[CONTRIBUTION FROM THE STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY]

CONTRIBUTIONS TO THE STUDY OF MARINE PRODUCTS. X. CLIONASTEROL

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Received April 15, 1942

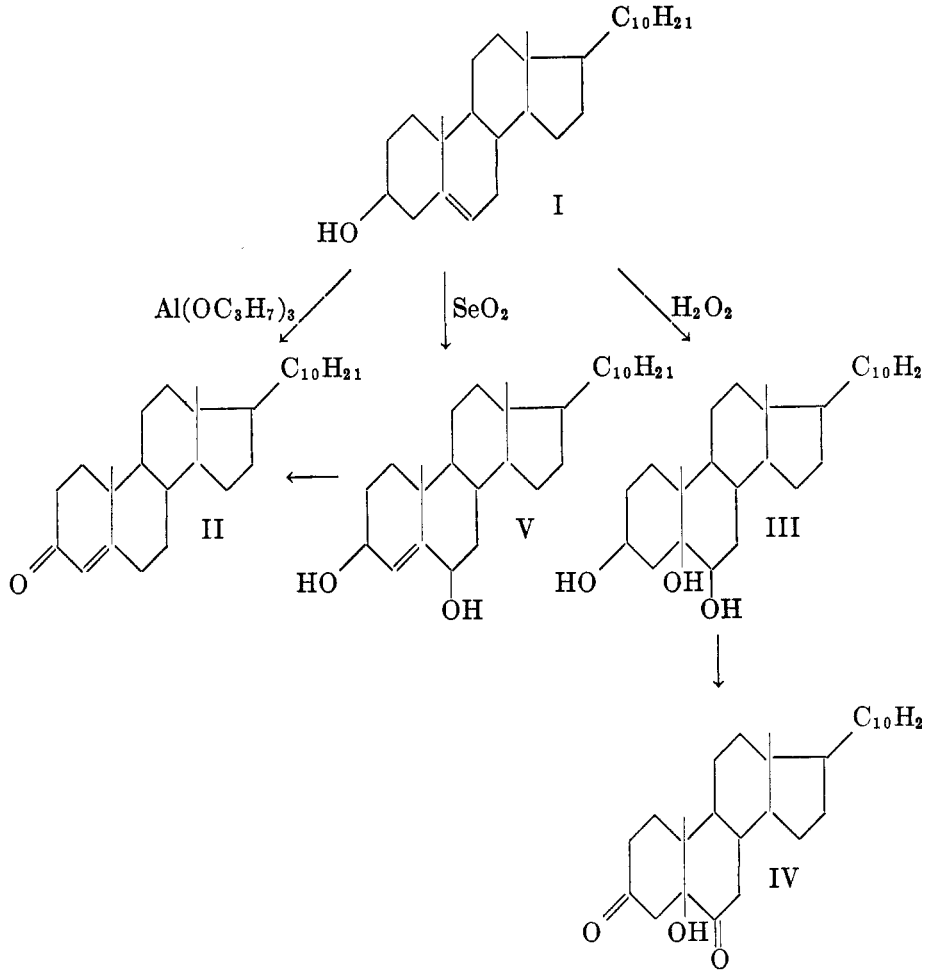
In a previous communication (1) it was pointed out that the sterol isolated by Dorée (2) from the marine sponge *Cliona celata* is a mixture of "phytosterol-like" sterols which may be separated into the mono-unsaturated clionasterol, $C_{29}H_{50}O$, and the di-unsaturated poriferasterol, $C_{29}H_{48}O$. Almost concurrently, Mazur (3) described the isolation of a sterol from the fresh-water sponge, *Spongilla lacustris*, the properties of which closely resemble those of clionasterol. Both Mazur and one of the present authors have therefore suggested the identity of the two sterols. In a more recent publication, Mazur (4) has now presented evidence intended to prove the identity of the spongilla sterol with 5,6-dihydrostigmasterol, and has implied that clionasterol is also identical with the latter. It is the purpose of the present publication to show that Mazur has incorrectly interpreted his results, and to prove the presence of a 5,6-double bond in clionasterol.

The properties of clionasterol and spongilla sterol are so different from the 5,6-dihydrostigmasterol prepared by Marker and Wittle (5) as to make their identity extremely unlikely. Since Marker's dihydrostigmasterol has been obtained by the reduction of stigmastenone it might be argued that it differs from the sponge sterol in the configuration at C^5. This, however, can not be the case since both sterols supposedly yield the same stigmastanol upon catalytic hydrogenation, which indicates identical configuration in the ring system.

Wallis and collaborators (6) have recently shown that Mazur's conclusions contradict the modern theories of optical rotatory power as applied to steroids, and have suggested on the basis of their calculations that the two sponge sterols possess a double bond in the 5,6-position. In a preliminary communication (7), the present authors have shown that they have arrived independently at the same conclusion on the basis of experimental evidence.

The presence of a 5,6-double bond in clionasterol (I) has been convincingly demonstrated by a series of oxidation reactions. Thus the Oppenauer oxidation of clionasterol yields clionastenone, $C_{29}H_{48}O$, (II), $[\alpha]_D^{25}$ + 80°, which shows the typical absorption spectrum of an α,β-unsaturated ketone. This observation coupled with the fact that conversion of the alcohol to the ketone is accompanied by a strong shift of the optical rotation in the positive direction suggests that the usual oxidation of a 5,6-en-3-ol to a 4,5-en-3-one has taken place. The 2,4-dinitrophenylhydrazone of clionastenone shows a specific rotation of + 232°, and the corresponding derivative of cholestenone one of + 241°. Such unexpectedly high rotations seem to indicate that these derivatives are heterocyclic compounds rather than hydrazones.

Oxidation of clionasterol with hydrogen peroxide gives a triol, $C_{29}H_{52}O_3$, in which the presence of an inert, tertiary hydroxyl group is indicated by the formation of a diacetate and dibenzoate. Formation of this triol is best explained by assuming the addition of two hydroxyl groups to a 5,6-double bond to give 3,5,6-trihydroxyclionastane (III). Oppenauer oxidation of the triol yields clionastanol-5-dione-3,6 (IV).



Rosenheim (8) and Butenandt (9) have shown that the oxidation of cholesterol with selenium dioxide gives rise to the formation of the two allylic isomers: 4-hydroxycholesterol, $[\alpha]_D - 60°$, and cholestene-4,5-diol-3,6, $[\alpha]_D + 6°$. Oxidation of clionasteryl acetate with selenium dioxide by Butenandt's method gives an acetate which upon hydrolysis yields a diol, $C_{29}H_{50}O_2$, $[\alpha]_D^{25} + 8°$. The relative insolubility of the diol and its positive rotation indicate that it is clionasten-4,5-diol-3,6 (V). Under the influence of acids the diol is dehydrated to

clionastenone (II), a reaction which is analogous to the dehydration of the selenium dioxide oxidation products of cholesterol to cholestenene.

In their theoretical considerations concerning the location of the double bonds in sponge sterols, Wallis and collaborators (6) have made the assumption that clionasterol and poriferasterol differ only in the presence of the double bond in the side chain of the latter. This assumption is justified, for catalytic hydrogenation of clionasterol gives a saturated sterol, $C_{29}H_{52}O$, which is identical with poriferastanol. In this connection it is of interest to note that Mazur (4) found that the slowness of this reaction indicates reduction of a 5,6-double bond rather than a 21,22-double bond which is known to absorb hydrogen readily.

EXPERIMENTAL[1]

Clionastenone. Eight grams of clionasterol and 9 g. of aluminum isopropoxide were dissolved in a mixture of 350 cc. of toluene and 85 cc. of cyclohexanone, and the solution was refluxed for two hours. After cooling, the mixture was shaken with 100 cc. of 3 *N* sulfuric acid, and the toluene layer washed successively with water, 5% sodium carbonate solution, and water. After drying, the toluene was removed *in vacuo*, and the remaining yellow oil digested with small amounts of methanol at 0°. A crystalline material was thus obtained which melted at 77–78° after several recrystallizations from methanol. The ketone was then distilled in a high vacuum and again recrystallized from methanol. The purified material melted at 79°; $[\alpha]_D^{25}$ + 80.2° (24.7 mg. in 3 cc. of chloroform).

Anal. Calc'd for $C_{29}H_{48}O$: C, 84.4; H, 11.7.
Found: C, 84.4; H, 11.7.

The 2,4-dinitrophenylhydrazone was prepared by refluxing 100 mg. of clionastenone with 10 cc. of Brady's reagent (10) for 30 minutes, and recrystallizing the crude product from alcohol and chloroform. It crystallizes in red needles of m.p. 227–228°, $[\alpha]_D^{25}$ + 232° (50.9 mg. in 3 cc. of chloroform).

Anal. Calc'd for $C_{35}H_{52}N_4O_4$: C, 70.0; H, 8.8; N, 9.45.
Found: C, 70.6; H, 8.85; N, 9.6.

Cholestenone 2,4-dinitrophenylhydrazone showed $[\alpha]_D^{25}$ + 240.8 (31.0 mg. in 3 cc. of chloroform.)

Clionastane-3,5,6-triol. To a solution of 5 g. of clionasterylacetate in 25 cc. of glacial acetic acid was added 5 cc. of 30% hydrogen peroxide in 0.5-cc. portions over a period of ten minutes. On each addition a flocculent precipitate was formed which subsequently went into solution. The mixture was then heated on the steam-bath for three hours, filtered from a small amount of precipitate, and poured into ice-water. Sodium chloride was added to facilitate the precipitation of the acetate mixture. The precipitate was collected and hydrolyzed by a 10% solution of potassium hydroxide in alcohol. Water was then added, and the triol filtered, dried, and recrystallized from a benzene-methanol mixture until the melting point remained constant at 237–238°.

Anal. Calc'd for $C_{29}H_{52}O_3$: C, 77.16; H, 11.7.
Found: C, 77.2; H, 11.7.

Triol monoacetate. Three hundred sixty milligrams of triol was refluxed with 5 cc. of acetic anhydride for one hour. The acetate which separated on cooling was recrystallized repeatedly from an ether-methanol mixture. On heating it softens at 235° and melts at 238°.

Anal. Calc'd for $C_{31}H_{54}O_4$: C, 75.9; H, 11.1.
Found: C, 76.0; H, 10.8.

[1] The authors are greatly indebted to Merck and Co., Rahway, N. J., for the preparation of the sponge sterols used in this investigation.

Triol diacetate. One hundred milligrams of triol was refluxed for 90 minutes with 4 cc. of acetic anhydride and 1 g. of fused sodium acetate. The diacetate which separated on cooling was filtered, washed with water and methanol, and crystallized from methanol until the melting point remained constant at 128–129°.

Anal. Calc'd for $C_{33}H_{56}O_5$: C, 74.4; H, 10.6.
Found: C, 74.05; H, 10.4.

Triol dibenzoate. Two cubic centimeters of benzoyl chloride was added to a solution of 225 mg. of triol in 5 cc. of dry pyridine. After standing at room temperature for eight hours the solution was poured into 2 *N* sulfuric acid, and the precipitate extracted with ether. After washing with water and carbonate solution, the extract was evaporated to dryness and the residue recrystallized from absolute alcohol. The dibenzoate melted at 225–228°.

Anal. Calc'd for $C_{43}H_{60}O_5$: C, 78.6; H, 9.2.
Found: C, 78.4; H, 9.35.

Clionastenol-5-dione-3,6. To a suspension of 400 mg. of triol in 30 cc. of dry acetone was added a solution of 1 g. of aluminum isopropoxide in 60 cc. of dry benzene, and the mixture was heated under reflux for ten hours. It was then cooled, 1 *N* sulfuric acid was added, and the benzene layer was washed with water and dried. Upon evaporation of the extract *in vacuo* there remained a colorless solid which was recrystallized several times from acetone. The diketone crystallized in small needles, and melted at 189–191°.

Anal. Calc'd for $C_{29}H_{48}O_3$: C, 78.3; H, 10.9.
Found: C, 78.0; H, 11.0.

Clionasten-4,5-diol-3,6. A solution of 4 g. of clionasterol in 100 cc. of acetic anhydride was heated for 30 minutes to 105–110°. With rapid stirring, a solution of 2.5 g. of selenium dioxide in 5 cc. of water was then added dropwise. After two hours the reaction product was precipitated with water, filtered, and washed repeatedly with hot water. The solid was then extracted with acetone, the extract filtered and poured into a 10% solution of potassium cyanide in water. After standing for several hours the mixture was extracted with ether, and the ether extract evaporated to dryness. The dark oily residue was refluxed for one hour with a 5% solution of potassium hydroxide in methanol. During the hydrolysis a copious crystalline precipitate appeared. It was filtered after cooling and crystallized first from a large volume of absolute ethanol, and then from ether by extraction from a thimble. The diol melted at 231–232°; $[\alpha]_D^{25}$ + 8.3° (47.0 mg. in 3 cc. of pyridine). It is difficultly soluble in most organic solvents with the exception of pyridine.

Anal. Calc'd for $C_{29}H_{50}O_2$: C, 80.9; H, 11.7.
Found: C, 81.1; H, 11.8.

Diol dibenzoate. The dibenzoate was prepared by treating the diol in pyridine solution with benzoyl chloride as described above. After several recrystallizations from absolute methanol it melted at 206–207°.

Anal. Calc'd for $C_{43}H_{58}O_4$: C, 80.8; H, 9.15.
Found: C, 80.6; H, 9.4.

Conversion of the diol to clionastenone. A small sample of the diol was refluxed with Brady's solution for five minutes. A 2,4-dinitrophenylhydrazone was formed which was recrystallized from a mixture of alcohol and chloroform. It melted at 229° and gave no depression with clionastenone-2,4-dinitrophenylhydrazone.

Hydrogenation of clionasteryl acetate. Eight hundred milligrams of acetate in 100 cc. of glacial acetic acid was hydrogenated at 60–70° in the presence of 0.3 g. of platinum oxide. The absorption of hydrogen was complete after 45 minutes. The reduction product gave a negative Liebermann test. The acetate was recrystallized from alcohol, m.p. 139°, $[\alpha]_D^{25}$ + 17.8° (30.4 mg. in 3 cc. of chloroform). Mixed with poriferastyl acetate of m.p. 140° it melted at 139.5–140°.

The alcohol prepared by hydrolysis of the acetate melted at 140–140.5°, $[\alpha]_D^{25}$ + 25° (30.4 mg. in 3 cc. of chloroform). Mixed with poriferastanol of m.p. 142–143° it melted at 141.5–142.5°.

The ketone prepared by oxidation of the alcohol with chromic anhydride melted at 159.5–160°; $[\alpha]_D^{25}$ + 43.1° (28.1 mg. in 3 cc. of chloroform). Mixed with poriferastanone of m.p. 161° it melted at 160.5–161°.

SUMMARY

Clionasterol has been shown to possess a double bond in the 5,6-position. It has been converted into clionastenone, clionastane-3,5,6-triol, clionastane-5-ol-dione-3,6, clionastene-4,5-diol-3,6, and poriferastanol. The structure of spongilla sterol has been discussed.

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