from cyclohexane yielded 180 g. (82%) of product, m.p. 96–97°.

β,β-Di-*n*-propyltropic Acid (β-Hydroxy-α-phenyl-β-*n*-propylcaproic Acid).—To an ether solution of isopropylmagnesium bromide prepared in the usual manner from 217 g. (1.75 moles) of isopropyl bromide and 42.5 g. (1.75 moles) of magnesium metal, there was added 157.9 g. (1 mole) of sodium phenylacetate. The suspension was stirred and refluxed 48 hours. Then an ether solution of 200 g. (1.75 moles) of di-*n*-propyl ketone was added dropwise over a three-hour period. The reaction mixture containing the thick, semi-solid complex was stirred overnight, cooled and hydrolyzed by the gradual addition of excess dilute sulfuric acid whereupon partial separation of the product occurred. The solid thus formed was removed by filtration and subsequently dissolved in sodium carbonate solution. Acidification of the alkaline solution gave 173 g. of acid, m.p. 175–177°. Extraction of the ether layer of the original filtrate with carbonate solution and acidification of the basic extracts yielded another 67 g. of product, m.p. 168–170°. The two crops were combined and crystallized from dilute alcohol. There was thus obtained 170 g. (68%) of material, m.p. 176–177°.

Diethylaminoethyl β,β-Pentamethylenetropate Hydrochloride (Diethylaminoethyl β-Hydroxy-α-phenylcyclohexaneacetate Hydrochloride).—An isopropyl alcohol solution of 23.4 g. (0.1 mole) of β,β-pentamethylenetropic acid and 13.5 g. (0.1 mole) of diethylaminoethyl chloride was refluxed four hours. The solvent was removed at reduced pressure and the viscous residue triturated with dry ether. The product slowly solidified and was then collected by filtration and dried. This material weighed 35.8 g. (97%) and melted at 129–132°. Crystallization from an isopropyl alcohol–ether mixture gave 26.5 g. (72%) of product, m.p. 138–139°.

The methiodide salt was obtained by treatment of an ether solution of the free base with methyl iodide. The crystalline solid which separated melted at 138–138.5° after recrystallization from absolute alcohol. Prolonged boiling of the alcohol solution resulted in some decomposition of the product.

Anal. Calcd. for $C_{21}H_{34}INO_3$: C, 53.05; H, 7.20; N, 2.94. Found: C, 53.06; H, 7.07; N, 2.72.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE POLYTECHNIC INSTITUTE OF BROOKLYN AND THE DEPARTMENT OF BIOCHEMISTRY OF THE JEWISH HOSPITAL OF BROOKLYN]

A Study of α-Cholesterylene[1,2]

BY JOSEPH L. OWADES[3] AND ALBERT E. SOBEL

A new product of pyrolysis of a steroid sulfate was shown to be identical to the long known but uncharacterized α-cholesterylene. Based on absorption spectra, molecular weight determination, peracid titrations, selenium dehydrogenation, bromination, maleic anhydride addition, ozonolysis, color tests, thermal stability and failure to react with mercuric acetate, the structure 3,6′-bis-2,4-cholestadiene is proposed for this hydrocarbon.

Although the existence of α-cholesterylene has been known for over a hundred years, information on its structure and properties has been very meager. The authors became interested in the structure of α-cholesterylene during an investigation of steroid sulfates. In studying the pyrolysis of the aluminum salt of cholesteryl sulfate[4] (I), one of the products was found to be identical to the α-cholesterylene prepared by Zwenger in 1848[5] by the action of 80% sulfuric acid on cholesterol.

Zwenger's reaction may be described as a steroid color test, in which α-cholesterylene is an end-product. To the best of our knowledge, no end-product of a steroid color reaction has been characterized prior to the work reported here. For this reason, and because it had been postulated that steroid sulfates may be intermediates in sterol metabolism,[4] this study of α-cholesterylene was undertaken.

Past knowledge about α-cholesterylene consisted of a good ultimate analysis by Zwenger,[5] a molecular weight determination (cryoscopic in naphthalene) by Mauthner and Suida,[6] the optical rotation and an uncharacterized bromide by Eck,[7] and the melting point obtained by each of these investigators. The molecular weight and rotation are at variance with the results reported here.

In addition to the above work, two other reactions have been reported to yield α-cholesterylene: the action of zinc chloride[7] and of phosphorus pentoxide[8] on cholesterol. The identity of these products with α-cholesterylene was presumed on the basis of similar melting points. However, these reactions in our hands gave compounds that differed from α-cholesterylene in rotation (Table I), ultraviolet (Fig. 1) and infrared (Fig. 2) absorption spectra.

The structure of α-cholesterylene proposed as a result of this investigation (II) is based on the following evidence.

The unusually high melting point (290–300°) and its non-volatility at 270° at 0.2 μ pointed qualitatively to a compound of dimensions greater than C_{27}. The Rast determination presented some difficulties because of the low solubility and slow rate of dissolution of the steroid in molten camphor, but it finally yielded a molecular weight corresponding to a dimer. The steroid is not soluble in cyclopentadecanone (Exaltone).

The presence of the steroid nucleus was demonstrated by the isolation of 3′-methyl-1,2-cyclopen-

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[1] Presented at the 117th Meeting of the American Chemical Society, Philadelphia, Penna., April, 1950.

[2] Abstracted from the dissertation submitted to the Faculty of the Graduate School of the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[3] The Fleischmann Laboratories, Standard Brands, Inc., 810 Grand Concourse, New York 51, N. Y.

[4] A. E. Sobel, P. Owades and J. L. Owades, THIS JOURNAL, **71**, 1487 (1949).

[5] C. Zwenger, *Ann.*, **66**, 5 (1848).

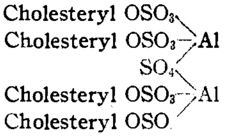
[6] J. Mauthner and W. Suida, *Monatsh.*, **17**, 29 (1896).

[7] J. C. Eck and R. L. Van Peursem, *Iowa State Coll. J. of Science*, **13**, 115 (1939).

[8] T. Wagner-Jauregg, T. Lennartz and H. Kothny, *Ber.*, **74B** 1513 (1941).

TABLE I

	$\alpha_D(CHCl_3)$
α-Cholesterylene (cholesterol + H_2SO_4)	− 26°
α-Cholesterylene (alum. chol. sulfate)	− 26.3°
Hydrocarbon (cholesterol + P_2O_5)	+ 89°
Hydrocarbon (cholesterol + $ZnCl_2$)	+308°



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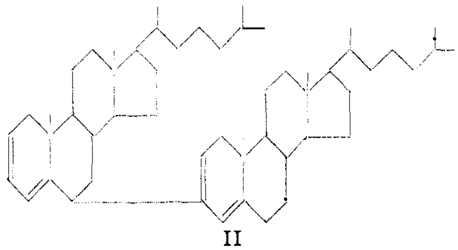

II

tanophenanthrene (Diels hydrocarbon) after selenium dehydrogenation.[9] The possibility of ring opening or contraction during the formation of the bisteroid was thus precluded.

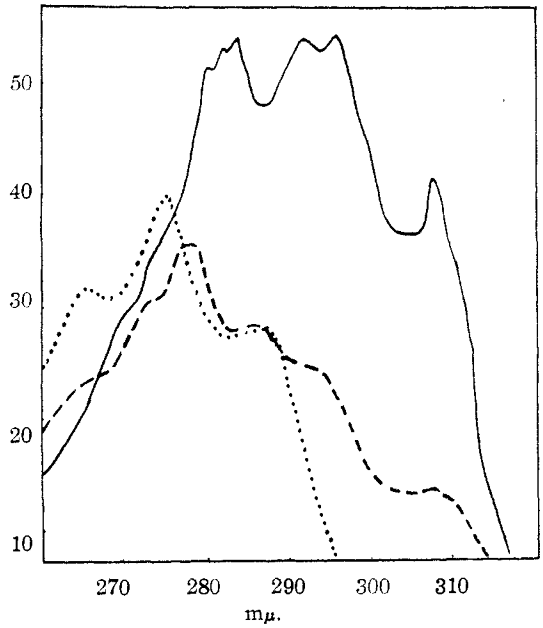

Fig. 1.—. . . . , α-Cholesterylene; - - - -, hydrocarbon (cholesterol + P_2O_5); ——, hydrocarbon (cholesterol + $ZnCl_2$).

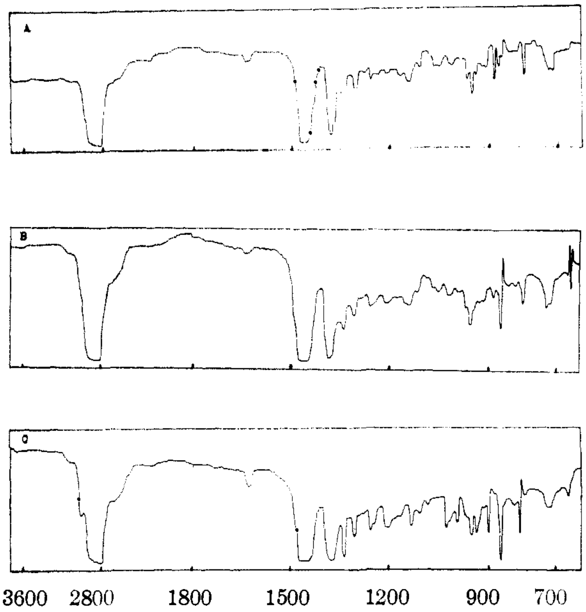

Fig. 2.—A, α-Cholesterylene; B, hydrocarbon (cholesterol + P_2O_5); C, hydrocarbon (cholesterol + $ZnCl_2$).

The existence of four double bonds in two insulated systems of homoannular conjugated unsaturation was shown in several ways. Perbenzoic and peracetic acid titrations revealed the presence of four double bonds. A tetrabromide was isolated (2,4-cholestadiene gives a dibromide). The ultraviolet absorption spectrum (Fig. 1) showed a maximum at 275 mμ. The application of Woodward's rules,[10] as extended by Fieser,[11] to the proposed structure (taking the average of 273 and 278 mμ for the two chromophoric moieties) (II) gave a calculated maximum at 275 mμ. Further evidence for the placement of each set of double bonds in one ring was the isolation of crystalline maleic anhydride adducts (a mono- and a diadduct were obtained).

The location of the double bonds in ring A (and not ring B) was indicated in three ways and confirmed by a fourth. The first was the ultraviolet absorption maximum, which was 8 mμ lower than that calculated for a 5,7-dienic system. The second was a negative Tortelli–Jaffe test.[12] And the third was treatment with mercuric acetate, which gave no products with ultraviolet absorption characteristic of a conjugated triene and allowed recovery of most of the starting material. A 5,7-diene (III) would have been expected to dehydrogenate to a conjugated triene.[13] The confirmatory evidence was ozonolysis followed by oxidative cleavage of the ozonide, which gave a keto acid (IV). A steroid unsaturated in ring B (III) would have given a diketone (V).

The ozonolysis also helped establish the points of linkage of the sterol nuclei. Of the three probable sites of attachment—3,3′-, 3,6′- or 6,6′-, the 3,3′-dimer was eliminated by the absorption spectrum. The 6,6′-dimer VI on ozonolysis, should have given a keto acid which was also a γ-diketone (VII). The ketone obtained was not a γ-diketone, as shown by the failure to cyclize to a pyrrole on treatment with ammonia. Further, a 6,6′-bisteroid should have given a keto acid that was dimeric itself (VII), whereas the compound obtained was monomeric. The points of attachment, therefore, are most probably the 3,6′-positions (II). The stability of α-cholesterylene to pyrolysis at 360°, and to boiling acetic anhydride and decalin also contraindicated the 6,6′-linkage, since this bond should be rela-

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(9) O. Diels, W. Gadke and P. Kording, *Ann.*, **459**, 1 (1927).

(10) R. Woodward, THIS JOURNAL, **64**, 72 (1942).

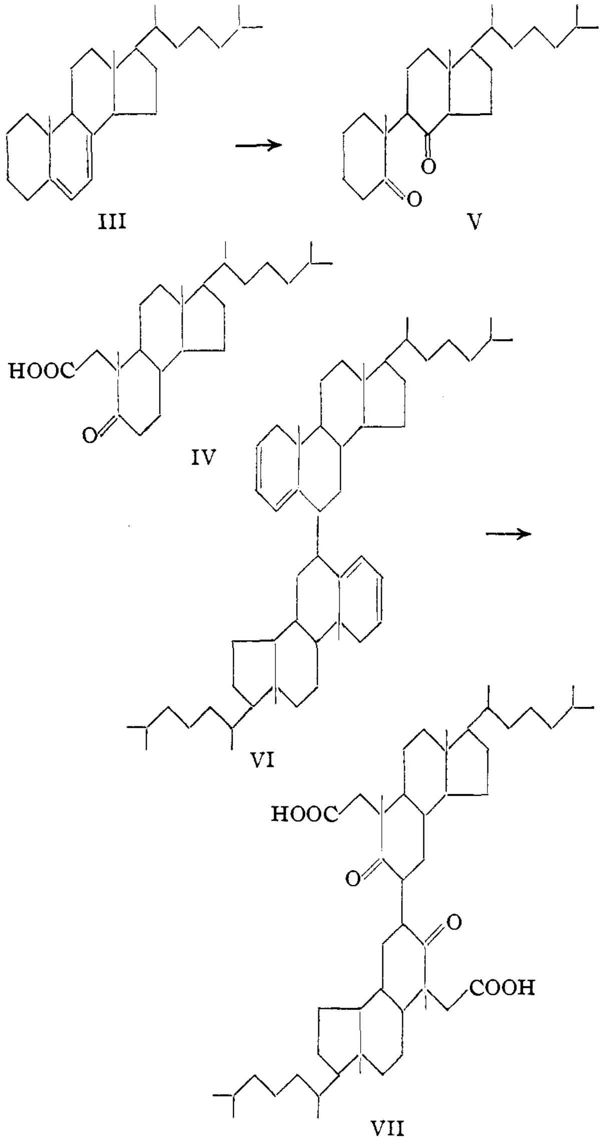
(11) L. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publishing Company, New York, N. Y., 1949, p. 184.

(12) M. Tortelli and E. Jaffe, *Chem. Zentr.*, **39**, 14 (1915).

(13) D. H. R. Barton, private communicaiton.

tively unstable thermally in accordance with Schmidt's rule.[14]

The formula suggested is in accordance with all the known facts. The necessary loss of hydrogen may be accounted for by the reduction of the sulfuric acid to sulfur dioxide (which was detected), or by hydrogen transfer under the influence of the acid or the pyrosulfate.[15]



Experimental

α-**Cholesterylene. (a) From Cholesterol.**—A modification of the method of Zwenger[5] as given by Eck[7] was used. Forty ml. of 1:1 sulfuric acid was added to 10 g. of cholesterol and the mixture heated in a water-bath at 65°. Sixty ml. of concentrated sulfuric acid was added in 1 min. with agitation. After 2 min. the liquid was decanted and the residue washed several times with water. One hundred ml. of water was added and the suspension allowed to stand for 24–48 hr. After neutralizing with ammonia, the suspension was washed by centrifugation twice with 50% alcohol and once with 95% alcohol. The residue was air-dried and extracted with ether for 24 hr. The ether-insoluble matter is crude α-cholesterylene. The yellow solid was recrystallized three times from chloroform, pyridine and toluene. The light yellow crystals softened at 290° and liquified at 300°. The crystals were dissolved in petroleum ether and chromatographed over alumina. About 5 l. of solvent was required for 100 mg. of steroid. White crystals were recovered, melting point unchanged, $\alpha_D -26°$ (c 0.1, chloroform).

Anal. Calcd. for $(C_{27}H_{43})_2$: C, 88.21; H, 11.79; mol. wt., 734. Found: C, 88.09; H, 11.83; mol. wt. (Rast), 880, 865.

(b) From Aluminum Cholesteryl Sulfate.—Ninety-two grams of aluminum cholesteryl sulfate[4] was heated at a pressure of 1 mm. to 138° for one-half hour. The mass was extracted with benzene and with alcohol. The residue was extracted with chloroform for 16 hr. After evaporation of the solvent, the crystals were purified as in (a), yield 1.1 g., m.p. 290–300°, no depression on admixture with material prepared as in (a); $\alpha_D -26.3°$ (c 0.1, chloroform).

Selenium Dehydrogenation.—A mixture of 1.7 g. of α-cholesterylene and 3.4 g. of selenium was heated at 340° for 70 hr. The residue was extracted with ether for 30 hr., the solvent removed and the residue distilled at 1 mm. pressure. The fraction distilling between 210–240° was taken up in petroleum ether and chromatographed over alumina. The eluate containing the blue-violet fluorescent band, on evaporation, gave, after crystallization, white crystals of Diels hydrocarbon, m.p. 126–127°, mixed melting point with an authentic specimen prepared from cholesterol showed no depression, absorption maximum at 258 mμ (log E_m 4.8).

Bromination.—A suspension of 0.211 g. of α-cholesterylene in 80 ml. of carbon tetrachloride was treated with 5 ml. of a 5% solution of bromine in carbon tetrachloride. After 24 hr. the solution was evaporated in a vacuum and the residue recrystallized five times from ether–methanol, m.p. 130–132° (dec.).

Anal. Calcd. for $(C_{27}H_{43}Br_2)_2$: C, 61.48; H, 8.22; Br, 30.30. Found: C, 61.53; H, 8.13; Br, 29.95.

Maleic Anhydride Adducts.—A xylene solution of 4 g. of maleic anhydride and 1 g. of α-cholesterylene was refluxed for 20 hr. The solvent was removed, and the residue dissolved in hot ethyl acetate, filtered, treated with methanol and cooled. The crystalline precipitate that formed was recrystallized twice from ethyl acetate–methanol and dried in a vacuum, m.p. 180–190°.

Anal. Calcd. for $C_{58}H_{88}O_3$: C, 83.59; H, 10.64. Found: C, 82.92; H, 10.48.

The white, ethyl acetate-insoluble material recovered above was recrystallized twice from toluene and dried at 100°, m.p. 268–273°.

Anal. Calcd. for $(C_{31}H_{45}O_3)_2$: C, 79.95; H, 9.74. Found: C, 80.63; H, 10.34.

Attempted Dehydrogenation with Mercuric Acetate.—One-half gram of α-cholesterylene dissolved in 300 ml. of chloroform was treated with a solution of 0.90 g. of mercuric acetate in 20 ml. of acetic acid and the solution stirred, under nitrogen, for 48 hr. The solution was filtered, and the filtrate concentrated in vacuum. Four crystalline crops were removed during the evaporation, and the mother liquor taken to dryness. All 5 crops were examined spectrophotometrically; the first four were unreacted α-cholesterylene, the fifth was amorphous but showed no absorption above 285 mμ.

Ozonolysis.—A solution of 0.40 g. of α-cholesterylene in 200 ml. of chloroform was ozonized for 10 min. (the rate of ozone flow was about 0.02 mole/hr.). The chloroform was replaced with acetic acid by distillation at room temperature under vacuum. The ozonide was oxidatively cleaved with hydrogen peroxide (0.6 g. of 30% peroxide) in sulfuric acid (0.04 g.) by refluxing for 2 hr. The solution was diluted with 200 ml. of water and extracted three times with chloroform. The chloroform was washed with water, dried with magnesium sulfate and evaporated to dryness. The residue, after crystallization from aqueous alcohol, melted at 95–100° (dec.).

Anal. Calcd. for $C_{25}H_{42}O_3$: C, 76.87; H, 10.84; neut. equiv., 390; mol. wt., 390. Found: C, 76.72; H, 10.98; neut. equiv., 392; mol. wt., 420.

The 2,4-dinitrophenylhydrazone melted at 135°, ultraviolet absorption maximum at 364 mμ.

Anal. Calcd. for $C_{31}H_{46}O_6N_4$: C, 65.31; H, 8.13; N, 9.83. Found: C, 65.19; H, 7.98; N, 9.62.

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[14] O. Schmidt, *Chem. Revs.*, **17**, 137 (1935).

[15] V. N. Ipatieff, H. Pines and R. C. Olberg, THIS JOURNAL, **70**, 2123 (1948).