ACTA CHEMICA SCANDINAVICA 4 (1950) 512 – 517

Chemical Investigations on *Pteropoda*

Isolation of a New Sterol, Pteropodasterol

KJELL BAALSRUD

Institutt for Organisk Kjemi, Norges Tekniske Høgskole, Trondheim, Norway

As far as we know species of the *Pteropoda* (order of the *Gastropoda*) have never before been investigated from a chemical point of view.* Through courtesy of "Fiskeridirektoratets Havforskningsinstitutt", Bergen, two species have now been collected for investigation, *viz.*, *Clione limacina* Phipps and *Limacina helicina* Phipps, the former being a naked, the latter a scaled pelagic snail.

The samples were collected the 28th July 1947 in Magdalena Bay on Svalbard by Konsulent Finn Devold to whom the author is very indepted for the material.

As shown in Table 1 the *L. helicina* contained some more water than *C. limacina*. The shell of *L. helicina* seems to consist of organic material. Compared with data from other molluscs given by Bergman [1], these pteropods seem to be among the less fatrich.

Table 1.

	Limacina helicina	*Clione limacina*
Dry weight (fat free)	9.68 g/100 g	6.88 g/100 g
Ash (525°)	3.07 g/100 g	2.71 g/100 g
Fat	0.72 g/100 g	0.68 g/100 g
Iodine number	166	167
Unsaponifiable matter	10.7 g/100 g fat	13.9 g/100 g fat
Sterol (as cholesterol)	62.1 g/100 g unsap.	40.8 g/100 g unsap.

* G. Rosenfeld (*Wiss. Meeresunters. Abt. Helgoland*, 5 (1904) 57) dealing with fat sources of the sea, found in *Limacina arctica* (= *L. helicina*) 7.3 g fat/100 g dry material with iodine number 164. 8.

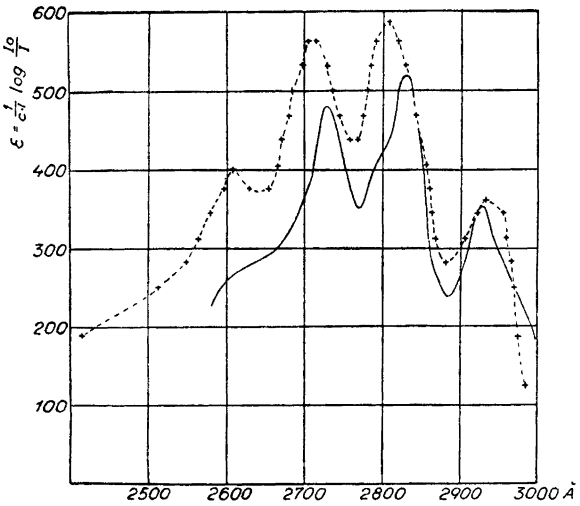

Fig. 1. Ultraviolet absorption spectra.
– – – Sterol from Clione limacina
——— 4 % ergosterol

The fats, extracted with alcohol and ether, were dark brown and viscous. The unsaponifiable matters were both semicrystalline solids with a bright yellow-red colour. Further examinations of the material were concentrated on the sterol fraction.

Till now cholesterol has been found in gastropods. These pteropods seem to be the first exception, since no cholesterol could be traced. For instance an etheral solution of the sterol fraction gave no precipitate on addition of bromine in acetic acid, and in no fractions any low melting acetate could be traced.

Molluscs are often found to contain more than one sterol. Therefore, different means were tried to separate the sterol fraction of *L. helicina* into different compounds. However, fractional crystallization and chromatography of the free sterol, its acetate and its 3,5-dinitrobenzoate always gave fractions with corresponding melting points and optical rotations the only contamination being small amounts of a sterol with conjugated double bonds.

Equally the sterol fraction of the *C. limacina* proved to contain only one sterol. Further the two sterols proved to be identical. The properties of this sterol indicate that it is a new compound which is later refered to as pteropodasterol.

Table 2.

Pteropodasterol	m. p. 132°	$[\alpha]_{20}^{D}$ — 40°
Pteropodasteryl acetate	— 136°	— — 47°
Pteropodasteryl 3,5-dinitrobenzoate	— 198.5°	— — 15°

The 3,5-dinitrobenzoate is one of the most suitable compounds for determination of the molecular formula. The analysis of this compound indicates the formula $C_{27}H_{46}O$ for the free sterol, but the range of melting points of the fractions analysed (m. p. 192°—198.5°) makes it probable that the samples were still impure. The physical constants of the hydrogenated sterol and its acetate were the same as those of stigmastanol as shown below.

Table 3.

Stigmastanol	Stanol		Stanyl acetate		
from	m. p.	$[\alpha]_{20}^{D}$	m. p.	$[\alpha]_{20}^{D}$	Ref.
Stigmasterol	135.4—36—37°	+ 24.8°	128—29.8°	+ 15.3°	2
»	135°	+ 28°	128°	+ 15.7°	3
Fucosterol	134.5—36°	+ 24.7°	128—30°	+ 15.1°	4
Spongillasterol	134—35°	+ 23.3°	129°	+ 11.5°	3
Pteropodasterol	134.5—35.5°	+ 22.3°	128—28.5°	+ 10.3°	

Pteropodasterol has been found to contain two double bonds. Hence the formula is $C_{29}H_{48}O$ and the carbon skeleton is the same as that of stigmasterol. The position of the double bonds has — in lack of material — not been investigated chemically. The methods of molecular rotation differences (M. R. D.) worked out by Barton [5,6], show that one double bond is in the 5,6-position and the other must be in the side chain. In Table 4 are given the molecular rotations of pteropodasterol and its M. R. D's according to Barton's nomenclatur, compared with those of related sterols.

The provitamine D content of the sterol fraction was determined by its UV absorption. In a sterol fraction, isolated from the unsaponifiable matter as the digitonin complex, and split according to Schønheimer and Dam [7], the provitamine content was found to be 4.5 %. By chromatography a fraction was obtained in the upper part of the column containing 10 % provitamine. Biological activity was not tested.

DISCUSSION

It is well known that vertebrates may synthezise their own sterols. Very little is known about the sterol metabolism of lower animals. Certain facts point to the possibility that lower animals may contain sterols of exogen

Table 4.

The M. R. D. according to Barton 1945 [2]

	$[M]_D^{20}$ sterol	$[M]_D^{20}$ acetate	$[M]_D^{20}$ 3,5-dinitrobenzoate	Δ^1	Δ^2
Chalinasterol	− 167	− 202		− 35	
Clionasterol	− 153	− 192	− 85	− 39	+ 68
Spongillasterol	− 174	− 219	− 109	− 45	+ 65
Poriferasterol	− 206	− 241	− 133	− 35	+ 73
Stigmasterol	− 202	− 241	− 133	− 39	+ 69
Pteropodasterol	− 165	− 214	− 91	− 49	+ 74

The M. R. D. according to Barton 1946 [3]

	$[M]_D^{20}$ sterol	$[M]_D^{20}$ acetate	Δ^1	Δ^2
Pteropodastanol	+ 94	+ 47		
Pteropodasterol	− 165	− 214	+ 259	+ 261
	found by Barton		$+ 251^{+10}_{-9}$	$+ 243^{+27}_{-18}$

origin, as stated by Bergmann [1]. All gastropods have been found to contain cholesterol, alone or together with another sterol. The occurence of a new sterol in two pelagic snails is worth notice. These pteropods are carnivorous, mainly feeding on *Calanus finmarchicus* and other crustaceans. Crustaceans are found to contain cholesterol. Thus the pteropods investigated are mainly feeding on a C_{27}-sterol whereas they contain a C_{29}-sterol. This leads to the conclusion that they contain no exogen sterol. These pteropods have their own metabolism since their organism seems to be able to exclude exogen sterol and synthezise its own one. Their metabolic product, however, is another one than that of other gastropods.

EXPERIMENTAL

Limacina helicina. The contents of a heat conserved glass jar were first extracted 4 times with ether, then an equal amount of ethanol was added, and the extraction with ether repeated 3 times more. The iodine number (175) of the fraction from the last three extractions, as determined by the micro method of Kaufmann [8], was higher than that of the first four ones (148). No fractionation effect could, however, be seen regarding the contents of unsaponifiable matter and of sterol. Half of the raw material was heat conserved, the rest was conserved with ammonium sulphate. No difference could be observed between the two parts of material preserved in these ways. The bulk of material was first denaturated and dried with ethanol, and then extracted 4 times with ether.

The fat was of a dark brown colour. This colour followed the fatty acids after saponification. Glycerol was determined as *iso*propyliodide [9] in a quantity corresponding to 82 % of the fatty acids calculated as triglycerides. Fat content 0.55—0.88 g/100 g wet material, fatfree residue 9.7 g/100 g wet material, and ash 3.07 g/100 g wet material. Unsaponifiable matter 10.7 g/100 g fat. The amount of sterol was determined as the digitonin complex and calculated as cholesterol; according to the work of Schønheimer and Dam [7] it was not found correct to calculate as pteropodasterol without special investigations. Found 62.1 g/100 g unsaponifiable matter.

Pteropodasteryl acetate. The bulk of unsaponifiable matter was extracted repeatedly with boiling methanol, and the sterol fraction recrystallized from methanol, m. p. 120.5 —22.5°. The crystals were dissolved in petroleum (b. r. 65—70°) and chromatographed. Different adsorbents were tried. Magnesium oxide was found to be the best one and was used in these experiments. The samples were brought on the column with petroleum and developed with petroleum-benzene 1 : 1. After crystallization from methanol the free sterol melted at 127—29.5°. The sterol was acetylated in boiling acetic anhydride. The acetate was crystallized twice from methanol and chromatographed. 8 fractions were collected and each fraction crystallized from methanol. All fractions melted in the range 131—34.5°, the m. p. of the highest melting fraction being 133—34.5°, $[\alpha]_D^{20}$ — 47.0° (41.1 mg in 3.14 ml, α_D — 1.23°). All optical rotations were measured in chloroform.

Pteropodasterol. The pure acetate was saponified. After recrystallization from methanol the free sterol was obtained, m. p. 130.5—32°, $[\alpha]_D^{20}$ — 39.9° (37.5 mg in 3.13 ml, α_D — 0.95°).

Pteropodasteryl 3,5-dinitrobenzoate. The free sterol was heated on a waterbath for 1 hour with a slight excess of 3,5-dinitrobenzoyl chloride in pyridine. The ester was crystallized from methanol and chromatographed, the highest melting fraction melting at 195.5—98.5°, $[\alpha]_D^{20}$ — 14.9° (45.2 mg in 3.12 ml, α_D — 0.43°).

Clione limacina. The material was treated in the same way as that of *L. helicina.* After extraction with alcohol and ether a dark brown, viscous fat was obtained in an amount of 0.68 g/100 g raw material, iodine number 167. The fatfree residue was dried in a vacuum, 6.88 g/100 g wet material, ash 2.71 g/100 g wet material. Unsaponifiable matter amounted to 13.9 g/100 g fat, and sterol content 40.8 g/100 g unsaponifiable matter (calculated as above).

Pteropodasteryl acetate. After some crystallizations from methanol, the sterol was chromatographed, acetylated and recrystallized from methanol, m. p. 134.5—36°, $[\alpha]_D^{20}$ — 47.9° (60.6 mg in 3.12 ml, α_D — 1.85°), and for another fraction prepared in the same way $[\alpha]_D^{20}$ — 47.1° (42.2 mg in 3.12 ml, α_D — 1.27°).

Pteropodasterol. The acetate was saponified, and the sterol crystallized from methanol, m. p. 131.5—32°, $[\alpha]_D^{20}$ — 40.4° (75.0 mg in 3.12 ml, α_D — 1.93°).

Iodine number of the free sterol has been determined according to the micro method of Kaufmann [8]. Dam [10] has found this method useful in the case of sterols.

	Iodine number	
Found for pteropodasterol	119	corresponding to 1.93 double bonds
Found for cholesterol	66.9	calculated 66

Pteropodasteryl 3,5-dinitrobenzoate. The free sterol was heated on a waterbath for 1 hour with a slight excess of 3,5-dinitrobenzoyl chloride in pyridine. The ester was re-

crystallized from methanol, added a little ether and chromatographed, m. p. 193—94.5°, $[\alpha]_D^{20}$ — 14.7° (34.6 mg in 3.12 ml, α_D — 0.32°).

Analysis of pteropodasteryl 3,5-dinitrobenzoate:

Found for *L. helicina*	C 70.13, 70.51	H 8.43, 8.34
» » *C. limacina*	» 70.35, 70.88, 70.37	» 8.42, 8.14, 8.46
Calculated for $C_{34}H_{48}O_6N_2$	» 70.34	» 8.29
» » $C_{35}H_{50}O_6N_2$	» 70.71	» 8.42

Mixed melting points of the acetates and the 3,5-dinitrobenzoates of the sterols from the two pteropods were 132—33.5° and 192.5—94.5° (turned clear 196°) respectively.

Pteropodastanyl acetate. 202.1 mg of the acetate was hydrogenated in acetic acid with a catalyst of palladium on barium sulphate. The acetate absorbed 20 ml hydrogen, corresponding to 1.93 double bonds. After recrystallization from methanol the hydrogenated substance showed m. p. 128—28.5°, $[\alpha]_D^{20}$ + 10.3° (66.45 mg in 3.12 ml, α_D + 0.46°).

Pteropodastanol. The free stanol was obtained after saponification of the saturated acetate and recrystallized from acetone, m. p. 134.5—35.5°, $[\alpha]_D^{20}$ + 22.3° (58.6 mg in 3.12 ml, α_D + 0.83°).

SUMMARY

The pteropods *Clione limacina* Phipps and *Limacina helicina* Phipps have been investigated. Both species have been found to contain a new sterol which is referred to as pteropodasterol.

The author wishes to express his sincere gratitude to the head of the laboratory, Prof. Dr. techn. N. A. Sørensen for his valuable advice and interest for this work.

REFERENCES

1. Bergmann, W., and Low, E. M. *J. Org. Chem.* **XII** (1947) 67.
2. Bengtson, B. E. *Z. physiol. Chem.* **237** (1935) 46.
3. Mazur, A. *J. Am. Chem. Soc.* **63** (1941) 2442.
4. Coffey, D. H., Heilbron, I. M., and Spring, F. S. *J. Chem. Soc.* (1936) 738.
5. Barton, D. H. R. *Ibid.* (1945) 813.
6. Barton, D. H. R. *Ibid.* (1946) 512.
7. Schønheimer, R., und Dam, H. *Z. physiol. Chem.* **215** (1933) 59.
8. *Handbuch der Lebensmittelchemie.* **IV**. Berlin (1939) p. 108.
9. *Ibid.* **VII**. Berlin (1939) p. 308.
10. Dam, H. *Nogle Undersøgelser over Sterinernes biologiske Betydning.* København (1933) p. 35.

Received February 8, 1950.