Biochemistry of sphingolipids

VII. Separation of isomeric sphingosine bases as their dinitrophenyl derivatives

At present a great variety of conditions has been used for the cleavage of sphingolipids. The most commonly employed reagent, acidic methanol, generally gives good yields of total long-chain base but also leads to the formation of undesirable by-products of sphingosine. During our comparative study on the formation of some degradation products in various hydrolytic procedures of sphingolipids special interest was taken in the erythro- and threo-sphingosines[1].

It seems likely that some threo-isomer is formed by inversion during acid hydrolysis. However, this does not rule out the possibility that some of this isomer occurs naturally.

Several authors [2–4] have reported the separation of the free bases of erythro- and threo-sphingosine. In earlier papers[5–7] we have described the use of thin-layer chromatography for the characterization of DNP (dinitrophenyl)-derivatives of sphingosines and phytosphingosines on aluminium oxide. Although this technique is

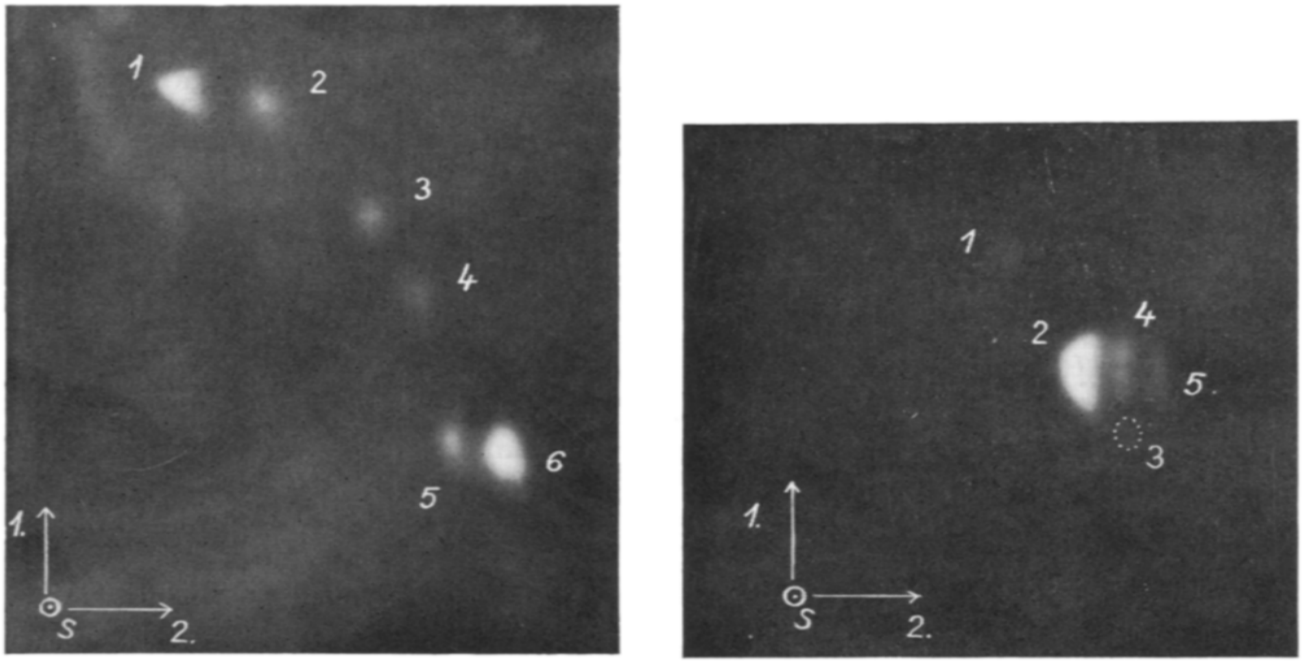

Fig. 1. Two-dimensional thin-layer chromatography of DNP-derivatives of erythro- and threo-sphingosines. Adsorbent: silica gel G impregnated with sodium tetraborate; 1st dimension: chloroform–methanol (90:10); 2nd dimension: methanol–tetralin–water (90:10:10) (upper phase) after impregnation of the layer with 5% tetralin. Detection: U.V. light (254 nm). DNP-derivatives: 1 = C_{20}-dihydrosphingosine; 2 = C_{18}-dihydrosphingosine; 3 = erythro-C_{18}-sphingosine; 4 = threo-C_{18}-sphingosine; 5 = C_{20}-phytosphingosine; 6 = C_{18}-phytosphingosine; S = start.

Fig. 2. Sphingosine bases of human blood serum sphingomyelins after aqueous methanolic HCl hydrolysis according to GAVER AND SWEELEY[8]. Experimental conditions are the same as in Fig. 1. 1 = C_{18}-dihydrosphingosine; 2 = erythro-C_{18}-sphingosine; 3 = threo-C_{18}-sphingosine; 4 = C_{17}-sphingosine; 5 = C_{16}-sphingosine; S = start.

very useful for the identification of these substances no resolution of isomeric sphingosines was obtained.

A two-dimensional thin-layer chromatographic method is reported here for the qualitative separation of isomers on silica gel impregnated with sodium tetraborate.

The borate-impregnated plates (18 × 18 cm) were prepared from a slurry of silica gel G (E. Merck) and a half-saturated solution of sodium tetraborate in water. The plates were dried at room temperature overnight. The solvent systems used for developing the chromatograms were chloroform–methanol (90:10) in the first dimension and methanol–tetralin–water (90:10:10) after impregnation with tetralin in the second dimension as described earlier[5–7]. The spots were visualized under ultra-violet light (Fig. 1).

The two-dimensional system here described was superior to the system on Al_2O_3 layers in which no separation of the isomers was obtained. Separations on non-borate silica gel were also unsuccessful. The excellent resolving power, rapidity and simplicity makes this modification very useful for the analysis of mixtures of isomeric sphingosines with other long-chain bases.

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