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On the Occurrence of 1-*O*-(2-Methoxyalkyl)glycerols and 1-*O*-Phytanylglycerol in Marine Animals

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1-*O*-(2-Methoxyalkyl)glycerols were isolated from the lipids of herring fillets, Baltic herring fillets, mackerel fillets, marine crayfish, fresh-water crayfish, shrimps, sea mussels, and cod liver oil. They were found both in the neutral lipids and in the phospholipids in considerably higher amounts than in mammalian tissues. The methoxy-substituted glycerol ethers had a high content of ethers with 16 carbon atoms in the long alkyl chains. The compounds with 16 and 18 carbon atoms together usually constituted over 90 %. The composition is roughly similar in the neutral lipids and in the phospholipids. A poly-unsaturated compound, 1-*O*-(2-methoxydocosahexaenyl)glycerol was found in the neutral lipids of mackerel fillets and cod liver oil and in the phospholipids of shrimps. A glycerol ether with a phytanyl chain was isolated from the liver oil of cod caught in the Baltic sea. The content and composition of the unsubstituted glycerol ethers have been determined for comparison.

2-Methoxy-substituted glycerol ethers were first isolated from Greenland shark liver oil.[1] They were also found in human milk, cow's milk, sheep's milk, and different human tissues, *viz.* red bone marrow, red cells, blood plasma, and in a uterine carcinoma.[2] The methoxy-substituted glycerol ethers had antibiotic activity and inhibited the dissemination and growth of several experimental tumours in mice.[3,4] As they occur in a relatively high concentration in shark liver oil compared to the minute amounts found in the mammalian tissues studied, it was of interest to determine the content of 2-methoxy-substituted glycerol ethers in different marine animals. The distribution of the compounds between the neutral lipids and the phospholipids was also studied.

MATERIAL AND METHODS

The material studied were fillets from herring (*Clupea harengus*), Baltic herring (*Clupea harengus*), mackerel (*Scomber scomber*), marine crayfish (*Nephrops norvegicus*), fresh-water crayfish (*Astacus fluviatilis*), shrimps (*Pandalus borealis*), and sea mussels (*Mytilus edulis*). Only the edible parts of the crayfishes, shrimps and sea mussels were included in the samples investigated. Both a commercial sample of cod liver oil and liver oil from cod caught in the Baltic sea were analyzed.

The extraction of the lipids, the separation of neutral lipids and phospholipids, the isolation of glycerol ethers and their isopropylidene derivatives, gas chromatography and mass spectrometry were performed as earlier described.[2] As before,[2] ethanol was used instead of methanol in some experiments in order to exclude the possibility of artefacts from methanol treatment.

RESULTS AND DISCUSSIONS

The methoxy-substituted glycerol ethers like the unsubstituted ones occur both in the neutral lipids and in the phospholipids (Table 1). The percentage of both groups of glycerol ethers is usually much higher in the phospholipids than in the neutral lipids. Due to the large proportion of neutral lipids in herring, Baltic herring, and mackerel, about the same quantities of glycerol ethers are found in the two lipid fractions of these species.

The content of methoxy-substituted glycerol ethers is higher in the neutral lipids and especi-

Table 1. The content of unsubstituted and methoxy-substituted glycerol ethers in the neutral lipids (N) and the phospholipids (P) isolated from marine animals.

Material	Lipids % (w/w)	Neutral lipids (N) and phospholipids (P) in total lipids % (w/w)		Unsubstituted glycerol ethers [a] in N and P % (w/w)		Methoxy-substituted glycerol ethers [a] in N and P % (w/w)	
		N	P	N	P	N	P
Herring fillets	17.3	94.4	5.6	0.05	0.57	0.02	0.14
Baltic herring fillets	10.4	91.4	8.6	0.07	0.54	0.01	0.14
Mackerel fillets	20.7	95.5	4.5	0.06	1.10	0.02	0.22
Marine crayfish	2.3	53.9	46.1	1.20	1.30	0.17	0.35
Fresh-water crayfish	4.3	69.6	30.4	0.65	1.70	0.07	0.06
Shrimps	2.8	47.6	52.4	1.20	1.70	0.08	0.19
Sea mussels	2.1	54.9	45.1	1.10	1.90	0.08	0.47
Cod liver oil, commercial sample	100	100		0.05		0.03	
Liver oil from cod caught in the Baltic sea	100	100		0.05 [b]		0.02	

[a] The figures should be multiplied by a factor of about 2.0–2.5 to get the content of the original glycerol ether lipids in the lipid fractions. [b] In addition this cod liver oil contained 0.03 % phytanyl glycerol ethers.

ally in the phospholipids of the marine animals studied than in the lipids of the mammalian tissues investigated.[2] The principal components of the methoxy-substituted glycerol ethers are the same in the marine animals as in the mammalian tissues. The compounds with 16 and 18 carbon atoms in the long hydrocarbon chains amount to over 90 % of the methoxy-substituted glycerol ethers of both the neutral lipids and the phospholipids (Table 2). The compounds with C_{16} chains, especially, are usually found in high concentrations. Only small amounts of methoxy-substituted glycerol ethers with less than 16 carbon atoms in the long alkyl chains are found. A poly-unsaturated methoxy glycerol ether, 1-*O*-(2-methoxydocosahexaenyl)glycerol, was found in the neutral lipids of mackerel fillets, in the liver oil from the cod caught in the Baltic sea, and in the phospholipids of shrimps. Such a poly-unsaturated methoxy-substituted glycerol ether was first isolated from Greenland shark liver oil[5] and has also been found in the lipids of human red blood cells.[2]

The content of unsubstituted glycerol ethers in the lipids of the herring, Baltic herring and mackerel fillets (Table 1) is comparable with the amounts of these glycerol ethers found in the mammalian tissues.[2] A somewhat higher content of unsubstituted glycerol ethers was found in the crayfish, shrimps, and mussels. Cod liver oil contains small quantities of glycerol ethers compared to the large amounts found in shark liver oil.[1,6] The composition of the unsubstituted glycerol ethers of the neutral lipids and of the phospholipids is roughly similar (Table 3). The compounds with even-numbered long hydrocarbon chains are the principal components and among them those with 16 and 18 carbon atoms dominate. The unsubstituted glycerol ethers of the neutral lipids of herring fillets, mackerel fillets, crayfish, shrimps, and sea mussels contain more saturated C_{16} and C_{18} components than cod liver oil. Fairly large amounts of C_{14} compounds are present in the unsubstituted glycerol ethers of both the neutral lipids and the phospholipids of herring and mackerel fillets.

Table 2. The percentage composition of methoxy-substituted glycerol ethers in neutral lipids (N) and phospholipids (P) from marine animals.

Long chain component	Herring fillets		Baltic herring fillets		Mackerel fillets		Marine crayfish		Fresh-water crayfish		Shrimps		Sea mussels		Cod liver oil, commercial sample	Liver oil from cod caught in the Baltic sea
	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	N
14:0	0.3	0.2					0.9	0.7	1.4	1.4						tr.
14:1							0.1									0.9
15:0		0.3	1.7		tr.		2.5	0.4	2.2	1.3						0.2
15:1							1.1	0.4								0.7
16:0	30.0	41.7	29.5	36.6	46.9	54.6	49.6	27.2	53.1	32.3	45.4	23.5	51.7	66.3	4.4	3.3
16:1	37.1	24.4	21.4	17.7	32.4	28.0	31.0	40.6	2.4	9.8	30.7	42.7	8.7	10.9	55.7	15.1
17:0	1.7	2.0	1.0	1.1	1.0	0.9	2.2	1.6	3.6	3.4	1.9	0.9	2.9	1.7	1.2	1.3
17:1	3.2	3.4	4.0	4.1	3.0	3.7	2.4	4.4	2.8	2.5	3.7	4.6		1.0	2.4	5.7
18:0	4.5	5.1	3.4	1.6	1.4	0.8	4.2	0.9	28.2	16.7	7.3	0.9	20.5	9.0	0.9	0.8
18:1	20.1	21.6	39.0	35.1	14.8	12.0	4.1	23.3	4.4	28.1	8.1	25.7	5.5	7.4	34.5	69.5
19:0	0.4	0.3		0.3		tr.	0.3	0.1	0.9	1.0	1.1	0.4	1.3	0.5	0.1	0.5
19:1	0.5	0.3		0.5	tr.		0.4	0.2	0.3	1.4	0.4	0.6	tr.	tr.	0.2	0.3
20:0	1.6	0.2	tr.	0.3			0.6	0.1	0.6	0.9	1.4	tr.	8.1	3.2	0.1	
20:1	0.2	0.2		0.9	tr.		tr.	0.1	0.1	1.2		0.3	1.3			
20:2																
21:0	0.1	tr.														
21:1	tr.	0.1														
22:0	0.2	tr.		0.4												
22:1	0.1	0.2		1.4			0.6								0.5	
22 poly					0.5							0.4				1.7

Table 3. The percentage composition of unsubstituted glycerol ethers in neutral lipids (N) and phospholipids (P) from marine animals.

Long chain component	Herring fillets		Baltic herring fillets		Mackerel fillets		Marine crayfish		Fresh-water crayfish		Shrimps		Sea mussels		Cod liver oil, commercial sample	Liver oil from cod caught in the Baltic sea
	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	N
12:0 and 1			0.5										0.9		1.4	1.6
14:0	15.2	14.3	13.9	16.8	12.0	6.5	3.8	17.3	4.1	2.6	4.8	5.0	3.0	8.5	5.5	1.2
14:1	5.9	3.9	6.4	3.8	1.6	1.1	0.6	2.8			0.5	0.2			15.5	4.9
15:0	2.2	2.6	1.2	1.7	2.3	1.9	3.2	6.6	tr.	tr.	2.8	3.1	0.9	2.7	0.5	tr.
15:1	tr.	1.3					1.6	5.1							1.9	1.1
16:0	41.0	36.2	57.7	52.2	43.7	40.5	36.4	27.5	41.5	48.0	44.5	50.9	20.8	51.2	13.4	3.6
16:1	13.1	10.8	3.9	3.9	6.6	3.4	12.6	24.3	5.9	8.7	8.4	10.3	5.9	2.8	19.2	14.7
17:0	0.8	3.2	0.1	1.1	2.4	2.3	2.7	1.1	3.6	3.7	2.9	3.6	4.8	5.4	1.8	
17:1	0.5	1.3					2.2	1.2	2.2	2.1				tr.	2.2	1.7
18:0	3.3	4.6	1.6	2.7	4.6	9.5	10.8	4.3	29.3	24.5	10.9	13.0	41.6	17.5	2.7	2.3
18:1	14.1	19.2	8.8	15.9	22.6	31.4	12.4	5.4	10.4	7.3	10.3	10.7	9.3	4.4	23.2	33.3
18:2			tr.				2.4	1.2						2.1		
19:0	0.3	tr.			0.1	0.1	0.5	0.3	1.3	1.7	0.2	0.1	1.1	0.3	0.1	
19:1	0.3	0.3	0.1		0.2	0.9	1.2	0.5	0.4	0.3	0.9	0.7	tr.	0.1	0.7	0.1
20.0	0.2	0.3	0.4	0.3	0.3	0.2	0.6	0.2	0.4	0.4	0.4	0.2	0.1	0.1	0.2	33.4 [a]
20:1	2.8	1.8	4.1	1.2	3.1	1.6	5.9	1.8	0.9	0.7	12.3	2.2	11.0	4.6	8.2	0.7
20:2							0.7	0.1								0.6
21:0	tr.	tr.											tr.		0.1	
21:1	tr.	tr.	0.2		0.2						0.3	tr.			0.1	0.1
22:0	tr.	tr.											0.4	0.1	0.7	
22:1	0.3	0.2	1.1	0.4	0.3	0.6	2.4	0.3	tr.	tr.	0.8	tr.	0.2	0.2	2.6	0.3
22 poly																0.4

[a] Phytanyl 33.3.

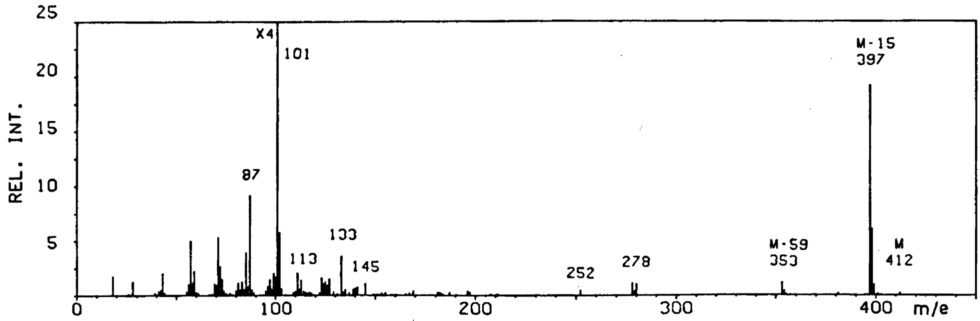

Fig. 1. Mass spectrum of the isopropylidene derivative of phytanylglycerol, isolated from cod liver oil.

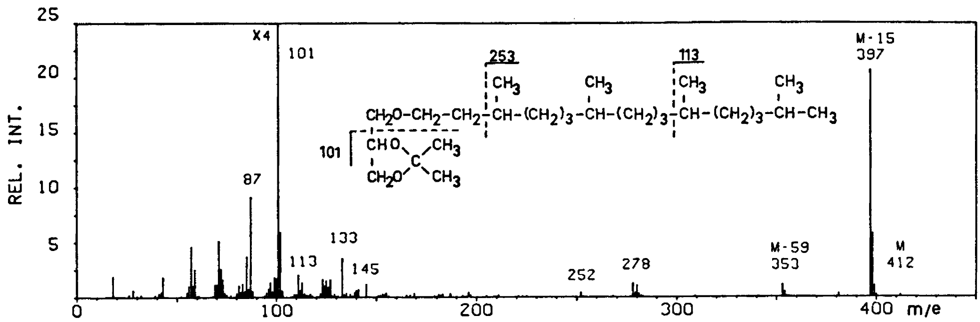

Fig. 2. Mass spectrum of synthetic 1-*O*-phytanyl-2,3-*O*-isopropylideneglycerol.

In the liver oil from cod caught in the Baltic sea a glycerol ether with a phytanyl chain was found. The phytanyl glycerol ether amounted to about a third of the whole glycerol ether mixture. A somewhat higher R_F-value in thin-layer chromatography (TLC) than for batyl alcohol both before and after acetonation indicated that the compound could be a glycerol ether with a branched hydrocarbon chain. This was supported by the IR spectrum of the compound, which showed strong bands at about 3450 (OH), 2960 (CH_3), 1365 – 1375 doublet ($C(CH_3)_2$), and 1100 cm^{-1} (C – O – C).[7] The mass spectrum indicated a molecular weight of 412 for the isopropylidene derivative. For comparison 1-*O*-phytanyl-2,3-*O*-isopropylideneglycerol and 1-*O*-phytanylglycerol were synthesized from dihydrophytol and isopropylideneglycerol in the same manner as earlier described.[8] The IR spectra of the synthesized compounds were identical with those of the compounds from cod liver oil, both as free glycerol ethers and as isopropylidene derivatives. The R_F-values in TLC were also identical. Analyses by GLC – MS of the isopropylidene derivatives gave the same retention times and identical mass spectra (Figs. 1 and 2).

A diphytanyl glycerol ether isolated from *Halobacterium cutirubrum* has been studied in detail by Joo *et al.*[7] They found that the diether had the unusual L-configuration. They also found small amounts of α-monophytanylglycerol ($[\alpha]_D - 0.95°$) which, however, had been formed from the diether during acid hydrolysis.[9] Unfortunately the amount of the phytanyl ether isolated from cod liver oil was not enough for determination of the optical rotation.

The phytanyl chain probably originates from chlorophyll. The composition of the glycerol ethers is thus obviously dependent on the diet of the animals. The glycerol ethers of the fresh-water crayfish are somewhat more saturated than those of the marine crayfish. This is also true for Baltic herring fillets compared to the herring fillets. These differences might be related to different dietary intake or other environmental factors, *e.g.* water temperature.

REFERENCES

1. Hallgren, B. and Ställberg, G. *Acta Chem. Scand. 21* (1967) 1519.
2. Hallgren, B., Niklasson, A., Ställberg, G. and Thorin, H. *Acta Chem. Scand. Ser B 28* (1974) 1029.
3. Boeryd, B., Hallgren, B. and Ställberg, G. *Brit. J. Exp. Pathol. 52* (1971) 221.
4. Boeryd, B., Hallgren, B. and Ställberg, G. *Abstracts, XIth International Cancer Congress*, Florence 1974, p. 139.
5. Hallgren, B., Ställberg, G. and Thorin, H. *Acta Chem. Scand. 25* (1971) 3781.
6. Hallgren, B. and Larsson, S. *J. Lipid Res. 3* (1962) 31.
7. Joo, C. N., Shier, T. and Kates, M. *J. Lipid Res. 9* (1968) 782.
8. Ställberg, G. *Chem. Scr.* (1974). *In press.*
9. Kates, M., Park, C. E., Palameta, B. and Joo, C. N. *Can. J. Biochem. 49* (1971) 275.

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