ment un signal à 3,64 ppm, qui pourrait être attribué au méthyle du groupement $-COOCH_3$.

La fortuitine aurait ainsi la formule brute $C_{53}H_{95}O_{11}N_7$ (poids moléculaire 1005) et la composition élémentaire C, 63.26; H, 9.45; N, 9.75; OCH_3, 3.08, en assez bon accord avec les valeurs trouvées.

Rappelons que le premier peptidolipide de ce genre, extrait de *Nocardia asteroides*, a été décrit par GUINAND, MICHEL ET LEDERER[2,3] et que LANÉELLE ET ASSELINEAU[4] viennent d'en rapporter un autre, isolé de *M. paratuberculosis* (*M. Johnei*).

Ces peptidolipides, ainsi que les peptido-glycolipides des Mycobactéries (mycoside C_1[5], mycoside C_m[6]), cires D de *M. tuberculosis var. hominis*[7], contiennent des D-amino-acides[8,9]. La fortuitine semble être le premier peptidolipide bactérien qui ne contient que des L-aminoacides.

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Structure of sulfatides

While studying the action of various acidic and alkaline reagents on sulfatides, we found them to be very resistant to alkali[1]. After refluxing a solution of sulfatides in 1 N sodium methoxide in methanol for 90 min, the sulfatides were recovered in high yield, and moved like the original material when chromatographed on thin layers of silica gel G. The peaks at 1240 and 820 cm^{-1}, characteristic of the sulfate group, were present in the infrared spectrum of the recrystallized product. Moreover, no 3,6-anhydro-D-galactose dimethylacetal could be detected with Seliwanoff's reagent in a methanolyzate of the alkali-treated sulfatide nor on paper chromatograms of the carbohydrate fraction of the methanolyzate, previously separated from the fatty

esters and the bases by elution from a charcoal-Celite column with 80 % aq. methanol. Methylgalactoside, however, appeared as the main component of this fraction.

Because, on the one hand, THANNHAUSER *et al.*[2] had concluded from methylation studies that the sulfate group of sulfatides is at C-6 of the galactose moiety of the molecule, and because, on the other hand, a galactopyranoside bearing a sulfate group at C-6 would be expected to form readily a 3,6-anhydro derivative by elimination of the sulfate under alkaline conditions[3], we have reinvestigated the position of the sulfate group by methylation. This has led us to the conclusion that the sulfate group of sulfatides is located at C-3 of the galactopyranoside moiety of the compound.

Shortly after we had reached this conclusion, the recent note by YAMAKAWA *et al.*[4] describing the identification of methyl 2,4,6-trimethylgalactoside in a methanolyzate of permethylated sulfatide by gas–liquid chromatographic analysis and thus demonstrating that the sulfate group is at C-3, came to our attention.

Because our results provide further evidence confirming the finding of YAMAKAWA in contradiction to the conclusion of THANNHAUSER, we wish to report briefly our experimental data.

A sulfatides preparation, isolated according to LEES *et al.*[5], was methylated repeatedly with methyl iodide and silver oxide at room temperature. Methanol and chloroform were used as auxiliary solvents during the first two methylations, chloroform alone afterwards. After methanolysis, the carbohydrate fraction obtained by elution with 80 % methanol from a charcoal-Celite column was hydrolyzed with 2 N H_2SO_4. Paper chromatography using the upper phase of a mixture of *n*-butanol–ethanol–water (5:1:4) as solvent and paper electrophoresis in borate buffer as pH 10 (ref. 6) indicated that the main component was 6-methyl-D-galactose (R_F, 0.30; migration, 0.90). Trace amounts of galactose (R_F, 0.17; migration, 0.91) and of two different dimethylgalactoses (R_F, 0.45; migration, 0.21 and R_F, 0.50; migration, 0.31) were also detected. By addition of phenylhydrazine and methanol to this fraction, 6-methyl-D-galactose phenylhydrazone crystallized; the m.p. (175–177°) was not depressed in admixture with an authentic sample and the infrared spectrum was identical with that obtained from the synthetic compound.

After eight repetitions of the methylation a faint spot corresponding to a trimethylgalactose could be detected on paper chromatograms and paper electropherograms in addition to 6-methyl-D-galactose and the two dimethylgalactoses. Its R_F value, 0.66, was clearly different, after long runs, from the values obtained for authentic 2,3,4-trimethylgalactose (R_F, 0.64) and 2,3,6-trimethylgalactose (R_F, 0.68). Detection was made with aniline phthalate. For electropherograms, 20 % of glacial acetic acid was added to the reagent.

In another experiment, sulfatides were methylated for 12 h with methyl iodide and silver oxide using dimethylformamide as solvent[7]. In this case, 2-methyl-D-galactose (R_F, 0.33; migration, 0.43) appeared as the main component of the sugar mixture obtained after methanolysis and hydrolysis. As judged by the intensity of the spots a fairly large proportion of unchanged galactose was still present in the mixture which contained also two dimethylgalactoses and a trimethylgalactose, inseparable on paper chromatograms from the compounds obtained in the previous experiment. A very neat separation of the trimethylgalactose from the 2,3,4 and 2,3,6 synthetic isomers was achieved by chromatography on paper impregnated with dimethylsulfoxide using benzene as solvent[8]. (R_F values are variable from one run

to another.) By using a two-dimensional system of chromatography followed by electrophoresis on Whatman No. 3 paper, a trace amount of 6-methyl-D-galactose was also shown to be present in the mixture.

When the sulfatides which had been methylated eight times in the absence of dimethylformamide were methylated once more in the presence of dimethylformamide, the trimethylgalactose was the main component of the mixture of sugars obtained after hydrolysis, and was contaminated only by very small amounts of the two dimethylgalactoses. After heating this carbohydrate fraction for 2 h at 75° in a sealed tube with aniline in ethanol, the anilide of 2,4,6-trimethyl-D-galactose was obtained as long needles melting at 176–176.5°. The compound showed mutarotation from $[\alpha]_D^{27}$ −89° to +38° in acetone (*c*, 0.57). The sulfur content of 2.9% of the methylated sulfatide and the presence in its infrared spectrum of the peaks at 1240 and 820 cm^{-1} indicated that the sulfate group had not been split off during the methylation. This was confirmed by the fact that no tetramethylgalactose was detected in the hydrolyzate. In contrast, tetramethylgalactose was found to be the main carbohydrate component in a hydrolyzate of methylated phrenosine. Very small amounts of 2,3,6-trimethylgalactose (R_F, 0.68) could also be detected in this case.

It is thus demonstrated that the sulfate group is located at C-3 of the galactopyranoside moiety of sulfatides.

The striking influence of the solvent on the course of the methylation of sulfatides, and the difficulty of achieving complete methylation, as well as the remarkable stability toward alkali of the sulfate group at C-3, are questions under further investigation.

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