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A selective staining test for the sensitive detection of some rare amino acids

Generally, in the location of amino acids on chromatograms, ninhydrin reagents are used, some of which have been modified for polychromatic detection[1,2]. However, the need sometimes arises, as in comparative phytochemical studies, for more selective reagents to detect all members of a group of compounds or an individual amino acid[1,3].

Comparatively few selective staining tests, however, are available in this field. *o*-Diacetylbenzene[4,5] has been used for the location of proteinogenic amino acids[5–8], aliphatic and aromatic primary amines[9,10], peptides and native protein[5]. *o*-Diacetylbenzene has been used in histochemical protein detection[11–13] and in topochemical studies of cellular proteins[14,15].

This paper describes the use of *o*-diacetylbenzene in a very sensitive and selective staining test for the detection of the four non-proteinogenic amino acids, L-α,β-diaminopropionic acid, L-α-amino-β-ureidopropionic acid (L-albizziine), L-mimosine and D-azetidine-2-carboxylic acid, separated by thin-layer or paper chromatography.

Materials and methods

Origin of materials and fine chemicals. All the materials and fine chemicals used were commercially available: MN 300 HR-cellulose (Macherey, Nagel & Co., Düren, G.F.R.); Selecta-cellulose No. 144 Avicel (Schleicher & Schüll, Dassel, G.F.R.); Selecta chromatographic paper No. 2043 bMgl (Schleicher & Schüll); *o*-diacetylbenzene (Schuchardt, Munich, G.F.R.); L-α,β-diaminopropionic acid monohydrochloride (Calbiochem, Los Angeles, U.S.A.); L-α-amino-β-ureidopropionic acid (L-albizziine) (Koch-Light Laboratories, Ltd., Colnbrook, Bucks., Great Britain); L-mimosine (Calbiochem); and D-azetidine-2-carboxylic acid (Calbiochem).

Preparation of cellulose layers. The MN 300 HR-cellulose plates were prepared as described earlier[2]. Microcrystalline cellulose powder (Avicel) (15 g) was suspended in 50 ml of water and homogenized for 1 min. The slurry was spread over four glass plates (20 × 20 cm) at a thickness of 250 μm, and dried overnight at ambient temperature in the horizontal position. Chromatographic papers and thin layers were marked as described earlier[2].

Standard solutions. Stock solutions (1 m*M*) of amino acids were prepared using 30% aqueous ethanol as the solvent.

Mobile phase. n-Butanol–glacial acetic acid–water (12:3:5) was used as the mobile phase.

Spray reagent. The reagent, consisting of a 0.3% solution of *o*-diacetylbenzene in methanol, was sprayed on to the thin layer or paper. The chromatograms were evaluated at room temperature under visible and UV light.

Results and discussion

The test was applied to the selective detection of L-α, β-diaminopropionic acid, L-α-amino-β-ureidopropionic acid (L-albizziine), L-mimosine and D-azetidine-2-carboxylic acid. All the usual proteinogenic amino acids and a great number of non-proteinogenic amino acids were proved[2] to react with *o*-diacetylbenzene, but not in a

comparatively selective manner as with the above four compounds. These other amino acids give a blue-violet colour in visible light. A positive fluorescent reaction (360 nm) was not found. In order to estimate the sensitivity of the selective staining test, the rare compounds were separated one-dimensionally on thin layers of MN 300 HR-cellulose and microcrystalline cellulose, and by paper chromatography. After development, the plates and papers were dried at room temperature. The amino acids were located by using *o*-diacetylbenzene in visible light (red-violet background) and UV light (360 nm; grey background). Colour reactions obtained by both means of detection are shown in Table I. The colours are stable for at least several weeks. For a positive test in visible light, larger amounts of the amino acids or a longer time of colour development were required. For the detection of separated compounds on the thin layers under UV light, the time after spraying was varied (Table II). This location

TABLE I

COLOUR REACTIONS GIVEN BY RARE AMINO ACIDS

Compound	*Colour reactions*	
	Visible light	*UV light (360 nm)*
L-α,β-Diaminopropionic acid	Sulphur yellow	Pale blue
L-Albizziine	Yellow-brown	Dark blue
L-Mimosine	Green	Yellow-green
D-Azetidine-2-carboxylic acid	Pink	Lilac

TABLE II

MINIMUM AMOUNTS OF AMINO ACIDS DETECTED BY *o*-DIACETYLBENZENE AND EVALUATED IN UV LIGHT (360 nm)

Compound	*Amount (μM)*	*Time after spraying (h)*
L-α,β-Diaminopropionic acid	0.001	0.5
L-Albizziine	0.05	20.0
L-Mimosine	0.001	2.0
D-Azetidine-2-carboxylic acid	0.05	20.0

method is most sensitive for L-α,β-diaminopropionic acid and L-mimosine, which could be detected on chromatograms spotted with a concentration of 0.001 μM. The sensitivity of the test is reduced on paper chromatograms. Larger amounts of L-albizziine and D-azetidine-2-carboxylic acid or a longer time after spraying are required for a positive test.

Different acidic or basic mobile phases used for the one-dimensional development did not influence the colour reaction.

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