J. exp. mar. Biol. Ecol., 1980, Vol. 47, pp. 25–33
© Elsevier/North-Holland Biomedical Press

MARINE STEROLS. XII. THE STEROLS OF SOME PELAGIC MARINE CRUSTACEANS

JAMES A. BALLANTINE and JOHN C. ROBERTS
Department of Chemistry, Institute for Marine Studies, University College of Swansea, Wales

and

ROBERT J. MORRIS
Institute of Oceanographic Sciences, Wormley, Surrey, England

Abstract: The sterol composition of some pelagic crustaceans (three species of pelagic euphausiids, one species of a bathypelagic marine mysid and one species of an estuarine mysid, two species of pelagic marine copepods, and one species of a bathypelagic marine copepod) have been determined by gas chromatographic–mass spectrometric techniques. The euphausiids appear to have developed an almost unispecific requirement for cholesterol, irrespective of trophic level, whilst the mysids show a considerable variation in sterol composition. The two near-surface copepod species contain a significantly greater variety of sterols than their bathypelagic counterpart. The results are compared with the known sterol compositions of other crustaceans in the marine food web and with phytoplankton. The extent of modification of dietary sterols after ingestion is discussed.

INTRODUCTION

Whilst the sterol chemistry of some of the marine and freshwater crustaceans has received considerable attention (see reviews by Goad, 1976, 1978; Morris & Culkin, 1977) comparatively few data are available on the sterol composition of pelagic marine copepods, euphausiids, and mysids. One species of euphausiid (*Nyctiphanes norvegica*) has been analysed by Idler & Wiseman (1971), one species of mysid (*Neomysis intermedia*) by Teshima & Kanazawa (1971), and one species of copepod (*Euchaeta japonica*) by Lee *et al.* (1974).

These crustacean groups are major components of the marine ecosystem. The copepods especially dominate the marine zooplankton in numbers and together with the other small crustaceans provide the link between the primary producers in the marine food web, the phytoplankton, and the larger carnivorous plankton. Knowledge of their sterol chemistry and hence their contribution to the pool of sterols in the marine food web is necessary for a proper understanding of the relative requirements of sterols by marine organisms and the biochemical potential for sterol synthesis-modification within the various trophic levels. Similarly such knowledge is necessary if the geochemical pathways of sterol diagenesis during and after sedimentation of dead organisms are to be confidently identified.

This paper presents the sterol composition of three species of pelagic to meso-

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

pelagic euphausiids, one species of a bathypelagic marine mysid and one species of an estuarine mysid, two species of pelagic marine copepods and one species of a bathypelagic marine copepod. The paper compares the sterol composition with those of other crustaceans in the marine food web and with phytoplankton and attempts to draw some conclusions as to whether the composition of the crustacean sterols is controlled metabolically by the animal as a result of its own specific physiological or biochemical requirements, or merely represents the type of dietary input, the animal having little control over its sterol make-up.

Material, Methods and Results

The animals were caught by net during biological cruises of *R.R.S. Discovery* to the northeastern Atlantic. They were sorted immediately by hand and where possible were kept alive for 3 h in clean, filtered sea water at 8 °C under low light conditions so that they might clear their guts. They were then frozen under nitrogen at −25 °C in specially cleaned glass jars. They were kept frozen at −25 °C until analysis.

Total lipids were extracted from the animals and the free sterol and sterol ester fractions crudely separated by methods previously described (Ballantine *et al.*, 1975, 1976). These fractions were then saponified with 10% potassium hydroxide in ethyl alcohol for 24 h under nitrogen in the dark and the non-saponified fraction purified by preparative TLC. The separated sterols which originated from the free and bound fractions were then determined using an SE30 0.3% textured glass bead column (Table I).

The sterols were identified and their relative amounts determined by the GLC of their trimethylsilyether (TMSE) derivatives on 1% Dexsil 300 GC and 1% Silar 5CP (2.5 m × 4 mm i.d. at 260 and 220 °C, respectively). These columns were coupled

TABLE I

Details of samples.

Species	Position caught	Depth of net (m)	Total lipid (% wet wt)	Sterol (% total lipid)	Sterol ester (% total lipid)
Meganyctiphanes norvegica M. Sars	25 °N; 17 °W	250–0	1.9	3.4	0.007
Thysanopoda subaequalis Boden	20 °N; 23 °W	1000–0	1.2	4.4	0.036
Stylocheiron abbreviatum G.O. Sars	25 °N; 17 °W	250–0	1.7	3.3	–
Eucopia sculpticauda Faxon	37 °N; 25 °W	1000–950	9.7	0.6	0.002
Neomysis integer (Leach)	Totton Marsh (Southampton)	surface	1.9	4.3	0.012
Labidocera acutifrons (Dana)	20 °N; 22 °W	surface	1.3	3.6	0.026
Calanus finmarchicus (Gunnerus)	60 °N; 20 °W	surface	2.7	0.7	–
Bathycalanus sp.	38 °N; 28 °W	1258–1264	7.7	0.2	–

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

through a silicone membrane separator to a fast scanning MS9 mass spectrometer, as previously described (Ballantine *et al.*, 1975). The results of the GLC-MS analysis upon the TMSE are tabulated in Tables II and III.

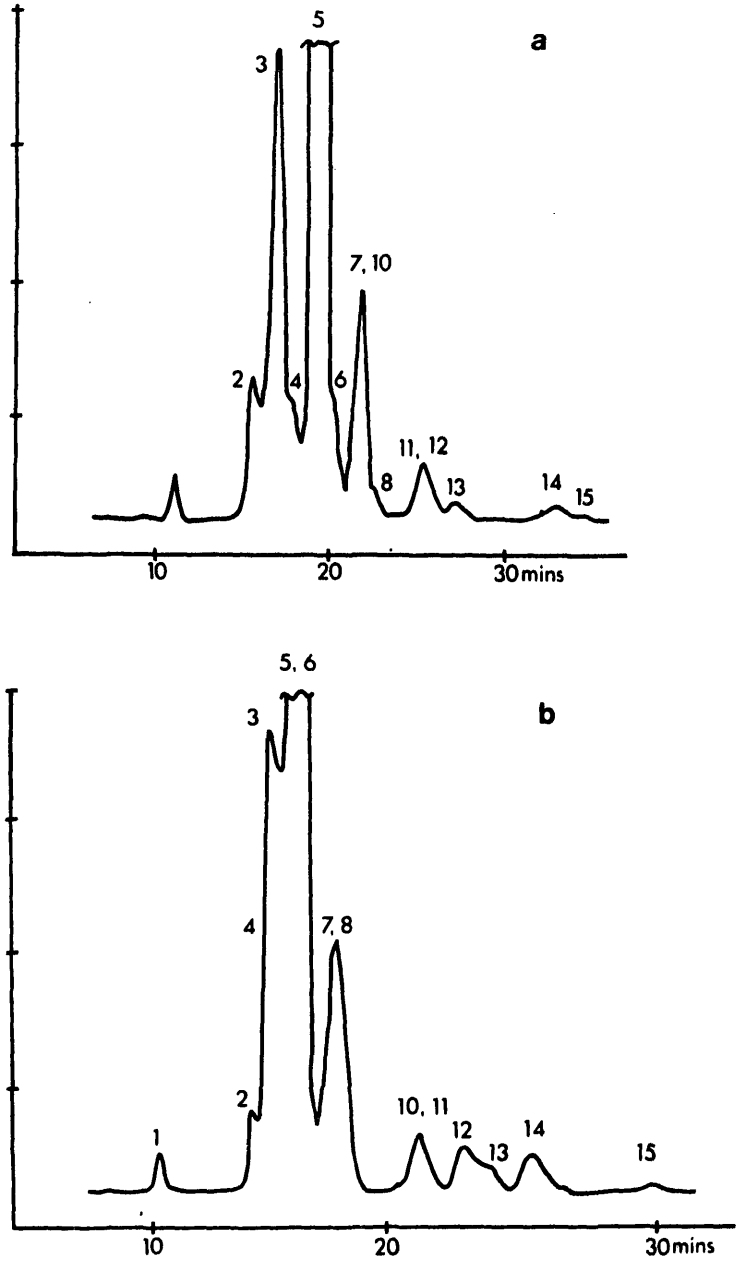

Fig. 1. Sterol profile of *Labidocera acutifrons* on Dexsil 300 GC (a) and Silar 5CP (b).

Fig. 1 represents the sterol profile of *Labidocera acutifrons*[1], which has been selected as an example because it contained almost all of the marine sterols found in the pelagic marine crustaceans. The TMSE derivatives of *L. acutifrons* were observed

[1] For taxonomic authorities see Table I.

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

TABLE II

Sterol composition of samples: tr, trace.

Species	Sterols[a]											
	1	2	3	4	5	6	7	8	9	10	11	12
Meganyctiphanes norvegica	–	–	0.6	–	98.2	–	–	–	–	–	0.2	0.2
Thysanopoda subaequalis	–	–	1.1	–	96.6	–	–	–	tr	–	0.2	0.5
Stylocheiron abbreviatum	–	–	1.7	–	96.0	–	–	–	2.1	tr	tr	tr
Eucopia sculpticauda	–	–	0.5	–	96.1	–	–	–	–	–	0.5	0.8
Neomysis integer	–	–	3.2	–	88.0	–	0.8	–	–	1.0	1.0	2.9
Labidocera acutifrons	0.7	1.4	14.3	tr	73.2	tr	4.2	0.6	–	1.2	0.3	1.6
Calanus finmarchicus	2.7	1.5	14.1	tr	45.4	–	3.0	–	–	27.7	1.5	–
Bathycalanus sp.	–	–	16.9	–	80.5	–	–	–	–	–	0.2	0.5
Thalassionema nitzochioides (Ballantine *et al.*, 1979b)	1.5	tr	22.7	–	59.6	–	3.6	–	–	5.0	2.9	2.2

[a] *Key list for sterols*

Sterol	1	26C5,22E	22(E)-24-norcholesta-5,22-dien-3β-ol
	2	27C5,22E*	occelasterol
	3	27C5,22E	22(E)-cholesta-5,22-dien-3β-ol
	4	27C,22E	22(E)-5α-cholest-22-en-3β-ol
	5	27C5	cholesterol
	6	27C	5α-cholestan-3β-ol
	7	28C5,22E	22(E),24(ε)-24-methylcholesta-5,22-dien-3β-ol
	8	28C,22E	22(E),24(ε)-24-methyl cholest-22-en-3β-ol
	9	27C5,7	cholesta-5,7-dien-3β-ol
	10	27C5,24(25)	desmosterol
	11	28C5	24(ε)-24-methylcholest-5-en-3β-ol
	12	28C5,24(28)	24-methylene cholesterol
	13	29C5,22E	22(E),24(ε)-24-ethylcholesta-5,22-dien-3β-ol
	14	29C5	24(ε)-24-ethylcholest-5-en-3β-ol
	15	29C5,24(28)Z	24(28)-24-ethylcholesta-5,24(28)-dien-3β-ol
	16	Unknown	

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

as eight partly resolved peaks in both the Dexsil and Silar chromatograms. By multiple mass spectrometric scanning of each of these peaks it was established that 14 different marine sterols were present in the extracts and their retention times were determined.

TABLE III

GL-MS characteristics of the sterol TMSE derivatives of *Labidocera acutifrons*.

Sterols	Identity[a]	Dexsil RRT	Silar RRT	M^+	Characteristic fragment ions[b]
1	26C5, 22E	0.63	0.65	442	427, 372, 352, 337, 313, 281, 255, *129*
2	27C5, 22E[c]	0.84	0.88	456	441, 366, 351, 327, 255, 215, 213, *129*
3	27C5, 22E	0.89	0.93	456	441, 366, 351, 327, 255, 215, 213, *129*
4	27C 22E	0.93	0.90	458	443, 374, 346, 345, *257*, 217, 215
5	27C5	1.00	1.00	458	443, 368, 353, 329, 275, 255, 247, *129*
6	27C	1.04	0.97	460	445, 370, 355, 306, 305, 217, 216, *215*
7	28C5, 22E	1.10	1.22	470	455, 380, 365, 341, 337, 255, 129, *125*
8	28C 22E	1.14	1.09	472	457, 374, 346, 345, *257*, 217, 215
10	27C5 24(25)	1.10	1.34	456	441, 366, 351, 343, 327, 255, 253, *129*
11	28C5	1.29	1.32	472	457, 382, 367, 343, 289, 261, 255, *129*
12	28C5, 24(28)	1.29	1.43	470	455, 386, 380, 365, 343, 341, 257, *129*
13	29C5, 22E	1.36	1.49	484	469, 394, 379, 355, 351, 255, 139, *129*
14	29C5	1.61	1.61	486	471, 396, 381, 357, 275, 255, 213, *129*
15	29C5, 24(28)Z	1.69	1.92	484	386, 371, 296, 281, 257, 255, 213, *129*

[a] For key see Table II.
[b] Only ions above m/z 125 are quoted in this table and the base peak is italicized.
[c] Occelasterol.

The sterols were identified (Table III) by a combination of their GLC retention times on the two different liquid phases and their characteristic mass spectrometric fragmentation patterns, which were compared with the GC-MS data for authentic sterol TMSE derivatives and for TMSE derivatives of known marine sterols obtained from other marine organisms as previously described (Ballantine *et al.*, 1975, 1976, 1977, 1978, 1979a, b). It must be noted that neither the GLC nor MS analysis is able to determine the stereochemistry at C24 in the sterol side chain and there was insufficient sample available to determine the stereochemistry by other means.

In this way sterols 1, 3, 5, 6, 7, 10, 11, 12, 13, 14 and 15 were identified (Tables II and III) as common sterols which were present in many marine organisms. Sterol 2 was identified as occelasterol obtained from an annelid by Kobayashi & Mitsuhaski (1974) and from the jellyfish *Pyrosoma* sp. by Ballantine *et al.* (1977). Sterol 4 was identified as 22-dehydrocholestanol and Sterol 8 was neospongesterol (or its C_{24} epimer) both of which were isolated by Erdman & Thomson (1972) from the sponge *Hymeniacidon perleve* and which were shown to be present in jellyfish (Ballantine *et al.*, 1975) and tunicates (Ballantine *et al.*, 1977). Sterol 9 was a minor sterol in the extracts of *Stylocheiron abbrevicatum* and was easily identified as cholesta-5,7-dien-3β-ol by comparison with authentic sterol.

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.