Details of this work will be published later. The authors are indebted to Zellstoff-Fabrik Waldhof, Mannheim, Germany, for the generous gift of the cerebrin from *Torulopsis utilis*.

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Received November 10, 1965.

Studies on Sphingosines

10. Use of Trimethylsilyl Ethers for the Gas Chromatography and Mass Spectrometry of Sphingosines*

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At present about 20 sphingosines are known,[1] differing in chain length, number of hydroxyls, and unsaturation (for nomenclature, see Ref. 1). To study the metabolism of these substances methods for the microscale identification and estimation of the different components must be available. The recent development of instruments which combine a gas chromatograph and a mass spectrometer [2-4] has afforded excellent opportunities for both structural analysis and estimation of isotopes in biosynthetic studies.[5] The present communication describes the application of this type of analysis to trimethylsilyl ethers of saturated dihydroxy sphingosines.

Use of trimethylsilyl ethers for gas chromatography of hydroxy compounds has appeared frequently in the literature the last few years, *e.g.* Refs. 6, 7. We have found these derivatives helpful also for sphingosine analysis [8] and recently Gaver and Sweeley used the same derivatives for the gas chromatography of sphingolipid hydrolysates.[9] However, when acid has to be used to free the sphingosines (which is the case for all lipids except ceramides) there is a problem of interference of by-products of trihydroxy sphingosines (tetrahydrofuran derivatives [10]) and of allylic group containing sphingosines (nucleophilic substitution,[11,8] isomerization,[12] and dehydration [12] products). This is illustrated in Fig. 1. For plant tissues, where no allylic sphingosines have yet been found, the problem is limited to one product of

* Communication 9 in this series is Ref. 1. The results of the present communication were presented at *The 9th International Conference on the Biochemistry of Lipids* at Nordwijk, Holland, September 5th–10th, 1965.

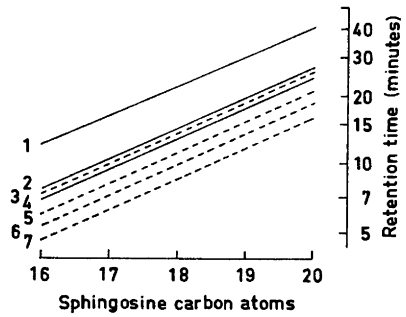

Fig. 1. Retention time of trimethylsilyl ethers of sphingosines (fully drawn lines) and some of their by-products (dotted lines) as a function of carbon chain length. The lines represent saturated trihydroxy sphingosines (1), saturated dihydroxy sphingosines (2), allylic isomers of monoenic dihydroxy sphingosines (3), monoenic dihydroxy sphingosines (4), methyl ethers of allylic dihydroxy sphingosines and tetrahydrofuran derivatives of trihydroxy sphingosines (5), and two monohydroxy compounds (6 and 7). The dienic dihydroxy sphingosines,[1] which are poorly separated from the monoenic analogues, have not been included in the figure. The gas-liquid separation of the combination instrument was made on a 2 m × 3.5 mm i.d. column packed with silanized 80–100 mesh Gas-Chrom P coated with 6 % silicone, at 207°C and a helium pressure of 1 kg/cm².

each trihydroxy homologue.[1] However, in animal tissues, where allylic sphingosines are always present, the series of by-products of each allylic homologue interferes with other sphingosines. In spite of efforts to minimize the by-product formation [9,8] studies of minor components, radioactivity measurements or mass spectrometry are linked with interpretation difficulties. By using dinitrophenyl (DNP) derivatives the contaminating by-products can be quantitatively removed [13] and the trimethylsilyl ethers of the purified DNP sphingosine fraction analyzed.[8] These compounds are, however, not suited for the combined mass spectrometric analysis. An alternative is to saturate the allylic group before the degradation of the sphingolipid. In this way all by-products except the tetrahydrofuran derivatives are avoided and a quantitative gas chromatography of saturated dihydroxy and trihydroxy sphingosine homologues is made possible.[8] Although this is valid also for glycosphingolipids [8] the following example is an adoption for sphingomyelins (where so far no trihydroxy sphingosines have been found). The unsaturated compounds can be quantified [8] as aldehydes after lead tetraacetate oxidation of a purified DNP sphingosine fraction.[13] However, for sphingomyelins the enzymatic splitting [14,15] of the phosphorylcholine moiety followed by alkaline hydrolysis of the amide bond may prove useful. [8]

5–10 mg of sphingomyelins in 5 ml of ethanol are hydrogenated for 60 min at atmospheric pressure and room temperature with about 5 mg of platinum oxide as catalyst. The catalyst is removed on Munktell's filter 00H and the evaporated filtrate is dephosphorylated by refluxing for 8 h in 0.5 ml of acetic acid-acetic anhydride, 4:1 (v/v). The reagents are evaporated and the residue is refluxed for 5–6 h in 2 ml of conc. hydrochloric acid-methanol, 16:80 (v/v). The hydrolysate is then partitioned according to Folch, the lower phase evaporated and the total sphingosines separated from fatty acids by chromatography on silicic acid.[13] 0.1 ml of a freshly prepared reagent consisting of 1.0 ml of dry pyridine, 0.2 ml of hexamethyldisilazane and two drops of trimethylchlorosilane is refluxed for 15 min at 120°C with up to 2 mg of sphingosine hydrochlorides. The reagents are evaporated in a stream of nitrogen and the residue taken up in 5 % pyridine in heptane for injection. The gas chromatographic-mass spectrometric instrument used was designed by Professor Stina Ställberg-Stenhagen of this Institute.[4]

The chromatogram of sphingosines of human plasma sphingomyelins [16] is shown in the upper right corner of Fig. 2. In elution order saturated dihydroxy sphingosines with 16 (12 %), 17 (2 %), and 18 (86 %) carbon atoms are indicated. No other homologues or trihydroxy sphingosines were found. In human brain sphingomyelins only 1 % of the C_{16}-sphingosine and traces of the C_{17}-sphingosine were found. The mass spectrum shown is of the C_{16}-sphingosine and the fragmentation is indicated in the figure. The parent mass is not seen due to an easy loss of one of the methyl groups.[17] The peak at mass 73 is due to the trimethylsilyl ion [17] and mass 75 is a rearrangement product.[17]

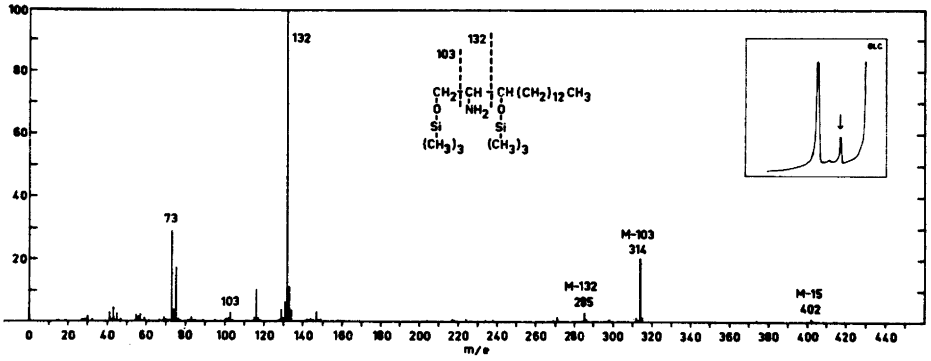

Fig. 2. Mass spectrum of the trimethylsilyl ether of the saturated dihydroxy C_{16}-sphingosine of human plasma sphingomyelins. In the upper right corner the gas chromatogram of total saturated sphingosines is shown. See text for further details.

The differences between the dihydroxy homologues are found in the higher mass range of the spectra: the parent mass minus 15, minus 103, and minus 132 are, for the C_{17}-sphingosine 416, 328, and 299, respectively, and for the C_{18}-sphingosine 430, 342, and 313, respectively. For metabolic experiments it should be possible to measure ^{15}N in the base peak quotient 132/133.

Details of this work and its further extension will be published later.

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Received November 10, 1965.