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THIN-LAYER CHROMATOGRAPHY OF QUATERNARY AMMONIUM SALTS ON ALUMINA LAYERS*

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SUMMARY

A thin-layer chromatographic technique using basic alumina layers and development with acetone-water (85:15 or 9:1) has been devised for the discrete separation of salts of compounds containing a single quaternary ammonium group. Quaternary ammonium cations were separated on basic alumina layers using chloroform-methanol-ammonia (6:3:1) as developing solvent and on acid alumina layers using single and two-fold development with chloroform-methanol mixtures. The effect of additional quaternary ammonium groups and free carboxylic groups on the chromatographic properties of compounds on alumina layers was also studied.

Using these systems efficient anion and cation separations of tetramethylammonium, tetraethylammonium, long-chain alkyl (C₁₂-C₁₈) trimethylammonium, choline, acetylcholine, N,N-dimethylpyrrolidinium, N,N-dimethylpiperidinium, N-methylpyridinium, berberine, columbamine, coptisine, jatrorrhizine, palmatine, betaine methyl ester, carnitine methyl ester, stachydrine methyl ester, homostachydrine methyl ester and trigonelline methyl ester salts were obtained.

INTRODUCTION

Whilst investigating the constituents of *Maerua* species, family Cappariaceae, we found that the tetramethylammonium chloride and nitrate present in several species¹ were well separated on basic and neutral alumina layers. In order to study further the chromatographic properties of quaternary salts on alumina layers we utilised compounds of biological and pharmaceutical interest which were readily available in our laboratories.

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Thin-layer chromatography (TLC) of quaternary ammonium salts has been carried out previously on silica gel² and impregnated cellulose layers³. The methods described involved, respectively, time-consuming pre-washing and activation procedures to reduce tailing due to ion exchange, or impregnation of the cellulose layers with aqueous stationary phase, followed by careful drying to give a standard water-cellulose ratio.

Separation of quaternary ammonium cations has been carried out on neutral alumina layers using acidic solvent mixtures⁴⁻⁶, and on acid alumina layers, neutral solvent mixtures have been used to separate synthetic curares⁷ and naturally occurring protoberberinium compounds^{8,9}.

Using air-dried, acid and basic alumina layers, we have developed chromatographic methods which give rapid and discrete separations of compounds containing a single quaternary ammonium group and their salts.

MATERIALS AND METHODS

Compounds

Compounds containing a single quaternary ammonium group. Tetramethylammonium chloride, tetraethylammonium bromide, choline chloride and acetylcholine chloride were supplied by British Drug Houses; dodecyltrimethylammonium bromide, trimethyltetradecylammonium bromide, hexadecyltrimethylammonium bromide and trimethyloctadecylammonium bromide by Schuchardt, München; and N,N-dimethylpyrrolidinium iodide and N,N-dimethylpiperidinium iodide by K and K Laboratories. N-Methylpyridinium iodide was synthesised. Berberine chloride, columbamine chloride, coptisine chloride, jatrorrhizine chloride and palmatine chloride were isolated⁹.

Compounds containing two or three quaternary ammonium groups. Hexamethonium bromide (Vegolysen) was obtained from May and Baker; suxamethonium chloride injection B.P. (Scoline) from Allen and Hanburys; tubocurarine chloride B.P. from Burroughs Wellcome*; and gallamine triethiodide injection (Flaxedil) from May and Baker.

Compounds containing a single quaternary ammonium group and a free carboxylic acid group. Betaine hydrochloride was supplied by Koch-Light and carnitine hydrochloride by Sigma. Stachydrine hydrochloride (N,N-dimethylpyrrolidine-2-carboxylic acid hydrochloride) and homostachydrine hydrochloride (N,N-dimethylpiperidine-2-carboxylic acid hydrochloride) were synthesised. Trigonelline hydrochloride (N-methylpyridine-3-carboxylic acid hydrochloride) was supplied by Fluka.

Compounds containing a single quaternary ammonium group and methylated carboxylic acid group. Betaine methyl ester chloride, carnitine methyl ester chloride, stachydrine methyl ester chloride, homostachydrine methyl ester chloride, and trigonelline methyl ester chloride were synthesised.

Preparations of methyl ester chlorides of compounds containing a single quaternary group and a free carboxylic acid group

Each compound was dissolved separately in methanol, excess methyl iodide was

* Regarded by some workers¹⁰ as only containing a single quaternary ammonium group.

added and the mixture refluxed for 1 h. The mixture was evaporated to dryness using a vacuum rotary evaporator and the residue dissolved in 75% methanol. The resultant solution of methyl ester iodide was then passed through a column containing either Amberlite IRA-400 (Cl⁻ form) or acid alumina for column chromatography (Woelm) to obtain the corresponding chloride. Recrystallisation was carried out from methanol-ether.

Preparation of salts

Each of the quaternary ammonium compounds listed above was dissolved separately in 50% methanol and each solution was treated with excess freshly-prepared, moist silver oxide to obtain the corresponding quaternary ammonium hydroxide. The resultant mixture was filtered and small portions of filtrate were treated separately with either ethanol saturated with hydrogen chloride, dilute hydrobromic acid, dilute hydriodic acid, dilute sulphuric acid, dilute phosphoric acid, dilute acetic acid or ethanol saturated with trinitrophenol. The salts so formed were precipitated by adding ether-acetone (75:25) and recrystallised from methanol-ether.

Alternatively, quaternary ammonium bromide, iodide, nitrate and picrate salts were prepared directly on the thin-layer plate using metathetical reactions². These were achieved by applying the quaternary ammonium chloride and potassium bromide, potassium iodide, potassium nitrate and potassium picrate, respectively, to the same points at the origin.

Sorbents

The following sorbents were used: Alumina Oxide G, for TLC, Merck; Basic Alumina, for TLC, Woelm; and Acid Alumina, for TLC, Woelm.

Thin-layer chromatography

Air-dried alumina layers, 500- μ wet thickness, were prepared on 20 \times 20 cm glass plates by suspending alumina in ethanol-water (9:1). Solutions of the quaternary ammonium salts in 50% methanol were applied to the plate or the metathetical reactions described previously were utilised to prepare a particular salt directly on the plate and development to a height of 15 cm from the point of application was carried out using the super-saturated method of STAHL¹¹. The solvents used for the separation of the salts of compounds containing a single quaternary ammonium group on basic alumina layers were acetone-water (85:15), or acetone-water (9:1) for those materials having high R_F values in the former solvent. Separation of the cations was more successful on basic alumina layers with chloroform-methanol-ammonia sp. gr. 0.88 (6:3:1) as developing solvent, or on acid alumina layers by single development with chloroform-methanol (4:1) or by single or double development with chloroform-methanol (85:15). The separated materials were detected using either Dragendorff's reagent (Munier and Macheboeuf modification), which gave red, violet, magenta or orange spots, or 2% iodine in methanol, with an overspray of saturated sodium nitrite solution in each case, when intensification of spots or detection of impurities was required.

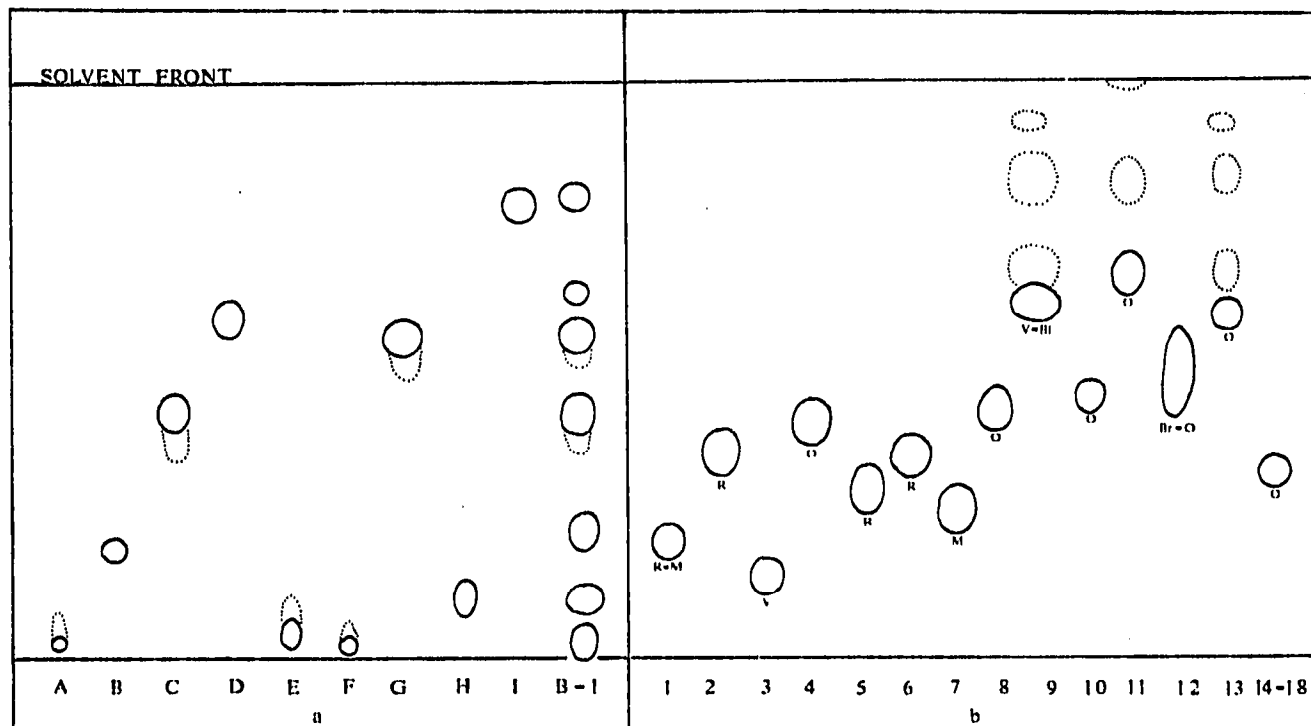
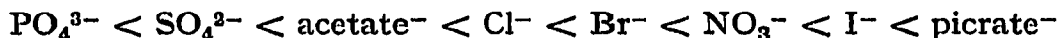


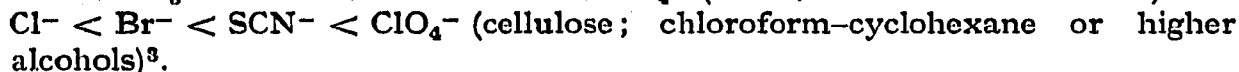
Fig. 1. TLC of quaternary ammonium salts. Sorbent: basic alumina. Solvent: acetone-water (85:15). Colour produced with modified Dragendorff's reagent: Bl = black; Br = brown; M = magenta; O = orange; R = red; V = violet. Samples in (a): A = tetramethylammonium hydroxide; B = chloride; C = bromide; D = iodide; E = sulphate; F = phosphate; G = nitrate; H = acetate and I = picrate. Samples in (b): 1 (=B) = tetramethylammonium chloride; 2 = tetraethylammonium chloride; 3 = choline chloride; 4 = acetylcholine chloride; 5 = N,N-dimethylpyrrolidinium chloride; 6 = N,N-dimethylpiperidinium chloride; 7 = N-methylpyridinium chloride; 8 = long-chain alkyl (C_{12} , C_{14} , C_{16} , C_{18})-trimethylammonium chlorides; 9 = berberine chloride; 10 = columbamine chloride; 11 = coptisine chloride; 12 = jatrorrhizine chloride; 13 = palmatine chloride; 14 = betaine methyl ester chloride; 15 = carnitine methyl ester chloride; 16 = stachydrine methyl ester chloride; 17 = homostachydrine methyl ester chloride; and 18 = trigonelline methyl ester chloride.

RESULTS AND DISCUSSION

The chromatogram of tetramethylammonium salts (Fig. 1a) illustrates the order of anion separation in all compounds containing a single quaternary ammonium group and methyl esters of compounds containing a single quaternary ammonium group and a free carboxylic acid group examined, the order being:



This order may be compared with those observed in other TLC systems



The main difference between our results and those of GORDON² is the position of the nitrate anion in relation to the halide anions. Our order of anion separation is the same as the order of preferential solvation of anions by water in aqueous dioxane¹³,

the order of miscibility of quaternary ammonium salts with polar associated liquids¹⁴ and the separation would seem to be largely dependent on the hydrogen bond strengths of the anions¹⁵, as discussed by GORDON².

Using basic alumina layers, little or no tailing of spots was seen, with the exception of protoberberinium alkaloid salts and discrete separation of the salts was obtained. With neutral alumina, separation was similar to that illustrated (Fig. 1a), but pronounced tailing of the spots was observed, whereas on acid alumina layers all the salts move close together, close to the solvent front.

Salts of compounds containing two or three quaternary ammonium groups did not separate in accordance with the anion present on basic, neutral or acid alumina layers using acetone-water mixtures. When applied to basic or neutral alumina layers all the salts gave long streaks from R_F 0.0-0.5, whereas on acid alumina, although the cations are separated in a similar way to that described by FIORI AND MARIGO⁷ using chloroform-methanol (4:1), the salts were not separated. In this class of quaternary ammonium compound, polarity and molecular size may play a significant role in the lack of separation of their salts.

If a free carboxylic acid group is introduced into the molecule, as in betaine, carnitine, stachydrine, homostachydrine and trigonelline, it is no longer possible to separate the salts by the method described. All the salts of each compound have the same R_F value and they remain on or near the point of application on acid, neutral and basic alumina layers, the greatest adsorption affinity being for acid alumina and the least for basic alumina. It would appear that the adsorption affinity of the carboxylic acid group over-rides that of the anions since, on methylation of the

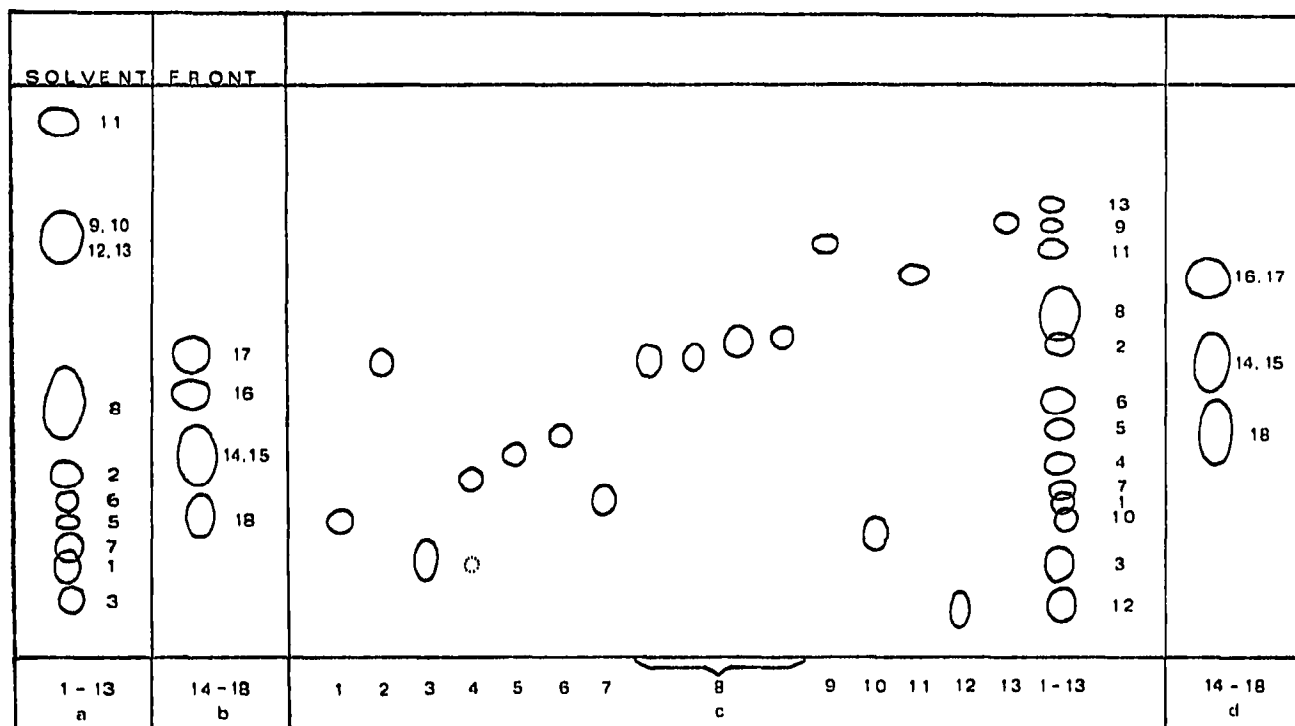


Fig. 2. TLC of quaternary ammonium cations. Sorbents: (a and b) basic alumina; (c and d) acid alumina. Solvents: (a and b) chloroform-methanol-ammonia (6:3:1), (c and d) chloroform-methanol (85:15). Samples: as in Figs. 1a and b.

carboxylic acid group, the salts of the resultant methyl esters show the same distribution pattern as illustrated (Fig. 1a).

It will be seen that the cations of the compounds containing a single quaternary ammonium group and compounds containing a single quaternary ammonium group and a methylated carboxylic acid group are not well separated on basic alumina using acetone-water (85:15) (Fig. 1b) and it was found that these were separated more successfully using chloroform-methanol-ammonia sp. gr. 0.88 (6:3:1) as the developing solvent (Figs. 2a and b). This solvent did not separate the anions greatly but gave a discrete separation of most cations except those of some protoberberinium alkaloids, betaine and carnitine methyl esters and those containing long-chain alkyl groups (C_{12} - C_{18}) which, whilst separated from the other cations used, did not separate one from another. The solvent is also unsuitable for choline esters as the basic conditions are sufficient to cause hydrolysis.

The cations may also be separated using acid alumina layers with chloroform-methanol (4:1) as developing solvent⁷. This system gave good separation of many of the compounds containing a single quaternary ammonium group (Fig. 1d), although some upward tailing on both air-dried and activated layers was observed in cations having R_F values above 0.5, with consequent overlapping when separating mixtures. This upward tailing was increased if higher alcohols were substituted for methanol, whilst good separations and spot shapes were achieved by reducing the proportion of methanol in the solvent mixture and using single development (Figs. 2c and d) or double development for more efficient resolution of lower spots. No breakdown of acetylcholine was observed, but as with basic alumina layers, the cations containing long-chain alkyl groups (C_{12} - C_{18}) were not separated from one another. All salts of a particular cation were found to move to the same height and it was shown, by subsequent elution and chromatography on basic alumina layers with acetone-water (85:15), that the salts were converted to the corresponding chloride. Anion exchange takes place, therefore, during the separation on acid alumina layers and these can only be used for the chromatographic confirmation of cation identity and not for prior separation of the cations when knowledge of the original anions is required.

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