On the Sulfate Containing Lipids of Human Kidney

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The only known sulfolipids of mammalian origin are the sulfate esters of cerebrosides, also called sulfatides. Hitherto they have only been isolated from brain and kidney [1,2]. The presence of sulfate in lipid extracts from other organs has however been proved [3].

In infantile metachromatic leucodystrophy, a sulfatidosis [2,4,5], relatively large amounts of sulfatides are excreted in the urine. Thus it should be of interest to study the sulfate containing lipids of kidney. The study was initiated by isolating these lipids from human kidneys.

Human kidneys, showing no gross abnormalities, were taken at autopsies from patients age 60 to 90 years, 24 to 48 h after death. Capsules and renal pelvis were removed, and the kidneys were coarsely ground in a meat mincer, then washed several times with 0.9 % NaCl to remove blood, homogenized in a Waring blendor and lyophilized. The dry tissue (542 g) was extracted twice with chloroform-methanol (C–M) 2:1 (v/v) 20 ml/g, at room temperature and at boiling point. The combined extracts were concentrated to a small volume and subjected to a mild alkaline hydrolysis as described by Schmidt *et al* [6]. After acidification to pH 4 the lipids were extracted from the hydrolysate with C–M 2:1 (v/v). This extract was evaporated to dryness, dissolved in C–M 98:2 and chromatographed on twelve 85 g silicic acid columns. Elution scheme: I. Chloroform 10 ml/g, II. C–M 96:4 (v/v) 5 ml/g, III. C–M 9:1 (v/v) 10 ml/g, IV. C–M 4:1 (v/v) 10 ml/g, V. C–M 2:1 (v/v) 10 ml/g, VI. Methanol 10 ml/g. Fractions III, IV, and V, which contained 75 % of the sulfate in the original extract, were then chromatographed on diethylaminoethyl-(DEAE)-cellulose [7]. By this the acid lipids were separated from the neutral ones. From the acid lipid fraction two main sulfate containing components, A and B, could be isolated. This was achieved by preparative thin layer chromatography [8] on 2 mm thick layers of Silica Gel G, fraction A having the highest R_F-value (solvent: Chloroform-methanol-water 60:35:8/by vol/). Each fraction was further purified by rechromatography twice on 1 mm thick layers of the same adsorbent, and then by chromatography on Florisil, from which they were eluted with C–M 2:1. Fraction B, however, was still contaminated by a phosphorus-containing component, most of which was removed by chromatography on 1 mm thick layers of Silica Gel G with propanol-water 70:30 (v/v) as solvent.

Fraction A (185 mg) had a molar ratio hexose:sulfate: nitrogen of 1.12:1:1.08 (hexose [9], as galactose: 18.4 %, sulfate [10]: 8.77 %, nitrogen [11]: 1.38 %, ash *: 11.7 %, phosphorus: 0.06 %), while fraction B (29.5 mg) had the hexose:sulfate:nitrogen quotient 1.99:1:1.53 (hexose, as galactose:glucose 1:1:24.1 %, sulfate: 6.45 %, nitrogen: 1.44 %, ash *: 11.7 %, phosphorus: 0.35 %). Both fractions showed the presence of hexosamine and sialic acid in trace amounts only. Half the amount of fraction B was then subjected to a final purification by rechromatography on Florisil. The resulting product, fraction B_1 (9.4 mg) was much purer (hexose, as galactose: glucose 1:1: 30.3 %, sulfate: 7.75 %, nitrogen: 1.56 %, phosphorus:

* This analysis was performed at the Micro Analysis Laboratory, Upsala.

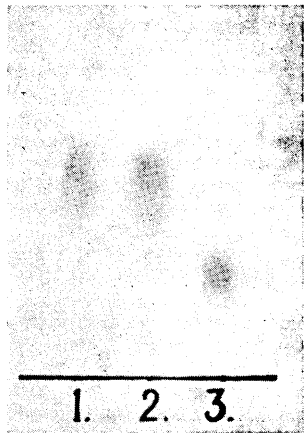

Fig. 1. Paper chromatography of sulfate containing lipids. 1. Brain sulfatides *. 2. Fraction A. 3. Fraction B.

* This was a gift from Dr. L. Svennerholm of this department.

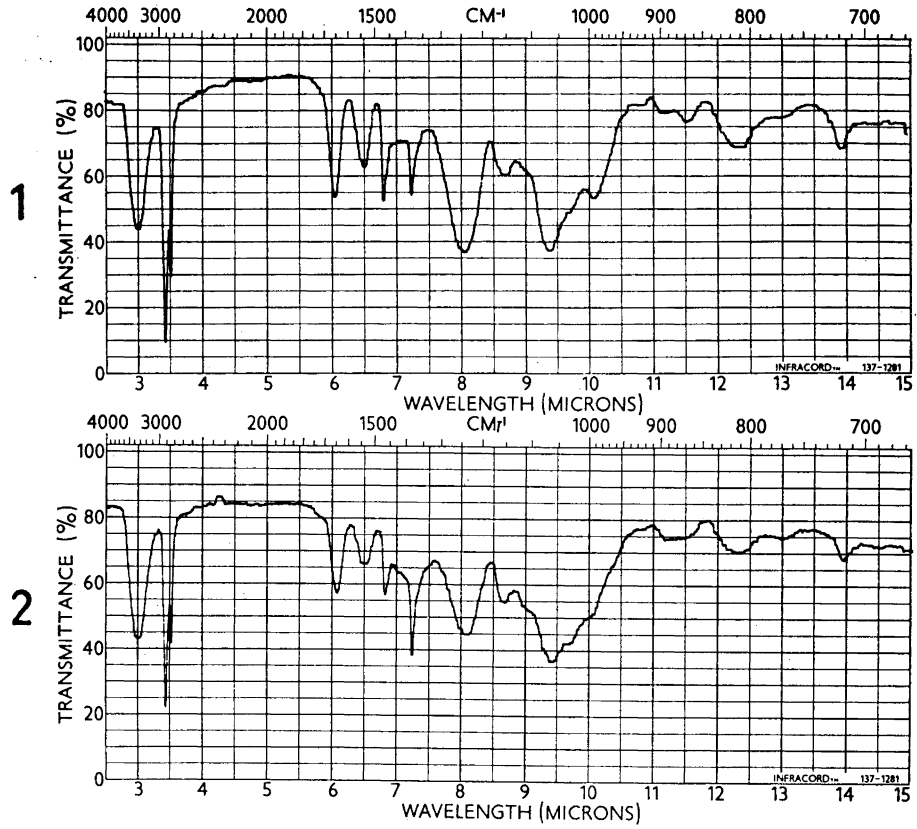

Fig. 2. IR-spectra of sulfate containing lipids from human kidney, dispersed in 300 mg KBr. The spectrograms were recorded in a Perkin Elmer Infracord Spectrophotometer. 1. Fraction A (1.1 mg). 2. Fraction B_1 (0.9 mg).

0.12 %). The analytical results gave a hexose: sulfate:nitrogen ratio of 2.08:1:1.38.

Both fraction A and fraction B showed one double spot at thin layer chromatography on Silica Gel G with the following solvents: chloroform-methanol-water 60:35:8 (by vol), and 65:25:4 (by vol), and propanol-water 70:30 (v/v) [12]. At paper chromatography with the solvent tetrahydrofuran-diisobutylketone-water 45:5:6 (by vol) [13] the two fractions were obtained as single spots which stained metachromatically [14] with cresyl violet. At all chromatographies fraction A had the same R_F-value as brain sulfatides (Fig. 1).

IR-spectra of the two lipids both show the absorption around 1240 cm^{-1}, which is characteristic for sulfatides. The absorption at 1120 – 1030 cm^{-1} is, however, much stronger in fraction B_1, which is in agreement with the higher hexose content is this fraction (Fig. 2).

Paper chromatography of the sugar components showed that fraction A only contained galactose, while fraction B contained glucose and galactose in about equimolar amounts.

The presence of sphingosine bases in both fractions has been demonstrated by paper and thin layer chromatography * [15].

* These analyses have kindly been performed by Dr. K. A. Karlsson of this department.

Two different types of sulfolipids have thus been isolated from human kidney. In regard to the analytical results one of them has the same chemical composition as brain sulfatides, acyl-sphingosine-galactose-sulfate. The other is probably an acyl-sphingosine-glucose-galactose-sulfate, which structure would best agree with the sugar and sulfate values found. The nitrogen content is on the other hand almost 40 % too high. Probably this is due to impurities, as even small contaminating amounts of any substance with a high nitrogen content would add a considerable error to the nitrogen determination.

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Optical Rotatory Dispersion and Configuration of *Solanum* Alkaloids

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Two isomeric series of *Solanum* alkaloids (aminoketal alkaloids) are known. They can be represented by tomatidine and 5α-solasodan-3β-ol and are usually characterized in the same manner as the steroid sapogenins by the prefixes neo- and iso-, respectively. The close relationship to the sapogenins has been established in various ways [1] and both compounds have the same structure and stereochemistry except for the spiroaminoketal side chain.

Schreiber [2] showed that the two series differ in configuration at C_{25} and related the configuration at this center to L(+)-α-methylglutaric acid for tomatidine and to D-(−)-α-methylglutaric acid for 5α-solasodan-3β-ol. The two series can thus be referred to as 25 L and 25 D, respectively.

Arguments advanced in the sapogenin field concerning the stereochemistry of the spiroketal side chain have been considered valid also in the case of the aminoketal alkaloids (*cf.* Ref. [1]). Both tomatidine and 5α-solasodan-3β-ol have been described as 22α-compounds, and the only difference between them should be that the C_{25}-methyl group is axial in tomatidine (structure 1) and equatorial in 5α-solasodan-3β-ol (structure 2). However, there remains the possibility that the alkaloids of the two series are both 22β-compounds (3 and 4) or that they differ in configuration at C_{22} as well as at C_{25}. A difference in configuration at C_{22} will then cause the methyl groups at C_{25} in alkaloids of both series to be either equatorial (2 and 3) or axial (1 and 4). According to Schreiber [2] tomatidine should be represented by structure 3 and 5α-solasodan-3β-ol by structure 2. This alternative is also

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