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On the Neutral Glycolipids of Human Kidney

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Studies of glycolipids have mostly been limited to the CNS, but some of the glycolipids of spleen,[1,2] erythrocytes,[3,4] and liver [5] have been isolated and described. The neutral glycolipids of blood serum have also recently been studied.[6] From these studies it seems probable that the neutral glycolipids in hitherto studied organs outside the CNS consist mainly of four different sphingolipid fractions, *viz.* ceramide-mono-, di-, and trihexosides and an aminoglycolipid fraction. Except the neutral glycolipids the presence of acid lipids containing sulfate [7] and sialic acid [8] has been established.

Informations about these lipids in normal kidneys are scanty. An immunologically active aminoglycolipid has, however, been isolated by Rapport, Graf and Schneider.[9] It thus seemed appropriate to isolate and describe the different glycolipid fractions of human kidney. A preliminary description of the sulfate containing lipids of this organ has recently been given.[10]

Human kidneys, showing no gross abnormalities, were taken at autopsies from patients aged 60 to 90 years, 24 to 48 h after death. The first steps of the preparation — removal of blood, homogenization, freeze-drying, lipid extraction, mild alkaline hydrolysis of the lipids, and chromatography of the alkalistable lipids on silicic acid columns — were performed exactly as earlier described.[10] The effluent from the silicic acid columns was divided into five fractions; one containing mainly the free fatty acids released by the alkaline hydrolysis, three crude glycolipid fractions, and finally one fraction containing the sphingomyelins with only traces of glycolipids. Thin layer and paper chromatography showed the presence of four main glycolipid components, which had been partially separated. Each of the three crude glycolipid fractions was passed through a DEAE-cellulose column, whereby the acid lipids were removed. The four neutral glycolipid fractions (I—IV) were then isolated by rechromatography on silicic acid columns in chloroform-methanol (C—M) mixtures followed by preparative thin layer chromatography on 1 mm thick layers of silica gel G, C—M—W 65:25:4 (by vol.). Two of the fractions, (I and II) — later shown to be the ceramide-monohexosides and the ceramide-dihexosides — were, however, still heavily contaminated. The monohexosides were, therefore, further purified by chromatography on Florisil in C—M 2:1 (v/v) and then rechromatographed on silicic acid in C—M 9:1 (v/v). The dihexsosides were purified by chromatography on silicic acid in C—M 4:1 (v/v).

The four fractions contained by now 70.1 % of the hexose in the original crude glycolipid fractions. (7.2 % was in the acid lipid fraction, 5.6 % was represented by an unidentified glycolipid, 7.3 % had been taken for analyses, 1.8 % was in the form of smaller mixed fractions not worked up, and 8.2 % were losses not accounted for). Before the final analyses the lipids were dissolved in small volumes of hot methanol and precipitated in the cold.

The analytical results are shown in Table 1. The presence of sphingosine bases in the fractions was demonstrated by paper and thin layer chromatography.* [11,12]

In regard to the analytical data the four fractions can be identified as ceramide-monohexosides, ceramide-dihexosides (glu-gal 1:1), ceramide-trihexosides (glu-gal 1:2) and amino-glycolipids(glu-gal-galam 1:2:1). The glucose-galactose ratios are only approximative, obtained by a subjective valuation of colour intensities of paper chromatogram spots. The R_F-values of the fractions at thin layer chromatography on silica gel G, solvent: C—M—W 65:25:4 (by vol.) [13] and at paper cromatography, solvent: tetrahydrofuran-di-isobutylketone-water 45:5:6 (by vol.),[14] were also the same as for corresponding lipids isolated from blood serum.**

From 542 g freeze-dried kidney tissue was isolated 115 mg monohexosides (I), 223 mg dihexosides (II), 568 mg trihexosides (III), and 1003 mg aminoglycolipides (IV). These weights were obtained before precipitation from methanol, when

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

** These substances were gifts from Dr. L. Svennerholm of this institute.

Table 1.

Glycolipid fraction	Paper chromatography of sugars * [16]	Hexose [17] %	Hexosamine [18] %	N [19] %	P %	Molar ratio hexose-nitrogen
I	glu (+) gal +	20.3 as gal	0	1.76	0.03	1:1.11
II	glu + gal +	37.7 as glu-gal 1:1	0	1.49	0.05	2:1.02
III	glu + gal ++	47.6 as glu-gal 1:2	0	1.30	0.01	3:1.05
IV	glu + gal ++ galam +	35.8 as glu-gal 1:2	11.9	2.11	0.11	3:2.27

* The sugars were liberated by hydrolysing the lipids with 2 N HCl for 2 h at 100°C in closed tubes.

the lipids still contained between 10 to 20 % impurities, but were chromatographically pure. Taking into account the different losses during the preparation, the percentage distribution could be calculated to be about 5–7 % (I). 10–12 % (II), 25–30 % (III), and 45–50 % (IV) of total neutral glycolipids.

It is of interest to compare this ratio with the results obtained from other organs. Thus in liver [5] and spleen [2] the dihexosides, in erythrocytes the aminoglycolipids,[4] in blood serum the mono- and dihexosides,[6] and in brain the monohexosides are the dominating neutral glycolipids. Each organ seems thus to have its own pattern. A remarkable fact is that the monohexosides of kidney are mainly galactocerebrosides. In other organs outside CNS glucocerebrosides dominate.[6]

As can be judged from the data presented by Rapport *et al.*[9], the immunologically active aminoglycolipid fraction isolated by them is probably the same as the one here described.

Recently it was reported that Fabry's disease was a sphingolipidos with the accumulation of large amounts of ceramide-trihexosides in the kidneys.[15] Like the trihexosides from normal kidney they had a glucose-galactose ratio of 1:2. The composition of the normal fatty acids also agrees very well with the present trihexosides,

Table 2. Fatty acid composition of the trihexoside fraction in per cent.

$C_{14:0}$ 0.46; $C_{16:0}$ 4.55; $C_{16:1}$ 0.18; $C_{17:0}$ 0.13; $C_{18:0}$ 2.29; $C_{18:1}$ 0.34; $C_{19:0}$ 0.13; $C_{20:0}$ 6.06; $C_{20:1}$ 0.75; $C_{21:0}$ 1.12; $C_{22:0}$ 23.22; $C_{22:1}$ 3.24; $C_{23:0}$ 2.43; $C_{23:1}$ 0.65; $C_{24:0}$ 26.27; $C_{24:1}$ 28.18; C_{25} traces; C_{26} traces.

Table 2.* Available data thus suggest that the trihexosides accumulated in kidneys in Fabry's disease have the same components as those normally present.

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Perchloric Acid Catalysed Acylation of Benzofurans

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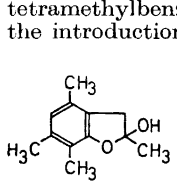
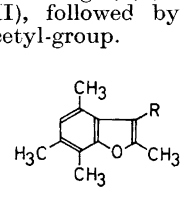
An attempt to convert 2-hydroxy-2,4,6,7-tetramethyl-2,3-dihydrobenzofuran (I)[1] into the corresponding 2-acetoxy compound with acetic anhydride in the presence of perchloric acid gave a yellow crystalline compound, which on treatment with water was converted to colourless 2,4,6,7-tetramethyl-3-benzofuranyl methyl ketone (III). Titration of the acid water phase indicated the simultaneous formation of 0.5 mole of perchloric acid per mole of the ketone III. Thus, instead of the intended acetylation of the 2-hydroxyl-group in I dehydration occurred forming 2,4,6,7-tetramethylbenzofuran (II), followed by the introduction of a 3-acetyl-group.



I



II R=H

III R=COCH$_3$

The yellow product that precipitated from the reaction mixture was obviously a molecular compound of the ketone III with perchloric acid in a mole ratio of 2:1. This interpretation of the reaction was supported by the formation of the same yellow product when 2,4,6,7-tetramethylbenzofuran (II) or the ketone III was treated with acetic anhydride/perchloric acid under the same conditions as compound I. That acylation of 2,4,6,7-tetramethylbenzofuran (II) occurred in the 3-position and not in the 5-position has not been shown experimentally but is very probable since it is known that electrophilic substitution takes place in the 3-position in benzofurans that are substituted in the 2-position with an electron releasing group (*e.g.* CH_3-).[2] 2-Benzofuranyl methyl ketone, which was obtained on acylation of benzofuran with acetic anhydride/perchloric acid did not give any isolatable perchloric acid adduct.

Acylation of benzofurans is often difficult because they polymerise in the presence of Friedel-Crafts catalysts.[3] Successful acylations have been carried out with, for instance, boron trifluoride [4] or stannic chloride [2,4] but the yields reported are lower than those now obtained with perchloric acid. Perchloric acid and perchlorates have recently been used as catalysts, inter alia in the acylation of furan.[5]

Molecular compounds of aromatic ketones with perchloric acid are known with mole ratios of both 1:1 and 2:1 (*e.g.* Refs.[6-8]). The tendency to form such compounds varies widely even in related types of compounds; *e.g.* 2-methoxychromones give crystalline addition products with perchloric acid but 4-methoxycoumarins do not.[8]

Experimental. Compounds I and II were prepared according to Ref.[1] and the acylation reagent ($Ac_2O/HClO_4$ in ethyl acetate) according to Ref.[9]

2,4,6,7-Tetramethyl-3-benzofuranyl methyl ketone (III) and its molecular compound with perchloric acid. A mixture of 2-hydroxy-2,4,6,7-tetramethyl-2,3-dihydrobenzofuran (I, 174 mg) and the acylation reagent (1.5 ml) precipitated a crystalline substance after about 15 min. The mixture was cooled to 0° and the product was filtered off and washed with ethyl acetate, giving pale yellow needles (116 mg), m.p. 110–150° (decomp.). (Equiv. wt. by titration with NaOH: 536. Calc. for a molecular compound 2 $C_{14}H_{16}O_2 \cdot HClO_4$: 533.01).

The mother liquor was washed with bicarbonate solution and evaporated. The residue