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THIN-LAYER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF TERTIARY AROMATIC AMINES AND THEIR N-OXIDES

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SUMMARY

Thin-layer chromatographic (TLC) systems for the separation of 4-substituted N-ethyl-N-methylanilines and their N-dealkylation and N-oxidation products are described. Various TLC systems for the separation of 2-, 3- and 4-substituted pyridines and their N-oxides are also described. Various detection systems were utilized for revealing the spots of the compounds on the chromatograms. None of the reagents used was specific for the detection of pyridine-N-oxides, but tetracyanoethylene was found to be a very sensitive and specific spray reagent for the detection of N,N-dialkylaniline-N-oxides.

INTRODUCTION

Several workers have described the paper chromatography (PC) and thin-layer chromatography (TLC) of nitrogen oxidation products (hydroxylamines, hydroxamic acids, N-oxides)[1–3] and the possible use of chromogenic reagents for their identification[1,3,4]. These methods have helped to elucidate the widespread natural occurrence of alkaloid-N-oxides in several plant species[5,6]. The realization that the metabolic N-oxidation of certain nitrogenous drugs and foreign compounds leads to the formation of carcinogenic or toxic metabolites has resulted in extensive work on various aspects of nitrogen oxidation[7,8]. Even though chromatographic methods have been used extensively in these studies, hitherto the data were available only from diverse sources. However, a recent review has collated these data on the isolation, identification and quantitation of various types of N-oxidation products using chromatographic techniques[9].

We have studied the metabolism of a range of structural types of nitrogenous compounds with simple structures in order to help to elucidate the occurrence and

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enzymology of metabolic N-oxidation[10–12]. These studies invariably required the use of sensitive chromatographic methods for the identification and quantitation of various metabolites. In this paper, the chromatographic systems used for the separation and detection of tertiary amines, their N-oxides and N-dealkylated products are described.

MATERIALS AND METHODS

TLC separations were performed on 20 × 20 cm aluminium sheets pre-coated with a 0.2-mm layer of silica gel 60 F_{254} (E. Merck, Darmstadt, G.F.R.). Stock solutions of compounds to be chromatographed (20 mg in 10 ml of ethanol) were prepared and 10-μl volumes of these solutions were spotted on to the plates using disposable micropipettes (Camlab, Cambridge, Great Britain). Ascending TLC was performed in all instances, in a glass chamber (TLC Chromotank; Shandon, London, Great Britain) saturated with the respective solvent system. The solvent front was run to a height of 15 cm, allowing a 5-min equilibration time before removal of the plate from the tank. The compositions of the solvent systems used are given in Table I. Chloroform, acetone, ethanol and methanol (BDH, Poole, Great Britain) were dried and redistilled prior to use. Cyclohexane, toluene, diethylamine and ammonia solution (specific gravity 0.880) were used as purchased (BDH). All of the spray reagents, except tetracyanoethylene (TCNE) and 3-methyl-2-benzothiazolone hydrazone hydrochloride (MBTH), were purchased from BDH. TCNE and MBTH were purchased from Aldrich (Gillingham, Great Britain).

TABLE I

SOLVENT SYSTEMS USED IN THE SEPARATION OF PYRIDINES, AROMATIC AMINES AND THEIR N-OXIDES

Compound	*Solvent system*	*Components*
Pyridines and their N-oxides	S1	Chloroform
	S2	Ethyl acetate
	S3	Acetone
	S4	Ethanol
	S5	Chloroform–ethanol–ammonia (sp.gr. 0.88) (95:4:1)
	S6	Ethyl acetate–ethanol–ammonia (sp.gr. 0.88) (90:5:1)
	S7	Acetone–ethanol–ammonia (sp.gr. 0.88) (95:4:1)
	S8	Chloroform–ethanol–diethylamine (80:30:1)
	S9	Chloroform–ethanol–ammonia (sp.gr. 0.88) (100:8:0.5)
	S10	Acetone–ethanol–diethylamine (95:5:1)
Aromatic amines and their N-oxides	S11	Cyclohexane
	S12	Toluene
	S13	Chloroform
	S14	Methanol
	S15	Cyclohexane–toluene (75:15)
	S16	Cyclohexane–toluene–diethylamine (75:15:10)
	S17	Chloroform–methanol (80:20)
	S18	Chloroform–methanol (90:10)
	S19	Chloroform–methanol (60:40)
	S20	Chloroform–methanol–ammonia (sp.gr. 0.88) (80:20:0.5)

The substituted-pyridine series and the 4-substituted-anilines were in part commercial products from Aldrich and Koch-Light (Colnbrook, Great Britain). The pyridine-N-oxide series was synthesized according to methods already described[13]. The N-ethyl- and N-methyl-4-substituted-anilines and the N-ethyl-N-methyl-4-substituted-anilines were prepared by methods developed in this laboratory[11]. The N-ethyl-N-methyl-4-substituted-aniline-N-oxides were synthesized by methods described in the literature[14].

The compounds listed in Table IV were obtained as follows: chlorpromazine and its N-oxide from Mr. G. Navas, Department of Pharmacy, Chelsea College, London, Great Britain; quinoxaline and its N-oxide from Dr. D. Case, ICI, Macclesfield, Great Britain; 2,2′-bipyridyl and its two N-oxides from Dr. J. Haginiwa, Faculty of Pharmaceutical Sciences, Chiba University, Chiba, Japan; and codeine, tropine, atropine and hyoscine and their N-oxides from Dr. J. D. Phillipson, School of Pharmacy, London, Great Britain. The N-oxides of dimethylamphetamine, nicotine, phendimetrazine, N,N-dimethylaniline and N,N-diethylaniline were synthesized in this laboratory.

RESULTS

The results obtained from this investigation on the pyridine and aniline series are summarized in Tables II and III. Whilst, results for some other tertiary amine-N-oxides are given in Table IV. The initial TLC data were obtained using pure solvents (S_1–S_4, S_{11}–S_{14}) arranged in the tables according to their polarity. Solvent systems of intermediate polarity were obtained by mixing different amounts of these pure solvents (S_5–S_{10}, S_{15}–S_{20}). By careful manipulation of the solvents described, the development of solvent systems specific for the separation of the compounds of interest was possible. The following solvent systems were the most useful for TLC: (a) S_9 and S_{10} for the substituted pyridines and their N-oxides, (b) S_{16} for primary, secondary and tertiary anilines and (c) S_{20} for the separation of the tertiary aniline N-oxides from the other anilines. The R_F values reported for the 100 compounds and 20 solvent systems listed in Tables II and III are averages of at least two determinations in each instance. The R_F values obtained clearly reflect the effects that the substituted functional groups have on the adsorption affinity and partitioning behaviour of the series of compounds tested.

The Royal Horticultural Society (RHS) colour chart was used to identify and record the colour reactions for the 15 different reagents used (see Table V). An abbreviated form of the RHS colour chart is shown in Table VI. A — sign in Tables II–IV indicates a negative reaction whereas a —(+) sign is ascribed to a colour too weak to be positively identified. Unless otherwise stated, the colours described were observed 10 min after spraying with the detection reagent.

DISCUSSION

Most of the detection reagents chosen were standard colour reagents that are used mainly for detecting aromatic amines and phenols. The use of Koenig reagent for the detection of pyridines is well documented and was not therefore adopted in this investigation. Although this reagent is very sensitive for pyridines, it does not

TABLE II

TLC SEPARATION AND DETECTION OF A SERIES OF PYRIDINES AND THEIR N-OXIDES

For solvent systems, see Table I. For detection systems, see Tables V and VI.

$R_F \times 100$*										Compound
S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	
18	32	70	90	82	74	89	100	85	88	Pyridine
0	0	3	20_T	21	3	16	48	28	14	Pyridine-N-oxide
18	39	73	77	81	78	91	100	86	90	3-Methylpyridine
1	0	3	24_T	27	3	17	65	36	16	3-Methylpyridine-N-oxide
19	46	78	81	82	83	93	100	89	93	3-Ethylpyridine
2	0	4	36_T	35	8	22	80	42	19	3-Ethylpyridine-N-oxide
ND	ND	ND	ND	ND	ND	ND	ND	91	98	3-Fluoropyridine
ND	ND	ND	ND	ND	ND	ND	ND	50	41	3-Fluoropyridine-N-oxide
36	80	100	100	91	100	100	100	94	100	3-Chloropyridine
5	3	20	43_T	47	23	48	83	53	46	3-Chloropyridine-N-oxide
38	80	100	100	92	100	100	100	95	100	3-Bromopyridine
5	3	20	46_T	49	27	50	84	58	48	3-Bromopyridine-N-oxide
16	35	80	65	80	78	91	97	87	90	3-Acetylpyridine
2	1	9	22	27	9	28	57	35	24	3-Acetylpyridine-N-oxide
3	10	45	67	31	50	75	63	36	73	3-Aminopyridine
0	0	1	37_T	3	4	10	19	5	8	3-Aminopyridine-N-oxide
2	6	56	73	20	34	76	70	29	74	3-Acetamidopyridine
0	0	2	30_T	4	3	12	29	8	10	3-Acetamidopyridine-N-oxide
40	67	97	82	90	84	98	100	94	95	3-Cyanopyridine
5	3	36	37_T	42	19	63	68	49	61	3-Cyanopyridine-N-oxide
0	4	50	60	11	22	71	50	18	68	Nicotinamide
0	0	2	17_T	2	2	15_T	19_T	4	13_T	Nicotinamide-N-oxide
0	0	2	8_T	0	0	0	2	0	0	Nicotinic acid
0	0	0	3_T	0	0	0	0	0	0	Nicotinic acid-N-oxide
0	24	78	84	9	31	56	58	16	53	3-Hydroxypyridine
0	0	2	30_T	0	0	0	3	0	0	3-Hydroxypyridine-N-oxide
2	5	56	74	15	23	76	68	26	71	3-Pyridylcarbinol
0	0	3	25_T	2	5	11	24	5	8	3-Pyridylcarbinol-N-oxide
ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1-(3-Pyridyl)ethanol
ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1-(3-Pyridyl)ethanol-N-oxide
0	25	78	100	44	47	86	100	82	83	3-Dimethylaminopyridine
0	8	10	60	10	3	15	67	32	12	3-Dimethylaminopyridine-N-oxide
5	12	60	62	75	31	82	95	85	81	Nikethamide***
0	0	5	19	31	3	30	70	44	26	Nikethamide-N-oxide
2	4	62	63	35	25	81	78	89	79	N-Monoethylnicotinamide
0	0	5	27	10	2	25	47	46	21	N-Monoethylnicotinamide-N-oxide
3	3	23	34	51	10	54	83	67	51	Cotinine
0	0	2	6	12	1	8	34	17	7	Cotinine-N-oxide
2	3	20_T	23_T	68	18	65	70	64	66	Nicotine
0	0	1	6_T	36	1	16	47_T	30	15	Nicotine-1-N-oxide
15	33	78	83	84	57	91	100	88	87	2-Methylpyridine
3	3	5	37_T	43	5	31	67	44	23	2-Methylpyridine-N-oxide
15	36	65	73	90	55	85	98	89	83	4-Methylpyridine
1	2	3	21	31	2	14	56	38	11	4-Methylpyridine-N-oxide
15	45	71	76	89	60	87	98	89	85	4-Ethylpyridine
1	2	3	30	35	4	19	69	45	15	4-Ethylpyridine-N-oxide
17	64	85	81	91	72	89	100	92	88	Quinoline
3	4	21	48	55	15	54	80	67	49	Quinoline-N-oxide
15	56	79	79	89	67	87	100	89	86	Isoquinoline
2	2	8	46	48	10	37	76	59	32	Isoquinoline-N-oxide

* T = Tailing; ND = not determined.

** Chromatogram heated at 100° for 5 min after spraying.

*** N,N'-Diethylnicotinamide.

Detection system

D_1	D_2	D_3	D_4	D_5**	D_6	D_7	D_8	D_9	D_{10}	D_{11}	D_{12}	D_{13}	D_{14}	D_{15}
102B	86A	—	1D	—	—	—	—	—	29C	4B	—	—(+)	—	112A
95A	86A	—	1D	—	—	—	—	—	—	—	—	—(+)	—	—
102B	86A	—	1D	—	—	—	—	—	29C	4B	—	—(+)	—	112A
95A	86A	—	1D	—	—	—	—	—	—	—	—	—(+)	—	—
102B	86A	—	1C	—	—	—	—	—	29C	4B	—	—(+)	—	112A
95A	86A	—	1C	—	—	—	—	—	—	—	—	—(+)	—	112A
102B	86A	—	1C	—	—	—	—	—	—	4B	—	—	—	—
95A	86A	—	—	—	—	—	—	—	—	155C	—(+)	—	—	—(+)
102B	86A	—	1D	—	—	—	—	—	—	4B	—	—(+)	—	—
95A	86A	—	1D	—	—	—	—	—	—	—	—	—(+)	—	—
102B	86A	—	1D	—	—	—	—	—	—	4B	—	—(+)	—	—
95A	86A	—	1D	—	—	—	—	—	—	—	—	—(+)	—	—
102B	86A	—	1D	173D	—	—	—	—	30D	4B	—	—(+)	—	—
95A	86A	—	1D	177D	199D	—	—	—	30C	—	—	—(+)	—	—
96B	86A	—	1D	173D	37D	5C	50C	—	26B	4B	—(+)	121C	—	112A
102B	86A	166D	1C	202D	—	5B	50B	—	—	—	—(+)	82D	—(+)	—(+)
95A	96B	—	1D	202C	202D	5C	50D	—	165D	4B	—	—(+)	—	—(+)
102B	86A	—	1D	—	—	5C	50C	—	—	—	—	—(+)	—	—(+)
95A	86A	—	—	—	198D	—	—	—	164D	4B	—	—(+)	—(+)	112A
102B	86A	—	1D	—	—	—(+)	—	—	—	—	—	—(+)	—	—
95A	86A	—	1D	202C	—	—(+)	—	—	187C	4B	—	—(+)	—	112A
102B	86A	—	—	—	—	—	—	—	—	—	—	—(+)	—	—
102B	86A	—	—	—	—	—	—	—	164D	4B	—	121D	—	—(+)
95A	86A	—	—	—	—	—	—	—	—	—	—	—(+)	—	—(+)
102B	86A	11C	1D	98B	93B	—	—	—	—	4B	—	121D	—	112A
95A	86A	166D	1D	148C	202D	—	—	—	—	—	—	—(+)	—	—
102B	86A	—	—	—	—	—	—	—	165D	4B	—	—(+)	—	112A
95A	86A	—	—	—	—	—(+)	—(+)	—	—	—	—	124C	—	—
102B	86A	11C	1D	202C	202D	—	—	—	—(+)	4B	—	158C	—	100C
95A	86A	11D	1D	202D	101D	—(+)	50D	—	—	—	—	124D	—	—
102B	86A	202D	1C	202C	—	—	—	—	—	4B	—(+)	82D	137D	—(+)
95A	86A	166D	1D	—	—	—	—	—	—	—	—(+)	—	—	—
102B	86A	—	1D	—	—	—	—	—	187C	4B	—	121D	—	112A
95A	86A	—	1D	—	—	—	—	—	—	—	—(+)	—(+)	—	—(+)
102B	86A	—	1D	202C	—	—	—	—	—(+)	4B	—	—	—(+)	112A
95A	86A	—	1D	—	—	—	—	—	—	—	—	—	—	—
102B	86A	—	—	202C	—	—	—	—	187C	4B	—(+)	—(+)	—(+)	112A
95A	86A	—	—	—	—	—	—	—	—	—	—	124C	—	112A
102B	86A	—	1D	202C	—	—	—	—	187C	4B	—	121C	—	92C
95A	115D	11B	1C	186B	48D	—	—	—	—	155C	—	48D	—(+)	—(+)
102B	86A	—	—	—	—	—	—	—	165D	4B	—	121C	—	92C
95A	86A	—	1D	—	202D	—(+)	—(+)	—	—	—	—	124C	—	112A
102B	86A	—	—	—	—	—	—	—	164D	4B	—	121C	—	92C
95A	86A	—	—	—	—	—(+)	—(+)	—	21C	—	—	—(+)	—	—
102B	86A	—	1D	—	—	—	—	—	—(+)	4B	—	121C	—	112A
95A	86A	—	—	—	—	—(+)	—	—	—	—	—	124C	—	—
102B	86A	—	1D	—	—	—	—	—	—(+)	4B	—(+)	121C	—	109D
95A	86A	11B	1C	—	—	—(+)	—(+)	—	—(+)	—	—(+)	162B	173D	112A
102B	86A	—	1D	—	—	—	—	—	168C	4B	—	121C	—	109D
95A	86A	11B	1C	—	—	—(+)	—(+)	—	—(+)	—	—	82D	—	—(+)

TABLE III

TLC SEPARATION AND DETECTION OF A SERIES OF 4-SUBSTITUTED ANILINES AND THEIR N-OXIDES

For solvent systems, see Table I. For detection systems, see Tables V and VI.

$R_F \times 100$										*Compound*
S_{11}	S_{12}	S_{13}	S_{14}	S_{15}	S_{16}	S_{17}	S_{18}	S_{19}	S_{20}	
0	13	60	78	3	29	87	89	90	90	Aniline
2	34	76	82	7	63	90	90	90	90	N-Methylaniline
2	37	84	82	8	77	90	90	90	90	N-Ethylaniline
3	43	91	82	13	92	90	90	90	90	N-Ethyl-N-methylaniline
0	0	0	36	0	0	9	24	24	42	N-Ethyl-N-methylaniline-N-oxide
0	9	51	77	3	32	87	88	88	88	4-Toluidine
0	24	69	82	5	65	90	90	90	90	N-Methyl-4-toluidine
0	27	77	82	5	81	90	90	90	90	N-Ethyl-4-toluidine
0	23	83	82	5	93	90	90	90	90	N-Ethyl-N-methyl-4-toluidine
0	0	0	37	0	0	11	27	38	44	N-Ethyl-N-methyl-4-toluidine-N-oxide
0	10	53	77	3	33	89	88	88	88	4-Ethylaniline
0	27	73	82	7	67	90	90	90	90	N-Methyl-4-ethylaniline
0	27	78	82	5	83	90	90	90	90	N-Ethyl-4-ethylaniline
0	13	50	77	3	19	83	87	87	90	4-Fluoroaniline
2	31	69	82	6	48	90	90	90	90	N-Methyl-4-fluoroaniline
2	35	80	82	7	64	90	90	90	90	N-Ethyl-4-fluoroaniline
3	37	87	82	11	91	90	90	90	90	N-Ethyl-N-methyl-4-fluoroaniline
0	0	0	36	0	0	8	23	35	38	N-Ethyl-N-methyl-4-fluoroaniline-N-oxide
0	20	61	78	4	17	87	87	87	87	4-Chloroaniline
4	50	81	82	11	42	90	90	90	90	N-Methyl-4-chloroaniline
4	56	86	82	13	58	90	90	90	90	N-Ethyl-4-chloroaniline
7	71	94	82	24	90	90	90	90	90	N-Ethyl-N-methyl-4-chloroaniline
0	0	0	37	0	0	9	26	38	42	N-Ethyl-N-methyl-4-chloroaniline-N-oxide
0	22	63	78	4	15	87	87	87	88	4-Bromoaniline
4	53	83	82	13	41	90	90	90	90	N-Methyl-4-bromoaniline
4	59	87	82	15	57	90	90	90	90	N-Ethyl-4-bromoaniline
7	75	94	82	27	91	90	90	90	90	N-Ethyl-N-methyl-4-bromoaniline
0	0	0	35	0	0	13	30	41	49	N-Ethyl-N-methyl-4-bromoaniline-N-oxide
0	24	67	78	4	15	89	88	88	88	4-Iodoaniline
5	57	85	82	15	41	90	90	90	90	N-Methyl-4-iodoaniline
5	65	88	82	17	58	90	90	90	90	N-Ethyl-4-iodoaniline
7	79	94	82	31	91	90	90	90	90	N-Ethyl-N-methyl-4-iodoaniline
0	0	0	37	0	0	11	27	38	43	N-Ethyl-N-methyl-4-iodoaniline-N-oxide
0	3	33	75	0	25	86	87	87	88	4-Anisidine
0	7	43	82	0	51	90	90	90	90	N-Methyl-4-anisidine
0	5	43	82	0	67	90	90	90	90	N-Ethyl-4-anisidine
0	7	49	74	0	88	90	90	90	90	N-Ethyl-N-methyl-4-anisidine
0	0	0	37	0	0	11	24	38	41	N-Ethyl-N-methyl-4-anisidine-N-oxide
0	3	34	75	0	24	83	85	87	87	4-Phenetidine
0	7	43	82	0	59	90	90	90	90	N-Methyl-4-phenetidine
0	5	43	80	0	73	90	90	90	90	N-Ethyl-4-phenetidine
0	4	49	77	0	91	90	90	90	90	N-Ethyl-N-methyl-4-phenetidine
0	0	0	37	0	0	11	25	40	45	N-Ethyl-N-methyl-4-phenetidine-N-oxide
0	16	68	79	3	22	90	90	90	90	4-Aminobiphenyl
0	42	86	82	3	48	90	90	90	90	N-Methyl-4-aminobiphenyl
0	44	86	81	7	67	90	90	90	90	N-Ethyl-4-aminobiphenyl
0	57	93	82	11	91	90	90	90	90	N-Ethyl-N-methyl-4-aminobiphenyl
0	0	0	38	0	0	12	27	42	47	N-Ethyl-N-methyl-4-aminobiphenyl-N-oxide

* Chromatogram heated at 100° for 5 min after spraying.

Detection system

D_1	D_2	D_3	D_4	D_5*	D_6	D_7	D_8	D_9	D_{10}	D_{11}	D_{12}	D_{13}	D_{14}	D_{15}
102B	—	—	173D	156D	118B	5B	44D	49C	6A	—	—	—	—	—
102B	—	189C	118D	118A	118B	4D	41D	28C	158B	43C	—	—	—	—
102B	—	189C	118D	118A	118B	4D	39D	2[illegible]C	158A	43C	—	—	—	—
102B	—	—	188D	128C	118B	—	−(+)	4[illegible]	−(+)	43C	—	88D	—	—
102C	—	—	1D	202C	—	−(+)	87D	—[illegible]	—	177D	115C	—	173D	111B
102C	—	162D	31D	164C	35D	5B	44D	5B	13B	—	—	—	—	—
102B	—	—	186D	196D	92C	5B	37C	5B	158B	—	—	—	—	—
102B	—	—	189D	196D	92C	4D	39D	5B	158A	—	—	—	—	—
102B	—	—	155C	128C	92C	—	−(+)	4D	−(+)	—	—	−(+)	—	—
102C	—	—	155C	202C	—	−(+)	87D	—	—	144A	115C	—	173D	111B
102B	—	162D	31D	164C	35D	5B	44D	5B	13B	—	—	—	—	—
102B	—	—	73D	196D	92C	4D	37B	5B	158B	—	—	—	—	—
102B	—	—	189D	196D	92C	4D	39D	5B	158A	—	—	—	—	—
102B	—	—	173D	156D	35D	5B	44D	5B	6A	—	—	—	—	—
102B	—	189D	118D	118A	92C	4D	41D	5B	158B	—	—	—	—	—
102B	—	189D	118D	118A	92C	4D	39D	5B	158A	43D	—	—	—	—
102B	—	—	155C	128C	92C	—	−(+)	4D	−(+)	—	—	−(+)	—	—
102C	—	—	—	202C	—	−(+)	87D	—	—	197A	115C	—	173D	111B
102B	86A	162C	173D	164C	35D	5B	47C	5B	6A	—	—	—	—	—
102B	82A	189D	155C	122C	92C	4D	43C	5B	158B	—	—	—	—	—
102B	82A	189D	155C	122C	92C	4D	41D	5B	158A	—	—	—	—	—
102B	82A	162D	155C	128C	92C	—	−(+)	4D	−(+)	—	—	−(+)	—	—
102C	—	—	8D	202C	—	−(+)	87D	—	—	177D	115C	—	173D	111B
102B	86A	162D	173D	164C	35D	5B	47C	5B	6A	—	—	—	—	—
102B	82A	189D	155C	122C	92C	5B	43C	5B	158B	—	—	—	—	—
102B	82A	189D	155C	122C	92C	4D	41D	5B	158A	—	—	—	—	—
102B	82A	162D	155C	128C	92C	—	−(+)	4D	−(+)	—	—	—	—	—
102C	—	—	8D	202C	—	−(+)	87D	—	—	177D	115C	—	173D	111B
102B	86A	162C	173D	164C	35D	5B	47C	5B	6A	—	—	—	—	—
102B	82A	189D	155C	122C	92C	1A	57D	5B	158B	43C	—	—	—	—
102B	82A	189D	155C	122C	118B	4D	41D	5B	158A	43C	—	—	—	—
102B	82A	162D	155C	128C	92C	—	−(+)	161A	−(+)	—	—	100D	—	—
102C	—	—	—	202C	—	−(+)	87D	—	—	174B	115C	−(+)	173D	111B
102B	—	183D	174C	187C	35D	5B	44C	5B	16A	—	—	—	—	—
102B	—	88D	73D	128C	122C	4D	37B	5B	163B	—	—	—	—	—
102B	—	88D	77D	128C	122C	4D	39D	5B	158A	—	—	—	—	—
102B	—	201D	117B	118C	121D	—	−(+)	161A	−(+)		—	100D	—	—
102C	—	—	—	202C	—	−(+)	87D	—	—	174B	115C	−(+)	173D	111B
102B	—	183D	174C	187C	35D	5B	44C	5B	16A	—	—	—	—	—
102B	—	88D	73D	128C	122C	4D	37B	5B	163B	—	—	—	—	—
102B	—	88D	77D	128C	122C	4D	39D	5B	158A	—	—	—	—	—
102C	—	201D	117B	118C	121D	—	−(+)	161A	−(+)	—	—	100D	—	—
102C	—	—	—	202C	—	−(+)	87D	—	—	174B	115C	−(+)	173D	111B
95A	—	162D	173D	164C	35D	5B	182A	5B	13B	—	—	—	—	—
95A	—	88D	73D	196D	92C	4D	57D	5B	158D	—	—	—	—	—
95A	—	88D	73D	196D	35D	4C	51D	5B	158A	—	—	—	—	—
95A	—	162D	56B	118C	156D	—	−(+)	161A	−(+)	—	—	−(+)	—	—
95A	—	—	—	202C	—	−(+)	87D	—	—	144D	115C	—	173D	111B

TABLE IV

DETECTION OF SOME TERTIARY AMINE-N-OXIDES ON THIN-LAYER CHROMATOGRAMS

For detection systems, see Tables V and VI.

Compound	*Detection system*							
	D_1	D_2	D_8	D_{11}	D_{12}	D_{13}	D_{14}	D_{15}
Aliphatic-N-oxides								
Chlorpromazine	102B	—	—	39C	—(+)	39C	—	112A
Chlorpromazine-N-oxide	102B	—	—	39C	—(+)	39C	—	112A
Dimethylamphetamine (+-isomer)	—	—	—	—	—	—	—	112A
Dimethylamphetamine (−-isomer)	—	—	—	—	—	—	—	112A
Dimethylamphetamine-N-oxide	—	—	—	—(+)	111D	—	—	153D
Alicyclic N-oxides								
Codeine	—(+)	—	—	—	—	—	—	111B
Codeine-N-oxide	—(+)	185A	—	—(+)	—	—	—	—(+)
Tropine	—(+)	—	—	—	—	—	—	92C
Tropine-N-oxide	—(+)	—	—	—(+)	—	—	—	92C
Atropine	—(+)	—	—	—(+)	—	—	—	111B
Atropine-N-oxide	—(+)	—	—	—	115D	—	—	—
Hyoscine	—(+)	—	—	—	—	—	—	112A
Hyoscine-N-oxide	—(+)	—	—	—	115D	—	—	—
Nicotine	102B	86A	—	4B	—	121C	—	92C
Nicotine-1′-N-oxide	95A	185A	—(+)	—(+)	115C	39D	—	111B
Phendimetrazine	—	—	—	—	—	—	—	—
Phendimetrazine-N-oxide	102B	155A	—	—(+)	115D	—	—	111B
Arylamine-N-oxides								
N,N-Dimethylaniline	102B	—	—	43C	—	87C	—	—
N,N-Dimethylaniline-N-oxide	102B	—	87D	177D	115C	—(+)	173D	111B
N,N-Diethylaniline	102B	—	—	43C	—	87C	—	—(+)
N,N-Diethylaniline-N-oxide	102B	—	87D	177D	115C	—	—(+)	111B
Heterocyclic N-oxides								
2,2′-Bipyridyl	102B	102B	—	—(+)	49B	—	186D	—
2,2′-Bipyridyl-mono-N-oxide	95A	—	—	—(+)	—	—	—	—
2,2′-Bipyridyl-1,1′-di-N-oxide	95A	102B	—	—	—	—	—	—
Quinoxaline	—	—	—	—	—	—	—	—
Quinoxaline-N-oxide	102B	185A	—	—(+)	—	—	—	—

give a colour with pyridine-N-oxides[10]. The use of TCNE was first described for the detection of aromatic compounds in paper chromatography[15] and its use as a chromogenic reagent in TLC has subsequently been reported[16,17]. The use of reduced sodium nitroprusside has been described for the detection of aromatic amine N-oxides[4]. The use of these reagents was extended to the detection of a wide range of N-oxidation products which were of specific interest in the biological work carried out in this laboratory.

Where necessary, the use of each detection system is described separately in order to simplify the discussion.

Ultraviolet light, D_1 and D_2

After running the plates they were air dried and examined under UV light of wavelength 254 and 366 nm (using a portable Hanovia UV lamp). As indicated in Table II, at 254 nm the pyridine-N-oxides had a characteristic colour which was

TABLE V

DETECTION SYSTEMS USED FOR THE IDENTIFICATION OF A SERIES OF HETEROCYCLIC AND AROMATIC AMINES AND THEIR N-OXIDES

Detection system	*Components*
D_1	Ultraviolet light, 254 nm
D_2	Ultraviolet light, 366 nm
D_3	Iron(III) chloride (Fe, 2% aqueous solution)
D_4	Potassium permanganate (Mn, 0.1% aqueous solution)
D_5	2,6-Dichloro-*p*-benzoquinone-4-chlorimine (DCQ, 0.2% in ethanol)
D_6	2,6-Dibromo-*p*-benzoquinone-4-chlorimine (DBQ, 0.4% in ethanol)
D_7	4-Dimethylaminobenzaldehyde (DAB, 0.33%) in 50% HCl (Ehrlich reagent)
D_8	4-Dimethylaminocinnamaldehyde (DAC, 0.2% in 1 *M* HCl (50 ml) and ethanol (50 ml)
D_9	Diazotised 4-nitroaniline (DpNA, 0.5% in 2 *M* HCl (5 ml) mixed with 5% aqueous sodium nitrite (0.5 ml)
D_{10}	Picryl chloride (PC, 1%) in ethanol, sprayed and chromatogram placed into a chamber with ammonia
D_{11}	Tetracyanoethylene (TCNE, 0.5% in ethyl acetate)
D_{12}	Reduced sodium nitroprusside (SN), 1.2% aqueous solution (10 ml) reduced with sodium borohydride to give a clear, deep red solution and 0.8 ml of acetic acid (1 *M*) added. After 2 min, 5 ml of water added
D_{13}	3-Methyl-2-benzothiazolone hydrazone hydrochloride (MBTH, 0.35% methanolic solution) and iron(III) chloride solution (1% solution in 0.5 *M* HCl)
D_{14}	Iron(II) thiocyanate (FTC) reagent. Iron fillings (100 mg) were added to 25 ml of a 4% ammonium iron(II) sulphate solution in 0.5 *M* H_2SO_4. After 15 min, the supernatant was added to a 1.33% solution of ammonium thiocyanate in acetone
D_{15}	Cobalt thiocyanate (CTC), 10% aqueous solution

TABLE VI

ROYAL HORTICULTURAL SOCIETY COLOUR CHART (IN ABBREVIATED FORM) USED TO RECORD COLOURS

Code	*Colour*	*Code*	*Colour*
1A– 13D	Yellow	155A–155D	White
14A– 23D	Yellow-orange	156A–156D	Greyed white
24A– 29D	Orange	157A–157D	Green-white
30A– 35D	Orange-red	158A–158D	Yellow-white
36A– 56D	Red	159A–159D	Orange-white
57A– 74D	Red-purple	160A–162D	Greyed yellow
75A– 79D	Purple	163A–177D	Greyed orange
80A– 82D	Purple-violet	178A–182D	Greyed red
83A– 88D	Violet	183A–187D	Greyed purple
89A– 98D	Violet-blue	188A–198D	Greyed green
99A–110D	Blue	199A–199D	Greyed brown
111A–124D	Blue-green	200A–200D	Brown
125A–143D	Green	201A–201D	Grey
144A–154D	Yellow-green	202A–202D	Black

different from that of the parent pyridines. Coupled with the difference in R_F values of these two groups of compounds, examination under UV light at 254 nm can be used for the identification of amounts of the N-oxides as small as 5 μg. However,

it is difficult to use UV light of 254 nm for the identification of the tertiary anilines and their N-oxides because (a) the colour under UV light is not sufficiently different to aid identification and (b) about 50 μg are required for positive detection of the N-oxide. With the arylamine-N-oxide the N–O bond tends to withdraw electrons from the ring, thereby reducing its aromaticity and consequently its UV absorption. Conversely, the N–O bond of the pyridine-N-oxide series tends to increase the aromaticity of the ring, enhancing the UV absorption [log ε (methanol) for pyridine = 3.62; log ε (methanol) for pyridine-N-oxide = 4.15 (refs. 10 and 18)].

Dimethylaminobenzaldehyde (DAB) and dimethylaminocinnamaldehyde (DAC), D_7 and D_8

As expected, DAB was not found to be a particularly useful reagent for the detection of the pyridine series; only those pyridines with amino, acetamido and hydroxy substituents gave a yellow colour. The primary anilines all gave a yellow colour immediately on spraying, while the secondary anilines developed a colour only on heating the plate at 100° for 5 min. This treatment did not, however, develop any coloured products with the tertiary anilines and only a faintly discernible reaction with their N-oxides was evident.

DAC did not give a characteristic colour with the pyridine series using TLC. However, after paper chromatography, spraying with DAC produced a red-violet colour which developed after 24 h with all the pyridine-N-oxides (and the tertiary aniline-N-oxides) but not with the parent bases. A red colour developed immediately after spraying the primary anilines with DAC on aluminium TLC plates*. The secondary anilines and the tertiary aniline-N-oxides gave this colour only on heat treatment (100° for 5 min), whereas the tertiary anilines failed to form coloured products. In this way, DAC proved to be a selective detection reagent for the tertiary aniline-N-oxides.

Both DAB and the vinylogue DAC have aldehyde functional groups which are believed to form coloured condensation products with available hydrogen atoms of the amino group, and tertiary anilines therefore do not react. However, the tertiary aniline-N-oxides do yield coloured complexes after treatment under acidic conditions and it may be that dye formation proceeds following oxidative N-dealkylation.

Tetracyanoethylene (TCNE), D_{11}

TCNE formed yellow complexes with all of the pyridine bases but failed to give any colour with the corresponding N-oxides. The tertiary aniline-N-oxides all gave brilliant colours on spraying with TCNE, which further intensified on standing although the corresponding tertiary anilines (with the exception of N-ethyl-N-methylaniline and N-ethyl-N-methyl-4-iodoaniline) failed to produce any colour until a few hours later. The primary anilines and most of the secondary anilines did not give a colour immediately on spraying with TCNE. It is clear that TCNE is a useful selective chromogenic reagent for detecting tertiary aniline-N-oxides. The red colour that developed with the two tertiary anilines previously mentioned was clearly different from that of their respective N-oxides and did not interfere with identification.

* The colours described for DAC treatment were obtained on commercially available aluminium TLC plates, but not on laboratory-prepared glass TLC plates. It seems that a metal catalyst is necessary for the chromogenic reactions.

Reduced sodium nitroprusside (SN), D_{12}

The sodium nitroprusside spray (according to Ziegler and Pettit[4]) failed to reveal any of the pyridine series or their N-oxides. The primary, secondary and tertiary anilines failed to react with this spray, although the tertiary aniline-N-oxides all gave a characteristic blue colour, indicating its usefulness for this group of N-oxides.

Fe(D_3), Mn(D_4), DCQ(D_5), DBQ(D_6), DpNA(D_9), PC(D_{10}), MBTH(D_{13}), FTC(D_{14}) and CTC(D_{15})

With these detection reagents, the most interesting results were as follows. Fe(D_3) produced colours with those pyridines that have amino and hydroxy functional groups. In addition, both quinoline-N-oxide and isoquinoline-N-oxide gave a pale yellow colour. A variety of colours occurred with the primary, secondary and tertiary anilines but none developed with the tertiary aniline-N-oxides. Mn(D_4) produced a yellow colour on a contrasting blue background with most of the pyridine series and their N-oxides. This reagent gave a variety of pale colours with all of the anilines and the tertiary aniline-N-oxides. DCQ(D_5) and DBQ(D_6) proved to be useful chromogenic reagents for differentiating between the primary, secondary and tertiary anilines and tertiary aniline-N-oxides. PC(D_{10}) produced positive colours for most of the pyridine bases but no colours with the pyridine-N-oxides. This reagent gave intense yellow colours with the primary anilines, pale yellow colours with the secondary anilines but no positive colours for the tertiary anilines or their N-oxides. FTC(D_{14}) and CTC(D_{15}) gave greyed orange and blue colours, respectively, with all of the tertiary aniline-N-oxides.

CONCLUSIONS

From Table II, it is apparent that none of the reagents used in this investigation was specific for the detection of the pyridine-N-oxides on TLC plates. However, as discussed previously, examination under UV light at 254 nm coupled with their characteristic R_F values in TLC solvent systems has proved a successful method for the identification of microgram amounts of these N-oxides in biological fluids. DAC, TCNE, SN, FTC and CTC were found to be specific reagents for the detection of the tertiary aniline-N-oxides; DAC and TCNE gave intense colours immediately on treatment with small amounts (5–10 μg) of these N-oxides, whilst SN, FTC and CTC tended to be less sensitive and larger amounts (about 100 μg) on a TLC plate were required for positive detection. These sprays have been found to be useful for the routine detection of these N-oxides in biological fluids. DAC, MBTH and FTC did not detect the aliphatic and alicyclic N-oxides listed in Table IV although CTC was of general use in the detection of both types of amines and their N-oxides. The results obtained with TCNE and SN indicate that they may be of use in the detection of aliphatic and alicyclic N-oxides.

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