CHROMATOGRAPHIC BEHAVIOUR OF ORGANIC ACIDS ON DOWEX 1-×10

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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, and a list was later presented[2] of the R_F values of more than 120 acids. The method was used successfully to monitor the separation by chromatography on anion exchange resins of mixtures of organic acids obtained by oxidative degradation of large molecules (humic acids derived from coal), and the list of R_F values allowed tentative identification of the component acids to be made. The acids were eluted with formic acid from a column of Dowex 1 (Dow Co. Inc.) in the formate form, and it was noted that the molarity of formic acid required to elute a particular acid was characteristic of that compound.

The elution of organic acids from columns of ion exchange resins is a complex procedure. In general acids of similar constitution are eluted in order of increasing ionisation constant, the weakest acid emerging first[3]. However, aromatic acids are generally eluted after aliphatic acids and the presence of a long aliphatic chain can lead to elution of a weak acid after a stronger acid having a short carbon chain.

LAWSON AND PURDIE[4] examined the elution of several aliphatic carboxylic acids from the anion exchange resin Dowex 1, and found no difference in the order of elution when using Dowex 1-×2 or the more highly cross-linked Dowex 1-×10. In the present investigation 94 organic acids were eluted from Dowex 1-×10 with formic acid of gradually increasing concentration, under standard conditions; the results are shown in Table I, which includes the molarity of formic acid over which each acid was eluted, and also the recovery obtained. Table II shows the R_F values of those organic acids used in the present work which were not included in the previous communication[2]; with the exception of oxamic acid these acids showed R_F values consistent with their basicities.

The molarity of formic acid at which a particular acid began to be eluted was considered to be the significant value. The degree of tailing, measured by the range of molarity over which an acid was eluted, probably depended on the solubility of the organic acid in the formic acid and hence on the amount used, and also possibly on the slope of the elution gradient employed.

It is evident from the results that structure and acid strength play important roles in the elution pattern. There seems to be no clearly defined general relationship between the molarity of formic acid required to elute a particular acid and its pKa_1

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TABLE I

ORDER OF ELUTION OF ORGANIC ACIDS FROM DOWEX 1-×10

Acid	pKa_1[5-9]	*Formic acid molarity range*	*Weight*	
			applied (mg)	*recovered (mg)*
Picolinic	6.39	0.0–0.8	116	109
Nicotinic	4.82	0.05–1.6	103	103
Glycollic	3.83	0.05–1.8	214	212
Isonicotinic	4.84	0.1–0.8	66	62
1,3,4,5-Tetrahydroxycyclohexanecarboxylic (quinic)	—	0.3–1.0	88	86
Succinic	4.13	0.5–2.1	123	113
Glutaric	4.34	0.5–2.2	102	96
Glyoxylic	3.3	0.8–1.7	109	82
DL-Malic	—	0.9–2.5	103	102
Ethylsuccinic	4.08	0.9–3.5	101	99
Methylsuccinic	—	1.1–2.2	67	84
Adipic	4.41	1.1–2.7	117	115
cis-Dimethylsuccinic	—	1.1–3.2	84	85
Itaconic	—	1.2–2.8	138	138
Pimelic	4.48	1.4–3.1	113	108
Propane-1,2,3-tricarboxylic	—	1.5–4.0	132	131
meso-Tartaric	3.11	1.6–2.8	110	113
2-Aminobenzoic	2.05	1.6–3.6	79	87
cis-Methylethylsuccinic	—	1.7–4.3	120	125
trans-Methylethylsuccinic	—	1.7–6.5	89	88
Diaminoethanetetraacetic	—	1.8–3.2	102	96
Butane-1,2,4-tricarboxylic	—	2.0–3.3	99	114
Diglycollic	—	2.1–3.6	95	96
Oxamic	—	2.3–4.6	103	84
Suberic	4.51	2.3–4.8	59	55
Pyridine-3,4-dicarboxylic	2.64	2.5–4.0	107	97
Citric	3.13	2.6–4.3	149	150
Sulphanilic	—	2.9–4.2	91	77
Malonic	2.75	2.9–4.8	121	123
Pyrrole-2-carboxylic	—	2.9–5.0	84	38
Pyridine-2,3-dicarboxylic (quinolinic)	2.44	3.1–4.7	85	85
DL-Tartaric	2.96	3.1–5.2	111	111
Pyridine-2,5-dicarboxylic	—	3.3–4.8	96	95
Mandelic	3.41	3.5–5.4	104	103
Pyridine-3,5-dicarboxylic	—	3.6–5.3	92	90
Fumaric	3.02	3.6–5.6	105	106
Azelaic	4.55	3.9–6.4	125	120
Butane-1,2,3,4-tetracarboxylic	—	4.2–5.9	85	83
Tetrahydroxysuccinic	—	4.2–5.9	88	15
Cholic	—	4.3–8.7	85	89
Phenylacetic	4.32	4.5–7.0	94	74
Ethylmalonic	2.96	4.8–5.4	87	79
Tartronic	—	4.8–6.3	80	79
2-Oxoglutaric	—	4.9–6.7	91	87
Nitrilotriacetic	—	4.9–6.7	85	80
Pyridine-2,6-dicarboxylic	—	5.2–6.8	106	106
Benzoic	4.17	5.2–7.6	126	64
Homophthalic	3.72	5.4–8.0	70	66
3-Phenylpropionic	4.66	5.5–8.4	85	57
3,4-Dihydroxybenzoic	—	5.5–8.8	124	125
Pyrazole-3,5-dicarboxylic	—	6.0–7.9	97	104
Methylmaleic	2.42	6.1–7.1	91	81

(*continued on p. 49*)

TABLE I (*continued*)

Acid	pKa_1^{5-9}	*Formic acid molarity range*	*Weight*	
			applied (mg)	*recovered (mg)*
p-Toluic	4.37	6.3–10.2	77	52
o-Toluic	3.91	6.4– 9.0	70	23
Mesoxalic	—	6.5– 9.4	97	100
m-Toluic	4.27	6.9–10.9	117	75
4-Nitrobenzoic	3.44	7.3–11.1	105	104
Isophthalic	3.28	8.0–13.0	72	69
Pyrazine-2,3-dicarboxylic	2.21	8.1–11.0	77	74
Phthalic	3.00	8.4–11.2	101	98
4-Hydroxycinnamic	—	8.5–11.4	92	94
2,3,4-Trihydroxybenzoic	—	8.5–12.0	119	119
2,5-Dihydroxybenzoic	—	8.6–12.0	105	101
Cinnamic	4.43	9.1–12.5	86	87
2,4,6-Trimethylbenzoic	3.44	9.1–22.3	105	104
2,4-Dihydroxybenzoic	3.22	9.2–13.0	111	112
Diphenic	3.5	9.3–11.4	106	101
Benzene-1,2,3-tricarboxylic	2.82	9.3–11.9	102	98
Oxalic	1.27	9.4–14.1	108	106
2-Hydroxybenzoic	2.90	9.5–12.5	122	114
3,5-Dinitrobenzoic	2.82	9.6–12.0	80	75
Benzene-1,3,5-tricarboxylic	2.52	10.3–13.4	105	106
2-Hydroxycinnamic	—	11.2–13.9	114	110
4-Methylhydrocinnamic	—	11.2–14.3	30	23
4-Methylcinnamic	4.56	11.5–15.7	93	89
Benzene-1,2,4-tricarboxylic	3.12	11.7–13.7	83	82
Naphthalene-1-acetic	4.24	11.8–14.9	81	83
2-Hydroxy-3-methylbenzoic	—	12.0–15.2	82	78
4-Benzoylbenzoic	—	12.1–15.2	61	53
4-Hydroxyisophthalic	—	12.2–17.3	100	101
Phthalonic	—	13.0–15.7	81	77
2,6-Dicarboxyphenylglyoxylic	—	13.1–15.6	88	87
α-Naphthoic	3.7	13.9–16.2	68	65
β-Naphthoic	4.15	14.1–18.7	111	102
Benzene-1,2,3,5-tetracarboxylic	2.38	14.3–17.5	98	89
2-Hydroxyisophthalic	—	15.0–17.8	96	95
3-Hydroxy-2-naphthoic	—	17.0–20.7	96	94
2,4,6-Trimethylphenylglyoxylic	—	17.4–20.2	125	119
Benzene-1,2,3,4-tetracarboxylic	2.06	17.4–20.6	81	69
Benzene-1,2,4,5-tetracarboxylic	1.92	19.4–23.0	95	105
Phenanthrene-9-carboxylic	—	21.5–23.5	64	66
Picric	0.8	25.0	111	113
Benzenepentacarboxylic	1.80	0–2 *M* sodium formate	61	60
Mellitic	1.40	0–2 *M* sodium formate	121	101

value, where this is known; this latter value is, however, useful in predicting the order of elution of structurally similar acids.

The results enable accurate predictions to be made of the ease with which any mixture of acids included in Table I may be separated. Also some information may be obtained concerning the class of compound to which a particular acid belongs; for example, none of the simple benzenecarboxylic acids is eluted with formic acid of strength less than 5.2 *M*. The difference between the strengths of formic acid required

TABLE II

R_F VALUES OF ACIDS NOT PREVIOUSLY REPORTED

Acid	*Source*[a]	*R_F value* ($\times$ 100)[b]					
		Solvent 1	*Solvent 2*	*Solvent 3*	*Solvent 4*	*Solvent 5*	*Solvent 6*
Oxamic	BDH	66–75	74–84	51–61	41–51	35–45	59–67
Pyrrole-2-carboxylic	Light	92–97	94–98	92–98	52–62	55–65	66–75
Mandelic	HW	95–98	96–100	92–97	62–71	70–77	75–82
4-Nitrobenzoic	BDH	97–100	97–100	96–100	65–72	72–80	76–84
4-Methylcinnamic	LH	99–100	99–100	94–100	74–82	76–85	82–89
Pyrazole-3,5-dicarboxylic[c]	Light	60–70	64–77	33–52	22–37	11–23	41–54
Pyrazine-2,3-dicarboxylic	Light	72–82	79–88	33––38[d] 51–69	27–41	16–25	50–61
2-Hydroxyisophthalic	Light	89–95	93–97	75–90	41–49	33–43	58–66
4-Hydroxyisophthalic	Light	89–95	92–98	89–97	45–54	40–51	63–71
Butane-1,2,4-tricarboxylic[c]	Light	73–81	81–88	72–82	19––23[d] 25–36	7–19	51–61
Butane-1,2,3,4-tetracarboxylic	Light	53–65	68–79	58–69	6–19	0–6	29–43
Methylsuccinic	LH	88–95	92–98	83–92	40–50	31–44	60–73

[a] BDH: The British Drug Houses Ltd., Poole, Dorset, Great Britain; HW: Hopkin and Williams Ltd., Chadwell Heath, Essex, Great Britain; Light: L.Light and Co. Ltd., Colnbrook, Bucks., Great Britain; LH: courtesy of Dr. L. HORTON. All acids were of laboratory reagent quality.

[b] Solvent 1: Ethyl formate–98% formic acid–water (12:5:3 v/v) containing bromophenol blue (0.015% w/v) 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/v) 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/v) and sodium acetate (0.05% w/v). Solvent 4: Ethanol–water–0.880 ammonia (35:13:2 v/v) containing thymol blue (0.03% w/v). Solvent 5: Ethanol–buffer (7:3 v/v) containing chlorophenol red (0.03% w/v). Solvent 6: Ethanol–buffer (1:1 v/v) containing chlorophenol red (0.03% w/v). Buffer: An aqueous solution of ammonia and ammonium carbonate, 1.5 *N* with respect to each.

[c] Acid applied as ammonium salt.

[d] –– between numbers indicates a spot of low intensity.

to elute different acids determines the shallowness of elution gradient required to achieve complete or partial separation; the lower limit of shallowness will depend upon the sensitivity of the paper chromatographic monitoring technique[3]. Experience with the complex mixtures of acids obtained by oxidative degradation of coal has shown that it is possible to achieve at least partial separations of acids eluted by similar strengths of formic acid; these include DL-malic from *meso*-tartaric, DL-tartaric from quinolinic, and succinic from adipic acid. These separations were achieved by means of a carefully selected elution gradient after tentative identification of the acids by paper chromatography.

METHOD

A glass column (50 cm $\times$ 0.7 cm diam.) was filled with Dowex 1-$\times$10 anion exchange resin (100–200 mesh, 10 g) and converted to the formate form by eluting with 2 *M* sodium formate solution. The column was then washed with 25 *M* formic acid to remove any soluble impurities, and finally washed with water. Most of the acids were put on to the column individually as aqueous solutions; those sparingly soluble in water were put on as their sodium or ammonium salts. The purity and

sources of the acids not listed in Table II were as given previously[2]. All the acids were thoroughly dried *in vacuo* over phosphorus pentoxide before use. In order to speed up the investigation some acids which from previous experience were known to separate efficiently were put on to the resin column in pairs.

The acids were eluted by gradient elution with formic acid of strength increasing gradually from 0 to 25 *M*, using a method similar to that described by BOCK AND LING[10]. In each separation approximately 92–95 10 ml fractions were collected with the aid of an automatic fraction collector, and were monitored by paper chromatography, using the method and one of the acidic solvents described previously[1]. The range of molarity over which each acid was eluted was determined by titration to phenolphthalein with *N* or *N*/10 sodium hydroxide of samples (1.00 ml) of the fractions immediately preceding and following those in which the eluted acid was detected. The latter were combined and concentrated by distillation *in vacuo* from a water bath maintained at 45° ± 2°. The concentrates were then evaporated to dryness *in vacuo* in a desiccator over silica gel; and the residues were finally dried over phosphorus pentoxide. Neither benzenepentacarboxylic acid nor mellitic acid could be eluted from the resin with 25 *M* formic acid, but were removed by gradient elution with 0–2 *M* sodium formate solution, which provided a greater concentration of formate ions. Because of the high concentrations of cations in the eluate fractions it was not possible to monitor the elutions by paper chromatography or to determine readily the range of formate ion concentration over which each acid was eluted. To determine the recoveries each total eluate was passed down a column of cation exchange resin (Zeo-Karb 225; Permutit Ltd.) to remove sodium ions before being concentrated as described above.

The recovery of the acids by these techniques (see Table I) was very good in most cases. Low recovery (*e.g.* with benzoic acid) was attributed to the volatility of the acid in steam which resulted in some loss during the concentration stage. Some recoveries were apparently greater than the theoretical amount; the increase in weight was due to the tenacious retention by some of the acids of the formic acid eluant, which could not be removed completely by the standard drying conditions employed. Low recoveries were not thought to arise by incomplete elution of the acids from the anion exchange resin, nor high yields by incomplete separation; although the same column of resin was used repeatedly for successive experiments with different acids, paper chromatographic examination showed no evidence of contamination of any eluted acid with another.

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SUMMARY

The molarity of formic acid has been determined that is required to elute each of 94 organic acids under standard conditions from the anion exchange resin Dowex 1-×10. The R_F values in six solvent mixtures are presented of several organic acids not included in a previous communication.

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