Studies on Sphingosines

16. The Chemical Structure of a Dienic Long Chain Base of Human Blood Plasma Sphingomyelins

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A suggestion for a dienic long chain base of blood plasma sphingomyelins was first made in 1959.[1] A periodate oxidation product was shown by gas chromatography on polyester columns to be more retained than hexadecenal and to change to hexadecanal after hydrogenation. No attempts were made to characterize the proposed parent base. Some years later a dinitrophenyl (DNP) derivative was isolated from the same source and suggested to be a C_{18} derivative with an allylic group and double bonds in the 4 and 14 positions.[2,3] However, the compound was prepared from an acid hydrolysate, known to contain by-products, and was not pure. Therefore the structure was redetermined on a pure fraction isolated by mild, non-acid procedures.

Human blood plasma sphingomyelins [2] were quantitatively degraded by a combination of enzymatic and alkaline hydrolysis.[4] Fatty acids and long chain bases were separated on silicic acid [3] and part of the bases hydrogenated with platinum oxide as catalyst. Natural and hydrogenated bases were converted to their corresponding DNP-derivatives [3] and freed from reagent products by chromatography on silicic acid. The DNP-derivatives of the natural fraction were then subjected to preparative thin layer chromatography on silver nitrate containing silica gel.[4] The mixture separated into three fractions, one fast moving fraction with saturated dihydroxy bases, one major, intermediate fraction with monoenic dihydroxy bases and some minor unidentified compounds (see Fig. 1), and one slow moving fraction with dienic dihydroxy compounds. The last fraction was finally separated on reversed phase paper chromatography [5] and shown to be practically one component.

On infrared spectroscopy in chloroform this component had a *trans* double bond absorption of the same intensity as DNP-

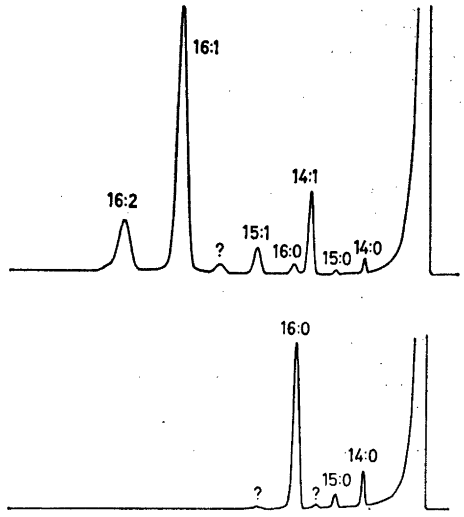

Fig. 1. Gas chromatograms of aldehydes derived from human blood plasma sphingomyelin long chain bases, before (upper) and after (lower) catalytic hydrogenation.

sphingosine, indicating only one *trans* double bond. The aldehyde derived from lead tetraacetate oxidation [3] had a molecular weight of 236 (by mass spectrometry [6]), consistent with hexadecadienal. After potassium permanganate oxidation a C_{10} dicarboxylic acid and smaller amounts of C_9 and C_8 dicarboxylic acids were identified. After heating in hydrochloric acid the pure DNP-derivative showed the same chromatographic by-product pattern as DNP-sphingosine, indicating the presence of an allylic group. By mass spectrometry of the trimethylsilyl ethers of the hydrogenated bases the C_{18} compound was assigned a 1,3-dihydroxy-2-amino structure.[6]

The DNP-derivatives of the hydrogenated bases were analyzed by thin layer chromatography on borate impregnated layers [4] with synthetic references of *erythro* and *threo* dihydrosphingosine (kindly supplied by H. E. Carter and D. Shapiro). Only traces of *threo* compounds could be seen. As the total long chain base fraction contains 15 % of the dienic compound (Fig. 1 and Table 1), this should have an *erythro* configuration. Although the optical rotation has not yet been measured, the

Table 1. Sphingosine composition of human blood plasma sphingomyelins. In the shorthand designations used d stands for dihydroxy; the number before the colon indicates carbon chain length and the number after the colon number of double bonds. See text for further details.

Aldehyde identified	Parent base	Relative amounts Natural bases	Hydrogenated bases
Tetradecanal	d16:0	1	11
Tetradecenal	d16:1	10	0
Pentadecanal	d17:0	0.3	5
Pentadecenal	d17:1	5	0
Hexadecanal	d18:0	1.5	80
Hexadecenal	d18:1	65	0
Hexadecadienal	d18:2	15	0
Unidentified	Unidentified	2	4

configuration of carbon atom 2 is analogous to sphingosine [7] and probably D.

The purified DNP-derivatives of total natural and hydrogenated bases were oxidized with lead tetraacetate and the aldehydes produced quantitatively analyzed by gas chromatography on Reoplex 400 columns (Fig. 1 and Table 1). The lower sphingosine homologues have been identified before.[3,6] The unidentified peaks may be monoenic, branched chain [8] dihydroxy bases.

Based on the above reported findings the dienic long chain base may be given the following structure: D-*erythro*-1,3-dihydroxy-2-amino-4, 14(*cis*, *trans*)-octadecadiene. In analogy with sphingosine the *trans* double bond is probably in the 4 position. The unusual location of the extra double bond close to the methyl end of the long chain base may have a special biological meaning.

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1. Sweeley, C. C. and Moscatelli, E. A. *J. Lipid Res.* **1** (1959) 40.
2. Karlsson, K.-A. *Biochem. J.* **92** (1964) 39P.
3. Karlsson, K.-A. *Acta Chem. Scand.* **18** (1964) 2395.
4. Karlsson, K.-A. *To be published.*
5. Karlsson, K.-A. *Acta Chem. Scand.* **18** (1964) 565.
6. Karlsson, K.-A. *Acta Chem. Scand.* **19** (1965) 2425.
7. Carter, H. E. and Humiston, C. G. *J. Biol. Chem.* **191** (1951) 727.
8. Carter, H. E., Gaver, R. C. and Yu, R. K. *Biochem. Biophys. Res. Commun.* **22** (1966) 316.

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Bacterial Carotenoids

XXVI.* C_{50}-Carotenoids. 2. Bacterioruberin

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The principal carotenoid of several halophilic bacteria, α-bacterioruberin, was assigned the structure *1* on the basis of earlier work in this laboratory.[1-3] However, 1,1'-dihydroxy-3,4,3',4'-tetradehydro-1,2,1',2'-tetrahydrolycopene (*1*, $C_{40}H_{56}O_2$) has recently been synthesized by Schneider and Weedon and a direct comparison with natural α-bacterioruberin revealed that the two compounds are not identical.[4,5] The structure of bacterioruberin (the prefix α should be reserved for carotenoids containing an α-cyclogeranylidene ring and will be omitted) is now being re-investigated using improved methods including NMR and mass spectrometry.

The molecular formula $C_{50}H_{76}O_4$ has been established by high resolution mass spectrometry. The previously reported physical data for bacterioruberin have been confirmed, as has the absence of primary, secondary and allylic hydroxyl groups and functional groups susceptible to hydride reduction: the infrared data preclude functional groups other than tertiary hydroxyl groups. Experiments involving silylation [6] and dehydration (with phosphorus oxychloride [7]) demonstrated the presence of four tertiary hydroxyl groups. The silylation reaction was shown to comprise four consecutive steps by isolation of three

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