the crystalline N-*p*-nitrophenyl-D-glucosylamine, m.p. and mixed m.p. 185°, and $[\alpha]^{25}D$ −188° in pyridine (*c* 1); lit.[30] m.p. 184°, $[\alpha]D$ −192° (pyridine).

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[CONTRIBUTION FROM THE FISHERIES RESEARCH BOARD OF CANADA, CHEMISTRY SECTION OF THE TECHNOLOGICAL STATION]

Marine Sterols. IV. 24-Dehydrocholesterol: Isolation from a Barnacle and Synthesis by the Wittig Reaction

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A new sterol, 24-dehydrocholesterol, has been isolated from the barnacle *Balanus glandula*. It represents 34.2% of the total sterols of the barnacle. The major component (59.8%) is cholesterol. The new sterol also has been synthesized *via* the Wittig reaction and the synthetic product shown to be identical with the natural one. A previously reported sterol obtained by the dehydrohalogenation of 25-bromocholesterol and assigned the structure 25-dehydrocholesterol has been shown to be a mixture of 40% 25-dehydrocholesterol and 60% 24-dehydrocholesterol.

A previous investigation of the sterols of molluscs resulted in the identification of 24-methylenecholesterol as a major sterol component of several species of molluscs.[1,2] Its subsequent synthesis *via* the Wittig reaction has been reported.[3,4] Our study of the sterols of marine invertebrates has now been extended to a crustacean, the barnacle *Balanus glandula*. To the best of our knowledge cholesterol is the only sterol which has been isolated and characterized from crustaceans.[5-10] The infrared spectra of crude barnacle sterols demonstrated the absence of any significant quantity of 24-methylenecholesterol. Chromatography of the azoyl esters[11] of the crude sterol mixture resulted in three zones. The sterol of the fastest moving zone was identified as cholesterol and represents 59.8% of the total sterols. The middle zone which represents 6% of the total sterol will be the subject of a future report. The slowest moving zone (34.2%) when hydrolyzed was found to be contaminated with a small amount of $\Delta^{5,7}$-sterol which was removed with maleic anhydride. The purified sterol and its derivative had properties different from those of any known sterol. Hydrogenation of the sterol with Adams catalyst in glacial acetic acid produced cholestanol with the uptake of 2 moles of hydrogen and demonstrated that the original sterol was a readily reducible cholestadienol. The modified Liebermann–Burchard reaction[12] showed that the cholestadienol had a double bond in the 5-position and that the second double bond made no significant contribution to the reaction, thus eliminating most nuclear double bonds from consideration. Ozonolysis resulted in the isolation of acetone as its 2,4-dinitrophenylhydrazone in good (51%) yield and completed the identification of the sterol as 24-dehydrocholesterol. The synthesis of this sterol *via* the Wittig reaction from 3β-acetoxy-5-cholenaldehyde and triphenylphosphine-isopropylidene was carried out in a manner analogous to that previously reported for the synthesis of 24-methylenecholesterol and 25-dehydrocholesterol. The properties of the synthetic sterol and its derivatives were identical with those of the sterol from barnacle.

With respect to the comparative biochemistry of marine invertebrates the question of the significance of 24-dehydrocholesterol in barnacles must await a comprehensive study of the sterols of other crustaceans. However, by analogy with Mollusca the results of this report already appear of interest. In Mollusca, as we ascend the evolutionary scale, we find a high percentage of Δ^7-sterols in chiton,[13,14] large quantities of 24-methylenecholesterol in Pelecypoda[1,2] and almost entirely cholesterol in Gastropoda and Cephalopoda.[15] Descending the evolutionary scale in Crustacea we find that cholesterol is the principal if not the only sterol (other than small amounts of provitamin D) in Malacostraca (crabs, shrimps, etc.);[13] Cirripedia (barnacle) has been shown here to contain cholesterol as a major component but also 24-dehydrocholesterol in large amounts. It remains to see whether cholesterol either continues to decrease or is absent in Ostracoda, Copepoda and Branchiopoda. The day may not be too far distant when the sterols of a marine invertebrate will be predictable from their position in the evolutionary scale. From the bio-

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chemical viewpoint there is already evidence that unsaturation of the cholesterol sidechain may be an important factor in cholesterol metabolism.[16-18] Further studies on lower forms of marine life may well provide a means of isolating possible intermediates. A sterol, "desmosterol," has been isolated as a minor component (2%) of the sterols of chick embryo.[18] This sterol (m.p. 121.2°) is a cholestadienol. The authors suggest that it is 24-dehydrocholesterol, though sufficient evidence to prove the identity is lacking. Now that the synthetic sterol is available it should be possible to establish the identity or non-identity of "desmosterol" and 24-dehydrocholesterol. We recently reported the synthesis of 25-dehydrocholesterol[3] and showed that it is not identical with two products prepared by dehydrohalogenation and assigned this structure.[19,20] We had available for comparison an acetate of one of the earlier preparations which showed no apparent evidence of deterioration. Part of the infrared spectrum of this compound was published by us and it showed no significant absorption at 890 cm.$^{-1}$ characteristic of a terminal methylene group. The only conclusion that could be reached at that time was that the earlier preparations were probably 24-dehydrocholesterol. However, the properties of the synthetic product reported in this paper show that this is not the case (Table I). One of the earlier investigators (Dr. Dauben) has synthesized a new batch of the sterol in question and kindly supplied us with a sample so that we might resolve the problem.[21]

TABLE I

PROPERTIES OF 24- AND 25-DEHYDROCHOLESTEROL

	24-Dehydrocholesterol *via* Wittig reaction		25-Dehydrocholesterol *via* Wittig reaction		"25-Dehydrocholesterol" *via* dehydrohalogenation	
	M.p., °C.	$[\alpha]_D$	M.p., °C.	$[\alpha]_D$	M.p., °C.	$[\alpha]_D$
Sterol	117–118	−37.9°	133	−40.7°	120.5–121.5	−40.2°
Acetate	99–100	−38.8	112	−44.4	92.5–93.5	−42.8
Benzoate	131	−14.0	126	−16.7		

The infrared spectrum of the new preparation has a significant absorption at 890 cm.$^{-1}$. We have recorded spectra of synthetic 24-dehydrocholesterol, 25-dehydrocholesterol prepared *via* the Wittig reaction, and the new product prepared by dehydrohalogenation and found the latter to be a mixture composed of 40% 25-dehydrocholesterol and 60% 24-dehydrocholesterol. It must be concluded that both the earlier preparations were originally a similar mixture and must have undergone extensive changes on storage. However, it is also probable that the composition of such a sterol mixture would vary with the reaction conditions and the number of recrystallizations.

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Experimental[22]

Preparation of Crude Sterols.—In a preliminary experiment ground-up fresh barnacles (22.5 lb.) were extracted by shaking with three batches of acetone. The first extract was heated to boiling before shaking. The mass was finally washed with ether and the combined extracts were evaporated to dryness. Moisture (38.13%) was determined on an aliquot of wet barnacles, and the oil content (0.83%) was determined on a sample of the dried material. The non-saponifiable portion (4.28 g.) was chromatographed on a silicic acid:Celite column, 7.5 cm. × 10 cm., and the sterols (1.77 g.) eluted with methylene chloride after washing the chromatogram thoroughly with Skellysolve C and Skellysolve C–benzene 1:1. To obtain larger quantities of sterol, 233 lb. of fresh barnacles were extracted 3 times with hot acetone in a pilot plant. The oil (300 g.) gave 93 g. of non-saponifiables and 12.6 g. of crude sterols when chromatographed as described.

Chromatography.—The azoyl esters of the crude sterol mixture were chromatographed on silicic acid:Celite, using 5.5:1 Skellysolve C–benzene developer as previously described.[1] Three zones developed, all well separated from each other. Starting with the uppermost zone the composition was: zone 1, 34.2%; zone 2, 6.0%; and zone 3, 59.8%.

Zone 1 Acetate.—The azoyl ester was crystallized from benzene–ethanol and hydrolyzed. Calculated from the ultraviolet spectrum the sterol contained 0.85% of provitamin D. The acetate was treated with maleic anhydride in the usual manner[23] to remove provitamin D. The residue from the maleic anhydride treatment was acetylated, and the acetate, crystallized from aq. ethanol, had m.p. 101.5°, $[\alpha]^{24}_D$ −39.2° (*c* 2.5 in $CHCl_3$).

Anal. Calcd. for $C_{29}H_{46}O_2$: C, 81.63; H, 10.87. Found: C, 81.59; H, 10.75.

The sterol was crystallized from aq. methanol, m.p. 117°, $[\alpha]^{22}_D$ −38.7° (*c* 2.6 in $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{44}O$: C, 84.31; H, 11.53. Found: C, 84.35; H, 11.44.

The benzoate was crystallized from acetone, m.p. 129°, $[\alpha]^{22}_D$ −15.4° (*c* 3.1 in $CHCl_3$). *Anal.* Calcd. for $C_{34}H_{48}O_2$: C, 83.55; H, 9.90. Found: C, 83.86; H, 9.58.

Molecular rotational differences: 24-Dehydrocholesterol, Δ^{Ac} −18; Δ^{Bz} +74; Δ^5-sterols, Δ^{Ac} −35 ± 16; Δ^{Bz} +81 ± 16.

Reduction of Zone 1 Acetate.—The acetate (16.3 mg.) was reduced in glacial acetic acid with platinum oxide catalyst. The hydrogen uptake was 1.78 ml. The theoretical amount for two double bonds is 1.71 ml. The reduced acetate was crystallized from aq. methanol, m.p. 111°, $[\alpha]^{22}_D$ +13.6° (*c* 3.4 in $CHCl_3$), no m.p. depression with cholestanyl acetate, m.p. 111°.

Anal. Calcd. for $C_{29}H_{50}O_2$: C, 80.87; H, 11.70. Found: C, 80.91; H, 11.79.

The sterol was crystallized from aq. methanol, m.p. 141°, $[\alpha]^{22}_D$ +23.6° (*c* 2.7 in $CHCl_3$), no m.p. depression with cholestanol, m.p. 142°. *Anal.* Calcd. for $C_{27}H_{48}O$: C, 83.43; H, 12.45. Found: C, 83.35; H, 12.57.

Ozonolysis.—Zone 1 sterol (530 mg.) was suspended in 10 ml. of acetone-free acetic anhydride–acetic acid (4:1) and cooled in an ice-bath. Ozone was passed through the solution until all of the solid had disappeared. Water and zinc dust were then added and the mixture was heated in a water-bath in order to decompose the ozonide. The volatile fraction was steam distilled into a water trap and the contents of this trap in turn steam distilled into a 2,4-dinitrophenylhydrazine solution. A 2,4-dinitrophenylhydrazone precipitated immediately. The crude product (175 mg.) was recrystallized twice from aqueous ethanol and melted at 128°, mixed m.p. 128° with the 2,4-dinitrophenylhydrazone of acetone (m.p. 128°).

Anal. Calcd. for $C_9H_{10}N_4O_4$: C, 45.38; H, 4.23. Found: C, 45.34; H, 4.38.

The 2,4-dinitrophenylhydrazone derivatives of the ozonolysis product and of that of acetone were chromatographed in isopropyl alcohol–water (1:1) on Whatman No.

(22) Melting points are uncorrected. Optical rotations were measured by means of a Rudolph precision polarimeter. Infrared spectra were recorded with a Beckman IR 4 instrument.

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1 filter paper treated with 5% olive oil in chloroform. The descending chromatograms were run for 2.5 days.[24] The derivatives were also run both ascending and descending in methanol–isoöctane (1:1) on untreated paper.[25] The two 2,4-dinitrophenylhydrazones had identical R_f values differing from those of formaldehyde, acetaldehyde and isobutyraldehyde.

Isopropyltriphenylphosphonium Bromide.—A pressure bottle was charged with 3.1 g. of isopropyl bromide and 6.6 g. of triphenylphosphine and was heated at 150° for one day. The crystalline precipitate weighed 6 g. It was recrystallized from a small amount of ethanol with ether, m.p. 238–239°.

Triphenylphosphine Isopropylidine Reagent.—The reagent was prepared from 3.08 g. (8 mmoles) of isopropyltriphenylphosphonium bromide, 12.9 ml. (8 mmoles) of an 0.62 *N* butyllithium solution and 25 ml. of anhydrous ether by shaking overnight at room temperature in a pressure bottle under nitrogen.

3β-Acetoxy-5-cholenaldehyde.—Ethyl 3β-acetoxy-thiol-5-cholenate was prepared according to the method of Levin, *et al.*,[26] The acid chloride of 4 g. of 3β-acetoxy-5-cholenic acid was prepared and treated with ethyl mercaptan in pyridine, giving 3 g. of thiol ester, m.p. 98–101°. The thiol ester was reduced[27] with deactivated Raney nickel (30 g. of nickel alloy activated in the usual manner and partially deactivated with acetone) to give a crude product melting at 132–140°. After purification over the bisulfite addition product, 1.36 g. of aldehyde was obtained, m.p. 140–142°.

Synthesis of 24-Dehydrocholesterol Acetate.—To the alkylidene reagent in the pressure bottle was added under nitrogen 840 mg. of 3β-acetoxy-5-cholenaldehyde, m.p. 140–142°. The bottle was sealed and shaken at room temperature for one hour. It then was heated at 65° for 6 hours. The reaction product was filtered and the solid material washed several times with moist ether. The ether extracts were evaporated and the residue was acetylated and chromatographed on a silicic acid:Celite column (7.5 cm. × 14 cm.). By-products from the reaction were eluted with Skellysolve C–benzene (1:1). Benzene then eluted 205 mg. of crude 24-dehydrocholesterol acetate. The acetate was recrystallized several times from aq. ethanol, m.p. 99–100°, $[\alpha]^{22}D$ −38.8° (*c* 2.6 in $CHCl_3$), mixed m.p. 100° with the natural product.

The sterol was crystallized from aq. methanol, m.p. 117–118°, $[\alpha]^{22}D$ −37.9° (*c* 2.1 in $CHCl_3$), mixed m.p. 117° with the natural product.

The benzoate was crystallized from aq. acetone, m.p. 131°, $[\alpha]^{22}D$ −14.0° (*c* 2.7 in $CHCl_3$), mixed m.p. 130° with the natural product.

Zone 3 Sterol.—The azoyl ester was crystallized from benzene–ethanol and hydrolyzed to the sterol which was crystallized twice from aq. methanol, m.p. 148°, $[\alpha]^{22}D$ −40.2° (*c* 3.0 in $CHCl_3$), mixed m.p. 148° with cholesterol.

Anal. Calcd. for $C_{27}H_{46}O$: C, 83.87; H, 11.99. Found: C, 83.82; H, 11.92.

The acetate crystallized from aq. ethanol, m.p. 116°, $[\alpha]^{22}D$ −47.0° (*c* 3.2 in $CHCl_3$), mixed m.p. 116° with cholesteryl acetate. *Anal.* Calcd. for $C_{29}H_{48}O_2$: C, 81.25; H, 11.29. Found: C, 81.34; H, 11.23.

The benzoate was crystallized from acetone, m.p. 145°, $[\alpha]^{22}D$ −14.2° (*c* 3.1 in $CHCl_3$), mixed m.p. 145° with cholesteryl benzoate.

The infrared spectrum and the modified Liebermann–Burchard reaction were identical to those of authentic cholesterol.

Acknowledgment.—We are indebted to CIBA Pharmaceutical Products, Inc., Summit, N. J., for a sample of methyl 3β-hydroxy-5-cholenate, and to the Upjohn Co., Kalamazoo, Mich., for a sample of ethyl 3β-hydroxy-5-thiolcholenate. Mr. A. P. Ronald of our Instrumentation section recorded the infrared spectra and obtained many of the analytical data.

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[CONTRIBUTION FROM STERLING-WINTHROP RESEARCH INSTITUTE]

D-Homosteroids. I. Derivatives of D-Homoetiocholan-3α-ol-11,17a-dione

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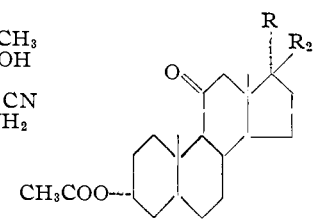
A number of 11-oxygenated-D-homosteroids have been synthesized from D-homoetiocholan-3α-ol-11,17a-dione. Several of these compounds possessed interesting endocrinological activity.

The detailed studies carried out by Ruzicka and Heusser[1] have shown a number of interesting correlations of endocrinological activity between homologous pairs of 11-desoxy-steroids and 11-desoxy-D-homosteroids. These and related observations stimulated our interest in the preparation of a series of 11-oxygenated-D-homosteroids, leading eventually to the synthesis of D-homopregn-4-ene-17aα,21-diol-3,11,20-trione 21-acetate (D-homocortisone acetate).[2] The present paper presents the synthesis of a series of derivatives of D-homoetiocholan-3α-ol-11,17a-dione.

D-Homoetiocholan-3α-ol-11,17a-dione 3-acetate has been prepared by Wendler, Taub and Slates[3] through application of the Tiffeneau ring-enlargement reaction[4] to the two epimeric 17-aminomethyletiocholane - 3α,17 - diol - 11 - ones. A similar procedure was followed in our work; however, we were able to isolate the two isomeric 17-cyanoetiocholane-3α,17-diol-11-one 3-acetates (Ia and Ib) and their corresponding 17-acetates IIa and IIb. We assigned the configuration at C_{17} in our compounds on the basis of the relative rates of acetylation of the 17-hydroxy group in the two

Ia, R = CN; R_2 = OH
IIa, R = CN; R_2 = $OCOCH_3$
IIIa, R = CH_2NH_2; R_2 = OH
Ib, R = OH; R_2 = CN
IIb, R = $OCOCH_3$; R_2 = CN
IIIb, R = OH, R_2 = CH_2NH_2



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