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Note

Separation of some tyrosine, tryptophan and phenylalanine derivatives by thin-layer chromatography

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Investigations on the pathways of synthesis of natural plant growth hormones from the aromatic amino acids tyrosine[1], tryptophan[2,3] and phenylalanine[1,4] have led to a need for the development of separation techniques for postulated intermediates of related chemical structure. These metabolites are extracted from the plant and fractionated into neutral, acidic or basic ether fractions[2]. From these organic fractions, some tryptophan and phenylalanine metabolites have previously been separated by paper chromatography[2–4] and thin-layer chromatography (TLC)[3,4].

This paper describes the separation by TLC of a more complete list of authentic aromatic metabolites which may be involved in auxin biosynthesis. We include the description of the resolution of the 2,4-dinitrophenylhydrazone derivatives of ketone- or aldehyde-containing compounds[3] which have been shown to be very labile to extraction procedures in the underivatized form[5].

EXPERIMENTAL

Authentic standards

Phenylacetic acid was purchased from Aldrich, cinnamic acid from Eastman, benzoic acid from Fisher, benzaldehyde and *p*-hydroxybenzaldehyde from ICN Pharmaceuticals, phenylacetaldehyde and *p*-coumaric acid from K & K Labs., 3-indolylacetic, 3-indolylpropionic, 3-indolylacrylic and 3-indolyllactic acids from Nutritional Biochemical Corp., phenylpropionic and *p*-hydroxyphenylpropionic acids from Pfalz and Bauer and tryptophol from Regis. 3-Indolylaldehyde, *p*-hydroxyphenylethanol and *p*-hydroxyphenylacetaldehyde were synthesized by M. T. Ceska, Department of Chemistry, Carleton University, Ottawa, Canada. All other standards were bought from Sigma.

Solvent systems

The solvent systems used for the separation of the authentic aromatic substances are listed in Table I.

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TABLE I

SOLVENT SYSTEMS USED FOR TLC SEPARATION

No.	*Components*	*Development time (h)*
S1	Isopropanol–57% ammonia solution water (10:1:1)	6
S2	Chloroform acetic acid (9:1)	3
S3	Benzene–1,4-dioxane acetic acid (90:25:4)	3
S4	*n*-Butanol–ethanol–57% ammonia solution water (4:4:1:1)	5
S5	Isopropanol–57% ammonia solution–water (85:15:10)	6
S6	Benzene–ethyl acetate–acetic acid (90:5:5)	1
S7	Benzene	1
S8	Methanol–57% ammonia solution–water (80:10:10)	6
S9	Ethanol–57% ammonia solution–water (100:18:10)	3
S10	Chloroform–acetic acid (95:5)	3
S11	Benzene–methanol–acetic acid (45:8:4)	2
S12	Benzene–methanol (75:25)	2
S13	Carbon tetrachloride–acetic acid (50:1)	2
S14	Carbon tetrachloride–acetic acid (9:1)	2
S15	Isopropanol 1-butanol–*tert.*-butanol–57% ammonia solution–water (40:20:20:10:10)	5
S16	Carbon tetrachloride–acetic acid (4:1)	2
S17	Chloroform–ethyl acetate–formic acid (105:100:25)	4
S18	Ethyl acetate isopropanol–57% ammonia solution (45:35:20)	5
S19	Chloroform–carbon tetrachloride–methanol (2:1:1)	3
S20	Chloroform–ethanol (13:7)	4
S21	Ethyl acetate–isopropanol water (65:24:1)	5
S22	Benzene–methanol–acetic acid (75:23:2)	2

Development

The TLC plates used were 20 × 20 cm silica gel 60 plastic-backed plates with F-254 fluorescent indicator, layer thickness of 0.2 mm (E. Merck, Darmstadt, F.R.G.). Each chromatogram was developed at 20°C over a distance of 15 cm in a 7 × 25 × 25 cm glass chamber lined with filter-paper and pre-saturated for 1 h. All solvent systems were freshly prepared and used for a maximum of two developments.

TABLE II

R_F VALUES OF AUTHENTIC STANDARDS FOR ACIDIC TYROSINE-DERIVED METABOLITES

The compounds were developed on silica gel 60 F_{254} pre-coated plastic plates 20 × 20 cm (Merck) over a distance of 15 cm, in glass chambers previously saturated for 1 h. STR means streaks.

Compound	*Solvent*				
	S1	*S2*	*S3*	*S4*	*S5*
p-Hydroxyphenyllactic acid	0.28	0.05	STR	0.14	0.26
p-Coumaric acid	0.33	0.50	0.44	0.17	0.32
p-Hydroxyphenylacetic acid	0.28	0.35	0.40	0.12	0.28
p-Hydroxybenzoic acid	0.28	0.43	0.48	0.10	0.28
p-Hydroxyphenylpropionic acid	0.35	0.47	0.50	0.08	0.35

TABLE III

R_F VALUES OF AUTHENTIC STANDARDS FOR ACIDIC TRYPTOPHAN-DERIVED METABOLITES

Conditions as in Table II.

Compound	*Solvent*								
	S1	*S10*	*S12*	*S17*	*S18*	*S19*	*S20*	*S21*	*S22*
3-Indolyllactic acid	0.35	0.04	0.08	0.40	0.33	STR	STR	0.25	–
3-Indolylacrylic acid	0.40	0.36	0.38	0.70	0.36	0.57	0.78	0.78	0.55
3-Indolylacetic acid	0.35	0.39	0.32	0.72	0.32	0.50	0.75	0.75	0.53
3-Indolylcarboxylic acid	0.32	0.33	0.42	0.73	0.30	0.57	0.75	0.82	0.50
3-Indolylpropionic acid	–	0.42	0.36	–	–	–	–	–	0.37

TABLE IV

R_F VALUES OF AUTHENTIC STANDARDS FOR PHENYLALANINE-DERIVED METABOLITES

Conditions as in Table II. DEC means decomposes.

Compound	*Solvent*												
	S3	*S4*	*S5*	*S6*	*S8*	*S9*	*S10*	*S11*	*S12*	*S13*	*S14*	*S15*	*S16*
Phenyllactic acid	0.19	0.27	0.49	0.00	0.73	0.77	0.65	0.18	0.05	0.00	0.39	0.28	0.04
Cinnamic acid	0.52	0.24	STR	DEC	0.73	STR	0.50	0.69	0.28	0.08	0.37	0.25	0.35
Phenylacetic acid	0.51	0.23	0.30	0.27	0.69	0.68	0.46	DEC	0.36	0.60	0.33	0.22	0.32
Benzoic acid	0.53	0.23	0.34	DEC	0.72	0.72	0.50	0.68	0.36	0.10	0.40	0.24	0.41
Phenylpropionic acid	0.56	0.25	0.35	0.33	0.68	0.70	0.55	–	0.42	0.09	0.39	0.25	0.35

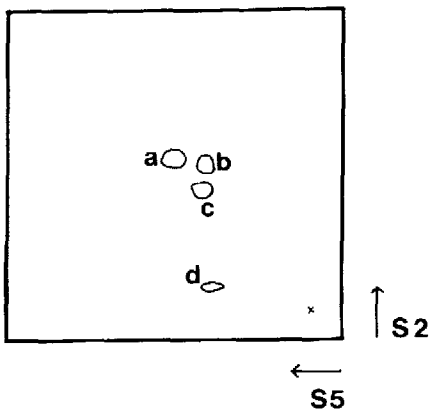

Fig. 1. Description of the location of *p*-hydroxyphenyl acids after two-dimensional chromatography, first in S2, then in S5. (a) Combined location of *p*-coumaric and *p*-hydroxyphenylpropionic acids; (b) *p*-hydroxybenzoic acid; (c) *p*-hydroxyphenylacetic acid; (d) *p*-hydroxyphenyllactic acid.

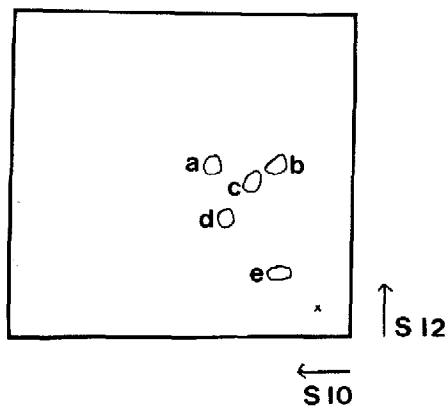

Fig. 2. Description of the location of indolic acids after two-dimensional chromatography, first in S12, then in S10. (a) 3-Indolylpropionic acid; (b) 3-indolylcarboxylic acid; (c) 3-indolylacrylic acid; (d) 3-indolylacetic acid; (e) 3-indolyllactic acid.

TABLE V

R_F VALUES FOR BASIC AND NEUTRAL AUTHENTIC SUBSTANCES

Conditions as in Table II.

Type	*Compound*	*Solvent*		
		S1	*S3*	*S5*
Basic	*p*-Hydroxyphenylethylamine	0.42	0.00	0.40
	Phenylethylamine	–	0.05	0.48
	Tryptamine	0.58	–	0.52
Neutral	*p*-Hydroxyphenylethanol	–	0.52	–
	Phenylethanol	–	0.65	–
	Tryptophol	0.70	0.25	–

Detection

For visual detection of indole compounds on the TLC plates, the modified Van Urk–Salkowski reagent[6] was used and for *p*-hydroxyphenyl substances the plates were sprayed with Folin–Ciocalteu reagent[7]. Phenyl compounds were detected by examination under UV light (254 nm). The dinitrophenylhydrazone derivatives were easily seen under normal light owing to their yellow colour.

RESULTS AND DISCUSSION

Tables II–IV summarize the results obtained with solvent systems found to give partial separation of the authentic standards of the acidic derivatives of tyrosine, tryptophan and phenylalanine. It was observed that the use of a second dimensional

TABLE VI

R_F VALUES OF 2,4-DINITROPHENYLHYDRAZONE DERIVATIVES OF AUTHENTIC SUBSTANCES

All chromatograms were run with three successive developments in the same dimension.

Compound	*Solvent*	
	S6	*S7*
p-Hydroxyphenylpyruvic acid	0.17	0.34
	0.28	0.50
p-Hydroxybenzaldehyde	0.46	0.10
p-Hydroxyphenylacetaldehyde	0.80	0.34
Phenylpyruvic acid	0.38	0.34
	0.42	0.46
Benzaldehyde	0.87	0.57
Phenylacetaldehyde	0.68	0.52
	0.77	
3-Indolylpyruvic acid	0.13	0.33
	0.18	0.46
3-Indolylaldehyde	0.52	0.04
3-Indolylacetaldehyde	0.08	0.78

development was more appropriate for optimal resolution of tyrosine and tryptophane metabolites (Figs. 1 and 2). Tyrosine metabolites were separated by development in the first dimension with S2 and in the second dimension with S5. Complete resolution of *p*-coumaric and *p*-hydroxyphenylpropionic acids could not be achieved (Fig. 1). The five homologous compounds derived from tryptophan were, however, resolved using S12 first, followed by S10 in the second dimension (Fig. 2). Kaldewey[8] reported the separation of these indole acids in S10 using one development; however, we found the resolution of 3-indolylacetic acid from 3-indolylacrylic acid to be optimized by using first S12, then S10 as a second-dimensional solvent system. Partial resolution of the phenylalanine metabolites was obtained with S16 in one dimension; we could not achieve a complete separation of cinnamic and phenylpropionic acids using a second dimension development.

Table V illustrates the separation of basic metabolites from the three aromatic amino acids which was obtained using S5, and the separation of the neutral metabolites resolved by S3. The R_F values of ketone- or aldehyde-containing compounds derivatized to their 2,4-dinitrophenylhydrazones[3] appear in Table VI; after three successive developments in the same dimension, all pyruvic acids present two distinct bands corresponding most probably to their *syn*- and *anti*-isomers. The double band observed for phenylacetaldehyde in S6 is hypothesized to be due to the separation of a monomeric and polymeric form of the molecule[9].

CONCLUSIONS

The chromatographic systems described allow a rapid separation of many authentic derivatives of tyrosine, tryptophan and phenylalanine. These techniques were used with plant extracts[1,5] and shown, as checked by gas–liquid chromatography, to give a satisfactory separation of the endogenous substances as well.

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