Sterols and Other Unsaponifiable Substances in the Lipids of Shell Fishes, Crustacea and Echinoderms. XVII. Mono-unsaturated Sterol Components of the Starfish, Asterina Pectinifera

By Yoshiyuki TOYAMA and Toru TAKAGI

(Received November 15, 1955)

In the 16th report[1] of this series, hitodesterol, which was first separated from *Asterina pectinifera* by Matsumoto and Toyama[2], was re-examined with the result that this sterol was found to be identical with α-spinasterol. It was further indicated that a di-unsaturated C_{28}-sterol, possibly of the $\Delta^{7,22}$-type, is also present in a lesser amount in this starfish.

The present study is concerned with mono-unsaturated sterol components of this starfish. As described in the 16th report, the steryl acetate mixture of this starfish was subjected to repeated recrystallizations by which hitodesteryl acetate was eventually separated as the highest melting fraction. In the present study, the steryl acetate mixture recovered from mother liquors of these recrystallizations was fractionally crystallized, and two fractions, the fraction I of m.p. 157–158°C and the fraction II of m.p. 118–119°C, were separated. The acetate fraction I and its free sterol and benzoate were found to agree with the corresponding derivatives of Δ^7-spinastenol in their properties. Also the properties of the $\Delta^{8(14)}$-and Δ^{14}-isomers prepared from the fraction I were found to accord with those of the corresponding isomers of Δ^7-spinastenyl acetate. Accordingly the sterol of the fraction I was recognized as Δ^7-spinastenol. Regarding the sterol of the fraction II, the results of our examination of the properties of the acetate, free sterol, benzoate and two isomers ($\Delta^{8(14)}$- and Δ^{14}-isomers) showed its identity with Δ^7-cholestenol, which was previously found in *Asterias amurensis*[3]. Thus it is indicated that Δ^7-spinastenol and Δ^7-cholestenol are present in the mono- unsaturated sterol components of *Asterina pectinifera*. It should, however, be noted that the possibility of the presence of Δ^7-sterols of the C_{28}-series is not excluded, though such sterols of the C_{28}-series could not be separated in the present study. Although Δ^7-spinastenol has recently been found in oats[4] and wheat[5], the present study is the first instance in which the occurrence of this sterol in the animal kingdom is demonstrated.

It was reported in previous studies by the authors that sterols of *Coscinasterias acutespina*[6] as well as of *Luidia quinaria*[7] consist mainly of mono-unsaturated Δ^7-sterols of the C_{28}- or C_{29}-series. A steryl acetate fraction,

TABLE I

PROPERTIES OF Δ^7-SPINASTENOL

	Fraction from *Asterina pect.*		Fraction from *Luidia quinaria*		Δ^7-Spinastenol[8]	
	m.p. (°C)	$[\alpha]_D^\circ$	m.p. (°C)	$[\alpha]_D^\circ$	m.p. (°C)	$[\alpha]_D^\circ$
Free sterol	145–147	+8.4	145–147	+9	144–145	+11
Acetate	157–158	+6.1	157	+6	156–157	+ 8
Benzoate	178–180	+15	177	——	180.5	+13
$\Delta^{8(14)}$-Isomer	112–113	+20	115	——	112–113	+23
Acetate of $\Delta^{8(14)}$-Isomer	113–114	+14	111–112	+18	116–117	+12
Δ^{14}-Isomer	123–124	——	——	——	127.5	+36.5
Acetate of Δ^{14}-Isomer	86– 87	+26	——	——	86.5	+24.3

1) Y. Toyama and T. Takagi, This Bulletin, 28, 469 (1955).
2) T. Matsumoto and Y. Toyama, *J. Chem. Soc. Japan*, 64, 1069 (1943).
3) T. Matsumoto and T. Wainai, *J. Chem. Soc. Japan, Pure Chem. Sect.*, 75, 756 (1954); Y. Toyama and T. Takagi, This Bulletin, 27, 421 (1954).
4) D.R. Idler et al., *J. Am. Chem. Soc.*, 75, 1712 (1953).
5) D.R. Idler, A.A. Kandutsch and C.A. Baumann, *J. Am. Chem. Soc.*, 75, 4325 (1953).
6) Y. Toyama and T. Takagi, *J. Chem. Soc. Japan, Pure Chem. Sect.*, 76, 243 (1955).
7) Y. Toyama and T. Takagi, This Bulletin, 27, 39 (1954).
8) D. Larsen and F.W. Heyl, *J. Am. Chem. Soc.*, 56, 2663 (1934); D.H.R. Barton and J.D. Cox, *J. Chem. Soc.*, 1948, 783.

m.p. 157°C, obtained in the previous study on *Luidia quinaria* was not closely studied at that time due to the scarcity of the material, but this fraction is very likely to consist mainly of Δ^7-spinastenyl acetate. In Table I, the melting points and specific rotations of the free sterol and its derivatives of the Δ^7-spinastenol fractions from *Asterina pectinifera* and *Luidia quinaria* are compared with those reported for Δ^7-spinastenol by previous authors.

Experimental

1. Δ^7-Spinastenyl Acetate Fraction.—As described in the 16th report, the solid material (49 g.) separated from the unsaponifiable matter of the acetone-soluble oil extracted from the starfish, *Asterina pectinifera*, 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). This was subjected to repeated recrystallizations from acetone until eventually a crude hitodesteryl acetate fraction (2.2 g.) of m.p. 182–183°C was obtained after thirteen recrystallizations. In the present study, a steryl acetate fraction (4.8 g.), m.p. 153–156°C, recovered from the mother liquors of the 5th–9th recrystallizations was fractionally crystallized from acetone, and the main fraction obtained was fractionated further. After several repetitions of fractionation, a fraction (1.6 g.) of m.p. 157–158°C was obtained. The melting point of this fraction was unaltered by further recrystallizations, and all fractions obtained by a further fractionation had the same melting point.

The fraction, m.p. 157–158°C, had $[\alpha]_D^{22}=+6.1°$, saponification value 123.9 and iodine value by the perbenzoic acid method 58.7 (calcd. for $C_{71}H_{52}O_2$: saponification value 122.8; iodine value 55.6). The change of color developed in the Liebermann-Burchard reaction for this fraction with the period of reaction is shown by the curve A in Fig. 1 in which the absorption at 620 mμ is plotted against the period of reaction. The curve A is quite similar to the curve for a typical Δ^7-sterol. Saponification of this fraction gave free sterol which had m.p. 145–147°C and $[\alpha]_D^{22}=+8.4°$ after recrystallization from acetone. Benzoate prepared from the free sterol had m.p. 178–180°C, $[\alpha]_D^{24}=+15°$ and saponification value 108.9 (calcd. for $C_{36}H_{54}O_2$: 108.1).

The acetate fraction of m.p. 157–158°C was dissolved in glacial acetic acid, palladium catalyst was added to the solution, and the mixture was shaken in an atmosphere of hydrogen for seven hours. Absorption of hydrogen did not occur, but an isomerization product was obtained, which showed m.p. 113–114°C and $[\alpha]_D^{24}=+14°$ after recrystallization from methanol. The change of color vs. the period of reaction in the L. B. reaction for this product is shown by the curve B in Fig. 1 which closely resembles the curve for a $\Delta^{8(14)}$-sterol. Saponification of this product gave free sterol which had m.p. 112–113°C and $[\alpha]_D^{25}=+20°$ after recrystallizations from methanol and acetone.

The $\Delta^{8(14)}$-isomer (acetate) obtained above was dissolved in chloroform, and dry hydrogen chloride was passed through the solution for two hours. The product obtained had m.p. 86–87°C and $[\alpha]_D^{27}=+26°$ after recrystallization from ethanol. Saponification of this product gave free sterol which, recrystallized from methanol, had m.p. 123–124°C. The change of color vs. the period of reaction in the L. B. reaction for this sterol is shown by the curve C in Fig. 1 which is similar to the curve for a typical Δ^{14}-sterol.

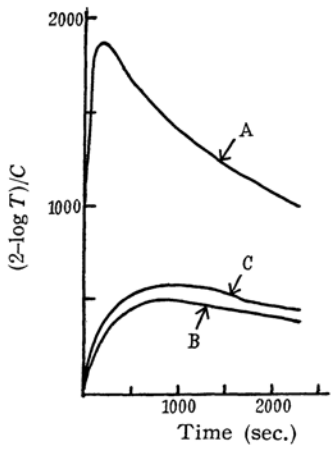

Fig. 1. Liebermann-Burchard reaction for Δ^7-spinastenyl acetate fraction.
T: Transmittance, C: Concentration (10^{-3} mol.)
Curve A for Δ^7-spinastenyl acetate fraction.
Curve B for $\Delta^{8(14)}$-isomer (acetate)
Curve C for Δ^{14}-isomer (free sterol)

2. Δ^7-Cholestenyl Acetate Fraction.—The acetone-ether filtrate separated from the crude steryl acetate (26.5 g.) was concentrated, and a further quantity (2.5 g.) of crude steryl acetate fraction was obtained. This fraction and the fractions recovered from the mother liquors of the 1st and 2nd recrystallizations of the crude steryl acetate (26.5 g.) were united. The united material (9.3 g.), m.p. 105–126°C, was fractionally crystallized from methanol, yielding the 1st crop (1.3 g.) of m.p. 130–132°C and the 2nd crop (6.0 g.) of m.p. 119–123°C. The 2nd crop was separated further into the following fractions by fractional crystallization from methanol: the 1st fraction (0.9 g.) of m.p. 125–127°C; the 2nd fraction (0.5 g.) of m.p. 123–124°C; the 3rd fraction (0.6 g.) of m. p. 120–121°C; the 4th fraction (0.8 g.) of m.p. 119–120°C; the 5th fraction (0.6 g.) of m.p. 119–120°C; the 6th fraction (2.6 g., recovered from the final filtrate) of m.p. 118–119°C. A further fractional crystallization of the 6th fraction gave fractions of the same melting point, 118–119°C. This frac-

tion had $[\alpha]_D^{28}=-2.0°$, saponification value 130.0 and iodine value by the perbenzoic acid method 62.0 (calcd. for $C_{29}H_{48}O_2$: saponification value 130.8; iodine value 59.2). The L. B. reaction for this fraction is shown by the curve A in Fig. 2. Free sterol obtained by saponification of this fraction had m.p. 122–123°C and $[\alpha]_D^{19}=\pm 0$ after recrystallization from methanol. Benzoate prepared from the free sterol had m.p. 153–155°C, $[\alpha]_D^{19}=+4°$ and saponification value 112.6 (calcd. for $C_{34}H_{50}O_2$; 114.3) after recrystallization from acetone.

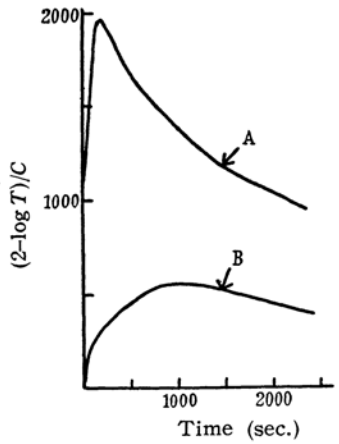

Fig. 2. Liebermann-Burchard reaction for Δ^7-cholestenyl acetate fraction.
T: Transmittance, C: Concentration (10^{-3} mol.)
Curve A for Δ^7-cholestenyl acetate fraction.
Curve B for $\Delta^{8(14)}$-isomer (acetate)

The isomerization product obtained by shaking a solution of this fraction (acetate) in an atmosphere of hydrogen for five hours in the presence of palladium catalyst showed m.p. 77–78°C and $[\alpha]_D^{21}=+9°$ after recrystallization from methanol. The L. B. reaction for this product is shown by the curve B in Fig. 2. Saponification of this product gave free sterol of m.p. 119–120°C and $[\alpha]_D^{21}=+20°$ after recrystallization from methanol.

Summary

In a continuation of the study on sterol components of *Asterina pectinifera*, the present study is concerned with mono-unsaturated sterol components. The steryl acetate fraction recovered from the mother liquors of the recrystallizations, which were carried out for the separation of hitodesteryl (α-spinasteryl) acetate in a previous study, was subjected to fractional crystallizations by which two fractions, Δ^7-spinastenyl acetate fraction and Δ^7-cholestenyl acetate fraction, were eventually separated. Accordingly it has been found that the sterol mixture of *Asterina pectinifera* contains Δ^7-spinastenol and Δ^7-cholestenol besides hitodesterol.

Department of Applied Chemistry
Faculty of Engineering
Nagoya University, Nagoya