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Structure determination of the polymorphism of acylgalactosylceramide in rat brain by gas chromatography / mass spectrometry and proton magnetic resonance

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This paper describes the structure of acylcerebrosides isolated from rat brains. Three fractions (acylglycosylceramides I, II, III) were resolved according to their decreasing R_F values on TLC. GLC analysis of acylglycosylceramides II and III indicates that their ester-linked fatty acids are short and rather unsaturated, while amide-linked fatty acids are longer and hydroxylated. Sugar GLC analysis indicates that acylglycosylceramides II and III contain only galactose. To determine the substitution position of the acyl group on the galactose moiety, the free hydroxyl groups of acylglycosylceramide were protected with dihydropyran, deacylated and subjected to permethylation. The methylated galactoside acetates obtained after hydrolysis and reduction were then analyzed by gas chromatography / mass spectrometry. Acylglycosylceramides II and III turned out to be complex mixtures of 2-*O*-acyl-, 3-*O*-acyl-, 4-*O*-acyl- and 6-*O*-acylgalactosylceramides. Moreover, the abundance of α -methylgalactoside reveals the existence of unsubstituted galactose, suggesting that some ester-linked fatty acids could be esterified to the hydroxyl group of hydroxy fatty acids linked to sphingosine. NMR spectrometry was used to confirm this ester linkage. The key spectral feature of the fatty acid-galactose linkage (4.45 ppm) did move to 4.15 ppm after saponification of acylglycosylceramide II; on the other hand, acylglycosylceramide III contained only the spectral feature 4.15 ppm, corresponding to a high percentage of unsubstituted galactose and consistent with the presence in the molecule of a fatty acid esterified by the ω -OH group of the hydroxy fatty acid (3.95 ppm).

Introduction

A glycolipid fraction of lower polarity than cerebrosides has been isolated from mammalian brains [1–5] and resolved mainly into four galactolipids. These glycolipids were demonstrated to be galactosylceramides combined with an ad-

ditional fatty acid through an ester linkage. The acylcerebrosides isolated from human, whale and bovine brains are, in fact, a mixture of 2-*O*-acyl-, 3-*O*-acyl-, 4-*O*-acyl- and 6-*O*-acylgalactosylceramides [5].

The presence of ester cerebrosides has also been reported in hog stomach [6] and rat and pig epidermis [7–13].

In the latter, the acylcerebrosides were first identified as acylglucosylceramides [9], suggesting that esterification was via the sugar ring. However,

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