FATTY OILS OF AQUATIC INVERTEBRATES. XXIV.

FATTY OILS OF THE ASCIDIANS, *SARCODIDEMNOIDES MISAKIENSE* AND *CYNTHIA KARASBOJA* WITH A PARTICULAR REFERENCE TO THEIR STEROLS

SUKETAKA ITO, TORU TAKAGI and YOSHIYUKI TOYAMA

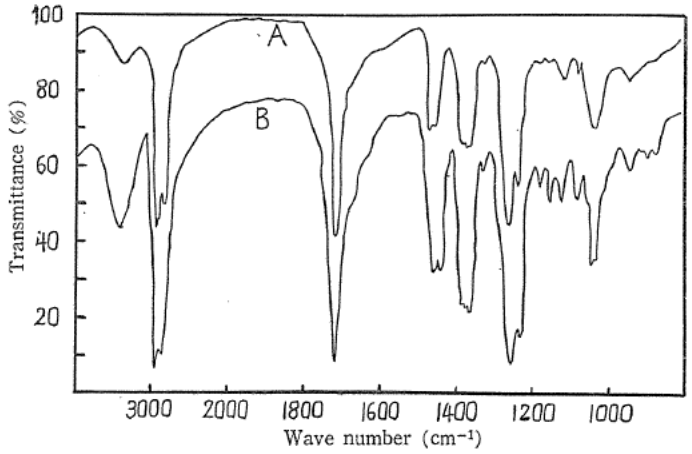
Department of Applied Chemistry

(Received October 28, 1959)

Among the animals of Protochordata, there are only two species, *Styela plicata*[1] and *Cynthia roretzi*,[2,3] whose lipid components have hitherto been studied. Sterols of *S. plicata* were found to contain cholesterol as a major component, while poriferasterol, clionasterol and 7-dehydrostigmasterol were separated from sterols of *C. roretzi*.

This paper records the results of our study on the fatty oils extracted from two lots of the ascidian, *Sarcodidemnoides misakiense* Oka et Willey and a lot of the ascidian, *Cynthia karasboja* Oka. The fatty acids from *S. misakiense* and *C. karasboja* are characterized by their comparatively high content of saturated acid as compared with those of common marine animal oils having an iodine value of the same level.

The crude sterol mixture from *S. misakiense* was separated into several fractions by fractional crystallization and chromatographic fractionation. Optical rotation and Liebermann-Burchard reaction indicated the presence of saturated sterol in some fractions. The saturated sterol fraction separated from one lot of *S. misakiense* by the Anderson-Nabenhauer method was found to consist chiefly of cholestanol but the saturated sterol fraction separated from the other lot of *S. misakiense* seemed to contain another saturated sterol besides cholestanol. Animals whose sterols are known to contain saturated sterols in a large proportion have hitherto been confined to those of the Porifera, *i.e.* sponges. Cholestanol was separated from the sterol mixture of *Suberites compacta*,[4] *Suberites domuncula*,[4] *Microciona porifera*,[5] *Stylotellata heliophila*,[5] *Chondrilla nucla*,[6] *Terpios fugax*,[7] *Terpios zeteki*,[7] an Aptos sp.,[7] *Weberella bursa*,[7] *Polymastia infrapilosa*,[7] and *Reniera japonica*.[8] Another saturated sterol, aptostanol, was reported to occur in the sterol mixture of *Radiella sol*,[7] *Weberella bursa*[7] and *Polymastia infrapilosa*.[7] The sterol of *S. misakiense* in this study is the first instance that animal sterol other than sponge sterol is found to contain saturated sterol in a large proportion. The saturated sterol in the total sterol of *S. misakiense* is estimated at 20–40% from the results of the treatment by the Anderson-Nabenhauer method. It may be mentioned here that although the absorption in the region 1240 cm^{-1} due to the acetoxyl group of cholestanyl acetate has been reported to show an unbranched band,[9] the same absorption observed in our study (Fig. 1) shows a distinctly branched band for the specimen prepared from cholestanol separated from *S. misakiense* and also for the specimen prepared by hydrogenation of chole-



A: Cholestanyl actate from *S. misakiense*
B: Cholestanyl acetate prepared by hydrogenation of cholesteryl acetate

FIG. 1. Infrared spectra of cholestanyl acetate.

steryl acetate.

The presence of clionasterol in the sterol mixture of *S. misakiense* was also indicated in this study.

The sterol mixture of *C. karasboja* appeared to contain chiefly Δ^5-sterol from its melting point, optical rotation and iodine value and also from properties of its acetate and benzoate, while saturated sterol could not be detected. It is remarkable that although *S. misakiense* and *C. karasboja* belong to the same order Ascidacea, saturated sterols occur only in the sterols from *S. misakiense* in a large proportion.

Experimental

1. Fatty oils and their fatty acids

Two lots of *Sarcodidemnoides misakiense* Oka et Willey and a lot of *Cynthia karasboja* Oka used in this study were caught in Sugashima, Toba-shi, Mie-ken. Fresh animals were extracted with ethanol and ether. The combined extract (lipid) was refluxed with ten times its weight of acetone for a while and then cooled down to ordinary temperature, the acetone-insoluble material (phosphatide) was removed by filtration, and the fatty oil was obtained from the acetone filtrate. All oils were a dark reddish brown viscous liquid. Date of catch, yields of lipid and fatty oil, characteristics of fatty oil and fatty acids, sterol content in unsaponifiable matter and $\Delta^{5,7}$-sterol content in total sterol are shown in Table 1.

The saturated acids (%) in the total fatty acids for *S. misakiense* oil No. 1 and *C. karasboja* oil were calculated from the saturated methyl ester content (%) in the methyl ester of total fatty acids determined by the permanganate acetone oxidation method. The neutralization values of saturated acids were calculated from the saponification values of the corresponding methyl esters. The results are shown in Table 2. The fatty acids from *S. misakiense* oil No. 1 and *C. karasboja* oil were isomerized under the condition of 21% KOH-ethylene glycol,

180°C and 15 minutes with a current of nitrogen, and the ultraviolet absorption values of the isomerization product were measured. The composition of polyethenoid acids calculated from the ultraviolet absorption data are shown in Table 2.

TABLE 1. Fatty Oils and Their Fatty Acids

	S. misakiense		*C. karasboja*
	No. 1	No. 2	
Date of catch	July, 1958	June, 1958	July, 1958
Weight of fresh animals (kg)	6.56	ca.4	2.42
Lipid (g)	15.7	11.5	7.6
Lipid (%)	0.24	0.29	0.31
Fatty oil (g)	12.7	7.5	6.9
Fatty oil (%)	0.19	0.19	0.29
Acid value	35.7	84.0	34.3
Saponification value	142.0	140.4	166.8
Iodine value	148.1	122.0	178.7
Unsaponifiable matter (%)	26.7	32.0	21.3
Neutralization value of fatty acids	—	186.3	183.2
Iodine value of fatty acids	152.2	130.6	195.6
Sterol content in unsaponif. matter (%)	54.4	64.9	56.9
$\Delta^{5,7}$-Sterol content in total sterol (%)	4.65*	17.69	11.2

* Determined with crude steryl acetate.

Notes: Percentage yields of lipid and fatty oil are expressed on the basis of fresh animals. The sterol content in unsaponifiable matter was determined by the digitonide method. The $\Delta^{5,7}$-sterol content in total sterol was calculated from the ultraviolet absorption values of sterol digitonide. Iodine values were determined by the Wijs method for fatty oils and fatty acids and by the pyridine sulphate dibromide method for unsaponifiable components.

TABLE 2. Composition of Fatty Acids

Acid	Wave length (mμ)	*S. misakiense* oil (No. 1)			*C. karasboja* oil		
		Sp. extinct. coefficient	%		Sp. extinct. coefficient	%	
			A	B		A	B
Hexaethenoid	374	1.80	6.1	6.1	2.67	9.1	9.1
Pentaethenoid	346	9.68	15.8	9.1	12.6	20.1	11.6
Tetraethenoid	315	14.3	5.8	8.3	20.0	9.7	12.9
Triethenoid	269	14.5	1.6	3.9	22.1	4.1	7.0
Diethenoid	234	15.4	3.3	4.5	23.9	6.8	7.5
Saturated			32.2			36.9	
Neutr. V. of sat. acids			206.5			202.8	

Notes: The figures in the column A were obtained by taking pentaethenoid acids as C_{22}, while those in the column B were obtained by taking pentaethenoid acids as C_{20}.

2. Sterol

(i) *S. misakiense.* The unsaponifiable matter (3.3 g, I.V. 92.1) from No. 1 oil (12.5 g) was recrystallized from thirty times its weight of methanol, giving a crude sterol (0.81 g) of m.p. 117.5°–119°C and I.V. 85.8. Its acetate, m.p. 108.5°–110.5°C and $\Delta^{5,7}$-diene content 4.65%, was subjected to four recrystallizations from

methanol, giving a small amount of crystals having m.p. 112°–114°C which was still raised by a further recrystallization. Concentration and cooling of the mother liquors from the 1st and 2nd recrystallizations formed scaly crystals and granular precipitate (*A*). On adding methanol to the mixture and heating to 60°C, the scaly crystals dissolved. The granular precipitate which remained undissolved was removed by decantation. Concentration and cooling of the solution formed again scaly crystals and a lesser amount of granular precipitate. The mixture was treated as before in order to remove the granular precipitate as completely as possible. After several repetitions of these treatments, two lots of scaly crystals were obtained from the mother liquors of the 1st and 2nd recrystallizations, respectively: (*B*) 180 mg, m.p. 109.5°–112°C and (*C*) 100 mg, m.p. 109.5°–111°C, $[\alpha]_D^{19} = +11.5°$. Scaly crystals (*B* and *C*) showed a very weak Liebermann-Burchard reaction. After treating scaly crystals three times with sulphuric acid by the Anderson-Nabenhauer method, the residue was recrystallized from methanol, giving a saturated steryl acetate fraction of m.p. 110.5°–112°C and $[\alpha]_D^{19} = +12.5°$ in the case of *B* and a saturated fraction of m.p. 110°–112°C in the case of *C*. Saponification of the saturated acetate fractions gave a free sterol which showed m.p. 135°C and $[\alpha]_D^{15} = +22.0°$ after recrystallization from methanol. The benzoate prepared from the free sterol was subjected to repeated recrystallizations from ethanol and acetone, giving needles of m.p. 133.5°–135°C. The acetate of m.p. 110.5°–112°C showed no depression of melting point when mixed with cholestanyl acetate (m.p. 110°–111°C) prepared by hydrogenation of cholesteryl acetate with the use of platinum black as catalyst. Infrared spectra of the acetate of m.p. 110.5°–112°C and those of cholestanyl acetate are quite alike as shown in Fig. 1.*

The steryl acetate fraction in the form of granular precipitate (*A*), 140 mg in total, was subjected to repeated recrystallizations from methanol, giving crystals of m.p. 133°–135°C. The benzoate prepared from the saponification product (free sterol) of these acetate crystals gave, after repeated recrystallizations from ethanol and acetone, needles of m.p. 137.5°–139°C which developed a blue color in the course of solidification as is the case with clionasteryl benzoate. The mother liquor from the 1st recrystallization of the unsaponifiable matter was concentrated and cooled, yielding a crystalline fraction (0.3 g) of m.p. 116°–122°C and $[\alpha]_D^{15} = -41.4°$. The benzoate of this fraction gave needles of m.p. 137.5°–139°C and $[\alpha]_D^{15} = -18.2°$ after recrystallizations from ethanol and acetone, which developed a blue color in the course of solidification. Saponification of this benzoate gave a free sterol of m.p. 133°–134°C after recrystallization from methanol.

The unsaponifiable matter (2.25 g) from No. 2 oil (7.3 g) was recrystallized from 80 cc of methanol giving a crude sterol (1.12 g) of m.p. 120°–123°C and $\Delta^{5,7}$-sterol content 17.93%. Acetylation of the crude sterol gave an acetate of m.p. 120°–123°C and $\Delta^{5,7}$-sterol content 15.26%. This acetate (1.0 g) was subjected to chromatographic fractionation to separate a $\Delta^{5,7}$-steryl acetate using an adsorption column of alumina, 2.7 cm in diameter and 42 cm in height, *n*-hexane as solvent and developer, and *n*-hexane containing ethanol as eluant. The results are shown in Table 3.

* Infrared spectra were taken by the KCl tablet method.

TABLE 3. Chromatography of Steryl Acetate Mixture from *S. misakiense* Oil (No. 2 Oil)

Fraction No.	Yield (mg)	M.p. (°C)	$\Delta^{5,7}$-Diene content (%)
1	35	90– 98	—
2	55	80–105	—
3	180	92–105	—
4	145	102–110	—
5	208	109–118	2
6	166	110–118	7
7	151	125–128	18
8	68	125–130	41

The fraction 1 in Table 3 showed the highest $\Delta^{5,7}$-diene content, but its yield was too small to permit a further fractionation of it. The fractions 1–3 were united, and 230 mg of the united material of $[\alpha]_D^{10} = +7.3°$ and I.V. 23.9 was treated twice with sulphuric acid by the Anderson-Nabenhauer method, giving a faintly yellowish clored residue (*D*, 150 mg) which was negative for the Liebermann-Burchard reaction. Three recrystallizations of this residue from methanol gave 27 mg of crystals of m.p. 115°–117°C and $[\alpha]_D^{12} = +14.0°$. This acetate was negative for the Liebermann-Burchard reaction and showed infrared absorptions resembling those of cholestanyl acetate, but it had a higher melting point than cholestanyl acetate (m.p. 111°C) suggesting that another saturated steryl acetate besides cholestanyl acetate is present in this fraction. The saturated acetate (110 mg) recovered from the mother liquor of recrystallization of the residue (*D*) had m.p. 111°–112°C and S.V. 129.2. This was saponified and the product was recrystallized from methanol, giving a free sterol (35 mg) of m.p. 136°–138°C and $[\alpha]_D^{15} = +24.4°$. The benzoate prepared from a recovered fraction of free sterol showed m.p. 135° (turbid)-155°C(clear) and $[\alpha]_D^{17} = +18.5°$ after recrystallization from acetone.

(ii) *C. karasboja.* The unsaponifiable matter (1.4 g) of I.V. 103.7 from No. 2 oil (6.7 g) was recrystallized from methanol, giving a crude sterol (0.28 g) of m.p. 120.5°–122.5°C, $[\alpha]_D^{28} = -21.2°$ and I.V. 122.1. The crude sterol was subjected to six recrystallizations giving eventually a small amount of crystals of m.p. 128°–129°C. The acetate prepared from the crude sterol had m.p. 123.5°–125°C and $[\alpha]_D^{28.5} = -34.1°$. The melting point was raised to 128°–129°C after repeated recrystallizations from methanol. The recovered acetate fraction was saponified and the product (free sterol) was converted to the benzoate which showed m.p. 145°–145.5°C after four recrystallizations from ethanol and acetone. The crude steryl acetate gave no residue after the treatment by the Anderson-Nabenhauer method.

Summary

Fatty oils were extracted from two lots of *Sarcodidemnoides misakiense* and one lot of *Cynthia karasboja*, and their characteristics were determined. The fatty acids from *S. misakiense* and *C. karasboja* had a comparatively high content of saturated acids as compared with those of common marine animal oils having an iodine value of the same level. Sterols from *S. misakiense* were subjected to

recrystallization, chromatography and sulphuric acid treatment by the Anderson-Nabenhauer method. The saturated sterols in total sterols were estimated at about 20–40%. The saturated sterol fraction from one lot of *S. misakiense* was found to consist chiefly of cholestanol, but the saturated sterol fraction from the other lot appeared to contain another saturated sterol besides cholestanol. The presence of clionasterol in sterols from *S. misakiense* oil was also indicated. Sterols from *C. karasboja* oil seemed to consist chiefly of Δ^5-sterol while saturated sterol could not be detected.

References

1) W. Bergmann, M. J. McLean and D. Lester: *J. Org. Chem.* 8, 271 (1943).
2) M. Kita and N. Wada: Paper presented at the Fat Symposium, Nagoya, November, 1955.
3) T. Takagi, T. Maeda and Y. Toyama: *Memoirs Faculty of Engineering, Nagoya Univ.* 8, 169 (1956).
4) W. Bergmann, D. H. Gould and E. M. Low: *J. Org. Chem.* **10**, 570 (1945).
5) W. Bergmann and F. H. McTigue: *J. Org. Chem.* **10**, 580 (1945).
6) W. Bergmann and F. H. McTigue: *J. Org. Chem.* **13**, 738 (1948).
7) W. Bergmann, F. H., McTigue, E. N. Low, W. M. Stokes and R. J. Feeney: *J. Org. Chem.* **15**, 96 (1950).
8) M. Kita: *Reports of Industrial Research Institute, Osaka Pref.* No. 18, 50 (1957).
9) R. N. Jones, P. Humphries, F. Herling and K. Dobriner: *J. Am. Chem. Soc.* **73**, 1215 (1951).