Sterols and Other Unsaponifiable Substances in the Lipids of Shell Fishes, Crustacea and Echinoderms. XVI. Reinvestigation of Hitodesterol and its Identity with α-Spinasterol

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(Received April 11, 1955)

Hitodesterol[1], m.p. 167–168°C, was found first in *Asterina pectinifera* and subsequently in *Asterias amurensis* (formerly *Asterias rollestoni*) in this laboratory. The formula for this sterol was deemed at that time to be $C_{28}H_{42}O$ or $C_{29}H_{44}O$, its tetra-unsaturation being inferred mainly from its iodine value determined by the pyridine sulfate dibromide method. It became, however, known that Δ^7-sterols generally give abnormally high iodine values by halogen absorption methods, and after having had experienced such an abnormally high iodine value for corbisterol, a $\Delta^{5,7,22}$-sterol of the C_{29}-series, the authors came to believe that hitodesterol shouldbe a di-unsaturated $\Delta^{7,22}$-sterol of the formula $C_{29}H_{48}O$ or $C_{28}H_{46}O$ which has a close resemblance to spinasterol, stellasterol and chondrillasterol, and is possibly identical with one of them[2]. Matsumoto and Wainai[3] have recently come to a similar conclusion that hitodesterol is a $\Delta^{7,22}$-sterol of the C_{29}- or C_{28}-series, the derivatives of which have the closest resemblance to the corresponding derivatives of α-spinasterol. Although a di-unsaturated sterol, named stellasterol, of m.p. 149–150°C, was reported to occur in *Astropecten auruntiacus*[4], this sterol appears not to be a uniform com-

1) T. Matsumoto and Y. Toyama, *J. Chem. Soc. Japan*, **64**, 1069 (1943); T. Matsumoto, M. Yajima and Y. Toyama, ibid., **64**, 1203 (1943).

2) Y. Toyama and T. Takagi, This Bulletin, **27**, 39 (1954).

3) T. Matsumoto and T. Wainai, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **75**, 1147 (1954).

4) A. Kossel and S. Edelbacher, *Z. physiol. Chem.*, **94**, 264 (1915).

pound. Bergmann and Stansbury[5] separated from *Asterias forbesi* a sterol mixture which was found to consist of $\Delta^{7,22}$- and Δ^{7}-sterols of the C_{28}-series, and they proposed to retain the name stellasterol for the former and assign the name stellastenol to the latter. Although a complete separation of these two sterols was not attained, ozonolysis of a mixture of stellasteryl and stellastenyl acetates yielded *d*-methylisopropylacetaldehyde which was identified as its 2, 4-dinitrophenylhydrazone of m.p. 119–120°C and $[\alpha]_D^{24}=+14.1°$. Hence these authors regarded stellasterol as a C–24 epimer of 5-dihydroergosterol.

The present paper records the results of our reinvestigation on hitodesterol. As for sterol components other than hitodesterol, such as mono-unsaturated sterols, in *Asterina pectinifera*, our investigation is now in progress, and the results will be reported in near future.

The crude sterol mixture of the star fish, *Asterina pectinifera*, was acetylated, and the acetylation product was subjected to repeated recrystallizations from acetone by which a steady rise of the melting point was brought about until finally a steryl acetate fraction (A) of m.p. 182–183°C was obtained. This fraction was found to be still contaminated with a minor amount of steryl acetate having a lower melting point, but the melting point and specific rotation of this fraction was not altered substantially by further recrystallizations. This fraction showed a saponification value which agrees with the calculated value for $C_{31}H_{50}O_2$ and an iodine value corresponding to 1.9 double bonds per molecule by the perbenzoic acid method, and was regarded as hitodesteryl acetate in the following. The melting points and specific rotations of hitodesterol and its derivatives are compared with the corresponding compounds of α-spinasterol, chondrillasterol and 5-dihydroergosterol in Table I.

The Liebermann-Burchard test for hitodesteryl acetate developed a blue coloration in which the change of absorption at 620 mμ with the reaction period was quite similar to that of the Δ^{7}-sterol type. A further evidence for the presence of double bond between C–7 and C–8 in hitodesterol was afforded by converting hitodesteryl acetate by the action of mercuric acetate to a product which showed the absorption characteristic to $\Delta^{7,9(11)}$-steryl acetates.

In order to obtain an insight into the structure of the side chain attached to C–17 of hitodesterol, its acetate was subjected to ozonolysis. Among the products of ozonolysis, *l*-ethylisopropylacetaldehyde was identified as its 2, 4-dinitrophenylhydrazone; m.p. 116–117°C and $[\alpha]_D^{14}=-4.6°$. Hence hitodesterol is established as a $\Delta^{7,22}$-sterol of the C_{29}-series and is identical with α-spinasterol, both having the same optical configuration at C–24.

Steryl acetate fractions having melting points between 171°C and 175°C were recovered from the mother liquors of the recrystallizations which were performed to obtain hitodesteryl acetate. These fractions were united, and the united fraction (B) was subjected to ozonolysis. 2, 4-Dinitrophenylhydrazones prepared from the products of ozonolysis were separated into several fractions, including a fraction of m.p. 115–116°C together with a small amount of a fraction of m.p. 124–126°C. The scarcity of the material did not permit us to determine the optical rotation. While the fraction of m.p. 115–116°C was regarded as 2, 4-dinitrophenylhydrazone of ethylisopropylacetaldehyde, the fraction of m.p. 124–126°C was considered to be 2, 4-dinitrophenylhydra-

TABLE I

COMPARISON OF HITODESTEROL AND SOME RELATED STEROLS

	Free sterol		Acetate		Benzoate		3, 5-Dinitrobenzoate	
	m.p. (°C)	$[\alpha]^{\circ}_{D}$	m.p. (°C)	$[\alpha]^{\circ}_{D}$	m.p. (°C)	$[\alpha]^{\circ}_{D}$	m.p. (°C)	$[\alpha]^{\circ}_{D}$
Hitodesterol	167–168	−3.0	182–183	−5.8	197–199	+3.8	195	±0
Hitodesterol[6]	167–168	±0	181–182	±0	—	—	—	—
Hitodesterol[7]	168–169	−2.6	182–183	−2.8	198.5	±0	195	±0
α-Spinasterol[8]	168–169	−3.6	186	−4.5	200	+1.8	—	—
α-Spinasterol[9]	167–168	−3	185	−5	201	+2	—	—
Chondrillasterol[10]	168–169	−1.1	175–176	−1	194–195	+4.0	—	—
5-Dihydroergosterol[9]	176	−19	181	−19	200	−10	—	—

5) W. Bergmann and H. A. Stansbury, Jr., *J. Org. Chem.*, 9, 281 (1944).
6) l.c., 1).
7) l.c., 3).
8) L. F. Fieser, M. Fieser and R. N. Chakravarti, *J. Am. Chem. Soc.*, 71, 2226 (1949).
9) D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, 1948, 1354.
10) W. Bergmann and F. H. McTigue, *J. Org. Chem.*, 13, 738 (1948).

zone of methylisopropylacetaldehyde. Accordingly the steryl acetate fraction (B) seems to contain a small proportion of the acetate of C_{28}-sterol, which gives methylisopropylacetaldehyde by ozonolysis, besides hitodesteryl acetate.

It is thus seen from the foregoing that the di-unsaturated sterol components of *Asterina pectinifera* consist mainly of hitodesterol, although C_{28}-sterol, possibly $\Delta^{7,22}$-sterol, seems to be present in a lesser amount. Whether this sterol of the C_{28}-series corresponds to stellasterol or not, remains undetermined in the present study. The fact that hitodesterol constitutes the main component of the di-unsaturated sterols of *Asterina pectinifera*, whereas the di-unsaturated sterol of *Asterias forbesi* was found by Bergmann and Stansbury to be stellasterol, is by no means difficultly explicable if it is taken into consideration that the content of some sterol components in the total sterol mixture of the same species of aquatic invertebrates often differs widely as has been found in the case of corbicula, chiton and *Asterias amurensis*[11]. It may also be worthy of mention that some sterols formerly found only in the vegetable kingdom have been found to occur not seldom in aquatic invertebrates too. Thus the occurrence of brassicasterol in oyster[12], mussel[13], corbicula[14] and clam[15], and β-sitosterol in *Spisula sachalinensis*[16] and *Ophiopholis aculeata*[17] has been reported in previous studies, and now the occurrence of α-spinasterol or hitodesterol in *Asterina pectinifera* has been demonstrated in the present study.

Experimental

Several lots of the sun-dried star fish, *Asterina pectinifera* (Müller et Troschel) were united. The united material, 15.7 kg. in total, was cut into small pieces and extracted with ether, yielding 268 g. (1.7%) of a dark reddish orange viscous lipid. On treating the lipid with 2.7 liters of acetone, an acetone-soluble oil (180 g.) with the following constants were obtained: d_4^{20} 0.9441, n_D^{20} 1.4906, acid value 92.0, saponification value 117.5, iodine value (Wijs method) 173.9, unsaponifiable matter 44.06%.

Recrystallization of steryl acetate mixture.—The unsaponifiable matter of the acetone-soluble oil was separated by saponification of the oil with alcoholic potash followed by extraction of the diluted soap solution with ether. It was a reddish orange mixture of solid and liquid, and contained 43.5% of sterol (digitonide method). The unsaponifiable matter (72 g.) was recrystallized from 200 cc. of acetone under cooling, and the solid material (49 g.) obtained was refluxed with acetic anhydride. The acetylated product was then recrystallized from 200 cc. of acetone-ether, yielding 26.5 g. of crystalline solid (crude steryl acetate mixture). Further recrystallization of this material from acetone brought about a steady rise of the melting point and iodine value as shown in Table II.

TABLE II

RECRYSTALLIZATION OF STERYL ACETATE MIXTURE

No. of recrystallization	Steryl acetate crystallized out		Steryl acetate recovered from mother liquor	
	Yield (g.)	m.p. (°C)	m.p. (°C)	I.V. by the perbenzoic acid method
2	19.5	141–147	110–117	—
4	14.0	145–151	132–134	—
6	8.5	159–165	142–145	71.2
8	5.0	168–172	153–158	89.5
11	2.8	178–179	167–168	—
12	2.5	182–183	171–173	99.6
13	2.2	182–183	173–175	

The fraction (A) obtained after 13 recrystallizations had $[\alpha]_D^{12} = -6.0°$*. A portion (0.67 g.) of this fraction was subjected to further recrystallizations by which the melting point, 182–183°C, was unaltered, but the melting point of the material recovered from the mother liquor of recrystallization was raised until finally 0.1 g. of a uniform steryl acetate of m.p. 182–183°, $[\alpha]_D^{18} = -5.8°$ and iodine value by the perbenzoic acid method 108.1 was obtained. The fraction (A) had saponification value 124.6 which agrees with the calculated value (123.4) for $C_{31}H_{50}O_2$, and its iodine value (106.9) by the perbenzoic acid method corresponded to 1.9 double bonds per molecule (I.V. calcd. for $C_{31}H_{50}O_2$, 111.6).

Hitodesterol and its derivatives.—Saponification of the fraction (A) gave free sterol which showed m.p. 167–168°C and $[\alpha]_D^{12} = -3.0°$ after recrystallization from acetone. Benzoate prepared from the free sterol by using benzoyl chloride and pyridine had m.p. 197–199°C, $[\alpha]_D^{11} = +3.8°$ and saponification value 109.2 (calcd. for $C_{36}H_{52}O_2$, 108.6) after recrystallization from acetone. 3,5-Dinitrobenzoate prepared from the free sterol was light yellow laminae of m.p. 195°C and $[\alpha]_D^{11} = \pm 0$.

11) Cf. Y. Toyama and T. Takagi, This Bulletin, **27**, 421 (1954).
12) T. Matsumoto and Y. Toyama, *J. Chem. Soc. Japan*, **65**, 310 (1944).
13) W. Bergmann and R. C. Ottke, *J. Org. Chem.*, **14**, 1085 (1949).
14) T. Matsumoto and Y. Toyama, *J. Chem. Soc. Japan*, **65**, 258 (1944).
15) Y. Toyama, T. Takagi and T. Tanaka, This Bulletin, **26**, 154 (1953).
16) Y. Toyama and T. Takagi, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **75**, 1238 (1954).
17) Y. Toyama and T. Takagi, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **76**, 237 (1955).

*) Optical rotations were measured with the samples dissolved in chloroform.

Calcd. for $C_{36}H_{50}N_2O_6$: N, 4.62%. Found: N, 4.45%.

The change of color developed in the Liebermann-Burchard reaction for the fraction (A) with the period of reaction is shown in Fig. 1 in which the absorption at 620 mμ is plotted against the period of reaction. The curve for hitodesteryl acetate is quite similar to the curve for a typical Δ^7-sterol.

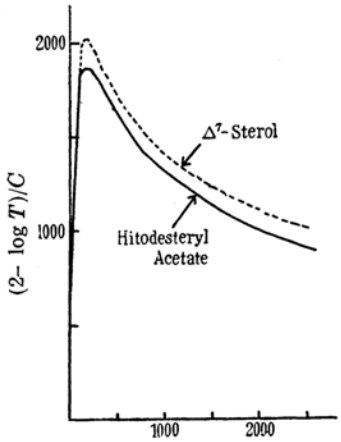

Fig. 1. Libermann-Burchard test for hitodesteryl acetate. T: Transmittance, C: Concentration (10^{-3} mole)

A solution of 0.1 g. of the fraction (A) was mixed with a solution of 0.3 g. of mercuric acetate in 10 cc. of glacial acetic acid, and the mixture was stirred for twelve hours. The product, after two recrystallizations from acetone, showed m.p. 168°C and $[\alpha]_D^{15}=+31.5°$. It exhibited an ultraviolet absorption in ethanol, as shown in Fig. 2, with the characteristic absorption maxima of $\Delta^{7,9(11)}$-sterol; $\log \varepsilon_{235}=4.02$, $\log \varepsilon_{243}=4.07$ and $\log \varepsilon_{250}=3.88$.

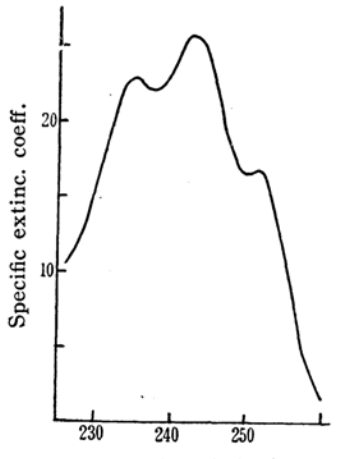

Fig. 2. Absorption curve of the conjugated product of hitodesteryl acetate.

Ozonolysis of hitodesteryl acetate and a fraction of lower melting point.—One g. of the fraction (A) was suspended in 20 cc. of glacial acetic acid, and a current of ozonized oxygen was passed through the suspension. After the suspension had become a clear solution, ozonization was continued for ten minutes more. One g. of zinc dust and a few drops of silver nitrate solution were then added, and the mixture was agitated for thirty minutes. Zinc was then removed by filtration. The liquid was diluted with water, and the solution was subjected to steam distillation. The distillate was collected in a trap containing 500 cc. of a 0.2% solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid. An orange yellow crystalline precipitate was formed in the trap, which was filtered after standing for twenty four hours. The precipitate (191 mg.) was dissolved in 10 cc. of chloroform, and the solution was passed through a 2 cm. x 22 cm. column of silica gel. The adsorption zone was then eluted with isohexane, yielding three eluates of 300 cc. each. Isohexane was distilled off from each eluate. The residues from the first, second and third eluates were 94 mg., 43 mg. and 15 mg. with m.p. 109–112°C, 108–111°C and 95–106°C, respectively. On recrystallizing the first and second fractions from ethanol, 118 mg. of orange yellow needles of m.p. 116–117°C and $[\alpha]_D^{14}=-4.6°$ were obtained. The melting point was unaltered by a further recrystallization. Its absorption curve in ethanol exhibited a maximum and a minimum characteristic to 2,4-dinitrophenylhydrazone of saturated aliphatic aldehyde as shown in Fig. 3 ($k_{359}=23.6$ and $k_{300}=1.6$).

Calcd. for $C_{13}H_{18}N_4O_4$: C, 53.05; H, 6.16; N, 19.04%. Calcd. for $C_{12}H_{16}N_4O_4$: C, 51.42; H, 5.76; N, 19.99%. Found: C, 53.03; H, 6.28; N, 18.48%.

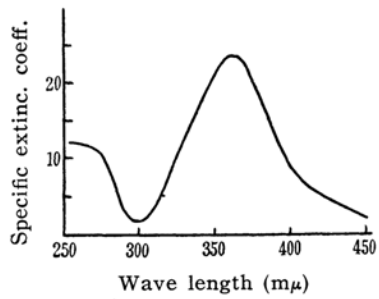

Fig. 3. Absorption curve of 2,4-dinitrophenylhydrazone.

Steryl acetate fractions, which were recovered from the mother liquors of recrystallizations in Table I and had melting points between 171°C and 175°C, were united. The united fraction (B), 0.7 g., was subjected to ozonolysis, and crystalline precipitates of 2,4-dinitrophenylhydrazone (90 mg.) were chromatographed in a similar way as described above, excepting that the adsorption zone was eluted first with isohexane and then with isohexane containing 1% of ether. The precipitates were thus separated into five fractions, the

fifth fraction being obtained by extraction of the silica gel with ethyl acetate. The first fraction (16 mg.) gave, on recrystallization from ethanol, orange yellow needles of m.p. 124–126°C. Calcd. for $C_{12}H_{16}N_4O_4$: N. 19.99%. Calcd. for $C_{13}H_{18}N_4O_4$: N, 19.04%. Found: N, 19.71%.

Recrystallization of the third, fourth and fifth fractions, 15 mg., 10 mg. and 25 mg., respectively, gave orange yellow needles of m.p. 115–116°C, 114–115°C and 114–115°C, respectively. These were united and once more recrystallized from ethanol giving orange yellow needles of m.p. 115–116°C. Calcd. for $C_{13}H_{18}N_4O_4$: N, 19.04%. Calcd. for $C_{12}H_{16}N_4O_4$: 19.99%. Found: N, 18.91%.

Summary

1. A fraction having m.p. 167–168°C and consisting mainly of hitodesteryl acetate was separated from the steryl acetate mixture of *Asterina pectinifera*. Hitodesterol was found to have an ethylenic linkage between C–7 and C–8. Among the products of ozonolysis of hitodesteryl acetate, *l*-ethylisopropylacetaldehyde was identified in the form of its 2, 4-dinitrophenylhydrazone. Hence hitodesterol has been found to be a $\Delta^{7,22}$-sterol of the C_{29}-series and identical with α-spinasterol.

2. Among the products of ozonolysis of a lower melting steryl acetate fraction, the presence of a small amount of methylisopropylacetaldehyde besides ethylisopropylacetaldehyde was indicated. Hence it appears that a C_{28}-sterol having at least an ethylenic linkage between C–22 and C–23, possibly of $\Delta^{7,22}$-type, is also present in a lesser amount in the unsaturated sterol components of *Asterina pectinifera*.

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