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Note

Simultaneous determination of methyl esters of α-hydroxy and nonhydroxy fatty acids from brain cerebroside by fused-silica capillary gas chromatography

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α-Hydroxy fatty acids are widely distributed in the complex lipids of biological materials. In particular, glycosphingolipids of nerve tissues contain most abundantly long-chain hydroxy fatty acids as well as straight-chain fatty acids [1]. Thus, when glycosphingolipids such as cerebroside and sulfatide from nerve tissue are methanolyzed with methanolic hydrochloride for the purpose of determining the fatty acid composition by gas—liquid chromatography (GLC), a mixture of methyl esters of hydroxy and nonhydroxy fatty acids is obtained, and they cannot be fully resolved on a packed column. Therefore, prior to GLC analysis, the methyl esters of hydroxy fatty acids have to be separated from those of nonhydroxy fatty acids with a column of Florisil [2] or silicic acid [3], or by preparative thin-layer chromatography (TLC) [4]. Furthermore, if packed columns are used for GLC, the determination of the fatty acid composition becomes time consuming because it is necessary to use two columns of different polarities to resolve fully the various classes of fatty acids. Recently, flexible fused-silica capillary columns have become available [5], which are highly efficient compared not only with packed columns but also with glass capillary columns, and they are easy to use [6, 7]. This report describes the determination of all classes of fatty acids of glycosphingolipids from nerve tissue in a single injection by the use of a fused-silica capillary column without prior separation or further derivatization.

MATERIALS AND METHODS

A Shimadzu GC-5A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a solventless injection system and a flame ionization detector was used,

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and modified to accept the fused-silica capillary column. The column was an OV-101 coated fused-silica capillary column, 25 m × 0.25 mm I.D., supplied by Shimadzu. The conditions of operation were: column temperature, 280°C (isothermal); carrier gas, nitrogen; flow-rate, 0.5 ml/min; split ratio, 1 : 8. Nitrogen gas was purified by passing it through stainless tubing packed with Molecular Sieve 5A. The chromatograms were recorded and the percentage composition of individual peaks calculated with a Chromatopack C-R1A (Shimadzu).

Cerebroside and sulfatide were isolated from crude lipid extracts of bovine brain white matter in our laboratory and proved to be pure enough for further analysis by TLC. Glycolipids were methanolyzed in 3% methanolic hydrochloride at 100°C for 3 h, and the methyl esters of fatty acids were extracted with petroleum ether (b.p. 30—60°C). An aliquot of the total fatty acid methyl esters thus obtained was separated into the hydroxy and nonhydroxy components on a silica gel H (Merck, Darmstadt, G.F.R.) plate which was prepared in our laboratory, using as solvent petroleum ether—diethyl ether (80:30, v/v) [4]. The two components were visualized with iodine vapor, scraped off separately from the plate, extracted and finally purified through a small column of silica beads. Standard samples of methyl esters of hydroxy and nonhydroxy fatty acids were products of Applied Science Labs. (State College, PA, U.S.A.).

RESULTS AND DISCUSSION

Fig. 1a clearly shows that baseline resolution was obtained for each of the 23 major fatty acids contained in brain cerebroside on a fused-silica capillary column. Fig. 1b and c are chromatograms of nonhydroxy and hydroxy fatty acid methyl esters, respectively, isolated by preparative TLC. Comparing Fig. 1a with Fig. 1b and c, it is evident that all of the nonhydroxy and hydroxy components from cerebroside were fully resolved on a single column. Fig. 2a, b and c also shows that total fatty acid methyl esters from sulfatide were well resolved on a single column as observed for those of cerebroside. This degree of separation of the methyl esters has not been attained with a packed column under any chromatographic conditions. In general, with a packed column of a nonpolar liquid phase such as SE-30 or OV-1, $C_{n:0}$ nonhydroxy and $C_{(n-1):1}$ hydroxy acid series, and $C_{n:1}$ nonhydroxy and $C_{(n-2):0}$ hydroxy acid series overlap or appear as one peak with leading or tailing shoulders*. For example, $C_{22h:0}$ and $C_{24:1}$, and $C_{25:0}$ and $C_{24h:1}$ which are contained in significant amounts in brain cerebroside were unresolved, respectively, on a packed column, so that proportions of total nonhydroxy and total hydroxy fatty acids could not be calculated by a single chromatography.

Table I compares percentage compositions of individual fatty acids calculated from chromatograms shown in Figs. 1 and 2. It is clear that values for a mixture of total fatty acids show good agreement with values from a separate chromatogram after preparative TLC. An earlier study [8] on the fatty acid composition of bovine brain lipids showed the relatively higher

*The numbers before and after the colon refer to the number of carbon atoms and double bonds, respectively, and h before the colon indicates hydroxy fatty acid.

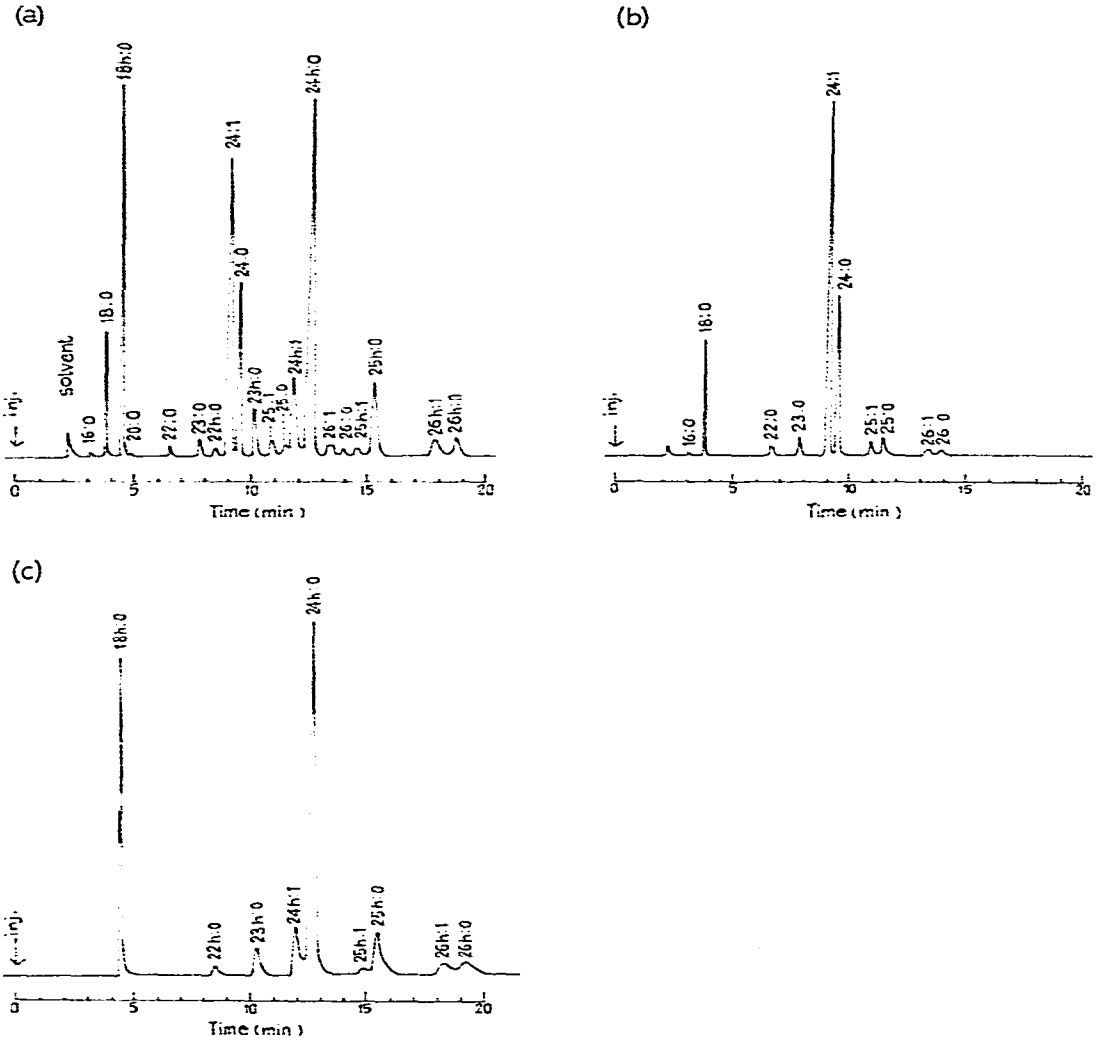

Fig. 1. Gas chromatograms of fatty acid methyl esters from bovine brain cerebroside on an OV-101 coated fused-silica capillary column. The analytical conditions were as described in Materials and methods. (a) Total fatty acid methyl esters from cerebroside; (b) nonhydroxy fatty acid methyl esters isolated by preparative TLC; (c) hydroxy fatty acid methyl esters isolated by preparative TLC. Individual esters were identified by comparison with standard samples and with the aid of a diagram of log retention times.

concentration of 16:0, 18:1, 22:0 and 24h:1 acids, and a lower concentration of 24:0 and 24:1 and 24h:0, compared with the present findings. This discrepancy may primarily be due to the difference in materials used, that is, whole brain or white matter.

For the analytical conditions used in the present study there are still some improvements to be made; the required operating temperature was too close to the maximum allowable temperature, and peak width became greater with longer chain length, particularly for hydroxy fatty acids. Besides, the life-time of the column has to be more carefully investigated, although the same column

TABLE I

PERCENTAGE DISTRIBUTION OF FATTY ACIDS OF BRAIN CEREBROSIDE AND SULFATIDE BASED ON CHROMATOGRAMS AFTER CHROMATOGRAPHY ON AN OV-101 COATED FUSED-SILICA CAPILLARY COLUMN

The column was run isothermally, 280°C, and percentages of individual peaks were calculated with a Chromatopack C-R1A. Values are averages of three determinations and expressed as mean ± S.D.

Fatty acids	Cerebroside		Sulfatide	
	Total*	Separated**	Total*	Separated**
16:0	0.6 ± 0.11	0.3 ± 0.09		
18:0	8.0 ± 0.28	9.1 ± 2.13	1.6 ± 0.22	1.3 ± 0.14
20:0	0.5 ± 0.17	0.2 ± 0.06		
22:0	2.0 ± 0.38	1.8 ± 0.34	0.6 ± 0.16	0.9 ± 0.42
23:0	3.6 ± 0.56	2.5 ± 0.33	1.6 ± 0.44	2.0 ± 0.66
24:0	24.2 ± 0.99	22.9 ± 1.44	24.7 ± 2.26	26.5 ± 3.82
24:1	47.2 ± 4.90	53.9 ± 0.77	62.5 ± 5.38	57.6 ± 1.88
25:0	4.2 ± 1.33	3.5 ± 0.40	3.1 ± 1.14	4.6 ± 1.18
25:1	4.9 ± 0.74	3.5 ± 0.38	3.3 ± 0.60	3.9 ± 1.59
26:0	2.3 ± 0.59	1.1 ± 0.27	1.2 ± 0.54	1.6 ± 0.72
26:1	2.5 ± 0.70	1.2 ± 0.19	1.4 ± 0.20	1.7 ± 0.97
18h:0	17.1 ± 1.79	20.2 ± 4.14	4.7 ± 0.70	3.2 ± 0.22
22h:0	1.7 ± 0.34	1.3 ± 0.49		0.7 ± 0.03
23h:0	5.6 ± 0.78	4.2 ± 1.10	4.7 ± 0.31	3.0 ± 0.12
24h:0	41.5 ± 2.92	45.0 ± 3.21	52.9 ± 3.98	60.3 ± 1.75
24h:1	11.4 ± 0.36	9.6 ± 0.99	12.0 ± 0.25	8.4 ± 0.40
25h:0	11.0 ± 0.70	10.0 ± 1.55	13.0 ± 2.04	14.0 ± 0.43
25h:1	2.3 ± 0.41	1.5 ± 0.23	2.7 ± 0.18	1.3 ± 0.08
26h:0	4.6 ± 0.64	4.1 ± 1.06	5.1 ± 1.19	5.3 ± 0.30
26h:1	4.8 ± 0.65	4.0 ± 0.47	4.9 ± 0.14	3.8 ± 0.31
OH%***	63.8%		17.8%	

*Values were obtained from chromatograms of total fatty acid methyl esters (Figs. 1a and 2a), and expressed as percentages of total nonhydroxy or total hydroxy fatty acids.

**Values were obtained from chromatograms of methyl esters of nonhydroxy (Figs. 1b and 2b) and hydroxy (Figs. 1c and 2c) fatty acids separated by preparative TLC, and expressed as percentages of individual components.

***Weight percentage of hydroxy fatty acids of total fatty acids from cerebroside or sulfatide, obtained from chromatograms in Fig. 1a or Fig. 2a, respectively.

had been used for almost daily analysis over a period of several months without serious loss of column efficiency. In practice, we checked correction factors with reference mixtures of nonhydroxy fatty acid series, and found that the variation of those values was so minor that it did not significantly affect the percentage composition of the fatty acids from biological materials. We think, therefore, that the life-time of a fused-silica capillary column is fairly satisfactory, although its precise comparison with a packed column was not done for an operation period of longer than a year. Previously, Ackman [9] and Slover and Lanza [10] intensively discussed the problems encountered in GLC analysis using a glass capillary column. Recently, the more efficient fused-silica column has become more generally available and many of the limitations dis-

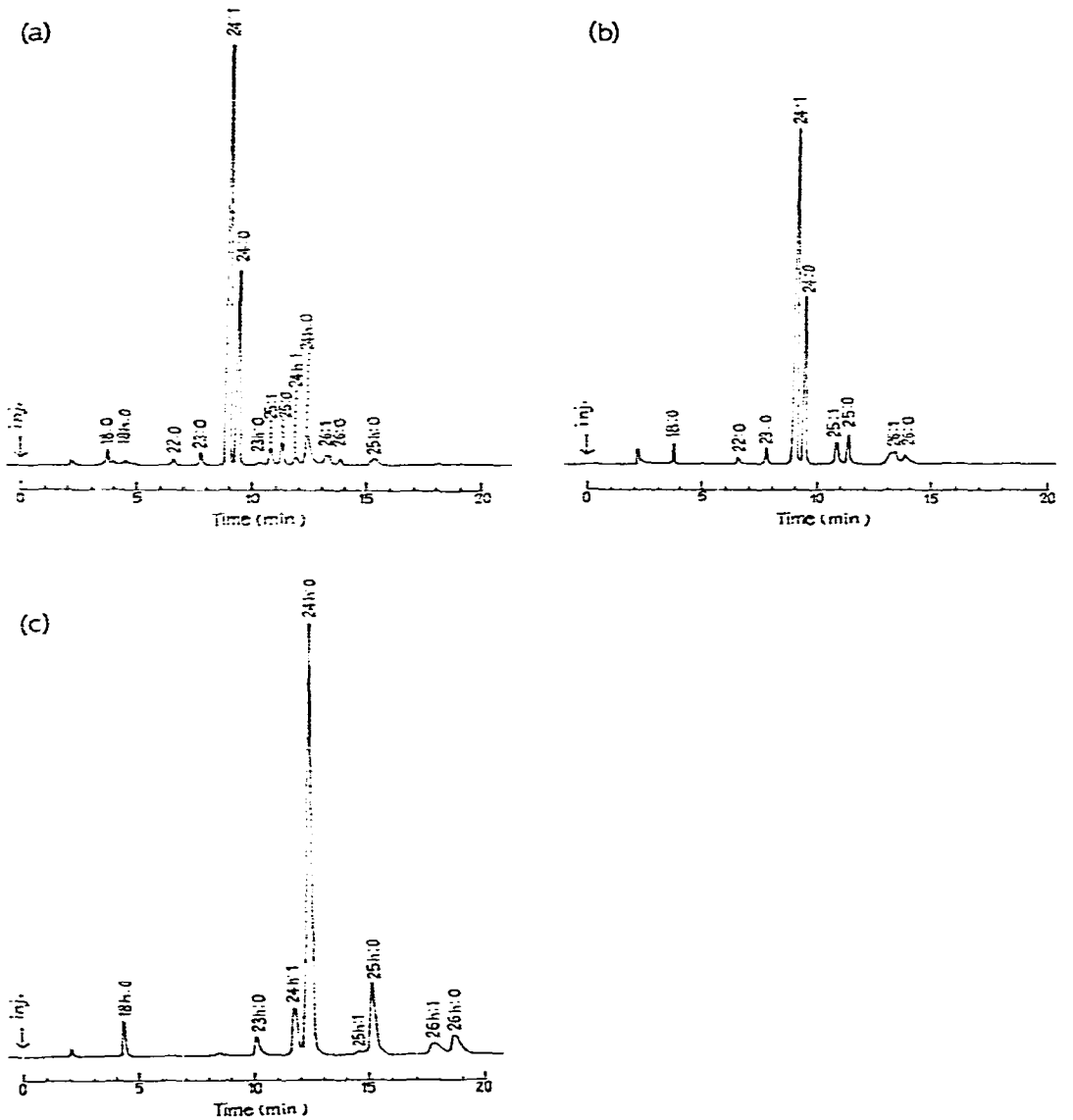

Fig. 2. Gas chromatograms of fatty acid methyl esters from bovine brain sulfatide on an OV-101 coated fused-silica capillary column. (a) Total fatty acid methyl esters from sulfatide; (b) nonhydroxy fatty acid methyl esters isolated by preparative TLC; (c) hydroxy fatty acid methyl esters isolated by preparative TLC. For other details see the legend to Fig. 1.

cussed above will probably be resolved in the near future [7]. Regardless of some limitations pointed out above, there are still some great advantages compared with a conventional method using a packed column: no pretreatment such as preparative TLC or derivatization is required and a fused-silica capillary column provides efficient and rapid separation of fatty acid methyl esters containing hydroxy and nonhydroxy components (within 20 min for the C_{26} hydroxy acid series). Previously, Tschöpe [11] reported the simultaneous

analysis of normal and α-hydroxy fatty acids on a packed column with ethylene glycol succinate. Prior to GLC, however, hydroxy fatty acids had to be converted to their acetyl derivatives, and, in addition, a considerably long time for chromatography was required (e.g. 30 min at least, for C_{22} hydroxy fatty acid). Recently, on a fused-silica capillary column a mixture of fatty acid methyl esters containing a few classes of hydroxy (chain length shorter than C_{16}) as well as nonhydroxy components has been separated [12]. However, this was a standard mixture, not biological material, and a fairly long analytical time was still required compared with that in the present study. To our knowledge, this is the first report of the simultaneous and full resolution of a mixture of hydroxy and nonhydroxy fatty acids from biological sources by GLC, and we believe that fused-silica capillary columns will become more widely used because of their high efficiency as well as the ease of handling.

REFERENCES

1 J. Eichberg, G. Hauser and M. Karnovsky, in G.H. Bourne (Editor), The Structure and Function of Nervous Tissue, Vol. III, Academic Press, New York, 1969, p. 185.
2 Y. Kishimoto and N.S. Radin, J. Lipid Res., 1 (1959) 72.
3 J.S. O'Brien and G. Rouser, Anal. Biochem., 7 (1964) 288.
4 Y. Tamai, H. Kojima, F. Ikuta and T. Kumanishi, J. Neurol. Sci., 35 (1978) 59.
5 R. Dandeneau, P. Bente, T. Rooney and R. Hiskes, Amer. Lab., 11 (1979) 61.
6 W. Jennings, J. High Resolut. Chromatogr. Chromatogr. Commun., 3 (1980) 601.
7 S.R. Lipsky, W.J. McMurray, M. Hernandez, J.E. Purcell and K.A. Billeb, J. Chromatogr. Sci., 18 (1980) 1.
8 J.S. O'Brien and G. Rouser, J. Lipid Res., 5 (1964) 339.
9 R.G. Ackman, Prog. Chem. Fats Other Lipids, 12 (1972) 165.
10 H.T. Slover and E. Lanza, J. Amer. Oil Chem. Soc., 56 (1979) 933.
11 G. Tschöpe, Hoppe-Seyler's Z. Physiol. Chem., 354 (1973) 1291.
12 C.W. Moss, S.B. Dees and G.O. Guerrant, J. Clin. Microbiol., 12 (1980) 127.