Sterols and Other Unsaponifiable Substances in the Lipids of Shell Fishes, Crustacea and Echinoderms. XIV. Unsaponifiable Components Other Than Corbisterol in the Lipid of Corbicula

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It was indicated in the 6th report[1] of this series that a di-unsaturated sterol, apparently brassicasterol, and cholesterol in addition to corbisterol occur in the sterols from *Corbicula leana*. The present paper is concerned with a further study on the unsaponifiable components of the lipid of corbicula.

The sterol mixture separated from the unsaponifiable substances of the lipid of corbicula was converted into the steryl acetate mixture, and the latter was subjected to repeated recrystallizations until a small amount of corbisteryl acetate was finally obtained. From the mother liquors of recrystallizations the following three fractions of steryl acetate were recovered: the fraction I of m.p. 125-127°, the fraction II of m.p. 116-119° and the fraction III of m.p. 100-103°.

The fraction I was fractionally crystallized from ethanol, and the least soluble fraction thus obtained was treated with maleic anhydride to remove the component with conjugated double bonds (corbisteryl acetate) completely in form of its maleic anhydride adduct. The portion with no cunjugated double bonds (A) was subjected to a further fractional crystallization by which nine fractions (see Table II) were separated. The lst fraction consisted of an acetate of C_{29} F_2-sterol which was regarded as identical with poriferasteryl acetate. The free sterol from this acetate showed, however, a considerably lower melting point than that reported for poriferasterol. The 8th fraction consisted of an acetate of C_{29} F_1-sterol which was found to be identical with clionasteryl acetate. Hydrogenation of the 2nd fraction gave poriferastanyl (clionastanyl) acetate. These results indicate that the non-conjugated portion (A) consists chiefly of the acetates of poriferasterol and clionasterol. A portion of the fraction I was also fractionated by way of bromide. Debromination of the ether-insoluble bromide yielded a steryl acetate which was recognized as poriferasteryl acetate, but the free sterol obtained by saponification of this acetate had a melting point which was also considerably lower than that reported in the literature. Although it requires a further study on this point, it does not necessarily follow from the lower melting points of the specimens obtained in these experiments that they are inferior in purity.

The fraction II was fractionated by way of bromide, and there was obtained a bromide fraction which, after debromination, gave cholesteryl acetate. The fraction III showed m.p. 111-113° after one recrystallization and was considered to consist substantially of cholesteryl acetate.

Table I records the properties of the sterols obtained in these experiments.

It is seen from the foregoing results that the sterol mixture from corbicula contains poriferasterol, clionasterol and cholesterol in addition to corbisterol, but the presence of the F_2-sterol, apparently brassicasterol, which was found in the sterol mixture from corbicula described in the 6th report, could not be indicated. Hence another steryl acetate fraction obtained from corbicula described in the 8th report[4] was examined. This fraction was recovered from the mother liquors of recrystallizations which were performed to separate corbisteryl acetate from the steryl acetate mixture. It was then treated with maleic anhydride, by which corbisteryl acetate was completely removed in form of its maleic anhydride adduct. The non-conjugated fraction was separated and purified by recrystallization, yielding brassicasteryl acetate of m.p. 150.5-151.5° and $[\alpha]_D^{24} = -63.6°$. Accordingly brassicasterol constitutes a component of the sterol mixture from corbicula described in the 8th report. These facts appear to be ascribed to a very wide variance in the relative contents of poriferasterol, clionasterol and brassicasterol in the sterol mixtures from the different samples of corbicula; the sterol mixture from corbicula described in these experiments contains a relatively large amount of poriferasterol and

1) T. Matsumoto and Y. Toyama, *J. Chem. Soc. Japan*, **65**, 256 (1944).

2) M. Kita and Y. Toyama, *J. Chem. Soc. Japan*, **70**, 451 (1949).

TABLE I

	Free sterol		Acetate		Benzoate	
	m.p.,°C	$[\alpha]_D^0$	m.p.,°C	$[\alpha]_D$	m.p.,°C	$[\alpha]_D^0$
C_{29} F_2-sterol from I	141—142	—	147—148	−53.1	140.5—141	−22.2
C_{29} F_2-sterol from I, by way of bromide	145—146	−50.9	146—147	−54.6	—	—
Poriferasterol[2,3]	155—156	−49.7	146.5—147	−53	139.5—140.5	−21.9
C_{29} F_1-sterol from I	137—138	−37.1	135—136	−42.2	133—135	−17.6
C_{29} F_1-sterol from I, by way of bromide	136—137	−38.0	132—133	−40.8	—	—
Clionasterol[2]	137.5—138.5	−37	137	−41.9	134.5—135	−16.8
Stanol from I, obtained by hydrogenation	143—144	+24.5	140.5—141.5	+15.4	—	—
Poriferastanol[2]	143—144	+24.7	140—141	+16.3	—	—
Sterol from II, by way of bromide	145—146	—	114—115	−44.2	—	—
Cholesterol	148	−38	114	−43		

clionasterol but it contains brassicasterol in such a small amount that its presence could not be demonstrated, whereas the sterol mixture from corbicula described in the 8th report, like that described in the 6th report, contains a larger amount of brassicasterol. A similar fact that the content of certain sterol components in the sterol mixture from shell fish of the same species varies widely for each sample of shell fish was noted in a previous study on the sterol components of chiton in which the content of γ-cholestenol differs widely for each sample of chiton.

After separating the sterol mixture from the crude unsaponifiable substances, there was obtained a viscous oily portion (B), which was contaminated with acidic and saponifiable substances. The acetylated product of the portion (B) was subjected to a fractional distillation. There was an indication of decomposition during the distillation, yielding a total distillate in a yield of about 56% of the acetylated product. A higher fraction was saponified, the product was dissolved in acetone, the solution was cooled, and there was obtained a crystalline solid which was found to consist chiefly of a mixture of batyl and chimyl alcohols. Hydrogenation of the remainder of the saponified product gave a small amount of solid material which was considered to contain substantially batyl alcohol. Accordingly the saponified product appears to contain also an unsaturated member (selachyl alcohol) corresponding to batyl alcohol. Lower fractions were saponified, giving a product which contained a methanol-insoluble portion consisting possibly of hydrocarbons. On treating the methanol-insoluble portion with sulfuric acid, the bulk of it was converted into a tarry matter. A closer examination of the components other than alcohols of batyl alcohol series in the viscous oily portion (B) could not be performed in these experiments. However, the unidentified components including hydrocarbons were suspected to contain more or less decomposition products which might have been formed in the course of distillation.

Experimental

Preparation and Fractionation of Steryl Acetate Mixture.—Boiled meat (37.75 kg.) of corbicula was vacuum-dried at 65-75°, giving 11.44 kg. of dried material, from which 1.13 kg. of lipid was extracted with trichloroethylene.* The lipid was saponified with alcoholic potash, the diluted soap solution was treated with ether in the usual way, and there were obtained 188 g. (16.6% of lipid) of unsaponifiable substances. These were crystallized from 3.5 litres of methanol, affording 113 g. of crystalline solid (crude sterol mixture) of m.p. 132-135°. The steryl acetate mixture prepared from crude sterol was subjected to repeated recrystallizations from ethanol by which 0.3 g. of corbisteryl acetate, m.p. 151.5-152°, was separated. Concentration of the mother liquors from the 3rd and subsequent recrystallizations, the 2nd recrystallization and the 1st recrystallization gave the following three fractions of steryl acetate, respectively: (I) 42 g., m.p. 125-127°; (II) 17.2 g., m.p. 116-119°; (III) 7.2 g., m.p. 100-103°.

Fractionation of Fraction I.—A portion (32 g.) of the fraction I was subjected to a further fractional crystallization from ethanol, by which

3) F. R. Valentine, Jr. and W. Bergmann, *J. Org. Chem.*, **6**, 452 (1941).

4) W. Bergmann and R. J. Feeney, *J. Org. Chem.*, **14**, 1078 (1949); W. Bergmann, F. H. McTigue, E.M. Low, W.M. Stockes and R.J. Feeney, ibid., **15**, 96 (1950).

* The fatty acid components of this lipid were studied in a previous paper by the present authors. Cf. Y. Toyama and T. Tanaka, *J. Chem. Soc. Japan*, **74**, 1016 (1953).

the least soluble fraction (5.0 g.) of m.p. 138-139° and $k_{282}=1.14$ was obtained. This was heated with maleic anhydride (1.5 g.) and xylene (30 cc.) for 10 hours at 140° in a sealed tube. After distilling off xylene under vacuum, the reaction product was saponified with alcoholic potash, and the unreacted sterol was extracted from the diluted maleate solution with ether. The ether extract (4.6 g.) showed m.p. 135-136° and $k_{282}=0$. The acetate prepared from the ether extract was fractionally crystallized from ethanol and acetone with the results given in Table II.

TABLE II

Fraction	Yield, g.	m. p., °C
1	0.82	147–148
2	0.27	145.5–146.5
3	0.27	145–146
4	0.27	142–143
5	0.39	142–143
6	0.33	139.5–140.5
7	0.48	138–139
8	0.81	135–136
9	0.84	132–133

The first fraction had $[\alpha]_D^{28}=-53.1°$,* saponification value 124.5 and iodine value by the perbenzoic acid method 110.0 (calculated for $C_{31}H_{50}O_2F_2$: saponification value 123.4, iodine value 111.6). Saponification of this fraction gave a free sterol which had m. p. 141–142° after recrystallization from ethanol. The benzoate prepared form the free sterol had m.p. 137–138° (turbid), 140.5–141° (clear) and $[\alpha]_D^{25}=-22.2°$ after recrystallization from ethanol.

The 8th fraction had $[\alpha]_D^{25}=-42.2°$, saponification value 123.9 and iodine value by the perbenzoic acid method 58.2 (calculated for $C_{31}H_{52}O_2F_1$: saponification value 122.8, iodine value 55.6).

The free sterol had m.p. 137–138° and $[\alpha]_D^{20}=-37.1°$ after recrystallization from ethanol. The benzoate had m.p. 133–135° and $[\alpha]_D^{20}=-17.6°$. The melted benzoate developed a blue-violet color in the course of its solidification.

The second fraction was hydrogenated at 60–70° in glacial acetic acid using palladium black catalyst. The product, recrystallized from ethanol, was negative for the Liebermann-Burchard reaction, and had m.p. 140.5–141.5° and $[\alpha]_D^{20}=+15.4°$. The free stanol had m. p. 143–144° and $[\alpha]_D^{20}=+24.5°$ after recrystallization from ethanol.

Fractionation of Fraction I by Way of Bromide.—A portion (10 g.) of the fraction I was dissolved in 150 cc. of ether and brominated at −10–−15°, and the insoluble bromide (1.26 g.) formed was filtered. This bromide, after being recrystallized from chloroform-methanol, had m. p. 190–191° (with decomposition), $[\alpha]_D^{12}=-42.8°$, and Br-content 42.05% (calculated for $C_{31}H_{50}O_2Br_4$ 41.28%). Debromination of this bromide gave a steryl acetate of m. p. 146–147° and $[\alpha]_D^{20}=-54.6°$.

The free sterol had m. p. 145–146° and $[\alpha]_D^{20}=-50.9°$ after recrystallization from ethanol. The ether solution separated from the ether-insoluble bromide was freed from the excess of bromine and concentrated under vacuum, and the product was fractionally precipitated by adding methanol to the ether solution. Debromination of the first precipitate (2.6 g.) and recrystallization of the debrominated product gave a steryl acetate having m. p. 132–133°, $[\alpha]_D^{20}=-40.8°$, saponification value 123.9 and iodine value* 59.6. The free sterol had m. p. 136–137° and $[\alpha]_D^{20}=-38.0°$ after recrystallization from ethanol.

Fractionation of Fractions II and III.—A portion (12.5 g) of the fraction II was dissolved in 150 cc. of ether and brominated at about −10°, and the insoluble bromide (0.5 g.) formed was filtered. The filtrate was freed from the excess of bromine and concentrated under vacuum, and the bromide was fractionally precipitated by adding methanol to the ether solution. Six precipitates were separated. Debromination of the second precipitate and recrystallization of the debrominated product gave a steryl acetate of m. p. 114–115°, $[\alpha]_D^{12}=-44.2°$, saponification value 131.1 and iodine value 64.6 (calculated for $C_{29}H_{48}O_2F_1$: saponification value 130.9, iodine value 59.2). The free sterol melted at 145–146°. Hydrogenation of the steryl acetate in glacial acetic acid in the presence of platinum black gave a stanyl acetate which had m. p. 107–108° and $[\alpha]_D^{20}=+11.9°$.

The fraction III showed m. p. 111–113° after one recrystallization from ethanol.

Separation of Brassicasterol from Sterol Mixture Described in the 8th Report.—The sterol mixture from corbicula was converted into the steryl acetate mixture, and the latter was repeatedly recrystallized to separate corbisteryl acetate (see the 8th report). From the mother liquors of recrystallization, a steryl acetate fraction (2.5 g.) of m. p. 142–143.5° and $k_{282}=2.63$ was recovered. In order to remove corbisteryl acetate completely from this fraction, it was treated with maleic anhydride. The yield of the non-conjugated fraction was about 0.6 g. due to a loss during the treatment. Recrystallization of the non-conjugated fraction from ethanol and acetone gave a steryl acetate (0.2 g.) of m. p. 150.5–151.5°, $[\alpha]_D^{24}=-63.6°$, saponification value 127.8 and iodine value 109.7 (calculated for $C_{30}H_{48}O_2F_2$: saponification value 127.3, iodine value 115.2). The free sterol from this acetate had m. p. 143.5–144.5° and $[\alpha]_D^{20}=-60.1°$ after recrystallization from ethanol.

* All rotations were taken in chloroform.

* Unless otherwise stated, the iodine values were determined by the pyridine sulfate dibromide method.

Components Other Than Sterols.—The methanol filtrate separated from the crude sterol mixture of the unsaponifiable substances contained a dark greenish-brown viscous liquid having acid value 13.3 and saponification value 41.6. This was acetylated, and a portion (35 g.) of fhe acetylated product was fractionally distilled with the results shown in Table III. There was an indication of decomposition during the distillation, and a conspicuous evolution of gas was noticed.

TABLE III

Fraction	b. p., °C/2 mm Hg	Yield, (g.)	n_D^{20}	Saponification value	Iodine value
1	—145	0.7	1.4695	133.8	54.1
2	145—170	0.9	1.4694	132.9	53.2
3	170—200	2.4	1.4671	138.9	48.2
4	200—210	3.8	1.4657	175.7	44.8
5	210—225	9.2	1.4657	187.8	42.8
6	225—227	1.8	1.4757	182.6	51.5
7	227—230	0.9	1.4827	171.8	61.4
Residue and loss		15.3			

The fractions 4, 5 and 6 were refractionated, and a fraction (3.5 g.) of b. p. 220–225°/2 mm Hg was separated. This fraction was saponified, the product was dissolved in acetone, and the solution was cooled with ice. The crystalline solid separated from the solution had iodine value 3.8, and recrystallization of this solid from acetone yielded a product (0.3 g.) of m. p. 56–57° and acetyl saponification value 266.8 (calculated for $C_{19}H_{40}O_3$: 280.1; calculated for $C_{21}H_{44}O_3$ 261.8). The oily liquid recovered from the acetone filtrate was hydrogenated at 60–70° in ethanol in the presence of Raney nickel. The hydrogenation product, after being recrystallized from 90% ethanol had m. p. 53.5–54.5°, iodine value 3.2 and acetyl saponification value 253.6.

The fractions 2 and 3 were saponified. On treating the product (2.0 g.) with 20 cc. of methanol, there was obtained 0.14 g. of methanol-insoluble portion. This was dissolved in 7 cc. of hexane, and the hexane solution was washed with an equal volume of concentrated sulfuric acid. The sulfuric acid layer became colored dark brown, and there was formed a tarry matter between the hexane and sulfuric acid layers. The hexane solution was washed several times with concentrated sulfuric acid followed by successive washing with a dilute caustic soda solution and water. After distilling off hexane from the hexane solution, there remained a small amount (30 mg.) of pale yellow liquid.

Summary

Among the sterol components of the unsaponifiable substances of corbicula, poriferasterol, clionasterol and cholesterol in addition to corbisterol are present. Among the unsaponifiable components other than sterols, the presence of a mixture of chimyl and batyl alcohols is indicated. The unsaponifiable components appear to contain also unsaturated members (selachyl alcohol series) corresponding to batyl alcohol series. Besides these components, there occur some other liquid components which contain possibly hydrocarbons. But these unidentified components appear to contain more or less the decomposition products which might have been formed during the distillation.

The sterol mixture obtained from corbicula described in the 8th report, like the sterol mixture from corbicula described in the 6th report, has been found to contain brassicasterol as a di-unsaturated sterol.

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