Studies on Sphingosines

7. The Existence of C_{18}- and C_{20}-Phytosphingosine in Animal Tissues*

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C_{18}-Phytosphingosine,[2] C_{18}-dehydrophytosphingosine [2] and C_{20}-phytosphingosine [3] have so far been found only in plants. Sphingosines containing an allylic group (C_{16}-,[1] C_{17}-,[1] C_{18}- and C_{20}-sphingosine [4]) have, on the other hand, been isolated only from animals. C_{18}-Dihydrosphingosine, long known to exist in animals, has recently been discovered in plant tissues.[5,6] The present communication gives evidence for the existence of phytosphingosines in animal tissues.

The animal phytosphingosines have been isolated from ceramides of hair (human and animal) and from cerebrosides of human kidney [7] (kindly provided by E. Mårtensson of this Department). The ceramides were subjected to alkaline and the cerebrosides to acid hydrolysis. The sphingosine fraction was purified, converted to dinitrophenyl (DNP) derivatives, fractionated and the isolated fractions characterized as described earlier.[1] The results are shown in Fig. 1 and Table 1.

* Communication 6 in this series is Ref.[1]

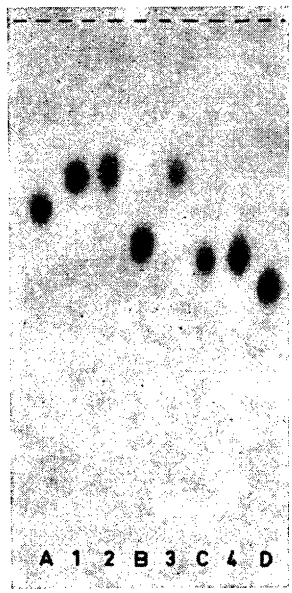

Fig. 1. Chromatogram showing dinitrophenyl derivatives of C_{18}-phytosphingosine from corn (1), C_{18}-phytosphingosine from hair (2), C_{18}-phytosphingosine from kidney (3) and C_{20}-phytosphingosine from hair (4). For comparison dinitrophenyl derivatives of the following substances [1] have been co-chromatographed: C_{16}-sphingosine (A), a mixture of C_{17}-sphingosine and two dienic C_{18}-sphingosines (B), C_{16}-dihydrosphingosine (C) and C_{18}-sphingosine (D). For chromatographic details, see Ref.[10]

Table 1. Characterization results of sphingosine fractions. (The fractions are the same as in Fig. 1. In parentheses percentage peak areas are shown.)

Fraction number	Acids from permanganate oxidation	Aldehydes from tetra-acetate oxidation	Infrared absorption in chloroform	Acid degradation
1	15:0 (85)	15:0 (99)	Strong 9.4 μ	Neg.*
2	15:0 (86)	15:0 (91)	Strong 9.4 μ	Neg.
3	—	15:0 (88)	Strong 9.4 μ	Neg.
4	17·0 (88)	17:0 (94)	Strong 9.4 μ	Neg.

* The inability to produce anhydro structures is in agreement with other results.[11]

Fraction 1 is C_{18}-phytosphingosine isolated from corn phosphatides (General Biochemicals, Ohio). The results from fraction 2, isolated from hair, and fraction 3, isolated from kidney, are consistent with a C_{18}-phytosphingosine structure whereas those of fraction 4, isolated from hair, suggest a C_{20}-phytosphingosine structure. The degradation with sodium periodate and lead tetra-acetate can be used to differentiate between DNP-sphingosines and DNP-phytosphingosines. DNP-C_{17}-dihydrosphingosine [1] and DNP-C_{18}-phytosphingosine both give pentadecanal on oxidation with lead tetra-acetate. On periodate oxidation, however, only DNP-C_{18}-phytosphingosine yields this product.

The cerebrosides from kidney contain C_{18}-sphingosine, C_{18}-dihydrosphingosine, one not yet fully characterized fraction and C_{18}-phytosphingosine. The last makes up about half of the total fraction. Ceramides from hair contain C_{18}- and C_{20}-sphingosine, C_{18}- and C_{20}-dihydrosphingosine and C_{18}- and C_{20}-phytosphingosine. The last two make up 5—20 % of the total fraction. C_{18}-Phytosphingosine has also been chromatographically detected in adult human brain cerebrosides but only in amounts corresponding to a few promille of the total sphingosines.

The practical absence of phytosphingosines from the metabolically inert [8] brain cerebrosides, the co-existence of C_{18}-phytosphingosine and C_{18}-sphingosines in kidney cerebrosides and C_{18}- and C_{20}-phytosphingosine and C_{18}- and C_{20}-sphingosines in hair ceramides suggests a metabolic relationship between phytosphingosines and sphingosines. In spite of the lack of experimental evidence it is proposed that sphingosines are formed from dihydrosphingosines [9] with phytospingosines as intermediates. If this is the case, the last step does not exist in plants. This problem will be explored as well as its connections with the chemical problems of the sphingolipidoses.

The recently disovered sphingosines in human blood [1] make likely the natural existence of the corresponding phytosphingosines: C_{16}- and C_{17}-phytosphingosine as well as C_{18}-phytosphingosine with one double bond, *e.g.* isomers of dehydrophytosphingosine. [2] No attempt has yet been made to verify this.

Details of this work will be published later. The author is indebted to Professor Herbert E. Carter, University of Illinois, for the generous gift of phytosphingosine and dehydrophytosphingosine used as reference compounds in the present investigation.

1. Karlsson, K.-A. *Acta Chem. Scand.* **18** (1964) 2395.
2. Carter, H. E. and Hendrickson, H. S. *Biochemistry* **2** (1963) 389.
3. Prošťenik, M. and Stanaćev, N. Ž. *Chem. Ber.* **91** (1958) 961.
4. Majhofer-Oreščanin, M. and Prošťenik, M. *Croat. Chem. Acta* **33** (1961) 219.
5. Stodola, F. H., Wickerham, L. J., Scholfield, C. R. and Dutton, H. J. *Arch. Biochem. Biophys.* **98** (1962) 176.
6. Carter, H. E., Hendry, R. A., Nojima, S., Stanaćev, N. Ž. and Ohno, K. *J. Biol. Chem.* **236** (1961) 1912.
7. Mårtensson, E. *Acta Chem. Scand.* **17** (1963) 2356.
8. Davison, A. N., Morgan, R. S., Wajda, M. and Payling Wright, G. *J. Neurochem.* **4** (1959) 360.
9. Brady, R. O., Formica, J. V. and Koval, G. J. *J. Biol. Chem.* **233** (1958) 1072.
10. Karlsson, K.-A. *Acta Chem. Scand.* **18** (1964) 565.
11. O'Connell, P. W. and Tsien, S. H. *Arch. Biochem. Biophys.* **80** (1959) 289.

Received November 18, 1964.