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CHROMATOGRAPHY OF SOME INDOLES ON FORMAMIDE-TREATED PAPER*

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SUMMARY

The radial chromatography of a wide range of indole compounds on formamide-treated paper has been studied. The R_F values and suitable methods for the detection of approximately 150 indole derivatives are reported.

In general, the procedure gives excellent results with most indoles, particularly with relatively non-polar compounds.

INTRODUCTION

The importance of many indole compounds in a wide variety of biological processes has long been recognized, but much of the progress that has been made in the past 20 years or so in plant, animal and microbial indole biochemistry has undoubtedly been due to the development and widespread use of various chromatographic techniques.

A vast number of publications on the paper chromatography of indole compounds have appeared since some of the first papers on this subject were published in the 1950s[1–6]. An extensive bibliography of publications dealing with indole chromatography can be found in the surveys by Macek and co-workers[7–9].

Widespread use has been made of various methods of modifying the properties of the stationary phase in paper chromatography. A procedure that has proved to be of significant value has been that of increasing the polarity of the surface with a non-volatile highly polar solvent. Propylene glycol, dimethylformamide and form-

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amide have been widely used for this purpose. The development of such chromatograms is usually carried out with a non-polar solvent, saturated with the stationary phase[10,11].

Some use has been made of systems of this type for the paper chromatography of certain indole compounds in the past both by the present authors and by other workers[6,12–16]. This paper reports the results of an extensive study of the chromatographic behaviour of a large number of indole derivatives on formamide-treated paper using several different solvent systems. In view of the better resolution and short development times which usually accompany the radial method of chromatography, this procedure was employed throughout the investigation.

MATERIALS AND METHODS

Materials

Indoles. The indole compounds were purchased from commercial sources whenever possible. In some instances, purification of the indole derivative by either recrystallisation or high-vacuum distillation was carried out, after preliminary chromatographic examination of the product had indicated it to be impure. Where the required compounds were not available they were prepared in our laboratories by methods described in the literature. The commercial sources or appropriate literature references are indicated in the tables of R_F values.

Solvents. Commercially available solvents of the highest quality were used in all instances and, if necessary, were redistilled prior to use.

Formamide. The "pH"* of the reagent-grade formamide used both for the stationary phase modification or solvent preparation was adjusted to 7 by the cautious dropwise addition of either concentrated aqueous ammonia or 98% formic acid.

Paper chromatographic procedure

Apparatus. A modified Kawerau (Shandon Scientific Co., London, Great Britain) circular chromatography apparatus was employed. The glass capillary solvent feed supplied with the apparatus was found to be unsatisfactory and was replaced with a soft cotton yarn (1/8-in.) wick (the cotton yarn was washed with acetone prior to use to remove fluorescent material). The solvent was contained in a 6-cm petri dish.

Stationary phase. Whatman No. 1 paper discs (26 cm) were dipped in a 30% solution of formamide ("pH" 7, see above) in acetone; blotted free of excess formamide solution, and allowed to dry in air for 20–30 min prior to use.

Solvent systems. S_1**, decalin (freshly distilled), saturated with formamide; S_2***, methylcyclohexane, saturated with formamide; S_3***, light petroleum (BDH, Poole, Great Britain, AnalaR grade, b.p. 60–80°), saturated with formamide; S_4***,

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* Although the exact meaning of "pH" for a non-aqueous system may be in doubt, the procedure used in this investigation ensures the approximate neutrality of the stationary phase.

** S_1 and S_2 were saturated with formamide by adding a small amount to the solvent, centrifuging the mixture (which separated out only with difficulty) several times and finally using the clear supernatant layer.

*** S_2, S_3, S_4 and S_{12} were saturated with formamide by adding a small amount to the solvent in question, shaking the mixture vigorously, allowing it to stand and the clear supernatant layer was decanted and used.

cyclohexane, saturated with formamide: S_5*, carbon tetrachloride, saturated with formamide: S_6, trichloroethylene, freshly distilled, containing 0.1% of formamide**: S_7***, tetralin, freshly distilled, saturated with formamide: S_8, *o*-dichlorobenzene, containing 0.1% of formamide: S_9, chloroform–benzene (1:1), containing 0.4% of formamide: S_{10}, benzene, containing 0.1% of formamide: S_{11}, ethyl acetate–chloroform (1:4), containing 1% of formamide: S_{12}, 1,1-dichloroethane, freshly distilled, containing 0.25% of formamide: S_{13}, 1,2-dichloroethane, containing 0.8% of formamide: S_{14}, *s*-tetrachloroethane, freshly distilled, containing 0.2% of formamide: S_{15}, furan, freshly distilled, containing 0.1% of formamide: S_{16}, chloroform, containing 0.8% of formamide: S_{17}§, ethyl acetate–benzene (1:3), saturated with formamide: S_{18}, dichloromethane, containing 0.9% of formamide.

Chromogenic reagents

(a) Ehrlich's reagent. *p*-Dimethylaminobenzaldehyde (1 g) was dissolved in a mixture of concentrated hydrochloric acid (30 ml) and ethanol (30 ml) and the solution diluted to 100 ml with water.

(b) 2,4-Dinitrophenylhydrazine reagent. 2,4-Dinitrophenylhydrazine (1.0 g) was dissolved in concentrated hydrochloric acid (30 ml) and the solution diluted to 1000 ml with methanol.

(c) Gibb's reagent. N,2,6-Trichloro-*p*-quinoneimine (2 g) was dissolved in ethanol (100 ml).

(d) Diazotized p-nitroaniline. A 0.3% solution of *p*-nitroaniline in 8% (w/v) hydrochloric acid (25 ml) was diazotized with sodium nitrite solution (1.5 ml of a 5% solution) immediately prior to use.

(e) Nitrose reagent (i.e., nitrite/nitric acid reagent). Sodium nitrite (1 ml of a 5% aqueous solution) was added to a solution of concentrated nitric acid (5 ml) in acetone (45 ml) and the mixture was used directly.

Procedure

Suitably sized spots§§ and adequately intense colour reactions could usually be obtained by applying *ca.* 10–20 µl of solutions (1 mg/ml) of the indole derivatives (in diethyl ether, chloroform or methanol) to the origin of the formamide-treated chromatographic papers. The chromatography was carried out at room temperature: the solvent migrated *ca.* 10–12 cm radially in approx. 25–35 min. In most instances, the spots due to the indole compounds could be located either by viewing the developed chromatograms in ultraviolet light or by spraying them with Ehrlich's reagent. In cases where the compounds were neither coloured nor fluorescent or did not react with this particular chromogenic reagent, one of the other reagents described above was used.

* S_5 was saturated with formamide by adding a small amount to the solvent: the mixture was shaken, allowed to stand, and the clear lower layer separated and used directly.

** In all instances, the percentage of formamide indicated as being added to the solvent is measured by volume.

*** See footnote ** on p. 250.

§ See footnote *** on p. 250.

§§ In radial chromatography, the "spots" usually appear as arc-shaped zones.

TABLE I

NEUTRAL INDOLES

Compound	*Source*[*]	*Detection*[**]	*Rf value*[***] *in solvent system*[§]																
			S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}	S_{15}	S_{16}	S_{17}	S_{18}
Indole	A	Ehr	38	39	45	44	65	76	81	80	87	86	91	89	90	90	SF	88	91	SF
2-Methylindole	B	Ehr	56	56	61	58	80	87	87	91	92	90	94	94	93	SF	SF	SF	SF	SF
3-Methylindole	A	Ehr	65	65	66	67	83	89	90	92	93	93	94	SF	SF	SF	SF	SF	SF	SF
5-Methylindole	B	Ehr	57	57	60	60	77	86	88	88	91	91	92	94	93	SF	SF	SF	SF	SF
1,2-Dimethylindole	C	Ehr	96	95	95	96	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
2,3-Dimethylindole	C	Ehr	77	77	77	77	91	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
2,5-Dimethylindole	C	Ehr	72	71	74	74	84	92	94	94	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
2-Phenylindole	C	Ehr	71	63	67	67	86	92	SF	94	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
1-Methyl-2-phenylindole	D	Ehr	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
4-Chloroindole	C	Ehr	49	44	50	48	70	79	87	86	88	88	92	90	91	90	SF	SF	94	94
5-Chloroindole	C	Ehr	48	44	52	46	71	80	90	87	88	90	92	89	92	90	SF	88	SF	SF
Indole-3-methanol	C	Ehr	21	20	23	21	51	73	67	75	85	80	91	85	90	92	95	90	87	SF
5-Methoxyindole	B	Ehr	26	26	31	27	58	75	73	79	86	83	90	86	89	92	95	91	89	93
4-Benzyloxyindole	B	Ehr	56	52	55	55	86	93	SF	95	95	94	95	SF	95	SF	SF	SF	SF	SF
5-Benzyloxyindole	B	Ehr	58	51	54	55	79	91	95	94	96	94	SF	SF	SF	SF	SF	SF	98	SF
6-Benzyloxyindole	B	Ehr	60	55	60	58	88	93	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
7-Benzyloxyindole	B	Ehr	84	81	82	83	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
5,6-Dimethoxyindole	18	Ehr	6	6	7	7	34	64	47	65	83	69	90	84	88	92	93	92	81	94
5,6-Dimethoxy-2-methylindole	18	Ehr	10	11	13	11	49	76	60	78	90	78	95	91	92	96	96	96	86	98
5,6,7-Trimethoxyindole	19	Ehr	35	35	39	34	77	87	80	90	93	92	SF	93	94	SF	SF	SF	92	SF
5-Methoxy-2-carbethoxyindole	B	Ehr	46	42	48	44	81	90	89	92	93	92	SF	SF	SF	SF	SF	SF	SF	SF
5-Benzyloxy-2-carbethoxyindole	B	Ehr	66	52	58	59	90	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
5-Methoxyindole-3-carboxaldehyde	B	DNPH	0	0	0	0	7	16	16	24	42	28	73	44	62	71	59	62	57	75
5-Benzyloxyindole-3-carboxaldehyde	B	DNPH	0	0	0	0	14	44	48	61	75	67	90	80	87	92	87	87	85	94
Indole-3-carboxaldehyde	B	DNPH	0	0	0	0	3	14	19	25	38	27	75	44	61	63	54	56	59	71
Indole-3-acetaldehyde	B	DNPH	8	7	8	6	24	45	64	47	74	57	90	61	83	88	90	86	82	91

3-Acetylindole	C	Gib	0	0	0	0	6	21	19	31	47	34	77	53	69	76	63	70	66	80
1-Acetylindole	D	Ehr	79	78	79	78	91	93	94	95	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
1,3-Diacetylindole	20	Ehr	27	25	30	25	69	89	SF	92	SF	89	SF	SF	SF	SF	SF	SF	SF	SF
1-(3-Indolyl)-3-butanone	C	Ehr	15	13	17	15	49	76	64	80	87	84	92	90	92	95	SF	93	90	SF
2-Methylene-1,3,3-trimethylindole-*o*-carboxaldehyde	C	DNPH	31	21	23	23	70	89	71	89	94	87	SF	SF	SF	SF	SF	SF	86	SF
Indole-3-aldehyde oxime	C	Gib	0	0	0	0	0	5	6	7	15	11	56	14	34	33	33	22	46	40
Indole-3-aldehyde thiosemicarbazone	C	DNPH	0	0	0	0	0	0	0	0	5	0	32	7	18	27	10	12	18	23
Indole-3-aldehyde azine	C	Gib	0	0	0	0	4	15	46	39	49	44	83	25	80	78	67	61	80	85
Ethyl 3-indoleacetate	B	Ehr	27	25	31	30	68	81	76	83	92	87	92	90	92	SF	SF	95	93	SF
Ethyl 3-indoleglyoxylate	C	Gib	0	0	0	0	11	40	33	51	69	55	91	69	82	88	83	85	80	91
Ethyl 2-methyl-3-indoleglyoxylate	C	Gib	0	0	0	0	14	48	45	69	79	70	91	79	87	90	88	90	87	95
Indole-3-acetamide	E	Ehr	0	0	0	0	0	0	0	0	8	8	30	8	20	32	13	24	14	30
3-Indoleacetonitrile	B	Gib	5	4	6	3	17	48	52	60	74	71	86	85	86	88	90	83	84	90
3-Cyanoindole	21	Gib	0	5	6	6	8	28	45	41	61	52	84	68	75	77	78	72	78	85
3-Cyano-2-methylindole	21	Gib	7	5	6	7	17	47	67	61	77	70	92	80	86	87	87	85	90	93
3,3-Diindolyl disulphide	22	Ehr	5	0	4	4	20	46	77	71	82	84	SF	87	92	93	SF	86	94	SF
3-Thiocyanoindole	23	Gib	11	6	9	8	21	52	61	68	77	70	89	80	88	89	94	82	87	SF
3-(2-Methyl-2-nitrovinyl)indole	C	S.I.	5	4	5	4	16	49	55	71	75	70	90	75	88	90	83	83	84	92
7-Azaindole	C	Gib	21	19	30	19	43	56	51	55	71	63	82	71	76	81	SF	82	79	88

* The compounds used in this investigation which were commercially available were obtained from the following sources: A = Eastman Organic Chemicals, Rochester, N.Y., U.S.A.; B = Regis Chemical Co., Chicago, Ill., U.S.A.; C = Aldrich, Milwaukee, Wisc., U.S.A.; D = California Corporation for Biochemical Research, San Diego, Calif., U.S.A.; E = Mann Labs., New York, N.Y., U.S.A.; F = Sandoz Pharmaceuticals, Basel, Switzerland, G = Fisher Scientific, Pittsburgh, Pa., U.S.A.; H = Koch-Light, Colnbrook, Great Britain; I = Sigma, St. Louis, Mo., U.S.A.; J = Schwarz/Mann, Orangeburg, N.Y., U.S.A.; K = Nutritional Biochemicals (ICN Pharmaceuticals), Cleveland, Ohio, U.S.A. In instances where the compounds were not commercially; available, they were synthesized in the laboratory; the appropriate literature reference is given in each case.

** Chromogenic reagents (and other methods) used for the detection of indole compounds on developed chromatograms: Ehr = Ehrlich's reagent; DNPH = 2,4-dinitrophenylhydrazine; Gib = Gibb's reagent; DPNA = diazotised *p*-nitroaniline; N/N = nitrite/nitric acid reagent; Fluor. = fluorescent in ultraviolet light; S.I. = coloured substance, self-indicating.

*** $R_F \times 100$ values; SF = the compound travels with or very close to the solvent front.

§ The compositions of solvent systems S_1–S_{18} are given in the Materials and methods section.

TABLE II

HYDROXY- AND ACETOXYINDOLES

Compound	*Source*[a]	*Detection*[b]	*R_F value*[c] *in solvent system*[s]																
			S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}	S_{15}	S_{16}	S_{17}	S_{18}
4-Hydroxyindole	**	Ehr	0	0	0	0	0	0	8	5	10	8	46	13	30	24	27	15	48	31
5-Hydroxyindole	**	Ehr	0	0	0	0	0	0	6	5	10	9	48	12	27	25	27	17	42	31
6-Hydroxyindole	**	Ehr	0	0	0	0	0	8	16	11	22	17	61	25	46	42	45	31	61	49
7-Hydroxyindole	**	Ehr	0	0	0	0	4	7	16	11	17	14	61	25	43	37	43	28	57	51
4-Hydroxyskatole	24	Ehr	0	0	0	0	5	9	23	15	27	25	61	30	47	43	58	33	68	57
5-Hydroxyskatole	24	Ehr	0	0	0	0	0	9	16	12	21	18	56	24	42	41	47	28	57	51
6-Hydroxyskatole	24	Ehr	0	0	0	0	5	9	16	12	22	18	59	24	43	41	48	30	61	52
7-Hydroxyskatole	24	Ehr	0	0	0	0	6	10	28	17	32	26	71	37	56	58	62	46	71	68
5,6-Dihydroxyindole	25	Ehr	0	0	0	0	0	0	0	0	0	0	14	0	7	5	6	0	12	7
5,6-Dihydroxy-N-methylindole	14	Ehr	0	0	0	0	0	5	0	7	11	8	48	18	37	34	38	28	40	45
5,6-Dihydroxy-2-methylindole	18	Ehr	0	0	0	0	0	0	0	0	3	2	22	5	11	9	12	6	17	12
5,6-Dihydroxy-N-ethylindole	14	Ehr	0	0	0	0	0	10	5	11	19	13	61	27	48	45	55	41	54	59
5,6-Dihydroxy-N-isopropylindole	14	Ehr	0	0	0	0	4	16	8	16	28	21	70	39	58	58	69	53	64	69
7-Iodo-5,6-dihydroxyindole	14	Ehr	0	0	0	0	0	0	0	4	6	6	32	9	21	15	21	11	37	23
7-Iodo-5,6-dihydroxy-2-methylindole	18	Ehr	0	0	0	0	0	0	0	9	11	8	48	16	31	26	34	21	44	43
7-Iodo-5,6-dihydroxy-N-methylindole	26	Ehr	0	0	0	0	9	21	6	27	34	27	68	41	59	60	65	56	68	69
7-Iodo-5,6-dihydroxy-N-ethylindole	14	Ehr	0	0	4	5	18	31	9	39	48	40	77	57	70	72	77	68	80	80

7-Iodo-5,6-dihydroxy-N-isopropylindole	14	Ehr	4	5	7	29	44	14	50	60	52	85	70	79	81	86	79	85	87
7-Bromo-5,6-dihydroxy-N-methylindole	27	Ehr	4	0	4	3	10	20	24	24	33	25	64	41	53	56	51	49	60	64
5,6-Diacetoxyindole	14	Ehr	0	0	0	0	11	37	23	45	69	51	84	79	88	90	84	90	79	SF
5,6-Diacetoxy-N-methylindole	14	Ehr	16	18	22	20	72	92	76	95	SF	92	95	SF	SF	SF	SF	SF	SF	SF
5,6-Diacetoxy-N-ethylindole	14	Ehr	29	31	36	36	84	96	86	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
5,6-Diacetoxy-N-isopropylindole	14	Ehr	44	45	50	51	90	SF	92	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
7-Iodo-5,6-diacetoxyindole	14	Ehr	8	8	7	8	47	76	66	85	88	83	94	92	SF	SF	SF	SF	89	SF
7-Iodo-5,6-diacetoxy-N-methylindole	14	Ehr	45	44	47	44	91	95	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
7-Iodo-5,6-diacetoxy-N-ethylindole	14	Ehr	59	55	57	61	97	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
7-Iodo-5,6-diacetoxy-N-isopropylindole	14	Ehr	75	72	72	79	97	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
7-Bromo-5,6-diacetoxy-N-methylindole	27	Ehr	42	43	47	41	87	SF	91	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
3,5,6-Triacetoxyindole	28	Ehr	0	0	0	0	7	32	15	37	66	43	86	75	86	92	88	86	70	94
3,5,6-Triacetoxy-N-methylindole	27	Ehr	10	10	11	8	64	91	65	92	95	89	SF	SF	SF	SF	SF	SF	93	SF
3,5,6-Triacetoxy-N-ethylindole	29	Ehr	17	17	20	16	77	SF	75	95	SF	93	SF	SF	SF	SF	SF	SF	SF	SF
3,5,6-Triacetoxy-N-isopropylindole	29	Ehr	26	28	31	28	86	SF	82	SF	SF	97	SF	SF	SF	SF	SF	SF	SF	SF
7-Iodo-3,5,6-triacetoxyindole	28	Ehr	6	2	4	5	25	69	51	82	88	76	92	90	93	SF	SF	SF	89	SF
7-Iodo-3,5,6-triacetoxy-N-methylindole	30	Ehr	34	22	26	25	81	94	90	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
7-Iodo-3,5,6-triacetoxy-N-ethylindole	29	Ehr	51	35	40	41	87	SF	92	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
7-Iodo-3,5,6-triacetoxy-N-isopropylindole	29	Ehr	65	48	53	57	91	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
7-Bromo-3,5,6-triacetoxy-N-methylindole	18	Ehr	27	28	31	31	87	95	88	SF	SF	95	SF	SF	SF	SF	SF	SF	99	SF

....., § See Table I.

§§ Prepared by the hydrogenolytic debenzylation of the corresponding benzyloxyindoles which were available commercially (Regis Chemical Co.).

TABLE III

INDOLE ACIDS

Compound	*Source**	*Detection***	*R_F value*** in solvent system§*																	
			S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}	S_{15}	S_{16}	S_{17}	S_{18}
Indole-2-carboxylic acid	C	Ehr	0	0	0	0	0	0	0	5	16	12	46	22	29	30	38	23	44	37
Indole-3-carboxylic acid	H	Ehr	0	0	0	0	0	0	4	4	8	7	39	13	21	22	20	15	34	27
Indole-3-acetic acid	A	Ehr	0	0	0	0	0	0	4	4	13	6	44	4	30	34	27	23	32	21
Indole-3-propionic acid	B	Ehr	0	0	0	0	0	0	8	8	24	15	62	6	46	51	47	39	51	47
Indole-3-butyric acid	B	Ehr	0	0	0	0	3	0	14	14	37	26	72	11	58	64	63	54	64	63
5-Methoxyindole-2-carboxylic acid	C	Ehr	0	0	0	0	0	0	0	4	7	11	39	20	30	35	45	27	40	41
5-Methylindole-2-carboxylic acid	I	Ehr	0	0	0	0	2	0	9	11	23	19	57	34	38	41	54	35	50	46
5-Hydroxyindole-3-acetic acid	B	Ehr	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	3	0
5-Benzyloxyindole-3-acetic acid	C	Ehr	0	0	0	0	0	2	18	18	40	28	77	40	72	76	67	60	67	64
Indole-3-lactic acid	I	Ehr	0	0	0	0	0	0	0	0	2	0	17	0	9	10	7	5	9	6
Indole-3-acrylic acid	I	Ehr	0	0	0	0	0	0	7	5	11	9	55	8	31	36	39	22	43	26
Indole-3-glyoxylic acid	B	Gib	0	0	0	0	0	0	0	0	0	4	16	5	9	7	7	6	9	9
N-Acetylindole-3-acetic acid	††	Gib	0	0	0	0	6	5	30	30	49	46	74	52	66	72	76	68	69	78

*–***, § See Table I.

†† Prepared by Dr. O. Hutzinger.

TABLE IV

INDOLE BASES, ALKALOIDS AND DERIVATIVES

Compound	*Source**	*Detection***	*R_F value**** *in solvent system*§																	
			S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}	S_{15}	S_{16}	S_{17}	S_{18}
Tryptamine	A	Ehr	0	0	0	0	0	11	6	8	8	9	19	17	21	7	12	12	9	9
5-Hydroxytryptamine (serotonin)	C	Ehr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N-Acetyl-5-methoxytryptamine	B	Ehr	0	0	0	0	0	11	19	14	36	14	69	32	52	74	49	70	34	74
5-Hydroxy-N,N-dimethyltryptamine (bufotenine)	E	Ehr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Indoline (2,3-dihydroindole)	H	Ehr	77	75	77	77	90	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
Harmine	E	Gib	0	0	0	0	6	41	12	13	29	12	66	18	41	29	24	43	23	57
Harmane	E	Gib	7	5	6	5	12	57	23	34	54	24	85	39	58	64	80	75	61	75
Harmol	E	Gib	0	0	0	0	0	0	0	0	0	0	21	0	4	33	0	6	7	6
Norharmane	E	Gib	6	4	4	0	15	42	39	36	58	34	83	46	62	68	84	72	64	79
Harminic acid	E	Gib	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Harmalol	E	Gib	0	0	0	0	0	12	9	7	16	9	50	15	24	30	14	44	16	45
Reserpine	D	Gib	0	0	0	0	31	90	49	61	93	70	SF	86	95	SF	SF	SF	92	SF
Ergotamine	E	Gib	0	0	0	0	0	29	21	30	69	40	90	68	87	94	66	94	53	95
Ibogaine	E	Gib	20	15	13	19	38	80	47	59	67	57	84	65	74	64	91	79	55	86
Yohimbine	K	Gib	0	0	0	0	0	45	7	9	27	8	71	19	46	45	25	57	20	66
Bulbocapnine	E	Gib	8	5	4	6	36	86	40	55	77	50	90	67	83	90	94	93	45	94
Lysergic acid	F	Ehr	0	0	0	0	0	0	0	0	0	0	9	0	0	7	0	12	0	6
Lysergic acid amide (ergine)	F	Ehr	0	0	0	0	0	0	0	0	6	0	27	12	10	16	5	17	10	29
Isolysergic acid amide (isoergine)	F	Ehr	0	0	0	0	0	9	6	7	19	7	47	13	28	45	15	41	14	58
Lysergic acid monoethylamide (LAE)	F	Ehr	0	0	0	0	0	12	0	9	15	7	45	11	27	60	15	51	17	51
Lysergic acid diethylamide (LSD)	§§	Ehr	0	0	0	0	9	47	18	41	68	32	85	58	74	93	66	92	54	92
Lysergic acid monomethylamide (MLD)	F	Ehr	15	11	9	9	53	88	66	83	94	71	SF	89	93	SF	92	SF	75	SF
Lysergic acid morpholide (LSM)	F	Ehr	0	0	0	0	0	24	7	17	39	14	68	24	59	89	37	85	29	83

*–***, § See Table I.

§§ See Acknowledgements.

TABLE V

TRYPTOPHAN AND DERIVATIVES

Compound	*Source*[*]	*Detection*[**]	*R_F value*[***] *in solvent system*[§]																
			S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}	S_{15}	S_{16}	S_{17}	S_{18}
Tryptophan	K	Ehr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tryptophan methyl ester	B	DPNA	0	0	0	0	11	35	13	23	40	31	52	41	49	65	31	65	37	67
Tryptophan ethyl ester	B	DPNA	6	0	0	0	16	53	17	40	63	47	75	61	65	81	39	80	55	83
Tryptophan *n*-butyl ester	B	DPNA	8	11	13	12	37	75	35	54	78	61	86	75	80	90	50	89	73	SF
Tryptophan *n*-octyl ester	B	DPNA	35	23	27	30	56	88	62	73	91	75	SF	90	90	SF	88	SF	88	SF
N-Acetyltryptophan	K	Ehr	0	0	0	0	0	0	0	0	0	0	17	0	4	7	0	6	4	11
N-Acetyltryptophan ethyl ester	E	Ehr	0	0	0	0	5	27	19	25	64	36	83	64	73	89	76	87	65	92
5-Hydroxytryptophan	B	Ehr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5-Benzyloxytryptophan	H	Ehr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6-Benzyloxytryptophan	B	Ehr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5-Methyltryptophan	B	Ehr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N-Acetyltryptophonamide	E	Ehr	0	0	0	0	0	0	0	0	0	1	11	0	4	9	0	6	4	11
N-Carbobenzoxytryptophan	L	Ehr	0	0	0	0	0	0	6	4	19	7	59	20	42	56	37	48	48	65
Tryptophol	B	Ehr	0	0	0	0	0	0	0	0	7	0	18	14	8	11	0	10	9	20

[*],[**],[§] See Table I.

TABLE VI

INDOXYLS, OXINDOLES AND ISATINS

Compound	*Source**	*Detection***	*R_F value**** *in solvent system*§																
			S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}	S_{15}	S_{16}	S_{17}	S_{18}
3-Acetylindoxyl	H	Ehr	14	13	16	15	43	65	63	68	82	77	89	85	87	91	94	87	87	94
5-Bromoindoxyl-O-acetate	D	Ehr	20	16	21	19	55	76	82	83	90	87	94	92	92	94	95	94	94	SF
Indican	D	Gib	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxindole	C	Gib	0	0	0	0	15	31	23	26	54	37	75	50	65	78	71	77	58	79
3-Ethyl-N-methyloxindole	C	Gib	76	76	78	82	92	94	85	SF	95	93	SF	95	94	98	SF	SF	SF	SF
3,3-Bis(4-hydroxyphenyl)indoxyl	C	Gib	0	0	0	0	0	0	0	0	0	0	34	0	4	7	0	5	30	8
Isatin	G	S.I.	0	0	0	0	0	7	9	11	21	11	54	25	42	54	41	46	36	64
5-Bromoisatin	C	S.I.	0	0	0	0	0	6	16	19	29	19	61	32	53	53	54	48	52	68
5-Nitroisatin	C	S.I.	0	0	0	0	0	0	0	5	5	5	30	16	25	23	22	19	23	35
N-Acetylisatin	C	S.I.	0	30	38	31	82	91	79	90	SF	91	90	90	92	92	SF	97	89	SF
5,6-Dichloroisatin	C	S.I.	0	0	0	0	6	11	42	36	47	37	68	44	62	62	74	60	68	76
Adrenolutin (5,6-dihydroxy-N-methyl-indoxyl)	30	Fluor.	0	0	0	0	0	0	0	0	0	0	13	0	5	7	6	6	6	10
Noradrenolutin (5,6-dihydroxyindoxyl)	28	Fluor.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N-Ethylnoradrenolutin (N-ethyl-5,6-dihydroxyindoxyl)	29	Fluor.	0	0	0	0	0	0	0	0	0	0	20	0	4	6	5	8	6	13

*,**,***,§ See Table I.

RESULTS AND DISCUSSION

The R_F values (radial) obtained for approximately 150 indole compounds in eighteen different solvent systems on formamide-treated paper are given in Tables I–VI. Each R_F value reported is the average of at least six determinations.

The indole compounds have been classified into groups according to certain chemical characteristics. Table I gives the R_F values of 45 neutral indoles; Table II, the R_F values of 37 mono- and dihydroxy- and acetoxyindoles; Table III, the R_F values of 13 indole acids and their derivatives; Table IV, the R_F values of 23 indole bases, including some indole alkaloids; Table V, the R_F values of 14 tryptophan derivatives; and Table VI, the R_F values of 14 indoxyls, oxindoles and isatins. The poor reproducibility of R_F values on impregnated papers sometimes observed when this chromatographic technique is employed[17] was not encountered in this investigation. This was undoubtedly due to the careful and meticulous use of standardized techniques when the impregnation and drying of the papers was carried out and the use of a standardized purified impregnant and pure solvents throughout.

It can be seen from Table I that most simple neutral indoles run very well on formamide-treated paper, the R_F values of individual compounds increasing, as expected, with polarity of the developing solvent. For example, indole itself has an R_F value of *ca.* 0.4 in non-polar saturated hydrocarbon solvents while it has an R_F value of *ca.* 1.0 in relatively polar solvents such as furan or dichloromethane. In general, alkyl substitution increases the R_F value of the indole compound in all the solvent systems investigated, whereas indoles with polar groups such as hydroxyl, formyl (*i.e.* aldehyde), amino or ester groups have lower R_F values than the corresponding unsubstituted indole. Table II indicates that mono- and dihydroxyindoles can be chromatographed satisfactorily in this type of system provided that reasonably polar developing solvents are used; as would have been expected, the 5,6-dihydroxyindoles are slower running than the corresponding monohydroxy compounds. An N-alkyl group increases the R_F value of the dihydroxyindole, the effect increasing with the size of the substituent on the nitrogen. As would have been anticipated, the less polar O-acetyl derivatives have higher R_F values than the corresponding hydroxy compounds.

Preliminary investigations had shown that a number of indole acids and their derivatives can be chromatographed satisfactorily on formamide-treated paper, provided that reasonably polar solvents are used[13], as these compounds do not migrate in saturated hydrocarbon solvents and non-polar chlorinated solvents such as carbon tetrachloride. The present investigation has considerably extended the range of indole acids and running solvents studied with this type of system over those reported earlier[13].

In Table IV, the results obtained with a number of basic indole compounds, including a number of indole alkaloids, are given. In general, the results were not as good as those obtained with the acidic or neutral indoles. The best results were obtained with chlorinated hydrocarbon solvents; problematical amounts of streaking were sometimes observed when benzene-containing solvents were used.

The best results for the lysergic acid derivatives, including the well known hallucinogen LSD, were obtained with solvents S_{12}, S_{15}, S_{17} and S_{19}. This type of system had been successfully employed previously for chromatographic studies with other ergot alkaloids and lysergic acid derivatives[31].

Table V indicates the results obtained with tryptophan and a number of tryptophan derivatives: while these systems gave satisfactory results with tryptophan esters and some acyltryptophans, the results obtained with tryptophan itself and certain hydroxytryptamine derivatives were not very satisfactory.

As can be seen from Table VI, chromatographic systems of this type are suitable for the chromatography of a number of simple indoxyls, oxindoles and isatins and their derivatives. These chromatographic systems were not particularly useful for the chromatography of the highly fluorescent 5,6-dihydroxyindoxyls such as noradrenolutin and adrenolutin, as slow decomposition of the compounds appeared to take place during the chromatographic separations.

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REFERENCES

1 S. P. Sen and A. C. Leopold, *Physiol. Plant.*, 7 (1954) 98.
2 M. D. Armstrong, K. N. F. Shaw, M. J. Gortatowski and H. Singer, *J. Biol. Chem.*, 232 (1958) 17.
3 R. J. Block, E. L. Durrum and G. Zweig, *A Manual of Paper Chromatography and Paper Electrophoresis*, Academic Press, New York, 1958, p. 313.
4 R. M. Acheson, R. M. Paul and R. V. Tomlinson, *Can. J. Biochem. Physiol.*, 36 (1958) 295.
5 J. B. Jepson, in I. Smith (Editor), *Chromatographic Techniques, Clinical and Biochemical Applications*, Heinemann Medical Books, London, 1958, p. 114.
6 Ž. Procházka, V. Šanda and K. Macek, *Collect. Czech. Chem. Commun.*, 24 (1959) 2928.
7 K. Macek, I. M. Hais, J. Gasparič, J. Kopecký and V. Rábek, *Bibliography of Paper Chromatography, 1957–1960, and Survey of Applications*, Publishing House of the Czechoslovak Academy of Sciences, Prague, 1962, p. 382.
8 K. Macek, I. M. Hais, J. Kopecký and J. Gasparič, *Bibliography of Paper and Thin-Layer Chromatography, 1961–1965, and Survey of Applications*, Elsevier, Amsterdam, 1968, p. 513.
9 K. Macek, I. M. Hais, J. Kopecký, J. Gasparič, V. Rábek and J. Churáček, *Bibliography of Paper and Thin-Layer Chromatography, 1966–1969, and Survey of Applications*, Elsevier, Amsterdam, 1972, p. 465.
10 O. Sibliková, in I. M. Hais and K. Macek (Editors), *Paper Chromatography, A Comprehensive Treatise*, Publishing House of the Czechoslovak Academy of Sciences, Prague, and Academic Press, New York, 1963, p. 363.
11 Ž. Procházka, in K. Macek and I. M. Hais (Editors), *Stationary Phase in Paper and Thin-Layer Chromatography*, Elsevier, Amsterdam, 1965, pp. 173 and 218.
12 Ž. Procházka, in I. M. Hais and K. Macek (Editors), *Paper Chromatography, A Comprehensive Treatise*, Publishing House of the Czechoslovak Academy of Sciences, Prague, and Academic Press, New York, 1963, p. 609.
13 R. A. Heacock and M. E. Mahon, *Can. J. Biochem. Physiol.*, 38 (1960) 1500.
14 R. A. Heacock, M. E. Mahon and B. D. Scott, *Can. J. Chem.*, 39 (1961) 231.
15 R. A. Heacock and M. E. Mahon, *Can. J. Biochem. Physiol.*, 41 (1963) 487.
16 R. A. Heacock and M. E. Mahon, *Can. J. Biochem. Physiol.*, 41 (1963) 2381.
17 J. Churáček, *J. Chromatogr.*, 33 (1968) 45.

18 R. A. Heacock, O. Hutzinger, B. D. Scott, J. W. Daly and B. Witkop, *J. Amer. Chem. Soc.*, 85 (1963) 1825.
19 R. D. Morin, F. Benington and L. C. Clark, *J. Org. Chem.*, 22 (1957) 331.
20 J. E. Saxton, *J. Chem. Soc.*, (1952) 3592.
21 R. Majima, T. Shigematsu and T. Rokkaku, *Ber. Deut. Chem. Ges.*, 57 (1924) 1453.
22 W. Carpenter, M. S. Grant and H. R. Snyder, *J. Amer. Chem. Soc.*, 82 (1960) 2739.
23 M. S. Grant and H. R. Snyder, *J. Amer. Chem. Soc.*, 82 (1960) 2742.
24 R. A. Heacock and O. Hutzinger, *Can. J. Chem.*, 42 (1964) 514.
25 R. J. S. Beer, K. Clarke, H. G. Khorana and A. Robertson, *J. Chem. Soc.*, (1948) 2223.
26 R. A. Heacock and B. D. Scott, *Can. J. Chem.*, 38 (1960) 508.
27 J. Harley-Mason, *J. Chem. Soc.*, (1950) 1276.
28 R. A. Heacock and B. D. Scott, *Experientia*, 17 (1961) 347.
29 R. A. Heacock and B. D. Scott, *Can. J. Chem.*, 38 (1960) 516.
30 J. D. Bu'Lock and J. Harley-Mason, *J. Chem. Soc.*, (1951) 712.
31 R. A. Heacock and M. E. Mahon, *Can. J. Chem.*, 36 (1958) 1550.
32 K. Macek, in I. M. Hais and K. Macek (Editors), *Paper Chromatography, A Comprehensive Treatise*, Publishing House of the Czechoslovak Academy of Science, Prague, and Academic Press, New York, 1963, p. 588.