde; (8) heptyl aldehyde; (9) octyl aldehyde.

Fig. 2. Thin-layer chromatogram of some aromatic and terpenoid aldehyde hydrazones of 2-hydrazinobenzothiazole on Silica Gel F_{254}. Solvent: light petroleum (b.p. 30–40°)–ethyl acetate–acetic acid (88:10:2). Hydrazones of: (1) hydroxycitronellal; (2) salicylaldehyde; (3) anisaldehyde; (4) *p*-tolualdehyde; (5) benzaldehyde; (6) cuminyl aldehyde; (7) citral; (8) citronellal.

zones are crystalline except those of isovaleraldehyde and citral, which are obtained as waxy solids.

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1 F. C. HUNT, *J. Chromatog.*, 35 (1968) 111.
2 E. SAWICKI AND T. W. STANLEY, *Microchim. Acta*, 4 (1960) 510.

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