gives a fairly rapid positive test; $[\alpha]_D^{27°} = -7.85°$ (1.905% in absolute ethanol, $\alpha = -0.15°$).

Anal. Calcd. for $C_{30}H_{64}NO_7P$: P, 5.32; N, 2.41; glycerol, 15.8; choline, 20.62. Found:[17] P, 5.30; N, 2.42; glycerol, 15.5, choline, 20.6. N/P, 1.01; choline/P, 0.99.

Hydrolysis of plasmalogen. A 0.1-g. sample was heated to boiling on a steam bath with 10 ml. of ethanol and 5 ml. of concentrated hydrochloric acid. Fifteen ml. of water and an additional 2 ml. of hydrochloric acid were added and the heating continued for a few minutes. The mixture was left standing at room temperature overnight when a flocculent precipitate was formed. This was collected by centrifugation and dissolved in low-boiling petroleum ether. The acid solution remaining from the plasmalogen hydrolysis was extracted three times with 25 ml. each of low-boiling petroleum ether. All extracts were combined, dried over anhydrous sodium sulfate, and the solvent was evaporated *in vacuo*. The residue consisted of a mixture of aldehydes in form of a soft wax, 50 mg. (89%). Its infrared spectrum showed a strong carbonyl band at 5.84 microns.

A significant part of the aldehyde mixture rather rapidly polymerized to form a product quite difficultly soluble in ethanol. Recrystallization of the polymer from ethanol gave needle-shaped crystals, m.p. 74–77°. The polymers of hexadecanal and octadecanal melt near 73°.

The soluble, unpolymerized part of the aldehyde fraction was treated with Brady's reagent in the standard manner. The 2,4-dinitrophenylhydrazones were purified by chromatography over alumina in a benzene solution and by two recrystallizations from 80% ethanol; m.p. 90–105°. The dinitrophenylhydrazone of hexadecanal has been reported to melt at 105–107°, and its higher homologs somewhat higher.

Anal. Calcd. for $C_{24}H_{40}N_4O_4$: C, 64.29; H, 8.93. Found: C, 64.81; H, 8.82.

Unsaturation. The unsaturation of the unhydrolyzed plasmalogen and the 2,4-dinitrophenylhydrazones of the aldehyde mixture was determined according to Yasuda's method[22] with 0.02*N* pyridine sulfate dibromide as the brominating agent. The average of two closely agreeing determinations were as follows: plasmalogen: iodine number, 10; double bonds per mole, 0.24; aldehyde-2,4-dinitrophenylhydrazones: iodine number, 13; double bonds per mole, 0.23.

NEW HAVEN, CONN.

[CONTRIBUTION NO. 1490 FROM THE STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY]

Contributions to the Study of Marine Products. XLVII 22-Dehydrocholesterol[1]

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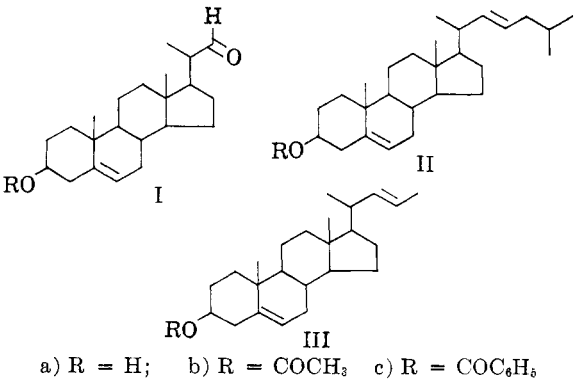
22-Dehydrocholesterol and 3β-hydroxy-$\Delta^{5,22}$-choladiene have been prepared by means of the Wittig reaction.

Among naturally occurring sterols with a methyl or ethyl group at the 24 position of their side chain those with a Δ^{22}-*trans*-oriented double bond are relatively common. The best known examples are ergosterol and stigmasterol. One might expect on biogenetic grounds that the corresponding derivative of cholesterol is also present in natural sterol mixtures. As yet, however, the natural occurrence of 22-dehydrocholesterol (II), while often suspected, has not been convincingly established. In connection with the synthesis of other sterols now in progress in this laboratory this unknown sterol has now been prepared, not only in order to obtain a reference sample to guide isolation studies but also to obtain starting material for the preparation of the ergosterol analog of cholesterol and its irradiation products.

The sterol was prepared from 3β-acetoxy-5-cholen-22-al (1) by means of the Wittig reaction which had previously been used with conspicuous success in the synthesis of 24-methylenecholesterol,[2,3] 24-[4,5] and 25-dehydrocholesterol.[3,5] Although the Wittig reaction is known to be nonstereospecific,[6] in the present synthesis the interaction between the aldehyde and the ylide generated



a) R = H; b) R = $COCH_3$ c) R = COC_6H_5

(1) This investigation was supported by a research grant, Nonr 253(00) from the Office of Naval Research.

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from triphenylphosphonium iodide[7] afforded mainly if not exclusively the *trans*-isomer (III). Its infrared spectrum showed the strong peak at 10.30μ which is associated with *trans*-oriented double bonds and which has been used to assign the *trans*-configuration to the Δ^{22}-double bond in the side chain of such naturally occurring sterols as ergosterol and stigmasterol.[8-10] In contrast, the spectrum showed no absorption in the 14-μ region which might have pointed to the presence of a *cis-oriented* double bond in the side chain.[11]

Upon treatment with bromine, the steryl acetate rapidly formed a nicely crystalline tetrabromide which afforded unchanged starting material upon debromination. Catalytic hydrogenation of the acetate gave cholestanyl acetate. This reaction proves that no significant inversion has taken place at C-20 either during the preparation of the aldehyde or its reaction with the Wittig reagent. As shown in Table I the melting points of 22-dehydrocholesterol and its derivatives are quite similar to those reported for β-sitosterol and derivatives. The melting points also fall within the range of those reported for many ill-defined sterol mixtures and their respective derivatives.[12] The possible occurrence of 22-dehydrocholesterol may therefore have easily been overlooked. As expected the 22-dehydrocholesterol is somewhat more levorotatory than cholesterol. The average difference between the molecular rotations of the two sterols and their respective derivatives is -70, a figure which is in good agreement with the Δ^{22}-value of 61 $\pm$ 20 reported for the C-24 methyl and ethyl homologs.[14]

TABLE I

COMPARISON OF MELTING POINTS

	22-Dehydrocholesterol	β-Sitosterol
Sterol	133.5–134	137[12]
Acetate	126–126.5	127[12]
Benzoate	146–147	146[13]

In another application of the Wittig reaction the previously unknown 3β-hydroxy$\Delta^{5,22}$-choladiene (III) has also been prepared. Reaction of the aldehyde (I) and the ethyl-ylide afforded a mixture of *cis* and *trans* isomers, and treatment with iodine was necessary to afford the pure trans isomer (III).

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EXPERIMENTAL

Triphenylisoamyl phosphonium iodide. A solution of 10 g. of triphenylphosphine in 50 ml. of anhydrous toluene was heated in a pressure flask with 11.3 g. of pure isoamyl iodide at 115° for 24 hr. The precipitate was filtered, recrystallized several times from aqueous ethanol, and finally thoroughly dried *in vacuo;* 11.92 g., m.p. 174–176° (rep.[7] 174°); λ_{max} 6.97 and 10.02μ.

22-Dehydrocholesteryl acetate (IIb). A solution of 3.16 g. of 3-β-acetoxybis-nor-5-cholen-22-al (1)[15] in 25 ml. of absolute ether was added to the ylide generated from 11.75 g. of triphenylisoamyl phosphonium iodide, 23.0 ml. of 1.11*N* butyllithium solution,[16] and 35 ml. of anhydrous ether in a pressure flask. The contents were stirred magnetically for about 1 hr. at room temperature, after which the flask was capped and heated to 65° for 15 hr. After cooling, the excess reagent was decomposed with moist ether, the solution evaporated to dryness, and the residue heated to 95° for one hr. with 20 ml. of acetic anhydride and 20 ml. of pyridine. The mixture was diluted with water and the crude acetate thus obtained was chromatographed in hexane on silicic acid–Celite (2:1). The acetate (1.5 g.) was eluted by hexane-benzene (1:1). A 1.23 g. sample of the acetate m.p. 126° was dissolved in 5 ml. of ether, and the solution mixed with 9 ml. of a 10% solution of bromine in glacial acetic acid. A precipitate formed readily (1.42 g.), a part of which was recrystallized from ethyl acetate. The 22-dehydrocholesteryl acetate tetrabromide thus obtained melted with decomposition at 188–189°; λ_{max} 5.76 and 8.08μ in KBr.

Anal. Calcd. for $C_{29}H_{46}Br_4O_2$: C, 46.67; H, 6.21. Found: C, 46.62; H, 6.54.

The acetate tetrabromide was suspended in 25 ml. of ether and 1 ml. of glacial acetic acid. Zinc dust (400 mg.) was added, and the mixture stirred for 10 min. The ether was then decanted from the zinc which was washed several times with ether. The combined extracts were washed with water and dried over anhydrous sodium carbonate. The solution was evaporated to dryness and the residue chromatographed in hexane on neutral alumina (Brockmann II). Elution with hexane-benzene (1:1) gave the acetate (IIb) which was recrystallized several times from methanol; 0.55 g.; m.p. 126–126.5°; $(\alpha)_D^{23}$ $-63.2°$; (C = 1.15 in $CHCl_3$); λ_{max} 5.76, 7.95, 10.30, and 12.52μ in KBr.

Anal. Calcd. for $C_{29}H_{46}O_2$: C, 81.63; H, 10.87. Found: C, 81.88; H, 10.80.

22-Dehydrocholesterol (IIa). The free sterol was obtained by hydrolysis of the acetate with potassium hydroxide in ethanol. It was recrystallized several times from methanol; m.p. 133.5–134°; $(\alpha)_D^{27}$ $-57.3°$; (C = 1.22 in $CHCl_3$); λ_{max} 2.97, 7.29, 7.34, 10.29, and 12.51μ in KBr.

Anal. Calcd. for $C_{27}H_{44}O$: C, 84.31; H, 11.53. Found: C, 84.21; H, 11.35.

Benzoylation of the sterol with benzoyl chloride in pyridine gave the benzoate (IIc). It was recrystallized from acetone; m.p. 146–147° with a play of colors which clears at 172°; $(\alpha)_D^{28}$ $-29.2°$; (C = 0.65 in $CHCl_3$) λ_{max} 5.82, 7.89, 8.00, 10.30, and 14.05μ in KBr.

Anal. Calcd. for $C_{34}H_{48}O_2$: C, 83.55; H, 9.90. Found: C, 83.66; H, 9.68.

Hydrogenation of 22-dehydrocholesteryl acetate. A 100-mg. sample of the acetate dissolved in 40 ml. of glacial acetic acid was shaken with 50 mg. of platinum oxide in a hydrogen atmosphere of 14-lb. pressure. After 2 hr. the catalyst was removed by filtration and the solvent evaporated under re-

(15) The authors are very much indebted to Dr. M. E. Herr, of the Upjohn Co., Kalamazoo, Mich., for the gift of a generous amount of stigmasterol and for explicit directions to convert it to the aldehyde. The aldehyde obtained showed m.p. 113–115°; $(\alpha)_D$ $-60.3°$; λ_{max} 3.68, 5.75, 5.77, 7.90, and 8.03 μ.

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duced pressure. After two recrystallizations from methanol, the residue gave cholestanyl acetate, m.p. 108–109°, which gave no depression of the melting point when mixed with an authentic sample. Its infrared spectrum was undistinguishable from that of the reference material.

3β-Acetoxy-5,22-choladiene (IIIb). A solution of 5.0 g. of triphenylphosphine in 50 ml. of anhydrous toluene was heated in a pressure flask with 10.2 g. of freshly distilled ethylbromide at 105° for 24 hr. The precipitate was filtered and dried; 6.35 g., m.p. 202–205°, reported 202–205°[17]; λ_{max} 6.90, 6.99, and 10.03μ.

A solution of 1.68 g. of 3β-acetoxybisnor-5-cholen-22-al (I)[15] in 25 ml. of anhydrous ether was added to the ylide generated from 3.35 g. of the triphenylethylphosphonium bromide and 7.0 ml. of 1.3*N* butyllithium in 25 ml. of absolute ether in a pressure flask. The mixture was then treated as described above, and the crude acetate was chromatographed on silicic acid: Celite and recrystallized from methanol; 0.74 g. m.p. 117–122°. The infrared spectrum showed a strong band at 10.27μ indicative of a *trans*-oriented double bond,[8-10] and a weak band at 10.02μ indicative of a *cis* double bond.[11]

The mixed acetates were refluxed with 100 ml. of benzene and 0.35 g. of iodine for 6 hr. The solution was cooled, washed with a solution of sodium thiosulfate, dried, and evaporated to dryness. The residue was dissolved in hexane, and the solution chromatographed over neutral alumina (Brockmann II). Hexane eluted a small fraction containing halogen. The main fraction was eluted with hexane-benzene (9:1). It was recrystallized three times from methanol; m.p. 127–129°, $(\alpha)_D^{26}$ −72.2° (C = 0.64 in $CHCl_3$); λ_{max} 5.76, 8.02, and 10.27 μ in KBr.

Anal. Calcd. for $C_{26}H_{40}O_2$: C, 81.20; H, 10.46. Found: C, 80.85; H, 10.56.

$\Delta^{5,22}$-Choladien-3β-ol (IIIa). Hydrolysis of the acetate with potassium hydroxide in ethanol gave the sterol which was recrystallized from methanol; m.p. 117–117.5°; $(\alpha)_D^{25}$ −65.8°; (C = 0.59 in $CHCl_3$); λ_{max} 2.96, 10.28, and 12.50μ in KBr.

Anal. Calcd. for $C_{24}H_{38}O$: C, 84.15: H, 11.18. Found: C, 83.94; H, 11.25.

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NEW HAVEN, CONN.

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Hydroxylated Codeine Derivatives

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The application of osmium tetroxide to the introduction of hydroxyl groups in the codeine and neopine series is described. Lithium aluminum hydride or sodium borohydride reduction of 8,14-dihydroxydihydrocodeinone leads to epimeric dihydroxydihydrocodeines. Analogous treatment of 14-hydroxycodeinone yields *true* 14-hydroxycodeine.

The observation that 3,4 dimethoxy-$\Delta^{6,7}$-13-ethylhexahydrophenanthrene (a degradation product of dihydrothebaine)[3] reacted smoothly with osmium tetroxide to yield, after hydrolysis of the osmate ester, the corresponding 6,7-glycol, led to the present study of the action of this reagent on the following morphine derivatives containing an alicyclic unsaturated center in ring C: desoxycodeine-C, codeine methyl ether, acetyl codeine, acetyl isocodeine, and acetyl neopine. It was of interest to pursue this investigation for several reasons: (1) the possibility of arriving at pharmacologically interesting substances was apparent; (2) conceivably this approach could improve upon earlier hydroxylation attempts (of codeine) where low yields were reported[4]; and (3) a route to the unknown 8,14-dihydroxylated neopine[5] (VI) might be afforded.

The considerable number of codeine and codeinone derivatives (containing one or more new hydroxyl groups in ring C) together with their ED_{50} values, relative to codeine, are shown in Table I. It will be noted that enhanced activity is elicited principally by those substances derived from 14-hydroxycodeinone VII. None of the presently reported derivatives showed significant analgesic activity; with the exception of 7-hydroxydihydroco-

TABLE I

Compound	ED_{50} (Mice)[a]
10-Hydroxycodeine	50.4
14-Hydroxycodeine	17.2
10-Hydroxydihydrocodeine	22.5
14-Hydroxymorphinone	42.2
14-Hydroxydihydromorphinone	0.17
14-Hydroxydihydromorphine	1.05
14-Hydroxydihydrocodeinone	1.4
8-Hydroxydihydrocodeinone	>150
14-Hydroxycodeinone	6.1
8,14-Dihydroxydihydromorphinone	6.3
8,14-Dihydroxydihydrocodeinone	16.7
7-Hydroxydihydrocodeine	None[b]
7,8-Dihydroxydihydrocodeine	>400
7,8-Dihydroxydihydrocodeine methyl ether	>100
8,14-Dihydroxydihydrocodeine	>200

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[a] We are indebted to Dr. Nathan B. Eddy, of this Laboratory, for permission to use these unpublished data. Codeine ED_{50} = 14.2. (ED_{50} is the dose which is effective for 50% of the test subjects.) [b] Fatal dose (LD_{50} = 50).