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THIN-LAYER SOLUBILIZATION CHROMATOGRAPHY

II. KETONES

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SUMMARY

A series of eight high-molecular weight ketones is separated by development on thin layers of starch-bound anion-exchange resin with wash liquids composed of aqueous methanol or acetone. The results are compared to those on ion-exchange papers, and the effects of a number of variables on the results are studied.

INTRODUCTION

The first paper of this series[1] introduced a new method for the chromatographic separation of organic nonelectrolytes by development with aqueous solutions of organic compounds on thin layers composed of a polystyrene ion-exchange resin plus binder. The technique was termed thin-layer solubilization chromatography and was applied to the separation of a group of phenols on starch-bound layers of Dowex 50W-X8(H^+) with aqueous methanol as the wash liquid.

Although other workers have used thin layers containing ion-exchange resins, we are aware of no other work employing layers composed of only strongly-acidic or strongly-basic polystyrene resin plus binder. BERGER *et al.* originally employed thin layers containing equal parts of ion-exchange resin and cellulose powder plus plaster binder for separations of halides and organic dyes[2]. They later omitted the binder and reported that layers formed from an aqueous slurry of cellulose MN 300 and resin (1:6, w/w) adhered well after air drying[3]. They also used layers of chelating resins to separate alkali-metal ions[4] and juxtaposed layers of anion- and cation-exchange resins (including cellulose) to separate various mixtures of ions[5,6]. HUETTENRAUCH[7] separated the components of vitamin B complex on a layer of Wofatit CP 300 weakly-acidic resin, PARIHAR *et al.*[8] separated various nitro organic compounds on layers of Amberlite 400 or 45 containing no binder and DRAGULESCU *et al.*[9] separated niobium and tantalum oxalates on layers of MN 300 DEAE-cellulose mixed with Dowex 1.

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This paper reports the application of our method to the separation of a series of uncombined high molecular-weight ketones, studies of several variables involved in the method and a comparison of the results with those obtained when the same ketones were subjected to solubilization chromatography on ion-exchange papers containing the same resin[10].

EXPERIMENTAL

Procedures for the preparation of the layers, spotting of the initial zones and ascending development of the plates in saturated N-chambers were exactly as described before[1].

In most cases, the strongly-basic quaternary-ammonium anion-exchange resin, Amberlite CG-400, 200–400 mesh (Rohm and Haas Co.), was used as supplied in the chloride form. Layers were 200 μ thick on 8 × 8 inch glass plates. For conversion of the resin to other forms, an appropriate amount of resin was stirred in a large beaker with distilled water, the bulk of the resin was allowed to settle and the fines were decanted off. This procedure was repeated 3 times. The resin was then packed into a column[11] through which an appropriate ionic solution was passed until selective qualitative tests indicated that conversion was complete. The column was washed with distilled water, the resin removed and used to prepare the layers. For comparison, some runs were also made with layers prepared from chloride-form resin from which the fines had been removed.

Developing solutions were prepared by proper dilution of reagent-grade solvents. Individual test solutions of the ketones were 1% in methanol (v/v), except for Nos. 3 and 5 (see Table I) which were 0.5%. Mixtures were prepared on the plate by successive application of individual samples to the same area of the layer, with drying in between.

TABLE I

VALUES OF R_F ON CG-400 ANION-EXCHANGE LAYERS

(a) R_F of front; (b) R_F of rear.

Ketone		*Methanol concn.:*					*Acetone concn.:*	
		12.0 M	*14.0 M*	*17.0 M*	*19.0 M*	*22.0 M*	*6.0 M*	*8.0 M*
(1) Phenyl-2-propanone	(a)	0.42	0.53	0.68	0.72	0.76	0.66	0.75
	(b)	0.32	0.45	0.59	0.62	0.68	0.48	0.61
(2) 4'-Methylacetophenone	(a)	0.37	0.48	0.70	0.74	0.81	0.58	0.73
	(b)	0.27	0.40	0.60	0.64	0.75	0.42	0.63
(3) *trans*-4-Phenyl-3-buten-2-one	(a)	0.25	0.35	0.59	0.63	0.69	0.57	0.57
	(b)	0.15	0.26	0.47	0.52	0.61	0.37	0.04
(4) 1-Phenyl-1,3-butanedione	(a)	0.13	0.21	0.38	0.42	0.60	0.37	0.55
	(b)	0.06	0.16	0.31	0.33	0.52	0.33	0.39
(5) Hexanophenone	(a)	0.15	0.27	0.58	0.64	0.83	0.18	0.57
	(b)	0.09	0.20	0.50	0.54	0.76	0.00	0.30
(6) Phenyl-2-thienyl ketone	(a)	0.11	0.17	0.35	0.38	0.58	0.26	0.56
	(b)	0.04	0.11	0.30	0.31	0.50	0.14	0.36
(7) 4'-Phenylacetophenone	(a)	0.05	0.15	0.34	0.38	0.59	0.21	0.46
	(b)	0.00	0.06	0.25	0.29	0.51	0.13	0.33
(8) 2-Tridecanone	(a)	0.07	0.20	0.70	0.85	1.0	0.16	0.77
	(b)	0.01	0.12	0.57	0.73	0.90	0.00	0.08

Five μl of each ketone solution was developed over a distance of 15 cm. Development times were approx. 60 min for layers prepared from the commercial resin and 30–40 min for layers prepared from resin with the fines removed.

All of the ketones except 4 and 6 were detected by spraying the air-dried plates with a fresh, basic solution of 3,5-dinitrobenzoic acid in methanol[1,12]. The zones initially appear blue on a red-purple background. The background fades with time, and the zones become more prominent. Although the blue color is stable with time, it is best to mark the zone positions immediately because the zones spread somewhat after spraying. Ketones 4 and 6 were detected before spraying by viewing under ultraviolet light in the dark. They appear as light brown spots on a bright yellow background.

RESULTS AND DISCUSSION

R_F values are reported for the leading and trailing edges of each zone so that separation possibilities are apparent from the data. The ketones will often be referred to by the numbers listed in Table I.

Preliminary studies and separations

Methanol was initially chosen as the wash liquid because it had proved successful for the separation of a series of ketones by column solubilization chromatography[13] and for the separation of this same series of ketones by paper solubilization chromatography[10]. Other wash liquids were evaluated, but none proved so good as methanol for achieving the desired separations. Acetone is a stronger developer than methanol on a molar basis, and two pairs of ketones not separable with any molarity of methanol were separated with 6 *M* acetone (see below). Higher molarities of acetone gave irregularly shaped zones, however. No useful degree of differential migration was found with any other wash-liquid system, and many caused cracking of the layers. Those tried include various molarities of methyl ethyl ketone and acetic acid and other more complex three- and four-component systems.

Table I shows R_F values for the individual ketones on chloride-form layers prepared from the resin as received with different molarities of methanol and acetone as the developer. In both cases there is a general increase in the R_F value for each ketone as the molarity of wash liquid increases. This trend was expected and is consistent with earlier results of column[13], paper[10,14,15] and thin-layer[1] solubilization chromatography. The sequence of the ketones was not the same with each molarity of methanol, however. The developed zones had lengths of approx. 1.5 cm in the methanol wash liquids and were regularly shaped. With acetone, the zones were less compact.

Comparison between R_F values for the same ketones on the Amberlite CG-400 resin layers and paper loaded with Amberlite IRA-400(Cl^-) resin[1] with methanol wash liquids shows that at a given molarity of methanol, the resin paper has less affinity for each ketone and yields generally more diffuse zones. This is to be expected because the ion-exchange paper is in effect an ion-exchange layer diluted with cellulose. The selectivity for and sequence of the ketones in the two media are also quite different, indicating that interactions with the cellulose in the resin paper may be significant.

Based on the results in Table I, separations of binary mixtures of most of the

ketones were planned and performed. Table II shows the molarity of methanol or acetone which was used in each case to achieve these separations. It is seen that only 4 out of 28 pairs of ketones could not be separated reliably with either 14, 19 or 22 *M* methanol, and that 2 of these pairs are separable by development with 6 *M* acetone. Some ternary mixtures were also resolved, for example ketones 2, 3 and 7 with 14 *M* methanol and ketones 5, 6 and 8 with 19 *M*. In every case, the R_F values of the ketones were the same whether a pure sample or in a mixture with one or more other ketones.

TABLE II

WASH LIQUIDS GIVING RELIABLE SEPARATIONS OF KETONE PAIRS

Ketone mixture[a]	*Methanol concn. (M)*	*Acetone concn. (M)*
1–2	—	—
1–3	14	
1–4	14 or 19	
1–5	14	
1–6	14 or 19	
1–7	14 or 19	
1–8	14	
2–3	22	
2–4	14 or 19	
2–5	14	
2–6	14 or 19	
2–7	14 or 19	
2–8	14	
3–4	14 or 19	
3–5	22	
3–6	14 or 19	
3–7	14 or 19	
3–8	19 or 22	
4–5	19	
4–6	—	6
4–7	—	6
4–8	19	
5–6	14 or 19	
5–7	14 or 19	
5–8	22	
6–7	—	—
6–8	19	
7–8	19	

[a] See Table I for identification of ketones.

In the earlier study with this same group of ketones on ion-exchange paper, only ketones 7 and 8 could not be separated from each other[1]. This pair is easily separated with 19 *M* methanol on ion-exchange layers. Mixtures of ketones 1–2 and 6–7, neither of which are separated on layers with either methanol or acetone, can be separated with 8 *M* and 14 *M* methanol, respectively, on ion-exchange paper[1]. Because of the similarity between the techniques used to develop the layers and papers, these differences in selectivity are most probably due to the differences in the nature of the stationary phases.

Variables affecting R_F values

The R_F values in Table I are the average of 5 "identical" runs for each ketone with 14 and 19 *M* methanol and at least 2 replications for the other wash liquids. The reproducibility of the runs was excellent, as indicated by the fact that the extremes of the R_F values (*i.e.*, the highest R_F value for the front of the zone and the lowest R_F for the rear in any of the replications for a given ketone) were very similar to the means. For example, the respective extremes for ketones 1–8 in 14 *M* methanol for 5 runs were 0.55, 0.44; 0.49, 0.40; 0.39, 0.23; 0.21, 0.15; 0.27, 0.20; 0.19, 0.08; 0.16, 0.06; and 0.27, 0.09 (compare to means in Table I).

To determine the effect of the size of the initial zone, 5, 10 and 15 μl spots of each test solution were developed with 14 and 19 *M* methanol. The spots were applied in 5-μl increments with drying in between. A slight, general increase in both the front and rear R_F values with increasing concentration was noted.

By use of the variable-thickness spreader, layers of 500 μ and 100 μ were cast for comparison with the usual 200-μ layers. In the thicker layers, zones developed with 14 and 19 *M* methanol were both longer and thinner and had generally higher R_F values. The ketones with the highest R_F values showed a relatively greater increase in movement. Development times were the same. In the thinner layers, the initial and developed ketone zones were more diffuse, and R_F values were generally decreased. Development times for 14 and 19 *M* methanol increased from 60 to 115 min.

After preparation, the layers were normally allowed to stand in air overnight before use. However, no change in R_F values was noted if the layers were stored for 1 or 2 weeks before use or if they were used as soon as 50 min after casting. Any shorter waiting period resulted in a non-adherent layer.

TABLE III

VALUES OF R_F ON LAYERS IN DIFFERENT IONIC FORMS (FINES REMOVED) WITH 14 AND 19 *M* METHANOL AS WASH LIQUIDS

(a) R_F of front; (b) R_F of rear.

Ketone[a]		R_F							
		Cl^- form		*SO_4^{2-} form*		*Br^- form*		*I^- form*	
		14.0 M	*19.0 M*	*14.0 M*	*19.0 M*	*14.0 M*	*19.0 M*	*14.0 M*	*19.0 M*
(1)	(a)	0.63	0.85	0.62	0.81	0.54	0.73	0.38	0.57
	(b)	0.51	0.73	0.50	0.70	0.41	0.65	0.27	0.46
(2)	(a)	0.57	0.87	0.55	0.86	0.50	0.78	0.40	0.63
	(b)	0.48	0.76	0.45	0.74	0.40	0.69	0.29	0.52
(3)	(a)	0.50	0.82	0.51	0.80	0.42	0.72	0.27	0.55
	(b)	0.40	0.67	0.39	0.70	0.31	0.60	0.16	0.47
(4)	(a)	0.26	0.55	0.29	0.44	0.30	0.55	0.22	0.50
	(b)	0.16	0.48	0.15	0.32	0.18	0.45	0.00	0.00
(5)	(a)	0.38	0.82	0.60	0.78	0.50	0.73	0.39	0.61
	(b)	0.29	0.71	0.50	0.68	0.41	0.67	0.29	0.53
(6)	(a)	0.25	0.57	0.32	0.65	0.21	0.48	0.13	0.40
	(b)	0.15	0.45	0.21	0.57	0.11	0.38	0.06	0.26
(7)	(a)	0.20	0.57	0.26	0.67	0.16	0.46	0.13	0.37
	(b)	0.09	0.46	0.10	0.55	0.09	0.36	0.05	0.21
(8)	(a)	0.30	1.0	0.30	0.92	0.37	0.95	0.32	0.85
	(b)	0.11	0.82	0.15	0.82	0.15	0.82	0.03	0.66

[a] See Table I for identification of ketones.

To determine the effect of changing the ionic form of the resin, a column procedure, described above, was used to effect conversion from chloride to the desired form. In order to get an adequate flow of solution, even with pressure applied to the top, it was necessary to decant the fines from the resin before packing it into the column. With both 14 and 19 *M* methanol as the wash liquid, the ketones moved generally farther but in about the same sequence on layers prepared from chloride-form resin with the fines removed. This can be seen by comparing the data in Table III, which are the average values for five separate runs, to those in Table I. The order of decreasing affinity for the layers is, with some exceptions, $I^- > Br^- > Cl^-$, the same as on anion-exchange papers in these forms. There is no clear trend evident on the sulfate-form layers. The sequence of the R_F values for the ketones is about the same in every case, and separation possibilities are not improved by changing the form of the resin layer. In every case, however, development times were reduced to about 30 min on these converted layers.

Preliminary evaluation of cation-exchange layers

A few preliminary results have been obtained with the same ketones on layers of starch-bound Amberlite CG-120 sulfonic acid cation-exchange resin. The preparation of consistently smooth layers composed of this resin has been more difficult because the consistency of slurry seems to be more critical. Moreover, neither the 3,5-dinitrobenzoic acid spray nor numerous other reagents we have tried will detect the ketones on these layers. Some success has been achieved with 10% ethanolic phosphomolybdic acid plus heating, but even this is not entirely reliable. Runs we have made with methanol wash liquids indicate some differential migration of the ketones in relatively compact zones.

Mechanism of thin-layer solubilization chromatography

Thin-layer solubilization chromatography is a partition process closely related to paper solubilization chromatography, the mechanism of which has been previously discussed[10]. The major difference is the absence of the cellulose matrix in the layers and the presence instead of a relatively small percentage of starch binder. It is likely that differences between the two techniques are due to interactions between the solutes and/or wash liquids with the cellulose in the ion-exchange papers, interactions which are absent in the ion-exchange layers.

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