Thin-layer chromatography of imino-acids

Imino-acids in plants are of three main types, based on the 4-, 5-, and 6-membered ring systems of azetidine, pyrrolidine and piperidine, respectively. Azetidine-2-carboxylic acid is widely distributed in the *Liliaceae* and occurs in a few species of *Agavaceae*, but is otherwise a rare plant component[1]. Of the pyrrolidine compounds, 4-hydroxyproline is a constituent of a few plant proteins, and proline is of widespread occurrence. Several piperidine compounds have been isolated from plants, including piperidine-2-carboxylic acid[2,3], 5-hydroxypiperidine-2-carboxylic acid[4], 4-hydroxypiperidine-2-carboxylic acid[5] and 1,2,3,6-tetrahydropyridine[3]. In several of the papers describing the characterisation of these piperidine compounds, evidence has been obtained by comparing the chromatographic characteristics of the unknown compound with synthesised reference compounds[2-6]. The chromatographic methods described have been with paper, but the solvent systems used have given only small differences in R_F values between the compounds, and hence the time of development required for effective separation of the compounds was lengthy. In recent work on *Salix fragilis* L. leaf galls, a new imino-acid was encountered, probably based on piperidine. For the study of this compound, thin-layer chromatographic methods were developed which gave discrete and rapid separations of the different imino-acids.

Experimental and results

Air dried silica gel G (Merck) layers, 250 μ thick, on glass plates 20 × 20 cm were used. The test compounds, in aqueous solution, were applied near one corner of the plate, the spot was dried with a hot air blower and developed using the supersaturated method of STAHL[7]. Two-way chromatograms were prepared using solvent systems that have been used successfully for the separation of amino-acids[8]. For the first direction, development was with chloroform–methanol–17 % ammonia (2:2:1, v/v), and for the second direction the solvent used was either (a) propanol–water (64:36, w/w), (b) *n*-butanol–acetic acid–water (60:20:20, w/w), or (c) phenol–water (4:1, w/w). Development in each direction was for 15 cm from the point of application of the spot. The spots were detected with an 0.1 % w/v solution of ninhydrin in acetone and with an 0.2 % w/v solution of isatin in *n*-butanol containing 4 % acetic acid. The colours were developed by heating the plates at 100° for 10 min (Table I). The compounds examined were piperidine-2-, piperidine-3-, and piperidine-4-carboxylic acids;

TABLE I

IMINO-ACID COLOURS PRODUCED WITH ISATIN AND NINHYDRIN

Compound	*Isatin*	*Ninhydrin*
Piperidine-2-carboxylic acid	blue-green	mauve to blue
Piperidine-3-carboxylic acid	blue-green	mauve to blue
Piperidine-4-carboxylic acid	blue-green	mauve to blue
4-Hydroxypiperidine-2-carboxylic acid	weak green	yellow changing to blue
5-Hydroxypiperidine-2-carboxylic acid	blue-green	mauve to blue
Compound from *Salix fragilis* galls	blue-green	mauve to blue
4-Hydroxyproline	blue-green	yellow changing to pink
Proline	green-blue	yellow

4-hydroxy- and 5-hydroxypiperidine-2-carboxylic acids; 4-hydroxyproline, proline and an imino-acid from *Salix fragilis* leaf galls, probably based on piperidine. Using the two-way chromatographic systems described, all the imino-acids tested were easily separated and clearly identified (Fig. 1).

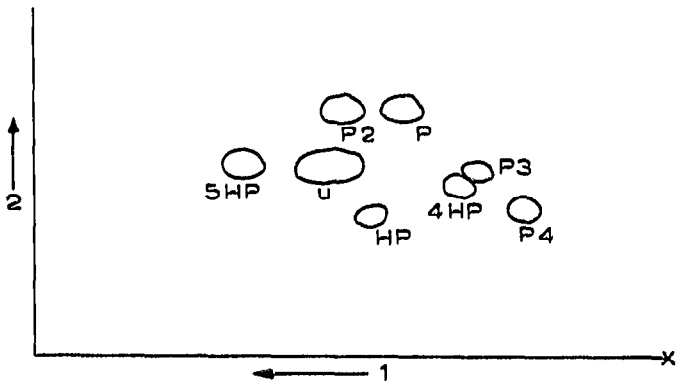

Fig. 1. Thin-layer chromatogram of imino-acids. 5HP = 5-hydroxypiperidine-2-carboxylic acid; 4HP = 4-hydroxypiperidine-2-carboxylic acid; P2 = piperidine-2-carboxylic acid; P3 = piperidine-3-carboxylic acid; P4 = piperidine-4-carboxylic acid; P = proline; HP = 4-hydroxyproline; u = compound from *S. fragilis* galls. Solvent systems; (A) chloroform–methanol–17% ammonia (2:2:1, v/v) and (B) phenol–water (4:1, w/w).

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