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# FATTY ACID AND STEROL COMPOSITION OF THE ANTARCTIC AMPHIPOD *THEMISTO GAUDICHAUDII* GUERIN 1828

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**Abstract**—1. A number of 56 different fatty acids and 7 sterols was found and identified in *Themisto gaudichaudii*.

2. 20:5($n$–3), 22:6($n$–3), 16:0, 18:1($n$–9), 16:1($n$–7) and 18:1($n$–7) were the dominating fatty acids in total lipids, phospholipids and triacylglycerols.

3. *T. gaudichaudii* contains an extremely high content of 20:5($n$–3) (21.1%) and 22:6($n$–3) (15.9%) in total fatty acids.

4. The sterol fraction contained cholesterol, desmosterol and 22-dehydrocholesterol as main components.

## INTRODUCTION

*Themisto gaudichaudii* Guerin 1828 is known to occur in swarms comparable to Antarctic krill (*Euphausia superba* Dana). It lives as a carnivorous amphipod of 11–20 mm length with a normal life cycle of 1 year and has a very marked diurnal vertical migration pattern (Everson and Ward, 1980). This amphipod species is limited to Antarctic waters and has an all year round circumpolar distribution.

*Themisto gaudichaudii* forms a part of the diet of Antarctic fishes (Takahashi and Nemoto, 1984), the sei whale (Bottino, 1978) and penguins (Jazedzeewski, 1981). The valid name of this species of the genus *Themisto* (Amphipoda: Hyperiidea) is *Themisto gaudichaudii*, but the former name *Parathemisto gaudichaudii* is still in use (Schneppenheim and Weigmann-Haas, 1986).

Bottino reported in 1978 the lipid composition of a sample of *T. gaudichaudii* from the stomach content of a caught sei whale. A high content of free fatty acids (17.7% of total lipids) demonstrated that the lipids of the sample must have decomposed considerably. A fatty acid analysis of another sample of this amphipod caught in 1972 in the Pacific sector of the Antarctic ocean reported by the same author exhibited the following major fatty acids: 22:6($n$–3) (17.3%), 20:5($n$–3) (16.7%), 18:1($n$–9) (16.7%) and 16:0 (14.6%). An analysis of the fatty acid composition of the triacylglycerols and phospholipids or an analysis of the sterol fraction of freshly caught *T. gaudichaudii* specimen is not reported.

During the Antarctic expedition 1985 with FRV *Walther Herwig* a sample of living *T. gaudichaudii* was caught and immediately prepared on board for analysis later. Subsequently a fatty acid and sterol analysis was performed.

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## MATERIALS AND METHODS

*Themisto gaudichaudii* was caught in Antarctic waters off Elephant Island on March 17 1985 on 61°59′S and 56°43′W with the FRV *Walther Herwig* using a rectangular midwater trawl (RMT 1 + 8). The haul contained besides the amphipods some salps, many copepods and a few euphausiids (*Thyanoessa macrura*, *Euphausia superba* and *Euphausia frigida*). 68 specimens of living *T. gaudichaudii* were drained (drained weight 4.86 g) on a sieve for 2 min, followed by homogenizing in a 20-fold excess of dichloromethane:methanol (2:1, v/v). The crude homogenate was stored at a temperature of −25°C until further treatment at land.

The homogenate was treated according to Folch *et al.* (1957) using less toxic dichloromethane instead of chloroform (Oehlenschläger, 1986). The lipids were redissolved in dichloromethane:methanol 1:1, v/v) and stored under a nitrogen atmosphere at −25°C. The lipids were separated into classes by thin layer chromatography (TLC) using HPTLC-plates (E. Merck) developed with $n$-hexane:diethylether:glacial acetic acid (50:50:1, v/v).

Fatty acid methyl esters (FAME) of total lipids and of triacylglycerols (TAG) and phospholipids (PL) were prepared with 14% boron trifluoride in methanol (Morrison and Smith, 1964). Trimethylsilylation of sterols was carried out as described by Ballantine *et al.* (1980). FAMEs were purified by TLC prior to gas chromatographic analysis (GLC). GLC-separations were run on a wall coated open tubular (WCOT) fused silica column (50 m) coated with CP Sil 5 CB (Chrompack), temperature programmed from 140 to 300°C (3°C/min) using a Packard 428 gas chromatograph equipped with a FID and a Shimadzu C-R1B integrator. Helium was used as a carrier gas at a flow rate of 1 ml/min with a split ratio of 100:1.

Gas chromatography/mass spectrometry (GLC/MS) of FAMEs was performed on a HP 5985 quadrupole mass spectrometer, ionization energy 70 eV, ion source temperature 200°C, column: 25 m WCOT coated with CP Sil 5, temperature programmed from 140 to 280°C (4°C/min), trimethylsilyl (TMS) sterols were analyzed using the same procedure with the exception that the GLC separation was run isothermic at 270°C. Individual FAME and TMS sterols were identified by co-chromatography with standards, by

comparison with calculated equivalent chain length (ECL) values (Fricke *et al.*, 1984) and by mass spectrometric data. To ensure identification of fatty acids samples were hydrogenated and re-chromatographed.

## RESULTS AND DISCUSSION

### *Fat content*

With 2.37% of wet weight the fat content of *T. gaudichaudii* was lower than the value reported by Bottino (1978), who found 8.9% in the sample collected from the sei whale stomach.

In the TLC separation of the lipid classes TAG, sterols and PL were found as major components. Free fatty acids as well as mono- and diacylglycerols were not found demonstrating that the preparation method chosen has led to no significant lipolytic decomposition of the original lipids.

Table 1. Fatty acid composition of total lipids, phospholipids, and triacylglycerols of *Themisto gaudichaudii*. Data are expressed as wt% of total fatty acids and represent means and standard deviation of at least three separate experiments

| FAME | $M^+$ | ECL | Total lipids | PL | TAG |
|---|---|---|---|---|---|
| 12:0 | 214 | 12.0 | 0.12 ± 0.03 | tr. | 0.15 ± 0.02 |
| 13:0 br | 228 | 12.7 | 0.04 ± 0.01 | tr. | tr. |
| 13:0 | 228 | 13.0 | 0.04 ± 0.01 | tr. | tr. |
| 14:0 | 242 | 14.0 | 3.96 ± 0.39 | 1.43 ± 0.09 | 4.83 ± 0.18 |
| 14:1 (*n*–?) | 240 | 13.6 | 0.10 ± 0.02 | tr. | 0.10 ± 0.02 |
| 14:1 (*n*–?) | 240 | 13.7 | 0.19 ± 0.04 | tr. | 0.21 ± 0.04 |
| 14:1 (*n*–?) | 240 | 13.8 | 0.08 ± 0.01 | tr. | 0.09 ± 0.01 |
| 15:0 br | 256 | 14.6 | 0.41 ± 0.03 | 0.22 ± 0.02 | 0.43 ± 0.02 |
| 15:0 br | 256 | 14.7 | 0.25 ± 0.03 | tr. | 0.34 ± 0.07 |
| 15:0 | 256 | 15.0 | 0.35 ± 0.02 | 0.26 ± 0.02 | 0.38 ± 0.02 |
| 15:1 br (*n*–?) | 254 | 14.4 | 0.15 ± 0.04 | tr. | 0.19 ± 0.02 |
| 15:1 br (*n*–?) | 254 | 14.5 | 0.08 ± 0.01 | tr. | 0.10 ± 0.02 |
| 16:0 br | 270 | 15.6 | 0.18 ± 0.04 | 0.11 ± 0.03 | 0.21 ± 0.02 |
| 16:0 br | 270 | 15.7 | 0.20 ± 0.05 | 0.09 ± 0.02 | 0.29 ± 0.03 |
| 16:0 | 270 | 16.0 | 13.16 ± 1.14 | 15.04 ± 0.41 | 13.56 ± 0.35 |
| 16:1 (*n*–7) | 268 | 15.7 | 6.03 ± 0.62 | 2.35 ± 0.19 | 8.10 ± 0.29 |
| 16:1 (*n*–?) | 268 | 15.8 | 0.25 ± 0.02 | 0.09 ± 0.03 | 0.30 ± 0.01 |
| 16:1 (*n*–?) | 268 | 15.9 | 0.20 ± 0.01 | 0.12 ± 0.02 | 0.24 ± 0.02 |
| 16:3 (*n*–?) | 264 | 15.5 | 0.43 ± 0.10 | 0.15 ± 0.04 | 0.61 ± 0.11 |
| 16:4 (*n*–3) | 262 | 15.4 | 0.70 ± 0.09 | 0.18 ± 0.08 | 0.95 ± 0.08 |
| 17:0 br | 284 | 16.6 | 0.43 ± 0.05 | 0.43 ± 0.04 | 0.53 ± 0.08 |
| 17:0 br | 284 | 16.7 | 0.55 ± 0.11 | 0.34 ± 0.04 | 0.53 ± 0.09 |
| 17:0 | 284 | 17.0 | 0.26 ± 0.01 | 0.38 ± 0.01 | 0.21 ± 0.02 |
| 17:1 (*n*–?) | 282 | 16.8 | tr. | tr. | tr. |
| 17:1 (*n*–?) | 282 | 16.9 | 0.10 ± 0.01 | tr. | 0.12 ± 0.01 |
| 18:0 | 298 | 18.0 | 1.19 ± 0.09 | 1.56 ± 0.02 | 0.86 ± 0.01 |
| 18:1 (*n*–?) | 296 | 17.9 | 0.52 ± 0.01 | 0.39 ± 0.01 | 0.53 ± 0.01 |
| 18:1 (*n*–?) | 296 | 17.9 | 0.12 ± 0.04 | tr. | 0.10 ± 0.02 |
| 18:1 (*n*–9) | 296 | 17.7 | 10.76 ± 1.11 | 8.94 ± 0.31 | 12.60 ± 0.61 |
| 18:1 (*n*–7)* | 296 | 17.8 | 4.83 ± 0.33 | 4.75 ± 0.11 | 5.29 ± 0.41 |
| 18:2 (*n*–6) | 294 | 17.6 | 1.46 ± 0.18 | 1.44 ± 0.03 | 1.15 ± 0.10 |
| 18:3 (*n*–3) | 292 | 17.6 | 0.58 ± 0.07 | 0.40 ± 0.01 | 0.53 ± 0.13 |
| 18:4 (*n*–3) | 290 | 17.4 | 2.63 ± 0.25 | 0.89 ± 0.02 | 3.49 ± 0.12 |
| 19:0 | 312 | 19.0 | 0.17 ± 0.07 | 0.10 ± 0.02 | 0.13 ± 0.01 |
| 19:1 (*n*–?) | 310 | 18.6 | 0.30 ± 0.18 | 0.27 ± 0.03 | 0.11 ± 0.02 |
| 19:1 (*n*–?) | 310 | 18.7 | 0.53 ± 0.05 | 0.28 ± 0.09 | 0.27 ± 0.02 |
| 19:1 (*n*–?) | 310 | 18.9 | 0.15 ± 0.09 | tr. | 0.19 ± 0.01 |
| 20:0 | 326 | 20.0 | 0.35 ± 0.15 | 0.10 ± 0.02 | 0.17 ± 0.02 |
| 20:1 (*n*–9) | 324 | 19.7 | 3.27 ± 0.20 | 1.62 ± 0.07 | 4.28 ± 0.09 |
| 20:1 (*n*–7) | 324 | 19.8 | 0.99 ± 0.07 | 0.56 ± 0.04 | 1.23 ± 0.07 |
| 20:1 (*n*–?) | 324 | 19.9 | 0.18 ± 0.06 | 0.06 ± 0.01 | 0.10 ± 0.01 |
| 20:2 (*n*–6) | 322 | 19.6 | 0.26 ± 0.07 | 0.32 ± 0.02 | 0.26 ± 0.06 |
| 20:3 (*n*–6) | 320 | 19.4 | 0.78 ± 0.13 | 0.77 ± 0.05 | 0.81 ± 0.09 |
| 20:4 (*n*–6) | 318 | 19.2 | 0.33 ± 0.07 | n.d. | tr. |
| 20:5 (*n*–3) | 316 | 19.3 | 21.10 ± 0.97 | 26.60 ± 0.56 | 19.66 ± 0.69 |
| 21:5 (*n*–3) | 330 | 20.2 | 0.62 ± 0.26 | 0.37 ± 0.02 | 0.38 ± 0.02 |
| 22:0 | 354 | 22.0 | tr. | tr. | 0.10 ± 0.05 |
| 22:1 (*n*–11) | 352 | 21.6 | 0.69 ± 0.31 | 0.17 ± 0.02 | 0.87 ± 0.04 |
| 22:1 (*n*–9) | 352 | 21.7 | 0.73 ± 0.19 | 0.62 ± 0.03 | 0.66 ± 0.01 |
| 22:1 (*n*–7) | 352 | 21.8 | 0.28 ± 0.13 | 0.21 ± 0.01 | 0.19 ± 0.02 |
| 22:5 (*n*–3) | 344 | 21.2 | 0.69 ± 0.28 | 1.03 ± 0.14 | n.d. |
| 22:6 (*n*–3) | 342 | 21.1 | 15.90 ± 1.07 | 26.12 ± 1.61 | 11.62 ± 0.41 |
| 23:0 | 368 | 23.0 | tr. | tr. | tr. |
| 24:0 | 382 | 24.0 | tr. | tr. | tr. |
| 24:1 (*n*–?) | 380 | 23.7 | 0.16 ± 0.11 | 0.15 ± 0.01 | 0.06 ± 0.01 |
| 24:1 (*n*–?) | 380 | 23.8 | 0.48 ± 0.45 | 0.33 ± 0.02 | 0.21 ± 0.02 |
| Others | | | 1.69 | 0.76 | 1.68 |

*Contains phytanic acid; tr. = trace; br = branched; n.d. = not detected; $M^+$ = molecular weight of fatty acid methyl ester as determined by GLC/MS; ECL = equivalent chain length, calculated by plotting chain length (as carbon number) vs retention time on CP Sil 5CB; FAME = fatty acid methyl ester; PL = phospholipids; TAG = triacylglycerols.

Table 2. Sterol composition of *Themisto gaudichaudii*. Data are expressed as wt% of total sterols and represent means and standard deviation of at least three separate experiments

| Sterol | Relative retention time (cholesterol = 1.00) | $M^+$ | wt% |
|---|---|---|---|
| 24-Norcholesta-5,22-dien-3β-ol | 0.71 | 442 | 3.35 ± 0.47 |
| Cholesta-5,22-dien-3β-ol (22-dehydrocholesterol) | 0.93 | 456 | 12.99 ± 0.33 |
| Cholesterol | 1.00 | 458 | 44.29 ± 2.41 |
| 5α-Cholestan-3β-ol (desmosterol) | 1.08 | 456 | 30.77 ± 1.21 |
| Ergosta-5,22-dien-3β-ol (brassicasterol) | 1.10 | 470 | 2.73 ± 0.18 |
| Ergosta-5,24(28)-dien-3β-ol | 1.20 | 470 | 2.79 ± 0.32 |
| Others | — | — | 1.89 ± 0.31 |

$M^+$ = molecular weight of trimethylsilyl sterol as determined by GLC/MS.

*Fatty acids*

In this investigation a number of 56 individual fatty acids could be separated and identified in total lipids. Table 1 shows the fatty acid composition of total lipids, phospholipids and triacylglycerol. In total lipids the major fatty acids were: 14:0 (3.96%), 16:0 (13.16%), 16:1(*n*–7) (6.03%), 18:1(*n*–9) (10.76%), 18:1(*n*–7) (4.83%), 20:1(*n*–9) (3.27%), 20:5(*n*–3) (21.1%), 22:6(*n*–3) (15.9%). In phospholipids the amount of 20:5(*n*–3) and 22:6(*n*–3) was higher (26.6 and 26.12% respectively) compared with the content in total fatty acids while it was considerably lower in triacylglycerols (19.66 and 11.62% respectively) indicating that like in other marine crustaceans also in *T. gaudichaudii* polyunsaturated fatty acids are accumulated predominantly in PL. (*n*–7) fatty acids ranging from 16:1 over 18:1 and 20:1 to 22:1, which was also found in Antarctic krill (Fricke *et al.*, 1984) is present. The amount of polyunsaturated fatty acids in total lipids is extremely high (43.76%).

Dimethylacetals which may arise from 1-0-akl-1′-enyllipids (plasmalogens) during the transmethylation procedure could not be verified among the FAME.

The branched chain fatty acids with C = 15 were almost only found in the triacylglycerols. Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) has the same ECL like 18:1(*n*–7) and was detected and determined in amounts of 0.7% of total fatty acids after hydrogenization. 22:1(*n*–11) occurs mainly in TAG. In the chromatogram of the hydrogenated total fatty acids traces of $C_{23}$, $C_{26}$ and $C_{28}$—fatty acids were present.

The fatty acid composition of *T. gaudichaudii* resembles fairly the lipid composition of other Antarctic crustaceans like *Euphausia superba* (Fricke *et al.*, 1984) or the two carnivorous isopods *Serolis pagenstecheri* and *S. cornuta* (Clarke, 1984) or Arctic amphipods like *Gammarus oceanicus* and *Echinogammarus marinus* (Clarke *et al.*, 1985). The main difference between the Antarctic hyperiidea and the Arctic gammaridae is evident in the content of 18:1(*n*–9) which is much higher in the Arctic gammaridae (approx. 25 vs 10% in *T. gaudichaudii*). The fatty acid composition reported for *Serolis* isopods show if compared with *T. gaudichaudii* more 18:1(*n*–9) (21.5% in PL vs 9.84% in PL of *T. gaudichaudii*) but much less 22:6(*n*–3) (5.89% in PL and 3.38% in TAG vs 26.12 and 11.6%, respectively, in *T. gaudichaudii*). 20:4(*n*–6) which is only a minor fatty acid in *T. gaudichaudii* (0.33% in total fatty acids is present in *S. pagenstecheri* with approx. 1% (TAG) to 5% (PL) and in *S. cornuta* with 1 to 2% (PL and TAG).

*Sterols*

The sterol composition as given in Table 2 is dominated by the high levels of $C_{28}$-sterols as cholesterol (44%), desmosterol (30.8%) and 22-dehydrocholesterol (13%). Smaller amounts of $C_{29}$ sterols presumably from the diet are also present. Besides the sterols listed in Table 2 traces of other sterols with relative retention time (RRT) 1.13 ($M^+$ 472), RRT 1.33 ($M^+$ 470), RRT 1.45 ($M^+$ 456) and RRT 1.50 ($M^+$ 470) were found, but could not be identified because of the small concentration. The sterol composition of *T. gaudichaudii* is similar to that reported by Ballantine (1980) for the Arctic copepod *Calanus finnmarchicus* with 45% cholesterol and 28% desmosterol. Antarctic krill which lives in the same environment together with *T. gaudichaudii* exhibits cholesterol contents of more than 70% and less than 20% of desmosterol (Fricke *et al.*, 1984). The predominance of cholesterol and considerable amounts of desmosterol together with small amounts of other $C_{28}$ and $C_{29}$ sterols are typical of the sterol composition in crustaceans (Ikekawa, 1985). The presence of high levels of desmosterol in *T. gaudichaudii* supports the assumption that this amphipod like other crustaceans is not able to synthesize cholesterol *de novo* but can convert dietary sterols via desmosterol into cholesterol.

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