[CONTRIBUTION FROM THE STERLING CHEMISTRY LABORATORY, THE BINGHAM OCEANOGRAPHIC LABORATORY, AND THE BERMUDA BIOLOGICAL STATION FOR RESEARCH]

CONTRIBUTIONS TO THE STUDY OF MARINE PRODUCTS. XXVI.[1] STEROLS FROM SPONGES OF THE FAMILY SUBERITIDAE[2]

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During the past decade the sterols of numerous animals, belonging to many phyla, have been investigated in this laboratory. These studies have revealed the interesting fact that the greatest diversity of sterols is to be found among the most primitive animals such as sponges and coelenterates. As has been pointed out in another communication of this series[1], it now appears that the process of evolution in animals has been accompanied by a discontinuation of the use of a variety of sterols in favor of the practically exclusive use of cholesterol.

TABLE I

ORDERS AND FAMILIES OF SPONGES[4]

Order: HALICHONDRINA (Vosmaer)
Family: Hymeniacidonidae (de Laubenfels)
Order: HADROMERINA (Topsent)
Family: Choanitidae (de Laubenfels)
Family: Suberitidae (Schmidt)
Family: Clionidae (Gray)

Best known among the sterols of lower invertebrates are those of the sponges of which more than fifty species have been studied in this laboratory. At present eight sponge sterols have been well characterized, and the occurrence in sponges of several other sterols has been indicated. Numerous as they are, the available data do not yet suffice to establish well defined relationships between the sterol content of sponges and the taxonomy of Porifera in general. Within certain families, however, such relationships have already been observed, particularly in regard to the families listed in Table I.

It has been shown in a previous communication (2) that the sterols of *Sphe-ciospongia vesparia*, of the family Choanitidae, are a mixture of the *levorotatory* poriferasterol and clionasterol. A similar mixture has been isolated from *Cliona*

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[4] This classification is based on that proposed by de Laubenfels (1).

celata, a species belonging to the Clionidae. In contrast, two species of Suberitidae, *Suberites compacta* and *S. domuncula*, have afforded mixtures of the *dextrorotatory* cholestanol and neospongosterol (3). These preliminary observations at once pointed to a significant and readily detectable biochemical difference between the families of Choanitidae and Clionidae and the family of Suberitidae. Since then several other species belonging to the respective families have been investigated, and the nature of their sterols has been found in accord with the earlier observations. The lipoid contents of these sponges are listed in Table II.

TABLE II

COMPOSITION OF DRIED SPONGES

SPECIES	% OF TOTAL		% OF ORGANIC	% OF FAT	% OF UNSAP.
	Spicules	Organic	Fat	Unsapon.	Sterol
Spheciospongia	22	78	4.5	46	50
Anthosigmella	59	41	6	48	58
Cliona	16	84	9	27	58
Terpios fugax	11	89	13.5	23	82
Terpios zeteki	46	54	8.5	41	64
Aaptos	17	83	5	33	69
Radiella	73	27	11	37	55
Weberella	30	70	11	64	55
Polymastia	51	49	11	35	66

FAMILY: CHOANITIDAE

Spheciospongia sp.[5] This sponge is one of the most common, and because of its large size, one of the most conspicuous sponges of the Bermuda Archipelago. Its crude sterol is strongly levorotatory. Bromination of the steryl acetate afforded a high-melting tetrabromide which upon debromination gave poriferasteryl acetate, m.p. 145–146°; $[\alpha]_D - 51°$. Debromination of the more soluble dibromide led to the isolation of clionasteryl acetate, m.p. 136°; $[\alpha]_D - 45°$. It is estimated that the original sterol mixture contained about forty % of poriferasterol and more than fifty % of clionasterol.

Anthosigmella varians (Topsent). This sponge is exceedingly common in the waters near Virginia Key, Florida, where it was collected in 1945.[6] Perbenzoic acid titration of the crude, levorotatory, sterol mixture indicated the presence of about thirty % of a diunsaturated sterol. By separation over the acetate bromides, the former was identified as poriferasterol, m.p. 156°; $[\alpha]_D - 49°$, and the latter as clionasterol, m.p. 140°; $[\alpha]_D - 39°$.

[5] A description of this new species will be given by de Laubenfels in his forthcoming paper on the sponges of Bermuda.

[6] The authors express their gratitude to Dr. W. F. Smith, University of Miami, for his generous assistance in the collection of this sponge.

FAMILY: CLIONIDAE

Cliona carriboea Carter. The first lot of this sponge was obtained from the coast of Florida in 1943. The sponges were of a tubular shape with an average height of about twenty cm. and a diameter of about five cm. The mixture of sterols obtained from this sponge consisted of approximately forty % of poriferasterol, m.p. 156°; $[\alpha]_D$ −49.5°, and more than fifty % of clionasterol, m.p. 139°; $[\alpha]_D$ −40°.

A second lot of this sponge had been collected in the coastal waters of Bermuda. Unlike the tall species from Florida, the Bermuda variety was of a flat and irregular shape. Its sterol mixture also consisted of clionasterol and poriferasterol, but the latter represented less than ten % of the total.

FAMILY: SUBERITIDAE

Suberites suberea Montague. A few dried specimens of this sponge were obtained through the courtesy of the U. S. National Museum. They had been collected off the coast of Alaska prior to 1900. The sponge afforded a dextrorotatory sterol mixture. Bromination of the steryl acetate, m.p. 124°; $[\alpha]_D$ +14.5°, gave the characteristic "spongosteryl acetate bromide" of m.p. 150°, which has previously been shown to be an adduct of cholestanyl acetate and neospongosteryl acetate dibromide (3). It may therefore be assumed that the sterol mixture from this sponge is essentially of the same composition as that present in *Suberites compacta* and *S. domuncula.*

Terpios fugax (Duchassaing and Michelotti). Substantial quantities of this sponge were collected in 1946 and 1947 in Harington Sound and Walsingham Pond, Bermuda. Upon continuous extraction of the dried sponge with acetone, there separated from the boiling solvent considerable amounts of a white, powdery material, which will be discussed in a future publication. The properties of the crude sterol obtained from this sponge, $[\alpha]_D$ +14°, 0.2 double bonds, at first suggested the presence of "spongosterol", *i.e.* a mixture of cholestanol and neospongosterol (3). Repeated brominations of the steryl acetate, however, failed to yield the characteristic "spongosteryl acetate bromide". No difficulties were encountered in identifying cholestanol as the principal saturated component of the sterol mixture. The isolation of the unsaturated component in a reasonable state of purity has so far met with little success. An acetate fraction, enriched with unsaturated material, melted at 136.5°; $[\alpha]_D$ +8°. It appears at present that the unidentified, unsaturated sterol is slightly dextrorotatory, and that, like neospongosterol, it is unsaturated in the side chain only.

Terpios zeteki de Laubenfels. This sponge was received from the Hawaiian Islands through the courtesy of Dr. de Laubenfels. Upon extraction with acetone it yielded products identical with those described under *Terpios fugax.*

Aaptos sp.[5] A collection of this sponge was made in Harington Sound, Bermuda in 1947. Titration of the crude steryl acetate, m.p. 121–125°; $[\alpha]_D$ +13°, with perbenzoic acid showed the presence of about ten % of unsaturated material, which was at once removed according to the procedure of Anderson and Nabenhauer (4). The relatively high melting point, 122–124°, of the product thus ob-

tained indicated the presence of a saturated compound other than cholestanyl acetate. Repeated recrystallizations of the acetate mixture eventually gave a fraction melting constantly at 133.5°; $[\alpha]_D$ +14°. Hydrolysis of the acetate gave the stanol, m.p. 135°; $[\alpha]_D$ +22.2°. Analysis of the stanyl-*m*-dinitrobenzoate, m.p. 209–212°; $[\alpha]_D$ +15.6°, indicated an empirical formula of $C_{28}H_{50}O$ for the stanol. It is proposed to refer to this compound as aptostanol until its relation to known sterols has been established.

Concentration of the mother liquors from the recrystallization of the original acetate gave lower-melting fractions. These were combined, saponified, and the resulting stanol was benzoylated. Recrystallization of the benzoate eventually gave cholestanyl benzoate.

Radiella sol Schmidt. This sponge which is rather common in the deep waters of the Atlantic Ocean was collected by the research ship "Atlantis" during the summer of 1948. The high dextro-rotation of the crude sterol, $[\alpha]_D$ +20°, indicated that it consisted essentially of saturated material. The acetate, freed

TABLE III

COMPARISON OF APTOSTANOL AND HALICLONASTANOL

ORIGIN	STANOL M.P., °C. $[\alpha]^\circ_D$	ACETATE M.P., °C. $[\alpha]^\circ_D$	BENZOATE M.P., °C. $[\alpha]^\circ_D$	*m*-DINITROBENZOATE M.P., °C. $[\alpha]^\circ_D$
Aaptos	135.5 +22	133.5 +14		212 +16
Radiella	134.5 +22	134 +15	133.5 +19	— -
Weberella	135.5 +20	134.5 +12		208 +18
Haliclonasterol	137.5 +18	136.5 +11	133.5	218

from unsaturated material (4), melted at 131°; $[\alpha]_D$ +14.5°, and after a few recrystallizations at 133–134°; $[\alpha]_D$ +15°. It was converted to the stanol, m.p. 134.5°; $[\alpha]_D$ +22°, and the stanyl benzoate, m.p. 133.5°; $[\alpha]_D$ +19°. A comparison of the physical data of this stanol with those of aptostanol (Table III) suggests the identity of the two sterols.

The mother liquors from the original acetate did not yield any low-melting fractions indicative of the presence of cholestanyl acetate, and systematic recrystallization of the benzoate failed to give cholestanyl benzoate. It appears therefore that the sterol of *Radiella sol* consists essentially of aptostanol.

Weberella bursa (Müller). This sponge[7] was obtained from the Atlantic Ocean near Newfoundland. Recrystallization of the steryl acetate, freed from unsaturated material, eventually afforded a fraction, m.p. 134°; $[\alpha]_D$ +12°, which appeared to be identical with aptostanyl acetate (Table III). Saponification of the lower-melting acetate fraction, benzoylation of the resulting stanol, and recrystallization of the benzoate gave cholestanyl benzoate.

Polymastia infrapilosa Topsent. This sponge[7] was collected in the same localities as the one discussed above. The saturated fraction of the steryl ace-

[7] Identified by Mr. W. Hartman, Yale University.

tates melted at 111–117°; $[\alpha]_D$ +11°. Lack of material has so far prevented a satisfactory separation of the mixture. The isolation of a fraction of m.p. 125°; $[\alpha]_D$ +11.2°, indicated the presence of aptostanyl acetate. In addition the presence of cholestanol in the original mixture was clearly demonstrated.

DISCUSSION

The investigations described above prove convincingly the existence of relations between the sterol content of sponges and their conventional taxonomic features. Within the order of Hadromerina species belonging to the family of Suberitidae have all been found to contain saturated compounds as the principal constituents of their sterol mixtures. In contrast members of the families of Choanitidae and Clionidae have been shown to contain as their principal sterols the Δ^5-unsaturated clionasterol and poriferasterol. Since the saturated sterols are distinctly dextrorotatory, and since the Δ^5-unsaturated sterols are strongly levorotatory, the optical rotation of the sterol mixture from a sponge of the order Hadromerina may be used as an aid in its classification. The usefulness of sterol analysis in the identification and classification of sponges has already been demonstrated in several instances, one of which is of particular interest in the present connection.

The sponge *Anthosigmella varians*, which has been discussed above, had originally been identified as *Suberites distortus*. Serious doubts concerning the correctness of this identification were raised when it was found that the sterols from this sponge, unlike those from other Suberitidae, were strongly levorotatory. When a more detailed analysis revealed the presence in the mixture of clionasterol and poriferasterol the authors concluded that the sponge in question belonged to the families of Choanitidae or Clionidae rather than to the Suberitidae. The correctness of this conclusion was eventually established by de Laubenfels. In the course of a careful study of the freshly caught sponge, this investigator detected certain features, easily overlooked, which proved this sponge to be indeed a species of Choanitidae.

With the exception of *Radiella sol*, all sponges of the Suberitidae which have so far been investigated contain cholestanol as a substantial component of their sterol mixture. In sponges of the genus *Suberites* and possibly also of the genus *Terpios* this stanol is accompanied by feebly dextrorotatory, unsaturated sterols, devoid of cyclic unsaturation, such as neospongosterol (3). The sterol mixtures from other Suberitidae, such as *Aaptos*, *Weberella*, *Polymastia*, and *Radiella*, contain in addition to, or to the practical exclusion of, cholestanol a stanol of the order $C_{28}H_{50}O$, which has tentatively been named aptostanol. This compound is the second saturated sterol which has so far been found to occur in animals. At present it does not appear that aptostanol is identical with any one of the well-characterized stanols. As shown in Table III, its physical properties are reminiscent of those of the recently described haliclonastanol (5) and hence indicate the possible identity of the two stanols.

In a future revision of the families of the order of Hadromerina, cognizance should be taken of the fact that the sterols of Suberitidae are quite distinct

from those of Choanitidae and Clionidae. The saturated nature of the principal sterols of Suberitidae implies a sterol metabolism in these sponges which is different from that taking place in the sponges from the two other families in which unsaturated sterols predominate. This difference appears at least as significant as the more conventional taxonomic evidence. It suggests that in a regrouping of the species of Hadromerina those belonging to the Suberitidae should be set distinctly apart from those assigned to the other families.

Another sponge which is of interest in this connection is *Hymeniacidon heliophila*. This species had first been described as *Stylotella heliophila*, but had been referred by de Laubenfels (1) to *Hymeniacidon* because of its close resemblance to various European species of this genus. If this sponge were indeed a species of the genus *Stylotella* it would belong to the Suberitidae of the order Hadromerina (Table I), and one should expect it to contain a saturated sterol as the principal component of its sterol mixture. Otherwise it would belong to the family of Hymeniacodinidae of the order Halichondrina. It has been shown in a previous communication (6) that the principal sterol of this sponge is cholestanol. On the basis of this evidence it might therefore be argued that the sponge under consideration is indeed a species of Suberitidae and that it should be reassigned to this family. Such a transfer, however, will remain premature until more is known about the sterol content of other species of Hymeniacidon and also of the closely related Halichondria. It appears at present, however, that there exist closer biochemical relationships between *Hymeniacidon heliophila* and the Suberitidae, than between the latter and Choanitidae and Clionidae.

EXPERIMENTAL

All melting points are corrected. Unless stated otherwise, all optical rotations were taken in a 1-dm. tube, the sample being dissolved in 3.06 cc. of chloroform. In all but one case the sterols were obtained by the following method. The air-dried sponges were ground and then thoroughly extracted with acetone in a Soxhlet apparatus. After evaporation of the solvent, the residue was dissolved in benzene, and the water removed by codistillation. In all instances, varying amounts of smeary, brown, water-soluble material remained undissolved in the benzene. The benzene extract was then evaporated to dryness, and the residue dried to constant weight at 80°. This acetone–benzene soluble fraction is referred to as fat in Table II. The data in this table are all based on weights of crude sponge material from which the non-spicular ash has been subtracted as described in a previous communication (7). The saponification of the fat and the isolation of the sterol was carried out as previously described. The sterol content of an aliquot part of the non-saponifiable fraction was determined by precipitation with digitonin.

Spheciospongia sp. The crude steryl acetate, m.p. 133°; $[\alpha]_D$ −45°, upon titration with perbenzoic acid showed unsaturation corresponding to 1.4 double bonds. The acetate mixture was separated into *poriferasteryl acetate*, m.p. 145–146°; $[\alpha]_D^{23°}$ −51°, and *clionasteryl acetate*, m.p. 136°; $[\alpha]_D^{23}$ −45° by way of the bromides as described previously in connection with the separation of the sterols from *Spheciospongia vesparia* (2).

Anthosigmella varians. The crude steryl acetate melted at 132–133°; $[\alpha]_D^{20°}$ −45.5. Titration with perbenzoic acid showed unsaturation corresponding to 1.3 double bonds. Bromination of the acetate mixture in the manner described previously gave the difficultly soluble poriferasteryl acetate tetrabromide, m.p. 190–192°. Its identity was demonstrated by its conversion to *poriferasteryl acetate*, m.p. 146°; $[\alpha]_D^{24°}$ −52.6° (20.4 mg., α −0.35°), to poriferasterol, m.p. 154°; $[\alpha]_D^{24°}$ −49.1° (24.3 mg., α −0.39°) and poriferasteryl-*m*-dinitrobenzoate,

m.p. 227°; $[\alpha]_D^{25°}$ −22.2° (31.7 mg., α −0.23°). None of these compounds showed a depression of the melting point when mixed with authentic material.

Debromination of the soluble bromides afforded an acetate which after frequent recrystallization gave *clionasteryl acetate*, m.p. 139.5–140°; $[\alpha]_D^{26°}$ −42°. It was converted to clionasterol, m.p. 139.5–140°; $[\alpha]_D^{26°}$ −39.3° (26.5 mg., α −0.34°), and clionasteryl benzoate, m.p. 135–136°, $[\alpha]_D^{28°}$ −18.4° (28.3 mg., α −0.17°).

Cliona carriboea. A total of 17.8 g. of sterol was obtained from the Bermuda species. The sterol mixture was separated in the usual manner by way of the acetate bromides. The difficultly soluble bromide was identified as poriferasteryl acetate tetrabromide, m.p. 187° by its conversion to *poriferasteryl acetate*, m.p. 146–147°; $[\alpha]_D^{26°}$ −54.5° (30.2 mg., α −0.54°), poriferasterol, m.p. 153–154°; $[\alpha]_D^{26°}$ −50.5° (30.3 mg., α −0.50°), and poriferasteryl propionate, m.p. 125–126°; $[\alpha]_D^{26°}$ −51.5° (29.8 mg., α −0.50°).

Debromination of the soluble bromides gave *clionasteryl acetate*, m.p. 137.5°; $[\alpha]_D^{26°}$ −42.5° (30.8 mg., α −0.44°), which was converted to clionasterol, m.p. 137.5°; $[\alpha]_D^{26°}$ −38° (30.6 mg., α −0.38°), clionasteryl propionate, m.p. 118–119°; $[\alpha]_D^{25°}$ −42.4° (29.5 mg., α −0.41°), and clionasteryl benzoate, m.p. 139–140°; $[\alpha]_D^{25°}$ −38° (30.6 mg., α −0.38°).

The acetate obtained from 25 g. of sterol isolated from the Florida species showed unsaturation equivalent to 1.35 double bonds when titrated according to Rosenmund's (8) method. Upon separation of the acetates by way of the bromides results analogous to those described above were obtained with the exception that the yield of poriferasteryl acetate was substantially higher.

Suberites suberea. A total of 63.5 g. of the dried sponge was ground and exhaustively extracted first with ether and then with acetone. The residue from the combined extracts, (0.6 g.), was dissolved in 50 cc. of hot 80% ethanol, and the solution mixed with 80 cc. of a hot 1% solution of digitonin in 80% ethanol. The mixture was then refluxed until the digitonide began to separate. After twenty-four hours it was collected and washed with ethanol and ether; 560 mg., equivalent to 140 mg. of sterol.

The digitonide was refluxed with 15 cc. of acetic anhydride for two hours. The solution was then diluted with water, and the precipitated acetate filtered, washed with water and methanol, and recrystallized twice from methanol; m.p. 122–124°, $[\alpha]_D^{27°}$ +14.4° (20.1 mg., α +0.095°). To a solution of 0.1 g. of acetate in 0.5 cc. of anhydrous ether was added 1.1 cc. of a 5% solution of bromine in acetic acid. After standing in the refrigerator overnight, the mixture was filtered, and the solid washed with acetic acid and methanol, and dried; m.p. 140–145°. After recrystallization from ethyl acetate–methanol, the bromide melted at 155°. It gave no depression of melting point when mixed with an authentic sample of *"spongosteryl acetate monobromide"* (3).

Anal. Calc'd for $C_{29}H_{50}O_2 + C_{30}H_{50}Br_2O_2$: Br, 15.5. Found: Br, 14.8.

Terpios fugax. During the acetone extraction of the dried, ground sponge a white, powdery material separated from the boiling solvent. After twenty-four hours of extraction, the suspension was cooled and filtered. The insoluble material represented approximately 1.5% of the dry sponge. The solvent was then evaporated and the sterol, m.p. 131°; $[\alpha]_D$ +14°, isolated as described previously. Titration of the crude steryl acetate, m.p. 120–122°, with perbenzoic acid showed unsaturation corresponding to 0.2 double bonds. Bromination of the acetate under a variety of conditions failed to yield a difficultly soluble bromide.

Numerous recrystallizations of the crude steryl benzoate from dioxane, absolute ethanol, and chloroform–methanol gave *cholestanyl benzoate*, m.p. 135.2° (turbid liquid), 155° (clear); $[\alpha]_D^{24°}$ +23.6°. The benzoate was converted to cholestanol, m.p. 142–142.5°; $[\alpha]_D^{24°}$ +13.5° (38.6 mg., 1.32 cc., α +0.395). None of these compounds gave melting point depressions when mixed with authentic material.

Terpios zeteki. Extraction of this sponge with acetone gave results analogous to those described above. Repeated recrystallization of the crude steryl benzoate, m.p. 130–135°, 0.25 double bonds, from dioxane, chloroform–methanol, acetone, and ether afforded *cholestanyl benzoate*, m.p. 135° (turbid liquid), 155° (clear); $[\alpha]_D^{23°}$ +22.1.° (31 mg., α +0.22°). It was converted to cholestanol, m.p. 142–142.5°; $[\alpha]_D^{23°}$ +23.1° (26.5 mg., α +0.20°) and to cholestanyl acetate, m.p. 111–112°; $[\alpha]_D^{23°}$ +12.9° (29.0 mg., α +0.12°).

Aaptos sp. The crude sterol, 3.1 g., melted at 118–125°; $[\alpha]_D$ +19°. Upon acetylation a dark-red solution was obtained, and the precipitated acetate was also colored. The acetate was then dissolved in chloroform and the solution treated with Norit. After filtration and concentration, methanol was added to the solution. A reddish oil precipitated. It was immediately separated from the supernatant liquid, which upon concentration gave 1.75 g. of acetate, m.p. 121–125°; $[\alpha]_D$ +13°; 0.15 double bonds.

Aptostanyl acetate. To a solution of 1.7 g. of the above acetate in 35 cc. of carbon tetrachloride and 18 cc. of acetic anhydride was added dropwise and with constant stirring and cooling, about 1 cc. of conc'd sulfuric acid. After twenty minutes, water and more carbon tetrachloride were gradually added until a clear separation of layers had taken place. The carbon tetrachloride layer was then washed once with a sodium chloride solution and twice with a solution of sodium bicarbonate, dried, and evaporated to dryness. The residue was recrystallized once from acetic anhydride; 1.25 g., m.p. 122–124°. After six recrystallizations from ethanol, ether, and ether–methanol, the *acetate*, 250 mg., melted constantly at 132.5–133.5°; $[\alpha]_D^{28°}$ +14.0° (30.6 mg., α +0.14°).

Anal. Calc'd for $C_{30}H_{52}O_2$: C, 81.02; H, 11.79.
Found: C, 80.94; H, 11.86.

Aptostanol. Saponification of the above acetate gave the *stanol* which was recrystallized from ether–methanol; m.p. 134.5–135.5°; $[\alpha]_D^{27°}$ +22.2° (28.9 mg., α +0.21°).

Aptostanyl-m-dinitrobenzoate. A mixture of 65 mg. of the above stanol and 85 mg. of freshly prepared *m*-dinitrobenzoyl chloride was dissolved in a small volume of anhydrous benzene and two drops of pyridine. The mixture was refluxed for 90 minutes and then evaporated to dryness under reduced pressure. Recrystallization of the residue from chloroform–methanol, absolute ethanol, and ether–ethanol gave 60 mg. of the *m-dinitrobenzoate*, m.p. 210–212°; $[\alpha]_D^{28°}$ +15.6° (27.5 mg., α +0.14°).

Anal. Calc'd for $C_{35}H_{52}N_2O_6$: C, 70.44; H, 8.78.
Found: C, 70.36; H, 8.69.

Cholestanol. Concentration of the first mother liquors from the series of recrystallizations which led to aptostanyl acetate gave 470 mg. of an acetate of m.p. 116–118°. It was saponified, and the free sterol at once benzoylated. Five recrystallizations of the benzoate from chloroform–methanol, benzene–ethanol, ether–methanol, and ether led to 85 mg. of cholestanyl benzoate, m.p. 135° (turbid liquid), 154° clear; $[\alpha]_D^{22°}$ +22.0° (26.9 mg., α +0.19°). Saponification of the benzoate and acetylation of the resulting stanol gave cholestanyl acetate, m.p. 111.5–113°; $[\alpha]_D^{21°}$ +14.4° (29.7 mg., α +0.14°).

Radiella sol. The total amount of sterol obtained from less than 200 g. of the dry sponge was 0.65 g; m.p. 120–124°, $[\alpha]_D$ +20°. The sterol was benzoylated and the benzoate recrystallized several times from chloroform–methanol; m.p. 128–131°; $[\alpha]_D$ +14°. The benzoate gave a very faintly positive Liebermann-Burchard reaction.

The total benzoate was then saponified and the sterol, 0.5 g., converted to the acetate, m.p. 125–128°. It was then treated with carbon tetrachloride, acetic anhydride, and sulfuric acid as described above to eliminate unsaturated impurities. After three recrystallizations from ethanol and ethyl acetate, the purified *acetate* melted at 133–134°; $[\alpha]_D^{23°}$ +15.0° (28.6 mg., α + 0.14°). It gave no depression of the melting point when mixed with *aptostanyl acetate* described above.

Anal. Calc'd for $C_{30}H_{52}O_2$: C, 81.02; H, 11.79.
Found: C, 80.89; H, 11.95.

Saponification of the actate gave the *stanol*, m.p. 133.5–134.5°; $[\alpha]_D^{25°}$ +22.0° (24.0 mg., 3.09 cc., α +0.17°).

Benzoylation of the stanol with benzoyl chloride gave a *benzoate* of m.p. 132–133.5°; $[\alpha]_D^{25°}$ +19.0° (24.2 mg., α +0.15°).

Anal. Calc'd for $C_{35}H_{54}O_2$: C, 82.95; H, 10.73.
Found: C, 82.82; H, 10.92.

Fractionation of the residues obtained from the mother liquors of the recrystallizations of the acetate and benzoate failed to yield derivatives of cholestanol.

Weberella bursa. The acetate obtained from the crude sterol, 4.9 g., was freed from unsaturated material by the method described above, and the resulting product was recrystallized from ethanol, ether–methanol, and chloroform–methanol until the m.p. remained constant at 133–134.5°; $[\alpha]_D^{23°}$ +12.0° (24.5 mg., α +0.10°). The *acetate* did not give a depression of the melting point when mixed with *aptostanyl acetate*.

Anal. Calc'd for $C_{30}H_{52}O_2$: C, 81.02; H, 11.79.
Found: C, 80.85; H, 12.0.

The *stanol* obtained from the purified acetate melted at 134.5–135.5°; $[\alpha]_D^{24°}$ +19.6° (25.0 mg., α +0.16°).

The *m-dinitrobenzoate* of the stanol was prepared as described above; m.p. 208°; $[\alpha]_D^{25°}$ +18.0° (22.2 mg., α +0.13°).

Anal. Calc'd for $C_{35}H_{52}N_2O_6$: C, 70.44; H, 8.78.
Found: C, 70.32; H, 8.84.

Residues from the first mother liquors of the recrystallization of the acetate were saponified and the resulting stanol was benzoylated. Systematic recrystallization of the bezonate eventually yielded *cholestanyl benzoate*, m.p. 134° (turbid liquid), 154° (clear); $[\alpha]_D^{24°}$ +22.2° (27.6 mg., α +0.20°).

It was converted to cholestanol, m.p. 141–142°, and cholestanyl acetate, m.p. 113.5–115°; $[\alpha]_D^{24°}$ +14.5° (21.3 mg., 3.09 cc., α +0.10°).

Polymastia infrapilosa. A total of 85 g. of dry sponge gave 0.8 g. of a very crude sterol, m.p. 110–112°; $[\alpha]_D$ +6°. The acetate, m.p. 108–116°; $[\alpha]_D$ −1°, was freed from unsaturated material by the method described above. After several recrystallizations, an *acetate* of m.p. 125°, $[\alpha]_D^{21°}$ +11.2° was obtained. Lack of material prevented further purification of this product.

The residues from the acetate mother liquors were saponified and the resulting stanol was benzoylated. Repeated recrystallizations of the benzoate eventually yielded *cholestanyl benzoate*, m.p. 135° (turbid liquid), 149° (clear).

SUMMARY

1. The sterols of ten species of sponges of the order Hadromerina have been isolated and investigated.

2. It has been shown that the principal sterols from species of the families Choanitidae and Clionidae are levorotatory, and that those from species of the family Suberitidae are dextrorotatory.

3. Poriferasterol and clionasterol have been found to be the principal sterols of the sponges *Spheciospongia sp.* and *Anthosigmella varians* of the family Choanitidae, and of *Cliona carriboea* of the family Clionidae.

4. Cholestanol has been shown to occur in the following species of the family Suberitidae: *Terpios fugax*, *T. zeteki*, *Aaptos sp.*, *Weberella bursa*, and *Polymastia infrapilosa*.

5. A new saturated sterol, $C_{28}H_{50}O$, tentatively named aptostanol, has been isolated from *Aaptos sp.* The occurrence of this stanol in *Radiella sol*, *Weberella bursa*, and *Polymastia infrapilosa* has been indicated.

6. Attention has been called to the possible identity of haliclonastanol and aptostanol.

7. The significance of these observations in relation to the classification of sponges has been discussed.

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