GAS-LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY OF CEREBROSIDES AS TRIMETHYLSILYL ETHER DERIVATIVES

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Summary

Trimethylsilyl ether derivatives of ceramide monohexosides have been separated by gas-liquid chromatography according to carbon number. The derivatives are eluted from the gas chromatograph as intact molecules and can be analyzed by mass spectrometry with respect to the constituent long chain base, fatty acid and hexose.

Recent studies have shown that ceramides can be separated and identified as TMS[x] derivatives by GLC-mass spectrometry (1-3). Combined with prefractionation according to degree of unsaturation this method has recently been used to separate and identify molecular species of ceramides derived from sphingomyelins (4) and of free ceramides in human blood plasma (5). Cerebrosides have to our knowledge not previously been separated into molecular species. The present investigation shows that the GLC-mass spectrometry procedure used for ceramides (1, 2) can be applied to ceramide galactosides and ceramide glucosides.

METHODS

Ceramide galactosides were synthesized using psychosin (galactosyl sphingosine, Applied Sciences Laboratories, Inc., State College, Pa.) and the fatty acids

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[x]Abbreviations: GLC, gas-liquid chromatography; Glu-LCB 18:1-16:0, N-palmitoyl sphingosine glucoside; mu., mass unit(s); TGCU, triglyceride carbon units; TMS, trimethylsilyl.

16:0, 20:0 and 24:0 (The Hormel Institute, Lipids Preparation Laboratory, Austin, Minn., purity >99 %). Carbodiimide was used to activate the carboxylic acids (2).

Ceramide glucosides (from spleen of patient with Gaucher's disease) were kindly supplied by Dr. C.C. Sweeley. The ceramide hexosides were converted to trimethylsilyl ethers by the addition of 100 µl pyridine, 40 µl hexamethyldisilazane and 20 µl trimethyl chlorosilane. After one hour at room temperature the samples were evaporated to dryness and the residues were dissolved in 200 µl CS_2.

The ceramide hexoside derivatives were separated by gas liquid chromatography using an F & M Model 400, gas chromatograph. The column was a U-shaped 1.4 m x 3 mm i.d. glass column packed with 1 % OV-1 on 100-120 mesh Gas Chrom Q (Applied Sciences Lab., Inc.). The column temperature was 320° and the carrier gas (helium) had a flow rate of 90 ml/min. Flash heater and detector bath were kept at 330°. The retention time was expressed as triglyceride carbon units (TGCU). Trimyristin, tripalmitin and tristearin were found to give a straight line when triglyceride carbon units 45, 51 and 57 were plotted against the logarithm of the retention times.

Identification of the GLC fractions were made by combined GLC-mass spectrometry using the LKB model 9000. Scan limits were 1-850 m/e. The electron energy was 22.5 ev.

RESULTS AND DISCUSSION

GLC of ceramide galactosides containing the fatty acids 16:0, 20:0 and 24:0 gave single peaks for each compound with the retention times (expressed as TGCU) 47.0, 51.0 and 54.9 respectively. The four carbon difference between each cerebroside homologue thus gave corresponding difference in TGCU.

The main mass spectrometric data (recorded up to m/e 850) for the synthetic cerebroside derivatives are given in fig. 1. Ions suitable for identification of molecular species by providing information on the molecular weight and constituent LCB and fatty acid are summarized in Table 1. The LCB and fatty acid ions are formed by cleavage between C-2 and C-3 of the sphingosine moiety and are analogous to those earlier described for ceramides (2). Evidence on the nature of the ions

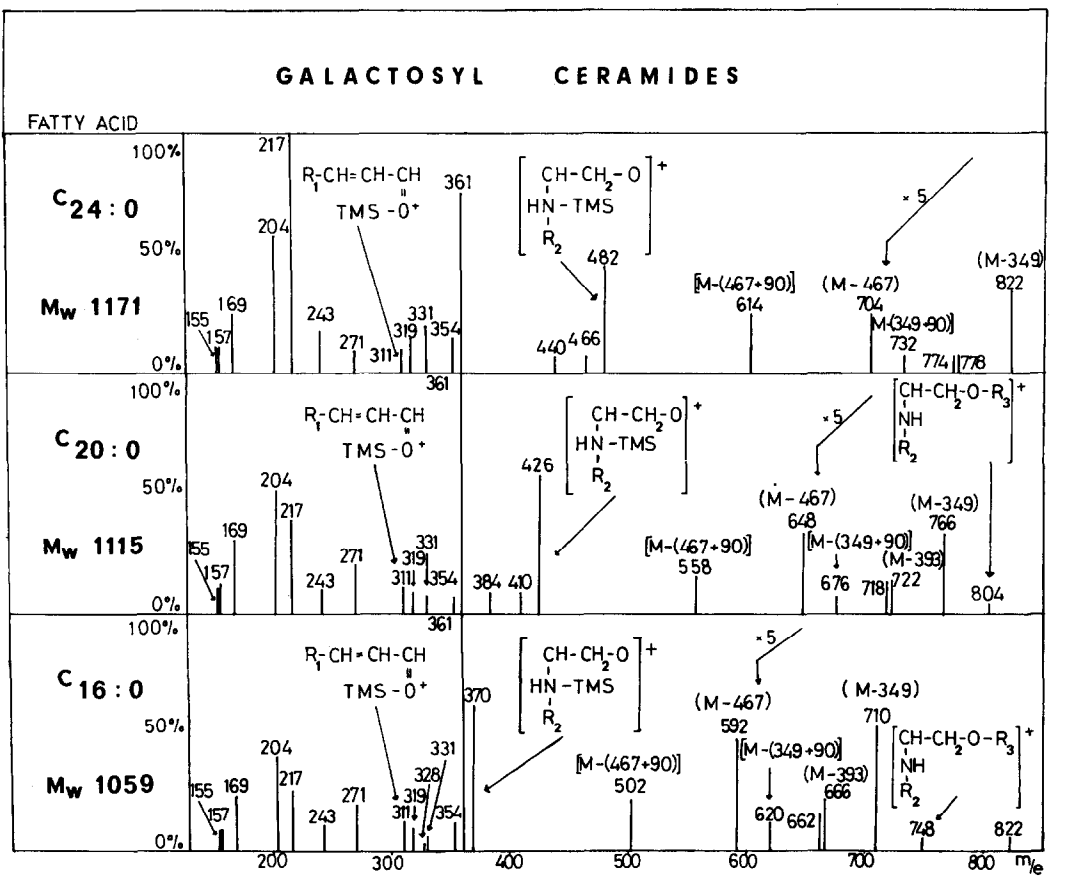

Fig. 1. Mass spectrometric data for trimethylsilyl ether derivatives of ceramide galactosides.

R_1 = $CH_3(CH_2)_{12}$

R_2 = fatty acyl group, 16:0, 20:0, 24:0

R_3 = TMS galactosyl

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given in Table 1 was obtained from the mass spectra of the homologous series of synthetic cerebrosides (Fig. 1) where ions containing the fatty acid moiety were shifted 56 m.u. between homologues. In addition mass spectrometric analysis of cerebrosides from mouse brain (6) containing e.g. stearic acid combined with either C_{18} sphingosine or C_{20} sphingosine were used to identify ions containing the part of the LCB where structural variations are found.

Several ions, viz. m/e 204, 217, 243, 271, 319 and 361 have earlier been

observed in the mass spectra of TMS derivatives of glycosides (7) and originate at least partly in the carbohydrate part of the molecule. These ions appear at the

Table 1 Ions suitable for structure determination of monohexoside ceramides.

LCB ion	Fatty acid ions	LCB and fatty acid ions
R_1-CH=CH-CH=$\overset{+}{O}$-TMS	$[CH(=NH-R_2)-CH_2-O-R_3]^+$	M-349
		M-393
		M-(349+90)
	$[CH(-HN\text{-}TMS-R_2)-CH_2-O]^+$	M-467
		M-(467+90)

R_1=$C_{13}H_{27}$ in C_{18}-sphingosine

R_2=Fatty acyl group

R_3=TMS-Galactosyl

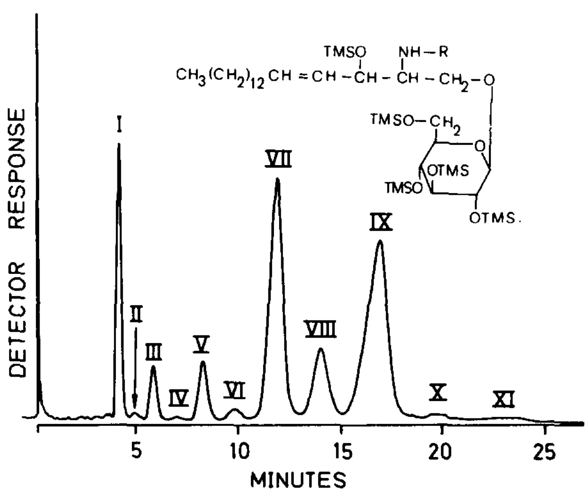

Fig. 2. Gas-liquid chromatogram of the trimethylsilyl ether derivatives of ceramide glucosides obtained from spleen of patient with Gaucher's disease.
Column: 1 % OV-1. Column temperature: 320°. Carrier gas: helium. R = acyl group. The mass spectrometric identification of the major peaks are given in table 2. The mass spectrum of peak I is shown in fig. 3.

Table 2 Gas chromatographic and mass spectrometric data on TMS derivatives of ceramide glucosides

Analyzed material	Triglyceride carbon units	LCB ion m/e	%	Fatty acid ions m/e	%	LCB and fatty acid ions m/e	%	Main constituent(s)
I[xx]	46.8	311	9	370	26	592	26	Glu-LCB 18:1-16:0
III	48.8	311	13	398	27	620	14	Glu-LCB 18:1-18:0
V	50.8	311	11	426	31	648	16	Glu-LCB 18:1-20:0
VII	52.8	311	10	452	3	672	3	Glu-LCB 18:1-22:1
				454	29	674	18	Glu-LCB 18:1-22:0
VIII	53.7	311	16	468	31	690	18	Glu-LCB 18:1-23:0
IX	54.8	311	11	480	5	702	4	Glu-LCB 18:1-24:1
				482	26	704	13	Glu-LCB 18:1-24:0

[xx] Peak number, see fig. 2.

same m/e values irrespective of the chain length of the LCB or the fatty acid.

The appearance of an LCB ion (m/e 311 in fig. 1) and a fatty acid ion representing the rest of the molecule (m/e 748 in the palmitoyl homologue) demonstrates that the TMS derivatives of the cerebrosides are eluted from the gas chromatograph as intact molecules.

An application of the GLC procedure is shown in fig. 2, where TMS derivatives of ceramide glucosides are separated. It is evident that compounds differing by one carbon atom can be separated completely. Mass spectra were recorded on peaks

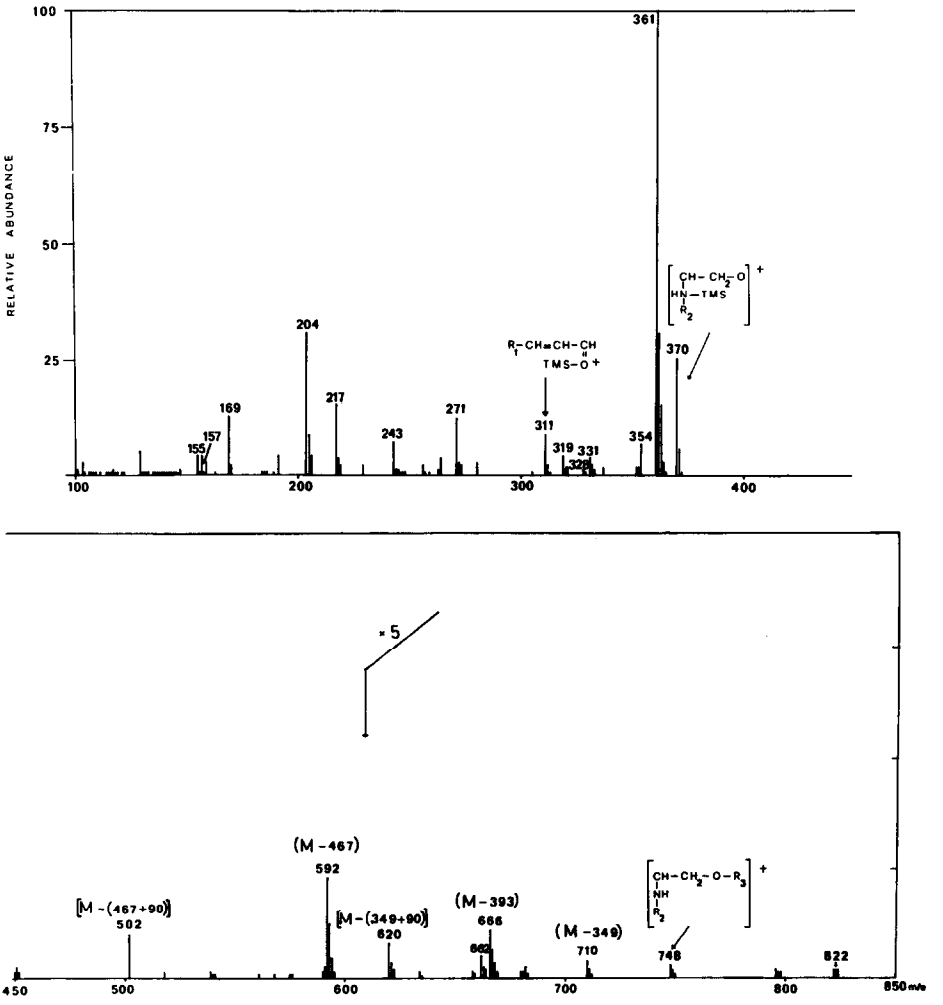

Fig. 3. Mass spectrum of peak I of fig. 1.

I-XI in fig. 2 and some of the data are summarized in Table 2. Most of the peaks are due to pure molecular species, consisting of sphingosine a saturated fatty acid and glucose and with no evidence for homologues of sphingosine. Fractions VII and IX contain derivatives with both saturated and monounsaturated fatty acids, which results in asymmetry of the peaks since the compounds containing monounsaturated fatty acids are eluted slightly before corresponding saturated analogue. The mass spectrum of the palmitoyl homologue recorded on peak I is shown in fig. 3. All of the ions described for ceramide galactosides are also present in the glucoside derivative, however intensity differences can be found (e.g. M-467 and M-689).

The analysis of intact molecular species of cerebrosides by GLC-mass spectrometry as described in this communication seems to offer new possibilities for studies of the structures and metabolism of glycosphingolipids.

Acknowledgements

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REFERENCES

1. Samuelsson, B. and Samuelsson, K., Biochim. Biophys. Acta, 164, 421 (1968).
2. Samuelsson, B. and Samuelsson, K., J. Lipid Res., 10, 41 (1969).
3. Casparrini, G., Horning, E.C. and Horning, M.G., Chem. Phys. Lipids 3, 1 (1969)
4. Samuelsson, B. and Samuelsson, K., J. Lipid Res. 10, 47 (1969).
5. Samuelsson, K., Biochim. Biophys. Acta 176, 211 (1969).
6. Samuelsson, K., Unpublished observations.
7. De Jongh, D.C., Radford, T., Hribar, J.D., Hanessian, S., Bieber, M., Dawson, G and Sweeley, C.C., J. Am. Chem. Soc., 91, 1728 (1969).