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Sphingoplasmalogens. A new type of sphingolipids

We have already reported[1] that the use of thin-layer chromatography[2] enabled detection of some new minor components in the brain cerebroside fraction purified from phospho- and sulpholipids. It appears now, that the minor components belong to a previously unknown class of sphingolipids.

Our first tentative proposal[1] was that the compounds differ from usual cerebrosides only in the presence of a new fatty acid, bound to the amino group of sphingosine. In contradiction to this suggestion, it appeared that *N*-palmitoylsphingosine galactoside* had the same mobility by thin-layer chromatography (R_F 0.21) as the natural or synthetic cerasine and dihydrocerasine. Hence, R_F values (0.62 and 0.56) of the minor components considerably higher than that of cerasine (0.21) could be explained only by substitution of one of the free hydroxyl groups by a long alkyl residue. This view was supported by the R_F values of minor components close to those of mono-*O*-palmitoylcerebrosides, obtained by acetylating cerebrone and cerasine (2 moles of palmitoyl chloride, pyridine, for 2 h at room temperature). However, the natural minor components differed from the synthetic mono-*O*-palmitoylcerebrosides in that the ester carbonyl band (1627 cm^{-1}) of their infrared spectra was absent (Fig. 1). The other difference was the complete stability of the minor components towards alkaline hydrolysis (0.5 N NaOH, 40°, 3 h). Analogous treatment of mono-*O*-palmitoylcerebrosides resulted in their complete destruction to the corresponding cerebrosides. This suggested that the minor components are not cerebroside *O*-acyl derivatives.

We isolated minor components from fraction A (ref. 2) on a preparative scale using thin-layer chromatography with the solvent system chloroform–methanol (85:15, v/v) (column chromatography still gives poor resolution[1]). The zones were detected by spraying the dry chromatogram with water[4]. Some 10 mg of each minor

* The substance was obtained from psychosine by the method of WEISS AND RAISMAN[3].

component were isolated as white amorphous substances. The samples were homogeneous by chromatography after crystallization from hot methanol.

One of the isolated minor components, corresponding to Spot 3 (Fig. 2), termed

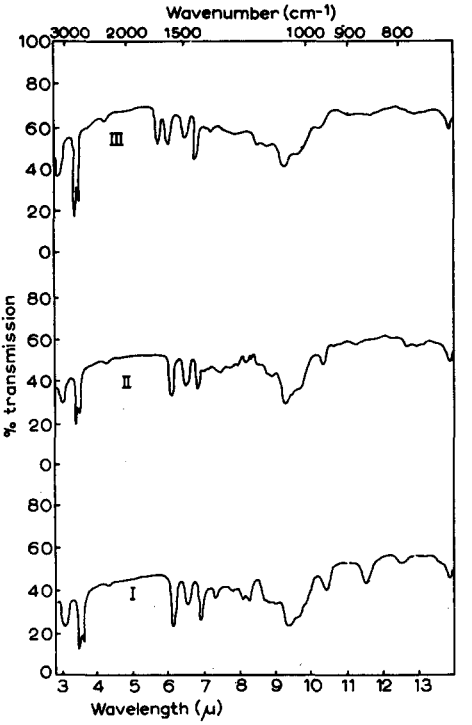


Fig. 1. Infrared spectrum of sphingolipids (KBr pellet). I, Sphingolipid 3; II, cerebrone; III, mono-*O*-palmitoylcerebrone.

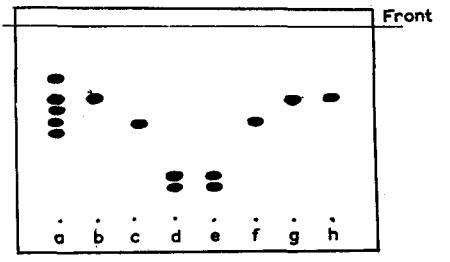


Fig. 2. Thin-layer chromatography of sphingolipids on silica gel. Solvent system: chloroform–methanol (85:15, v/v). Detection: conc. H_2SO_4 spray. a, 150 μg of Fraction A (ref. 2); b, 30 μg of Sphingolipid 3 isolated by thin-layer chromatography; c, 30 μg of Sphingolipid 1 isolated by thin-layer chromatography; d, cerebrone + cerasine (30 μg); e, Sphingolipid 3 after acid hydrolysis; f, Sphingolipid 1 after acid hydrolysis; g, Sphingolipid 3 after alkaline hydrolysis; h, 30 μg of mono-*O*-palmitoylcerasine.

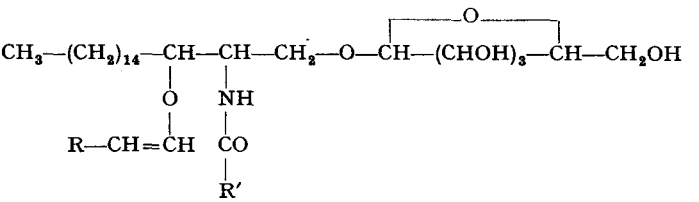
further as "Sphingolipid 3", was subjected to detailed analysis. Sphingolipid 3 released dihydrosphingosine on periodate oxidation and subsequent hydrolysis[5]; the base was identified by thin-layer chromatography[6]. Methanolysis (10% methanolic H_2SO_4, 100°, 4 h) afforded, besides dihydrosphingosine minor quantities of sphingosine. Acid

hydrolysis, (10 % aq. HCl, 80°, 8 h, neutralization by Dowex-2 X8, CO_3^{2-} form) afforded galactose and about 5 % glucose. Mild acid hydrolysis of Sphingolipid 3 in the presence of mercury catalyst (1 ml of 0.01 N H_2SO_4, 0.1 ml of 0.05 M $HgCl_2$, 100°, 15 min) resulted in a quantitative yield of dihydrocerebrone and dihydrocerasine mixture, the former being predominant. Acid lability of Sphingolipid 3 and its stability to alkaline hydrolysis resembled the behaviour of plasmalogens and suggested the presence of an alkenyl ether grouping.

The infrared spectrum of Sphingolipid 3 is very similar to that of cerebrone. However, its intense band at 970 cm^{-1} (Fig. 1), though not differing in intensity or position from that of the cerebrone double bond, cannot be due to the presence of the sphingosine residue, as more than 90 % of the base residues of the compound are represented by dihydrosphingosine. The intensity of this band was at the same time too high to relate it to the presence of unsaturated fatty acid, as the unsaturated fatty acid content of sphingolipids is usually less than 20 % (ref. 7).

Higher fatty aldehyde was detected in the acid hydrolysate of Sphingolipid 3 by the method of WITTENBERG *et al.*[8]. Quantitative determination was unsuccessful because of the much lower rate of hydrolysis than occurs with plasmalogens.

All the above data together suggest the following structure of Sphingolipid 3:



(1)

Sphingolipid 3 contained 13.8 % galactose[9]. Formula I ($R' = CH_3(CH_2)_{20}CHOH$, $R = C_{16}H_{33}$) requires 14.6 %. The sphingosine base:galactose ratio was equal to 1:1. Sphingosine base was determined spectrophotometrically[10].

Hence, one of the minor components of the cerebroside fraction is *O*-alkenylcerebroside, a representative of a new class of sphingolipids which we propose to term "sphingoplasmalogens". Our product, named Sphingolipid 3, is certainly not an individual compound, but a mixture of substances of general structure shown by the above formula and differing in substituents R and R'. This mixture is contaminated probably with some other lipids. In spite of this, we now claim the presence of this new class of sphingolipids in beef-brain tissue.

We have investigated, preliminary, the structure of Sphingolipid 1 (Fig. 2). Periodate oxidation and subsequent hydrolysis of this substance lead to the formation of a sphingosine base with a chromatographic mobility close to that of sphingosine *O*-methyl ether, and like this compound staining yellow when detected with ninhydrin[6]. Sphingolipid 1 contains galactose, less than 5 % glucose, and a fatty acid. It is inert towards mild acid and alkaline hydrolysis. Its infrared spectrum is similar to that of cerebroside. We tentatively suggest Sphingolipid 1 to be a previously unknown derivative of 3-*O*-alkylsphingosine or 3-*O*-alkyldihydrosphingosine.

Work is now in progress to isolate individual sphingoplasmalogens for the final elucidation of their structure and function.

We should like to express our thanks to Professor D. SHAPIRO (Israel) for the synthetic cerebrosides: cerasine, dihydrocerasine and cerebrone.

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