PROC. N.S. INST. SCI. (1979)
Volume 29, pp. 501-516

LIPIDS AND FATTY ACIDS OF COROPHIUM VOLUTATOR FROM MINAS BASIN*

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Corophium volutator from Minas Basin, Bay of Fundy, had about 1.2% lipid made up of 55.3% phospholipid, 31.4% triglyceride, and minor amounts of other lipids. This lipid composition is similar to that found in other estuarine and shoreline crustaceans not utilizing storage lipids during part of their annual cycle. The fatty acids of the phospholipids and triglycerides were those characteristic of marine lipids. Some interrelationships among monoethylenic fatty acids and among longer-chain polyunsaturated fatty acids of these 2 lipid classes are discussed. The more interesting aspects of these fatty acids were, however, found in a minor lipid class, thought to be sterol ester, for which comparative data are lacking.

Introduction

The burrowing amphipod *Corophium volutator* (Pallas), a common intertidal animal in Europe, is apparently limited in distribution in North America to the Bay of Fundy and the coast of Maine (Gratto 1978). In Minas Basin this animal is one of the most abundant invertebrate species, and it is the dominant inhabitant of fine sediments (Gratto 1978). Densities exceeding 20,000 individuals per m^2 are not uncommon (Boates 1978), and 73,000 per m^2 have been reported (Gratto 1978). This species is present the year round with maximum numbers in early summer following reproduction.

C. volutator is reported to be the most important food for shore birds in the area, and also forms part of the diet for groundfeeding fish. The mean calorific value of May samples, corrected for ash, was 5.01 cal per mg (Boates 1978). The lipid content of *C. volutator* is of the order of 1 to 1.5% (wet weight), and only about one-third is triglyceride. The energy contribution to shore birds must, therefore, rest on quantity of *C. volutator* available rather than on a high content of lipid.

Because of the obvious importance of *C. volutator* in the food web of Minas Basin, we undertook an examination of the lipids and fatty acids of this species, and the results are reported herein.

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Materials and Methods

C. volutator were collected in early December 1978, and again in early February 1979, near Starr's Point, Minas Basin. Three analyses (lots I-III) were conducted on the December sample and one analysis (lot IV) on the February sample. Lot I (8 individuals, 0.13 g) was placed in a centrifuge tube (10 ml, Teflon-lined screw cap) with distilled water (0.3 ml), MeOH (1.0 ml), and 50% NaOH (0.2 ml). The tube was flushed with nitrogen, capped, and heated for 1 h at 100°. After cooling, water (1.5 ml) was added, and the aqueous alcohol phase was extracted twice in situ with petroleum ether (1.5 ml per time). The petroleum ether extract was examined for sterols. The aqueous phase was acidified with 12N HC1 and the fatty acids were recovered into petroleum ether (2 extractions as described above) and transferred to a screw-capped tube. The petroleum ether was removed with a jet of nitrogen and MeOH (1 ml) and 14% BF_3-MeOH (1 ml) were added. The tube was flushed with nitrogen, heated for 1 h at 100°, and cooled. Water (2 ml) was added and the methyl esters recovered by 2 extractions with petroleum ether (1.5 ml per time). The combined petroleum ether solutions were washed once with H_2O (1 ml) and concentrated for GLC (gas-liquid chromatography) analysis of the methyl esters (Ackman 1972). About half the methyl ester sample was dissolved in EtOH and hydrogenated over Pt catalyst (Ackman & Burgher 1964) for quantitative (Ackman et al. 1967) and qualitative examination of minor branched chain fatty acids (Ackman & Sipos 1965; Ackman et al. 1967).

Lot II of *C. volutator* (0.61 g) was extracted in a screw-capped centrifuge tube with $CHCl_3$ (2 ml) and MeOH (1 ml), with intermittent agitation over a period of 3 h. Water (2 ml) was added, the separated $CHCl_3$ layer transferred to another tube, and the residual material re-extracted with additional $CHCl_3$ (1 ml) and MeOH (0.5 ml) for one-half hour. The $CHCl_3$ extracts were combined and centrifuged. The clear chloroform solution was removed and freed of solvent, first by nitrogen jet and then by exposure to high vacuum. The lipid recovered (1.3%) was used for lipid class analyses (Table I) by Iatroscan (see below).

A third lot (frozen en bloc, 2.8 g) was blended for 1 min in a Sorvall Omni-Mixer (50 ml cup) with $CHCl_3$ (20 ml) and MeOH (30 ml). The blend was filtered, the filter washed with $CHCl_3$ (10 ml), water (20 ml) was added to the filtrate, and after agitation the $CHCl_3$ layer was separated and evaporated to yield lipid at 0.7% of the weight of sample. This lipid was dissolved in $CHCl_3$ and streaked on a TLC (thin-layer chromatography) plate (Adsorbosil-5 Prekote, Applied Science Laboratories, pre-developed with ethyl acetate) and developed in petroleum ether:diethyl ether:acetic acid (85:15:1). Lipids (5 bands) were detected by spraying with 2, 7-dichlorofluorescein and viewing under UV light. The band at the origin was considered to be phospholipid with small amounts of pigments, etc. Above this was a definite band located opposite a standard cholesterol spot. A diffuse free fatty acid band was also identified by a standard. Above the strong triglyceride band (R_f 0.6) was an obvious but broad band (R_f 0.8), initially thought to be either or both of wax esters or sterol ester. Lipid bands were recovered from the silica gel with $CHCl_3$ (3 ml) followed with 3 ml of $CHCl_3$:MeOH (1:1). The pooled solvents were centrifuged lightly and transferred to a clean tube before evaporation. The fatty acids of the phospholipids and triglycerides were converted to methyl esters by heating with BF_3-MeOH (see above). The band of greatest R_f was saponified (see above) and the unsaponifiables were recovered separately from the fatty acids. The latter were converted to methyl esters (see below).

For lipid class analyses, Chromarods (Iatron Laboratories Inc., Tokyo, Japan) were spotted and developed for 20 min in petroleum ether:benzene:formic acid (80:20:1),

Table I. Lipid classes of *Corophium volutator* (w/w%) as determined by Iatroscan analysis of lot II material

	w/w%	Chromarod R_f
Phospholipid	55.3	0.00
Sterol	3.0	0.16
Triglyceride	31.4	0.35
Glyceryl ether?	4.9	0.48
Free fatty acid	5.0	0.58
Sterol ester, hydrocarbon*	0.4	0.92

*Combined in band of R_f 0.8 on TLC plate in proportions of 1:4.

dried briefly, and then redeveloped in petroleum ether:diethyl ether:formic acid (96:4:1) for 20 min before drying and analysis by Iatroscan (Sipos & Ackman 1978). The results are given in Table 1.

The total unsaponifiable material of lot I was examined for sterols by a gas-liquid chromatographic procedure (Kovacs et al. 1979). The unsaponifiable material from the most mobile band on TLC of lot III (see above) was recovered and examined by GLC, initially under conditions suitable for C_{16}-C_{20} hydrocarbons and later under conditions similar to those for sterols.

The respective methyl esters of fatty acids from triglycerides and phospholipids, after hydrogenation for quantitation studies, were also subjected to urea complex treatment (Ackman & Hooper 1968) to confirm the methyl-branched and isoprenoid structures.

Live animals of Lot IV (0.486 g), freed of water on filter paper, were extracted with $CHCl_3$-MeOH as described above for lot III. The lipid recovery was 1.72%. The broad top lipid band from a TLC plate was recovered for Iatroscan Chromarod examination and the triglyceride band was recovered, the lipid saponified, and the nonsaponifiable matter removed. The fatty acids were recovered and converted to methyl esters by heating for 1 h with 7% BCl_3 in MeOH (Brian et al. 1972). The analyses of these esters, hydrogenation, and urea complex treatment followed the procedures outlined above.

Results and Discussion

Lipids

The inclusion of water and inorganic particles evidently resulted in low lipid recovery from lot III, but lot IV (recovery 1.7%) and lot II (recovery 1.3%) may be taken as reasonable values for *C. volutator* collected in winter. The proportions of non-polar (phospholipid) material in the lipids of these animals was thus 0.7-0.9% (Table I). Note that in Figure 1 the phospholipid signal is attenuated by a factor of 10.

The lipids of many small pelagic crustacea have been investigated (Sargent et al. 1977; Lee & Hirota 1973) with particular reference to storage (depot) fats in the form of wax esters and triglycerides. Relatively little is known about isopods, etc. which

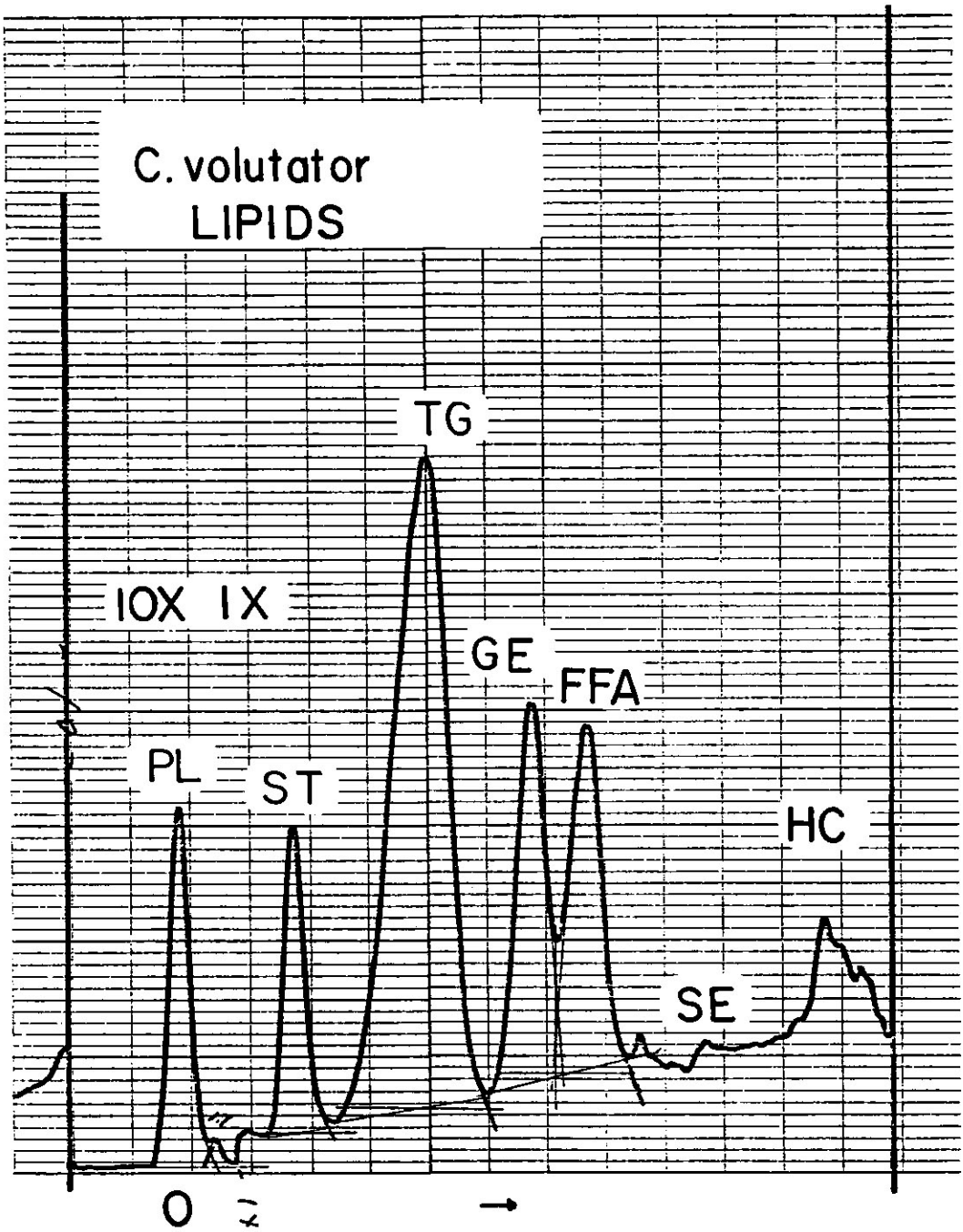

Fig 1. Recording of Chromarod separation of *C. volutator* lot III total lipids. Double development with first solvent of petroleum ether:benzene:formic acid in volume proportions 80:20:1 and second solvent of petroleum ether:diethyl ether:formic acid in volume proportions at 96:4:1. Note attenuations (X). Abbreviations are: PL = phospholipids, ST = sterols, TG = triglycerides, GE = glyceryl ethers, FFA = free fatty acids, HC = hydrocarbons, SE = sterol esters, 0 = origin. Arrow shows direction at development.

are found along the shoreline (e.g., Gopalakrishnan et al. 1977; Strong & Daborn 1978), but it seems probable that they are biochemically active for most of the year and therefore that lipid turnover (Morris et al. 1973; O'Rourke & Monroe 1976) would occur in winter. In Nova Scotia, the mud-dwelling amphipod *Pontoporeia femorata* had a total of 3.0% (wet weight basis) lipid, including 71.6% triglycerides and 15.9% polar (phospho-) lipid. However, this was an unusual case of lipid development (Paradis & Ackman 1976a; 1976b). In the same study the mysid, *Mysis stenolepsis*,was found to have 1.5% lipid with 38.9% as triglyceride and 37.2% as polar lipid.

Total lipid is quite variable in whole crustacea because of the importance of storage lipids (i.e., wax ester referred to above) in some pelagic species (Takahashi & Yamada 1976). Thus if the total lipid exceeds 1 to 2%, the proportion of phospholipid in total lipid will be depressed. The significance of this is that phospholipid is a functional lipid characteristic of membranes in muscle cells, gills, etc. (Sargent 1976; Chapelle 1978), and tends to be a reasonably high and constant proportion (0.5-1.5%) of the whole animal when muscle, gill, and other organs are a high proportion of sample (Zama et al. 1976; Martin & Ceccaldi 1977). A low level (1-2% wet weight) of total lipid seems to be typical of crustaceans with a fairly invariant habitat and thus not exposed to varying food intake and a need to accumulate substantial levels of depot fat to offset periods of poor food availability (Shul'men & Shchepkin 1974; Lawrence 1976). Under unusually favorable circumstances, high percentages of lipids (21-33% of dry weight) were reported for *Crangon crangon, Gammarus salinus, Neomysis integer, Praunus flexuosus,* and *Orchestia gammarella,* although further work showed that half these percentages might be more normal (Moore 1976). Phospholipid was relatively constant for each species, amounting to 24 to 33% of the lipid. The total percentage of lipid and polar-lipid class proportions of *C. volutator* (Table I) is thus not exceptional for estuarine crustaceans. The total sterols recovered from lot I animals were identified and measured as cholesterol, 85%; 22-dehydrocholesterol, 8%; 24-methylenecholesterol, 3%; and desmosterol, 4%. Cholesterol is usually the dominant sterol in crustaceans, but they lack the ability to biosynthesize cholesterol (O'Rourke & Monroe 1976) and other sterols may be stored as precursors or as substitutes.

The total lipid upper band (R_f 0.8 on TLC plates) had been presumed initially to be all sterol ester until a low recovery of fatty acids from this band in the lot II analysis was noted. This suggested the need for additional work (see lot IV treatment). On reexamination no obvious fatty alcohol component was noted and the fatty acids (Table II) do not provide evidence for any special type of lipid, although generally exogenous fatty acids are strongly represented. The GLC analysis of the intact lipid of R_f 0.8 conducted at high temperature showed a homologous series of unidentified compounds eluting somewhat ahead of cholesterol on the liquid phase OV-17. These were later tentatively identified as hydrocarbons by Iatroscan (Fig 2). This band is thought to be a mixture of sterol esters and longer chain hydrocarbons in relative proportions of 1:4. No other lipid class could be identified, but no special search was made for some possible components such as wax esters based on phytol (Withers & Nevenzel 1977). The presumed homologous series of hydrocarbons could be derived locally from plants (Nishimoto 1974), or even be included in the cuticle lipids of the *C. volutator* itself (cf. Hamilton et al. 1975; 1976; Lee 1975). The occurrence of hydrocarbons up to C_{30}, and of ketones to C_{24}, in pelagic crustaceae such as *Acanthomysis* sp has been thoroughly investigated (Yamada 1972) The origin of hydrocarbons in biological samples is extensively discussed in the literature. However, at the moment it is not possible to exclude the occurrence of these hydrocarbons as artifacts or contaminants (Whittle et al. 1974; Mironov & Shchekaturina 1976; Zsolnay 1977).

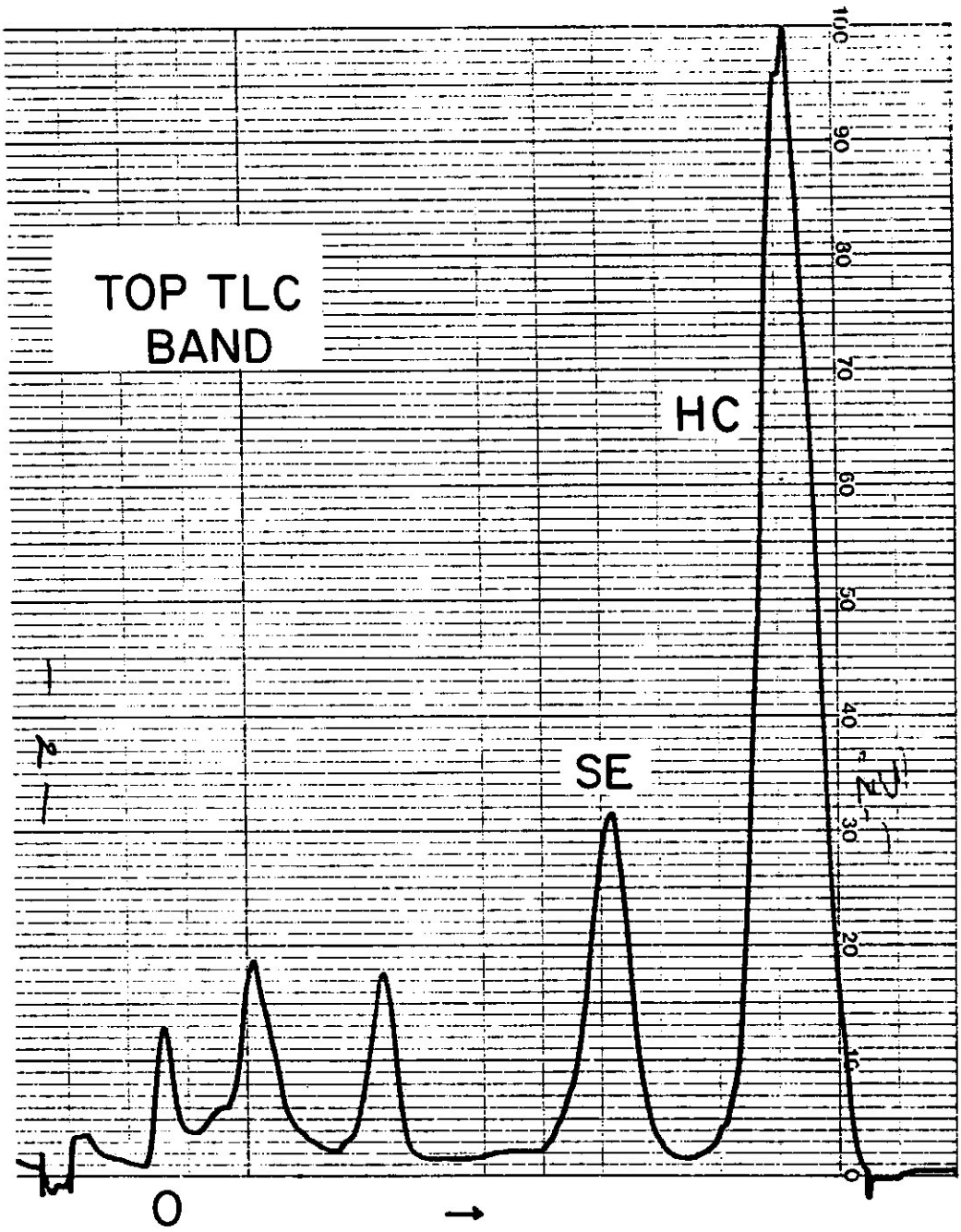

Fig 2. Recording of Chromarod separation of upper band recovered from TLC plate (see text) separation of *C. volutator* lot IV total lipids. Double development with first solvent of petroleum ether:benzene:formic acid in volume proportions of 86:14:1 and second solvent of petroleum ether:diethyl ether:formic acid in volume proportions of 97:3:1. Abbreviations are: SE = sterol esters, HC = hydrocarbons.

Fatty Acids

The proportions of some of the important saturated fatty acids in the TG (triglycerides) and PL (phospholipid) fractions show minor differences; the former has more 14:0 (and 12:0), the latter more 18:0 and also more 20:0, 22:0, and 24:0. In the respective lipids of the zooplankton *Meganyctiphanes norvegia* and *Thysanoessa inermis,* there was also much more 14:0 in the TG than in the PL (Ackman et al. 1970), but in other estuarine crustaceans there seemed to be little difference in 14:0 content of these lipid fractions (Morris et al. 1973; Moore 1976). The longer chain saturated acids, 20:0, 22:0, and 24:0, are seldom important in crustaceans (Morris 1973) except in surface lipids (Hamilton et al. 1976). The iso, anteiso, and straight-chain, odd-carbon fatty acids are also not commonly reported in the literature, but are present in proportions similar to those recorded for the above mentioned zooplankters. Except under special circumstances (Paradis & Ackman 1976a), the C_{15} and C_{17} acids, including branched chain acids, total about 1% each in lipids of almost all marine organisms (Ackman & Sipos 1965), the exceptions being the photoplankters (Ackman et al. 1968) and the seaweeds (Ackman & McLachlan 1977). There is every reason to assume that such fatty acids are normally biosynthesized in marine animals, but they also could possibly be contributed by endogenous bacterial lipids, and also by those living on detritrus etc. (Cranwell 1976). It seems likely that the more obvious presence of the isoprenoid acids in the phospholipids is related to structural similarities enabling these acids to mimic the longer chain saturated acids, especially 16:0 (palmitic acid). The upper-band fatty acids, probably from sterol esters, are relatively deficient in saturated fatty acids, but percentages of iso and anteiso odd-chain (C_{15}, C_{17}) acids seem to be higher than in the other lipid groups. Among the unknowns of Table II were a few minor components visible in both the raw and hydrogenated esters. In the phospholipid sample these clearly included aldehyde and/or acetal breakdown products (Ackman 1972) emerging very slightly after iso 14:0 and iso 16:0, indicating the original presence of plasmalogen lipids. Certain other minor components were the subject of further study (see below).

Two aspects of marine monoethylenic fatty acids are of special interest. In general a high proportion of 18:1ω7 to 18:1ω9 reflects de novo biosynthesis of 16:0 and of the associated 16:1ω7 which is then chain-extended to 18:1ω7. In the TG of lot III *C. volutator,* this is evidently taking place since 18:1ω7 at 3.35% exceeds 18:1ω9 at 2.98%. The TG of lot IV (Fig 3) confirm that 18:1ω7 is very important to *C. volutator,* and it can be concluded that the diet does not include a high proportion of preformed fatty acids, and that dietary carbon compounds are broken down and resynthesized into monoethylenic fatty acids as required. The second interesting aspect of monoethylenic fatty acid biochemistry is based on the recent conclusion that 20:1 and 22:1 fatty acids have no obligatory role in aquatic lipids except in the form of the corresponding fatty alcohols of copepod wax esters (Ackman et al. 1979; Ratnayake & Ackman 1979a; 1979b). Since exogenous 22:1ω11 of copepod origin is not freely available to *C. volutator,* only traces of the chain shortening products 18:1ω11 and 20:1ω11 are observed (Table II). In the lot IV TG (details not tabulated) 22:1ω11 was two-thirds of 22:1ω9 and accordingly 20:1ω11 was a higher proportion of the 20:1 isomers than in the TG of lot III.

An unusual component fatty acid from the upper band was tentatively identified from GLC retention data as a monounsaturated iso acid, since it disappeared on hydrogenation at the same time as iso 15:0 increased in proportion. A similar acid was noted in a Nova Scotian seaweed (Ackman unpubl.), and probably these acids may be more common than hitherto suspected (Fukushima et al. 1978). Iso acids in

Table II. Fatty acids (w/w%) recovered from whole *Corophium volutator* (lot I) and from individual lipid classes (lot III)

Fatty acids	Saponification	Extracted lipids		
Saturated acids	Total fatty acids	Triglyceride fatty acids	Phospholipid fatty acids	Upper band fatty acids
12:0	0.02	0.19	0.01	0.05
Iso 14:0	0.21	0.03	ND	0.03
14:0	2.38	3.12	1.01	2.28
Iso 15:0	0.18	0.28	0.05	0.32
Anteiso 15:0	0.07	0.17	0.02	0.23
15:0	0.54	0.61	0.25	0.65
Iso 16:0	0.08	0.11	0.06	0.13
16:0	18.25	16.24	18.40	9.07
Iso 17:0	0.51	0.39	0.65	1.17
Anteiso 17:0	0.14	0.10	0.28	0.82
17:0	0.76	0.43	0.72	0.28
Iso 18:0	0.05	0.02	0.09	0.03
18:0	1.73	0.67	2.14	1.25
19:0	0.05	0.03	0.10	0.03
20:0	0.08	0.07	0.26	ND
22:0	0.07	ND	0.42	ND
24:0	0.14	0.02	0.17	ND
4,8,12-TMTD	0.07	0.04	0.14	0.01
2,6,10,14-TDPD	0.14	0.02	ND	ND
3,7,11,15-TMHD	0.44	0.08	0.68	0.83
7-M-16:0	0.08	0.05	0.08	ND
Unknowns, total	1.56	0.51	5.21	2.22
TOTAL	27.1	23.2	29.5	17.4
Monoethylenic acids				
14:1ω9	0.03	0.02	0.01	0.51
14:1ω7	0.12	0.02	0.02	0.13
14:1ω5	0.06	0.10	0.01	0.11
16:1ω11	0.05	0.03	0.01	0.08
16:1ω9	0.08	0.20	0.01	4.95
16:1ω7	5.05	15.01	3.33	10.45
16:1ω5	0.06	0.16	0.03	0.10
17:1ω8	0.16	0.05	0.12	0.08
18:1ω13	0.05	0.05	0.01	0.07
18:1ω11	0.03	0.04	0.01	0.32
18:1ω9	8.54	2.98	9.18	10.94
18:1ω7	5.84	3.35	5.72	5.43
18:1ω5	0.10	0.08	0.10	0.28
19:1	0.26	0.27	0.29	0.31

20:1ω11	0.03	0.03	0.05	0.04
20:1ω9	1.36	0.32	2.02	0.84
20:1ω7	1.09	0.32	1.02	0.46
22:1ω11	ND	ND	0.04	ND
22:1ω9	0.08	0.03	0.38	0.08
22:1ω7	0.05	ND	0.32	ND
24:1ω9	0.02	0.01	0.14	ND
trans 16:1ω13?	0.05	ND	0.03	ND
16:1ω3?	0.11	0.03	0.01	ND
Iso 15:1?	0.02	ND	ND	0.25
TOTAL	27.1	23.1	23.9	36.2
Polyethylenic acids				
16:2ω6	0.49	0.80	0.02	0.18
16:2ω4	1.11	1.46	0.08	2.51
18:2ω7	0.05	0.02	0.02	0.06
18:2ω6	1.10	0.75	0.96	2.17
18:2ω4	0.50	0.55	0.31	0.43
20:2ω6	0.74	0.21	0.47	0.39
16:3ω4	2.91	3.98	0.34	2.82
18:3ω6	0.28	0.59	0.10	0.47
18:3ω4	0.03	0.03	0.01	0.08
18:3ω3	0.28	0.53	0.12	0.47
20:3ω6	0.07	0.06	0.19	0.30
20:3ω3	0.05	0.05	0.12	0.13
16:4ω3?	0.01	0.02	0.01	0.06
16:4ω1	1.65	1.70	0.25	0.93
18:4ω3	2.62	4.28	0.51	2.57
18:4ω1	0.04	0.08	0.02	0.17
20:4ω6	3.01	0.95	4.72	3.47
20:4ω3	0.14	0.27	0.10	0.36
22:4ω6	0.05	ND	0.12	ND
20:5ω3	25.83	30.61	21.32	27.45
21:5ω3	0.93	1.29	0.83	0.51
22:5ω6	0.56	0.19	1.70	0.19
22:5ω3	0.38	0.20	0.55	0.20
22:6ω3	7.05	5.07	18.16	3.00
TOTAL	49.9	55.6	54.1	51.4

*4,8,12-TMTD is trimethyltridecanoic acid,
2,6,10,14-TMPD is tetramethylpentadecanoic acid,
3,7,11,14-TMHD is tetramethylhexadecanoic acid, and
7-M-16:0 is 7-methylhexadecanoic acid found in esters after hydrogenation.

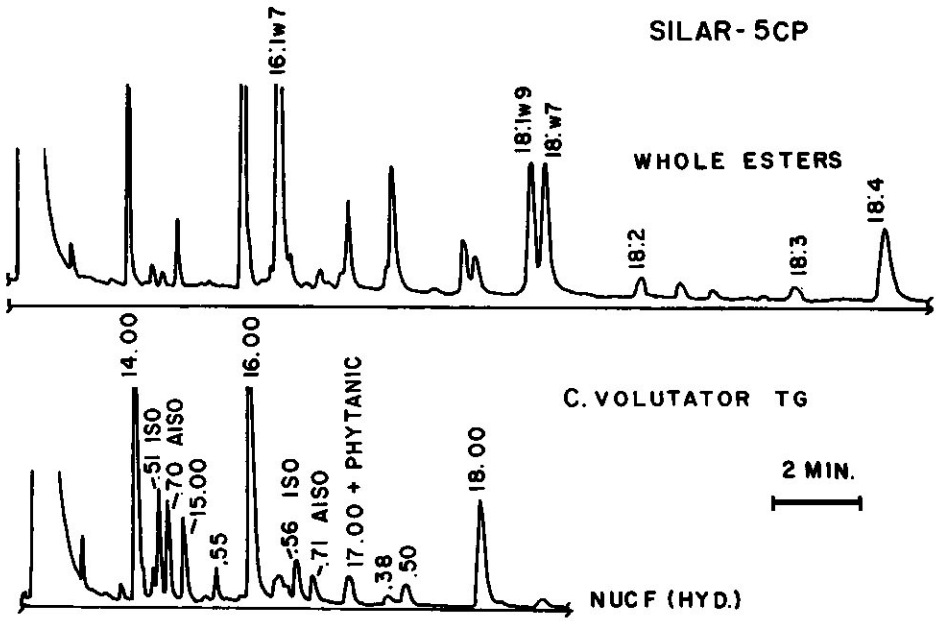

Fig 3. Comparison of parts of GLC charts for the C_{14} - C_{18} methyl esters of fatty acids from triglycerides of lot IV *C. volutator*. Open-tubular column, coating SILAR-5CP, at 170°. The upper section is of whole esters, the lower of the same esters after hydrogenation and 1 treatment with urea. Decimal numbers are FCL values for selected components (see Table III). Note triad peak between 16:0 and *iso* - 17:0 at ECL (16).56 in NUCF fraction.

some mammalian tissues can be associated with sterols (Hradec & Dusek 1968). Recent work on the omnipresent *Desulfovibrio desulfuricans* suggests a local origin for such fatty acids (Boon et al. 1977). The percentage of monoethylenic acids in the total fatty acids from this band was higher than in the other lipid classes, and among the components contributing to this total was a fatty acid tentatively identified as 16:1ω9 at 4.95% of the total. If the identification is correct, this is almost a unique occurrence of this acid in animal lipids as the 16:1 group of isomers is normally dominated by the 16:1ω7 isomer. The tentative GLC identification is supported by the importance of the probable 14:1 isomer (14:1ω9) arising from chain shortening of 16:1ω9, which in turn should arise by chain shortening of 18:1ω9. Alternatively this lipid could be involved in biosynthesis of monoethylenic fatty acids from saturated acids by one or more pathways which can start from 10:0, for example, with 18:1ω9 as the product (Gurr 1974). Traces of a 16:1ω3 acid were tentatively identified, also possibly originating in an aberrant fatty acid pathway.

The totals for polyunsaturated fatty acids (Table II) are all very similar but there are quite important differences in detail. The unusual fatty acids 16:2ω4, 16:3ω4, and 16:4ω1 all originate in phytoplankters (Ackman et al. 1968) or seaweeds (Ackman & McLachlan 1977) and cannot as far as is known be formed in animals. Not unexpectedly these acids accumulate in the TG and are much less important in the PL. Some are also observed to be more important in the upper-band fatty acids than in the PL. Conversely the fatty acid 'essential' to mammals (20:4ω6) is highest in

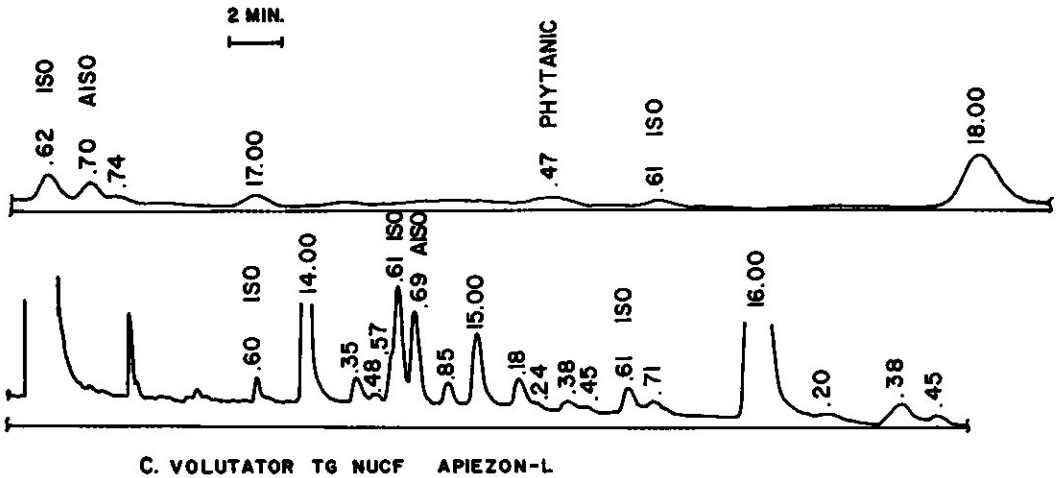

Fig 4. Part of GLC chart for methyl esters of fatty acids from triglycerides of lot IV *C. volutator* after hydrogenation and 1 treatment with urea. Open-tubular column, coating Apiezon-L, at 170°. Decimal numbers are FCL values for selected components (see Table III). Note (16).74 component, possibly a cyclopropanoid acid and compare with (17).38 component in Figure 3. Extra width of phytanic acid peak at (17).47 suggests two stereoisomers. Genuine *iso*-18:0 with ECL of (17).61 appears to be too small to match with peak at ECL (17).50 in Figure 3.

the PL, but is also important in the upper band. The precursor of 20:4ω6 is 18:2ω6 in both plants and animals, and as in most marine animal lipids (Moreno et al. 1979) does not accumulate (except in this case in the upper band lipid). The successor acid 22:5ω6 is also important in the PL. The vital role of 20:5ω3 and 22:6ω3 in marine PL is most apparent in the percentage figures for this lipid class, arguing for active control of these polyunsaturated acids by the animal (Moreno et al. 1979). On the other hand, in the triglycerides there is an accumulation of 20:5ω3 and 18:4ω3, both probably of exogenous plant origin, while 22:6ω3 is less important. The unusual 21:5ω3 component (Mayzaud & Ackman 1978) is also most obvious in the triglycerides, and seems to be present in the PL and TG in proportion to the percentage of 20:5ω3. This may be indicative of an exogenous origin for the 21:5ω3. It is interesting that freshwater crustaceans need and accumulate 20:5ω3 and 22:6ω3 (Takahashi & Yamada 1976; Teshima et al. 1976) at reduced levels, although these fatty acids are commonly thought of as 'marine' fatty acids. Their significance in lipids from species living in brackish waters is therefore difficult to evaluate.

Non-methylene-interrupted dienoic fatty acids, (Paradis & Ackman 1977; Pearce & Stillway 1977), did not appear to be present in significant quantities.

Minor and Unusual Fatty Acids

Several fatty acids other than those listed in Table II were apparent in the hydrogenated methyl ester sample from lot II neutral lipids. These were concentrated by urea complex treatment and the same procedure was later applied to the neutral lipids from lot IV. These had been freed of unsaponifiable matter and converted to methyl esters with BCl_3 instead of BF_3 in case cyclopropane structures were present. Retention data for all significant peaks on the liquid phases SILAR-5CP and Apiezan-L are recorded in Table III, cross-referenced where possible since a

Table III. Equivalent chain length data for analyses of minor components concentrated by urea complex treatment of products from hydrogenation of methyl esters of *Cor-ophium volutator* triglycerides (lot IV)

Equivalent chain lengths		Identification	
SILAR-5CP*	Apiezon-L	Structure	Status
13.53	13.60	Iso 14:0	Firm
14.00	14.00	14:0	Firm
14.15	14.35	4,8,12-trimethyltridecanoate	Firm
14.42(20%)	14.48	4-methyltetradecanoate	Possible
14.42(80%)	14.85	Unknown	
14.51	14.61	Anteiso 15:0	Firm
14.70	14.69	Iso 15:0	Firm
15.00	15.00	15:0	Firm
15.37	14.72	Cyclopropane	Possible
15.55	15.61	Iso 16:0	Firm
15.90	16.38	2,6,10,14 tetramethylpentadecanoate	Possible
16.00	16.00	16:0	Firm
16.31	16.38	7-methylhexadecanoate	Possible
16.36	15.71?	Cyclopropane	Possible
16.43	16.45	4-methylhexadecanoate	Possible
16.52	16.62	Iso 17:0	Firm
15.72	16.71	Anteiso 17:0	Firm
17.00 (50%)	17.00	17:0	Firm
17.00 (50%)	17.47	3,7,11,15 tetramethylhexadecanoate	Firm
17.38	16.74	Cyclopropane	Possible
17.50(80%)	17.17?	?	?
17.50 (20%)	17.61	Iso 18:0	Possible
?	14.57	?	?
?	15.18	?	?
?	15.24	?	?
?	15.38	?	?
?	15.45	?	?
?	16.20	2-methylhexadecanoate	Possible

*SILAR-5CP column No. 6153 and Apiezon-L column 907, both operated at 170°.

number of components coincide on the one column or the other. The complete identification of all of these components was not possible without further work, but the reproducibility of the chromatograms from 2 separate lots of *C. volutator,* with lipids converted to methyl esters by different reactions, suggests that the non-specific component data are worth recording as a starting point for future research. The Apiezon-L chromatogram chart showed several peaks not accounted for in the SILAR-5CP analysis. Because of the special reproducibility of retention data on Apiezon-L (Ackman 1972) these unknowns are included in Table III.

This cross-check on polar and non-polar columns is important in establishing the presence or absence of certain components. The component with ECL of 17.50 on SILAR-5CP could have been mistaken entirely for iso 18:0, but could not be accounted for quantitatively on Apiezon-L (ECL 17.61). This loss of the larger part of the peak argues for a degree of polarity, and a cyclopropanoid fatty acid was initially suspected, but authentic methyl 9,10-methylenehexadecanoate had an ECL on the SILAR column of 17.38 and would have emerged at about 16.74 on this Apiezon-L column (experimental on AP-L 907; see also literature data summarized by Ackman, 1972). Careful comparison of retention times showed that this unknown of ECL 17.50 was virtually absent among the NUCF components of the phospholipids, although the nearby suspected plasmalogen derivative emerging at ECL 17.55 on SILAR-5CP was confined to the phospholipids. On the other hand the corresponding peak of triglyceride origin at ECL 15.55 on SILAR-5CP could be verified as iso 16:0 by the same size peak on AP-L at ECL 15.61. Morris (1973) records a component listed as iso-18:0 for certain similar crustacea, suggesting that the unknown may be widespread in such lipids.

Other details were supported by circumstantial evidence, such as the width of the phytanic acid (3,7,11,15-tetramethylhexadecanoic) peak on Apiezon-L being greater than expected from that of adjacent single components. Two stereoisomers are therefore likely (Ackman 1972). The methyl esters of phytanic acid and 4,8,12-trimethyltridecanoic acid were definitely present in both polar lipid and triglyceride, but methyl 2,6,10,14-tetramethylpentadecanoate was obvious only in the triglycerides.

The methyl-branched fatty acids other than iso and anteiso are of considerable interest (possibly the triad peak of Fig 3), but their identification by gas-liquid chromatography only (Ackman et al. 1972; Smith & Duncan, 1979) is difficult. The cyclopropane fatty acids, if the identifications are correct, are clearly of bacterial origin (Brian et al. 1972; Oliver & Colwell 1973; O'Leary 1975; Lambert & Moss 1977; Ikemoto et al. 1978), but the amounts are very minor. There is no implication that the mud-dwelling *P. volutator* subsists on exogenous bacteria directly, but it is possible that it has gut bacteria capable of freeing or accumulating materials from plant detritus either directly or indirectly, in the latter case by stripping off the exoflora.

Acknowledgments

We gratefully acknowledge the assistance of J. Bromley, G. Gratto, and J.S. Boates for collection of the animals.

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