[CONTRIBUTIONS FROM THE STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY]

CONTRIBUTIONS TO THE STUDY OF MARINE PRODUCTS. VIII. THE STEROL OF SPONGES: CLIONASTEROL AND PORIFERASTEROL

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Cholesterol is the typical principal sterol of vertebrate animals; no exception to this rule has as yet been discovered. In contrast to this uniformity, marine invertebrates are characterized by a surprising diversity of principal sterols. In many species cholesterol is either partially or entirely replaced by other, as yet ill identified, sterols. Such sterols may be of the order C_{27}, as in the case of actiniasterol (1) or of the order C_{29}, as in the case of ostreasterol (2). There exists of course the possibility that sterols of other orders may yet be discovered. It has been suggested that this striking difference between vertebrates and marine invertebrates is due to a dependency on exogenous sterols by at least some of the members of the latter group of animals. Such an assumption would explain the presence of phytosterol-like sterols of the order C_{29} in herbivorous animals like the oyster and other bivalves (2) and the presence of cholesterol or other sterols of the order C_{27} in carnivorous animals like the sea anemones (1). Because of the paucity of available data, however, an accurate evaluation of this hypothesis is as yet impossible. At any rate, its application will have to be restricted to marine invertebrates, since other invertebrates with the possible exception of the fresh-water sponges (3) contain cholesterol as their principal sterol regardless of their feeding habits.

Studies on the sterols of sponges were the first to reveal the presence of sterols other than cholesterol in the animal kingdom. In 1904 Henze (4) described the isolation of spongosterol ($C_{27}H_{48}O$) from *Suberites domuncula*, and in 1908 Dorée (3) reported the presence of clionasterol ($C_{27}H_{46}O$) in *Cliona celata*. Many years later (1933) a third sterol was discovered in *Microciona prolifera* by Bergmann and Johnson (5). The presence of a different sterol in each of the species of sponges so far investigated is very surprising. It is conceivable that such a diversity of sterols is indeed typical for the phylum of *Porifera*, but it is also possible that these sterols are not uniform compounds but rather mixtures of some as yet unidentified sterols. Little credence only can be given the formulas which have been attributed to the sterols of sponges. They were proposed at a time when

the opinion prevailed that almost all naturally occurring sterols were of the order C_{27}. Since the recent methods for the accurate determination of the formulas of sterols have not yet been applied to the sterols of sponges, and since there was reason to suspect that these sterols, because of the feeding habits of sponges, were of the order C_{29} rather than C_{27}, the authors of the present communication have begun a reinvestigation of the sterols of sponges.

In a series of preliminary studies it was found that the loggerhead sponge, *Spheciospongia vesparia*, is a very suitable material for the preparation of larger quantitites of sponge sterols.[1] The air-dried sponge contains between one and one-half per cent of sterol. The bulk of sterols can be obtained directly by concentrating an acetone extract of the dried sponges. Additional quantities are obtained by the saponification of the residues from the mother liquors.

The sterol thus prepared showed a great similarity to clionasterol. Authentic clionasterol was therefore prepared from *Cliona celata*[2] according to the directions of Dorée (3). A direct comparison of the two sterols proved their identity. Further studies on clionasterol from either of the two sponges, however, revealed it to be a mixture. The absorption spectrum of the crude sterol indicated the presence of less than one per cent of a sterol with conjugated double bonds. Since the sponges had been dried in the open air it is quite probable that the bulk of the material of the nature of a provitamin D has been destroyed by oxidation. No attempt was made to isolate the remainder.

The principal constituents of the sterol mixture were identified as a mono- and di-unsaturated sterol. Their separation was effected by way of the acetate bromides according to the method of Windaus (6). The diunsaturated sterol, which represented about forty per cent of the sterol mixture is different from all other sterols which have so far been described. It is proposed to name this sterol poriferasterol because it appears to be a typical constituent of the marine representatives of the phylum *Porifera*. The formula for poriferasterol is $C_{29}H_{48}O$, as based on analyses of a series of derivatives. The properties and reactions of the monounsaturated sterol are reminiscent of those of the original clionasterol, and it is therefore proposed to retain this name for the monounsaturated sterol in the sponges under investigation. On the basis of analyses of a number of derivatives, the formula of clionasterol is to be changed from $C_{27}H_{46}O$ (3) to $C_{29}H_{50}O$. Hence in conformity with the working hypothesis concerning the sterols

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[2] The authors want to express their gratitude to Dr. M. W. de Laubenfels, Pasadena, California for the identification of this sponge.

of marine invertebrates the two principal sterols of at least some of the representatives of the phylum *Porifera* are of the order of plant sterols. Table I gives a comparison of the sterols which have so far been isolated from sponges.

After the present report on clionasterol had been submitted for publication, a brief communication by Mazur (7) appeared in which it is stated that the typical sterol of the fresh-water sponge, *Spongilla lacustris*, is also of the order $C_{29}H_{50}O$. The sterols of this and another fresh-water sponge, *Ephydatia fluviatilis*, have already been the subject of a preliminary investigation by Dorée (3). This author has produced significant, if not convincing evidence for the presence of cholesterol in the sterol mixture. Since all other non-marine invertebrates which have so

TABLE I

COMPARISON OF THE DIFFERENT STEROLS OF SPONGES

NAME	STEROL		ACETATE		BENZOATE		*m*-DINITRO-BENZOATE	
	m.p. °C	$[\alpha]_D$	m.p. °C	$[\alpha]_D$	m.p. °C	$[\alpha]_D$	m.p. °C	$[\alpha]_D$
Clionasterol (old) (3)	138	−37	134	—	141	—	—	—
Clionasterol (new)	138	−37	138	−42	135	−17	203	−14
Poriferasterol	156	−49	147	−53	141	−22	227	−22
Spongosterol (4)	124	−20	124	—	128	—	—	—
Microcionasterol (5)	127	−20	126	−25	143	−11	—	—
Spongillasterol (7)	137	−42	137	−48	137	−17	200	−18

far been investigated have been found to contain cholesterol, the question of the presence of this sterol in fresh-water sponges assumes special significance.

Poriferasterol

The formula $C_{29}H_{48}O$ for poriferasterol is based on analyses of its acetate tetrabromide, acetate dibromide, *m*-dinitrobenzoate, *o*-iodobenzoate, and the *m*-dinitrobenzoate of poriferastanol. The presence of two double bonds has also been demonstrated by titration with perbenzoic acid and catalytic hydrogenation. The acetate, propionate, benzoate, and phenylurethane of poriferasterol have also been prepared to facilitate its identification.

Partial debromination of poriferasteryl acetate tetrabromide according to Fernholz and Stavely (8) gave an acetate dibromide. In analogy with the partial debromination of stigmasteryl acetate tetrabromide this evidence seemed indicative of the presence of a double bond in the side chain

of the sterol molecule, probably between C_{21} and C_{22}. This appears unlikely, however, because all attempts have so far failed to obtain an aldehyde, identifiable as a semicarbazone, by the ozonization of poriferasterol or its acetate.

Catalytic hydrogenation of poriferasterol proceeds smoothly to give the saturated poriferastanol. In Table II the properties of this sterol are compared with those of stigmastanol, ostreastanol (2), and ergostanol. The data at once show that poriferastanol is different from stigmastanol. The figures for poriferastanol and ostreastanol are sufficiently similar to arouse suspicion of the identity of the two compounds. Because of the properties of its acetate tetrabromide, poriferasterol and hence poriferastanol are more readily obtainable in a pure state than ostreasterol and ostreastanol. The slight discrepancies between the data of the two sterols

TABLE II

COMPARISON OF PORIFERASTANOL WITH OTHER SATURATED STEROLS

SUBSTANCE	PORIFERASTANOL		STIGMASTANOL		OSTREASTANOL		ERGOSTANOL	
	m.p. °C	$[\alpha]_D$	m.p. °C	$[\alpha]_D$	m.p. °C	$[\alpha]_D$	m.p. °C	$[\alpha]_D$
Stanol	143	+25	137	+25	141	+24	144	+15
Acetate	141	+16	131	+15	137	+15	145	+6
m-Dinitrobenzoate	213	+17	215	+13	—	—	203	+13
Stanone	161	+47	157	+42	157	+42	164	+35

may conceivably be due to the presence of impurites in ostreastanol. In their studies on the constitution of brassicasterol, Fernholz and Stavely (9) have pointed out that notwithstanding improved analytical methods the establishment of correct formulas for sterols remains a difficult problem. These authors found that while analytical data favored the formula $C_{29}H_{48}O$ for brassicasterol, the identity of brassicastanol with ergostanol proved that $C_{28}H_{46}O$ is the correct formula. Because of the experiences of these authors, the possible identity of poriferastanol and ergostanol has been taken into consideration. While it is true that some of the data for poriferastanol are not unlike those of a slightly impure brassicastanol (ergostanol) (9), the significant difference in the melting points of the *m*-dinitrobenzoates proves that the two compounds are not identical.

Investigations which are in progress in this laboratory have furnished evidence demonstrating the presence of poriferasterol in invertebrates other than sponges. They have also made it probable that the stigmasterol-like products occasionally met with among the sterols of mollusks (10, 11) are impure poriferasterol.

Clionasterol

The separation of clionasterol from the sterol mixture is a difficult process and it is questionable whether a product of a high degree of purity has as yet been isolated. Clionasteryl acetate obtained by the debromination of the soluble acetate bromides contains some poriferasteryl acetate, which can only be removed by frequent rebrominations. During these separations considerable quantities of clionasteryl acetate undergo decomposition.

The formula $C_{29}H_{50}O$ for the new clionasterol is based on analyses of its *m*-dinitrobenzoate and *o*-iodobenzoate. In Table I the data for the new clionasterol are compared with those of the old clionasterol and other sponge sterols. There exists such a striking similarity between clionasterol and the sterol of the fresh-water sponge, *Spongilla lacustris*, as to make their identity highly probable. Sterols of the formula $C_{29}H_{50}O$, and of properties resembling those of clionasterol, are frequently met with in marine invertebrates. In many instances they occur associated with other sterols from which they are difficult to separate. Thus the sterols of the coral *Meandra areolata* (12) and the gorgonia, *Xiphogorgia*, are mixtures of cholesterol and a sterol, $C_{29}H_{50}O$, closely resembling clionasterol.

EXPERIMENTAL

All melting points are corrected.

Preparation of the sterol mixture. The air-dried sponges of *Spheciospongia vesparia* or *Cliona celata* were cut into small pieces and extracted with acetone in a large Soxhlet apparatus for 24 hours. The extract was then filtered and concentrated to about one-fourth of its original volume. Upon cooling, a copious, almost colorless crystalline precipitate appeared, which was filtered and washed with acetone and methanol. In another experiment the total oily extract of a large quantity of sponges was diluted with an equal volume of ethanol, and the crystalline precipitate was filtered and washed with alcohol.

The precipitate was refluxed for one hour with a 5% solution of potassium hydroxide in methanol. The mixture was then diluted with water and the non-saponifiable material extracted with ether in the usual manner. Between the ether and aqueous layer a small amount of material was found suspended which was identified as the potassium salt of a hydroxy acid, which possessed a strong negative rotation and which gave the usual color reactions for sterols. Acidification of the aqueous layer with mineral acid yielded only an insignificant amount of fatty acids. This indicated that the bulk of the sterols is present in the sponge in an unesterified form.

The oil remaining after the evaporation of the mother liquor of the first precipitate was saponified with an alcoholic solution of potassium hydroxide, and the non-saponifiable matter was isolated in the usual manner. The latter, which consisted principally of sterols, contained a small amount of a steam distillable oil of pleasant odor, resembling that of terpenes.

The non-saponifiable fraction prepared by either of the two methods was refluxed for one hour with acetic anhydride. After cooling, the acetates were filtered and washed with glacial acetic acid and methanol.

Poriferasterol acetate tetrabromide. Ten-gram lots of the crude sterol acetate were dissolved in 100 cc. of anhydrous ether, and 125 cc. of a 5% solution of bromine in glacial acetic acid was added. After 24 hours standing in the refrigerator, a copious precipitate of rhombohedral prisms had formed. It was filtered, washed with acetic acid and methanol, and dried in a desiccator. On the average, 6.2 g. of acetate tetrabromide was obtained, corresponding to 3.5 g. of poriferasteryl acetate or to the presence of 35% of poriferasterol in the sterol mixture. After several recrystallizations from chloroform and alcohol the bromide melted at 185° with decomposition; $[\alpha]_D^{24}$ −43.5° (21.4 mg. in 3.03 cc. of chloroform). The tetrabromide is a remarkably stable compound; an analytical sample which had been kept in a stoppered tube for eleven months showed no signs of decomposition and gave the same analytical results as before.

Anal. Calc'd for $C_{30}H_{48}Br_4O_2$: C, 47.4; H, 6.4; Br, 42.0.
$C_{31}H_{50}Br_4O_2$: C, 48.1; H, 6.5; Br, 41.3.
Found: C, 48.0; H, 6.6; Br, 41.2.

Poriferasteryl acetate dibromide. To 3.2 g. of the acetate tetrabromide in 50 cc. of dry benzene was added 2 g. of sodium iodide in 20 cc. of ethanol, and the mixture allowed to stand at room temperature for eighteen hours. The mixture was then shaken with sodium sulfite to remove free iodine, washed with water, and dried over sodium sulfate. Addition of methanol to the solution gave 1.2 g. of a crystalline precipitate. It was dissolved in chloroform, and the solution treated with Norit and filtered. Methanol was then added, and the precipitate was recrystallized several times from chloroform-methanol. Poriferasteryl acetate dibromide crystallizes in long flat prisms; on heating it begins to darken at 202° and melts with decomposition at 211–212°, $[\alpha]_D^{25}$ −31° (34.2 mg. in 3.03 cc. of chloroform).

Anal. Calc'd for $C_{30}H_{48}Br_2O_2$: C, 60.0; H, 8.1; Br, 26.6.
$C_{31}H_{50}Br_2O_2$: C, 60.6; H, 8.2; Br, 26.0.
Found: C, 60.4; H, 8.5; Br, 26.1.

Poriferasteryl acetate. Preliminary experiments proved that a complete debromination of the tetrabromide could not be effected by the method of Schoenheimer (13). The debromination was therefore carried out with zinc in glacial acetic acid. Since the bromide is only sparingly soluble in glacial acetic acid, it was dissolved in benzene (6.2 g. in 150 cc.), and glacial acetic acid (200 cc.) and zinc dust were then added. The solution was heated in such a manner as to permit the benzene to distil off gradually. After removal of the benzene the solution was refluxed for six hours with frequent addition of small amounts of zinc dust. The hot solution was then filtered and water was added to the filtrate to precipitate the acetate. The acetate was dissolved in ether, and the solution washed with sodium carbonate, water, dried over sodium sulfate, and evaporated to dryness. The remaining acetate was recrystallized several times from ether-methanol. It crystallizes in glistening plates, m.p. 146.5–147°, $[\alpha]_D^{24}$ −53.0° (31.6 mg. in 3.03 cc. of chloroform).

Anal. Calc'd for $C_{31}H_{50}O_2$: C, 82.0; H, 11.1.
Found: C, 82.0; H, 11.2.

Titration with perbenzoic acid. By titration with perbenzoic acid in the usual manner the acetate took up oxygen after 48, 72, and 96 hours corresponding to 1.92, 1.95, and 2.08 double bonds.

Poriferasterol. The acetate was saponified by refluxing with a 5% solution of potassium hydroxide in 95% alcohol. The sterol was recrystallized several times from alcohol and finally from ether. It crystallizes in plates from both solvents,

m.p. 155–156°, $[\alpha]_D^{24}$ −49.7° (54.6 mg. in 3.03 cc. of chloroform). The sterol gives the Salkowski and Liebermann-Burchard reaction.

Anal. Calc'd for $C_{29}H_{48}O$: C, 84.5; H, 11.7.
Found: C, 84.7; H, 11.3.

Poriferasteryl propionate. Poriferasterol was refluxed with an excess of propionic anhydride for 45 minutes. The propionate which separated after cooling was recrystallized several times from dilute alcohol, m.p. 125–125.5°, $[\alpha]_D^{24}$ −48.1° (41.9 mg. in 3.03 cc. of chloroform).

Anal. Calc'd for $C_{32}H_{52}O_2$: C, 82.0; H, 11.2.
Found: C, 82.0; H, 11.3.

Poriferasteryl benzoate. To a solution of poriferasterol in dry pyridine an excess of benzoyl chloride was added and the mixture kept at room temperature for eight hours. The benzoate was then precipitated with water, filtered, washed with water and alcohol, and recrystallized from alcohol. It crystallizes in plates; on heating it turns into a turbid liquid at 139.5–140.5° which becomes clear at 141.5°, $[\alpha]_D^{24}$ −21.95° (52.5 mg. in 3.03 cc. of chloroform).

Anal. Calc'd for $C_{36}H_{52}O_2$: C, 83.7; H, 10.2.
Found: C, 83.7; H, 10.0.

Poriferasteryl phenylurethane. To a solution of 0.25 g. of poriferasterol in 15 cc. of dry benzene, 3 cc. of phenyl isocyanate was added, and the mixture refluxed for three hours. The benzene and the excess isocyanate were removed *in vacuo* at 100°. The residue was extracted three times with high-boiling ligroin and then recrystallized several times from ethyl acetate-ethanol; needles, m.p. 191–192.5°, $[\alpha]_D^{24}$ −33.2° (32.8 mg. in 3.03 cc. of chloroform).

Poriferasteryl m-*dinitrobenzoate.* A solution of 0.5 g. of poriferasterol and 0.5 g. of *m*-dinitrobenzoyl chloride in 30 cc. of dry pyridine was heated on the steam-bath for three hours. The mixture was then poured into dilute sulfuric acid, and the precipitate was filtered, washed with dilute acid, water, and hot acetone. After four recrystallizations from ethyl acetate-ethanol the *m*-dinitrobenzoate showed the m.p. 227–228°, $[\alpha]_D^{24}$ −22.1° (38.7 mg. in 3.03 cc. of chloroform).

Anal. Calc'd for $C_{35}H_{48}N_2O_6$: C, 70.9; H, 8.2.
$C_{36}H_{50}N_2O_6$: C, 71.3; H, 8.3.
Found: C, 71.4; H, 8.4.

Poriferasteryl o-*iodobenzoate.* A solution of 0.25 g. of poriferasterol and 0.25 g. of *o*-iodobenzoyl chloride in 10 cc. of dry pyridine was heated for three hours on the steam-bath. It was then poured into dilute sulfuric acid and the solution extracted with ether. The ether extract was washed with a solution of sodium carbonate and water, dried over sodium sulfate, and evaporated to dryness. The residue was recrystallized several times from 95% alcohol; flat needles, m.p. 153–154.5°, $[\alpha]_D^{24}$ −25.3° (31.8 mg. in 3.03 cc. of chloroform).

Anal. Calc'd for $C_{35}H_{49}IO_2$: C, 66.9; H, 7.9.
$C_{36}H_{51}IO_2$: C, 67.3; H, 8.0.
Found: C, 67.3; H, 7.9.

Poriferastyl acetate. Two grams of poriferasteryl acetate of m.p. 144–145.5° in 100 cc. of glacial acetic acid was hydrogenated at 60–70° in the presence of 0.5 g. of platinum oxide. Two moles of hydrogen was taken up rapidly. After five hours the solution was filtered and concentrated to a small volume *in vacuo*. The Liebermann test of the crystalline residue was negative. The acetate was recrystallized from ethanol, m.p. 140–141°, $[\alpha]_D^{24}$ +16.3° (35.7 mg. in 3.03 cc. of chloroform).

Anal. Calc'd for $C_{31}H_{54}O_2$: C, 81.2; H, 11.9.
Found: C, 81.4; H, 11.8.

Poriferastanol. The acetate was hydrolyzed by refluxing with a 5% solution of potassium hydroxide in the usual manner. The sterol was recrystallized several times from alcohol and then from ether; plates from alcohol and needles from ether, m.p. 143–144°, $[\alpha]_D^{24}$ +24.7° (50.5 mg. in 3.03 cc. of chloroform). The carbon values were about 2% low due to solvent of crystallization.

Poriferastyl m-*dinitrobenzoate.* A solution of 0.25 g. of poriferastanol and 0.4 g. of *m*-dinitrobenzoyl chloride in 10 cc. of pyridine was heated on the steam-bath for three hours. The solution was poured into dilute sulfuric acid, and the precipitate was filtered, washed with water and hot acetone, and recrystallized several times from benzene-ethanol; colorless leaflets, m.p. 213–213.5°, $[\alpha]_D^{24}$ +17.1° (32.0 mg. in 3.03 cc. of chloroform).

Anal. Calc'd for $C_{36}H_{54}N_2O_6$: C, 70.8; H, 8.9.
$C_{35}H_{52}N_2O_6$: C, 70.4; H, 8.8.
Found: C, 70.8; H, 8.9.

Poriferastanone. To a solution of 0.24 g. of poriferastanol in 10 cc. of 95% acetic acid was added 0.15 g. of chromic anhydride in a small amount of water. The mixture was heated on the steam-bath with frequent shaking for 30 minutes, cooled, and treated with dilute sulfurous acid. The precipitate was filtered, washed with water, and recrystallized several times from ethanol; m.p. 161–161.5°, $[\alpha]_D^{24}$ +46.7° (25.2 g. in 3.03 cc. of chloroform).

Anal. Calc'd for $C_{29}H_{50}O$: C, 84.0; H, 12.2.
Found: C, 83.9; H, 12.3.

Clionasteryl acetate. A stream of nitrogen was blown through the mother liquor remaining after the removal of poriferasteryl acetate tetrabromide until some solid had separated. This solid, which contained large quantities of tetrabromide, was filtered off. Zinc dust was then added to the filtrate, which was first heated on the steam-bath to remove the ether and then refluxed for six hours. Additional amounts of zinc dust were added at frequent intervals. The acetate was dissolved in ether, and the solution washed with sodium carbonate and water, dried over sodium sulfate, and evaporated to dryness. During frequent recrystallizations from ether-methanol and absolute ethanol the melting point of the acetate rose gradually from 132–133° to 141°. Titration with perbenzoic acid and bromination showed the presence of poriferasteryl acetate. The acetate was therefore subjected to a second bromination according to the method described above. Tetrabromide, corresponding to the presence of about 10% of poriferasteryl acetate, was filtered off, and the mother liquor was debrominated as described above. Clionasteryl acetate purified in this manner showed the m.p. 137° and $[\alpha]_D^{24}$ −41.9° (19.0 mg. in 3.03 cc. of chloroform).

Anal. Calc'd for $C_{31}H_{52}O_2$: C, 81.5; H, 11.5.
Found: C, 81.3; H, 11.3.

Clionasterol. The acetate was saponified by a 5% solution of potassium hydroxide in 95% ethanol. The sterol was recrystallized several times from alcohol and finally from ether. The purest material showed the melting point 137.5–138.5°, $[\alpha]_D^{24}$ −37° (42.3 mg. in 3.03 cc. of chloroform). It gives the Liebermann-Burchard and the Salkowski reaction as cholesterol does. The solubility in different solvents is about the same as that of cholesterol.

Anal. Calc'd for $C_{29}H_{50}O$: C, 84.1; H, 12.2.
Found: C, 83.8; H, 12.0.

Clionasteryl propionate. Clionasterol was refluxed with an excess of propionic anhydride for 45 minutes. The propionate which separated after cooling was re-

crystallized three times from dilute alcohol, m.p. 117–118°, $[\alpha]_D^{22}$ −41.84° (49.9 mg. in 3.03 cc. of chloroform).

Anal. Calc'd for $C_{32}H_{54}O_2$: C, 81.7; H, 11.6.
Found: C, 81.7; H, 11.6.

Clionasteryl benzoate. To a solution of clionasterol in dry pyridine an excess of benzoyl chloride was added and the mixture kept at room temperature overnight. The benzoate was then precipitated with water, filtered, washed thoroughly with water and alcohol, and then recrystallized five times from hot alcohol, in which it is more soluble than cholesteryl benzoate. The benzoate crystallizes in needles. It melts sharply at 134.5–135° to a clear liquid. On cooling, the liquid benzoate turns bluish-green at 131° and purple at 116°. Solidification begins at 106°; $[\alpha]_D^{22}$ −16.8° (56.2 mg. in 3.03 cc. of chloroform).

Anal. Calc'd for $C_{36}H_{54}O_2$: C, 83.4; H, 10.5.
Found: C, 83.6; H, 10.3.

Clionasteryl phenylurethane. To a solution of clionasterol in dry benzene, phenyl isocyanate was added in excess and the mixture was refluxed for 3 hours. The solvent and the excess of phenyl isocyanate were removed *in vacuo* and the residue dried *in vacuo* at 100°. It was washed three times with boiling ligroin, and the remaining urethane was recrystallized from a mixture of ethyl acetate and ethanol until the melting point remained constant at 180.5–182°; $[\alpha]_D^{22}$ −29.36° (28.9 mg. in 3.03 cc. of chloroform).

Anal. Calc'd for $C_{36}H_{55}NO_2$: C, 80.9; H, 10.4; N, 2.6.
Found: C, 81.2; H, 10.5; N, 2.9.

Clionasteryl m-*dinitrobenzoate.* To a solution of clionasterol in dry pyridine an excess of *m*-dinitrobenzoyl chloride was added and the mixture heated on the steam-bath for 4 hours. It was then poured into dilute sulfuric acid, and the precipitate was filtered, washed with water and warm acetone. The *m*-dinitrobenzoate was recrystallized several times from ethyl acetate and a mixture of ethanol and benzene; m.p. 201–203°, $[\alpha]_D^{23}$ −13.95° (35.3 mg. in 3.03 cc. of chloroform).

Anal. Calc'd for $C_{36}H_{52}N_2O_6$: C, 71.0; H, 8.6; N, 4.6.
$C_{35}H_{50}N_2O_6$: C, 70.7; H, 8.5; N, 4.7.
Found: C, 70.9, 71.0, 70.8; H, 8.5, 8.6, 8.6; N, 4.8.

Clionasteryl o-*iodobenzoate.* To a solution of clionasterol in dry pyridine was added an excess of *o*-iodobenzoyl chloride, and the mixture was heated on the steam-bath for 3 hours. It was then poured into dilute sulfuric acid and the acid extracted with ether. The ether extract was washed with a solution of sodium carbonate and water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was then treated with hot ethanol, when all but a few drops of oily material went into solution. On cooling of the filtered solution, the *o*-iodobenzoate separated in the form of a gelatinous mass. It was recrystallized from ethanol until the melting point remained constant at 103.5–104.5°; $[\alpha]_D^{22}$ −19.76° (48.0 mg. in 3.03 cc. of chloroform).

Anal. Calc'd for $C_{36}H_{53}IO_2$: C, 67.1; H, 8.3.
$C_{35}H_{51}IO_2$: C, 66.7; H, 8.2.
Found: C, 67.1; H, 8.3.

SUMMARY

Clionasterol as isolated from the marine sponges *Spheciospongia vesparia* and *Cliona celata* is a mixture of a mono- and di-unsaturated sterol. The name clionasterol has been retained for the monounsaturated sterol which

represents about sixty per cent of the mixture. A number of derivatives of the new clionasterol have been described and it has been demonstrated that the formula is $C_{29}H_{50}O$ rather than $C_{27}H_{46}O$.

The name poriferasterol has been proposed for the diunsaturated sterol $C_{29}H_{48}O$. A number of derivatives of this new sterol have been described, and it has been shown that it is hydrogenated to a saturated sterol closely resembling ostreastanol.

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