Biochemistry of sphingolipids. XII. Paper chromatography of dinitrophenyl derivatives of sphingosines and their degradation products

In our previous papers[1–4] we have reported on the thin-layer chromatography of DNP (dinitrophenyl)-derivatives of sphingosines and phytosphingosines on aluminium oxide and silica gel impregnated with sodium tetraborate. This method was widely applied to the separation of long-chain bases prepared from sphingolipids of animal and plant origin.

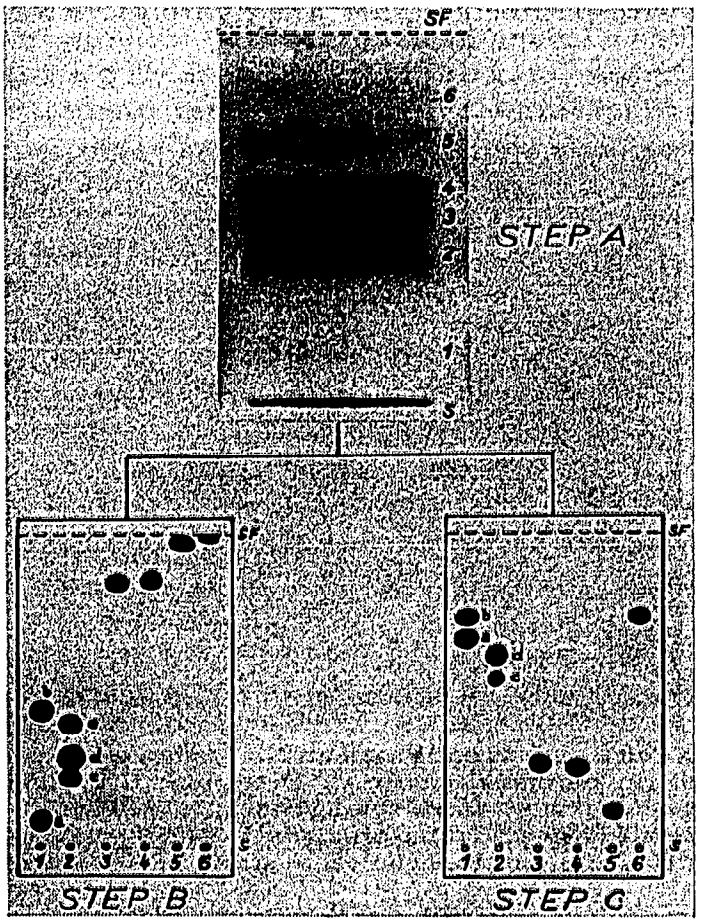
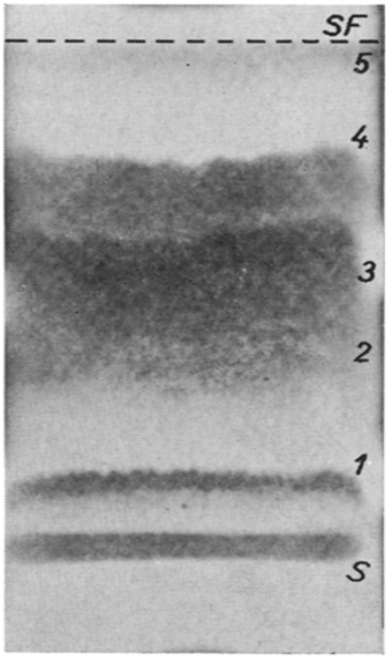


Fig. 1. Schematic representation of the paper chromatographic fractionation of DNP-sphingosine bases and their degradation products. Step A. Preparative chromatography of crude DNP-derivatives on Whatman No. 3 paper impregnated with silica gel. System: petroleum ether (b.p. 60–90°)–diethyl ether (65:35). Detection: U.V. light (254 nm). DNP-derivatives: 1 = phytosphingosines + unidentified degradation products I; 2 = sphingosines + dihydrosphingosines; 3 = O-methyl derivatives I (probably 3-O-methyl-sphingosines); 4 = O-methyl derivatives II (probably 5-O-methyl-Δ^3-sphingosines); 5 = unidentified degradation products II; 6 = 2,4-dinitrophenol. S = start; SF = solvent front. Step B. Separation of DNP-fractions after preparative isolation on Whatman No. 3 silica gel paper impregnated with sodium tetraborate in chloroform. 1a = Phytosphingosines; 1b = unidentified degradation products I; 2c = threo-sphingosines; 2d = erythro-sphingosines; 2e = dihydrosphingosines; 3 = O-methyl derivatives I; 4 = O-methyl-derivatives II; 5 = unidentified degradation products II; 6 = 2,4-dinitrophenol. Step C. Separation of DNP-fractions after preparative isolation on Whatman No. 3 paper impregnated with tetralin in methanol–tetralin–water (90:10:10; upper phase). 1a = C_{20}-phytosphingosine + unidentified degradation products I; 1b = C_{18}-phytosphingosine; 2c = C_{18}-dihydrosphingosine; 2d = C_{18}-sphingosine; 3 = O-methyl-derivatives I; 4 = O-methyl-derivatives II; 5 = unidentified degradation products II; 6 = 2,4-dinitrophenol.

During comparative studies on the formation of some degradation products in various hydrolytic procedures of sphingolipids[5], we have found paper chromatography a simple, rapid and valuable technique for the characterization of these substances.

Experimental

(*a*) *Hydrolysis of sphingolipids and preparation of DNP-derivatives.* 25 ml of freshly prepared methanol–sulfuric acid reagent (1.25 ml of conc. H_2SO_4 diluted to 25 ml with methanol) was added to a sample of 50 mg of human brain sphingolipids (without gangliosides). The mixture was heated for 18 h at 60–70° in a water bath. After removal of fatty acid methyl esters with petroleum ether, the hydrolysate was



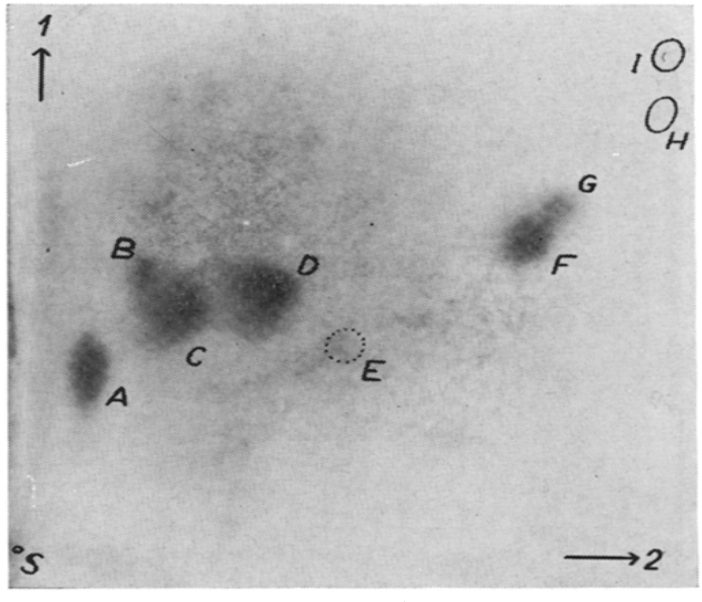


Fig. 2. Preparative chromatography of crude DNP-derivatives on Whatman No. 3 silica gel paper impregnated with sodium tetraborate in chloroform–methanol (100:0.5). Detection: U.V. light (254 nm). DNP-derivatives: 1 = phytosphingosines; 2 = threo-sphingosines; 3 = erythro-sphingosines; 4 = dihydrosphingosines + unidentified degradation products I; 5 = O-methyl-derivatives + unidentified degradation products II + 2,4-dinitrophenol; S = start; SF = solvent front.

Fig. 3. Two-dimensional paper chromatography of DNP-derivatives. 1st dimension: Whatman No. 3 silica gel paper; petroleum ether (b.p. 60–90°)–diethyl ether (65:35). 2nd dimension: chloroform after impregnation with sodium tetraborate. Detection: U.V. light (254 nm). A = Phytosphingosines; B = threo-sphingosines; C = erythro-sphingosines; D = dihydrosphingosines; E = unidentified degradation products I; F = O-methyl-derivatives I; G = O-methyl-derivatives II; H = unidentified degradation products II; I = 2,4-dinitrophenol. S = start.

made alkaline with potassium hydroxide. Sphingosine bases were then extracted with diethyl ether. The ether solution was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The DNP-derivatives were prepared according to KARLSSON[6].

DNP-derivatives of phytosphingosines were prepared from baker's yeast.

(*b*) *Preparative paper chromatography of DNP-derivatives on paper impregnated with silica gel (Step A).* The crude DNP-derivatives dissolved in diethyl ether were

spotted as a narrow band along the entire length of the starting line on Whatman No. 3 paper (18 cm × 18 cm) impregnated with silica gel. The chromatogram was developed in petroleum ether (b.p. 60–90°)–diethyl ether (65:35). After drying for a short time in air, the zones were located under ultraviolet light and continuously eluted with methanol.

(*c*) *Chromatography of individual fractions on silica gel paper impregnated with sodium tetraborate* (*Step B*). The eluates of all fractions obtained from preparative chromatography were evaporated at room temperature under nitrogen. After dissolving in a small volume of diethyl ether they were spotted on Whatman No. 3 silica gel paper impregnated with sodium tetraborate. The impregnation was performed by drawing the paper through an aqueous half-saturated solution of sodium tetraborate (22.4 g $Na_2B_4O_7 \cdot 10H_2O$ in 1000 ml water) and drying at 110–120° for 10 min. The chromatograms were run in chloroform or chloroform–methanol (100:0.5).

(*d*) *Chromatography of individual fractions in a reversed-phase system* (*Step C*). The fractions were separated on Whatman No. 3 silica gel paper impregnated with 2.5–5 % solution of tetralin in diethyl ether using methanol–tetralin–water (90:10:10, upper phase) as a solvent system.

Step A (Fig. 1) allows the separation of sphingosines, phytosphingosines and most of the degradation products. Step B is advantageous for the further subfractionation of sphingosines into isomers and differentiation of these from dihydrosphingosines. In addition, phytosphingosines are separated from the degradation products. Step C permits the identification of the homologous DNP-sphingosines, dihydrosphingosines and phytosphingosines.

The preparative chromatographic procedure on silica gel paper with or without impregnation with sodium tetraborate could be used for the quantitative estimation of each type of DNP-derivatives (Fig. 2)[5].

(*e*) *Two-dimensional chromatography*. The combination of the systems described above in a two-dimensional modification would appear to be useful for the rapid qualitative characterization of the constituents of crude DNP-derivatives extracts (Fig. 3).

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