(ii) with this method, knowledge of all the boiling points is not required but enough must be known to construct the curve for boiling points analogous to that shown in Fig. 2. Two retention times must also be known to enable the construction of the remainder of the retention time curve by geometric superimposition of the boiling point curve. From this construction retention times may be read directly for any member of the group.

The two methods depend upon precise measurement of retention times and an accurate knowledge of boiling points. In some cases where the boiling points are very close the retention times of a number of compounds may be indistinguishable.

Conclusions

Gas chromatographic examination of normal aliphatic ester isomers has revealed two relationships useful in the prediction of retention times. It is believed that these relationships can be extended not only to other similar series of esters but to any series of compounds which maintains a basic structure about which or within which a group of constant size is shifted in a regular fashion.

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[1] G. J. PIEROTTI, C. H. DEAL, E. L. DERR AND P. E. PORTER, *J. Am. Chem. Soc.*, 78 (1956) 2989.
[2] A. J. HAAGEN-SMITH, J. G. KIRCHNER, A. N. PRATER AND C. L. DEASY, *J. Am. Chem. Soc.*, 67 (1946) 1646.
[3] P. ISSENBERG AND E. L. WICK, *J. Agr. Food Chem.*, 11 (1963) 2.

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R_F values of some catecholamines, precursors and metabolites

In an effort to find a two-dimensional paper chromatographic system suitable for the radioautography of catecholamine metabolites of tyrosine and related compounds, the R_F(s) of various commercially obtained standards were determined in a number of solvent systems. All experiments were done with Whatman No. 1 paper and with diazotized sulfanilic acid and/or ninhydrin as the detecting reagents. Descending chromatography was used except where otherwise specified. The data are tabulated in Tables I and II. The preferred bidimensional system was methanol–butanol–benzene–water (4:3:2:1), run with the grain of the paper in the long direction, followed by *n*-butanol saturated with 1 *N* hydrochloric acid, a combination which gave reasonable resolution and excellently formed spots.

Certain of these solvents were also tried in solvent reversals such as used by WEISS AND ROSSI[1]. The results are given in Table III, along with the corresponding values

TABLE

R_F VALUES (× 100) OF SOME CATECHOLAMINES,

	S_1	S_2	S_3	S_4	S_5	S_6[t]	S_6[t*]	S_7	S_8[t]	S_9[t]
Tyrosine	55	76	47	49[t]	46	56	43	67	65	22
Tyrosine-O-sulfate	—	—	—	—	—	—	—	—	—	—
Tyramine	74	78	—	80	66	72	61	85	79	54
p-Hydroxyphenylacetic acid	65	79	65	66	65	72	60	80	80	—
3,4-Dihydroxyphenylalanine	47	68	40	54	36	46	37	59	62	5
3,4-Dihydroxyphenylacetic acid	59	67	57	60	57	66	56	76	80	—
Dopamine	71	79	66	73	58	66	54	75	72	36
Noradrenaline	68	78	61	71	54	65	55	77	65	18
Adrenaline	75	79	70	74	63	71	—	82	73	16
Metanephrine	72	76	70	73	64	70	—	80	81	—
Normetanephrine	72	80	68	73	62	68	—	81	72	—
3-Methoxytyramine	72	79	70	74	66	73	60	81	80	42

t = tendency to streaking; * = ascending
S_1 = *n*-butanol–pyridine–0.2 *N* sodium acetate (1:1:1); $13^1/_2$ h.
S_2 = *n*-butanol–pyridine–1 *M* sodium acetate (1:1:2); aqueous phase; $9^1/_2$ h.
S_3 = *n*-butanol–pyridine (1:1) saturated with 1 *M* sodium acetate; $14^1/_2$ h.
S_4 = *n*-butanol–pyridine–water (1:1:1); 13 h.
S_5 = benzene–methanol–butanol–pyridine–water (1:2:1:1:1); $9^1/_2$ h.
S_6 = methanol–water–pyridine (20:5:1); 11 h descending, 24 h ascending.
S_7 = toluene–ethyl acetate–pyridine–water–methanol (1:1:1:1:1); 7 h.
S_8 = toluene–ethyl acetate–methanol–water (1:1:1:1); aqueous phase; 7 h.
S_9 = water-saturated methyl ethyl ketone; 9 h.
S_{10} = *n*-butanol–ethanol–water (2:1:1); $13^1/_2$ h.
S_{11} = methanol–*n*-butanol–benzene–water (4:4:4:1); 8 h.
S_{12} = methanol–*n*-butanol–benzene–water (2:1:1:1); $15^1/_2$ h.
S_{13} = methanol–*n*-butanol–benzene–water (4:3:2:1); 12 h descending, 15–23 h ascending.
S_{14} = toluene–ethyl acetate–methanol–0.1 *N* HCl (1:1:1:1); 7 h.
S_{15} = methanol–amyl alcohol–benzene–2 *N* HCl (37:17.5:35:12.5); $9^1/_2$ h.
S_{16} = *n*-butanol saturated with 1 *N* HCl; 24 h descending, $26^1/_2$ h ascending.
S_{17} = *tert.*-butanol–acetone–formic acid–water (160:160:1:39); $9^1/_2$ h.
S_{18} = chloroform–acetic acid–water (2:1:1); aqueous phase; 7 h.
S_{19} = *n*-butanol–acetic acid–water (4:1:1); 10 h.
S_{20} = *tert.*-butanol–acetone–propionic acid–water (160:160:1:39); $8^1/_2$ h.
S_{21} = benzene–propionic acid–water (2:1:1); $8^1/_3$ h.

I

PRECURSORS AND METABOLITES

S_9*	S_{10}	S_{11}	S_{12}	S_{13}	S_{13}*	S_{14}	S_{15}	S_{16}	S_{16}*	S_{17}t	S_{17}t*	S_{18}	S_{19}	S_{20}	S_{21}
12	43	—	49	45	42	78	61	38	30	20	25	81	35	14	83
—	—	—	15	10	—	—	—	6	—	—	—	9	5	—	—
10	60	36	72	66	65	80	58	42	38	55	55	90	62	—	92
95	73	—	79	64	72	81	67	84	86	86	94	88	90	87	87
2	23	—	37	21	20	75	51	19	18	8	6	80	21	6	83
92	61	—	68	54	62	82	69	67	75	85	—	83	80	80	92
7	47	33	61	51	49	76	48	22	20	38	37	86	42	38	88
6	40	16	55	42	43	72	40	13	13	27	29	83	30	28	87
—	48	21	61	58	55	76	45	17	—	32	—	88	39	34	90
—	52	34	69	68	—	80	59	27	—	41	—	91	51	35	93
—	50	25	65	56	—	79	50	22	—	35	—	87	45	30	90
8	57	34	70	61	62	80	57	33	33	43	42	90	58	41	92

TABLE II

R_F VALUES (× 100) OF SOME ADDITIONAL TYROSINE AND CATECHOLAMINE METABOLITES

Compound	S_{12}	S_{13}	S_{16}	S_{19}
N-Acetyltyrosine	85	82t	52	81
O-Acetyltyrosine	64	49	33	47
3-Methoxytyrosine	63	53	26	37
Homovanillic acid	91	67	61	84
3-Methoxy-4-hydroxymandelic acid	59	39	48	69
3,4-Dihydroxymandelic acid	50	30	46	54

S_{12} = methanol–*n*-butanol–benzene–water (2:1:1:1); $15^1/_2$ h.
S_{13} = methanol–*n*-butanol–benzene–water (4:3:2:1); 12 h descending, 15–23 h ascending.
S_{16} = *n*-butanol saturated with 1 *N* HCl; 24 h descending, $26^1/_2$ h ascending.
S_{19} = *n*-butanol–acetic acid–water (4:1:1); 10 h.

TABLE III

COMPARISON OF CALCULATED AND EXPERIMENTAL R_N VALUES FOR SOME SOLVENT REVERSALS*

Compound	Experimental and (theoretical) R_N values (× 100)							
	S_7 followed by S_{19}			S_1 followed by S_{19}	S_7 followed by S_{20}	S_{19} followed by S_{12}	S_{12} followed by S_{19}	Phenol–HCl** followed by S_{19}
	$x^* = 0.23$	$x = 0.16$	$x = 0.30$	$x = 0.12$	$x = 0.49$	$x = -0.24$	$x = -0.19$	$x = 0.20$
Dopamine	25 (34)	48 (37)	35 (31)	30 (36)	50 (27)	35 (31)	63 (43)	31 (21)
Dopa	33 (42)	55 (43)	48 (40)	34 (34)	65 (52)	(22)	55 (33)	16 (18)
Adrenaline	31 (41)	56 (44)	44 (38)	38 (41)	51 (37)	33 (30)	67 (45)	40 (28)
Noradrenaline	34 (47)	53 (49)	48 (45)	37 (44)	60 (42)	28 (27)	66 (44)	20 (17)
Tyrosine	19 (36)	44 (38)	33 (33)	27 (31)	62 (51)	34 (29)	68 (38)	—
Tyramine	11 (18)	28 (22)	16 (14)	19 (21)	40	38 (35)	56 (39)	—
p-Hydroxyphenyl-acetic acid	−6 (−13)	1 (−6)	(−19)	−11 (−4)	−21 (−32)	36 (38)	33 (25)	73
3,4-Dihydroxyphenyl-acetic acid	−3 (−3)	12 (2)	(−9)	8 (−2)	(−24)	40 (42)	40 (29)	18 (−2)
Metanephrine	15 (27)	34 (31)	22 (24)	23 (29)	45 (35)	35 (32)	(43)	45 (31)
Normetanephrine	19 (34)	41 (37)	28 (31)	30 (34)	51 (42)	33 (31)	65 (44)	43 (28)
3-Methoxytyramine	10 (21)	29 (25)	(17)	18 (23)	45 (28)	(34)	(40)	39 (21)

* R_N, as defined by WEISS *et al.*[2], is the ratio of the distance of the spot from the initial starting line to the distance travelled by the first solvent; x is the solvent distance ratio. The calculated R_N is given in parentheses following the experimental R_N. For solvents see Table I.

** 88% aqueous phenol in an atmosphere of 1 *N* HCl; R_F data given by WEISS *et al.*[2] for this solvent used in calculating R_N.

calculated as indicated by WEISS *et al.*[2]. The spots produced by this technique are sharper than those obtained with ordinary one- or two-dimensional chromatography.

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[1] B. WEISS AND G. V. ROSSI, *Nature*, 195 (1962) 178.
[2] B. WEISS, G. V. ROSSI AND L. A. REBER, *Nature*, 197 (1963) 280.

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Temperature control in paper chromatography

In order to overcome the considerable variations in R_F values of paper chromatograms and the even more marked effects on reverse phase systems that occur with temperature variation, it is usual where this technique is employed extensively to resort to temperature controlled rooms. However, where it is used less frequently such measures may be inconvenient.

To achieve similar control, we have found that a suitably sized box, in our own experience accommodating three tanks of usual size, may be maintained at an even temperature over long periods, by simply using a hair drier (fitted with the usual heating coil) in series with a mercury contact thermometer. The general arrangement is illustrated in Fig. 1.

The provision of a collapsible side finishing flush with the bottom of the box, allows for ease of removal of a tank and for the side to be used as a small bench if required.

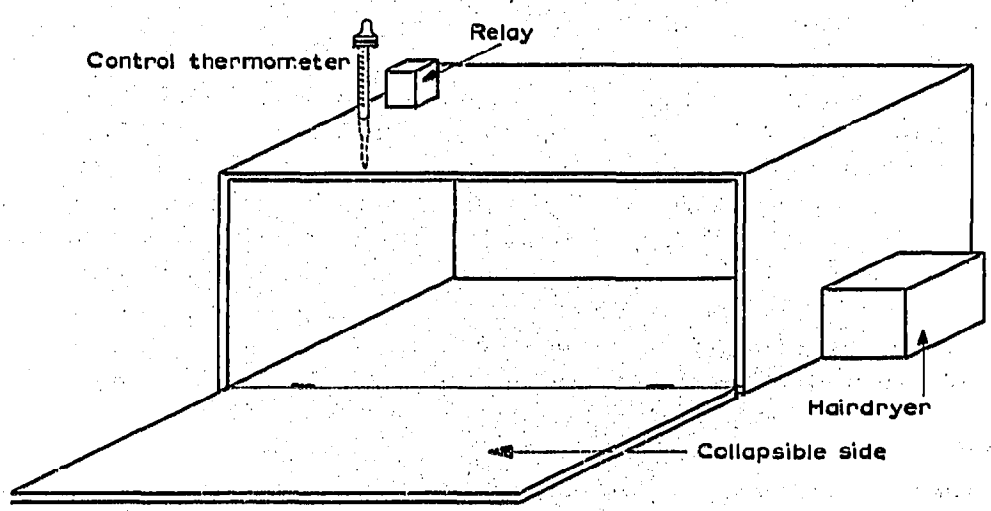

Fig. 1. Temperature control box.