Bull. Fac. Fish. Hokkaido Univ.
28(1), 40-46. 1977.

Occurrence of Branched- and Odd-chain Fatty Acids in a Mud-feeding Sea Urchin, *Strongylocentrotus franciscanus*

Kenji HAYASHI* and Toru TAKAGI*

Abstract

The fatty acid composition of non-polar lipids consisting mainly in triglycerides of the gonad and viscera in a mud-feeding sea urchin, *Strongylocentrotus franciscanus*, is reported. In both the gonad and viscera, the percentage of the pentaenes of C_{20} (ca. 20%), the monoenes of C_{16}, C_{18} and C_{20}, and the saturate of C_{16} (each ca. 10%) are found as the major components, and the unsaturated acids are more abundant than the saturated ones. The contents of *iso* and *anteiso* fatty acids are 4.0% in the gonad and 3.2% in the viscera, and those of normal odd-chain fatty acids ranging C_{13-23} are 7.4% in the former and 5.6% in the latter. These significant amounts of branched- and odd-chain fatty acids are possibly derived in part from the dietary sediments of the sea urchin.

Introduction

The occurrence in nature and biochemistry of branched-chain fatty acids[1,2], and the essential fatty acid activity of odd-chain fatty acids in fish[3] have already been reviewed. Branched- or odd-chain fatty acids are now known to occur as common minor constituents in the lipids of marine animals. Exceptionally, certain fishes have been very rich in odd-chain fatty acids in their lipids. The mullet, *Mugil cephalus*, of a vegetarian mud-grubbing species, normally contained 10–25% odd-chain fatty acids[4,5,6]. The smelt, *Osmerus mordax*, from one specific location, was found to contain relatively larger amounts of odd-chain fatty acids[7]. The same was also true for a typical mud-feeding species, the holothurian, *Scotoplanes theeli*, which contained comparatively larger amounts of odd- and branched-chain fatty acids[8]. It is well known that microorganisms metabolized relatively high amounts of branched-chain fatty acids[9,10]. The relatively high ratio of branched-chain to straight-chain fatty acids in the sediments was suggested to be of a bacterial origin[11].

In this paper, we report findings on the relatively high contents of the homologues of branched- and odd-chain fatty acids in a mud-feeding sea urchin, *Strongylocentrotus franciscanus*. In this country, the raw or salted gonad of algal feeding sea urchins, *Anthocidaris crassispina*, *S. intermedius* and *S. pulcherrimus*, are known to be more tasteful than those of the *S. franciscanus*; the latter tastes mud. There are several reports on the fatty acid composition of the algal feeding sea urchins, *S. nudus*[12], *Pseudocentrotus depressus*[12], *A. crassispina*[12,14,15], *S. inter-*

* *Laboratory of Chemistry of Fish Oil, Faculty of Fisheries, Hokkaido Uniuersity*
(北海道大学水産学部魚油化学講座)

medius[13] and *S. pulcherrimus*[12,16], however, there is little information on that of the sea urchin, *S. franciscanus*.

Experiments

Sea urchin, *S. franciscanus*, was collected at a depth of 20 m off-Moheji near Hakodate, Japan, in April, 1973. The specimens of 66 individuals were 65 cm in average test diameter, the spine being excluded and 87 g in average weight. The pooled gonad and viscera of the above specimens were 360 g and 510 g in total wet weight, respectively. All the lipids in the gonad and viscera were extracted by the method of Bligh and Dyer[17]. The non-polar lipids were separated from the whole by chromatography on silicic acid-Celite (2:1) columns with the elution of chloroform. The fatty acids were recovered after removal of the unsaponifiable materials and were esterified with boron trifluoride-methanol reagent[18]. The fatty acid composition of the non-polar lipids was determined by means of a YANAGIMOTO gas chromatograph (model G8) equipped with a dual hydrogen flame detector. The column used was 1.5 m×3 mm i.d., U shaped, of stainless steel, and packed with 10% diethyleneglycol-succinate (DEGS) on Chromosorb W AW. The oven temperature was 190°C and the inlet pressure of nitrogen 0.7 kg/cm^2. The fatty acid methyl esters were identified by comparison with known commercially available standards. An additional identification of both the saturated and unsaturated fatty acids was accomplished by a log plot of the retention times against the number of carbons in the chain, before and after hydrogenation of the fatty acid methyl esters. The part of fatty acid methyl esters has been hydrogenated in a n-hexane solution with an activated Raney-nickel catalyst, bubbling hydrogen at atmospheric pressure and at 40°C. The percentage of each fatty acid was calculated by measuring the area of the peaks. The analysis of the components of the non-polar lipids and unsaponifiable materials was accomplished by thin-layer chromatography (TLC); a thin layer of 0.25 mm silicic acid activated at 110°C for 60 min, a developing solvent: petroleum ether-ethyl ether-acetic acid (90:10:1, v/v) or petroleum ether-ethyl ether (50:50, v/v), and a reagent: 15% phosphomolybdic acid in ethanol.

Results and Discussion

The lipid contents and the characteristics of the non-polar lipids of the gonad

Table 1. *Lipid contents and the characteristics of the non-polar lipids of the gonad and viscera in the examined sea urchin.*

Part	Lipid content %	Non-polar lipid			
		Content %	A.V.*	I.V.**	U.S.M.*** %
Gonad	1.8	64.8	9.1	142.2	19.9
Viscera	0.9	56.1	—	144.4	25.1

* Acid value. ** Iodine value. *** Unsaponifiable materials.

Table 2. *Fatty acid composition of the non-polar lipids of the gonad and viscera in the examined sea urchin.*

Fatty acid	Weight percent	
	Gonad	Viscera
Iso 12:0	tr*	tr
12:0	tr	0.1
12:1	tr	tr
Iso 13:0	tr	tr
13:0	0.1	0.1
13:1	tr	tr
Iso 14:0	0.1	0.2
14:0	4.4	4.5
14:1	0.2	0.1
Iso 15:0	0.6	0.6
Anteiso 15:0	1.3	0.9
15:0	0.8	0.6
15:1	0.3	0.2
Iso 16:0	0.5	0.4
16:0	9.5	10.4
16:1	10.5	8.7
Iso 17:0	0.6	0.5
Anteiso 17:0	0.5	0.4
17:0	0.7	0.4
17:1	0.7	0.4
Iso 18:0	0.2	0.1
18:0	3.1	3.8
18:1	10.3	8.5
18:2	1.5	1.9
18:3	1.3	1.5
18:4	4.1	4.0
19:0	0.8	0.5
19:1	0.8	1.0
Iso 20:0	0.2	0.1
20:1	9.7	9.7
20:2	1.0	0.9
20:3	0.3	0.6
20:4	4.6	7.5
20:5	20.3	22.7
21:1	1.6	1.0
21:5	1.1	0.9
22:1	2.8	2.4
22:2	1.1	0.7
22:5	0.8	0.6
22:6	2.8	2.3
23:0	0.5	0.5
24:1	0.3	0.3

* Trace.

and viscera are given in Table 1. All the lipids of the examined species were in a viscous state with dark reddish brown for the gonad and with dark brown for the viscera. The lipid contents for the gonad yielded twice as much as those of the viscera, although the contents of both the gonad and viscera were lower than those of algal feeding sea urchins, *A. crassispina*[12] and *S. pulcherrimus*[16]. It was also noted that there is a higher lipid content in the gonad than in the viscera, as a rule. The non-polar lipid contents in the gonad were higher than those in the viscera, whereas the polar lipids were abundant in the viscera. These data agree with the results on the sea urchins, *S. pulcherrimus*[16] and *A. crassispina*[19]. The TLC analysis of non-polar lipids in both the gonad and viscera revealed that triglycerides were the predominant components. Kōchi[16] has also reported that the major components of the non-polar lipids in both the gonad and viscera of the algal feeding sea urchin, *S. pulcherrimus*, were triglycerides. The iodine values for both the gonad and viscera in the examined species were relatively higher than those of *A. crassispina*[14,19]. The results of the TLC analysis showed that sterins were the larger components of the unsaponifiable materials for both the gonad and viscera, while hydrocarbons and glyceryl ethers were the smaller components. Small amounts of fatty alcohols were detected in the viscera.

The fatty acid composition of non-polar lipids for the gonad and viscera in the examined species are given in Table 2. In addition, the comparisons of the saturated and unsaturated fatty acids based on the carbon numbers are listed in Table 3 for the gonad and in Table 4 for the viscera. As given in Table 2, the relatively predominant fatty acids for both the gonad and viscera were 20:5, 18:1, 16:1, 16:0 and 20:1 acids. And the major carbons in the fatty acids were C_{20}, C_{18} and C_{16} acids, and the unsaturated acids were more abundant than the saturated

Table 3. *Comparison of the saturated and unsaturated fatty acids of the gonad in the examined sea urchin.*

Fatty acid	Weight percent				
	Saturated	Unsaturated			Total
		Monoenoic	Dienoic	Polyenoic	
C12	tr(tr)*	tr**	—	—	tr
C13	0.1(tr)	tr	—	—	0.1
C14	4.5(0.1)	0.2	—	—	4.7
C15	2.7(1.9)	0.3	—	—	3.0
C16	10.0(0.5)	10.5	—	—	20.5
C17	1.8(1.1)	0.7	—	—	2.5
C18	3.3(0.2)	10.3	1.5	5.4	20.5
C19	0.8	0.8	—	—	1.6
C20	0.2(0.2)	9.7	1.0	25.2	36.1
C21	—	1.6	—	1.1	2.7
C22	—	2.8	1.1	3.6	7.5
C23	0.5	—	—	—	0.5
C24	—	0.3	—	—	0.3
Total	23.9(4.0)	37.2	3.6	35.3	100.0

* The numbers in parentheses indicate the branched-chain fatty acids.
** Trace.

Table 4. *Comparison of the saturated and unsaturated fatty acids of the viscera in the examined sea urchin.*

Fatty acid	Weight percent				
	Saturated	Unsaturated			Total
		Monoenoic	Dienoic	Polyenoic	
C12	0.1(tr)*	tr**	—	—	0.1
C13	0.1(tr)	tr	—	—	0.1
C14	4.7(0.2)	0.1	—	—	4.8
C15	2.1(1.5)	0.2	—	—	2.3
C16	10.8(0.4)	8.7	—	—	19.5
C17	1.3(0.9)	0.4	—	—	1.7
C18	3.9(0.1)	8.5	1.9	5.5	19.8
C19	0.5	1.0	—	—	1.5
C20	0.1(0.1)	9.7	0.9	30.8	41.5
C21	—	1.0	—	0.9	1.9
C22	—	2.4	0.7	2.9	6.0
C23	0.5	—	—	—	0.5
C24	—	0.3	—	—	0.3
Total	24.1(3.2)	32.3	3.5	40.1	100.0

* The numbers in parentheses indicate the branched-chain fatty acids.
** Trace.

acids, for both the gonad and viscera (Table 3, and Table 4). The monoenoic acids were greater in the gonad, whereas the viscera had more of the polyenoic acids. The typical mud-feeding holothurian, *S. theeli*[8], had a high percentage of 20:4 and 20:5 acids in the fatty acid composition, although in the examined mud-feeding sea urchin only the 20:4 acid was less than that of the holothurian. The fatty acid composition of the non-polar lipids in the gonad or viscera of the algal feeding sea urchins. *S. nudus*[12], *P. depressus*[12], *S. intermedius*[13], *A. crassispina*[12,14,15], and *S. pulcherrimus*[12,16], was reported to be relatively larger in 14:0 and 16:0 acids and smaller in 20:5 acid. In contrast, those of the examined mud-feeding sea urchin revealed larger 20:5 acid and smaller 14:0 and 16:0 acid contents.

A linear-log plot of the DEGS retention values of the methyl-branched peaks yielded two straight lines, corresponding to the *iso* and *anteiso* fatty acids in the fatty acid composition for both the gonad and viscera in the examined species. Branched-chain fatty acids in the sea urchin which was examined contained significant amounts, namely, 4.0% in the gonad and 3.2% in the viscera. *Iso* and *anteiso* fatty acids were about equal in amount. The branched-chain isomers with even numbers of carbon atoms were relatively abundant compared with those of the odd ones. The C_{15} and C_{17} branched-chain fatty acids were relatively abundant. Recently, Kōchi[16] has reported that the fatty acids in triglycerides of the major components of non-polar lipids obtained from the gonad and viscera in the algal feeding sea urchin, *S. pulcherrimus*, contained minor quantities of *iso* and *anteiso* fatty acids, namely, 0.8% in the gonad and 1.4% in the viscera. It has been reported that the typical mud-feeding species of the holothurian, *S. theeli*, contained comparatively larger amounts of 5.0% branched-chain fatty acids[8], and the same was also true for the sediments at 1.5–7.2%[11]. By a comparison of the ratios of even-chain saturated acids to the corresponding *iso* branched isomer for

C_{16} and C_{14}, Leo *et al.*[11] found ratios between 1 and 20 for the sediments, compared to the ratios between 100 and 500 for a variety of higher marine organisms[20]. Lewis[8] has also reported that the holothurian gave ratios between 2 and 11 for the C_{14}, C_{16}, and C_{18} acids. The same ratios calculated to C_{14}, C_{16}, and C_{18} acids for the examined sea urchin were between 16 and 44 for the gonad, and between 23 and 38 for the viscera, agreeing closely with the ratios of the sediments[11] and holothurian[8] and indicating a significant enrichment. The examined sea urchin fed upon the surface layer of the bottom sediments which was known to be rich in organic detritus and bacteria. Considering that bacteria lipids are noted for being rich in branched-chain fatty acids[9,10], it is probable that these acids in the examined sea urchin might stem from bacterial sources contained in its diet.

The contents of normal odd-chain fatty acids in the examined sea urchin were relatively higher, namely, 7.4% in the gonad and 5.6% in the viscera, compared to 0.5–3.7% in the non-polar lipids of the gonad in algal feeding sea urchins, *P. depressus*[12], *S. nudus*[12], *S. pulcherrimus*[12], and *A. crassispina*[12,14], and 4.9% in triglycerides for both the gonad and viscera in *S. pulcherrimus*[16]. The normal odd-chain fatty acids in the examined species were adequately approximated to the amounts of 7.0% in the mud-feeding holothurian, *S. theeli*[8], and of 3.6–6.4% in the sediments[11]. It is reported that the mullet, *M. cephalus*, of a vegetarian mud-grubbing species concentrated normally larger amounts, namely, 10–25% of normal odd-chain fatty acids[4,5,6], as compared to 1.8–3.6% for a variety of higher marine organisms[20]. Thus, it is presumed that the relatively high contents of normal odd-chain fatty acids in the examined mud-feeding sea urchin might also be related to its diet.

It is inferred that the characteristic fatty acid composition, described above, in the non-polar lipids of the examined mud-feeding sea urchin, differed from those of the algal feeding sea urchins, and the difference was attributed in part to the sediment components of the sea urchin diet.

Acknowledgment

We wish to thank Dr. Akira Fuji, Faculty of Fisheries, Hokkaido University, who identified the sea urchin sample.

References

1) Abrahamsson, S., Ställberg-Stenhagen, S., and Stenhagen, E. (1963). The higher saturated branched chain fatty acids. *In* Progress in the chemistry of fats and other lipids. Vol. VII, Part 1, pp. 1–164, Pergamon Press, Oxford.
2) SenGupta, A.K. (1972). Recent advances in the chemistry and biochemistry of methyl branched fatty acids. *Fette·Seifen Anstrichmittel* **74**, 693–705.
3) Aaes-Jørgensen, E. (1967). Fish oils as a source of essential fatty acids. *In* Fish oil; Their chemistry, technology, stability, nutritional properties, and uses. pp. 1–440, The Avi Publishing Company, Inc., Westport, Connecticat.
4) Reiser, R., Stevenson, B., Kayama, M., Choudhury, R.B.R., and Hood, D.W. (1963). The influence of dietary fatty acids and environmental temperature on the fatty acid composition of teleost fish. *J. Amer. Oil Chem. Soc.* **40**, 507–513.
5) Sen, N. and Schlenk, H. (1964). The structure of polyenoic odd- and even-numbered

fatty acids of mullet (*Mugil cephalus*). *Ibid.* **41**, 241–248.

6) Gruger, E.H., Nelson, R.W., and Stansby, M.E. (1964). Fatty acid composition of oils from 21 species of marine fish, freshwater fish and shell-fish. *Ibid.* **41**, 662–667.

7) Addison, R.F. and Ackman, R.G. (1970). Exceptional occurrence of odd-chain fatty acids in smelt (*Osmerus mordax*) from Jeddore Harbour, Nova Scotia. *Lipids* **5**, 554–557.

8) Lewis, R.W. (1967). Fatty acid composition of some marine animals from various depths. *J. Fish. Res. Bd. Canada* **24**, 1101–1115.

9) Shorland, F.B. (1962). The comparative aspects of fatty acid occurrence and distribution. *In* Comparative biochemistry. Vol. 3, Part A, pp. 1–102, Academic Press, New York.

10) Kaneda, T. (1963). Biosynthesis of branched chain fatty acids. *J. Biol. Chem.* **238**, 1222–1228.

11) Leo, R.F. and Parker, P.L. (1966). Branched-chain fatty acids in sediments. *Science* **152**, 649–650.

12) Kōchi, M. (1968). Studies on "Uni-Shiokara" VI. Fatty acid composition of lipid from sea-urchin gonad. *Bull. Shimonoseki Fish. Univ.* **17**, 9–16. (In Japanese with English abstract).

13) Fujino, Y., Negishi, T., and Umatani, K. (1970). Studies on lipids in the sea urchin egg. Part I. On the chemical change of lipids during curing with salt. *J. Food Science and Technology* **17**, 343–349. (In Japanese with English abstract).

14) Tsuyuki, H. and Itoh, S. (1971). Acetone-soluble lipids of sea urchin eggs salted in Korea. *Bull. Coll. Agr. & Vet., Med. Nihon Univ.* No. 28, 37–41. (In Japanese with English abstract).

15) Kōchi, M. (1974). Gas chromatographic analysis of fatty acids in non-polar lipid of sea-urchin gonad in combination with thin-layer argentation chromatography. *Bull. Shimonoseki Fish. Univ.* **22**, 95–104. (In Japanese with English abstract).

16) Kōchi, M. (1976). Distribution of isomeric eicosadienoic acid in lipid classes of a sea urchin. *Bull. Jap. Soc. Sci. Fish.* **42**, 219–225. (In Japanese with English abstract).

17) Bligh, E.G. and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, 911–917.

18) Morrison, W.R. and Smith, L.M. (1962). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J. Lipid Res.* **3**, 600–608.

19) Tsuyuki, H. and Mochizuki, A. (1966). Studies on lipids of sea urchin, *Anthocidaris crassispina*. *J. Food Science and Technology* **13**, 380–384. (In Japanese).

20) Ackman, R.G. and Sipos, J.C. (1965). Isolation of the saturated fatty acids of some marine lipids with particular reference to normal odd-numbered fatty acids and branched-chain fatty acids. *Comp. Biochem. Physiol.* **15**, 445–456.