PN 1207

Direct conversion of sulfatides into cerebrosides

When BLIX[1] in 1933 found equimolar amounts of fatty acid, sphingosine, galactose and sulfuric acid in a hydrolyzate of cerebronsulfuric acid, which he had isolated from brain; he concluded that this sulfatide was most likely a sulfuric ester of the corresponding cerebroside.

The position of the sulfuric ester group on C-6 of the galactose moiety of the sulfatide molecule was determined by THANNHAUSER, FELLIG AND SCHMIDT[2].

LEES *et al.*[3], by showing that the intact sulfatide contains no free amino group and no free reducing sugar group, substantiated the assumption that the fatty acid, sphingosine and galactose constituents are linked in the same way as in cerebrosides. The preparations of the expected products of partial splitting, ceramide and psychosine, which would have further confirmed the type of linkage between the constituents, were unsuccessful when the procedures used by KLENK[4] to prepare these substances from cerebrosides were applied to the sulfatides. However, glacial acetic acid at 100° for 90 min was found to release practically all the sulfur while the cerebroside portion of the molecule appeared to remain intact. No attempt was made to isolate the cebrosides.

Recently, JATZKEWITZ[5] has separated a sulfatide preparation into 2 components by column and thin-layer chromatography. The fatty acids and sphingosine-type bases were analysed and were found to be identical to the acids and bases constituting, respectively, the 2 cerebrosides phrenosine and cerasine. By non-selective sulfation of phrenosine and of cerasine with chlorosulfonic acid in pyridine, and chromatographic analysis of the resulting mixtures on thin layers of silica, one of several spots was found to migrate in both cases like the corresponding sulfatides.

Several authors have published infrared spectra of sulfatides[5]. The absorption at 1240 cm^{-1} has been used by WITMER AND AUSTIN[6] for the quantitative estimation of sulfatides. LLOYD AND DODGSON[7] observed the peak at 820 cm^{-1} which has been attributed to equatorial sulfuric esters of the primary alcohol group in hexopyranose rings, thereby confirming the conclusion of THANNHAUSER *et al.*[2].

Although all these data are compatible with the proposition that sulfatides are sulfuric esters of cerebrosides, no direct evidence has been given supporting this view.

We wish to report in this note the direct conversion of sulfatides into cerebrosides by treatment for 4 h at room temperature with 0.05 N anhyd. HCl in methanol according to the method of desulfation originated by KANTOR AND SCHUBERT[8]. The product of the reaction contained less than 0.1 % of sulfur. Chromatography of this material on thin layers of silica gel G showed 2 spots corresponding to the cerebrosides phrenosine and cerasine in 2 solvent systems: *n*-propanol–12.5 % aq. ammonia (8:2, v/v), and 80 % aq. phenol. Detection was made with the molybdate–$HClO_4$ reagent[9] and with orcinol in 60 % H_2SO_4, respectively. Sulfatides were absent. The splitting of the sulfuric ester is also evidenced by the disappearance in the infrared spectrum of both peaks at 1240 and 820 cm^{-1}. The spectrum of the desulfated substance is similar to that of the cerebrosides. Little degradation of the cerebroside part of the molecule seems to accompany this mild desulfation as the cerebrosides are recovered in a yield of about 70 % by chromatography on silicic acid columns as described by CARTER *et al.*[10].

This direct transformation of sulfatides into cerebrosides in high yield by a mild

procedure of desulfation proves that sulfatides are sulfuric esters of cerebrosides as proposed already by BLIX. Prolonged treatment leads to further transformations which are being studied.

The sulfatide used in this work was prepared by the method of LEES *et al.*[3]. It was chromatographed on silicic acid for further purification and contained no detectable phosphorus. Chromatography on thin layers of silica gel G revealed only the two sulfatides as described by JATZKEWITZ[5].

The great lability of sulfatides at room temperature in methanol containing small amounts of acid should be borne in mind whenever procedures for isolation of sulfatides involve treatment with alcohols. In this connection, it is of interest to note that in the course of investigations on the action of alkaline reagents on sulfatides, it appeared that they are very stable at high pH. This property, astonishing for compounds containing a hexopyranose ring bearing a sulfuric ester group in position 6, is now under study.

We are grateful to Dr. M. LEES for the gift of a sample of sulfatide, and to Dr. J. FOLCH-PI for his interest in this work.

This investigation was done during the tenure of an Established Investigatorship of the American Heart Association. It was supported by Research Grants B-2840 and B-130 from the National Institutes of Neurological Diseases and Blindness, National Institutes of Health.

McLean Hospital, Research Laboratory, Belmont, Mass., P. STOFFYN
and Department of Biological Chemistry, Harvard Medical School, A. STOFFYN
Boston, Mass. (U.S.A.)

[1] G. BLIX, *Z. Physiol. Chem.*, 219 (1933) 82.
[2] S. J. THANNHAUSER, J. FELLIG AND G. SCHMIDT, *J. Biol. Chem.*, 215 (1955) 211.
[3] M. LEES, J. FOLCH-PI, G. H. SLOANE STANLEY AND S. CARR, *J. Neurochem.*, 4 (1959) 9.
[4] E. KLENK, *Z. Physiol. Chem.*, 153 (1926) 74.
[5] H. JATZKEWITZ, *Z. Physiol. Chem.*, 320 (1960) 134.
[6] F. J. WITMER AND J. H. AUSTIN, *Mikrochem. Acta*, 4 (1960) 502.
[7] A. G. LLOYD AND K. S. DODGSON, *Biochim. Biophys. Acta*, 46 (1961) 116.
[8] T. G. KANTOR AND M. SCHUBERT, *J. Am. Chem. Soc.*, 79 (1957) 152.
[9] H. WAGNER L. HÖRHAMMER AND P. WOLFF, *Biochem. Z.*, 334 (1961) 175.
[10] H. E. CARTER, J. A. ROTHFUS AND R. GIGG, *J. Lipid Res.*, 2 (1961) 228.

Received October 31st, 1962