

Copper nitrate ^b		Ceric ammonium nitrate ^b /silica ^c	FeCl ₃ - K ₃ [Fe(CN) ₆] ^b /silica ^c	Methyl red-NaOBr ^b /silica ^c
silica ^c	Cellulose ^b			
no stain		no stain	faint blue ^e	faint orange
white	grey ^e	no stain		
no stain		no stain	no stain	no stain
no stain	faint grey	faint white	faint blue ^e	no stain
no stain	no stain	faint white	faint blue ^e	orange
white	white	no stain	faint white ^e	no stain
			faint blue ^e	no stain
			no stain	no stain
			white	no stain

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The thin-layer chromatography of the hydroxyindole-3-carboxylic acids*

The importance of hydroxyindoles in metabolic processes in plants and animals is now generally recognized¹⁻³. Recent progress in the development of new and improved methods, including chromatographic procedures, for the detection and determination of hydroxyindoles in biological materials have been reviewed by SANDLER^{4,5}.

Two of the main problems that are often encountered in the use of paper and thin-layer chromatographic procedures for the detection and identification of hydroxyindoles are the lack of suitable standard compounds and reliable data on their R_F values. The chromatographic behaviour of all four isomeric hydroxyindoles (hydroxylated in the benzene ring) has only been studied in a relatively few cases including indole⁶, skatole^{7,8}, tryptamine^{9,10}, tryptophan⁶, acetyltryptophan¹⁰ and oxindole¹¹. The 4-, 5-, 6- and 7-hydroxyindole-3-carboxylic acids have recently all been synthe-

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TABLE I

 R_F VALUES AND COLOUR REACTIONS^a OF INDOLE-3-CARBOXYLIC ACID AND THE HYDROXYINDOLE-

	<i>Indole-3-carboxylic acid derivative</i> (R_F values $\times 100$) ^b	
	<i>Indole-3-carboxylic acid</i>	<i>4-Hydroxyindole-3-carboxylic acid</i>
S_1^c	73	29
S_2^c	67	16
S_3^c	87	28
S_4^c	75	19
$S_5^{c,d}$	79	73
$S_6^{d,e}$	64	70
S_6^f	92	76
Gibb's reagent	Orange	Deep blue → Deep maroon (pink centre) (brown centre)
<i>p</i> -Dimethylaminocinnamaldehyde	Pale blue → dark blue	Pale blue-violet → pale blue
Chloranil	Grey-brown → pale brown	Grey-green
Van Urk's reagent	Yellow → purple	Pale blue
2,3-Dichloro-5,6-dicyano- <i>p</i> -benzoquinone	Olive (blue centre) → olive	Grey-blue → blue
Ehrlich's reagent	Pink	Deep blue
Diazotized sulphanilic acid	Pale yellow	Yellow

^a The colours reported are those observed after 30 min together with any significant changes which subsequently occurred when the plates were allowed to stand overnight after spraying.

^b The R_F values reported are the average of three determinations.

^c These R_F values were obtained using commercially available Merck precoated Silica Gel F₂₅₄ plates (thickness 0.25 mm).

sized by unambiguous routes in these laboratories¹² and this communication reports the results of some investigations into the TLC properties of this group of hydroxyindoles.

Experimental

Indolecarboxylic acids. Indole-3-carboxylic acid¹³ and the 4-, 5-, 6- and 7-hydroxyindole-3-carboxylic acids¹² were prepared by the methods described in the literature.

Thin-layer chromatographic procedures. The following plates were used: (a) commercially available Merck Silica Gel F₂₅₄ plates (20 × 20 cm, thickness 0.25 mm), (b) Merck Silica Gel GF₂₅₄ plates (20 × 20 cm, thickness 0.5 mm) prepared in the laboratory, and (c) commercially available Merck Cellulose F plates (20 × 20 cm, thickness 0.1 mm).

The solvents used were: (S_1) chloroform-methanol (7:3); (S_2) chloroform-dioxane-methanol (7:3:2); (S_3) chloroform-dimethylformamide-methanol (7:3:2); (S_4) diisopropyl ether-dimethylformamide-methanol (8:2:1); (S_5) diethyl ether-light petroleum*—acetic acid (75:25:2); (S_6) benzene-ethyl acetate-water-acetic acid (55:25:50:30).

The two-phase solvent system S_6 was only used with the cellulose plates. After the compounds being studied had been applied, the plates were exposed to the

* B.D.H. "AnalaR" grade (b.p. 60–80°).

CARBOXYLIC ACIDS

<i>Hydroxyindole-3-carboxylic acid</i>	<i>6-Hydroxyindole-3-carboxylic acid</i>	<i>7-Hydroxyindole-3-carboxylic acid</i>
	55	60
	44	51
	65	67
	47	54
	45	64
	32	51
	27	53
iolet	Grey-brown → brown	Blue → Grey-green (greenish centre) (yellow centre)
pale blue-violet → pale blue	Red-violet → light blue	Green (fades)
grey-red → grey	Grey → light brown	Grey-blue → pale brown
brown → light purple	Brown → pale brown	Blue (fades)
live	Olive-grey → olive	Brown → brown (pink centre)
iolet-purple	Grey-blue → pale blue	Pale blue → deep blue
orange-brown → orange	Yellow-brown → brown	Orange-red → brick-red

^a Double development used: during the first development the solvent was allowed to ascend 7.0 cm, the plate was removed from the tank, dried and then a second development, involving solvent rise of 14.0 cm, was carried out.

^b R_F values obtained on plates prepared in the laboratory using Merck Silica Gel F₂₅₄ (thickness 0.5 mm).

^c R_F values obtained on commercially available Merck precoated Cellulose F plates.

vapours of the aqueous phase for 1 h, and then quickly transferred to a tank containing the organic phase for development.

Detection. The following chromogenic reagents were prepared by the methods described in the literature (*cf.* refs. 14 and 15). (i) Gibb's reagent (neutral) (a 1% solution of N,2,6-trichloro-*p*-quinoneimine in ethanol). (ii) *p*-Dimethylaminocinnamaldehyde (a solution of *p*-dimethylaminocinnamaldehyde (2 g) in a mixture of 6 N HCl (100 ml) and ethanol (100 ml). This solution was diluted with 5 vol. of ethanol immediately prior to use). (iii) Chloranil (1% solution in chloroform). (iv) Van Urk's reagent (a solution of *p*-dimethylaminobenzaldehyde (0.125 g) in 100 ml of 65% H₂SO₄, containing 0.1 ml of 5% aqueous ferric chloride). (v) 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (a 1% solution in chloroform). (vi) Ehrlich's reagent (a 1% solution of *p*-dimethylaminobenzaldehyde in a mixture of hydrochloric acid (25 ml) and methanol (75 ml)). (vii) Diazotized sulphanilic acid (acid, in the presence of excess nitrous acid). A 5% aqueous solution of sodium nitrite (1.5 ml) was added to a solution of sulphanilic acid (1.5 ml, containing 9 g of sulphanilic acid in 90 ml of concentrated hydrochloric acid per liter of water); a further quantity (6 ml) of the aqueous sodium nitrite solution was then added. The solution was diluted to 50 ml with water after standing at room temperature for 5 min.

The developed chromatoplates were sprayed with one of the above seven chromogenic reagents and the colours noted firstly after 30 min and secondly after the plates had been allowed to stand overnight at room temperature.

Results and discussion

The R_F values obtained for indole-3-carboxylic acid and for the 4-, 5-, 6- and 7-hydroxyindole-3-carboxylic acids, using a number of different solvent systems and layers, are given in Table I. The colour reactions given by indole-3-carboxylic acid and the four hydroxyindole-3-carboxylic acids with six chromogenic reagents are also reported in Table I.

There is some information in the literature on the paper chromatographic properties of 5- and 6-hydroxyindole-3-carboxylic acids¹⁰⁻¹⁸, however the use of only a limited number of solvent systems and chromogenic reagents was reported.

The current investigation has shown that the four hydroxyindole-3-carboxylic acids can readily be separated from each other and from indole-3-carboxylic acid. The best solvent system found so far is S_6 . It is of interest to note that better separations of the four isomers and a reversal of the order of the R_F values for indole-3-carboxylic acid and 4-hydroxyindole-3-carboxylic acid can be obtained on plates prepared from Merck Silica Gel GF₂₅₄ powder as compared to Merck precoated silica gel plates. Very compact spots are obtained with this acid-containing system if a double development procedure is used (see footnotes to Table I).

The colour reactions of these compounds with several chromogenic reagents are presented in Table I. Most of the reagents listed give distinctively different colours with the five indole compounds under investigation. The colours observed tend to vary with the concentration of the compound and the time after spraying; careful comparisons with standard compounds are desirable for reliable identifications.

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