Comp. Biochem. Physiol. Vol. 85B, No. 1, pp. 131–134, 1986
Printed in Great Britain

0305-0491/86 $3.00 + 0.00
Pergamon Journals Ltd

1-O-ALKYLGLYCEROLIPIDS IN ANTARCTIC KRILL (*EUPHAUSIA SUPERBA* DANA)

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(*Received* 7 *November* 1985)

Abstract—1. Antarctic Krill contains 1-O-alkyllipids as minor components ranging from 0.3 to 0.6% of total lipid content.
2. 1-O-Alkyllipids were present in whole Krill samples and in isolated abdominal muscle as well.
3. In both phospholipids and neutral lipids the presence of 1-O-alkyllipids could be found.
4. A number of 16 individual species of 1-O-alkylglycerols could be separated by GLC and identified using GLC/MS after enzymatic and chemical degradation of total lipids.
5. The predominant alkyl chains found were: 16:0 (40.5–52.7%), 14:0 (10.9–13.0%) and 16:1 (8.3–11.8%, one of two isomers).
6. 1-O-Alk-1′-enyllipids (plasmalogens) and 2-O-alkyllipids were not detected.

INTRODUCTION

Small amounts of alkoxylipids, commonly referred to as glyceryl ethers or ether lipids, are present in the lipids of many marine animals (Mangold, 1979; Horrocks and Sharma, 1982). Though the alkoxylipids of fishes are investigated to some extent there is only limited information available on invertebrates and especially on crustaceans. The occurrence of alkenyl moieties (a metabolic succursor of alkyl groups) was shown by Dembitskii (1976 and 1979) in the phospholipids of marine invertebrates and by Isay *et al.* (1976) in crustaceans from tropical waters and other invertebrates (1984). The amounts found by Isay (1976) varied from 0.5 to 5.9% of total lipids. Clarke (1977) demonstrated that the Antarctic crustacean *Chorismus antarcticus* Pfeffer contained 1-O-alk-1′-enyl-glycerolipids in its polar lipids and also small amounts of 1-O-alkyl-2,3,-O-diacylglycerols. The presence of alkyldiacylglycerols in the Antarctic crustaceans *Serolis cornuta* and *Serolis pagenstecheri* (Isopoda) from 0.32% (males) to 1.48% (females) could be shown by the same author (1984).

Little information is present on the physiological function of 1-O-alkyllipids. Recently Demonopoulos *et al.* (1979) reported 1-O-alkyl-2-O-acetyl-*sn*-glycero-3-phosphocholine to be the platelet-activating factor (PAF) and Berdel *et al.* (1981) reviewed the anti-tumor action of alkyl-lysophospholipids.

While investigating the complete lipid composition of Antarctic Krill (*Euphausia superba* Dana) (Fricke *et al.*, 1984) we failed in finding plasmalogens but there was some evidence for the presence of 1-O-alkylglycerolipids in trace amounts. The samples investigated were frozen on board a research vessel in 1977 and 1981 and could only be investigated after some months of frozen storage. The amounts of free fatty acids found in these samples indicated that some degradation processes had taken place during storage. Therefore it was necessary to confirm and verify our findings. This was done during the 1985 expedition of the Federal Republic of Germany with FRV "Walther Herwig". Lipid extracts of freshly caught Krill were prepared on board.

MATERIALS AND METHODS

Krill samples were collected from the Scotia Sea in December 1977 (A) and from the Gerlache Strait in March 1981 (B) and in March/April 1985 (C–F) during the second (1977/78), the third (1980/81) and the fourth (1984/85) Antarctic expedition of the Federal Republic of Germany with FRV "Walther Herwig" using a 1219 mesh pelagic Krill net. In 1977 and 1981 Krill samples of 5 kg each were quick frozen on board and stored at −35°C until analyzed. Lipid extraction was performed according to Folch *et al.* (1957) after some months of frozen storage. Samples from 1985 were homogenized in a 20-fold excess of dichloromethane:methanol (2:1, v/v) immediately after catching (almost all specimens were alive until this procedure). In addition to the lipid extracts of whole Krill a sample of Krill muscle was prepared (F) on board during the 1985 expedition. This sample was prepared from the Krill muscles of approx. 80 specimens which were carefully removed from the exoskeleton by hand and then treated like the whole Krill samples. These crude extracts were stored at −30°C until further treatment.

Lipid separation and derivatisation

The alkylglycerolipids from the 1977 and 1981 samples were isolated after stepwise hydrolysis of the total lipids according to Pugh *et al.* (1977). Phospholipids and neutral lipids were separated by thin layer chromatography (TLC) on silica gel (E. Merck, Darmstadt, FRG) with diethylether:water (100:1, v/v). After incubation of phospholipids with phospholipase C (from *Bacillus cereus*, Boehringer,

Mannheim, FRG) using the procedure of Blank *et al.* (1975) the alkylglycerols were prepared from the phospholipids and the neutral lipids by concentrated methanolic hydrochloric acid (2 hr, 80°C). The alkylglycerols were isolated by TLC on silica gel using the above mentioned system. The 2,3-O-isopropylidene derivatives of alkylglycerols were produced after Hanahan *et al.* (1963) using acetone/perchloric acid.

The lipid extracts of the 1985 samples were treated according to Snyder *et al.* (1971) with Vitride (sodium-dihydro-bis-(2-methoxyethoxy)-aluminate) to form the free alkylglycerols.

Gas chromatography/mass spectrometry (GLC/MS)

The separation and identificatoin of alkylglycerols was achieved on a 25 m WCOT Silar 10°C (Packard Instruments) column, temperature programmed from 110 to 210°C, 3°C/min, and on a 50 m WCOT SIL 5 CB column (Chrompack), temperature programmed from 220 to 320°C, 3°C/min, on a Packard 428 gas chromatograph equipped with a FID and a HP 3371 integrator. Helium was used as a carrier gas with a flow rate of 1 ml/min, split ratio was 100:1.

GLC/MS of 1-O-alkyl-2,3-O-isopropylideneglycerols was performed with a HP 5985A quadrupole mass spectrometer, ionization energy 70 eV, ion source temperature 200°C, GLC column: 25 m WCOT CP Sil 5 (Chrompack). The identification of individual alkylglycerols was achieved by cochromatography of underivatised alkylglycerols and their isopropylidene derivatives as well using standard substances on a polar and a non-polar column and by the characteristic mass spectra of 1-O-alkyl-2,3-O-isopropylideneglycerols (m/e 101 and $M^+ - 15$). Quantification was attained using 1-O-heptadecylglycerol as internal standard added to the crude lipid extract.

RESULTS AND DISCUSSION

In all Krill samples investigated 1-O-alkylglycerolipids were found as minor lipid components. The amounts ranged from 0.3 to 0.6% of total lipid content of Antarctic Krill (Table 1). No differences were found between the lipid samples caught in different years and seasons. Also the differing treatments of the samples—frozen storage before lipid extraction vs lipid extraction of freshly caught material—showed no influence on the alkylglycerol content. From the qualitative investigation in the phospholipids and neutral lipids in the 1977 and 1981 Krill samples we found that 1-O-alkylglycerolipids were present in both phospholipids and neutral lipids. 2-O-Alkyllipids could not be detected in any sample.

The amount of 1-O-alkylglycerolipids is lower than reported by Isay (1976) for crustaceans from tropical regions and is similar to the content in the Antarctic benthic prawn *Chorismus antarcticus* and the Antarctic isopods *Serolis* in which Clarke (1977 and 1984) reported the presence of small amounts of 1-O-alkylglycerols. In *Chorismus a.* Clarke found also high amounts of 1-O-alk-1′-enyllipids. This was in agreement with the findings of Dembitskii (1976 and 1979) who showed the presence of plasmalogens in a number of marine invertebrates including crustaceans. During our analyses of the 1977 and 1981 Krill samples (Fricke *et al.*, 1984) and during the analyses of the 1985 samples no plasmalogens were found. This discrepancy might be explained by the use of hydrochloric acid fume for reaction thin layer

Table 1. Lipid content and 1-O-alkylglycerol content in total lipids of Antarctic Krill (*Euphausia superba* Dana). Data are expressed as wt % and represent means and standard deviation of at least three separate experiments

	A*	B†	C‡	D‡	E‡	F‡§
Lipid content 5‖	2.7 ± 0.2	6.2 ± 0.3	9.2 ± 0.3	8.3 ± 0.7	10.3 ± 0.4	5.7 ± 0.3
1-O-Alkylglycerol-content	0.43 ± 0.04	0.50 ± 0.02	0.53 ± 0.01	0.65 ± 0.03	0.58 ± 0.03	0.58 ± 0.01

*Krill caught in December 1977.
†Krill caught in March 1981.
‡Krill caught in March/April 1985. Krill samples extracted immediately after catching.
§Krill muscle analyzed.
‖On a wet weight basis.
*†Krill samples stored at −30°C.

Table 2. 1-O-Alkylglycerol composition in total lipids of Antarctic Krill (*Euphausia superba* Dana) samples. Data are expressed as wt % of total 1-O-alkylglycerols and represent means and standard deviation of at least three separate experiments. Numbering corresponds to peak-numbering in Fig. 1. Samples A–F, see explanation in Table 1

			Samples					
No.	$M^+ - 15$*	Alkyl-chain	A	B	C	D	E	F
1	311	14:1	1.5 ± 0.1	0.6 ± 0.1	1.1 ± 0.1	1.6 ± 0.2	1.0 ± 0.1	1.2 ± 0.1
2	313	14:0	12.3 ± 1.8	13.0 ± 1.3	11.4 ± 0.5	12.4 ± 1.0	10.9 ± 0.4	10.9 ± 1.0
3	325	15:1	0.7 ± 0.1	0.2 ± 0.1	3.1 ± 0.2	3.2 ± 0.2	3.2 ± 0.2	2.6 ± 0.1
4	325	15:1	0.9 ± 0.2	1.0 ± 0.7	2.1 ± 0.5	1.0 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
5	327	15:0 br†	0.9 ± 0.2	3.0 ± 0.3	4.4 ± 0.2	1.7 ± 0.2	2.2 ± 0.2	3.4 ± 0.2
6	327	15:0 br	0.7 ± 0.2	1.6 ± 1.0	1.5 ± 0.4	0.7 ± 0.1	0.8 ± 0.1	1.9 ± 0.1
7	327	15:0	2.6 ± 0.2	1.3 ± 0.7	1.5 ± 0.2	2.0 ± 0.3	1.7 ± 0.1	1.4 ± 0.2
8	339	16:1	8.6 ± 0.1	11.8 ± 1.8	8.3 ± 0.1	9.7 ± 0.5	9.1 ± 0.2	8.5 ± 0.2
9	339	16:1	4.0 ± 0.1	2.3 ± 1.4	3.2 ± 0.2	3.5 ± 0.1	3.1 ± 0.1	3.2 ± 0.1
10	341	16:0	44.1 ± 1.8	52.7 ± 6.0	40.5 ± 0.9	43.6 ± 2.0	42.1 ± 0.7	44.1 ± 1.4
11	353	17:1	0.9 ± 0.2	0.6 ± 0.4	0.9 ± 0.1	0.8 ± 0.2	0.6 ± 0.1	1.1 ± 0.2
12	355	17:0	1.1 ± 0.1	0.7 ± 0.4	1.1 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	0.8 ± 0.2
13	365	18:2	4.6 ± 1.7	1.5 ± 0.9	3.3 ± 0.2	3.2 ± 0.2	4.0 ± 0.4	3.3 ± 0.2
14	367	18:1	6.3 ± 0.6	3.0 ± 0.5	4.8 ± 0.3	5.2 ± 0.3	5.5 ± 0.6	5.1 ± 0.2
15	367	18:1	3.1 ± 0.3	4.4 ± 0.6	5.4 ± 0.4	5.4 ± 0.3	5.8 ± 0.7	5.9 ± 0.2
16	369	18:0	4.9 ± 0.5	1.7 ± 1.1	4.6 ± 0.2	4.7 ± 0.1	4.8 ± 0.1	4.8 ± 0.1
	Others		2.8	0.6	2.9	0.5	3.4	0.6

*Mass fragment used for identification of individual 1-O-alkylglycerols (arising from cleavage of methyl group from the isopropylidene derivative).
†br = branched chain.

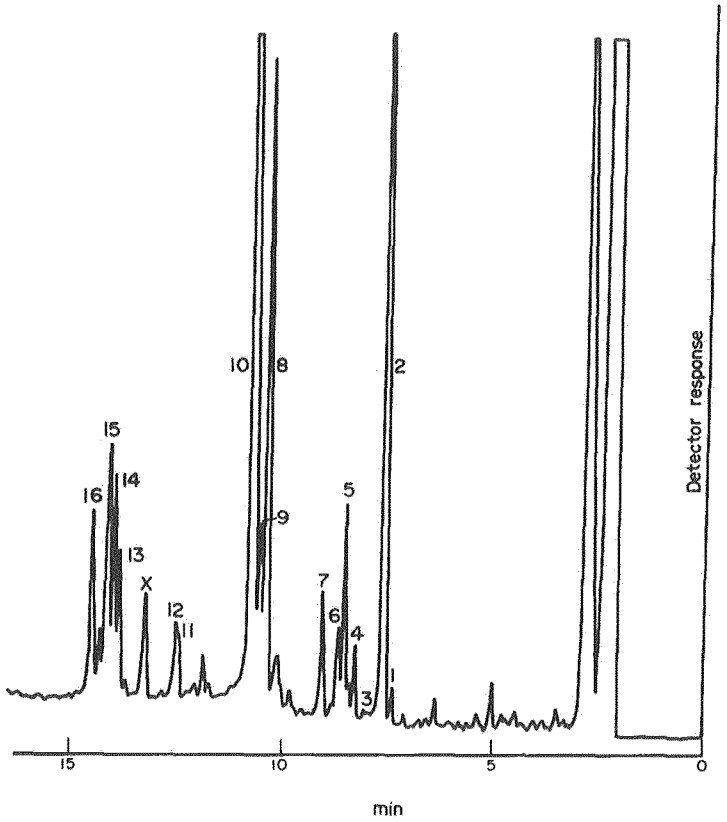

Fig. 1. GLC-separation of 1-O-alkylglycerols in Antarctic Krill (*Euphausia superba* Dana) (50 m WCOT column coated with SIL 5 CB, temperature programmed 220–320°C, 3°C/min). Peaks numbered 1–16 show individual 1-O-alkylglycerols as listed in Table 2. X is contamination by phthalic acid ester.

chromatography by Dembitskii and Clarke in estimating the amounts of plasmalogens. According to Touchstone (1984) this method can produce misleading results.

In Fig. 1 a separation of 1-O-alkylglycerols into individual species is shown. The quantification and mass spectrometrical identification of the components in Fig. 1 are given in Table 2.

In total a number of 16 different 1-O-alkylglycerols were found and identified by GLC/MS. The alkyl moieties 16:0 and 14:0 (with 44.09 and 12.33% of total alkyl chains) were dominant. The alkyl chains 16:1, 18:1 and 15:1 were each present in two isomeric forms. The positions of the double bonds of these chains could not be determined because of the very small amount of these alkyl chains which allowed no further analysis. The same problem arose for the two branched alkyl chains of 15:0. 15:0 and 17:0 are the only saturated and 15:1 and 17:1 the only unsaturated odd numbered alkyl chains. Only one double unsaturated alkyl chain (18:2) was found. Alkyl groups with more than 18 C-atoms or with more than two double bonds were absent.

All alkyl side chains found correspond to the acyl chains present in glycerolipids of Krill (Fricke *et al.*, 1984).

The 1-O-alkylglycerol composition within the different samples from 1977 to 1985 did not differ significantly. From the data in Table 2 it is evident that the frozen storage of samples A and B for several months had no influence on the 1-O-alkylglycerol composition. Furthermore this composition is not influenced by the total lipid content of the Krill samples ranging from 2.7 to 10.3% on a wet wt basis (Table 1). The 1-O-alkylglycerol composition and the 1-O-alkylglycerol content were very similar in the samples from whole Krill (A–E) and in the Krill muscle sample (F). These findings suggest that the 1-O-alkylglycerolipids form an integral component of the Krill and are not only located in the digestive tract where they possibly may be present from undigested diet.

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