purified to meet chromatographic standards. The polyamide film was made by following the earlier literature directions[10]. Visualization was achieved by irradiation of the chromatograms with ultraviolet light (2538 Å), after spraying with Rhodamine B solution[11].

Results and discussion

Table I summarizes the R_F values of eighteen N-benzyloxycarbonyl amino acids and three similar N-benzyloxycarbonyl amino acid ester derivatives in five different solvent systems. The spread of R_F values is sufficient for most purposes. It is planned to extend these results to other amino acid and peptide derivatives in the near future.

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1 B. O. HANDFORD, T. A. HYLTON AND B. WEINSTEIN, *Ohio J. Sci.*, 68 (1968) in press.
2 K. R. WOODS AND K.-T. WANG, *Biochim. Biophys. Acta*, 133 (1967) 369.
3 K.-T. WANG, I. S. Y. WANG, A. L. LIN AND C.-S. WANG, *J. Chromatog.*, 26 (1967) 323.
4 K.-T. WANG AND I. S. Y. WANG, *J. Chromatog.*, 27 (1967) 318.
5 P. SCHELLENBERG, *Angew. Chem.*, 74 (1962) 118.
6 E. EHRHARDT AND F. CRAMER, *J. Chromatog.*, 7 (1962) 405.
7 Z. PRAVDA, K. PODUŠKA AND K. BLÁHA, *Collection Czech. Chem. Commun.*, 29 (1964) 2626.
8 G. PATAKI, *J. Chromatog.*, 16 (1964) 553.
9 M. L. MASKALERIS, E. S. SEVENDAL AND A. C. KILBRICK, *J. Chromatog.*, 23 (1966) 403.
10 K.-T. WANG, I. S. Y. WANG AND A. L. LIN, *J. Chinese Chem. Soc. (Taiwan)*, 13 (1966) 77.
11 D. WALDI in E. STAHL (Editor), *Thin Layer Chromatography*, Academic Press, New York, 1965, p. 499.
12 I.U.P.A.C.-I.U.B. COMMISSION TENTATIVE RULES, *Biochemistry*, 5 (1966) 2485.

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Separation of simple indole derivatives by thin layer chromatography*

In recent years a number of adsorbent systems for thin layer chromatography (TLC) have been described for the successful separation of several compounds. The work reported here is an effort to compare the chromatographic behaviour of 23 simple indole derivatives, their R_F values and color reactions on Eastman Chromagram and Gelman Instant thin layer chromatography (ITLC) sheets with the standard chromatoplates.

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Materials

Glass plates 20 cm × 20 cm × 0.4 cm.

Spreader: standard Desaga type.

Adsorbent: Camag silica gel.

Chromagram sheets: Eastman Kodak Company.

Gelman-Instant thin layer chromatography ITLC sheets, Type SG: Gelman Instrument Company, Ann Arbor, Mich.

Chromatoplates. 25 g of silica gel were vigorously shaken with 50 ml distilled water for 50 sec and the slurry was poured into a Desaga spreader and applied to the plates to a thickness of 250 mμ. The plates were air dried for 10 min to set and then arranged on a rack and dried in an oven at 110° for 30 min. They were cooled and used immediately.

Chromagram. Precoated Eastman Chromagram sheets 20 cm × 20 cm with a 100 mμ layer of silica gel with fluorescent indicator incorporated were activated at 110° for 30 min to provide a highly active layer of adsorption before use.

Instant thin layer chromatography (ITLC). Similarly, Gelman ITLC Silica Gel type SG, with fluorescent indicator, sheets 20 cm × 20 cm were activated at 110° for 30 min and used.

Solvents. The following solvent systems were used (time required in minutes for development in parentheses):

(1) Chloroform–methanol–acetic acid, 75:20:5 (50)

(2) Chloroform–96 % acetic acid, 95:5 (40)

(3) Methyl acetate–isopropyl alcohol–ammonia, 45:35:20 (20)

(4) Isopropyl alcohol–ammonia–water, 100:5:10 (20).

Single dimension ascending chromatography was used. The volume of the solvent used for development was 100 ml. Usually fresh solvent systems were used for every six plates or with each new run.

Indole compounds were dissolved generally in 95 % ethanol or acetone and made up quantitatively to a concentration of 1 mg per ml.

Method

Aliquots equivalent to 0.5 to 5 μg of each indole compound were applied with a Hamilton syringe to the chromatoplates, Chromagram or ITLC sheets and developed by the ascending technique. The solvent was allowed to ascend to a height of 10 cm. The plates or sheets were then removed from the tanks and the solvent completely removed at room temperature, and sprayed with the appropriate detection reagent.

Fresh solutions of the following reagents were used to give color reactions for simple indole derivatives[1]:

(1) Van Urk's reagent. 1 g 4-dimethylaminobenzaldehyde is dissolved in 50 ml HCl (diluted 1:19) and 50 ml ethanol is added to the solution.

(2) Prochazka's reagent. 10 ml of formaldehyde solution 35 %, 10 ml pure HCl 25 %, and 20 ml ethanol.

(3) Ehrlich's reagent. 1% solution of 4-dimethylaminobenzaldehyde in 96% ethanol.

Results and discussion

The color reactions and mean R_F values of 23 indole compounds are given in

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TABLE I

R_F VALUES × 10 AND COLOR REACTIONS OF SIMPLE INDOLE DERIVATIVES

Solvents used: (A) Chloroform–methanol–acetic acid (75:20:5); (B) Chloroform–96% acetic acid (95:5); (C) Methyl acetate–isopropyl alcohol–ammonia (45:35:20); (D) Isopropyl alcohol–ammonia–water (100:5:10).

No.	*Indole derivative*	*Chromatoplates*				*Chromagrams*				*Color with Van Urk's and Ehrlich's reagent*
		A	*B*	*C*	*D*	*A*	*B*	*C*	*D*	
1	Indole	8.8	7.8	8.6	8.0	8.8	7.7	9.0	8.3	Violet
2	Skatole	9.2	7.4	9.0	7.1	8.9	7.5	9.2	8.2	Blue
3	5-Hydroxy-indole	7.6	2.2	8.8	7.6	7.8	2.8	8.9	8.0	Mauve
4	2-Methyl-indole	9.0	6.8	9.2	7.5	9.4	6.3	9.5	8.3	Pink
5	3-Methyl-indole	9.1	7.7	8.7	7.3	9.2	8.8	8.9	7.9	Blue
6	5-Methyl-indole	9.5	7.3	9.4	7.7	9.7	8.0	9.3	7.8	Pink
7	3-Indole-aldehyde	7.7	1.7	8.3	6.2	8.0	3.5	8.9	8.2	Pink
8	Indole-3-acetic acid	9.0	3.6	9.6	7.6	9.1	7.5	9.6	7.8	Blue
9	Indole-3-propionic acid	7.7	5.1	5.3	4.0	8.4	5.6	7.0	5.2	Blue
10	Indole-butyric acid	8.1	3.5	3.9	3.5	8.0	5.5	5.6	5.5	Blue
11	Indole-acrylic acid	7.1	2.5	3.2	4.7	7.3	3.5	4.2	4.5	Light brown
12	5-Hydroxy-indole-3-acetic acid	5.1	0.4	2.5	2.6	5.7	5.7	2.7	2.7	Blue
13	β-Indole-3L-DL-lactic acid	4.5	0.9	5.6	3.5	3.8	0.9	6.7	4.0	Blue
14	Tryptamine	2.4	1.5	7.6	4.2	3.5	2.7	7.7	4.0	Blue
15	5-Hydroxy-tryptamine	0.9	0	5.3	1.7	1.3	7.5	5.9	2.7	Blue
16	Gramine	1.6	0	7.4	0.8	3.2	*	*	*	Light beige
17	DL-Tryptophan	1.2	0	2.3	2.3	1.1	0	3.1	2.8	Blue
18	DL-Hydroxy-tryptophan	0	0	1.6	1.5	0	0	2.5	1.6	Blue-grey
19	5-Methyl-DL-tryptophan	0.9	0	3.0	2.7	1.4	0	4.1	3.7	Blue
20	Isatin	8.3	2.7	7.5	6.0	8.6	3.7	8.5	6.9	Blue
21	Anthranilic acid	7.8	3.9	4.9	3.8	8.5	6.2	6.8	4.2	Intense yellow
22	3-Hydroxy-anthranilic acid	6.6	*	3.6	3.3	6.8	1.2	3.6	3.5	Yellow
23	Xanthurenic acid	2.7	3.7	5.0	4.0	*	*	*	4.0	Dull brown

* Coloured spots not seen in any reagent.

Table I. Little variability in R_F values was experienced either in the chromatoplates or in Chromagram sheets. ITLC results were unsatisfactory. The boundary or border effect was pronounced, while it was negligible in the other two.

While no attempt was made to relate the R_F values to a reference substance, the empasis in this study was to correlate the chromatographic behaviour with the chemical constitution of the indole compounds on Chromagram sheets and ITLC in a variety of solvent systems previously used[1] for chromatoplates. The R_F values $\times$ 10 of acidic compounds usually ranged from 3.0 to 6.0, neutral indoles from 7.0 to 9.0, and the basic compounds were close to the starting point.

The acidic solvent system (chloroform–acetic acid) gave better separations followed by the alkaline system (methyl acetate–isopropyl alcohol–ammonia) for the chromatoplates and chromagrams. ITLC sheets have not been found to accomplish better separation in any of the above solvent systems. ITLC being uneven and extremely brittle presented some difficulty in handling. Increased amounts of material were required for spotting. The normal amount (100 ml) of solvent used for development in other systems was inadequate. Furthermore, it was difficult to determine visibly the distance the solvent had travelled. Poor separation with considerable tailing was experienced in all cases.

Uniformly coated chromagram sheets, in addition to giving satisfactory separations, have certain advantages over the conventional chromatoplates. Small amounts of material are sufficient for spotting and lesser amounts of solvent necessary for development, which travels much faster than in ITLC or chromatoplates. It is less time consuming to prepare the layers, neat and easy to handle, and the results are comparable to those obtained on chromatoplates. However, R_F values may depend upon the quality of adsorbent, the variability in thickness of layer, the quality and nature of solvent and the amount of samples.

Among the three detection reagents used in this study, color reactions by Van Urk's reagent were most specific. Reaction with Ehrlich's reagent gave lighter colors, which darkened overnight, whereas Prochazka's reagent was poor.

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1 E. STAHL, *Thin Layer Chromatography. A Laboratory Handbook*, Academic Press, New York, 1965, p. 483–502.

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