MASS SPECTROMETRY OF POLAR COMPLEX LIPIDS
ANALYSIS OF A SULFATIDE DERIVATIVE

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Summary. Acetylation of brain sulfatide followed by trimethylsilylation at elevated temperature produces a fully acetylated derivative with the sulfate group replaced by a trimethylsilyl substituent. The mass spectrum of this compound is dominated by peaks from the galactose residue, and a 3-position of the original sulfate group may be assigned.

Tissues with an increased sodium-ion transport, like brain, kidney, the rectal gland of elasmobranchs and the avian salt gland, have high levels of sulfatide (cerebroside-sulfuric acid ester) (1). The ratio of sulfatide concentration and the activity of the sodium and potassium dependent adenosinetriphosphatase (2) is approximately the same for the rectal gland and the salt gland (1). This may indicate that sulfatide is involved as a carrier or receptor in the hormone dependent sodium-ion transport. Assuming the sulfatide-enzyme ratio to be the same for cells with only self-supporting sodium-ion transport, one may calculate the sulfatide content of red blood cells of the cat (3) to be very low (1 μ g per g dry weight). For a closer examination of the role of sulfatide in these systems and also in subcellular fractions of the avian salt gland, it was necessary to develop a specific and sensitive method of analysis. Mass spectrometry is a method of choice as it has proved powerful in other lipid fields and allows analysis of submicrogram amounts of material. Mass spectra of trimethylsilyl derivatives of neutral glycosphingolipids were recently presented (4), and successful analysis of a silylated ganglioside has been reported (5). In the present work an investigation on sulfatide was made. Attempts to analyze this lipid with a retained sulfate portion, after treatment with diazomethane or after silylation, have so far been unsuccessful. However, acetylation of hydroxy groups followed by silylation at elevated temperature

results in a quantitative exchange of the sulfate for a trimethylsilyloxy group, the product giving a characteristic mass spectrum with an indication of the original sulfate group position.

Experimental. Thin-layer chromatographically pure bovine brain cerebroside and human brain sulfatide (lithium salt) were obtained by column chromatography. This procedure and the methods of analysis have been presented elsewhere (1, 6). Both lipids contained galactose as sole carbohydrate, and the long-chain bases were sphingosine and a few per cent of dihydrosphingosine. The fatty acid composition is heterogenous (7, 8), and the molecular species reproduced in the formulas of Fig. 1 and 2 makes up about 20 per cent of the cerebroside and about 15 per cent of the sulfatide species, respectively. Acetylation was performed with acetic anhydride-pyridine 1:1, v/v, at room temperature overnight, in a test tube with a teflon-faced screw cap. The reagent was evaporated under nitrogen and the samples sucked with a water pump for an hour. Both samples were treated for 1 hour at 120° C with closed caps with pyridine-hexamethyldisilazane-trimethylchlorosilane 4:2:1, v/v/v (9). The reagent was evaporated and the residue taken up in chloroform. For mass spectrometry a pyrophylite probe was dipped into the solvent (10 mg sample per ml). For a quantitative transfer of small amounts of sample, however, a quartz tube may be used, from which the solvent is evaporated. Mass spectra were recorded on an AEI MS 902 high resolution instrument with a direct inlet system, using an ionizing potential of 70 eV, ionizing current of 0.1 mA, and an ion source temperature of 220°C. Peaks below m/e 40 were not reproduced.

Results and discussion. Mass spectra of acetylated and silylated cerebroside and sulfatide are reproduced in Fig. 1 and 2, respectively. Published data on fully acetylated (10) and partially methylated and acetylated (11) carbohydrates may be used for the interpretation. Base peaks in both cases are at m/e 43, due to acetyl ions. The second largest peak of cerebroside is at m/e 331, due to a fragmentation at the glycosidic oxygen (see formula in Fig. 1). The corresponding peak of the sulfatide derivative is at m/e 361, the 30-unit increase in mass owing to an exchange of one of the acetoxy groups of galactose for a trimethylsilyloxy group. Except for this and the series (due to a heterogenous fatty acid portion) of molecular peaks above m/e 900, the two spectra look very similar. The major peaks are derived from the carbohydrate residues, and those at m/e 264, 169 and 109 exist in both spectra. The first of

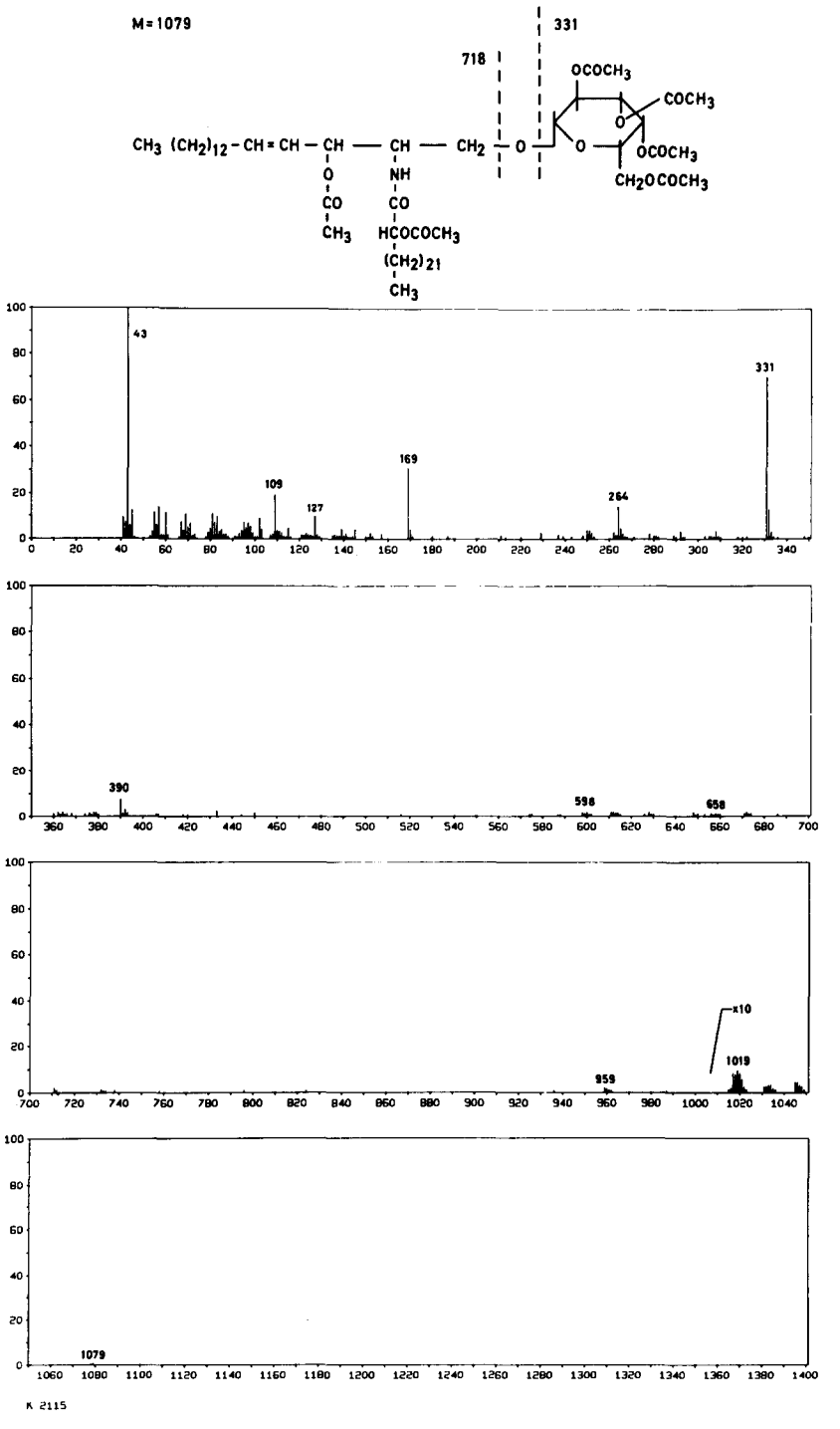

Fig. 1. Mass spectrum of an acetylated and trimethylsilylated mixture of bovine brain cerebrosides. The molecular species in the top formula contains galactose, sphingosine and a 24-carbon 2-hydroxy fatty acid.

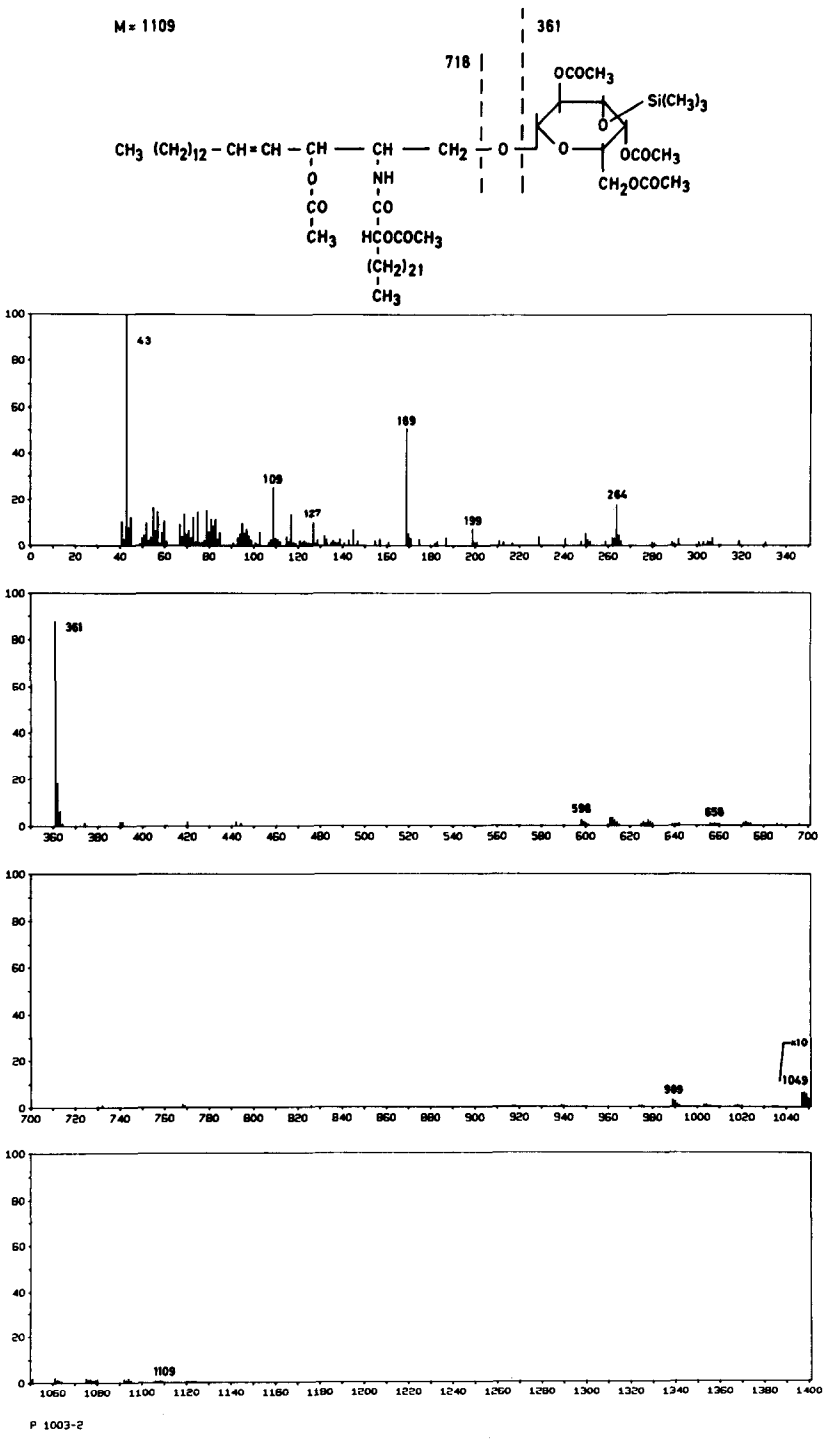

Fig. 2. Mass spectrum of an acetylated and trimethylsilylated mixture of human brain sulfatides. The molecular species in the top formula contains galactose (with an original sulfate group in 3-position), sphingosine and a 24-carbon 2-hydroxy fatty acid.

these has not yet been identified. The peak at m/e 169 has the elemental composition $C_8H_9O_4$, and is thus identical with the peak derived from fully acetylated hexoses (10), suggested to be 331-60-60-42 (60 is the mass of acetic acid and 42 that of ketene), which has a retained acetoxy group in position 6. 109 is obtained by an additional loss of acetic acid. A 6-position of the trimethylsilyloxy group (and thus of the original sulfate group) may thus be excluded, otherwise the peak at m/e 169 should have changed to 199 in Fig. 2 (present in low amounts). As the general appearance of the spectra suggests a pyranose structure (10), the original sulfate substituent therefore should be in position 2, 3 or 4. No reference spectra are yet available to differentiate conclusively between these possibilities. However, the ether bound silyl group is comparable to the methoxy group of isomeric, monomethylated hexapyranose acetates (11). On mass spectrometry of these, the substituent in 3-position (acetoxy or methoxy) is most easily lost, and in comparison with the peaks referred to above the 3-methoxy and the fully acetylated compounds look similar. The 2-, and probably the 4-, positional isomers give no peak at m/e 169. It therefore appears very probable that the spectrum in Fig. 2 is produced by a pyranose compound with a trimethylsilyloxy substituent in 3-position, having replaced the original sulfate group. The series of peaks around m/e 658 (718-60) indicate the glycosidic bond at C-1 of sphingosine. This is a confirmation of results obtained by use of classical methods (12). Information concerning the nature of the replaced substituent (sulfate), not obtained in this way, may be gained by e. g. atomic absorption (13) or flame (14) spectrometry.

The present method may be used in combination with e. g. thin-layer chromatography as a very sensitive means of sulfatide detection. In addition it offers a simplified way (15) of determining the sulfate group position, useful e. g. when sulfatides (monoglycosyl or diglycosyl derivatives) of different origins are compared.

The predominance of peaks derived from the polar portion, often obtained homogenous after chromatography of polar lipids, is encouraging and suggests possibilities for characterization of polar groups (containing e. g. carbohydrate or phosphate) of complex lipids in general, information of which is not easily obtained with present-day methods. In the case of fully acetylated diglycosylceramide of human brain the peak at m/e 331, derived from the terminal galactose residue (compare Fig. 1), has got 30-40% of the base peak (m/e 43) intensity. In trimethylsilyl derivatives (4, 5), galactosyl-

ceramide of bovine brain has the second largest peak (50-60% of base peak, m/e 73, intensity) at m/e 361, derived in an analogous manner to m/e 331 in Fig. 1 and having lost trimethylsilanol (451-90). The trimethylsilyl derivative of tetraglycosylceramide (globoside of human kidney) has an intense peak at m/e 420, which is the mass of the terminal N-acetyl hexosamine derivative. On the contrary, peaks obtained from the heterogenous, lipophilic portion are of low intensity (compare Fig. 1 and 2).

A successful direct analysis by mass spectrometry of mixtures of compounds like these will definitely broaden the use of this method in the study of natural products. However, before this can be demonstrated in detail, systematic works on known lipid structures (natural and synthetic) must be performed, including a search for the most suitable derivatives of analysis.

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