CONFIGURATION OF 2-HYDROXY ACIDS FROM BRAIN CEREBROSIDES DETERMINED BY GAS CHROMATOGRAPHY

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1. Introduction

Earlier work on the configuration of biologically occurring 2-hydroxy acids has been based on comparison of optical rotations (for review see [1]). Cerebronic acid has been chosen by most investigators. Synthetic DL-24h^2:0, resolved as the strychnine salt [2], 2L-hydroxy 4:0, 6:0, 8:0, 10:0 and 16:0, prepared by anodic coupling [3] and derivatives of D-lactic, D-mandelic and D-hexahydromandelic acids [4] have been used for the comparisons. The results indicate the D-configuration for cerebronic acid. The cerebronic acid used in these studies, however, was not free from homologs [5]. It has been assumed, but it has never been shown that the homologs have the same configuration as cerebronic acid [5]. This information was considered necessary in a GC-MS* study of synthetic ceramides containing Hfa [6]. In the present paper a recently published method for steric analyses by GC using (-)menthylformate esters [7] has been applied to long chain Hfa from brain cerebrosides. The results show that only one optical isomer of each of these acids is present and that this isomer has the D-configuration.

* The following non-standard abbreviations have been used: GC, gas chromatography; MS, mass spectrometry; Hfa, 2-hydroxy acids; 18h^2:0, 2-hydroxy stearic acid; Nfa, normal fatty acids; Hfa-Me, 2-hydroxy acid methyl esters; MC-Hfa-Me, 2-menthyloxycarbonyl fatty acid methyl esters; MC-D-Hfa-Me, 2D-menthyloxycarbonyl fatty acid methyl esters; TLC, thin layer chromatography.

2. Experimental

2.1. *Chemicals*

Bovine brain cerebrosides, grade II, Sigma Chemical Company, St. Louis, Mo. and racemic 14h^2:0, 16h^2:0, 18h^2:0, 20h^2:0, 22h^2:0 and 24h^2:0 from the sources listed in [6] were used. Optically active Hfa were prepared according to Horn and Pretorius [3]. They were purified by silicic acid chromatography before and after conversion to methyl esters. (-)Menthylchloroformate was prepared according to Westley and Halpern [7].

D-26h^2:0 was racemized by CrO_3-oxidation [8], purification of the 2-ketoester by silicic acid chromatography, reduction of the latter by $NaBH_4$ in methanol and purification of the DL-26h^2:0 by silicic acid chromatography.

2.2. *Preparation of (-)menthylformate esters*

100 μg Hfa-Me in 40 μl benzene, 60 μl (-)menthylchloroformate solution (about 1 μmol/μl) and 12 μl dry pyridine was left at room temperature for 30 min, after which time the reaction was complete. After addition of 2 ml benzene the solution was washed with 3 × 2 ml water. The residue, dissolved in CS_2 was quantitatively applied to a TLC-plate. This was developed with benzene-dioxane (97:3) and sprayed with 0.2% 2′,7′-dichlorofluorescein in ethanol. The zones were marked in UV light. MC-Hfa-Me diastereoisomers (the single spot with R_f 0.85 which separated from Hfa-Me (R_f 0.4)) were recovered from the plate by eluting the zone in a glass column with 10 ml diethyl ether. The dye

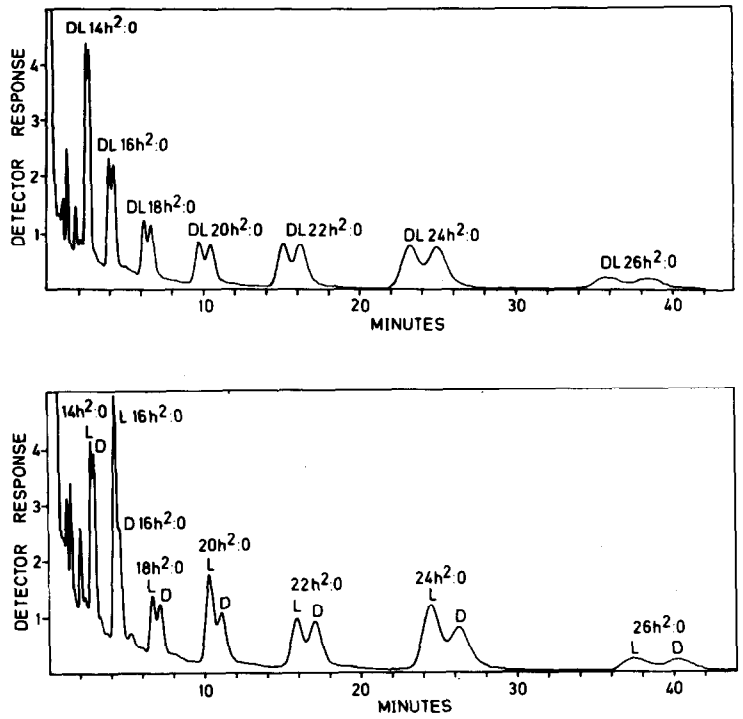

Fig. 1a. Gas chromatogram of MC,Me derivatives of racemic $14h^2$:0, $16h^2$:0, $18h^2$:0, $20h^2$:0, $22h^2$:0, $24h^2$:0 and $26h^2$:0 on 1.4% OV-210 at 250°C.

Fig. 1b. Gas chromatogram of the mixture of esters in fig. 1a after addition of MC-Me derivatives of L-$16h^2$:0, L-$20h^2$:0 and L-$24h^2$:0.

was not eluted. The MC-Hfa-Me were dissolved in 100 µl CS_2 for GC analysis.

2.3. *Gas chromatography*

Hfa-Me were analyzed at 230° in a U-shaped column (1.7 m × 3.5 mm i.d.) containing 1.5% SE-30 ultraphase (Pierce Chemical Company, Rockford, Ill.) on 100–120 mesh Gas Chrom Q. For analyses of MC-Hfa-Me, 1.4% OV-210* (Pierce Chemical Company) on 100–120 mesh Gas Chrom Q was used as stationary phase (column temperature 250°). The carrier gas was helium.

2.4. *Isolation and hydrogenation of cerebroside Hfa*

The cerebrosides were refluxed for 5 hr in methanol-concentrated HCl (5:1)**. Total fatty acid methyl esters were extracted with diethyl ether. Conversion to methyl esters was completed by treating the residue with diazomethane in ether, before separation of Hfa-Me from Nfa-Me on silicic acid column chromatography. The Hfa-Me were further purified by preparative TLC (solvent system: hexane-diethyl ether (85:15)). The zones were made visible and the substances recovered as described above. The Hfa-Me were dissolved in ethanol and catalytically hydrogenated with H_2 over platinum oxide.

3. Results

Fig. 1a shows a gas chromatogram on OV-210 of

* OV-210 is a trifluoropropyl silicone phase, similar in composition and separating characteristics to QF-1 but of greater thermal stability.

** Control experiments showed that no racemization occurred during the methanolysis.

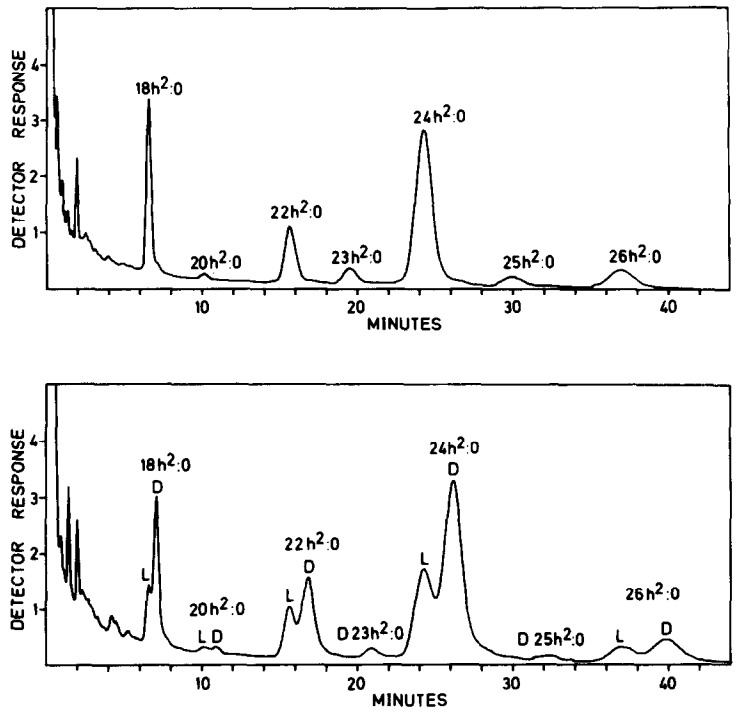

Fig. 2a. Gas chromatogram of MC,Me derivatives of hydrogenated Hfa from bovine brain cerebrosides on 1.4% OV-210 at 250°C.
Fig. 2b. Gas chromatogram of the mixture of esters in fig. 2a after addition of appropriate amounts of even numbered MC-DL-Hfa-Me.

the (-)menthylformate esters of racemic Hfa. Each acid gives two partially separated peaks. The MC-derivatives of optically active Hfa-Me give only one symmetrical peak. Optically active $16h^2$:0, $20h^2$:0 and $24h^2$:0 were prepared by Kolbe electrolysis from (-)malic acid. Consequently they have the same configuration as (-)glyceraldehyde, i.e. L (S according to the Cahn-Ingold-Prelog system). In fig. 1b appropriate amounts of the MC, Me-derivatives of these acids have been added to the mixture in fig. 1a. It is concluded that the first peak of each pair represents esters with the L-configuration and the second peak those with the D-configuration. Incidently, (-)menthylformate derivatives of Lα-phenylalkyl carbinols and Lα-amino acids [7] also have shorter retention times than the derivatives of the corresponding D-enantiomers.

Bovine brain cerebrosides were used as source of biological Hfa. The Hfa constituted about 35% of the total fatty acids. Individual acids were identified by GC on SE-30, using authentic Hfa-Me as standards. Unsaturated Hfa showed retention times some 0.3 carbon units less than their saturated analogs. Their identity was established by GC after hydrogenation of the Hfa-mixture. The Hfa composition is largely in agreement with earlier work on beef brain cerebrosides [9]. For steric analyses, the saturated Hfa-Me obtained by hydrogenation were used. Fig. 2a shows the analysis of MC-Hfa-Me on OV-210. Only one isomer of each acid is present. In fig. 2b appropriate amounts of even numbered MC-DL-Hfa-Me have been added. The esters of biological origin increase the slower emerging peak of each pair and consequently have the D-configuration. To determine the configuration of the odd numbered Hfa, for which no reference compounds were available, the retention times have been expressed as MC-L-Hfa-Me and MC-D-Hfa-Me carbon units (table 1), using the

Table 1
Retention data for odd numbered MC-Hfa-Me on OV-210

Hfa	Retention times	
	MC-L-Hfa carbon units	MC-D-Hfa carbon units
$23h^2$:0	23.4	23.0
$25h^2$:0	25.4	25.0

internal standards in fig. 2b for making the semilogarithmic plots. The correct C-values are obtained when the retention times are expressed as MC-D-Hfa-Me carbon units. The overall conclusion is that all major Hfa of bovine brain cerebrosides have the same configuration at C-2, viz. D or according to the Cahn-Ingold-Prelog system, R.

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References

[1] G.Rouser, J. Am. Oil Chem. Soc. 42 (1965) 569.
[2] A.Müller, Chem. Ber. 72 (1939) 615.
[3] D.H.S.Horn and Y.Y.Pretorius, J. Chem. Soc. (1954) 1460.
[4] K.Mislow and S.Bleicher, J. Am. Chem. Soc. 76 (1954) 2825.
[5] A.C.Chibnall, S.H.Piper and E.F.Williams, Bioch. J. 30 (1936) 100.
[6] S.Hammarström, B.Samuelsson and K.Samuelsson, J. Lipid Res. 10 (1969) in press.
[7] J.W.Westley and B.Halpern, J. Org. Chem. 33 (1968) 3978.
[8] S.Bergström, G.Aulin-Erdtman, B.Rolander, E.Stenhagen and S.Östling, Acta Chem. Scand. 6 (1952) 1157.
[9] J.S.O'Brien and G.Rouser, J. Lipid Res. 5 (1964) 339.