On a molar basis the limits of detection on unchromatographed papers were about the same for methionine, S-methyl-cysteine, N-acetyl-methionine, glycyl-methionine and cystathionine, whereas for α-hydroxy-γ-methylmercapto-butyric acid about twice as much was required for detection. The reagent was much less sensitive for lanthionine and methionine sulphoxide of which 10^{-3} M solutions were required. Homocystine constituted a special case: Iodine formation was observed with down to 2.5×10^{-4} M solution when the 10^{-2} M solution was diluted with 10^{-2} M HCl, whereas only bleaching of the reagent was observed when 70 % ethyl alcohol was used for dilution.

The modified iodoplatinate reagent is thus a sensitive detection agent for methionine and some other substances containing a thioether grouping, the notable exception being lanthionine which was only detected at relatively high concentrations. The usefulness of the reagent in detecting methionine-containing peptides will probably depend upon whether these also contain cysteine (reaction 4).

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Chromatographic Determination of Sulfatides

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In the study of the metabolism of glycolipids it was often important to detect and determine small amounts of sulfuric acid esters of glycolipids (sulfatides). The methods available for the determination of sulfatides — in general a quantitative estimation of lipid-bound sulfate — gave unreliable results with total lipid extracts of biological materials. Therefore, a specific method for the determination of lipid-bound sulfate was recently elaborated [1] in our department. The procedure has, however, some limitations: it is rather insensitive, inorganic sulfate may interfere, and it does not distinguish between different sulfate esters of glycolipids. The present method was elaborated as a sensitive supplement to the quantitative sulfate method at the isolation of sulfatides [2] and for the laboratory diagnosis in urinary sediments of an earlier little known inherited disorder [3], metachromatic leucodystrophy.

Experimental. Extraction of sulfatides. A total lipid extract of tissues or tissue fluids was obtained by extraction with 20 volumes of chloroform-methanol (C—M), 2:1, v/v. Urinary sediment was obtained by adjusting the pH of the urine to 5—6 and let the urine stand over night at + 4°. The sediment formed was collected by centrifugation and extracted with C—M 2:1. Nonlipid material was removed by phase partition as described for the determination of cerebrosides [4]. The solvent phase was evaporated and the lipids redissolved in fresh C—M 2:1.

Chromatography on Florisil. If the lipid extract contained more than ten times as much phospholipids as sulfatides most of them were removed in the following manner: 0.5 g of Florisil (magnesium trisilicate), 60—100 mesh, was added to the lipid extract not containing more than about 10 mg of lipids.

The extract was then slurried onto a small chromatographic column with 0.5 g Florisil and after several rinsings with C—M 2:1 the column was eluted with 25 ml of C—M 2:1. The total effluent was collected and evaporated.

Paper chromatography. An aliquot of the lipid extract, containing from 1—10 μg of sulfatides, was spotted on to the starting line of a Schleicher & Schüll 2045b paper, 12 × 48 cm. When urinary sediments were analysed an aliquot of the lipid extract, corresponding to 2 mg of urine creatinine, was applied on the paper. Four unknown samples and two standards of 5 and 10 μg of sulfatides were spotted on to each paper. The paper was developed for 3 h at 21° in tetrahydrofuran-diisobutylketone-water, 45:5:6, by vol. The sulfatides were visualized by dipping the papers into 0.02 % cresyl violet in 1 % aqueous acetic acid for 30 min at room temperature or for 10 min at 60°. Excess staining reagent was removed by rinsing the papers for 30 min each time in three changes of 1 % aqueous acetic acid. All chromatograms were evaluated when the papers were still wet. The sulfatides stained metachromatically; in low concentrations, up to about 2 μg of sulfatides, the spot was red-violet but in higher concentrations the spot became brown-red against a blue-violet to blue background. The intensity and size of the spots of the unknown samples were compared to the reference sulfatides on each paper and with reference chromatograms on which 0.1—100 μg of sulfatides had been developed. From the first running of the unknown sample the most suitable amount for a second run was estimated. The accuracy of the procedure was astonishingly good; the standard deviation was about ± 5 % in extracts where sulfatides constituted at least 1/10 of total lipids. The recovery of added sulfatides was 90—95 % for the addition of 0.1 mg sulfatides and 98 % for the addition of 1.0 mg to 5 mg of other lipids.

In the urinary sediments of subjects with metachromatic leucodystrophy there occurred besides the ordinary sulfatide, acyl-sphingosine-galactose-sulfate, a sulfolipid with a lower R_F-value (Fig. 1). It had the same R_F-value as the acyl-sphingosine-dihexoside-sulfuric acid ester isolated from human kidney by Mårtensson [5].

Other acid lipids gave also metachromatic spots with cresyl violet but no other lipids than those with sulfate esters gave the typical brown-red spots. Mauve coloured spots were given by phosphatidyl serine, inositol phospholipids and gangliosides. Serine phosphatides had in general a somewhat higher R_F-value than the sulfatides in this solvent and they gave a rather weak metachromasia. In brain and especially in blood serum, spleen and liver lipid extracts there were formed after mild alkaline hydrolysis lysocompounds of inositiol phospholipids which moved with about the same R_F-value as the slow running sulfolipid of kidney [5]. They took a strong red-violet colour with cresyl violet but the colour did not change to pure red although very large amounts were applied. If a sulfate ester lipid was run on the same paper this "false" metachromasia was easily differentiated from the true one [6]. Some fast moving gangliosides had about the same R_F-value as the slow running sulfolipids but they gave also in high concentrations a mauve colour. Di- and trisialogangliosides gave a more red colour but they remained at the starting line.

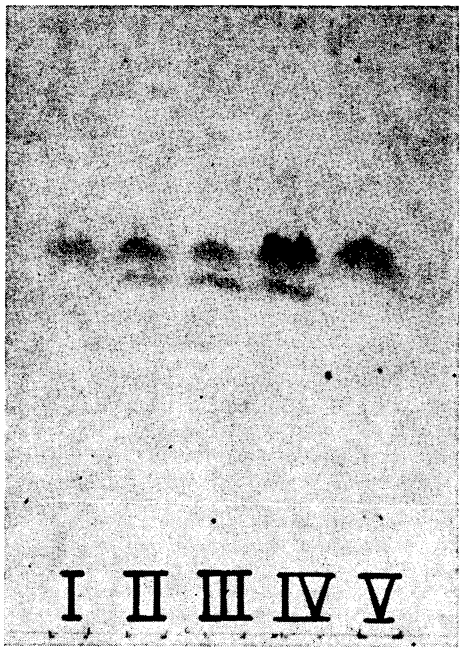

Fig. 1. Paper chromatogram of sulfolipids. I = 5 μg brain sulfatides, II and IV = human kidney extract [5], III lipid extract from urinary sediment of a subject with metachromatic leucodystrophy, V = 10 μg brain sulfatides.

Under normal conditions all these lipids were retained on the Florisil columns but they appeared, for instance, in the effluent at overloading of the column. For a positive identification of the sulfolipids it was, therefore, necessary to apply as much material on the paper that with the cresyl violet stain a brown-red metachromatic spot was obtained.

Summary. A simple procedure is described for the chromatographic determination of small amounts of sulfatides in biological materials. As little as 0.2 μg of sulfatides can be detected and the optimal concentration for the analysis is 1—10 μg of sulfatides.

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The Preparation of 3,3,5,5-Tetramethyl-1,2-dithiolane-4-one

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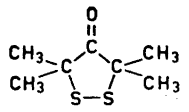
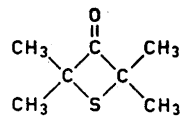
The unsubstituted 1,2-dithiolane-4-one (1,2-dithiacyclopentane-4-one) is too unstable to be isolated in the pure state. Schotte was, however, able to prepare its semicarbazone [1]. We wish to report here the synthesis of the tetramethyl-



I



II

substituted ketodisulphide (I), where the methyl groups have stabilized the ring system making it possible to isolate the compound by distillation.

As the starting material for the synthesis we have used α,α′-dibromodiisopropylketone. This compound has been prepared from diisopropylketone, bromine and phosphorus pentabromide by Faworsky [2]. We have simplified the synthesis and obtained the dibromoketone in a better yield by performing the bromination with bromine in hydrobromic acid.

At first we attempted to prepare the dimercapto compound corresponding to I by starting with α,α′-dibromodiisopropylketone and sodium hydrosulphide, but the only isolated product was the cyclic monosulphide II, 2,2,4,4-tetramethyl-3-thietanone [3]. However, the disulphide I could be obtained in a fairly good yield from the dibromoketone and sodium disulphide. It is a light yellow liquid (m.p. 13—14°) and shows a number of ultra-violet absorption peaks between 200 and 330 mμ.

Further work is in progress on the cyclic ketodisulphides.

Experimental. α,α′-Dibromodiisopropylketone. While stirring at room temperature, 80 g (0.5 mole) of bromine were added dropwise to 57 g (0.5 mole) of diisopropylketone mixed with 50 ml of hydrobromic acid (48 %). The mixture was heated to 55° and a further 80 g (0.5 mole) of bromine were added. The mixture was kept at this temperature with continued stirring for 3 h. The product was separated from the hydrobromic acid, washed with water, sodium bisulphite solution (10 %), sodium carbonate solution (5 %), water, and dried over anhydrous calcium chloride. The α,α′-dibromodiisopropylketone was collected at 84.5—86.5°/10 mm, n_D^{20} 1.5062. The yield was 109 g (80 %). α,α′-Dibromodiisopropylketone prepared according to Faworsky shows b.p. 84—85°/9 mm [2] and n_D^{20} 1.5062 [3].

3,3,5,5-Tetramethyl-1,2-dithiolane-4-one. 18 g of $Na_2S \cdot 9H_2O$ in a mixture of 200 ml of dimethyl sulphoxide and 30 ml of water were dissolved with stirring at 75°. 2.4 g of finely ground sulphur were then added, and heating