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Note

Biochemistry of sphingolipids

XXVII. Thin-layer microchromatography of DNP derivatives of long-chain bases on Silufol$_{UV\,254}$ sheets

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Plastic sheets pre-coated with a layer of silica gel or other adsorbents are commonly used for the thin-layer chromatographic (TLC) separation of various compounds. Many such sheets are now commercially available, *e.g.*, Eastman Chromagram Sheets (Eastman-Kodak, Rochester, N.Y., U.S.A.) and MN-Polygram sheets (Macherey, Nagel & Co., Düren, G.F.R.). All of these products consist of a layer of adsorbent bound with poly(vinyl alcohol) to a solvent-inert support of poly(ethylene terephthalate). In some instances a fluorescent indicator is added.

E. Merck (Darmstadt, G.F.R.) developed aluminium TLC sheets coated with silica gel, aluminium oxide, cellulose or polyamide layers. A similar type of sheet, Baker-Flex, was introduced by J. T. Baker (Phillipsburg, N.J., U.S.A.).

Another type of pre-coated sheet, Silufol$_{UV254}$, is manufactured by Glassworks Kavalier, Votice, Czechoslovakia. This type consists of a reflecting silica gel sheet with an inert inorganic luminiscent indicator which makes possible the highly improved detection of spots by virtue of a reflecting reinforced aluminium support. Starch is used as a binder.

In previous work[1], we used these sheets for the differentiation of dinitrophenyl (DNP) derivatives of long-chain bases according to the degree of unsaturation, after impregnation of the silica gel layer with Ag^+ ions. In the further studies reported here we have applied this method as a simple microtechnique for the separation of DNP derivatives of long-chain bases and their degradation products.

EXPERIMENTAL

Materials

DNP derivatives of long-chain bases were prepared from sphingolipids isolated from various human body tissues by the methods published earlier[2]. Standard samples of some degradation products originating from long-chain bases during hydrolysis in an acidic medium were prepared in our laboratory[3] or were a generous gift from Dr. K. A. Karlsson (University of Gothenburg, Sweden).

Chromatography

The following types of non-impregnated and impregnated sheets of Silufol$_{UV\ 254}$, Series 052168 (7.5 × 4 cm or 7.5 × 7.5 cm) were used: (a) non-impregnated sheets without previous activation; (b) sheets impregnated with 0.05 *M* sodium tetraborate were prepared as described for "classical" silica gel layers or paper chromatography[4,5]; (c) sheets impregnated with tetralin (reversed-phase chromatography) were prepared as described earlier[4,5].

Solvent systems

The following systems were used: 1, *n*-hexane–diethyl ether (20:80); 2, chloroform–methanol (95:5); 3, methanol–tetralin–water (90:10:10; upper phase).

Detection

The spots were located under UV light, the DNP derivatives appearing as dark spots on a green fluorescent background.

RESULTS AND DISCUSSION

Silufol$_{UV\ 254}$ sheets are very suitable for the resolution of DNP derivatives under various experimental conditions. We studied the separating power of these sheets in comparison with the results obtained in "classical" TLC and paper chromatography. It was found that the separation on Silufol$_{UV254}$ is very effective, with the advantages of the use of only minute amounts of the DNP derivatives, a reduced time of development and increased quality of resolution.

Using non-impregnated sheets without previous activation it was possible to obtain a good separation of many DNP derivatives of natural long-chain bases together with some of their degradation products (Fig. 1).

The impregnation of the silica gel layer with sodium tetraborate permits the resolution of *erythro* and *threo* isomers of 4-sphingenine, sphinganines and 4D-hydroxysphinganines (Fig. 2).

The two-dimensional technique on sheets partly impregnated with sodium tetraborate is very useful (Fig. 3). In this instance the separation of almost all derivatives was better than that obtained by one-dimensional TLC.

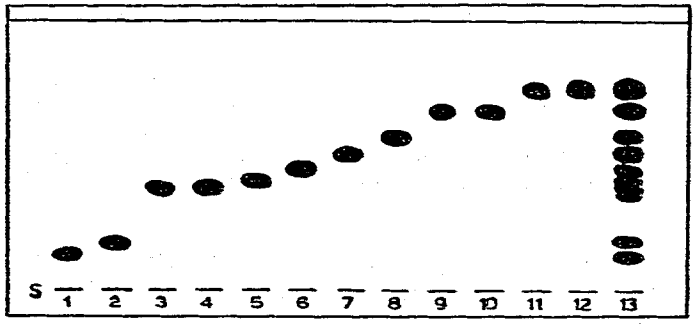

Fig. 1. Separation of DNP derivatives of long-chain bases and their degradation products on Silufol$_{UV254}$ sheets. Solvent system: *n*-hexane–diethyl ether (20:80). 1 = 5D-Hydroxy-3-sphingenine; 2 = 5L-hydroxy-3-sphingenine; 3 = 4-sphingenine; 4 = 4D-hydroxysphinganine; 5 = sphinganine; 6 = 3L-4-sphingenine; 7 = 5D-methoxy-3-sphingenine; 8 = 5L-methoxy-3-sphingenine; 9 = 3D-methoxy-4-sphingenine; 10 = 3L-methoxy-4-sphingenine; 11 = *cis*-3,5-sphingadiene; 12 = 3,5-sphingadiene; 13 = mixture of 1–12.

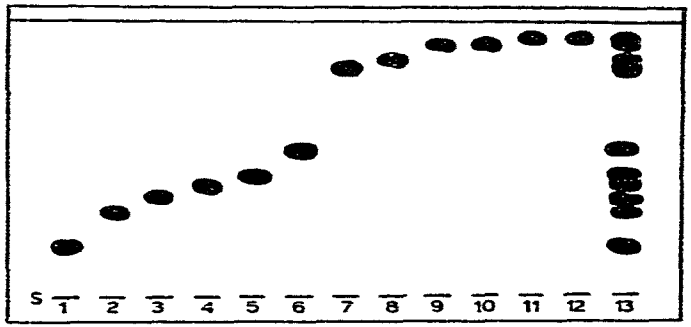

Fig. 2. Separation of DNP derivatives of long-chain bases and their degradation products on Silufol$_{UV254}$ sheets impregnated with 0.05 *M* $Na_2B_4O_7$. Solvent system: chloroform–methanol (95:5). 1 = 4D-Hydroxysphinganine; 2 = 3L-4-sphingenine; 3 = 4-sphingenine; 4 = 5L-hydroxy-3-sphingenine; 5 = 5D-hydroxy-3-sphingenine; 6 = sphinganine; 7 = 5L-methoxy-3-sphingenine; 8 = 5D-methoxy-3-sphingenine; 9 = 3L-methoxy-4-sphingenine; 10 = 3D-methoxy-4-sphingenine; 11 = *cis*-3,5-sphingadiene; 12 = 3,5-sphingadiene; 13 = mixture of 1–12.

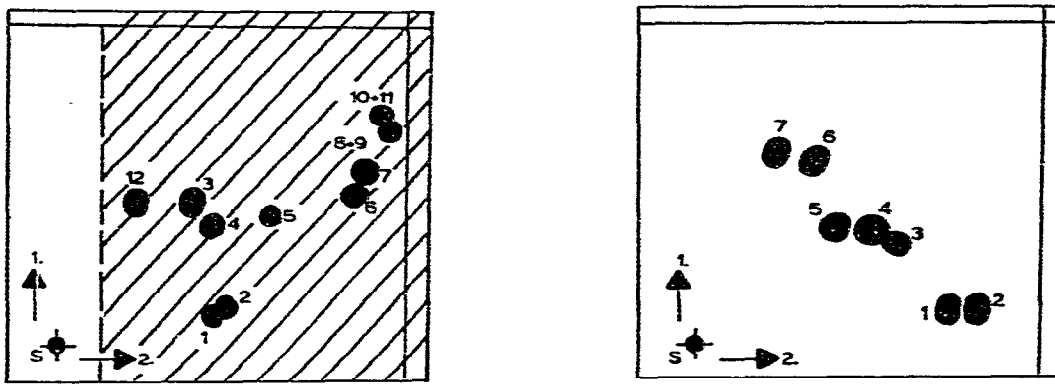

Fig. 3. Two-dimensional separation of DNP derivatives of long-chain bases and their degradation products on Silufol$_{UV254}$ sheets partly impregnated with 0.05 *M* $Na_2B_4O_7$ (hatched area). Solvent systems: 1st dimension, *n*-hexane–diethyl ether (20:80); 2nd dimension, chloroform–methanol (95:5). 1 = 5D-hydroxy-3-sphingenine; 2 = 5L-hydroxy-3-sphingenine; 3 = 3L-4-sphingenine; 4 = 4-sphingenine; 5 = sphinganine; 6 = 5D-methoxy-3-sphingenine; 7 = 5L-methoxy-3-sphingenine; 8 = 3D-methoxy-4-sphingenine; 9 = 3L-methoxy-4-sphingenine; 10 = *cis*-3,5-sphingadiene; 11 = 3,5-sphingadiene; 12 = 4-hydroxysphinganine.

Fig. 4. Two-dimensional separation of DNP derivatives of long-chain bases and their degradation products on a reversed-phase system. Solvent systems: 1st dimension (impregnation with 0.05 *M* $Na_2B_4O_7$), chloroform–methanol (95:5); 2nd dimension, methanol–tetralin–water (90:10:10, upper phase) after impregnation with tetralin. 1 = 4D-Hydroxyeicosasphingenine; 2 = 4D-hydroxysphingenine; 3 = 3L-4-sphingenine; 4 = 4-sphingenine; 5 = eicosasphingenine; 6 = sphinganine; 7 = eicosasphinganine.

Two-dimensional TLC on a layer impregnated with sodium tetraborate and with a reversed-phase system make possible the simultaneous separation of homologous and isomeric derivatives (Fig. 4).

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