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Separation of indole derivatives and catecholamines by thin-layer chromatography

Various papers have been published concerning thin-layer chromatographic (TLC) separation of tyrosine and tryptophan metabolites from norepinephrine (NE)[1–5] and serotonin[7–12]. GIESE *et al.*[5] succeeded in separating NE and its metabolites, CHOULIS[2] successfully separated dopamine, epinephrine and NE. SCHNEIDER AND GILLIS[10] achieved a satisfactory separation of dopamine, DOPA and NE by a two-dimensional run. SANDLER AND RUTHVEN[8] reported R_F values for catecholamines (CA) found with different solvent systems. COWLES *et al.*[3], AURES *et al.*[1] and PILLAY AND MEHDI[7] described the separation of indole compounds.

All the R_F values determined by the above authors were unsuitable, or alkali solutions had been used which contributed to the decomposition of the substances in question.

For a series of pharmacological and enzymic kinetic experiments, it appears desirable, however, to separate all NE and 5-HT precursors and metabolites by TLC. Recently FLEMING AND CLARK[4] reported a method which permits isolation and identification of CA by TLC. The required two-dimensional run in their method, however, is time-consuming and has additional difficulties connected with the evaluation of radioisotope experiments.

In this paper, a method is reported for the TLC separation of tyrosine, DOPA, Dopamine and NE, as well as the main metabolites normetanephrine and 3-methoxy-4-hydroxymandelic acid by a one-dimensional run. Isolation, identification and quantitative measurement of the tryptophan metabolites (serotonin route) is possible using the same technique.

For these substances, the solvent system of GIESE *et al.*[5] proved to be successful.

Experimental

Materials. 1, L-tyrosine, puriss; 2, L-3-(3,4-dihydroxyphenyl)alanine (DOPA), puriss; 3, 3-hydroxytyramine hydrochloride (dopamine); 4, L-norepinephrine hydrochloride; 5, D,L-normetanephrine hydrochloride; 6, D,L-4-hydroxy-3-methoxymandelic acid; 7, D,L-5-hydroxytryptophan (5-HTP); 8, D,L-tryptophan; 9, 5-hydroxytryptamine (serotonin); 10, 5-hydroxyindoleacetic acid (5-HIAA); 11, urea; 12, tryptamine; 13, dimethyltryptamine (DMT); 14, bufotenine; 15, 5-methoxyindoleacetic acid (5-MeOIAA); 16, indoleacetic acid (IAA); 17, melatonine. Suppliers were Fluka (1–3, 6–8, 10–17), Roth (5, 9), Merck (11) and Hoechst (4).

Solvent systems. (A) *n*-Butanol–5 *N* acetic acid (100:35). (B) Butan-2-one (ethyl methyl ketone)–acetone–2.5 *N* acetic acid (40:20:20).

Detection. The spots of the CA were visualized by spraying the plates with *p*-nitroaniline[13]. The indoles were sprayed with *p*-dimethyl aminocinnamic aldehyde (*p*-DMCA).

Thin-layer plates. For the indoles, using cellulose MN 300 (Macherey & Nagel, Düren, G.F.R.), plates were prepared by mixing 16 g cellulose and 100 ml water with an ESGE stirrer. Plates were coated by means of a Camag model to a thickness of

0.3 mm and were dried in the open air. For the CA, however, precoated cellulose plates, 20 × 20 cm, (Schleicher & Schüll, Dassel, G.F.R.) gave better results.

Separation of compounds. The tyrosine metabolites were dissolved in water at a temperature of 22°. No change in chromatographic behaviour could be found by attempting to dissolve all substances in 0.01 *N* HCl. Indoles were dissolved in a solution containing dilute HCl and methanol.

For the indoles, 50 ng in 10 μl were always applied, and for the CA, 1 μg in 10 μl.

The chambers with saturated solutions were protected against the influence of direct light. Chromatograms were ascending, one-dimensional.

Quantitative analysis of the indoles. Quantitative analysis was by remission or transmission in a Zeiss spectrophotometer for TLC (No. CA 2).

TABLE I

TLC BEHAVIOUR OF TYROSINE DERIVATIVES TOWARDS NE ON CELLULOSE-PREPARED PLATES (SOLVENT SYSTEM B)

Running time for one running procedure; 2–2 ½ h.

Substance	*hR_F values*	*Colour impression*
Tyrosine	29	Pink
DOPA	16	Blue–grey
Dopamine	52	Pale blue
Norepinephrine	38	Grey–greyish blue
Normetanephrine	47	Blue–lilac
3-Methoxy-4-hydroxymandelic acid	89	Lilac

Results and discussion

Table I shows the separation of the tyrosine derivatives on cellulose-precoated plates, from which it can be seen that the sharpness of separation of these compounds was excellent. The method was applicable to both mixed and pure substances. Variations in the distance of travel from the origin showed that a sharp separation could also be attained by shorter distances (12 cm; compared with the usual 18.5 cm).

Table II shows the separation of some tryptophan metabolites. For the examination of 5-HTP metabolism in the brain, we used solvent system A, which is suitable for the separation of radioactively labelled 5-HTP, 5-HT and 5-HIAA (unpublished results).

500 ng is quoted[11] as the lowest detection limit, when serotonin is stained with *p*-DMCA on silica gel. Polyamide plates are not suitable for indole visualization with *p*-DMCA because of the green staining on the layer[6]. Using the present method, 5 ng of serotonin can be detected using cellulose as sorbent. Quantitative densitometric analysis of serotonin is possible by transmission measurement down to 10 ng (to be published). This technique is not applicable to animal experiments, where urea and tryptophan are co-chromatographed, since their R_F values are unsuitable for the densitometric determination of serotonin.

A solvent system therefore had to be found for the determination of serotonin in tissue which satisfactorily separated the physiological substances 1–5 (Table II). Of the sixty solvent systems, which were tested on small-scale chromatograms

TABLE II

TLC SEPARATION OF TRYPTOPHAN METABOLITES OF THE INDOLE TYPE ON CELLULOSE PLATES (SOLVENT SYSTEMS A AND B)

The running time of the solvent system A is 2 h and of processing solution B it is 1 h, with a separation run of 12 cm.

No.	*Substance*	*hR_F values*		*Colour impression*[a]
		A	*B*	
1	D,L-5-HTP	15	35	Blue
2	D,L-Tryptophan	33	50	Lilac
3	Serotonin	35	65	Blue
4	5-HIAA	85	98	Blue
5	Urea	40	57	Red
6	Tryptamine	71	65	Lilac
7	DMT	72	71	Lilac
8	Bufotenin	47	75	Blue
9	5-MeOIAA	98	98	Blue
10	IAA	98	98	Lilac
11	Melatonin	98	98	Blue

[a] It is observed in the colour reaction that the replacement of HCl in position 5 in the indole ring by a hydroxy or methoxy group causes a change of colour from violet to blue.

(3 × 10 cm), it was found that alkaline solvent systems could not be used because of the sensitivity of the substances. Use of the alcohol- and ester-containing solvent systems had to be restricted, as serotonin formed a widely scattered spot. The combination of water, acetic acid and acetone yielded hR_F values around 98. We therefore used a mixture of a higher-molecular-weight ketone — ethyl methyl ketone — in which the solubility of the substances was reduced.

Solvent system B, obtained in this manner, is especially suitable for the separation of the substances 1–5 (Table II) and allows densitometric determination of serotonin and tryptophan. 5-HIAA is not suitable for densitometric determinations because of high R_F values, whereas 5-HTP is not suitable because of the widely scattered spotting. Experiments using this method in animal tissue, especially brain, are in progress.

The TLC separation of the NE precursors and main metabolites achieved may be a good method for some enzymic kinetic examinations of NE formation. The quantitative estimation of radioactive compounds on a single plate with a one-dimensional run seems possible.

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