R_F VALUES OF ORGANIC ACIDS IN SELECTED SOLVENTS

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In a previous communication[1] a method was described for the paper chromatography of organic acids in which the indicator was incorporated in the solvent mixture. Because of its rapidity and simplicity this method has found considerable use during the separation of complex mixtures of organic acids resulting from oxidative degradation of large molecules. The present paper includes a list of the R_F values of more than 120 acids, which has been compiled for reference purposes.

The acids were examined in six solvent mixtures, and the results are shown in Table I. The acids are grouped according to the number of carboxyl groups in the molecule and numbered consecutively from 1 to 128 to facilitate reference to them in the text. The chromatograms obtained using solvent 5 were examined in ultra-violet light (principal wavelength 3650 Å), and any acid giving a vivid fluorescence was

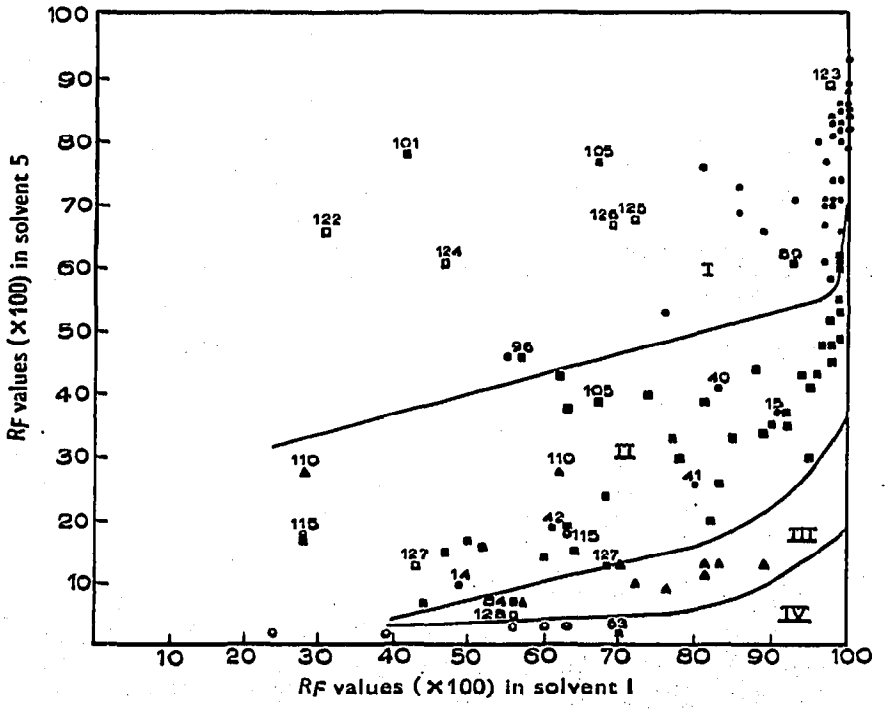

Fig. 1. R_F values in solvent 5 *versus* R_F values in solvent 1 for acids listed in Table I. ● monocarboxylic acids; ■ dicarboxylic acids; ▲ tricarboxylic acids; ○ tetra-, penta-, hexa-carboxylic acids; □ miscellaneous organic acids, inorganic acids. Band I: monocarboxylic acids. Band II: dicarboxylic acids. Band III: tricarboxylic acids. Band IV: tetra-, penta-, hexa-carboxylic acids.

TABLE I*

R_F VALUES OF ACIDS

Code No.	Acid	Source	Quality	R_F value × 100						Fluorescence in U.V. light
				Solvent 1	Solvent 2	Solvent 3	Solvent 4	Solvent 5	Solvent 6	
				Monocarboxylic acids						
1	Formic	1	AR	—	—	—	61–70	—	—	
2	Acetic	1	AR	—	—	—	61–71	—	—	
3	Propionic	2	LR	—	—	—	69–76	73–82	80–88	
4	*n*-Butyric	2	LR	—	—	—	73–80	81–90	85–93	
5	Isobutyric	1	GPR	—	—	—	72–79	83–91	87–95	
6	Isovaleric	1	GPR	—	—	—	75–82	86–93	93–99	
7	*n*-Caproic	1	GPR	—	—	—	77–87	*89–97	*90–98	
8	*n*-Caprylic	1	GPR	—	—	—	78–91	*90–99	*93–99	
9	*n*-Decanoic	1	GPR	—	—	—	77–92	*89–99	*90–100	
10	Glycollic	3		72–80	81–89	64–74	52–61	48–58	72–82	
11	Lactic	1	AR	85–92, 97–100	90–95, 98–100	77–87, 90– –96	60–67	60–71	74–83	
12	3-Hydroxy-*n*-butyric	2	LR	89–97	91–97	79–89	23–36, 58–66	67–74	76–85	
13	Quinic	1	GPR	50–60	65–73	38–50	50–57	41–50	65–73	
14	Glyoxylic	10		31–66	45–75	25–52	14–26	4–15	46–62	
15	Pyruvic	1	GPR	87–94	89–95	71–83	46–52	29–44	66–75	
16	2-Oxobutyric	3		93–100	96–100	82–94	59–68	69–84	73–86	
17	Levulinic			96–100	97–100	91–96	62–69	64–76	76–87	
18	Pivalic	2	LR	—	—	—	—	—	*92–98	
19	Trifluoroacetic	9		—	—	—	82–94	88–94	90–95	
20	5,5,5-Trifluorolevulinic	9		98–100	98–100	97–100	73–83	82–88	87–94	
21	Trichloroacetic	1	AR	—	—	—	81–94	81–90	87–96	
22	*trans*-Crotonic	2	LR	—	—	—	*70–78	*73–85	*84–93	
23	Tiglic	2	LR	—	—	—	—	—	*78–87	
24	2-Furancarboxylic	1	GPR	96–100	97–100	95–99	62–69	67–75	83–90	
25	Cholic	2	LR	99–100	99–100	*98–100	82–98	84–92	95–98	
26	Benzoic	1	AR	99–100	99–100	98–100	76–82	82–89	85–91	
27	Phenylacetic	8		98–100	99–100	96–100	71–80	75–84	84–90	
28	3-Phenylpropionic	2	LR	99–100	99–100	98–100	74–80	81–87	91–97	
29	*o*-Toluic	2	LR	98–100	98–100	97–100	78–85	78–88	84–92	
30	*m*-Toluic	2	LR	98–100	98–100	97–100	75–83	78–88	84–92	
31	*p*-Toluic	2	LR	98–100	98–100	97–100	73–81	78–88	84–92	

32	4-Methylhydrocinnamic	7		99–100	99–100	98–100	74–82	80–89	83–92	
33	2,4,6-Trimethylbenzoic	7		99–100	99–100	98–100	81–90	89–97	85–95	
34	*o*-Hydroxybenzoic	2	LR	98–100	99–100	97–100	75–83	81–88	88–93	Vivid blue
35	*m*-Hydroxybenzoic	8		95–99	96–100	96–100	57–65	62–71	70–79	
36	*p*-Hydroxybenzoic	8		94–99	96–99	95–99	51–59	55–66	64–74	
37	2-Hydroxy-3-methylbenzoic	2	LR	99–100	99–100	97–100	75–83	82–90	85–92	Vivid blue
38	2,4-Dihydroxybenzoic	2	LR	94–99	94–99	95–99	59–68	66–75	69–76	
39	2,5-Dihydroxybenzoic (Na salt)	2	LR	93–98	93–98	95–99	70–78	75–84	78–86	Vivid blue
40	3,4-Dihydroxybenzoic	2	LR	79–87	80–89	85–92	44–53	34–48	54–65	
41	2,3,4-Trihydroxybenzoic	1	GPR	75–85	76–88	81–90	0–49	0–52	0–65	
42	3,4,5-Trihydroxybenzoic	1	GPR	56–66	62–72	66–76	0–40	0–37	0–58	
43	2,4,6-Trimethylphenylglyoxylic	7		99–100	99–100	97–100	80–88	84–93	89–95	
44	4-Benzoylbenzoic	7		98–100	99–100	94–100	78–88	81–90	88–94	
45	*o*-Aminobenzoic	1	GPR	93–100	93–100	97–100	62–71	64–75	77–85	Vivid blue
46	Hippuric**	8		95–100	98–100	88–97	64–71	69–78	78–86	
47	*o*-Hydroxyhippuric**	8		96–100	98–100	93–98	51–57	54–62	70–76	
48	3,5-Dinitrobenzoic	1	GPR	99–100	99–100	98–100	71–79	77–86	81–90	
49	Pentafluorobenzoic	9		99–100	99–100	98–100	87–96	91–95	92–97	
50	Cinnamic			99–100	99–100	97–100	70–78	75–83	83–92	
51	2-Hydroxycinnamic	1	GPR	97–100	97–100	96–100	64–73	67–81	77–86	Vivid blue
52	4-Hydroxycinnamic	3		97–100	97–100	95–100	62–70	65–77	75–85	
53	1-Naphthoic	2	LR	96–100	96–100	97–100	73–80	79–86	85–94	
54	2-Naphthoic	2	LR	95–100	96–100	98–100	70–76	76–85	80–89	
55	Naphthalene-1-acetic	1	GPR	98–100	99–100	98–100	73–80	77–87	82–91	
56	1-Hydroxy-2-naphthoic	1	GPR	96–100	96–100	97–100	74–81	80–88	86–93	Vivid blue
57	3-Hydroxy-2-naphthoic	1	GPR	97–100	97–100	97–100	75–81	82–89	84–91	Vivid green
58	6-Bromo-2-hydroxy-naphthalene-3-carboxylic	3		99–100	99–100	99–100	77–87	89–96	86–94	Vivid green
59	Phenanthrene-9-carboxylic**	10		—	—	91–100	77–84	80–88	80–88	Blue
60	Pyridine-2-carboxylic	2	LR	81–90	87–95	57–69	58–70	62–76	72–83	
61	Pyridine-3-carboxylic	1	GPR	80–92	89–97	74–87	63–74	68–77	78–85	
62	Pyridine-4-carboxylic**	1	GPR	74–87	82–92	65–76	67–75	72–86	79–86	
	Dicarboxylic acids									
63	Oxalic	2	AR	63–76	72–84	47– –57–69	0–26	0–3	0–3	
64	Malonic	1	GPR	79–84	84–89	68–78	20–31	13–26	48–61	
65	Succinic	2	AR	81–88	85–92	76–85	32–44	25–40	60–72	
66	Glutaric	1	GPR	89–94	90–96	84–93	35–48	28–46	64–76	
67	Adipic	1	GPR	91–97	95–99	89–96	39–51	36–50	67–78	
68	Pimelic	1	GPR	97–100	98–100	94–99	44–55	42–55	71–82	

* For source and quality of acids and footnotes, see p. 74.

(continued on p. 72)

TABLE I (*continued*)

Code No.	Acid	Source	Quality	R_F value × 100						Fluorescence in U.V. light
				Solvent 1	Solvent 2	Solvent 3	Solvent 4	Solvent 5	Solvent 6	
69	Suberic	1	GPR	98–100	99–100	96–100	48–58	48–62	74–85	
70	Azelaic	2	LR	98–100	99–100	97–100	51–62	55–67	77–89	
71	Sebacic	1	GPR	99–100	99–100	97–100	55–65	62–74	81–92	
72	Ethylmalonic	2	LR	92–98	95–100	66– –72, 90–97	36–45	22–37	58–72	
73	Ethylsuccinic	7		96–100	96–100	93–98	45–54	41–54	66–76	
74	*cis*-Dimethylsuccinic	7		96–100	96–100	93–98	43–52	38–51	65–75	
75	*cis*-Methylethylsuccinic	7		95–100	97–100	92–100	47–59	45–59	67–77	
76	*trans*-Methylethylsuccinic	7		97–100	98–100	95–100	49–56	46–60	68–79	
77	Tartronic	10		54–65	69–81	52–62	26–35	9–19	52–65	
78	Malic (L or DL)			63–73	71–81	56–65	22–34	17–30	55–65	
79	Tartaric (D or L)	2	LR	46–57	58–71	35– –43–51	20–30	11–21	49–60	
80	*meso*-Tartaric	2	LR	42–52	55–66	33–41–46	19–28	10–19	47–57	
81	Tetrahydroxysuccinic	1	GPR	59–67	68–76	52–60	19–28	15–23	50–60	
82	Mucic**	1	GPR	*24–31, 45–55	*44–52, 61–76	*25–29, 37–44	*21–38	12–21	49–57	
83	Mesoxalic	10		35–52	53–69	22– –39, 42–52	18–29	3–10	42–54	
84	Oxosuccinic	3		50–62	66–82	35–45, 59–77	20–43	1–12	48–57	
85	2-Oxoglutaric	2	LR	76–85	82–89	63–75	33–43	33–45	65–75	
86	Meconic	1	GPR	56–69	65–80	47–60	44–53	30–45	57–66	
87	Diglycollic	3		72–84	83–91	63–77	38–48	22–38	64–76	
88	Iminodiacetic**	1	GPR	58–70	69–80	19–35– –49	28–38	10–20	56–65	
89	Tetrafluorosuccinic	9		90–95	94–98	60–81	55–65	55–66	81–89	
90	2-(Trifluoromethyl)-succinic	9		98–100	98–100	97–100	55–65	55–64	79–86	
91	Maleic	2	LR	80–86	87–92	59–83	35–42	20–32	59–70	
92	Fumaric	2	LR	85–92	90–95	84–95	41–51	27–41	68–78	
93	Methylmaleic	1	GPR	88–95	91–97	73–88	40–47	28–42	67–80	
94	Itaconic	2	LR	87–93	88–94	84–93	40–50	27–43	64–75	
95	Phthalic	1	GPR	92–98	94–98	89–96	45–54	34–47	65–75	
96	Isophthalic**	2	LR	*43–70	*46–76	89–99	49–56	40–52	66–76	
97	Terephthalic**	1	GPR	—	—	—	45–54	36–49	65–75	
98	Homophthalic	3		95–99	97–100	93–98	45–53	40–55	68–77	
99	Diphenic			98–100	98–100	96–100	53–63	57–67	77–86	
100	Phthalonic	2	LR	93–99	96–100	77–94	42–50	36–50	68–80	

101	2,2′-Dihydroxy-1,1-dinaphthyl-methane-3,3′-dicarboxylic	3		*39–45	*61–67	*40–46	*70–82	*74–82	*83–92	Green
102	Pyridine-2,3-dicarboxylic	3		70–83	76–89	42–50; 52–65	34–43	27–38	60–70	
103	Pyridine-2,5-dicarboxylic**	3		66–82	68–86	44–53; 54–66	40–50	34–45	61–72	
104	Pyridine-2,6-dicarboxylic**	3		81–94	87–95	38–53; 56–67	42–52	38–50	64–74	
105	Pyridine-3,4-dicarboxylic**	1	GPR	62–71	67–80	42–52; 69– –76	38–49; 71– –78	33–45; 73– –81	59–70; 79– –86	
106	Pyridine-3,5-dicarboxylic**	5		54–69	65–86	42– –70–89	40–52	35–51	64–79	
	Tricarboxylic acids									
107	Propane-1,2,3-tricarboxylic	2	LR	67–77	81–89	60–82	17–32	4–15	40–65	
108	Citric	1	AR	50–63	68–80	51–60	15–28	2–11	46–59	
109	2,6-Dicarboxyphenylglyoxylic	7		67–73, 85–92	73–80, 88–95	66–84	15–31	8–18	45–56	
110	Nitrilotriacetic**	1	GPR	0– –56–68	0– –62–84	10–36– –42	30–43	21–34	56–69	
111	*trans*-Aconitic	1	GPR	76–85	78–86	75–86	24–34	7–18	47–60	
112	Benzene-1,2,3-tricarboxylic	3		71–80	82–88	67–76	12–24	4–13	46–55	
113	Benzene-1,2,4-tricarboxylic	7		76–85	85–92	75–86	17–30	6–16	49–58	
114	Benzene-1,3,5-tricarboxylic	4		77–89	87–95	85–95	20–36	6–19	52–62	
	Tetracarboxylic acids									
115	Ethylenediaminetetra-acetic**	1	GPR	0– –55–70	0– –65–86	21–33– –46	27–39	11–24	53–68	
116	Benzene-1,2,3,4-tetracarboxylic	3		49–62	69–79	53–63	2–9	0–5	25–36	
117	Benzene-1,2,3,5-tetracarboxylic	7		53–66	70–79	58–66	3–11	0–5	30–40	
118	Benzene-1,2,4,5-tetracarboxylic	4		57–69	73–83	59–71	4–14	0–5	35–46	
119	Naphthalene-1,4,5,8-tetra-carboxylic	3		99–100	99–100	99–100	9–18, 63–72	0–6, 12–21, 28–34, 56–70	27–44, 62–72	
	Pentacarboxylic acid									
120	Benzenepentacarboxylic	11		32–45	57–67	26– –42, 49–56	0–5	0–4	12–23	
	Hexacarboxylic acid									
121	Mellitic	10		19–28	48–56	7– –22, 44–50	0–3	0–3	0–3	

(continued on p. 74)

TABLE I (*continued*)

Code No.	Acid	Source	Quality	R_F value × 100						Fluorescence in U.V. light
				Solvent 1	Solvent 2	Solvent 3	Solvent 4	Solvent 5	Solvent 6	
				Miscellaneous organic acids						
122	Sulphanilic	1	AR	15–47	50–70	7–32	59–66	62–69	75–84	
123	Picric	6	AR	93–100	94–100	82–94	76–89	84–94	84–93	
				Inorganic acids						
124	Hydrochloric	1	AR	44–50	62–68	43–49	57–66	56–66	75–84	
125	Nitric	1	AR	68–75	78–84	49–57	62–71	64–72	76–85	
126	Perchloric (K salt)	1	GPR	66–72	77–84	—	37–50	55–78	66–86	
127	Sulphuric	1	AR	38–48, 65– –71	58–70, 77– –81	37–49	24–36	9–16	52–63	
128	Orthophosphoric			52–60	65–75	28–41, 47–51	8–18	1–9	30–43	

Key

* Result obtained with double quantity (200 μg) of acid.
** Acid applied as ammonium salt.
– – between two numbers denotes a spot of weak intensity.

Qualities of acids:

AR = analytical reagent; LR = laboratory reagent; GPR = general purpose reagent.

Sources of acids:

1. Hopkin and Williams Ltd., Chadwell Heath, Essex, England
2. The British Drug Houses Ltd., Poole, Dorset, England
3. L. Light and Co. Ltd., Colnbrook, Bucks., England
4. Kodak Ltd., Liverpool, England
5. Aldrich Chemical Co. Inc., Milwaukee 10, Wisc., U.S.A.
6. Griffin and George Ltd., London, England
7. Courtesy of Dr. L. HORTON
8. Courtesy of Dr. S. P. JAMES
9. Courtesy of Dr. R. STEPHENS
10. Courtesy of Dr. F. DOBINSON
11. Prepared by authors.

noted. Acrylic acid (200 μg per spot) did not show up after chromatography with any of the solvents.

Fig. 1 shows a graph of R_F value in solvent 1 *versus* R_F value in solvent 5, for all acids which gave discernible spots when using these two solvents. The R_F value plotted was the mean of the minimum and maximum values shown in Table I. Acid 119, which gave four spots in solvent 5, was omitted. In general, the acids fell into bands according to the number of carboxyl groups in the molecule, in a manner similar to that illustrated by Howe[2] for different solvents. The following were exceptions: acids 14, 15, and 84 (2-oxoacids); acids 40, 41, 42, and 101 (di- and tri-hydroxyaromatic acids); and acids 63, 89, 96, 105, 110, and 115. Sulphanilic, picric, hydrochloric, nitric, and perchloric acids appeared in the monocarboxylic band, sulphuric acid in the dicarboxylic band, and orthophosphoric acid in the tricarboxylic band.

METHOD

The acids to be chromatographed were dissolved in water or ethanol and pipetted onto the starting line of the chromatogram, which was 2.5 cm from the edge of the paper. Acids that were not soluble in either of these solvents were applied as their ammonium salts. Approximately 100 μg of acid were employed for each spot; if a particular acid gave an unsatisfactory chromatogram then this weight was doubled. Mineral acids were applied as decinormal solutions (approx. 10 μl per spot). The diameter of each spot was limited to 1 cm. The solvent mixtures were prepared immediately before use from materials which had not been specially purified; equilibration was found to be unnecessary. The compositions of the six solvent mixtures are shown below.

Solvent 1: Ethyl formate–98% formic acid–water (12:5:3 v/v) containing bromophenol blue (0.015% w/w) and sodium formate (0.05% w/v).

Solvent 2: Ethyl formate–98% formic acid–water (2:1:1 v/v) containing bromophenol blue (0.015% w/w) and sodium formate (0.05% w/v).

Solvent 3: Ethyl acetate–glacial acetic acid–water (2:1:1 v/v) containing bromophenol blue (0.015% w/w) and sodium acetate (0.05% w/v).

Solvent 4: Ethanol–water–0.88 ammonia (35:13:2 v/v) containing thymol blue (0.03% w/w).

Solvent 5: Ethanol–buffer (7:3 w/v) containing chlorophenol red (0.03% w/v).

Solvent 6: Ethanol–buffer (1:1 w/v) containing chlorophenol red (0.03% w/v).

The buffer employed as a constituent of solvents 5 and 6 was an aqueous solution of ammonia and ammonium carbonate (1.5 *N* with respect to each). The ascending technique[3] of solvent flow was employed with Whatman No. 1 paper and 100 ml of the appropriate solvent, at a temperature of 18 ± 1°. Chromatography was always carried out in the machine direction of the paper. The length of solvent flow was limited to 20 cm from the starting line.

In solvents 1 and 2 the indicator moved with an R_F value of unity; in solvents 3, 4, and 6 the value was 0.95 and in solvent 5 it was 0.9. If it was necessary when using the last four solvents to locate acids with very high R_F values, the dried chromatograms were sprayed between the maximum R_F value of the indicator and an R_F value of unity with an ethanolic solution of the appropriate indicator.

After using solvent 4, the acids were most conveniently located by exposing the dried chromatogram to hydrogen chloride gas, when they became visible as yellow spots on a purple background. Trifluoroacetic, trichloroacetic, hydrochloric, nitric and perchloric acids, however, gave red spots.

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SUMMARY

The R_F values are reported of more than 120 organic acids, using a previously described paper chromatographic method and six solvent mixtures. The values may be grouped according to the number of carboxyl groups in the molecule.

REFERENCES

1 R. D. HARTLEY AND G. J. LAWSON, *J. Chromatog.*, 4 (1960) 410.
2 J. R. HOWE, *J. Chromatog.*, 3 (1960) 389.
3 R. J. WILLIAMS AND H. KIRBY, *Science*, 107 (1948) 481.