RESEARCH REPORTS

FATTY OILS OF AQUATIC INVERTEBRATES

XII. THE FATTY OILS OF *BUCCINUM* (*VOLUTHARPA*) *PERRYI*, *TEGULA* (*CHLOROSTOMA*) *ARGYROSTOMA SUBLAEVIS* AND *MYTILUS EDULIS* WITH A PARTICULAR REFERENCE TO THEIR STEROL COMPONENTS

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Of the three kinds of shellfish used for this study, *Buccinum* (*Volutharpa*) *perryi* and *Tegula* (*Chlorostoma*) *argyrostoma sublaevis* belong to the Gastropoda, while *Mytilus edulis* belongs to the Bivalvia. While there appears no literature on the fatty oil components of the former two, the fatty oil and lipid of *Mytilus edulis* have frequently been reported, and its fatty acid components were investigated by Lovern.[1] *M. edulis* is reported to contain a relatively large amount of provitamin D. According to a study by van der Vliet,[2] the provitamin D of *M. edulis* consists of 7-dehydrocholesterol, ergosterol, $\Delta^{5,7,22}$-cholestatrien-3-ol and a member of unknown structure in a proportion of 3 : 1 : 1–2 : 1.

The authors have procured the above mentioned three kinds of shellfish caught in Hokkaido, and examined their fatty oils with a particular reference to their sterol components. The acetates from the crude sterol mixtures of *B. perryi* and *T. arg. sublaevis* were found to have a little higher iodine values than cholesteryl acetate, and fractional crystallization of the crude steryl acetates gave fractions which had higher melting points than cholesteryl acetate. From these results it was indicated that the sterol components of *B. perryi* and *T. arg. sublaevis* consisted chiefly of cholesterol together with a lesser amount of other sterols. A fraction which was recognized as cholesteryl acetate was separated by fractional crystallization of the crude steryl acetates from both shellfish. Further, in the case of *T. arg. sublaevis*, cholesteryl acetate was also separated by bromination of a steryl acetate fraction followed by removal of the ether-insoluble bromide, formed in a quite minor quantity, and debromination of the ether-soluble bromide. The crude sterol mixture from *M. edulis* was fractionated by fractional crystallization of its acetate and fractional precipitation of brominated acetate, and a F_2-sterol fraction consisting mainly of poriferasterol was obtained from the ether-insoluble bromide fraction while three F_1-sterol fractions corresponding to clionasterol, β-sitosterol and cholesterol, respectively, were obtained from ether-soluble bromide fractions. The sterol components thus separated in this study are summarized in Table 1.

TABLE 1. Properties of Sterols and their Derivatives

	Free sterol		Acetate		Benzoate	
	m.p. (°C)	$[\alpha]_D^\circ$	m.p. (°C)	$[\alpha]_D^\circ$	m.p. (°C)	$[\alpha]_D^\circ$
B. perryi						
Sterol obtained by fract. crystallization of acetate	143.5–144.5	−38.6	114–115	−42.4		
T. arg. sublaevis						
Sterol obtained by fract. crystallization of acetate	142.5–143.5	−40.9	114–115	−43.9		
Sterol obtained from bromide	145–146	−38.3	113.5–114.5	—		
M. edulis						
Sterol from bromide I	150–152	−48.0	143–145	−51.5		
Poriferasterol[3]	155–156	−49.7	146.5–147	−53		
Sterol from bromide II	136–138	−37.5	135.5–137	−42.4	132–134	−17.6
Clionasterol[3]	137.5–138.5	−37	137	−41.9	134.5–135	−16.8
Sterol from bromide III	134–135.5	−34.8	122–124	−38.6		
β-sitosterol[4]	136–137	−36.6	125–126	−41.0		
Sterol from bromide IV	143–145	−39.1	114–115	−42.6		
Cholesterol	148	−38	114	−43		

Provitamin D contents of the crude sterol mixtures from *B. perryi*, *T. arg. sublaevis* and *M. edulis* were found to be about 1%. Such a low level of provitamin D content for *M. edulis* is remarkably different from the data reported by previous authors. However, this may be regarded as an instance of the characteristic feature repeatedly observed by the authors that shellfish of the same species caught at different localities often show a great variance in the content of certain sterol components.

In the case of *M. edulis*, the solid unsaponifiable fraction obtained after removal of sterols was recrystallized from acetone, giving a crystalline substance which was recognized as a mixture of batyl and chimyl alcohols. The unsaponifiable fraction obtained after removal of sterols and a greater part of other solid components was acetylated, and the product was fractionally distilled with the results that there was evidence to indicate the presence of a fraction of unsaturated alcohol having a high acetyl value, possibly of the selachyl alcohol series, together with some methanol-insoluble matter (hydrocarbon?).

Experimental

1. *Buccinum* (*Volutharpa*) *perryi*

The material used in this study is the dried meat* of *B. perryi* received in January, 1954. It was prepared from fresh shellfish caught in Hokkaido by lightly boiling the whole shellfish (5,468 g) with water, removing the shell, and drying the meat (3,480 g) under exposure to infrared radiation. The dried meat (990 g) was reduced to powder and extracted with ether giving 46 g (4.6% on the basis of dried meat) of an ether-extract (lipid) which was a viscous liquid of dark reddish orange color. The ether-extract was refluxed with about tenfold acetone for a

* All the dried meats from the three kinds of shellfish used in this study were received by the courtesy of Dr. M. Yamada, Faculty of Fisheries, Hokkaido University.

while, the solution was cooled to the ordinary temperature, the insoluble portion (phosphatide) was removed by filtration, and 22.3 g (48.5% on the basis of ether-extract) of an acetone-soluble oil was obtained. This was a viscous liquid of dark reddish orange color with some solid and had the following characteristics: d_4^{50} 0.9525, n_D^{50} 1.4858, acid value 32.6, saponification value 142.7, iodine value* 134.6, unsaponifiable matter 26.88%.

The fatty acids and unsaponifiable matter were separated from the acetone-soluble oil in the usual way. The fatty acids had a dark reddish orange color and solidified at the ordinary temperature: Neutr. V. 187.8, I.V. 130.4, ether-insoluble bromides 30.8%, solid acids (I.V. 28.8) by the lead salt ethanol method 29.9%.

The unsaponifiable matter was a yellowish orange crystalline solid with some liquid and had I.V. 80.4 and sterol content (digitonide method) 66.78%. Recrystallization of the unsaponifiable matter (4.7 g) from methanol yielded a crystalline solid (3.0 g) consisting of crude sterols. The crude sterol mixture had m.p. 142°–143°C, I.V. 74.6 and $\Delta^{5,7}$-sterol content 1.05%. The acetate mixture from the crude sterols showed m.p. 112°–115°C, S.V. 129.1 and I.V. 69.1. This was separated into seven fractions by fractional crystallization from methanol and ethanol. The first fraction had m.p. 118°–119°C, $[\alpha]_D^{25} = -43.4°$ † and S.V. 129.4. The succeeding fractions had lower melting points in turn, and the seventh fraction obtained in a quite minor amount had m.p. 106°–109°C. The sixth fraction which was obtained in a relatively large amount had m.p. 114°–115°C, $[\alpha]_D^{27} = -42.4°$, S.V. 130.1 and I.V. 64.2 (calcd. for cholesteryl acetate: S.V. 130.9, I.V. 59.2). Saponification of this fraction yielded a free sterol which, after recrystallization from methanol, had m.p. 143.5°–144.5°C, $[\alpha]_D^{25} = -38.6°$ and I.V. 70.2 and showed no depression of melting point on admixture with cholesterol.

2. *Tegula* (*Chlorostoma*) *argyrostoma sublaevis*

The material used in this study is the dried meat of *T. arg. sublaevis* received in October, 1953. This material (536 g) was prepared by drying the meat (1,383 g) from fresh whole shellfish (7,875 g), caught in Hokkaido, under exposure to infrared radiation. After the same treatment of this dried meat as in the case of *B. perryi*, 31 g (5.8% based on the dried meat) of an ether-extract and 26.1 g (84.2% based on ether-extract) of an acetone-soluble oil were obtained. The acetone-soluble oil was a viscous liquid with some solid and had a dark reddish orange color with green dash. It had the following properties: d_4^{40} 0.9269, n_D^{40} 1.4762, A.V. 66.3, S.V. 173.9, I.V. 119.6, Unsap. M. 15.67%. The fatty acids prepared from this oil solidified at the ordinary temperature and showed N.V. 195.2, I.V. 114.8, ether-insoluble bromides 26.3% and solid acids (I.V. 24.7) by the lead salt ethanol method 29.1%.

The unsaponifiable matter had I.V. 82.6 and sterol content 58.72%. The crude sterol mixture (1.8 g) obtained by recrystallization of the unsaponifiable matter (3.3 g) from methanol showed m.p. 140°–142°C, I.V. 77.8 and $\Delta^{5,7}$-sterol content 1.20%, and its acetate had m.p. 113°–116°C, S.V. 129.1 and I.V. 71.4.

Fractionally crystallizing the crude steryl acetate mixture from methanol and ethanol, six fractions were obtained. The first fraction had m.p. 120°–121°C, $[\alpha]_D^{25}$

* Iodine values were determined by the Wijs method for fatty oils and fatty acids and by the pyridine sulfate dibromide method for unsaponifiable components including sterols.

† All optical rotations were measured in chloroform.

$= -46.3°$, S.V. 130.8 and I.V. 69.8. The succeeding fractions had lower melting points in turn, and the sixth fraction obtained in a minor amount had m.p. 100°–104°C. The fourth fraction had m.p. 114°–115°C, $[\alpha]_D^{27} = -43.9°$, S.V. 130.8 and I.V. 66.6. Saponification of this fraction yielded a free sterol which, after recrystallization from methanol, showed m.p. 142.5°–143.5°C, $[\alpha]_D^{25} = -40.9°$ and I.V. 72.2. The third and fifth fractions were united and dissolved in ether. Bromine in acetic acid was added to this solution, and the ether-insoluble bromides formed in a quite minor amount were removed by filtration. After removal of excess bromine from the filtrate, methanol was added to the solution, and a white solid bromide of m.p. 115°–117°C and Br-content 27.51% (calcd. for $C_{29}H_{48}O_2Br_2$: Br 27.16%) was separated. Debromination of this bromide with zinc and acetic acid followed by recrystallization of the debrominated product from ethanol yielded a crystalline steryl acetate of m.p. 113.5°–114.5°C. The free sterol from this acetate had m.p. 145°–146°C and $[\alpha]_D^{29} = -38.3°$ after recrystallization from methanol.

3. *Mytilus edulis*

The material used in this study is the dried meat (1,818 g) of *M. edulis* received in December, 1952. It was prepared by sun-drying the meat (5,680 g) from fresh whole shellfish (8,865 g) caught in Hokkaido. The ether-extract (150 g, 8.3% on the basis of dried meat) was a viscous liquid of dark reddish orange color. On treatment with acetone this extract yielded 109 g (72.7% on the basis of ether-extract) of an acetone-soluble oil: d_4^{25} 0.9538, n_D^{25} 1.4825, A.V. 60.0, S.V. 188.0, I.V. 143.5, Unsap. M. 10.40%. The fatty acids prepared from the acetone-soluble oil had a dark reddish orange color, solidified at the ordinary temperature and showed N.V. 191.8, I.V. 147.8, ether-insoluble bromides 43.6% and solid acids (I.V. 26.8) by the lead salt ethanol method 25.5%.

The unsaponifiable matter obtained from the acetone-soluble oil was a crystalline solid containing some liquid, and contained 50.05% of sterols.

(*a*) *Sterol fraction.*—The crude sterol mixture (5.8 g) obtained by recrystallization of the unsaponifiable matter (11.7 g) from methanol had m.p. 135°–137°C, I.V. 95.1 and $\Delta^{5,7}$-sterol content 1.21%. The acetate from the crude sterol mixture had m.p. 108°–110°C, and its melting point was raised by recrystallization from ethanol and acetone. But the material was exhausted before its melting point has become constant. Three fractions of steryl acetate were recovered from mother liquors of recrystallization: (*A*) 1.4 g, m.p. above 130°C; (*B*) 1.3 g, m.p. between 121°C and 125°C; (*C*) 2.6 g, m.p. below 115.5°C. These three fractions were brominated in ether by adding a solution of bromine in acetic acid, and the ether-insoluble bromides formed were filtered off. After removal of excess bromine, the bromides remaining in the ether solution were fractionally precipitated by adding methanol to the solution. In this way, the acetate fraction *A* gave the bromide I (0.5 g) as ether-insoluble bromide and the bromide II (0.6 g) as a fraction of ether-soluble bromide. The acetate fractions *B* and *C* gave the bromides III (0.6 g) and IV (0.5 g), respectively, as fractions of ether-soluble bromide.

Recrystallization of the bromide I from chloroform-methanol yielded white laminae: m.p. 190°–192°C (decomp.), $[\alpha]_D^{15} = -40.3°$, Br-content 40.91% (calcd. for poriferasteryl acetate tetrabromide $C_{31}H_{50}O_2Br_4$: Br 41.28%). Debromination of this bromide gave a steryl acetate which, after recrystallization from ethanol, had m.p.

143°–145°C, $[\alpha]_D^{17} = -51.5°$C and S.V. 123.9 (calcd. for $C_{31}H_{50}O_2$: S.V. 123.4). The free sterol from this acetate showed m.p. 150°–152°C, $[\alpha]_D^{17} = -48.0°$ and I.V. 120.5 after recrystallization from methanol (calcd. for $C_{29}H_{48}O$: I.V. 123.0).

The bromide II had Br-content 26.83% (calcd. for $C_{31}H_{52}O_2Br_2$: Br 25.92%), and its debromination product showed, after recrystallization from ethanol, m.p. 135.5°–137°C, $[\alpha]_D^{17} = -42.4°$, S.V. 123.0 and I.V. 57.1 (calcd. for $C_{31}H_{52}O_2$: S.V. 122.8, I.V. 55.6). On saponification, this acetate yielded a free sterol which after recrystallization from methanol had m.p. 136°–138°C and $[\alpha]_D^{16} = -37.5°$, and its benzoate showed after recrystallization from ethanol m.p. 132°–134°C and $[\alpha]_D^{16} = -17.6°$. When the melted benzoate was allowed to stand for cooling, it developed a fine blue-purple color during its solidification.

The bromide III had m.p. 120°–122°C, and its debromination product showed after recrystallization from ethanol-methanol m.p. 122°–124°C, $[\alpha]_D^{15} = -38.6°$, S.V. 122.8 and I.V. 58.6. Saponification of this acetate yielded a free sterol of m.p. 134°–135.5°C and $[\alpha]_D^{15} = -34.8°$.

The bromide IV showed m.p. 115°–116°C and Br-content 28.02%. Its debromination product, after recrystallization from ethanol, had m.p. 114°–115°C, $[\alpha]_D^{14} = -42.6°$, S.V. 130.2 and I.V. 63.1. Saponification of the acetate yielded a free sterol of m.p. 143°–145°C and $[\alpha]_D^{11} = -39.1°$ which showed no depression of melting point on admixture with cholesterol. The steryl acetate of m.p. 114°–115°C was hydrogenated in acetic acid with platinum black as a catalyst, and the stanyl acetate obtained showed m.p. 106°–108°C and $[\alpha]_D^{11} = +10.2°$ after recrystallization from methanol (cholestanyl acetate[5]: m.p. 109°–110°C, $[\alpha]_D^{20} = +11.5°$).

(*b*) *Non-sterol fraction.*—Since the material remaining after removal of the crude sterol mixture from the unsaponifiable matter was contaminated more or less with saponifiable matter, it was freed from the saponifiable matter. In this way, 4.2 g of semi-solid unsaponifiable matter was recovered. This was recrystallized from acetone, and there was obtained 0.7 g of crystalline solid which showed still a positive Liebermann-Burchard reaction. Sterol was removed from this solid using digitonin, and 0.69 g of semi-solid substance showing no Liebermann-Burchard reaction was recovered. Recrystallization of this substance from acetone yielded laminae of m.p. 64°–66°C and acetyl V. 265.7 (batyl alcohol, $C_{21}H_{44}O_3$: m.p. 70°–71°C, acetyl V. 261.8; chimyl alcohol, $C_{19}H_{40}O_3$: m.p. 60.5°–61.5°C, acetyl V. 280.1).

The material remaining in the acetone filtrate of the initial recrystallization was recovered and acetylated. The product (3.5 g) was fractionally distilled with the results shown in Table 2.

TABLE 2. Fractional Distillation of Acetate

Fraction	b.p. (°C/4 mmHg)	Yield (g)	S.V.	I.V.
1	-200	0.20	136.7	45.8
2	200–225	0.20	153.8	43.6
3	225–235	0.42	182.0	41.7
4	235–250	1.05	191.0	46.8
5	250–274	0.53	152.4	82.9
Residue (diff.)	—	1.10	—	—

The fraction 4 in Table 2 was saponified, and the product was dissolved in acetone. On cooling the solution with ice, a minor amount of solid separated was filtered off. The residue obtained after distilling acetone from the filtrate was separated into the methanol-insoluble and methanol-soluble fractions. The methanol-soluble fraction had acetyl V. 251.9 and I.V. 68.3. Hydrogenation of this fraction with Raney-nickel as a catalyst yielded a solid which showed m.p. about 60°C after recrystallization from 90% ethanol.

Summary

Characteristics of the oils extracted from *Buccinum* (*Volutharpa*) *perryi*, *Tegula* (*Chlorostoma*) *argyrostoma sublaevis* and *Mytilus edulis* were determined.

Sterols of *B. perryi* and *T. arg. sublaevis* were found to contain cholesterol as a main component.

In the sterol components of *M. edulis*, the presence of poriferasterol, clionasterol, β-sitosterol and cholesterol was indicated. In the non-sterol components of *M. edulis*, the presence of batyl and chimyl alcohols and possibly also unsaturated alcohol of the selachyl alcohol series was indicated.

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