[CONTRIBUTION FROM THE STERLING CHEMISTRY LABORATORY AND THE BINGHAM OCEANOGRAPHIC LABORATORY, YALE UNIVERSITY]

CONTRIBUTIONS TO THE STUDY OF MARINE PRODUCTS. XVII. SPONGOSTEROL[1]

WERNER BERGMANN, DAVID H. GOULD,[2] AND EVA M. LOW

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In 1904, Henze (1) isolated from the Mediterranean sponge, *Suberites domuncula*, a new sterol which he named spongosterol. The unusual properties of this sterol left no doubt about its difference from cholesterol which until then had been regarded as the typical sterol of all animals. A few years later (2), the same author presented evidence which indicated spongosterol, m.p. 123–124°, $[\alpha]_D$ −19.6°, to be a saturated sterol of the formula $C_{27}H_{48}O$, and therefore an isomer of cholestanol and coprosterol. Notwithstanding its saturated character, spongosteryl acetate reacted readily with bromine in glacial acetic acid to form a monobromide, $C_{29}H_{49}BrO_2$, m.p. 156°, which Henze regarded as a substitution product. Since then several other new sterols have been isolated from animals, particularly marine invertebrates, but as far as the authors are aware, no further information on spongosterol has been published.

In connection with a systematic study of the sterols of sponges, now in progress in this laboratory, it appeared of particular interest to reinvestigate this unusual sterol. Modern knowledge of the optical properties of steroids makes appear quite unlikely the natural occurence of a levo-rotatory, saturated sterol. It seemed equally improbable that a saturated steryl acetate reacts readily with bromine to form a monobromide under the mild conditions used by Henze. Present circumstances made it impossible to obtain the Mediterranean sponge as source material for the isolation of spongosterol. A very close relative of this species, however, *Suberites compacta*, is quite common in certain regions of the coastal waters of New England. The dried sponge contains about 0.7% of a sterol the properties of which show such great similarity with those reported for spongosterol as to leave little doubt about the identity of the two sterols. (See Table I.) The most convincing piece of evidence rests on the fact, that like spongosteryl acetate, the present steryl acetate reacts readily with bromine in glacial acetic acid to give a monobromide melting at 156°. There existed, however, one significant discrepancy between the physical properties of the respective sterols. Spongosterol has been reported to be a levo-rotatory compound, $[\alpha]_D$ −19.6°; the present sterol, however, was found to be dextro-rotatory, $[\alpha]_D^{24}$ +18.2°. It appeared quite probable, therefore, that the direction of the rotation of spongosterol had been incorrectly reported. That such must have been the case became certain, when it was found that a sample of spongosteryl propionate, which one of the authors received from Professor Henze

[1] This communication describes work done by David H. Gould in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry, Yale University, 1944.

[2] Present address: National Oil Products Co., Harrison, N. J.

twelve years ago, showed a distinct positive rotation, $[\alpha]_D^{28}$ +8°. This sample showed no depression of the melting point when mixed with the propionate of the present sterol. This observation at once disposes of the controversial point of the natural occurrence of a levo-rotatory, saturated sterol.

Titrations with perbenzoic acid showed the presence of 0.2–0.4 double bonds in crude samples of spongosterol and its derivatives, and 0.4–0.5 double bonds in the purified samples. The results threw doubt upon the homogeneity of spongosterol, and suggested that the sterol was a mixture of a saturated and a mono-unsaturated compound, assuming the absence of significant quantities of more highly unsaturated sterols. Similar results were obtained by quantitative hydrogenation of spongosteryl acetate. These observations at once suggested the possibility that the reaction between spongosteryl acetate and bromine was due to the absorption of the reagent by the unsaturated component of the mixture. That such is indeed the case was shown by the fact that after treatment with catalytic hydrogen, spongosteryl acetate fails to react with bromine.

TABLE I

COMPARISON OF SPONGOSTEROL FROM *Suberites domuncula* and *S. compacta*

DERIVATIVE	*S. domuncula*		*S. compacta*	
	m.p. °C.	$[\alpha]_D$	m.p. °C.	$[\alpha]_D$
Sterol	124	−19.6	124	+18.2
Acetate	124		125	+8
Propionate	132[a]	+8[a]	131	+10
Benzoate	128		128	
Acetate monobromide	157		157	

[a] Data determined in this laboratory on Henze's original sample.

When considerable difficulties were encountered in bringing about a separation of the saturated and unsaturated components of spongosterol, attempts were carried out to isolate the saturated sterols by destructive elimination of the unsaturated material. The method of Anderson and Nabenhauer (3) which is usually employed with conspicuous success in the preparation of pure saturated sterols, failed to give satisfactory results. In the course of an investigation on the oxidation of spongosterol there was isolated among other products a dicarboxylic acid which proved to be identical with dihydro-iso-Diels acid (4). Since this acid is readily formed upon oxidation of cholestanol with chromic acid, the presence of this sterol in spongosterol was indicated. In order to isolate it in a pure state, spongosteryl acetate was treated with ozone, and the reaction mixture separated into neutral and acidic components. Saponification of the neutral fraction, which represented almost one-half of the starting material, gave cholestanol. Because the presence of such considerable quantities of cholestanol in a sponge was quite unexpected, great care was taken in proving the identity of the compound by direct comparison of several of its derivatives with those of authentic cholestanol, by its oxidation to cholestanone and dihydro-iso-Diels

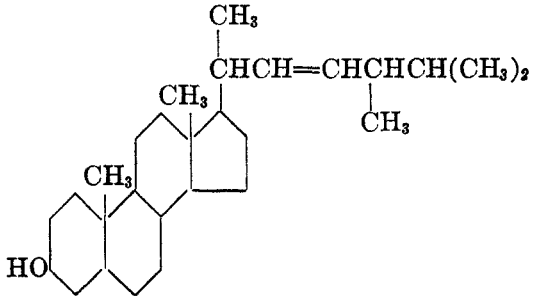
acid, and the conversion of the latter to the pyroketone (5). Cholestanol was also isolated by way of numerous recrystallizations of steryl benzoate prepared from crude spongosterol, where cholestanyl benzoate is eventually obtained as the least soluble fraction.

The evidence presented above clearly demonstrates that spongosterol is a mixture of cholestanol and an unsaturated component. In order to simplify further discussion, the unsaturated component will from now on be referred to as neospongosterol, and the name spongosterol will be retained to designate the cholestanol-neospongosterol mixture. Neospongosterol is not, as was at first suspected a simple dehydrocholestanol, for hydrogenation of spongosterol does not give a uniform product. The positive rotation of spongosterol and its derivatives contraindicated the presence in neospongosterol of a 5,8-double bond, which is known to confer a strongly negative rotation upon steroids. Equally unlikely appeared the presence of a cyclic bond anchored at C-8, because none of the various fractions gave a positive Tortelli-Jaffé reaction (6).

A definite clue to the structure of neospongosterol was derived from a further study of the ozonization of spongosteryl acetate. This reaction yielded apart from cholestanyl acetate, described above, a volatile aldehyde, and a monocarboxylic acid. The aldehyde was isolated in the form of its 2,4-dinitrophenylhydrazone, m.p. 118°, $[\alpha]_D^{24}$ +17.10°, which gave satisfactory analyses for a derivative of $C_5H_{11}CHO$. When mixed with a sample of the corresponding hydrazone of *l*-methylisopropylacetaldehyde, m.p. 124°, obtained from ergosterol, it melted at 118–122°. It appears, therefore, that, like the aldehyde obtained from starfish sterols (7), the present aldehyde is partially racemized *d*-methylisopropylacetaldehyde.

Hydrolysis of the acidic fragment obtained upon ozonization of spongosteryl acetate gave a monocarboxylic acid, $(C_{21}H_{35}O)COOH$, m.p. 274–276°. Its identity with β-3-hydroxy-bisnorallocholanic acid was demonstrated by its properties and analysis, and those of its methyl ester, m.p. 152° and acetoxymethyl ester, m.p. 130°, and by direct comparison with authentic samples.

It may be deduced on the basis of the evidence presented above, that neospongosterol is a 22,23-dehydrocampestanol (1), which as yet has not been described. The isolation of pure neospongosterol presented great difficulties due to its tendency and that of its derivatives to form stable molar adducts



(I) Neospongosterol

with cholestanol and its respective derivatives. Thus there was obtained upon frequent recrystallizations of spongosteryl benzoate a more soluble fraction, m.p. 121°, $[\alpha]_D^{24}$ +10.7°, the properties of which did not change upon further recrystallizations. Titration with perbenzoic acid showed the presence of 0.5 double bonds. Conversion of the benzoate to the acetate also gave a constant-melting product, m.p. 123°, $[\alpha]_D^{24}$ +30°, containing 0.5 double bonds as determined by titration with perbenzoic acid and catalytic hydrogenation. Ozonization of this acetate also yielded about forty-five per cent of cholestanyl acetate, and *d*-methylisopropylacetaldehyde and β-3-acetoxy-bisnorallocholanic acid.

The so-called spongosteryl acetate monobromide, the formation of which was at first difficult to explain, also proved to be molar adduct consisting of cholestanyl acetate and neospongosteryl acetate dibromide. Upon debromination it yields an acetate, m.p. 125°, which reacts with ozone to give cholestanyl acetate, the aldehyde and acid in approximately the same proportions as described above. As far as the authors are aware, the only similar adduct which has so far been described is the cholesterol-cholesterol dibromide compound (8) which is obtained upon addition of one-half mole of bromine to a solution of cholesterol.

Pure neospongosterol was eventually obtained by way of its acetate dibromide. This compound was obtained by means of a laborious series of treatments of the so-called monobromide with ether, in which the adduct apparently dissociates to some extent into its components, of which the dibromide is only sparingly soluble. The pure dibromide, m.p. 200° (dec.), gave satisfactory analyses for $C_{28}H_{47}OCOCH_3Br_2$. Upon debromination it gave neospongosteryl acetate, m.p. 141–142°, $[\alpha]_D^{24}$ 0°, which was converted into neospongosterol, m.p. 153°, $[\alpha]_D^{24}$ +10°, and neospongosteryl benzoate, m.p. 146°.

Ozonization of neospongosteryl acetate yielded two fragments, a volatile aldehyde and an acid. The former was isolated in the form of its 2,4-dinitrophenylhydrazone, m.p. 120°, $[\alpha]_D^{24}$ +18°, which proved to be identical with the partially racemized *d*-methylisopropylacetaldehyde described above. Saponification of the latter gave β-3-hydroxy-bisnorallocholanic acid.

Upon catalytic hydrogenation neospongosteryl acetate quickly absorbed one mole of hydrogen to give the dihydro acetate, m.p. 142°; $[\alpha]_D^{23}$ +16°. Its physical properties and those of dihydroneospongosterol, m.p. 145°, $[\alpha]_D^{26}$ +25.3°, are sufficiently similar to those of campestanyl acetate, m.p. 143°; $[\alpha]_D$ +18°, and campestanol (9), m.p. 147°; $[\alpha]_D$ +31°, as to suggest the identity of the respective compounds.

The evidence presented above justifies the formulation of neospongosterol as 22,23-dehydrocampestanol (I). The rotations of certain derivatives and fragments of neospongosterol suggest that the samples which have so far been obtained were accompanied by small amounts of its C-24 isomer, the as yet undescribed 22,23-dehydroergostanol.

The sponges *Suberites compacta* and *S. domuncula* are the first representatives of the animal world in which cholestanol has been found to be the principal sterol. Until now this sterol had only been observed as a minor impurity of

cholesterol isolated from normal vertebrate tissues and sera (10). Cholestanol is also the first well defined C_{27} sterol to be found in representatives of the phylum Porifera. Neospongosterol is the first example of a naturally occurring, unsaturated sterol in which the ring system is saturated. It is also the first representative of C_{28} sterols to be isolated from sponges.

EXPERIMENTAL[3]

All melting points are corrected. Unless stated differently, all optical rotations were taken in a 1-dm. tube, the sample being dissolved in 3 cc. of chloroform.

Isolation of spongosterol. One and one-half kg. of fresh sponge, *Suberites compacta*, was passed through a meat grinder and extracted four times with one liter of acetone. The insoluble material was then filtered, washed with acetone, transferred to a Soxhlet apparatus and thoroughly extracted with ether. The acetone extracts were combined and concentrated to a small volume, which was then extracted with ether. All ether extracts were then combined, dried, and evaporated. Dried to constant weight, the residue represented 15.6 g. of a waxy, deep green solid, corresponding to about 1% of the weight of the fresh sponge. The dried, insoluble residue of spongin-like material weighed 370 g., corresponding to about 25% of the fresh sponge.

For the isolation of larger quantities of fatty material, air and vacuum dried sponges were extracted for twenty-four hours with acetone in a large Soxhlet apparatus. Evaporation of the extracts gave a green wax, the weight of which amounted to 2.5–3% of the dry sponge.

The waxy material was refluxed for one hour with a 5% solution of potassium hydroxide in 80% ethanol, and the unsaponifiable matter was isolated in the usual manner. It represented 35–40% of the wax, or about 1% of the dry sponge. Quantitative determination with digitonin showed the presence of 70–71% of sterol in the unsaponifiable fraction. The sterol content of the wax was therefore about 25%, and that of the dry sponge about 0.7%.

The unsaponifiable material was treated with boiling methanol until all but a small amount of tarry, green material had gone into solution. The crystalline precipitate obtained upon cooling of the methanol extracts melted at 113–115°, and after several recrystallizations from methanol it melted at 123.5–124.5°; $[\alpha]_D^{24}$ +18.2°.

Spongosteryl acetate. Refluxing the sterol with acetic anhydride gave the acetate, which after five recrystallizations from ethanol, acetone, and ligroin melted at 125–126°; $[\alpha]_D^{28}$ +8.2° (32.7 mg., α +0.09°). Titration with perbenzoic acid and quantitative catalytic hydrogenation showed the presence of 0.45 double bonds.

Spongosteryl propionate. Refluxing the sterol with propionic anhydride gave the propionate, which after several recrystallizations from ethanol melted at 130–131°; $[\alpha]_D^{27}$ +10.8° (36.8 mg., 3.06 cc., α +0.13°). It showed no depression of the melting point when mixed with a sample of authentic spongosteryl propionate, m.p. 132–133°; $[\alpha]_D^{28}$ +8.1° (18.8 mg., 3.06 cc., α +0.05°).

Spongosteryl benzoate. This derivative was prepared by treating the sterol with benzoyl chloride in a pyridine solution. After several recrystallizations from ether-ethanol it melted at 128–129° to a turbid liquid which cleared up at 141–142°.

Anal. Calc'd for $C_{34}H_{52}O_2$: C, 82.87; H, 10.64.
$C_{35}H_{52}O_2$: C, 83.28; H, 10.38.
Found: C, 83.06; H, 10.46.

Spongosteryl acetate monobromide. To a solution of 9.6 g. of spongosteryl acetate in 60 cc. of anhydrous ether was added 100 cc. of a 5% solution of bromine in glacial acetic acid. Formation of a crystalline precipitate began almost immediately. After standing in a refrigerator overnight, the reaction mixture was filtered, and the solid washed with acetic acid and methanol, and dried in a desiccator. A total of 5.3 g. of material, m.p. 140–145°,

[3] The authors are greatly indebted to Dr. L. Ruigh, National Oil Products Co., Harrison, N. J., for the isolation of sterol from a large quantity of sponges.

was thus obtained. After two recrystallizations from ethyl acetate-methanol, and one from ethyl acetate, the bromide melted at 155–157°; $[\alpha]_D^{26}$ +6.5° (25.96 mg., α +0.055°).

Anal. Calc'd for $C_{29}H_{50}O_2 + C_{30}H_{50}Br_2O_2$: C, 68.58; H, 9.75; Br, 15.47.
Found: C, 67.85; H, 9.51; Br, 16.6.

Concentration of the mother liquor remaining after removal of the monobromide gave 2.7 g. of material, m.p. 122–125°. Debromination of the final mother liquor gave a halogen-free acetate, m.p. 113–115°.

Debromination of spongosteryl acetate monobromide. In a preliminary test it was observed that complete debromination could not be effected by means of sodium iodide. The bromide was therefore refluxed for 3 hours with zinc and glacial acetic acid, additional amounts of zinc dust being added at frequent intervals. The hot solution was then filtered, and water was added to the filtrate to precipitate the acetate. After two recrystallizations from ethanol and acetone it melted at 125–126°; $[\alpha]_D^{24}$ +9.0° (30.6 mg., α +0.09°). Titration with perbenzoic acid showed the presence of 0.45–0.50 double bonds.

Ozonization of spongosteryl acetate. Ozonizations were carried out with samples of spongosteryl acetate prepared either by acetylation of crude spongosterol or spongosterol purified by way of the benzoate, or by debromination of spongosteryl acetate monobromide. The results were the same in all cases. A stream of ozone was passed through a vigorously stirred suspension of one part of acetate in 18 parts of glacial acetic acid. The reaction was discontinued 30 minutes after all material had been dissolved. Two grams of zinc dust and a few drops of a 1% solution of silver nitrate were then added, and the mixture stirred for 30 minutes. The filtrate was diluted with twice its volume of water, and the mixture distilled until the temperature at the still head had reached 110°. The distillate contained the volatile fraction.

The liquid remaining in the still was diluted with water, and the precipitate filtered and dissolved in warm acetic acid. A 1% solution of chromic anhydride in 90% acetic acid was then added dropwise until no further reaction took place. After the excess chromic anhydride had been reduced by methanol, the solution was diluted with water and twice extracted with ether. The combined ether extracts were washed first with water and then with a 10% solution of potassium carbonate until the alkaline washings no longer gave a precipitate upon acidification. The ether layer was then washed with water, dried, and evaporated to dryness. The residue represented the neutral fraction.

The combined alkaline extracts were acidified with dilute hydrochloric acid and extracted three times with ether. The ether solution was then thoroughly washed with a 10% solution of potassium carbonate, and the combined alkaline extracts acidified with hydrochloric acid and extracted with ether. The ether extract was washed with water, dried, decolorized with Norit, and evaporated to dryness. The residue represented the acid fraction.

d-Methylisopropylacetaldehyde. A 1.5% solution of 2,4-dinitrophenylhydrazine in dilute hydrochloric acid was added to the distillate containing the volatile fraction. After standing overnight the flocculent precipitate was filtered, washed with water, and dried. It was then dissolved in 20 parts of benzene, and the solution percolated through a column of activated alumina. The hydrazone was eluted with benzene, and the eluate once more passed through a column of activated alumina. The benzene solution was evaporated to dryness, and the residue repeatedly recrystallized from dilute ethanol; m.p. 118°, $[\alpha]_D^{24}$ +17.1° (33.3 mg., α +0.19°).

Anal. Calc'd for $C_{12}H_{16}N_4O_4$: C, 51.42; H, 5.76.
Found: C, 51.82; H, 5.71.

β-3-Hydroxy-bisnorallocholanic acid. The acid fraction obtained during the ozonization of spongosteryl acetate was dissolved in a 5% solution of sodium hydroxide and heated on the steam-bath for one hour. The solution was then acidified and extracted with ether. The ether extract was washed with water, dried, and evaporated to dryness. After several recrystallizations from acetone, the residue gave β-3-hydroxy-bisnorallocholanic acid, melting with decomposition at 274–276°.

Anal. Calc'd for $C_{22}H_{36}O_3$: C, 75.81; H, 10.41.
Found: C, 75.28; H, 10.78.

Methyl ester of β-3-hydroxy-bisnorallocholanic acid. A suspension of the acid described above in ether was treated with diazomethane. After the solid material had gone into solution, the ether was evaporated and the residue recrystallized several times from dilute methanol. The ester melted at 151–152.5° and when mixed with an authentic sample[4] (m.p. 153.3–156°), it melted at 153.5–155.5°.

Anal. Calc'd for $C_{23}H_{38}O_3$: C, 76.19; H, 10.57.
Found: C, 76.18; H, 11.02.

Methyl ester of β-3-acetoxy-bisnorallocholanic acid. The ester described above was refluxed for forty-five minutes with a mixture of equal parts of acetic acid and acetic anhydride. After cooling, the solution was diluted with water, and the residue filtered and recrystallized several times from dilute methanol; m.p. 130°.

Anal. Calc'd for $C_{25}H_{40}O_4$: C, 74.21; H, 9.97.
Found: C, 74.24; H, 10.0.

Cholestanol. The neutral fraction obtained from the ozonization of spongosteryl acetate was refluxed for one hour with a 5% solution of potassium hydroxide in methanol. The free sterol was isolated in the usual manner, recrystallized from ethanol and benzoylated with benzoyl chloride in pyridine. After several recrystallizations from ethanol, the the benzoate thus obtained melted at 134–136° to a turbid iridescent liquid which became clear sharply at 154°, $[\alpha]_D^{24}$ +21.7° (30.01 mg., α + 0.217°). When mixed with authentic cholestanyl benzoate, the product showed no depression of the melting and clearing point.

Hydrolysis of the benzoate gave cholestanol, m.p. 141–142°, $[\alpha]_D^{24}$ +23.8° (28.84 mg., α +0.229°), which showed no depression of the melting point when mixed with authentic material. Acetylation of the sterol gave an acetate, m.p. 114–115° $[\alpha]_D^{24}$ +14.35° (30.31 mg. α +0.145°) which showed no depression of the melting point when mixed with dihydrocholesteryl acetate.

Oxidation of the sterol with chromic acid anhydride according to the method of Bruce (11) gave cholestanone, m.p. 127–128°, which gave no depression of the melting point when mixed with an authentic sample. A more vigorous oxidation of the sterol according to the method of Windaus and Uibrig (4) gave dihydro-iso-Diels acid, m.p. 194–196°, the melting point of which was not depressed by addition of an authentic sample.

Anal. Calc'd for $C_{27}H_{46}O_4$: C, 74.61; H, 10.67.
Found: C, 74.84; H, 10.65.

Methylation of the acid with diazomethane gave the dimethyl ester, m.p. 65–67°.

Anal. Calc'd for $C_{29}H_{50}O_4$: C, 75.28; H, 10.89.
Found: C, 75.66; H, 10.75.

Heating the dicarboxylic acid with acetic anhydride according to the method of Windaus and Dalmer (5) gave the pyroketone, norcholestanone, m.p. 99°.

Anal. Calc'd for $C_{26}H_{44}O$: C, 83.80; H, 11.91.
Found: C, 83.74; H, 12.22.

Recrystallization of spongosteryl benzoate. Fifty-eight and eight-tenths grams of spongosteryl benzoate, prepared from crude spongosterol, was recrystallized seven times from ethyl acetate and then three times from dioxane in a Skau tube. There was obtained 10.5 g. of a benzoate, m.p. 135° (turbid liquid), 155° (clear); $[\alpha]_D^{22}$ +22.6°. It gave no depression of melting point when mixed with authentic cholestanyl benzoate, m.p. 134–136°; $[\alpha]_D^{22}$ +22°. Hydrolysis of the benzoate gave cholestanol, m.p. 141–142°; $[\alpha]_D^{22}$ +23.6°, and acetylation of the latter gave cholestanyl acetate, m.p. 112–113°; $[\alpha]_D^{22}$ +13.3°. The two substances have no depression of melting points when mixed with authentic samples.

In working up the ethyl acetate mother liquors, there was obtained from the most soluble fraction a benzoate of m.p. 120°; repeated recrystallization did not raise the melting point above 121°; $[\alpha]_D^{24}$ +10.7° (22.5 mg., α + 0.39°). Titration with perbenzoic acid showed the presence of 0.5 double bonds.

[4] The authors are indebted to Dr. H. E. Stavely, Squibb Institute for Medical Research, for the gift of samples.

Hydrolysis of the benzoate gave a sterol, m.p. 119°; $[\alpha]_D^{24}$ +16.8° (29.3 mg., α +0.108°), which upon acetylation gave an acetate of m.p. 123°; $[\alpha]_D^{24}$ +3° (30.7 mg., α +0.03°). Treatment of the acetate with perbenzoic acid and catalytic hydrogen showed the presence of 0.5 double bonds. Bromination of the acetate by the method described above gave the bromide of m.p. 155°, and ozonization gave cholestanyl acetate, the aldehyde and acid, as described under the ozonization of spongosteryl acetate.

Neospongosteryl acetate dibromide. Isolation by recrystallization. Spongosteryl acetate monobromide, m.p. 154–157°, was triturated with ten parts of anhydrous ether. The bulk of material dissolved, and some crystalline material melting above 190° remained undissolved. The ether solution was concentrated, and the material which crystallized, was again treated with ether. A second crop of difficultly soluble, high-melting bromide was thus obtained. When this process was repeated once more, only a small crop of high-melting material remained undissolved. All mother liquors were then combined and evaporated to dryness. The residue, m.p. 135–150°, was recrystallized from absolute alcohol and ethyl acetate until a monobromide of m.p. 155–156° was obtained. This material was then treated with ether as described above, whereby further crops of high-melting material were isolated. The entire process was then repeated once more. In this manner a total of 1.2 g. of high-melting bromide was eventually obtained from 5 g. of starting material.

Isolation by chromatography. A sample of 2.3 g. of the acetate monobromide, m.p. 153–155°, dissolved in 50 cc. of low-boiling petroleum ether was passed through a column of activated alumina 35 cm. in height and 2.5 cm. in diameter. More petroleum ether was then passed through the column, and the percolate collected in fractions of 25 cc. each and evaporated to dryness. The first six fractions did not leave any residue. The next fifteen fractions (300 cc.) gave a total of 660 mg. of material which melted at 120–122° after one recrystallization from alcohol. A further fraction of 200 cc. gave only 50 mg. of residue, m.p. 115–125°. Benzene was then passed through the column. A 100-cc. fraction gave 950 mg. of a residue, which upon treatment with ether yielded 250 mg. of a difficultly soluble bromide, m.p. 195–200°. The column was finally washed with a mixture of equal parts of ethanol and ether. A 75-cc. fraction gave 380 mg. of residue, from which 170 mg. of high-melting bromide was obtained. This separation therefore yielded a total of 420 mg. of the desired bromide.

The various fractions of high-melting bromides were combined and twice recrystallized from ether by extraction through a thimble. Upon slow heating, the pure product decomposes at 200–205°.

Anal. Calc'd for $C_{28}H_{47}OCOCH_3Br_2$: C, 59.80; H, 8.36; Br, 26.53.
$C_{29}H_{49}OCOCH_3Br_2$: C, 60.40; H, 8.50; Br, 25.93.
Found: C, 59.73, 59.90; H, 8.30, 8.38; Br, 26.56.

Neospongosteryl acetate. One and one-half grams of the dibromide, 5 g. of zinc dust, and 100 cc. of glacial acetic acid was refluxed for three hours, small amounts of zinc dust being added from time to time. The hot solution was then filtered, and the zinc repeatedly extracted with hot acetic acid. The combined filtrate and washings were diluted with water to precipitate the debrominated material. After filtration, washing with water, and repeated recrystallization from ethanol, the acetate melted at 141–142°; $[\alpha]_D^{24}$ 0° (24.9 mg., α 0°).

Anal. Calc'd for $C_{28}H_{47}OCOCH_3$: C, 81.39; H, 11.38.
Found: C, 81.02; H, 11.35.

Neospongosterol. The acetate was refluxed for three hours with a 5% solution of potassium hydroxide in ethanol. The free sterol was isolated in the usual manner. After several recrystallizations from methanol and a mixture of ethyl acetate-ethanol it melted at 153°; $[\alpha]_D^{24}$ +10° (24.0 mg., α +0.08°).

Anal. Calc'd for $C_{28}H_{48}O$; C, 83.92; H, 12.08.
Found: C, 83.88; H, 12.10.

Neospongosteryl benzoate. The sterol was benzoylated in pyridine with benzoyl chloride.

After several recrystallizations from a mixture of ether and ethanol, and ethyl acetate; the benzoate melted at 146°.

Anal. Calc'd for $C_{28}H_{47}OCOC_6H_5$: C, 83.28; H, 10.38.
Found: C, 83.10; H, 10.27.

Dihydroneospongosteryl acetate. A solution of neospongosteryl acetate in ethyl acetate was hydrogenated at room temperature and atmospheric pressure with a platinum black catalyst. Hydrogen was absorbed rapidly, and the reaction came to a standstill after one mole had been consumed. The filtered solution was evaporated to dryness, and the residue recrystallized several times from ethanol; m.p. 142° $[\alpha]_D^{23}$ +15.8° (29.5 mg., α +0.157°). It showed no depression of the melting point when mixed with campestanyl acetate, m.p. 143°.

Anal. Calc'd for $C_{28}H_{49}OCOCH_3$: C, 80.02; H, 11.79.
Found: C, 80.40, H, 11.45.

Dihydroneospongosterol. The saponification of the acetate in the usual manner gave the sterol which after several recrystallizations from alcohol melted at 144.5°, $[\alpha]_D^{26}$ +25.3° (20.3 mg., α +0.17°).

Ozonization of neospongosteryl acetate. A 200-mg. sample of neospongosteryl acetate was ozonized in a manner analogous to the one used in the case of spongosteryl acetate. Treatment of the volatile fraction with 2,4-dinitrophenylhydrazine, purification of the precipitate by chromatography and recrystallization, gave 34 mg. of a hydrazone, m.p. 120°; $[\alpha]_D^{24}$ +18.0° (15.3 mg., 2 dm. tube, α + 0.184°). When mixed with the corresponding hydrazone of *l*-methylisopropylacetaldehyde, m.p. 124°, it melted at 120–122°.

Anal. Calc'd for $C_{12}H_{16}N_4O_4$: C, 51.42; H, 5.76.
Found: C, 51.76; H, 5.78.

The non-volatile fraction was oxidized with chromic acid anhydride, and the acid fraction was saponified and isolated in the manner described above. After recrystallizations from glacial acetic acid and acetone, the acid melted at 172°. It gave no depression of melting point when mixed with β-3-hydroxy-bisnorallocholanic acid of m.p. 175–176°.

SUMMARY

The sterol of the Atlantic sponge, *Suberites compacta*, has been shown to be identical with spongosterol, the sterol of the Mediterranean sponge, *Suberites domuncula*. Spongosterol is dextro- and not levo-rotatory, as had been originally reported by Henze (1).

It has been shown that spongosterol and its derivatives are molar adducts of a saturated and a mono-unsaturated sterol, and their respective derivatives.

The saturated component was obtained from spongosterol by means of destructive elimination of the unsaturated component. It was identified as cholestanol by direct comparison of a series of its derivatives with authentic material.

The unsaturated component which has been named neospongosterol, was isolated by way of its acetate dibromide. It was shown to be 22,23-dehydrocampestanol by its degradation to β-3-hydroxy-bisnorallocholanic acid and *d*-methylisopropylacetaldehyde, and by its catalytic hydrogenation to campestanol.

Spongosteryl acetate monobromide had been shown to consist of molar quantities of cholestanyl acetate and neospongosteryl acetate dibromide.

The significance of these observations has been discussed.

NEW HAVEN, CONN.

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