Sterols and Other Unsaponifiable Substances in the Lipids of Shell Fishes, Crustacea and Echinoderms. XV. Occurrence of $\Delta^{7:8}$-Cholestenol as a Sterol Component of Star Fish, Asterias amurensis Lütken

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It was shown in the 5th report[1] of this series that the unsaponifiable substances of the lipid of star fish,* *Asterias amurensis* Lütken, caught around Himagajima Island in Aichi Prefecture contained batyl alcohol as a non-steroid component and hitodesterol** as a sterol component. The present paper describes the results of our studies on the sterol components separated from the star fish of the same species, caught around the southern coast of Hokkaido. The acetate of crude sterol mixture was subjected to fractional crystallization, by which an acetate fraction having a constant melting point of 119—120° and other higher melting fractions were easily separated. The acetate of m.p. 119—120° had a saponification value and an iodine value which agreed closely with the calculated values for cholestenyl acetate. The melting points of the acetate, free sterol and benzoate were found to be close to those reported by previous authors for the corresponding derivatives of $\Delta^{7:8}$-cholestenol (γ-cholestenol). The identity of this star fish sterol with

1) M. Matsumoto, M. Yajima and Y. Toyama, *J. Chem. Soc. Japan*, **64**, 1203 (1943).

* The name of the species *Asterias amurensis* Lütken, was formerly designated as *Asterias rollestoni* Bell.

** Later investigation in our laboratory showed that this sterol has the formula $C_{28}H_{46}O$ or $C_{29}H_{48}O$, having a close resemblance to stellasterol, chondrillasterol and spinasterol, possibly identical with one of them. Cf. The 13th report of this series, Y. Toyama and T. Takagi, This Bulletin, **27**, 39 (1954).

Δ 7 : 8-cholestenol was demonstrated further by conversion of the steryl acetate to Δ 8 : 14-isomer (α-cholestenyl acetate) and Δ 7 : 8, 9-11-cholestadienyl acetate. The higher melting acetate fractions separated from the acetate fraction of m.p. 119—120° yielded, after further recrystallizations a fraction of m.p. 148—153°. Though this fraction was not a single steryl acetate, the easy conversion to its isomer and the saponification and iodine values of the latter appeared to indicate that the fraction of m.p. 148~153° contains predominantly the acetate of Δ 7 : 8-sterol of C_{28} or C_{29} series.

Comparing the above results with those obtained in the previous study, there exists a remarkable difference. While batyl alcohol was easily separated in the previous study, it was not so in the present study. This may be attributable to a far smaller content, if any, of batyl alcohol in the unsaponifiable substances of the present lipid sample. While di-unsaturated hitodesterol was found in the previous study, the sterol mixture of the present lipid sample, as a whole, consisted mainly of mono-unsaturated sterols which contained convincibly Δ 7 : 8-cholestenol. Analogous instances when striking differences exist within an individual lot of aquatic invertebrate of the same species in its unsaponifiable component have already been noted in the case of chiton[2] and corbicula[3]. These facts may possibly be mentioned as a characteristic feature of the unsaponifiable substances of the lipids of some aquatic invertebrates.

Experimental

Some sun-dried star fish, *Asterias amurensis* Lütken, used for these experiments were received from M. Yamada of the Faculty of Fisheries, Hokkaido University. The fish were caught around the southern coast of Hokkaido. The sun-dried material (952 g.) was cut into small pieces. Extraction with ether yielded 84 g. of a dark reddish orange viscous lipid. On treating the lipid with one liter of acetone, an acetone-insoluble oil (73 g.) with the following constants was obtained: d_4^{25} 0.9266, n_D^{25} 1.4733, acid value 71.2, saponification value 162.5, iodine value (Wijs method) 141.5, unsaponifiable matter 15.93%.

Unsaponifiable matter. — The unsaponifiable matter obtained from the acetone-soluble oil by the usual method was a reddish orange solid. The sterol content in the unsaponifiable matter was found to be 52.9% by the digitonide method. The unsaponifiable matter (8.5 g) was recrystallized from 80 cc. of ethanol, yielding a crude sterol (4.2 g.) of m.p. 115–117°. Acetylation of the crude sterol gave a crude steryl acetate of m.p. 115–118° and iodine value* 63.8. Four grams of this acetate was repeatedly recrystallized as shown in Table I.

TABLE I

No. of recrystallization	Solvent	Acetate, crystallized out		Acetate, recovered from mother liquor	
		Yield (g.)	m.p. (°C.)	m.p. (°C.)	Iodine V.
1	Acetone	2.5	125–129	115–116	60.1
2	,,	1.6	127–131	115–116	—
3	,,	0.7	127–137	121–122	66.1
4	Ethanol-ether	0.3	133–145	125–127	66.4
5	Acetone	0.25	140–147	125–132	—
6	,,	0.18	142–147	128–138	—
7	,,	0.12	148–153	135–138	—

Acetate of m.p. 119–120°.—The acetates recovered from the mother liquors of recrystallizations, Nos. 1, 2 and 3, in Table I were united and fractionally crystallized from acetone. After removing a small amount of high melting fraction, a fraction (1.4 g.) of m.p. 119–120° was separated. The melting point was unaltered by further recrystallization from acetone and ethanol-ether, and the fraction recovered from the mother liquors of further recrystallizations showed the same melting point. The fraction of m.p. 119–120° showed $[\alpha]_D^{20} = +5.1°$**, saponification value 131.7 and iodine value 60.5 (calculated for $C_{29}H_{48}O_2$: saponification value 130.8 and iodine value 59.2). Free sterol obtained by saponification of this fraction showed, after recrystallization from ethanol, m.p. 122–123° and $[\alpha]_D^{15} = +4.0$. Benzoate prepared from the free sterol showed, after recrystallization from acetone, m.p. 154–155° and $[\alpha]_D^{15} = +6.8°$.

Δ 8 : 14-Cholestenyl acetate.—The acetate fraction (0.12 g.) of m.p. 119–120° was dissolved in

2) Y. Toyama and T. Tanaka, This Bulletin, **26**, 497 (1953).

3) Y. Toyama and T. Tanaka, This Bulletin, **27** 264 (1954).

* Unless stated otherwise, the iodine values recorded in this paper were determined by the perbenzoic acid method. The pyridine sulfate dibromide method gave an enormously high iodine value of 185.4 for this steryl acetate.

** All rotation were measured with the samples dissolved in chloroform.

15 cc. of glacial acetic acid, and the solution was agitated for 3 hours in an atmosphere of hydrogen in the presence of palladium black. Hydrogen was not absorbed, but isomerization occurred, giving a product of m.p. 76–78° and $[\alpha]_D^{14}=+10.8°$. Free strerol from this product showed m.p. 118–120°.

Δ 7 : 8, 9 : 11-Cholestadienyl acetate.—A solution of 1 g. of the acetate fraction of m.p. 119–120° in 15 cc. of chloroform was mixed with a solution of 2 g. of mercuric acetate in 30 cc. of glacial acetic acid, and the mixture was allowed to stand for 18 hours with frequent stirring.[4] The product, after purification by fractional crystallization from methanol and acetone, showed m.p. 113–115° and $[\alpha]_D^{15}=+32.1°$. It exhibited an ultraviolet absorption curve as shown in Fig. 1

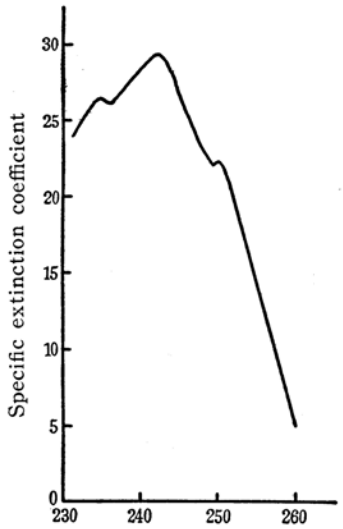

Fig. 1

4) Cf. W. Bergmann and P. G. Stevens, *J. Org. Chem.*, **13**, 10 (1948); W. V. Ruyle et al., *J. Am. Chem. Soc.*, **75**, 2604 (1953).

with the characteristic absorption maxima of 7 : 8, 9 : 11-conjugated sterol; log ε_{235} 4.05, log ε_{243} 4.10 and log ε_{250} 3.98 (Fieser[5] give 4.10, 4.15 and 3.96 for the corresponding values).

Higher melting acetate fraction.—The acetate fraction of m.p. 148–153° in Table I had iodine value 67.5. Free sterol from this fraction had m.p. 146–149° and $[\alpha]_D^{15}=+4.3°$. On treating this acetate fraction in a way similar to that described for the acetate fraction of m.p. 119–120°, it underwent isomerization with little hydrogen absorption. The isomerization product, after recrystallization from methanol, showed m.p. 111–113°, saponification value 125.1 (calculated : $C_{30}H_{50}O_2$ 126.7, $C_{31}H_{52}O_2$ 122.8) and iodine value 60.1 (calculated : $C_{30}H_{50}O_2$ 57.3, $C_{31}H_{52}O_2$ 55.6).

Summary

Sterol mixture of star fish, *Asterias amurensis* Lütken, caught around the southern coast of Hokkaido has been found to consist largely of mono-unsaturated sterols. By fractional crystallization of steryl acetate mixture, an acetate fraction of m.p. 119—120° and a higher melting fraction were separated. The fraction of m.p. 119—120° was identified with the acetate of Δ 7 : 8-cholestenol. The higher melting fraction was not a single steryl acetate, but it appeared to contain predominantly the acetate of Δ 7 : 8-sterol of C_{28} or C_{29} series.

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5) L. F. Fieser, *J. Am. Chem. Soc.*, **73**, 5007 (1951).