Mem. Fac. Fish., Kagoshima Univ.
Vol. 30 pp. 317～323 (1981)

Sterols and Fatty Acids of the Lab-lab and Snail from the Milkfish-Pond

Shin-ichi TESHIMA, Akio KANAZAWA and Akio TAGO*[1]

Abstract

The sterol and fatty acid compositions of the lab-lab and snail (family Ceritheidae) were analyzed in the interests of bio-ecological transport of dietary sterols and fish nutrition in the extensive fishpond.

The floating lab-lab contained cholesterol (40.2% of total sterols), 24-methycholesterol (14.0%), 24-methylcholesta-5, 22-dienol (17.3%), 24-ethylcholesterol (7.2%), and 24-ethylcholesta-5, 22-dienol (9.7%) as the major sterols. In the adhering lab-lab, 24-E-ethylidenecholesterol (13.3%) was the prominent sterol in addition to the above-mentioned five sterols. The snail contained cholesterol (54.9%), cholest-7-enol (5.9%), 24-methylcholesterol (12.6%), 24-ethylcholesterol (3.9%), cholesta-5, 22-dienol (6.7%), 24-methylcholesta-5, 22-dienol (9.4%), cholesta-5, 24-dienol (6.0%), and small amount of 24-norcholesta-5, 22-dienol (0.5%).

The fatty acid composition of floating lab-lab resembled that of adhering lab-lab. The two types of lab-lab contained palmitic (16:0) and palmitoleic (16:1) acids as the major fatty acids and substancial amounts of linoleic (18:2ω6, 3.6% and 6.1%) and linolenic (18:3ω3, 4.5%) acids. But, the lab-lab involved very low levels of eicosapentaenoic (20:3ω3) and docosahexaenoic (22:6ω3) acids.

In Philippines, fish-farmers have been rearing the milkfish, *Chanos chanos*, a marine fish subsisting on filamentous green algae, in extensive pond operations. The fishponds are generally set up on tidal flats near tidal streams, so they incidentally produce the crops such as the jumbo shrimp, *Penaeus monodon*, swamp crab, *Scylla serrata*, other species of fish etc[1]. A lab-lab is the so-called mixture of some algae and plankton which propagate thick in the fishponds. The adhering lab-lab is the conglomerate of alive organisms attached to the fish-net etc., whereas the floating lab-lab is regarded to be dead. Undoubtedly, the lab-lab is recognized to be an important food stuff to support the growth of milkfish and other animals in extensive fishponds.

In relation to the bio-ecological transport of dietary sterols, we intend to investigate the sterol composition of the lab-lab and snail (family Ceritheidae) which were collected from the fishpond in Philippines. Also, our interest was focussed on the fatty acid composition of the lab-lab, because recent investigations on fish nutrition have pointed out the unique aspects of essential fatty acid (EFA) requirements in

*1 Laboratory of Fisheries Chemistry, Faculty of Fisheries, University of Kagoshima, 4–50–20 Shimoarata, Kagoshima 890, Japan.

aquatic animals[2-5]: that is, in most fish they have been shown to require ω3-series of fatty acids such as linolenic (18: 3ω3), eicosapentaenoic (20: 5ω3), and docosahexaenoic (22: 6ω3) acids rather than ω6 series of acids such as linoleic (18: 2ω6) and arachidonic (20: 4ω6) acids.

The present paper deals with these results and discussion.

Materials and Methods

Samples analyzed The specimens of the lab-lab and snail (family Ceritheidae) were collected in Iloilo, Philippines, during August, 1978. The lab-lab was mainly composed of blue-green algae (about 50%) and other organisms such as some green algae, copepods, rotifers, diatoms, and *Euglena* sp.

Isolation of fatty acids and sterols The lab-lab and snail were directly saponified with ethanolic potassium hydroxide[6] at 80°C for 24 hr, and then separated into the unsaponifiable matter and fatty acid fractions by extraction with ether. The fatty acid fraction was subjected to determination of fatty acid composition. Sterols were isolated from the unsaponifiable matters by alumina column chromatography with hexane-benzene[7].

Determination of fatty acid composition The composition of fatty acids was determined by gas-liquid chromatography (GLC) on 10% DEGS (column 3 m×3 mm i.d., column temperature 190°C) as described previously[8].

Identification of sterol components The sterol components were preliminarily identified by comparison of relative retention times (RRT) to standards in GLC on 1.5% OV-17 (column 3 m×3 mm i.d., column temperature 245°C)[9]. Then, the acetate derivative of sterols were separated into the several fractions by thin-layer chromatography on 10% (w/w) $AgNO_3$-Kieselgel G with ethanol-free chloroform[10], and the fractions so obtained were subjected to GLC-Mass spectrometry to confirm the identity of sterol components. GLC-Mass spectrometry[11] was conducted with a column (2 m×2 mm i.d.) of 3.0% OV-1 at 270°C by using a Japan Electron Optics JEOL-JMS-300 mass spectrometer.

Results and Discussion

Sterols of the lab-lab Table 1 shows the yields of sterols from the floating and adhering lab-lab. Most of lab-lab sterols was present as desmethylsterols. Although the lab-lab contained small amounts of methylsterols which were less polar in TLC on Kieselgel G with chloroform-methanol (49: 1) than common desmethylsterols, further characterization of the components was not carried out in this study. The steryl acetates of desmethylsterols from the floating lab-lab gave nine peaks in GLC on 1.5% OV-17 and also separated into six bands of Rf 0.88, 0.78, 0.68, 0.50, 0.38, and 0.13 in $AgNO_3$-TLC. Table 2 indicates the sterol composition of the floating and adhering lab-lab. All sterols given in Table 2 showed the identical behaviour

Table 1. Yields of the unsaponifiable matters and sterols from the lab-lab and snail.

Fraction	Weight (mg)		
	Floating lab-lab	Adhering lab-lab	Snail
Unsaponifiable matters	53.4	48.6	13.0
Total sterols	6.3	4.2	1.2
Desmethylsterols	5.6	3.7	1.2
Methylsterols	0.7	0.4	0

on TLC and GLC, and the same mass spectra as authentic sterols or steryl acetates.

The most less polar band in $AgNO_3$–TLC was composed of five components which were elucidated by GLC-Mass spectrometry to be cholestanyl (**1**, RRT 1.01) in GLC on 3.0% OV–1), cholest-7-enyl (**2**, RRT 1.14), 24-methylcholestanyl (**3**, RRT 1.28), 24-ethylcholest-22-enyl (**4**, RRT 1.36), and 24-ethylcholestanyl (**5**, RRT 1.58) acetates. **1**: m/e 430 (M^+), 415, 370, 355, 316, 290, 276, 275, 257, 230, and 215. **3**: m/e 444 (M^+), 429, 384, 330, 290, 276, 275, 257, 230, and 215. **5**: m/e 458 (M^+), 443, 398 383, 344, 290, 276, 257, 230, and 215. The presence of the molecular ions at m/e 430, 444, and 458, together with the ions at m/e 257 (M^+–R–AcOH, R=side chain)[12] and the ions (M^+–C–1 to C–4) at m/e 316, 330, and 344, supported the structures of **1**, **3**, and **5**. **2**: m/e 428 (M^+), 413, 368, 315, 288, 255, 229, and 213. The intense molecular ion peak at m/e 428 (100%, relative intensity) and the ion at m/e 255 (M^+–R–AcOH, 40%) were indicative of C_{27}–Δ^7 steryl acetate[12]. **4**: m/e 456 (M^+), 413, 353, 344, 329, 315, 284, 269, 257, 255, and 215. Together with the molecular ion at m/e 456, the intense ions at m/e 257 (M^+–R–AcOH, 100%) and 215 (M^+–R–42–AcOH) showed that **4** had a saturated ring and C_{10} side chain involving one double. The position of side chain was determined to be located at C–22 by the ions[13] at m/e 315 (M^+–R–2H), 413 (M^+–43), 353 (M^+–43–AcOH), 284 (M^+–C–22 to C–29–1H–AcOH), and 269 (m/e 284–CH_3). Thus, **4** was identified as 24-ethylcholest-22-enyl acetate[14].

The secondary less polar band in $AgNO_3$–TLC (Rf 0.78) was composed of three components, cholesteryl (**6**, RRT 1.00 in GLC on 3.0% OV–1), 24-methylcholesteryl (**7**, RRT 1.27), and 24-ethylcholesteryl (**8**, RRT 1.58) acetates. **6**: m/e 368 (M^+–AcOH), 353, 275, 260, 255, 247, 228, and 213. **7**: m/e 382 (M^+–AcOH), 274, 261, 255, 228, and 213. **8**: m/e 396 (M^+–AcOH), 288, 275, 255, 228, and 213. The band (Rf 0.68) contained 24-ethylcholesta-5, 22-dienyl acetate (**9**, RRT 1.49). **9**: m/e 394 (M^+–AcOH), 379, 351, 282, 267, 255, 228, and 213. The band (Rf 0.50) contained 24-methylcholesta-5, 22-dienyl acetate (**10**, RRT 1.12). **10**: m/e 380 (M^+–AcOH), 351, 282, 267, 255, 188, and 213. The unresolved bands with Rf 0.38 were found to contain 24-norcholesta-5, 22-dienyl (**11**, RRT 0.66), cholesta-5, 22-dienyl (**12**, RRT 0.92), and 24-E-ethylidenecholesteryl (**13**, RRT 1.68) acetates. **11**: m/e 352 (M^+–AcOH), 337, 282, 267, 255, 253, 228, and 213. **12**: m/e 366 (M^+–

AcOH), 351, 282, 255, 253, 228, and 213. **13**: m/e 394 (M^+–AcOH), 379, 351, 281, 267, 255, 253, 228, and 213. The absence of molecular ion[12] and the ions[13] at m/e 282 due to the cleavage at C(20)–C(22) with one hydrogen transfer and the loss of acetic acid indicated that **11** and **12** were C_{26}–$\Delta^{5,22}$ and C_{27}–$\Delta^{5,22}$ steryl acetates, respectively. In conjunction with the RRT in GLC, **13** was characterized as 24-E-ethylidenecholesteryl acetate by the ions at m/e 296 (M^+–C–23 to C–29–1H–AcOH) and 281 (m/e 296–CH_3) characteristic of C–24 ethylidene steryl acetates. The most polar band (Rf 0.13) was identified as 24-methylenecholesteryl acetate (**14**, RRT 1.28). **14**: m/e 380 (M^+–AcOH), 296 (M^+–C–23 to C–28–1H–AcOH, 40%), 281 (m/e 296–CH_3), 255, 253, 228, and 213.

Table 2. Sterol composition of the floating lab-lab, adhering lab-lab, and snail.

	Sterol (as acetates)	RRT*1		Composition (%)		
		OV–17	OV–1	Floating lab-lab	Adhering lab-lab	Snail
	Cholestanol (**1**)	1.01	1.01	2.1	2.1	—
Saturated	24-Methylcholestanol (**3**)	1.29	1.28	0.2	0.3	—
	24-Ethylcholestanol (**5**)	1.61	1.61	0.2	0.8	—
	Cholesterol (**6**)	1.00	1.00	40.2	40.5	54.9
	Cholest-7-enol (**2**)	1.13	1.14	0.2	0.4	5.9
Monoene	24-Methylcholesterol (**7**)	1.28	1.27	14.0	6.3	12.6
	24-Ethylcholest-22-enol (**4**)	1.49	1.36	0.2	0.4	—
	24-Ethylcholest-5-enol (**8**)	1.59	1.58	7.2	8.5	3.9
	24-Norcholesta-5, 22-dienol (**11**)	0.66	0.66	0.5	0.8	0.5
	Cholesta-5, 22E-dienol (**12**)	0.94	0.92	3.1	5.5	6.7
	24-Methylcholesta-5, 22-dienol (**10**)	1.14	1.12	17.3	9.8	9.4
Diene	Cholesta-5, 24-dienol (**15**)	1.19	1.19	—	—	6.0
	24-Methylenecholesterol (**14**)	1.29	1.28	4.3	2.1	—
	24-Ethylcholesta-5, 22-dienol (**9**)	1.42	1.49	9.7	9.2	—
	24-E-Ethylidenecholesterol (**13**)	1.68	1.64	1.0	13.3	—

*1 Relative retention times of steryl acetates to cholesteryl acetate

The similar analyses of sterols from the adhering lab-lab also showed the occurrence of the same sterol components as those from the floating lab-lab (Table 2). As shown in Table 2, the sterol composition (%) of two types of lab-lab resembled each other, except for the occurrence of relatively large amounts of 24-E-ethylidenecholesterol in the adhering lab-lab. The present study showed that the floating and adhering lab-lab composed of mainly (50%) blue-green algae contained cholesterol (40.2 and 40.5% of total sterols), 24-methylcholesterol (14 and 6.3%), 24-ethylcholesterol (7.2 and 8.5%), 24-methylcholesta-5, 22-dienol (17.3 and 0.8%), and 24-ethylcholesta-5, 22-dienol (9.7 and 9.2%) as the prominent sterols.

Blue-green algae have been recognized to lack sterols in their tissues up to the last decade[15]. Later, the occurrence of sterols has been reported in all species examined. Cholesterol and 24-ethylcholesterol were detected in *Phormidium luridum*[16], *Anacyctis nidulans*[17], *Fremyella diplosiphon*[17], *Calothriv* sp[18]., *Nostoc commune*[18], and *Cyanidium caldarium*[18]. In the case of the blue-green alga, *Anabena cylindrica*[6], 24-methylcholesta-5, 22-dienol was found to be the exclusively major sterol (90%). Since the blue-green

Table 3. Fatty acid composition (%) of the floating lab-lab and adhering lab-lab.

Fatty acid	Composition (%)	
	Floating lab-lab	Adhering lab-lab
12:0	1.0	4.0
14:0	2.6	3.5
14:1	4.5	3.5
15:0	0.2	1.8
15:1	0.8	T*1
16:0	44.0	36.0
16:1	20.5	14.0
17:0	2.0	2.4
17:1	2.4	2.2
18:0	2.0	2.1
18:1ω9	4.6	3.1
18:2ω6	3.6	6.1
18:3ω6	1.5	2.1
18:3ω3	4.5	4.5
20:1ω9	0.9	3.0
20:2ω3	0.9	0.5
20:2ω6	0.5	0.6
20:3ω3	0.3	1.8
20:4ω6	0.6	0
20:4ω3	0.5	1.6
22:1ω9	T	0.1
20:5ω3	0.5	T
22:4ω6	0.3	T
22:4ω3	0.8	T
22:5ω6	0.6	3.1
22:6ω3	0.5	0.3
Saturates	51.8	49.8
Monoenes	33.7	29.4
Σω6	7.1	11.9
Σω3	8.0	8.7

*1 Less than 0.1%

algae contain in general cholesterol as the minor sterols, cholesterol occurring in the lab-lab was deduced to come from the zooplankton in the lab-lab. Whereas, 24-methyl- and 24-ethylsterols and their Δ^{22}-derivatives detected in the lab-lab were suspected to be mainly derived from the blue-green algae and other phytoplankton.

Sterols of the snail The snail contained cholesterol (54.9%), cholest-7-enol (5.7%), 24-methylcholesterol (12.6%), 24-ethylcholesterol (3.9%), cholesta-5, 22-dienol (6.7%), 24-methylcholesta-5, 22-dienol (9.4%), cholesta-5, 24-dienol (6.0%), and traces of 24-norcholesta-5, 22-dienol. Cholesta-5, 24-dienyl acetate (**15**): m/e 366 (M^+–AcOH), 351 (M^+–AcOH–CH_3), 342 (M^+–C–22 to C–27–1H), 255, 253, 228, 213, and 69. As compared with most gastropods reported in other studies[19,20], this snail was unique in the respect that it contained lesser amounts of cholesterol and considerably high amounts of other C_{27}-sterols such as cholest-7-enol and C_{28}- and C_{29}-sterols. Although it is difficult to withdraw definite reasons why the snail showed the unique sterol composition, we imagine that some sterols such as 24-methylcholesterol, 24-ethylcholesterol, and 24-methylcholesta-5, 22-dienol were partly derived from the lab-lab.

Fatty acids of the lab-lab Table 3 indicates the composition (%) of fatty acids of the floating and adhering lab-lab. In both two types of the lab-lab, palmitic (16: 0) and palmitoleic (16: 1) acids were the major fatty acids. In addition, the two types of the lab-lab contained substancial amounts of 18: 2ω6 and 18: 3ω3 but extremely low levels of 20: 5ω3 and 22: 6ω3. The sum of ω3-series of fatty acids amounted to 8.0 and 8.7% of total acids in the floating and adhering lab-lab, respectively. Except for *Tilapia zillii*[21], most fish has been shown to require ω3 fatty acids such as 18: 3ω3, 20: 5ω3, and 22: 6ω3 rather than ω6 fatty acids such as 18: 2ω6 and 20: 4ω6[2-4]. Especially, marine fish such as the red sea bream etc[4]. were demonstrated to require 20: 5ω3 but not 18: 3ω3. Therefore, the question araises whether the milkfish requires strictly 20: 5ω3 as essential fatty acids as well as other marine fish and possesses the ability for conversion of dietary 18: 3ω3 to 20: 5ω3 and 22: 6ω3, because the lab-lab, their primary food, contained very small amounts of 20: 5ω3 and 22: 6ω3. This point should be investigated in detail by the feeding trials and also by the tracer experiments in future.

References

1) Drews R. A.: in "Fish as Food" (ed. by Borgstrom G.), Vol. 1, Academic Press, New York and London, 1961, pp. 121–143.
2) Cowey C. B. and J. R. Sargent: *Comp. Biochem. Physiol.*, **57B**, 269–273 (1977).
3) Takeuchi T.: in "Yogyo to Shiryo-Shishitsu" (ed. by Japan. Soc. Sci. Fish.), Suisangaku Ser. No. 22, Koseisha Koseikaku, Tokyo, 1978, pp. 23–42.
4) Yone Y.: in "Yogyo to Shiryo-Shishitsu" (ed. by Japan. Soc. Sci. Fish.), Suisangaku Ser. No. 22, Koseisha Koseikaku, Tokyo, 1978, pp. 43–59.
5) Teshima S.: in "Yogyo to Shiryo-Shishitsu" (ed. by Japan. Soc. Sci. Fish.), Suisangaku Ser. No. 22, Koseisha Koseikaku, Tokyo, 1978, pp. 60–77.

6) TESHIMA S. and A. KANAZAWA: *Bull. Japan. Soc. Sci. Fish.*, **38**, 1197–1202 (1972).
7) TESHIMA S. and A. KANAZAWA: *Bull. Japan. Soc. Sci. Fish.*, **37**, 63–67 (1971).
8) TESHIMA S., A. KANAZAWA and H. OKAMOTO: *Mem. Fac. Fish. Kagoshima Univ.*, **25**, 41–46 (1976).
9) TESHIMA S., A. KANAZAWA and T. ANDO: *Mem. Fac. Fish. Kagoshima Univ.*, **20**, 131–139 (1971).
10) RUBINSTEIN I. and L. J. GOAD: *Phytochemistry*, **13**, 481–484 (1974).
11) TESHIMA S., A. KANAZAWA, S. HYODO and T. ANDO: *Comp. Biochem. Physiol.*, **64B**, 225–228 (1979).
12) KNIGHTS B. A.: *J. Gas-chromatog.*, **5**, 273–282 (1967).
13) WYLLIE S. G. and C. DJERASSI: *J. Org. Chem.*, **33**, 305–313 (1968).
14) ERDMAN T. R. and R. H. THOMSON: *Tetrahedron*, **28**, 5163–5173 (1972).
15) AUSTIN J.: in "Advances in Steroid Biochemistry and Pharmacology" (ed. by BRIGGS M. H.), Vol. 1, Academic Press, London, 1970, pp. 73–96.
16) DE SOUZA N. J. and W. R. NES: *Science*, **162**, 363 (1968).
17) REITZ R. C. and J. G. HAMILTON: *Comp. Biochem. Physiol.*, **25**, 401–416 (1968).
18) PAOLETTI C., B. PUSHPARAJ, G. FLORENZANO, P. CAPELLA and G. LERCKER: *Lipids*, **11**, 266–(1976).
19) IDLER D. R. and P. WISEMAN: *Int. J. Biochem.*, **2**, 516–528 (1971).
20) GOAD L. J.: in "Marine Natural Products, Chemical and Biological Perspectives" (ed. by SCHEUER P. J.), Vol. II, Academic Press, New York, San Francisco, and London, 1978, pp. 75–172.
21) KANAZAWA A., S. TESHIMA, M. SAKAMOTO and Md. A. AWAL: *Bull. Japan. Soc. Sci. Fish.*, **46**, in press (1980).