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Note

Thin-layer chromatography of methyl N-trimethyl-γ-aminobutyrate chloride and related compounds

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So far, only a few reports[1–4] on the application of thin-layer chromatography to aliphatic quaternary ammonium compounds have been published.

In this work, methyl N-trimethyl-γ-aminobutyrate chloride (MTB), which is a drug with excellent cholinergic action[5,6], has been separated from its structurally related compounds by a TLC method.

MATERIALS

The MTB used was the pharmaceutical material synthesized in this Institute and other related compounds shown in Table I were obtained by the following methods.

The acidic form (TB) was prepared by the hydrolysis of MTB in 1 *N* hydrochloric acid, and the esters (ETB, PTB and BTB) were prepared by the transesterification of MTB with the appropriate anhydrous alcohols contained in about 0.1 *M* equivalent hydrochloric gas. All of these reactions were carried out at 70° for 3 h.

METHODS

The TLC plates were prepared as follows. A slurry of 30 g of Kieselgel G (E. Merck, Darmstadt, G.F.R.) in 60 ml of water was spread on 10×20 cm glass plates to a thickness of 0.25 mm with a Camag (Muttenz, Switzerland) automatic applicator. The plates were dried at 120° for 1 h and stored in a desiccator.

A 1% aqueous solution of each compound was prepared and 1 μl of the solution (corresponding to 10 μg of each compound) was spotted at 2.5 cm from the edge of the plate. The plate was developed at about 25° with the solvent system ethyl acetate–formic acid–water (10:2:2) until the solvent front had travelled about 12 cm. About 40 min were usually required for the development.

The plate was then dried and spread with Dragendorff's reagent, and each of the compounds appeared as an orange spot.

TABLE I

THIN-LAYER CHROMATOGRAPHY OF MTB AND RELATED COMPOUNDS

General formula of MTB compounds: $[(CH_3)_3N^+-CH_2-CH_2-CH_2-COO-R]Cl^-$.
Plate: Kieselgel G, 0.25 mm layer, 120° for 1 h.
Developing solvent: ethyl acetate–formic acid–water (10:2:2).

Compound	*R*	R_F value*
Methyl N-trimethyl-γ-aminobutyrate chloride (MTB)	CH_3	0.21
N-trimethyl-γ-aminobutyrate chloride (TB)	H	0.17
Ethyl N-trimethyl-γ-aminobutyrate chloride (ETB)	C_2H_5	0.28
n-Propyl N-trimethyl-γ-aminobutyrate chloride (PTB)	C_3H_7	0.37
n-Butyl N-trimethyl-γ-aminobutyrate chloride (BTB)	C_4H_9	0.42

* Mean R_F values of five experimental results.

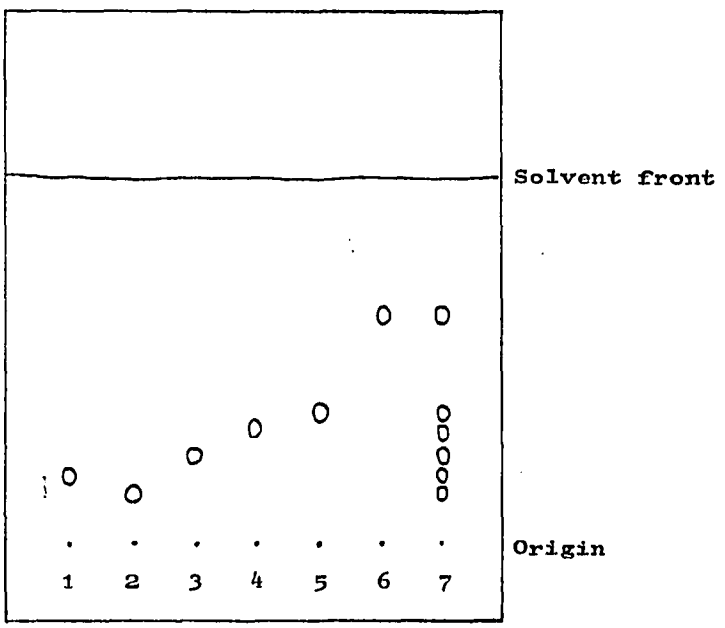

Fig. 1. Thin-layer chromatogram of MTB and related compounds. Plate: Kieselgel G, 0.25 mm layer, 120° for 1 h. Solvent system: ethyl acetate–formic acid–water (10:2:2). 1=MTB; 2=TB; 3=ETB; 4=PTB; 5=BTB; 6=crystal violet; 7=mixture.

RESULTS AND DISCUSSION

The R_F values and a typical chromatogram are shown in Table I and Fig. 1.

The detection limit was found to be *ca.* 1 μg for each compound. The only adsorbent used in our work was Kieselgel G, but heating of the TLC plates at various temperatures (105–150°) was also examined. Several developing solvents, such as acetone–36% hydrochloric acid (10:1)[1], ethyl acetate–formic acid–water[4]

TABLE II

R_{st} VALUES OBTAINED USING CRYSTAL VIOLET AS THE STANDARD MATERIAL

Compound	*R_F value**		*Rst value**	
	Mean	*Coefficient of variation (%)*	*Mean*	*Coefficient of variation (%)*
MTB	0.21	10.0	28	7.1
TB	0.17	10.4	23	7.5
ETB	0.28	8.1	37	6.2
PTB	0.37	7.0	49	3.9
BTB	0.42	6.0	56	3.7
Crystal violet	0.75	3.5	100	—

* Mean values of five experimental results.

in various proportions, *n*-butanol–acetic acid–water (60:20:20)[7], phenol–water (75:25)[7], benzene–ethanol (5:1)[8] and methanol[9], were also examined.

Heating the plates hardly affected the chromatograms, but the best separation was achieved with a plate heated at 120° for 1 h. On the other hand, the various developing solvents had a much greater effect, and sharp spots free from tailing were found only in the solvent system ethyl acetate–formic acid–water. Increasing the proportion of formic acid gave higher R_F values for the each compound, but did not improve the separation. The most favourable result was achieved with ethyl acetate–formic acid–water in the proportions 10:2:2.

The R_F values obtained in this TLC system showed some variations (Table II), but the separations were adequate for the identification of each compound.

The R_{st} values[10] were applied using crystal violet as the standard material, and the results were better in terms of reproducibility, as shown in Table II.

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