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Note

Normal- and reversed-phase ion-pair thin-layer chromatography with bifunctional (bolaform) bis(trimethylammonium) ion-pair reagents

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Compared to high-performance liquid chromatography (HPLC) the application of ion-pair techniques to the separation of ionisable compounds by thin-layer chromatography (TLC) has been limited (for some examples see refs. 1–5). As a result of this neglect many of the factors governing the separation of compounds by ion-pair TLC have remained poorly understood. However, it is now becoming clear there are major differences between ion-pair TLC and ion-pair HPLC[4,5]. As part of our continuing investigations of the practice of ion-pair TLC we briefly examined the properties of the bifunctional ion-pair reagent 1,12-bis(trimethylammonium)dodecane[6]. Whilst similar in many ways to conventional reagents (*e.g.* tetra-*n*-butylammonium) atypical results were observed with solvents containing in excess of 80% of methanol (in water). Use of such solvent systems with 1,12-bis(trimethylammonium)-dodecane led to a large reduction in R_F compared to solvents of, theoretically, lower eluotropic strength, and reversal of the migration order of the test compounds (a group of aromatic acids)[6].

These initial studies were limited to reversed-phase ion-pair TLC on paraffin-impregnated silica gel TLC plates coated with the reagent. In order to obtain a better understanding of the properties of these bifunctional (bolaform) ion-pair reagents we have prepared a short-chain bis(trimethylammonium) reagent [1,3-bis(trimethylammonium)propane] and compared this and 1,12-bis(trimethylammonium) dodecane with tetra-*n*-butylammonium and tetramethylammonium on silica gel, paraffin-impregnated silica gel and C_{18} bonded silica gel TLC plates. This has allowed us to compare the performance of the two bolaform ion-pair reagents with those of two more conventional reagents in a variety of different types of TLC system.

EXPERIMENTAL

Chemicals

The conventional ion-pair reagents used in this study were tetra-*n*-butylammonium bromide and tetramethylammonium chloride, which were purchased from Fluka (Buchs, Switzerland). The two bolaform ion-pair reagents 1,12-bis(trimethylammonium)dodecane bromide and 1,3-bis(trimethylammonium)propane bromide were synthesised from trimethylamine and the appropriate dibromoalkane[6].

The test acids, 2,4-dihydroxybenzoic acid, 2,6-dihydroxybenzoic acid, gentisic acid and salicylic acid, were obtained from Aldrich (Gillingham, U.K.). All test compounds were used as received.

Preparation of TLC plates

Silica gel and C_{18} bonded silica gel TLC plates (E. Merck) 20 × 10 cm incorporating a fluorescent indicator (254 nm) were obtained from BDH (Poole, U.K.). The plates were coated with an ion-pair reagent by dipping in a 0.1 *M* solution of the reagent in a suitable solvent as described elsewhere[4–6]. Similarly, paraffin-impregnated ion-pair-coated plates were prepared by dipping silica gel TLC plates in 0.1 *M* solutions of ion-pair reagent containing 7.5% (v/v) paraffin BP (Hills Pharmaceuticals, Burnley, U.K.)[4,5].

Chromatography

Ascending chromatography was performed in glass TLC tanks 20 × 20 × 5 cm using solvent systems of methanol–water for reversed-phase separations and dichloromethane–methanol for normal-phase chromatography.

For some experiments ion-pair reagents were also included in the developing solvent at a concentration of 0.1 *M*.

RESULTS AND DISCUSSION

Chromatography on ion-pair reagent coated plates

In our previous studies[6] using 1,12-bis(trimethylammonium)dodecane on TLC plates impregnated with paraffin we noted that, at high methanol concentrations in the solvent, there was a large reduction in the R_F of the test acids with a concommitant inversion of their migration order. Experiments with 1,12-bis(trimethylammonium)dodecane coated onto bare silica gel showed a number of similarities. Thus, whilst the R_F values attained by the test compounds were generally higher than on similar plates impregnated with both 1,12-bis(trimethylammonium)dodecane and paraffin, there was nevertheless a large reduction in R_F and a reversal of the migration order with high methanol concentrations in the mobile phase. Indeed, the R_F values for three of the test compounds (2,4-dihydroxybenzoic acid, gentisic acid and salicylic acid) were actually lower in 100% methanol than in any of the other solvent compositions tested. Interestingly, when these experiments were repeated on C_{18} bonded plates, R_F values increased in a regular way up to approximately 80% of methanol in the solvent. With higher concentrations of methanol spot shape deteriorated to such an extent that reliable measurement of R_F was not possible. However, no reduction in R_F of any of the test compounds was observed. These results are illustrated in Fig. 1.

The type of behaviour noted at high concentrations of methanol is not typical of that observed with non-bolaform ion-pair reagents such as tetra-*n*-butylammonium. In order to determine whether the type of chromatography observed with

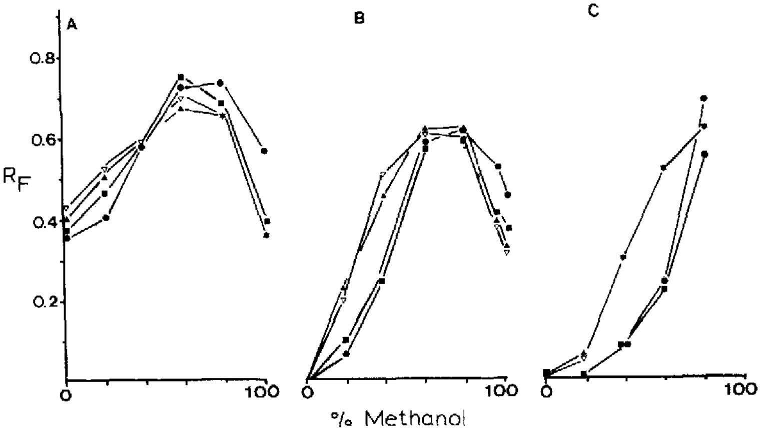

Fig. 1. Chromatography of 2,6-dihydroxybenzoic acid (●); salicylic acid (■); 2,4-dihydroxybenzoic acid (▲); and gentesic acid (▽) on silica gel (A), paraffin-coated silica gel (B) and C_{18} bonded silica gel (C) TLC plates coated with 1,12-bis(trimethylammonium)dodecane. Methanol–water based solvent systems were used.

1,12-bis(trimethylammonium)dodecane was characteristic of bolaform reagents or restricted to this reagent we also examined the properties of 1,3-bis(trimethylammonium)propane in the same systems. Although differences in R_F were noted, essentially similar results were obtained for 1,3-bis(trimethylammonium)propane compared to 1,12-bis(trimethylammonium)dodecane with a large reduction in R_F values and a reversal of migration order with solvents of high methanol content and paraffin-impregnated TLC plates. On bare silica gel good chromatography was not obtained with solvents containing water; however, with 100% methanol R_F values of 0.27, 0.54, 0.24 and 0.36 were obtained for 2,4-dihydroxybenzoic acid, gentisic acid and salicylic acid respectively.

This is the opposite of the order which would be predicted for conventional "tetra-*n*-butylammonium like" ion-pair reagents. This type of behaviour is probably directly related to the differences in structure between the bolaform and conventional reagents.

From these studies it appeared that, at high methanol concentrations, the reduction in R_F and reversal of migration order might reflect a change in the underlying separation mechanism from reversed to normal phase. In order to test this hypothesis the use of 1,3-bis(trimethylammonium)propane and 1,12-bis(trimethylammonium) dodecane for normal-phase ion-pair TLC on silica gel TLC plates was investigated. The conventional reagent tetra-*n*-butylammonium was also studied. For these experiments dichloromethane–methanol based solvent systems were used.

The results obtained with 1,3-bis(trimethylammonium)propane and 1,12-bis-(trimethylammonium)dodecane (Fig. 2) are of the type which would be predicted for a normal-phase system. Thus, with dichloromethane as solvent all of the test compounds remained at the origin. With increasing methanol content there was a steady increase in R_F with the migration order of the test acids the reverse of that seen with methanol–water "reversed-phase" systems. Similar results were obtained with tetra-

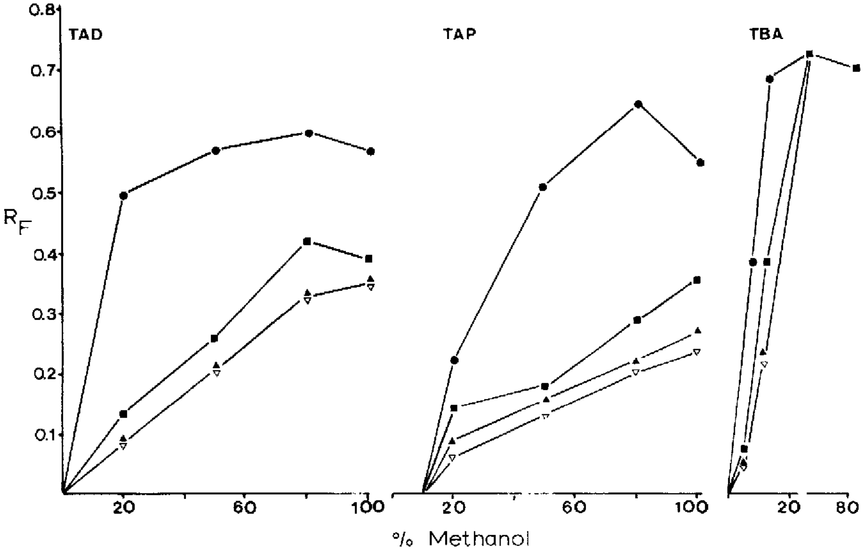

Fig. 2. Normal-phase ion-pair chromatography on silica gel coated with 1,12-bis(trimethylammonium) dodecane (TAD), 1,3-bis(trimethylammonium)propane (TAP) or tetra-*n*-butylammonium (TBA) of the four test acids (symbols as for Fig. 1) using methanol–dichloromethane based solvent systems.

n-butylammonium. However, it is noteworthy that, whilst the bolaform reagents gave good chromatography for all solvent compositions, tetra-*n*-butylammonium could only be used for normal-phase ion-pair TLC with solvents containing less than 20% methanol (Fig. 2).

Chromatography with ion-pair reagents in the solvent

The main reason for using TLC plates coated with the ion-pair reagents is that we have found in previous studies that the inclusion of the reagent in the solvent alone does not usually lead to acceptable chromatography[4] (see also ref. 3). The reason appears to be that the reagent migrates up the plate more slowly than the advancing solvent front, which therefore reaches and elutes the sample without the opportunity for ion-pair formation to occur. An exception to this general rule is tetramethylammonium, which is very water soluble and sufficiently polar to migrate with the solvent front. Tetramethylammonium is thus able to form an ion pair with the test acid. With tetra-*n*-butylammonium, ion-pairing is inefficient, often leading to the appearance of double spots corresponding to both ion-paired and non-ion-paired compounds. Given the high polarity and water solubility of the bolaform reagents we were curious to see whether, when included only in the solvent, an ion-pair effect would be observed. All four reagents (1,12-bis(trimethylammonium)dodecane, 1,3-bis(trimethylammonium)propane, tetra-*n*-butylammonium and tetramethylammonium) were dissolved in methanol–water (1:1) at a concentration of 0.1 *M*. This solvent system was then used to chromatograph the test acids on C_{18} bonded reversed-phase TLC plates. In the absence of any ion-pair reagent in the solvent all the test compounds chromatographed as long streaks. With 1,12-bis(trimethylam-

TABLE I

R_F VALUES FOR THE TEST ACIDS ON C_{18} BONDED REVERSED-PHASE TLC PLATES FOR ION-PAIR REAGENTS DISSOLVED IN THE SOLVENT OR COATED ONTO THE PLATES

Methanol–water (1:1) was used as solvent (with or without ion-pair). Ion-pair reagents: TAD = 1,12-bis(trimethylammonium)dodecane; TAP = 1,3-bis(trimethylammonium)propane; TBA = tetra-*n*-butylammonium; TMA = tetramethylammonium.

Compound	*Ion-pair reagent in solvent*				*Ion-pair reagent coated onto the plate*			
	TAD	*TAP*	*TBA*	*TMA*	*TAD*	*TAP*	*TBA*	*TMA*
2,4-Dihydroxybenzoic acid	0.56–0.45	0.65	0.65/0.2	0.61	0.52	0.64	0.33	0.63
2,6-Dihydroxybenzoic acid	0.24	0.44	streak	0.39	0.25	0.44	0.09	0.42
Gentisic acid	0.45	0.63	0.65	0.63	0.48	0.66	0.26	0.64
Salicylic acid	0.25	0.34	0.1	0.35	0.23	0.34	0.15	0.34

monium)dodecane and tetra-*n*-butylammonium 2,4-dihydroxybenzoic acid gave two spots (R_F values 0.56 and 0.45 for 1,12-bis(trimethylammonium)dodecane and 0.65 and 0.2 for tetra-*n*-butylammonium). With tetra-*n*-butylammonium (but not 1,12-bis(trimethylammonium)dodecane) 2,6-dihydroxybenzoic acid was also badly streaked. Gentisic and salicylic acid gave good spot shape with both reagents. Both 1,3-bis(trimethylammonium)propane and tetramethylammonium gave good spot shape for all four test compounds and appeared rather similar with roughly comparable R_F values (Table I). Of the four ion-pair reagents the long-chain bolaform compound 1,12-bis(trimethylammonium)dodecane gave the lowest R_F values, and thus formed the least polar "ion-pairs". On ion-pair reagent coated plates TBA gave the lowest R_F values (Table I), implying much less efficient ion-pair formation when present only in the solvent.

The R_F values obtained with 0.1 *M* ion-pair reagent in the mobile phase were, with the exception of TBA, similar to those observed following impregnation of the plates with a 0.1 *M* solution. A reduction in the concentration of ion-pair reagent in the solvent to 0.01 *M* gave very poor results with excessive streaking and double spots for all four test acids suggesting that insufficient reagent was present.

CONCLUSIONS

The two bolaform ion-pair reagents which we have investigated have some unusual and interesting properties. In particular, the reduction in R_F and reversal of migration order at high methanol content, properties which appear consistent with a change in chromatographic mechanism from reversed to normal phase, is not seen with "conventional" ion-pair reagents. This ability to use bolaform ion-pair reagents for both normal- and reversed-phase ion-pair TLC, over a wide range of solvent compositions, is a novel and potentially very useful property. The mechanism by which the reagents exert these effects remains unclear. However, the fact that the reduction in R_F and reversal of migration order observed on silica gel and paraffin coated silica gel plates are not seen on the C_{18} bonded phase implies that access to the silanols on the surface of the silica gel is important. Given the polarity of these reagents (as evidenced by their use in the solvent as well as coated onto the plates, and by their water solubility) it seems unlikely that they would easily gain entry into the highly lipophilic C_{18} stationary phase.

Further studies on ion-pair TLC with these compounds are underway. We are also intending to investigate their use for ion-pair HPLC where these new ion-pair reagents may also cast light on the mechanism(s) operating therein.

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