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Biochemistry of sphingolipids

XXV. Separation and identification of long-chain bases and their degradation products on silica gel layers impregnated with silver nitrate

Thin-layer chromatography on silver nitrate-impregnated silica gel is known to separate the unsaturated compounds according to the number of isolated double bonds, and according to the geometrical configuration of such double bonds (*cis* or *trans*). More recently the possibility of separating suitable positionally isomeric unsaturated compounds has also been reported[1,2].

The application of such impregnated layers to the separation of long-chain bases was briefly reported by KARLSSON[3]. Using this technique the differentiation of saturated, monoenoic and dienoic bases seems to be very effective.

The present communication deals particularly with the chromatography of the DNP(dinitrophenyl)-derivatives of the above-mentioned naturally occurring bases and at the same time with the unsaturated degradation products originating from acid hydrolytic conditions[4].

Experimental

Isolation of long-chain bases. The crude long-chain base extracts were obtained from a human brain sphingolipid mixture after hydrolysis according to GAVER AND SWEELEY[5]. A mixture of the bases containing the dienoic derivatives was isolated from human blood serum sphingomyelins by the action of phospholipase C (phosphatidylcholine phosphohydrolase EC 3.1.4.3) and subsequent hydrolysis with 1 *N* methanolic potassium hydroxide[6]. Other substances (*e.g.* DNP-derivatives of the various byproducts were kindly donated by Dr. K. A. KARLSSON, University of Gothenburg, Sweden).

Chromatography. Precoated silica gel sheets (Silufol UV 254, Sklárny Kavalier, Votice, Czechoslovakia) were impregnated with Ag^+-ions by immersion in a 5% aqueous silver nitrate solution and after drying briefly at laboratory temperature, activated for 30 min at 100–110°.

Samples were applied as solutions in diethyl ether 0.5 cm from the lower edge of the chromatograms (4 cm × 7.5 cm) and developed without previous saturation of the chamber in chloroform–methanol (95:5).

For the simultaneous separation of the homologous long-chain bases, a two-dimensional modification was performed. A part of the chromatogram (7.5 cm × 7.5 cm) was impregnated with $AgNO_3$ and run in chloroform–methanol (95:5) in the first dimension. After a short drying period at laboratory temperature the sheets were impregnated with a 5% solution of tetralin in diethyl ether and developed with methanol–tetralin–water (90:10:10; upper phase) for the second dimension. The spots were located under U.V. light.

Results and discussion

Fig. 1 illustrates a typical example of a one-dimensional chromatogram of saturated, monoenoic and dienoic long-chain bases. As can be seen, the mobilities of

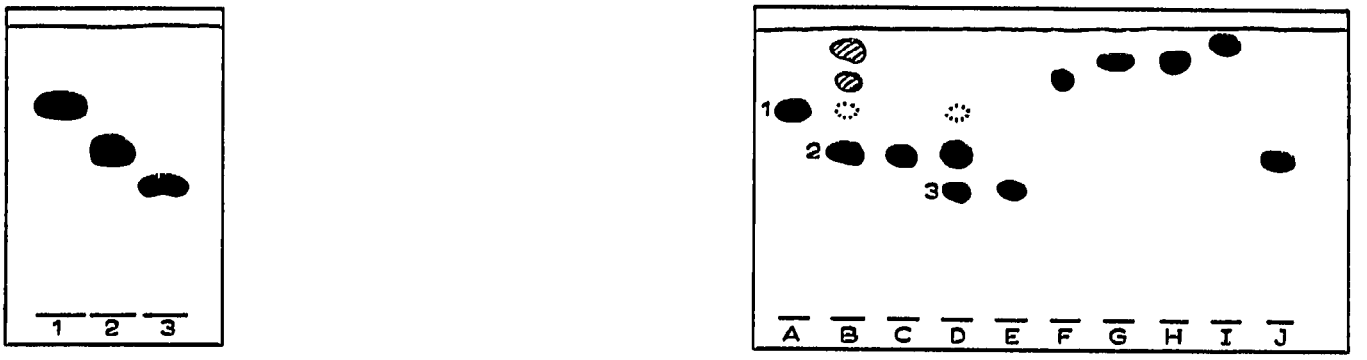

Fig. 1. Separation of DNP-derivatives of long-chain bases on silica gel sheets impregnated with silver nitrate in chloroform–methanol (95:5). (1) = Saturated bases (eicosasphinganine); (2) = monoenoic bases(4-sphingenine); (3) = dienoic bases (isolated from human blood serum sphingomyelins, *cis*-4,14-octadecadiene).

Fig. 2. Separation of DNP-derivatives of long-chain bases and their degradation products on silica gel sheets impregnated with silver nitrate in chloroform–methanol (95:5). (A) = Saturated bases; (B) = crude long-chain bases isolated from human brain sphingolipids after acid hydrolysis[5]; (C) = 3L-4-sphingenine; (D) = crude long-chain bases isolated from human blood serum sphingomyelins; (E) = 5D-hydroxy-3-sphingenine + 5L-hydroxy-3-sphingenine; (F) = *cis*-3,5-sphingadiene; (G) = 3,5-sphingadiene; H = 5D-methoxy-3-sphingenine + 5L-methoxy-3-sphingenine; (I) = 3D-methoxy-4-sphingenine + 3L-methoxy-4-sphingenine; (J) = 4D-hydroxy-sphingenine. (1) = Saturated bases; (2) = monoenoic bases; (3) = dienoic bases.

all three classes of compounds depend on the degree of unsaturation. The same effect has been observed many times with argentated thin-layer chromatograms of methyl esters of fatty acids or other related substances[1].

The resolution of other long-chain base degradation products originating from

Fig. 3. Two-dimensional separation of DNP-derivatives of long-chain bases and their degradation products on silica gel sheets partly impregnated with silver nitrate (hatched lines). Solvent systems: 1st dimension, petroleum ether (b.p. 60–90°)–diethyl ether (20:80); 2nd dimension, chloroform–methanol (95:5). (1) = 5D-Hydroxy-3-sphingenine + 5L-hydroxy-3-sphingenine; (2) = dienoic bases (*cis*-4,14-sphingadiene); (3) = monoenoic bases (4-sphingenine); (4) = saturated bases (sphingenine); (5) = 5D-methoxy-3-sphingenine; (6) = 5L-methoxy-3-sphingenine; (7) = 3D-methoxy-4-sphingenine + 3L-methoxy-4-sphingenine; (8) = *cis*-3,5-sphingadiene; (9) = 3,5-sphingadiene.

Fig. 4. Two-dimensional separation of DNP-derivatives of long-chain bases isolated from human blood serum sphingomyelins on silica gel sheets partly impregnated with silver nitrate (hatched lines). Solvent systems: 1st dimension, chloroform–methanol (95:5); 2nd dimension, methanol–tetralin–water (90:10:10; upper phase)after impregnation with tetralin. (1) = Sphingenine; (2) = eicosasphingenine(?); (3) = 4-sphingenine; (4) = 4-heptadecasphingenine(?); (5) = 4-hexadecasphingenine(?); (6) = *cis*-4,14-octadecasphingadiene.

different acid hydrolytic conditions is shown in Fig. 2. The most interesting aspect is the possibility of separating the two geometrical isomers of the 3,5-sphingadienes. These isomers are to be expected as the dehydration products of 4-sphingenine. In our system the *trans,trans* isomer has a higher mobility than the *cis,trans* isomer. This observation confirms the results of STROCCHI AND PIRETTI[8] obtained by thin-layer chromatography of the geometrical isomers of 9,12-octadecadienoic acid.

It can be seen from Fig. 2 that some derivatives have the same or very similar mobility in the system described and are not successfully separated (mainly several degradation products of 4-sphingenine). In such a case it is very useful to employ the two-dimensional technique. We have used a modification in which the chromatogram (partly impregnated with $AgNO_3$) was run in petroleum ether (b.p. 60–90°)–diethyl ether (20:80; 1st dimension) and then in chloroform–methanol (95:5; 2nd dimension) (Fig. 3)[7].

This solvent combination permits the characterization of practically all long-chain bases and their degradation products.

The separation on silica gel sheets impregnated with silver nitrate and tetralin allows a good resolution of the DNP long-chain base derivatives according to their degree of unsaturation and their position in the homologous series (Fig. 4).

Summarizing, an improved one- or two-dimensional microchromatographic procedure for DNP long-chain bases and their degradation products on Silufol UV 254 sheets impregnated with $AgNO_3$ is described. Some examples of the practical application of this technique to the identification of natural bases and their synthetic derivatives are shown. A more detailed report about further work with these novel precoated sheets will be published in our next paper[7].

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