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Note

A fluorogenic spray for the detection of phospholipids on thin-layer chromatograms

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In a previous paper[1], we reported the use of 1-anilinonaphthalene-8-sulphonate (ANS) as a fluorogenic spray reagent for the detection and fluorometric microdetermination of phospholipids on thin-layer chromatograms. The effect seemed to involve combination of ANS with apolar regions of positively charged and neutral lipids[2,3]. However, in a further study, for which we required very pure natural phospholipids, we found that ANS could not be removed from the lipid compound (particularly with phosphatidylethanolamine and lecithin) without use of an acidic environment, conditions we preferred to avoid because of the lability of some phospholipids. The presence of ANS in no way affected the results of gas chromatographic analysis for fatty acids.

As the strong interaction of ANS with lipids is probably due to bonding of the sulphonic acid group to the amino-group of the lipid, we experimented with use of the more inert N-phenyl-1-naphthylamine (NPN). After spraying the plates with an ethanolic solution of this dye, we were able to locate the separated compounds by using a short-wave UV lamp. The phospholipids could then be freed from any NPN by developing the plate with pure (ethanol-free) chloroform, as the fluorochrome moved with the front, leaving the phospholipids in place.

EXPERIMENTAL

Thin-layer plates

We used silica gel pre-coated plates (20 × 20 cm) with 0.25-mm layers for analytical and 2-mm layers for preparative separations, each without a fluorescent indicator (E. Merck, Darmstadt, G.F.R.). Before use, the plates were developed with methanol, dried at room temperature for 24 h and used without any activation.

Developing solvents

Lipid samples were applied to the plates as homogeneous lines with a Hamilton micro-syringe attached to a Desaga dispenser. One-dimensional chromatograms of phospholipids were developed with chloroform–methanol–water (65:30:4). The chromatographic chamber, which was lined with filter paper, was saturated with

the solvent vapour for 1 h before use. Chromatograms of neutral lipids were developed with hexane–diethyl ether–acetic acid (80:20:0.5).

All solvents were redistilled before use; chloroform was freed from methanol by filtration through an alumina column.

Fluorogenic spray

After development, the plates were air-dried and uniformly sprayed with a freshly prepared 0.1% ethanolic solution of NPN (twice recrystallised from ethanol and treated with charcoal). The lipid components appear as blue spots when viewed by short-wavelength UV radiation from a lamp with a Woods glass filter. The plates must be free from any organic solvent, as this can interfere with the fluorescence of the separated compounds. The sensitivity of this method is similar to that previously attained with ANS[1].

RESULTS AND DISCUSSION

The background of layers after being sprayed with NPN was practically zero, provided that no ethanol or developing solvent was present. As little as 1 nmole of egg lecithin, dipalmitoyl-lecithin, or sphingomyelin and 10 nmoles of egg phosphatidylethanolamine could easily be detected visually, and these values can be considered as reasonable detection limits. Instrumental detection, of course, would improve the limits.

In a previous paper[1] we described the method and gave results of the micro-determination directly on the plate. However, great care should be taken when using this method, as the fluorescence enhancement is affected not only by the polar end-group, but also by the composition of the side-chains. The intensity increases with increasing carbon number and decreases with increasing unsaturation[4]. Thus, if direct fluorometric micro-determination of an unknown phospholipid mixture is attempted, results will only be reliable if at least one separate phosphorus determination is made.

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