[CONTRIBUTION FROM THE STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY]

CONTRIBUTIONS TO THE STUDY OF MARINE PRODUCTS. XII. THE OXIDATION OF PORIFERASTEROL[1]

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As has been shown in a previous communication (1), the sterol mixture obtained from the marine sponges *Cliona celata* and *Spheciospongia vesparia* contains two principal components, the mono-unsaturated clionasterol, $C_{29}H_{50}O$, and the di-unsaturated poriferasterol, $C_{29}H_{48}O$. It has also been shown (2) that the two sterols are closely related to each other, since they both give the same poriferastanol upon catalytic hydrogenation. The poriferasterol may be separated from the sterol mixture by way of its difficultly soluble acetate tetrabromide. Partial debromination of this tetrabromide by the method of Fernholz and Stavely (3) gives poriferasteryl acetate dibromide. Since this behavior is quite analogous to that of stigmasteryl acetate tetrabromide, it was assumed that poriferasterol possesses one double bond in the ring system, and another in the side chain. The correctness of this assumption has now been established by the oxidation and ozonization of poriferasterol.

Like clionasterol (2), poriferasterol has a nuclear bond in the 5,6-position. This was first established by the oxidation of poriferasterol with aluminum isopropoxide and cyclohexanone. During the oxidation the usual shift of the double bond to the 3,4-position takes place to give poriferastenone, $(\alpha)_D^{25} + 57°$, which shows the typical absorption spectrum an α,β-unsaturated ketone. The ketone was characterized by its 2,4-dinitrophenylhydrazone, m.p. 232–234°, and its semicarbazone, m.p. 229–230°.

Because of the similarity of the behavior of the stigmasteryl and poriferasteryl acetate tetrabromide it was first assumed that the side chain double bond of poriferasterol occupied the 22,23-position.[2] Doubts as to the correctness of this assumption, however, were raised, when it was found that preliminary ozonization experiments with poriferasterol failed to give an aldehyde identifiable as a semicarbazone. Further investigations along this line have now demonstrated the correctness of the original assumption. Ozonization of poriferasteryl acetate 5,6-dibromide according to the directions of Fernholz and Stavely (4) gave an acid, which after debromination and hydrolysis yielded 3(β)-hydroxy-5,6-bisnorcholenic acid, m.p. 291–292°. The acid was identified by its physical properties and analysis, its methyl ester, m.p. 140°, and methyl ester acetate 137.5°, and by comparison with authentic material.

This observation proves what so far has been only an assumption, namely, that the sponge sterols are true sterols. It also proves definitely the presence

[1]The material in this paper constitutes part of a dissertation submitted by A. M. Lyon in partial fulfillment of the requirements for the Ph.D. degree, Yale University, June 1942.

[2]In a previous communication (1) this position has been erroneously called the 21,22-position.

of the hydroxyl group in the 3(β)-position, and the presence of a double bond in the 5,6-position. Since poriferasterol differs from clionasterol only by the presence of a double bond in the 22,23-position, the latter must be regarded as 22,23-dihydroporiferasterol. The results also demonstrate that poriferasterol differs from the isomeric stigmasterol only in the arrangement of the terminal seven carbon atoms of the side chain. All attempts to elucidate the structure of this final group have so far met with little success. Ozonization of poriferasterol yielded a volatile substance which gave fuchsin-aldehyde reaction. The fragment, however, was more water-soluble than the aldehydes obtained by the ozonolysis of stigmasterol and ergosterol, and it failed to give an insoluble semicarbazone. The volatile compound reacts readily with 2,4-dinitrophenylhydrazine to give a hydrazone, m.p. 113°, $(\alpha)_D^{25}$ 0°, which analyzed satisfactorily for a derivative of $C_7H_{14}O$. This derivative therefore represents the final seven carbon atoms of the side chain. Decomposition of the 2,4-dinitrophenylhydrazone with oxalic acid regenerated the volatile product, which again failed to give an insoluble semicarbazone, but reacted readily with 2,4-dinitrophenylhydrazine. Lack of material has made necessary a temporary abandonment of the study of this fragment.

In this connection it is of interest to note that Mazur (5) obtained upon ozonization of the spongilla sterol an aldehyde, $C_7H_{14}O$, which was identified as its 2,4-dinitrophenylhydrazone of m.p. 109°. It appears likely that this compound is identical with the corresponding substance obtained from poriferasterol, but it seems at present unlikely that it is identical with the 2,4-dinitrophenylhydrazone of ethylisopropylacetaldehyde. Since the data for the spongilla sterol lie between those of clionasterol and poriferasterol, and since a separation of a possible mixture by way of the acetate bromides had not been carried out, it appears likely that the spongilla sterol is a mixture of clionasterol and poriferasterol. Ozonization of such a mixture would lead to the formation of the fragment $C_7H_{14}O$, characterized by its 2,4-dinitrophenylhydrazone of m.p. 113°.

EXPERIMENTAL[3]

All melting points are corrected.

Poriferastenone. One gram of poriferasterol and 1 g. of aluminum isopropoxide were dissolved in a mixture of 35 cc. of dry toluene and 10 cc. of cyclohexanone, and the solution was refluxed for four hours. The ketone was then isolated as previously described (2). The crude ketone was dissolved in alcohol and treated with 10 cc. of a 1% digitonin solution to remove a small amount of unreacted sterol. The filtrate from the digitonide was evaporated to dryness *in vacuo* and the residue extracted with petroleum ether. The extract was taken to dryness, and the residue recrystallized several times from acetone; m.p. 111–112.5°; $(\alpha)_D^{25}$ + 56.7°; maximum of absorption in the ultraviolet 240 mμ.

Anal. Calc'd for $C_{29}H_{46}O$: C, 84.81; H, 11.30.
Found: C, 84.89; H, 11.62.

Poriferastenone 2,4-dinitrophenylhydrazone. It was prepared by refluxing equal parts of the ketone and 2,4-dinitrophenylhydrazine for several minutes in alcohol and adding a

[3]The authors are greatly indebted to Merck and Co., Rahway, N. J. for the preparation of the sponge sterols, and for a grant in aid of the investigation.

drop of conc'd hydrochloric acid. The hydrazone, which separated immediately, was recrystallized first from chloroform-ethanol and then twice from ethanol; m.p. 231.8–234.5°.

Anal. Calc'd for $C_{35}H_{50}N_4O_4$: C, 71.17; H, 8.53; N, 9.48.
Found: C, 71.17; H, 8.54; N, 9.53.

Poriferastenone semicarbazone. This derivative was prepared in the usual manner and recrystallized from alcohol; m.p. 229–230°.

Anal. Calc'd for $C_{30}H_{49}N_3O$: C, 77.03; H, 10.56.
Found: C, 76.89; H, 10.66.

3(β)-*Hydroxybisnorcholenic acid.* Poriferasteryl acetate was dissolved in chloroform, and sufficient bromine in chloroform was added to satisfy one double bond. After more than twice the theoretical amount of ozone had passed through the solution, which was cooled by an ice-bath, it was evaporated *in vacuo* below 35°, and the residue debrominated with zinc and glacial acetic acid. Water was then added, the mixture extracted with ether, and the extract washed with water and an excess of 2 *N* sodium hydroxide. The precipitate which formed on the interphase was washed with 2 *N* sodium hydroxide and ether. The salt was then decomposed with 6 *N* sulfuric acid, and the acid extracted with ether. The ether was evaporated and the residue refluxed with a 5% solution of potassium hydroxide in methanol. Water and 2 *N* sulfuric acid were then added and the mixture extracted with a large amount of ether. The ether residue was first recrystallized from ether in a thimble and then from glacial acetic acid, m.p. (decomp.) 291–292°. The mixed melting point with an authentic sample of 3(β)-hydroxybisnorcholenic acid showed no depression.

Anal. Calc'd for $C_{22}H_{34}O_3$: C, 76.23; H, 9.91.
Found: C, 75.76; H, 9.81.

3(β)-*Hydroxybisnorcholenic acid methyl ester.* The methyl ester was prepared from the acid with diazomethane and recrystallized from aqueous methanol, m.p. 140–141°.

Anal. Calc'd for $C_{23}H_{36}O_3$: C, 76.58; H, 10.10.
Found: C, 76.21; H, 10.06.

3(β)-*Acetoxybisnorcholenic acid methyl ester.* The methyl ester described above was allowed to stand overnight with pyridine containing 10% of acetic anhydride. The solvent was then removed under diminished pressure and the residue recrystallized from methanol; m.p. 137.5°. The mixed melting point with an authentic sample of 3(β)-acetoxybisnorcholenic acid methyl ester showed no depression.

Ozonization of poriferasteryl acetate. One gram of the acetate was suspended in 20 cc. of glacial acetic acid, which had been distilled three times from chromic anhydride. Ozone was passed through the suspension, which became clear after 15 minutes. After one hour the solution was poured into 100 cc. of water, and the mixture distilled into a solution of 2,4-dinitrophenylhydrazine sulfate until one-quarter of the original volume remained. The reagent solution was prepared by dissolving 0.5 g. of 2,4-dinitrophenylhydrazine in 2 cc. of concentrated sulfuric acid and diluting with 100 cc. of ethanol. An amorphous yellow precipitate formed, which was allowed to coagulate overnight. The crude product was purified by percolation of its benzene solution through activated alumina according to the directions of Mazur (5). It was recrystallized three times from alcohol; m.p. 113–114°; $(\alpha)_D^{25} \pm 0°$.

Anal. Calc'd for $C_{13}H_{18}N_4O_4$: C, 53.05; H, 6.16; N, 19.04.
Found: C, 52.85; H, 6.26; N, 19.24.

SUMMARY

Poriferasterol has been oxidized to poriferastenone. Poriferasterol has been degraded by ozonization to a C_{22} acid, identified as 3(β)-hydroxybisnorcholenic acid. A volatile C_7 fragment from the side chain has been isolated in form of its 2,4-dinitrophenylhydrazone. Clionasterol has been shown to be 22,23-dihydroporiferasterol.

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