Sterols and Other Unsaponifiable Substances in the Lipids of Shell Fishes, Crustacea and Echinoderms. XIII. Sterol Components of Star Fish, Luidia quinaria von Martens

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Among the sterol components of star fish, stellasterol ($C_{27}H_{44}O$, m.p. 149~150°, m.p. of its acetate 176~177°)[1] in *Astropecten aurantiacus* and asteriasterol (m.p. 70°)[2] in *Asterias forbesi* were known in earlier times. Later studies,[3] however, revealed that asteriasterol, like astrol[4] in *Astropecten aurantiacus*, is identical with batyl alcohol. Bergmann and Stansbury[5] separated a sterol fraction resembling stellasterol from *Asterias forbesi*, but they found that this fraction is not a single sterol but a mixture of two sterols, $C_{28}H_{46}O$ (F_2) and $C_{28}H_{48}O$ (F_1). Although neither components were separated from each other, both components were shown to have an ethylenic linkage at C-8, the di-unsaturated component having one more ethylenic linkage at the 22:23-position. These authors retained the name stellasterol for the di-unsaturated $C_{28}H_{46}O$, and designated the mono-unsaturated $C_{28}H_{48}O$ as stellastenol. According to Barton,[6] who examined the relations between the optical activities of these sterols and their esters, the ethylenic linkage common to stellasterol and stellastenol is located at the 7:8-position. One of the present authors, Toyama, and Matsumoto[7] separated hitodesterol (m.p. 167~168°, m.p. of its acetate 181~182°) from *Asterina pectinifera* (Müller et Troschel). Although the formula $C_{28}H_{42}O$ or $C_{29}H_{44}O$ was given for hitodesterol at that time, this formula has been found to be erroneous by later investigation in our laboratory[8] and hitodesterol was assumed now to have 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. Meanwhile, the occurrence

1) A. Kossel and S. Edelbacher, *Z. Physiol. Chem.*, **94**, 264 (1915).
2) J.H. Page, *J. Biol. Chem.*, **57**, 471 (1923).
3) l. c., (*J*).
4) T. Matsumoto, M. Yajima and Y. Toyama, *J. Chem. Soc. Japan*, **64**, 1203 (1943); W. Bergmann and H. A. Stansbury, Jb., *J. Org. Chem.*, **8**, 283 (1943).
5) W. Bergmann and H.A. Stansbury, Jb., *J. Org. Chem.*, **9**, 281 (1944).
6) Cf. L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene" 1949, p. 297.
7) T. Matsumoto and Y. Toyama, *J. Chem. Soc. Japan*, **64**, 1069 (1943).
8) Y. Toyama, not yet published.

of another sterol (m.p. 144~146°, m.p. of its acetate 160~162°) in *Asterina pectinifera* (*Patiria pectinifera*) was reported by Kuwata and Ban.[9] This sterol, named patiriasterol, was concluded to be identical with $\Delta^{22:23}$-ergostenol by these authors.

In this paper, the results of our studies on the sterol components of star fish, *Luidia quinaria* von Martens, are recorded. The sterol mixture, as a whole, consisted chiefly of mono-unsaturated sterol. The acetate of the sterol mixture was subjected to fractional crystallization, yielding several acetate fractions which had somewhat different iodine values by the perbenzoic acid method. The acetate fraction of higher melting point showed a higher iodine value than the acetate fraction higher iodine value than the acetate fraction of lower melting point, indicating the presence of a lesser amount of saturated and di-unsaturated sterols in addition to mono-unsaturated sterol in the original sterol mixture. By repeated fractional crystallizations of the acetate mixture, two sterol fractions I (m.p. 145~147°, m.p. of its acetate 157°) and II (m.p. 135°, m.p. of its acetate 149°) were obtained. Both fractions consisted of mono-unsaturated sterol. The acetates of the fractions I and II absorbed no hydrogen in the presence of palladium black in glacial acetic acid, but they underwent isomerization, as is the case with a $\Delta^{7:8}$-sterol. Bromination-debromination of the acetate of the fraction I resulted in the formation of 7:8, 9:11-conjugation which presented a further evidence for the presence of 7:8-ethylenic linkage in the fraction I.[10] Although the fraction II might consist of another $\Delta^{7:8}$-sterol which is different from the $\Delta^{7:8}$-sterol of the fraction I, it is also possible that both fractions consist of the same $\Delta^{7:8}$-sterol and differ only in their purity. Comparing the fraction I and patiriasterol, the melting points of free sterols and their corresponding esters are fairly close to each other, but they differ decisively in their behavior toward hydrogenation. While patiriasterol has its ethylenic linkage at the 22:23-position, the ethylenic linkage of the fraction I is located convincibly at the 7:8-position. On the other hand, the fraction I agrees with stellastenol in the position of ethylenic linkage, both having 7:8-ethylenic linkage, but it is not yet decided whether the sterol in the fraction I is C_{28}-sterol or C_{29}-sterol. For the sake of comparison, the properties of γ-spinastenol (C_{29}), $\Delta^{7:8}$-ergostenol, patiriasterol, and the sterol fractions I and II, together with some derivatives of the respective sterols are shown in Table I.

TABLE I

	Free sterol		Acetate		Benzoate		α-Isomer		Acetate of α-isomer	
	m.p., °C	$[\alpha]_D^\circ$	m.p., °C	$[\alpha]_D^\circ$	m.p., °C	$[\alpha]_D^\circ$	m.p., °C	$[\alpha]_D^\circ$	m.p., °C	$[\alpha]_D^\circ$
γ-Spinastenol (11)	144–145	+11	156–157	+8	180.5	+13	112–113	+23	116–117	+12
$\Delta^{7:8}$-Ergostenol (12)	148	−2	157–159	+4	180.5	+2	132	+11	109	0
Patiriasterol	144–146	+4.4	160–162	+4.2	175–176	+7.1	—	—	—	—
Sterol fract. I	145–147	+9	157	+6	177	—	115	—	111–112	+18
Sterol fract. II	135	+2	149	+2	171	—	114	—	115	+8

Experimental

A lot of star fish, *Luidia quinaria* von Martens, caught around the Osaki-Shimojima Island in the Inland Sea of Seto in August, 1952, were sun-dried. The dried material (1,030 g.) was cut into small pieces and then extracted with ether, yielding 29 g. (2.8%) of ether extract which was a viscous liquid of reddish orange color and deposited a large amount of solid in the winter season. On treating the ethereal extract with 300 cc. of acetone, 22.1 g. of acetone-soluble portion (fat) and 6.9 g. of acetone-insoluble portion (phosphatide) were separated. The acetone-soluble portion was a reddish orange oil having the following characteristics: d_4^{20} 0.9405, n_D^{20} 1.4740, acid value 111.8, saponification value 143.9, iodine value by the Wijs method 132.4, unsaponifiable matter 28.16%.

The acetone-insoluble portion gave only 0.3 g. of unsaponifiable matter by extraction of the saponified product with ether. The unsaponifiable matter (6 g.) obtained from the acetone-soluble

9) S. Kuwata and Shoichi Ban, *J. Pharm. Soc. Japan*, **69**, 212 (1949).

10) Cf. R. C. Anderson, R. Stevenson and F. S. Spring, *J. Chem. Soc.*, **1952**, 2901.

11) D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, **1948**, 783.

12) D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, **1948**, 1354.

portion was recrystallized twice from methanol, yielding 2.2 g. of crude sterol, which had m.p. 125~129°, iodine values 65.6 by the perbenzoic acid method and 177.4 by the pyridine sulfate dibromide method. The ultraviolet absorption spectra of the crude sterol indicated the absence of provitamin D. The steryl acetate (2.0 g.) prepared by acetylation of crude sterol had m.p. 135~141° and saponification value 127.1. This was subjected to repeated recrystallizations as shown in Table II.

TABLE II

No. of recrystallization	Solvent	Acetate, crystallized out		Acetate, recovered from mother liquor	
		Yield g.	m.p. °C.	m.p. °C.	Iodine v. by the perbenzoic acid method
1	Methanol	1.9	143–144	104–106	43.4
2	Ethanol	1.6	146–148	105–110	—
3	„	1.1	147–152	124–126	—
4	Acetone	0.5	156–159	147–149	—
5	„	0.25	156–159	147–149	62.6
6	„	0.10	162–165	157–159	70.7
7	„	0.08	162–165		
8	"	0.02	174–175	165–166	—

Iodine values, calculated: $C_{30}H_{50}O_2$ (F_1) 57.3, $C_{31}H_{52}O_2$ (F_1) 55.6

Sterol Fraction I. The acetate of m.p. 157–159°, recovered from the mother liquor of recrystallization in Table II, was fractionally crystallized from acetone, yielding 1st crop of m.p. 160°, 2nd crop of m.p. 157° and 3rd crop of m.p. 152°. The 2nd crop showed no change in its melting point by further recrystallizations from acetone and ethanol-ether and the final product had saponification value 124.9 (calculated, $C_{30}H_{50}O_2$ 126.7, $C_{31}H_{52}O_2$ 122.8), iodine values 63.5 by the perbenzoic acid method 190.1 by the pyridine sulfate dibromide method, and $[\alpha]_D^{10}$+5.6°. The free sterol obtained by saponification of the acetate had, after recrystallization from methanol, m.p. 145–147° and $[\alpha]_D^{10}$+9.0°. Benzoylation of the free sterol gave a benzoate of m.p. 177°.

A solution of the acetate of m.p. 152° with a palladium black catalyst in glacial acetic acid was shaken under the atmosphere of hydrogen for 3 hours at room temperature. Absorption of hydrogen did not occur at all, but the acetate underwent isomerization during this treatment, forming an isomer (α-isomer) which showed, after recrystallization from methanol, m.p. 111–112° and $[\alpha]_D^{25}$+18.1°. The free sterol obtained by saponification of this acetate melted at 115°.

The acetate (0.05 g.) of m.p. 152° was dissolved in 10 cc. of ether, and 5 cc. of a 2% solution of bromine in ether was added dropwise. After standing for one hour, a mixture of 0.25 g. of potassium iodide dissolved in a small amount of water and 10 cc. of ethanol was added to the solution of brominated product, and the mixture was allowed to stand for 48 hours in order to complete debromination. An aqueous solution of sodium bisulfite was then added, and the mixture was extracted with ether. The ethereal extract, after recrystallization from methanol, melted at 164° and exhibited the characteristic ultraviolet absorption spectra of 7:8, 9:11-conjugated sterol; $\log \varepsilon_{235}$ 3.91, $\log \varepsilon_{242}$ 3.88, $\log \varepsilon_{251}$ 3.80.

Sterol Fraction II. The acetate of m.p. 147~149°, recovered from mother liquors of recrystallization in Table II, was fractionally crystallized from acetone. After removing higher and lower melting fractions, a fraction of m.p. 148~149° was separated and further recrystallized from acetone, ethanol-ether and methanol-acetone until an acetate of a constant melting point of 149° was obtained. It had saponification value 123.4, iodine values 60.1 by the perbenzoic acid method and 170.9 by the pyridine sulfate dibromide method, and $[\alpha]_D^{12}$+2.2°. Saponification of the acetate gave a free sterol of m.p. 135° and $[\alpha]_D^{12}$+1.6°. Benzoylation of the free sterol gave a benzoate of m.p. 171°.

On shaking a solution of acetate of m.p. 149° with a palladium black catalyst in glacial acetic acid under the atmosphere of hydrogen for 3 hours, isomerization without absorption of hydrogen occurred, yielding an isomerized product (α-isomer) of m.p. 115° and $[\alpha]_D^{25}$+7.9°. Saponification of this product gave a free sterol of m.p. 114°.

Summary

Sterol mixture of star fish, *Luidia quinaria* von Martens, has been found to consist largely of mono-unsaturated sterol with a lesser amount of saturated and di-unsaturated sterols. By fractional crystallization of steryl acetate mixture, sterol fractions I (m.p. 145° and m.p. of its acetate 157°) and II (m.p. 135° and m.p. of its acetate 149°) were separated. Both fractions were found to consist of $\Delta^{7:8}$-sterol of C_{28} or C_{29} series. It requires some further research to decide whether each fraction consists of a different individual sterol or both fractions consist substantially of the same sterol, differing only in their purity.

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