Studies on Sphingosines

11. The Chemical Structure of Phytosphingosine of Human Origin and a Note on the Lipid Composition of the Yeast *Hansenula ciferrii**

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Recently, evidence was presented for the presence in human and animal tissues of phytosphingosine and related bases (trihydroxy bases).[2-4] The mass spectrum [1] of t18:0 ** from human kidney ceramide glucosides [5] is shown in Fig. 1. An identical spectrum was taken from t18:0 from the yeast *Torulopsis utilis*.[3] t20:0, indicated in Fig. 1, was identical with t20:0 from the yeast. Thus the chemical structures of trihydroxy bases of animal and plant origins are the same, namely 1,3,4-trihydroxy-2-amino compounds.

The configurations at carbon atoms 2 and 3 (D, D) are the same for t18:0 from corn [6] and d18:1 from ox brain.[7] As proposed by Kidder [8] and by us [2] this may indicate a direct biochemical transformation of the two types of bases into each other. Experiments are being carried out in several laboratories to test this hypothesis (personal communication from Charles C. Sweeley). The possibility of fatty acids as biosynthetic precursors was tested by Law *et al.*[9] These workers used a mutant [10] of the yeast *Hansenula ciferrii*, which may be considered a primitive form of sphingolipidosis, accumulating and excreting large amounts of acetylated d18:0 and t18:0. In whole cell cultures serine, palmitic, and hexadecenoic acid, to a less degree hydroxypalmitic acid, were incorporated into both bases.

The overproduction of bases in the yeast may be due to a deficiency of incorporation of bases into complex sphingolipids. The bases are detoxified by acetylation and excreted.[10] (After alkaline degradation 20 μg of the free bases in a spot test were demonstrated to arrest cell growth completely.) If bases are accumulated one may expect precursors to be present in detectable amounts. The fatty acid fraction (see Table 1) had an interesting composition in this connexion.

The total lipids of *Hansenula ciferrii* (F−60−10),[10] grown on slant cultures of YM-agar for five days, made up 7 % of the dry weight. After mild alkaline degradation [11] of the total lipids 29 % were free fatty acids and 42 % N-acetyl bases.

* Communication 10 in this series is Ref. 1.

** In the shorthand designations used throughout this paper h, d and t stand for hydroxy, dihydroxy and trihydroxy, respectively. The number before the colon indicates carbon chain length and the number after the colon the number of double bonds.

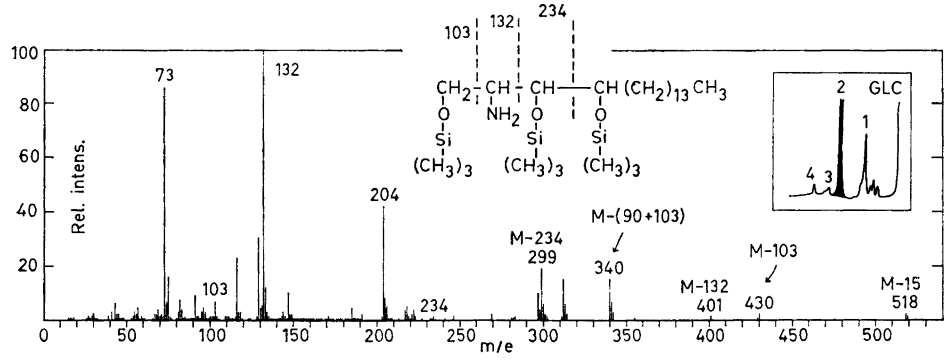

Fig. 1. Mass spectrum of the trimethylsilyl derivative of phytosphingosine from ceramide glucosides of human kidney. In the upper right corner the gas chromatogram of total sphingosines is shown; (1) is d18:1, (2) is t18:0, (3) is d20:1 and (4) is t20:0. (2) was taken into the mass spectrometer.[1] Fragment 204 is probably derived from carbon atoms 3 and 4.

The presence of D-2-hydroxy acids in the fatty acid fraction was indicated by infrared spectroscopy and by a slightly negative rotation in chloroform.[12] The fatty acids were esterified with methanol and separated on thin layer plates into normal esters and hydroxy esters. These were analyzed by gas chromatography, the hydroxy esters after conversion to trimethylsilyl or trifluoroacetyl derivatives. The acetyl bases were hydrolyzed in alkali[13] and the freed bases were isolated and characterized as dinitrophenyl derivatives[14] and also analyzed by gas chromatography as trimethylsilyl[1,2] and trifluoroacetyl[15] derivatives. As can be seen from Table 1 there is a correspondence between the fatty acids (except for oleic acid) and the bases. Although this may be a coincidence the fatty acid-base relationship[9] should be further elucidated.

Table 1. Fatty acid and base composition of *Hansenula ciferrii*. (See text for further details.)

Fatty acid	Relative amounts	Base	Relative amounts
18:1	42	—	—
h16:0	40	t18:0	89
16:0	13	d18:0	7
h18:0	1.2	t20:0	0.5
18:0	1	d20:0	0.4
h17:0	1.5	t19:0	0.1
17:0	0.2	d19:0	0.5
h15:0	0.2	t17:0	0.5
15:0	0.5	d17:0	1
14:0	0.1	d16:0	0.5

The configuration at carbon atom 4 of the trihydroxy bases of different human sphingolipid fractions[15] has not yet been established. These bases may well have another origin than the corresponding bases of plant tissues. For example, the allylic bases, not yet found in plant tissues, may be catabolized through trihydroxy bases by addition of water to the double bond (personal communication from M. Prostenik).

The author is indebted to E. Mårtensson for the kidney lipids and to L. J. Wickerham for the yeast.

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Received November 16, 1966.

Crystal Data of Some α-Hydroxyquinones Containing Intramolecular Hydrogen Bonds

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As a part of an investigation of molecules containing intramolecular hydrogen bonds and of intermolecular packing in polymorphous systems, the following crystallographic data have been obtained.

The unit cell parameters have been determined with estimated uncertainties of 0.1—0.2 % for the axes and 0.2° for the angles. The molecular structures of these compounds are being studied.