Comp. Biochem. Physiol., 1975, *Vol.* 50B, *pp.* 479 *to* 484. *Pergamon Press. Printed in Great Britain*

LIPID COMPOSITION OF TWO SPECIES OF ANTARCTIC KRILL: *EUPHAUSIA SUPERBA* AND *E. CRYSTALLOROPHIAS*

NESTOR R. BOTTINO

Department of Biochemistry and Biophysics, Texas A & M University, College Station, Texas 77843, U.S.A.

(*Received* 21 *February* 1974)

Abstract—1. The lipids of two Antarctic euphausiids were characterized.

2. In *Euphausia superba* complex lipids were the major lipid class followed by triglycerides.

3. In *E. crystallorophias* the complex lipids were also the major lipid class, but the second major constituent was waxes.

4. The complex lipids of both euphausiids consisted mostly of phosphatidylcholine with smaller amounts of phosphatidylethanolamine and lysophosphatidylcholine. The phospholipids of *E. crystallorophias* were less unsaturated than those of *E. superba*.

5. The waxes of *E. crystallorophias* were mostly esters of oleic (84%) and palmitoleic (10%) acids with *n*-tetradecanol (69%) and *n*-hexadecanol (28%).

INTRODUCTION

THE WORD "krill" in Norwegian means "whale food", and it is with this meaning that the term is generally used, involving the various crustaceans (mostly euphausiids, but also amphipods and decapods) which whales eat (Nemoto, 1970). However, when one refers to Antarctic krill, one generally means *Euphausia superba*, which is the most abundant and far better known species of krill in the Antarctic Oceans. *E. superba* is usually found in open, turbulent waters at the confluences of oceanic streams (Ivanov, 1970). But *E. superba* is rarely found in the colder waters in close proximity to the ice where the smaller *E. crystalorophias* seems to predominate (Knox, 1970). Both *E. superba* and *E. crystallorophias* are considered phytoplankton feeders (Mauchline & Fisher, 1969 for *E. superba*; Andriashev, 1968 and Knox, 1970 for *E. crystallorophias*). Both euphausiids are consumed by whales, seals, fish, penguins and petrels (Andriashev, 1968; Knox, 1970) thus occupying a central position in various food chains in open waters and in waters close to or under the ice.

During cruise 51 of the *USNS Eltanin* to the Ross Sea, January–February 1972, I was able to collect specimens of *E. superba* in stations 8, 9 and 11 (Fig. 1) and specimens of *E. crystallorophias* in practically all of the stations located along the Ross Ice Shelf (stations 11–18). Since the lipids of *E. superba* have been studied only superficially and those of *E. crystallorophias* have not been studied at all, I decided to scrutinize them in more detail. The results are the subject of the present report. A preliminary presentation of these results has been made (Bottino, 1973).

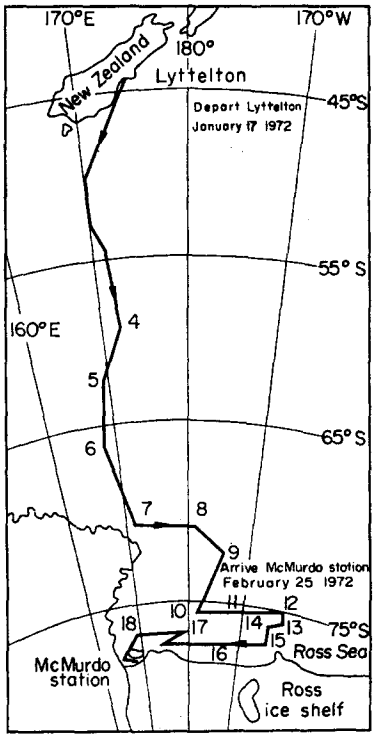

Fig. 1

MATERIALS AND METHODS

Euphausiids were collected with a 1 m mid-water trawl at depths from 0 to 300 m. Once on board the ship the samples were rapidly sorted by hand and extracted with the chloroform–methanol (2 : 1, v/v) mixture of

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Folch *et al.* (1957). Portions of 5–10 mg lipids were transesterified with methanol in the presence of boron trifluoride (American Oil Chemists' Society, 1970). The fatty acid methyl esters thus formed were separated from wax alcohols by thin-layer chromatography (TLC) on silica gel without binder (Adsorbosil-5, Applied Science Co., State College, Pa.) using a mixture of benzene–ethyl acetate (95 : 5, v/v) as developing solvent. Fatty acid compositions were determined by gas–liquid chromatography on a 6 ft × ⅛ in. column of siliconized polyethyleneglycol succinate (DGSS-X, Applied Science) 10% (w/w) on Gas Chrom P 100–120 mesh (Johns-Manville, Denver, Colo.) at 170°C. A dual flame Model GC-5 Beckman gas chromatograph (Beckman Co., Fullerton, Calif.) was used connected to an Infotronic digital integrator (Columbia Scientific Industries, Austin, Texas). Final results were calculated on a Hewlett–Packard Desk Programmable Calculator Model 7810A. Results were expressed as weight per cent. Fatty acids were identified by co-chromatography with known standards and by plotting relative retention times vs. chain length before and after hydrogenation. For quantitative fractionation of lipid classes 20–40 mg of lipids were separated by the method of Freeman & West (1966) modified as follows: (a) Silica gel without binder (Adsorbosil-5, Applied Science) was used instead of silica gel-G. (b) Acetic acid was eliminated from the solvent mixture No. 1 to simplify the drying between developments. (c) Quantitation of the spots was done by gravimetry and not by colorimetry of dichromate reduction. The gravimetric estimation required the use of the following procedure: Once separated by TLC and located with dichlorofluorescein spray, the spots were scraped off the plates, extracted six times with 4 ml (each) of a mixture of chloroform–methanol–acetic acid–water (50 : 39 : 1 : 10, v/v) (Arvidson, 1968). The extracts were filtered through fine pore sintered glass funnels into test-tubes, then 8 ml of 4 M ammonium hydroxide were added and the mixture shaken and centrifuged. The resulting upper phase, which contained the DCF, was discarded and to the lower phase was added 8 ml of 50% (v/v) methanol in water. After the addition of anhydrous sodium sulfate, the liquid was filtered through a fine pore sintered glass funnel containing a layer of anhydrous sodium sulfate. The filtrate was then evaporated under vacuum and the residue was weighed. Using known amounts of total lipids in each determination, it was possible to estimate the recovery. Any result showing less than 80 per cent recovery was discarded and the fractionation repeated. The fatty acids of the lipid classes were converted into their methyl esters and studied by GLC as indicated above. In the case of the wax fraction the methylation procedure using boron trifluoride catalyst was found inefficient and was replaced by a 1-hr reflux with a 2% solution of sulfuric acid in methanol. The alcohol components of the waxes were studied by GLC without any previous treatment. The major component of the alcohol mixture was also isolated by preparative GLC and examined with an LKB-9000 mass spectrometer. The spectrum was identical to that of authentic 1-n-tetradecanol. Other alcohols were identified by their GLC behavior in comparison with known standards and by plotting relative retention times vs. chain length.

Table 1. Fatty acids of Antarctic krill*

	E. superba		*E. crystallorophias*	
Fatty acid	Station 8	Station 11	Station 13	Station 16
	weight %			
12:0	0·3	0·2	0·2	tr
14:0	14·9	14·3	2·3	2·4
15:0 br†	0·3	0·2	0·1	
15:0	0·5	0·2	0·2	0·1
16:0	21·2	24·7	13·8	14·8
18:0	0·7	1·4	1·2	1·3
22:0	0·1	0·1		
14:1 (*n*−?)	0·3	0·2		
15:1 (*n*−?)	tr	0·1		
16:1 (*n*−7)	9·0	8·9	8·4	10·8
17:1 (*n*−8)	0·7	0·3	0·4	0·4
18:1 (*n*−9)	18·2	21·7	47·5	45·2
20:1 (*n*−9)	0·6	0·9	0·2	0·5
18:2 (*n*−6)	0·3	0·1		
18:2 (*n*−3)	2·6	2·0	3·3	2·7
20:2 (*n*−3)			0·1	
18:3 (*n*−6)	0·3	0·2	0·2	0·3
18:3 (*n*−3)	1·1	1·0	0·9	0·9
20:3 (*n*−3)	0·6	0·5	0·5	
18:4 (*n*−3)	2·2	3·3	1·5	0·9
20:4 (*n*−6)	0·5	0·4		0·7
20:4 (*n*−3)	0·5	0·2		0·1
22:4 (*n*−6)	0·2	0·3		
20:5 (*n*−3)	16·0	11·4	11·8	13·4
22:5 (*n*−3)	0·3	0·1	0·1	
22:6 (*n*−3)	8·6	7·3	7·3	5·5

RESULTS AND DISCUSSION

Table 1 shows the fatty acid compositions of the unfractionated lipids of *E. superba* and *E. crystallorophias*. The fatty acid patterns of the samples of *E. superba* were quite similar to each other and to that previously reported for another sample of the same species. In fact, differences among samples were only slightly higher than those found between duplicate analyses of the same sample (Bottino, 1974). Similarly, the fatty acid patterns of six samples of *E. crystallorophias* differed very little from each other (Bottino, 1974). Table 1 shows one example of this. However, there are noticeable quantitative differences between the fatty acid compositions of the two species. For example, the level of oleic acid is about twice as high in *E. crystallorophias* as in *E. superba*, while the reverse is true for the content of the saturated acids.

Previous studies from this laboratory (Bottino, unpublished) have demonstrated that complex lipids constitute 50 per cent or more of the lipids of most Antarctic plants and animals other than mammals. The data in Table 2 show that complex lipids are also the major lipid class in the two

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Table 2. The lipids of Antarctic krill

	E. superba		*E. crystallorophias*	
	Station 8 (1)*	Station 11 (2)	Station 13 (4)	Station 16 (2)
			weight %	
Waxes	—	—	44±10‡	20±1
Steroid esters	—	—	2±3	27±9
Triglycerides	8	36±6	—	—
Diglycerides	17	4±5	—	4±1
Complex lipids	54	58±14	53±8	42±8
PC†		48	46	
PE†		8	6	
Lyso PC		1	1	
PG†		1	—	
Unknown§	21	2±22	1±2	7±1

* Number of determinations in parentheses.
† PC, Phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.
‡ Weight per cent plus or minus the standard deviation.
§ R_f between those of triglycerides and diglycerides. The recovered amount of this fraction was too small for further characterization.

Table 3. Composition of the waxes of *E. crystallorophias*

	Fatty acids			Wax alcohols	
	Station 13	Station 16		Station 13	Station 16
	Waxes	Waxes	Steroid esters		
			weight %		
8:0	0·3		1·4		
10:0	0·1	0·1	0·6		
12:0		tr	0·4		0·1
13:0		tr	0·1	tr	tr
14:0		0·5	8·3	72·5	69·2
15:0 br	0·5				
15:0		0·1	0·3		
16:0	0·8	0·6	14·0	25·3	28·0
18:0	0·3	0·2	2·4	2·2*	2·2*
20:0					0·5
22:0					
11:1 (*n*−?)			0·1		
12:1 (*n*−?)			0·3	tr	tr
13:1 (*n*−?)	0·6		0·8		
14:1 (*n*−?)			0·3		
15:1 (*n*−?)	0·1	1·3	11·0		
16:1 (*n*−7)	8·8	11·2	14·0		
17:1 (*n*−8)	0·3	0·3	1·1		
18:1 (*n*−9)	84·1	83·1	22·1		
20:1 (*n*−9)	0·6	0·4			
18:2 (*n*−3)	3·1	2·2	1·9		
18:3 (*n*−3)	0·4		0·9		
20:3 (*n*−3)			0·3		
18:4 (*n*−3)			1·5		
20:5 (*n*−3)			16·5		
22:6 (*n*−3)			1·4		
Unknown			0·3		

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Table 4. Fatty acids of the complex lipids of Antarctic krill

	E. superba—Station 11				*E. crystallorophias*—Station 13			
	CL*	PC	PE	Lyso PC	CL	PC	PE	Lyso PC
				Weight %				
9:0	tr	0·1	0·2		0·1	tr	0·1	0·6
10:0		0·3	0·5	1·8		tr	1·4	2·7
11:0		0·1	0·2				0·1	0·6
12:0		tr	0·2	1·3			1·4	1·7
13:0	tr	0·1	0·2	4·2	tr	tr	1·6	6·2
14:0	3·0	3·4	2·6	8·3	2·9	4·2	4·6	11·1
15:0	0·7	1·1	1·5	5·9	0·9	1·0	1·2	5·2
16:0 br				1·6				
16:0	25·1	25·9	18·8	32·6	29·1	29·8	26·2	37·8
17:0				1·4			tr	
18:0	1·2	1·1	1·7	4·1	1·0	1·0	2·9	1·9
20:0			0·2			0·4		1·8
22:0	0·3	0·2		0·1	tr	0·1		2·0
11:1 (*n*−?)		0·1	0·1	2·3				3·9
12:1 (*n*−?)	0·1				0·1			2·2
13:1 (*n*−?)		tr	0·1	2·8			0·2	2·5
14:1 (*n*−?)	tr		tr	1·6				tr
15:1 (*n*−?)			0·1					
16:1 (*n*−7)	2·9	2·8	1·3	4·2	5·3	6·9	2·0	2·4
17:1 (*n*−8)	0·1	0·1		tr	0·2	0·2		
18:1 (*n*−9)	14·2	13·2	21·8	12·3	21·2	21·0	31·0	13·0
20:1 (*n*−9)	0·8	0·9	0·3	0·2		0·1		tr
22:1 (*n*−9)								0·5
18:2 (*n*−3)	2·3	2·4	1·4	1·1	2·1	2·5	1·3	0·7
21:2 (*n*−6)				0·1				
18:3 (*n*−6)	0·2	0·4			0·1			
18:3 (*n*−3)	1·6	1·8	0·8	0·2	1·2	1·5	0·3	0·4
20:3 (*n*−6)	0·1	0·1	0·1					
20:3 (*n*−3)	1·0	0·7	1·2	tr	1·4	0·3	0·3	
21:3 (*n*−3)	tr	3·8	4·5	1·6	1·1	8·4		
22:3 (*n*−3)	0·5	0·5	0·1		3·6			
18:4 (*n*−3)	3·8	4·7	0·6	1·2	1·7	2·3		tr
20:4 (*n*−6)	1·5	1·5	0·2	1·9	0·1	0·3		0·2
20:4 (*n*−3)		0·4	0·2	0·2				0·2
20:5 (*n*−3)	25·3	22·5	20·3	6·5	19·3	14·8	15·1	2·4
22:5 (*n*−3)	0·1							
22:6 (*n*−3)	15·2	11·8	20·8	2·5	8·6	5·2	10·3	tr
Saturated	30·3	32·3	26·1	61·3	34·0	36·5	39·5	71·6
Monounsaturated	18·1	17·1	23·7	23·4	26·8	28·2	33·2	24·5
Polyunsaturated	51·6	50·6	50·2	15·3	39·2	35·3	27·3	3·9

* CL, Complex lipids.
PC, phosphatidylcholine; PE, phosphatidylethanolamine.

the major complex lipid was phosphatidylcholine (PC), 49 per cent in *E. superba* and 46 per cent in *E. crystallorophias*. Phosphatidylethanolamine (PE) and lysophosphatidylcholine were also found in both euphausiids but in much lower concentrations. Unexpected large amounts of waxes were found in *E. crystallorophias* while none was found in *E.* were steroid esters.* The waxes of each sample of *E. crystallorophias* were isolated by TLC and the fatty acids and alcohols were examined independently by GLC. Both samples had very similar compositions, as shown by the data in Table 3. Two

* For the sake of simplicity in the following discussion

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monoenoic acids, oleic and palmitoleic, constituted about 94 per cent of the fatty acids of these waxes. Of the four alcohol components, about 70 per cent was tetradecanol and 28 per cent was hexadecanol. Thus the waxes of *E. crystallorophias* are less unsaturated than the copepod waxes examined by Lee *et al.* (1971a, b) and by Benson *et al.* (1972). The waxes of the present study resemble many other waxes of marine animals in their high content of oleic acid (Nevenzel, 1970) but differ from the rest in having tetradecanol as their major alcohol component. In most other marine waxes so far investigated, hexadecanol is the predominant alcohol. The fatty acids of the steroid esters of *E. crystallorophias* (Table 3) contain polyunsaturated fatty acids while those of the aliphatic waxes do not. Table 4 shows the fatty acids of the major phospholipids of *E. superba* and *E. crystallorophias*. About half of the fatty acids in *E. superba* PC and PE were polyunsaturated, mostly 20:5 and 22:6. Only about one-third of the fatty acids of the PC and PE of *E. crystallorophias* were polyunsaturated. Again, 20:5 and 22:6 were the predominant polyunsaturated acids.

With few exceptions, the experimental evidence indicates that the lipids of aquatic animals living at lower temperatures tend to be more polyunsaturated than those of aquatic animals living at higher temperatures (Johnson & Roots, 1964; Knipprath & Mead, 1966; Kemp & Smith, 1970). In the case of the lipids of *E. superba* and *E. crystallorophias*, a peculiar situation exists. Although it is true that the total lipids of *E. crystallorophias*, which lives in colder waters, are more unsaturated than those of *E. superba*, it is due to a higher level of monounsaturated acids. In fact, the phospholipids of *E. crystallorophias* are less unsaturated than those of *E. superba* (Table 4).

It is possible that the monoenoic waxes of *E. crystallorophias* may have a role in the adaptation of these animals to their extremely cold environment. Benson *et al.* (1972) have found that cold water copepods accumulate waxes. According to their views, the energy stored as waxes may be liberated slowly during periods in which food is scarce or not available. These terms certainly apply to *E. crystallorophias* since this euphausid not only lives in the cold waters near the ice but also under the ice, almost all year around.

SUMMARY

The lipids of specimens of *E. superba* and *E. crystallorophias* caught in various locations of the Ross Sea, Antarctica, were studied. The fatty acid composition of *E. superba* differed quantitatively from that of *E. crystallorophias*, the former consisting mostly of phosphatidylcholine with smaller amounts of phosphatidylethanolamine and lysophosphatidylcholine. The two species differ, however, in their neutral lipids, those of *E. superba* being triglycerides and diglycerides, those of *E. crystallorophias* being waxes, including steroid esters. The alcohols of the waxes were identified by mass spectrometry and gas–liquid chromatography, before and after hydrogenation, and found to be 69% n-tetradecanol and 28% n-hexadecanol. Wax fatty acids were about 84% 18:1 ($n-9$) and 10% 16:1 ($n-7$). The high levels of waxes in *E. crystallorophias* may be related to their year-round very cold environment.

Acknowledgements—I wish to thank Dr. Karl Dahm for the mass spectrographic analyses and Mrs. Claudia Wiltrout for her technical assistance. These studies were supported by a grant from the National Science Foundation (GV-30413).

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