Studies on Sphingosines

6. C_{16}- and C_{17}-Sphingosines, hitherto Unknown Sphingosines*

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As announced earlier [1] two new sphingosine fractions have been isolated from human plasma sphingomyelins. The composition of these has now been determined. In addition, chromatographic evidence for several other sphingosines has been obtained.

The sphingomyelins were isolated as described earlier.[1] The material was subjected to dephosphorylation according to Bevan *et al.*[3] Part of the material was catalytically hydrogenated prior to dephosphorylation. The reagent mixture was evaporated and alkaline degradation according to Carter *et al.*[4] performed. The hydrolysis mixture was acidified with hydrochloric acid and extracted with diethyl ether. In this way a quantitative recovery of sphingosines plus fatty acids was achieved. This mixture was separated on silicic acid into a fatty acid (eluant: 2 % methanol in chloroform) and a sphingosine (eluant: 75 % methanol in chloroform) fraction. The sphingosine fraction was then converted to the corresponding dinitrophenyl (DNP) derivatives by dissolving in dinitrofluorobenzene in ethanol and addition of buffer.[5] The ether extract was fractionated as described earlier.[6]

From the hydrogenated fraction no preparative by-products [7] were found, as expected in the absence of allylic groups. The original fraction, however, gave 30–40 % by-products of the total sphingosines. A smaller part of these, eluted before the main fraction on silicic acid, has not been fully characterized but is probably composed of dehydration products [7] of sphingosines. The dominating part, eluted after the main fraction on silicic acid, contains allylic rearrangement products [7] of sphingosines. The main fraction contains about one fourth of *threo* isomers.

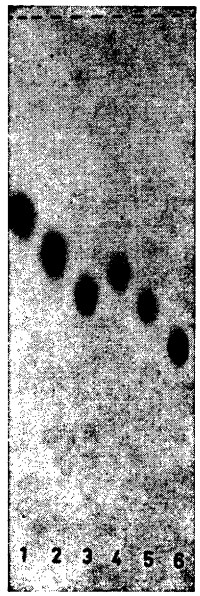

Fig. 1. Chromatogram showing dinitrophenyl derivatives of C_{16}-sphingosine (1), a mixture of C_{17}-sphingosine and two dienic C_{18}-sphingosines (2), C_{18}-spingosine (3), C_{16}-dihydrosphingosine (4), C_{17}-dihydrosphingosine (5) and C_{18}-dihydrosphingosine (6). For chromatographic details, see Ref.[6]

A partition chromatogram of the different isolated components is shown in Fig. 1. Fractions 1–3 are from the native and fractions 4–6 from the catalytically hydrogenated sphingomyelins. (In addition to these some slow moving fractions have been isolated. They make up about one third of the total and their chemical structures are under study.) The fractions were subjected to oxidation with potassium permanganate and lead tetraacetate and the acids and aldehydes produced were identified by means of gas chromatography. The infrared absorption in the *trans* double bond region together with acid degradation [7] indicate the presence of allylic groups. The results are collected in Table 1. Fraction 3 is C_{18}-sphingosine and fraction 6 C_{18}-dihydrosphingosine. The results from fraction 1 are consistent with a C_{16}-sphingosine structure, those from fraction 4 with a

* Communications 4 and 5 in this series are Refs.[1] and [2], respectively.

Table 1. Characterization results of sphingosine fractions. (The fractions are the same as in Fig. 1. In parentheses percentage peak areas are shown. In fraction 2 correction has been made for further degradation during the permanganate oxidation.)

Fraction number	Acids from permanganate oxidation	Aldehydes from tetra-acetate oxidation	Infrared absorption in chloroform	Acid degradation
1	12:0 (83)	14:1 (99)	10.3 μ	Pos.
2	13:0 (29)	15:1 (16)	10.3 μ*	Pos.
	10d:0 (56)	16:2? (78)		
	8d·0 (15)			
3	14:0 (83)	16:1	10.3 μ	Pos.
4	14:0 (70)	14:0 (98)	No 10.3 μ	Neg.
5	—	15:0 (94)	No 10.3 μ	Neg.
6	16:0 (76)	16:0 (97)	No 10.3 μ	Neg.

* Stronger than in fractions 1 and 3.

C_{16}-dihydrosphingosine structure and those from fraction 5 with a C_{17}-dihydrosphingosine structure. Fraction 2 is a mixture of several substances. The 13:0 acid and 15:1 aldehyde are consistent with the presence of a C_{17}-sphingosine structure. This is confirmed by the C_{17}-dihydrosphingosine obtained after catalytic hydrogenation (fraction 5). The dominating dicarboxylic acid (10d:0) indicates the presence of a structure with two double bonds. The chromatographic localizations of the DNP-fraction and the aldehyde, as well as the comparison after catalytic hydrogenation suggest a C_{18}-structure. This and the probable presence of an allylic group presents the structure of a C_{18}-sphingosine with double bonds in the 4—5 and 14—15 positions. The presence of a small amount of a dicarboxylic acid with eight carbon atoms (8d:0) indicates the probable existence of an isomer with the second double bond in the 12—13 position. The further separation of the mixture in fraction 2 is under investigation.

On sodium periodate oxidation [8] of the total sphingosines, aldehydes in the correct positions for C_{15}-sphingosine, C_{15}-dihydrosphingosine, C_{14}-sphingosine and C_{14}-dihydrosphingosine are obtained. However, as the total amount of these is less than 2 % and the by-products contaminate, no definite statement regarding the natural occurrence of these sphingosines can be made until they have been concentrated as undegraded structures.

The relative amounts of C_{18}-, C_{17}- and C_{16}-sphingosines are about 90 : 1—2 : 4—5, respectively. The C_{18}-sphingosines with two double bonds make up about 10—15 per cent of the total C_{18}-sphingosines. The C_{17}- and C_{16}-sphingosines are dominated by unsaturated structures.

Details of this work will be published later.

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