CHAPTER III

EXPERIMENTAL

I. Melting point

All melting points were determined on an electrothermal melting point apparatus and are uncorrected.

II. Dehydrating agent

Ether was dried over anhydrous sodium sulphate unless otherwise indicated.

III. Alumina for thin-layer chromatography

Merck's aluminum oxide G (after Stahl) was used for preparation of thin-layers of Alumina unless otherwise specified.

IV. Alumina for column chromatography

Merck's aluminum oxide, activity I and neutral, has been used for the preparation of alumiuna columns for chromatography.

V. Deactivated aluminum oxide for column chromatography

Merck's aluminum oxide, activity I and neutral was deactivated with water (5%) or 10% aqueous acid (5%) prior to the preparation of column chromatography.

VI. Silica-gel for thin-layer chromatography

Thin-layers of silica used in this work were prepared by using Merck's reagent silica gel H (after Stahl).

VII. Development of thin-layers

2', 7'-Dichlorofluorescein in methanol (5%) was used as spray reagent for revealing compounds on thin-layer of alumina or silica gel unless otherwise specified..

VIII. Infra-red spectra

The IR spectra have been determined in potassium bromide or nujol mulls on an Perkin-Elmer-421 or Perkin-Elmer-237 Grating Infra-red Spectrophotometer.

IX. Ultraviolet spectra

The UV spectra were recorded in 95% ethanol on a Unicam S.P. 800 Ultraviolet Spectrophotometer.

X. Nuclear magnetic resonance spectra

The NMR spectra were determined in trifluoroacetic acid with tetramethylsilane as internal standard on a Varian A 60 Spectrometer.

EXPERIMENTAL (Continued)

Preparation of 5α,6β-Dibromocholestan-3β-ol (43)

A solution of powdered sodium acetate (0.03 mole; 25 g.) and bromine (0.2 mole; 34 g.) in glacial acetic acid (600 ml.) was added to a stirred solution of commercial cholesterol (0.18 mole; 75 g.) in dry ether (1 l.) which was maintained at 20°, and the product was collected on a Buchner funnel. The cake was pressed down and washed with acetic acid until the filtrate was completely colorless. A second crop of satisfactory dibromide was obtained by adding water (400 ml.) to the combined filtrate and washings, collecting the precipitate, and washing it with acetic acid until it was colorless. The dibromide (97 g.; 83%) obtained in this way had m.p. 110.5 -115°.

Oxidation of 5α, 6β-Dibromocholestan-3β-ol (43)

The moist dibromide obtained from cholesterol (75 g.) was suspended in acetic acid (1l.) in a 3-l. round bottomed flask equipped with a stirrer and mounted over a bucket of ice and water that could be raised to immerse the flask. The suspension was stirred at room temperature (25-30°), and a solution, preheated to 90°, of sodium dichromate dihydrate (40 g.) in acetic acid (1l.) was added through a funnel. The mixture reached a temperature of 55-58° during the oxidation, and all the solid dissolved in 3-5 minutes. After a further 2 minutes, the ice bucket was raised

until the flask was immersed; stirring was then stopped, and the mixture was allowed to stand in the ice bath without disturbance for 10 minutes to allow the dibromoketone to separate in an easily filterable crystalline form. Stirring was again resumed and the temperature was raised to 25°. Water (400 ml.) was added and the contents of the flask cooled to 15°. The product was collected on a Buchner funnel, and the filter cake was drained until the flow of filtrate amounted to no more than 25 drops per minute. The solution was then released, and the walls of the funnel were washed down with methanol until the last drops of filtrate were completely colorless. The dibromoketone (80 g.), which consisted of shiny colorless plates, m.p. 71.5-73°dec., was dried to constant weight at room temperature in a dark cupboard. 5α, 6β-Dibromocholestan-3-one is reported (43) to have m.p. 73-75°dec.

Debromination of 5α, 6β-Dibromocholestan-3-one (43)

Moist 5α, 6β-dibromocholestan-3-one (80 g.) was suspended in ether (1l.) and acetic acid (12.5 ml.) in a 2l. round bottomed flask. The suspension was stirred mechanically, an ice bath was raised into position, and the temperature was brought to 15°. The ice bath was then lowered and fresh zinc dust (2.5 g.) was added. As the reaction was exothermic, the flask was again cooled and more zinc dust (17.5 g.) was added at such a rate that the temperature did not exceed 20°; this took about 5 minutes. The ice bath was then lowered, and the ethereal solution containing suspended zinc dust was stirred for a further 10 minutes. Stirring was continued

and pyridine (20 ml.) was added; this precipitated a wite zinc salt. The mixture was filtered through a Büchner funnel, and the filter cake was washed well with ether. The colorless filtrate was washed with water and then shaken thoroughly with 5% aqueous sodium bicarbonate solution until it was free from acetic acid as indicated by testing the ethereal solution with moist blue litmus paper. The solution was dried over magnesium sulphate and concentrated to a volume of about 500 ml.; methanol (250 ml.) was added, and the mixture was concentrated to a volume of approximately 600 ml. Crystallization was allowed to proceed at room temperature, then at 0-4°, and the large colorless prisms were collected by suction filtration. The cholest-5-en-3-one (46.75 g.) obtained had m.p. 117.5-119°. Fieser (43) has reported that the first crop m.p. 124-129° and the second crop m.p. 117-125°.

Methylation of Cholest-5-en-3-one (102)

Cholest-5-en-3-one (46.75 g.) in dry t-butanol (830 ml.) at 40° was treated with potassium (14.3 g.) in the same solvent (280 ml.). Methyl iodide (45.6 ml.) was added in one portion, and the mixture was refluxed for one hour. The resulting solution was evaporated to dryness in a rotatory evaporator and the solid was washed thoroughly with chloroform. The chloroform washings were concentrated to 150 ml., and 95% ethanol (50 ml.) was added. 4,4-dimethylcholest-5-en-3-one (28.45 g.,57%) separated from this solution as large colorless needles, which had m.p. 170-172°, on recrystallization from chloroform and 95% ethanol.

The IR spectrum of this compound (Fig.1, P. 81) shows absorption peaks at 1700 cm^{-1} (C=O stretching of cyclohexanone), 1648 cm^{-1} (C=C stretching vibrations), 800 and 850 cm^{-1} (CH stretching and bending vibrations of a trisubstituted alkene), 2940, 2860 and 1455 cm^{-1} (methylene stretching and bending vibrations), 1370 cm^{-1} (C-CH_3 banding), and 1360 and 1380 cm^{-1} (gem-dimethyl doublet).

Preparation of 4,4-Dimethylcholest-5-en-3-one Oxime

Hydroxylamine hydrochloride (3.71 g., 1.1 moles) was added to 4,4-dimethylcholest-5-en-3-one (20 g.) in pyridine (200 ml.) and the mixture heated under reflux for 3 hours. Water was added until no further precipitation occurred, and the solid was filtered off on a Buchner funnel, washed several times with water, and dried in an oven at 90-100°. Crystallization of the solid several times from chloroform and acetone gave 4,4-dimethylcholest-5-en-3-oue oxime (18.40 g., 88.5%) as colorless rods, m.p. 227-229°.

Found : C=81.40%; N=11.38%; H=3.31%

Calc. for $C_{29}H_{49}NO$: C=81.43%; H=11.55%; N=3.27%

Absorption peaks at 3300 cm^{-1}; and 850 and 785 cm^{-1} in the IR spectrum of this compound (Fig.2, P. 82) are due to OH stretching; and C-H stretching and bending vibrations of a trisubstituted alkene respectively.

Reduction of 4,4-Dimethylcholest-5-en-3-one Oxime with Sodium and Butanol-1

4,4-Dimethylcholest-5-en-3-one oxime (6.29 g.) in refluxing

butanol-1 (2 l.) was treated with sodium (59.3 g.), and the heating was continued for a further 3 hours. The excess of sodium was destroyed with ethanol, and the resulting solution was evaporated to dryness in a rotatory evaporator. Water (200 ml.) was added and the aqueous suspension was extracted several times with ether. The combined ether extracts were washed with water, and dried. Evaporation of the ether furnished an oil (7.68 g.) which was adsorbed on a column of aluminum oxide (800 g.), deactivated with water, from ether : light petroleum (1:9). Elution of the column was carried out as shown in Table XIV.

Reduction of 4,4-Dimethylcholest-5-en-3-one Oxime with Lithium Aluminum Hydride

4,4-Dimethylcholest-5-en-3-one oxime (8.97 g.) in ether (800 ml.), dried over lithium aluminum hydride, was added to a suspension of lithium aluminum hydride (8.5 g.) in dry ether (760 ml.) and the mixture was refluxed for 50 hours. The excess of lithium aluminum hydride was carefully destroyed with water at 0°, and the precipitated aluminum oxide was filtered off and continuously extracted with ether. The combined ether extracts were washed with water until the washings were neutral to litmus. After being dried, the ether extracts were evaporated to dryness to afford an oil (7.73 g.), which was adsorbed on a column of aluminum oxide (800 g.), deactivated with water, from ether:light petroleum (1:9). Elution of the column with mixture of ether and light petroleum afforded the products shown in Table XV.

Table XIV

Eluant	Number of 200 ml. fractions collected	Compound (As revealed by thin-layer chromatography)	Weight (g.)	M.P. (°C)
10% Ether in light petroleum	15	Oxime plus traces of 4,4-dimethylcholest-5-en-3α-ylamine, 4,4-dimethylcholest-5-en-3β-ylamine and two other spots	2.5085	-
20% Ether in light petroleum	4			
30% Ether in light petroleum	7			
40% Ether in light petroleum	2			
45% Ether in light petroleum	15	4,4-Dimethylcholest-5-en-3α-ylamine	1.8431	63-65°
45% Ether in light petroleum	4	Mixture of 4,4-dimethylcholest-5-en-3α-ylamine and 4,4-dimethylcholest-5-en-3β-ylamine	0.8097	-
45% Ether in light petroleum	5	4,4-Dimethylcholest-5-en-3β-ylamine	2.4296	90-93°

Table XV

Eluant	Number of 200 ml. fractions collected	Compound (As revealed by thin-layer chromatography)	Weight (g.)	M.P. (°C)
10% Ether in light petroleum	12	Oxime plus traces of 4,4-dimethylcholest-5-en-3α-ylamine, 4,4-dimethylcholest-5-en-3β-ylamine and two other spots	2.8687	-
20% Ether in light petroleum	8			
30% Ether in light petroleum	1			
30% Ether in light petroleum	4	4,4-Dimethylcholest-5-en-3α-ylamine	0.3247	63-65°
30% Ether in light petroleum	4	Mixture of 4,4-dimethylcholest-5-en-3α-ylamine and 4,4-dimethylcholest-5-en-3β-ylamine	0.6736	-
30% Ether in light petroleum	10	4,4-Dimethylcholest-5-en-3β-ylamine	3.0714	90-93°
40% Ether in light petroleum	10			

Thin-layer Chromatography of Reduction Product of 4,4-Dimethylcholest-5-en-3-one Oxime and 4,4-Dimethylcholestan-3-one Oxime

Thin-layers of adsorbent were prepared in the following manner; the thickness of each coating was not determined. A mixture of Merck's aluminum oxide G (20 g.) and water (40 ml.) was stirred until it became viscous, then it was applied to glass plates (20×20 cm.) using a Desaga spreader. After being dried at room temperature for one hour, the plates were activated at 125° for 30 minutes, cooled and stored in a desiccator until required for use. Two lines were described on each plate, one 2 cm. for one edge and parallel to it, which we shall refer to as the base line, and the other 15 cm. above this line and parallel to it, the upper line. The compounds (about 500 μg.) were dissolved in chloroform and applied as small spots to the base line of a plate at 4 cm. intervals. After the chloroform had evaporated, the plate was placed in a glass tank filled to a depth of 1 cm. with chloroform and the tank was covered with a glass cover. The eluting solvent immediately began to climb up the plate at a fast rate which gradually became slower as the distance between the solvent front and the base line increased. The time taken for the solvent front to reach the upper line was greater than 45 minutes. When the solvent front had reached the upper line, the plate was removed from the tank, the solvent allowed to evaporate, and the plate sprayed with a solution of 2', 7'-dichlorofluorescein in methanol (5%) to reveal the compounds.

Preparation of 4,4-Dimethylcholest-5-en-3α-ylamine Hydrochloride

Concentrated hydrochloric acid was added to the solution of 4,4-dimethylcholest-5-en-3α-ylamine in ether until it was acidic. The amine hydrochloride was filtered off on a Büchner funnel, washed with water until the washings were neutral to litmus and dried in an oven at 90-100°. Crystallization of 4,4-dimethylcholest-5-en-3α-ylamine hydrochloride several times from absolute ethanol furnished colorless plates, m.p. 255° dec., $[\alpha]_D$ = -51.43° (C=0.63; $CHCl_3$). The yields of 4,4-dimethylcholest-5-en-3α-ylamine hydrochloride obtained from the oxime by reduction with lithium aluminum hydride, and sodium and butanol-1 are shown in Table XVI.

Table XVI

Method of reduction	4,4-Dimethylcholest-5-en-3α-ylamine hydrochloride
Sodium and butanol-1	1.6904 g. (25.55%)
Lithium aluminum hydride	0.3028 g. (3.18%)

Found : C=77.16%; H=11.45%; N=3.06%; Cl=7.99%

Calc. for $C_{29}H_{52}NCl$: C=77.37%; H=11.64%; N=3.11%; Cl=7.88%

Strong peaks in the IR spectrum of this compound (Fig. 3, P.83) at 2920, 2840 and 1455 cm^{-1} are due to CH_2 stretching and bending vibrations; 1600 and 1500 cm^{-1} to C-NH_3^+ stretching and deformation vibrations; 1385 cm^{-1} to gem-dimethyl; 1365 cm^{-1} to C-CH_3 deformation vibrations; and 835 and 790 cm^{-1} to CH (of a trisubstituted alkene) stretching and bending vibrations.

The UV spectrum of the above compound had $\lambda_{max.}$ 208 mμ (ϵ = 1652)(Fig. 19, P. 99).

The NMR spectrum of this compound (Fig. 15, P. 95) shows signals at 9.21τ ($3H_s$; C_{18}), 9.09τ ($6H_d$, J=6 cps; C_{26}, C_{27}), 9.03τ ($3H_s$, J = 7 cps; C_{21}), 8.58τ ($3H_s$; C_{19}), 8.71 ($6H_s$; C_{30},C_{31}), 6.49τ ($1H_s$; C_3), and 4.19τ ($1H_s$; C_6).

Preparation of 4,4-Dimethylcholest-5-en-3β-ylamine Hydrochloride

4,4-Dimethylcholest-5-en-3β-ylamine hydrochloride was prepared from 4,4-dimethylcholest-5-en-3β-ylamine in a similar way to that used for preparation of the 3α-epimer. Crystallization of the hydrochloride several times from methanol affored needles, m.p. 265° dec., $[\alpha]_D$ = -52.64° (C=0.42; $CHCl_3$). Yields of this amine hydrochloride obtained on reduction of the oxime are shown in Table XVII.

Table XVII

Method of reduction	4,4-Dimethylcholest-5-en-3β-ylamine hydrochloride
Sodium and butanol-1	2.5122 g. (37.92%)
Lithium aluminum hydride	3.2044 g. (33.90%)

Found : C = 77.55%; H=11.74%; N=2.99%; Cl=7.71%

Calc. for $C_{29}H_{52}NCl$: C=77.35%; H=11.64%; N=3.11%;Cl=7.88%

This compound possessed IR absorption maxima at 2920,2840 and 1460 cm^{-1} (CH_2 stretching and bending vibration), 1590,1565 and 1510 cm^{-1} ($C-NH_3^+$ stretching and bending vibrations), 1390 and 1360 cm^{-1}

(gem-dimethyl), 1370 cm^{-1} ($C-CH_3$ deformation vibrations), and 835 and 790 cm^{-1} (stretching and bending vibrations of CH of a trisubstituted alkene)(Fig. 4, P.84).

A maxima in the UV absorption curve was observant at 207 mμ (ϵ = 1358)(Fig. 20, P.100).

Its NMR spectrum (Fig.16, P.96) shows signals at 9.21τ ($3H_s$; C_{18}), 9.08τ ($6H_d$, J = 6 cps; C_{26},C_{27}), 9.03τ ($3H_s$, J = 8 cps; C_{21}), 8.68τ ($6H_s$; C_{19}, C_{30}), 8.78τ ($3H_s$;C_{51}), 6.72τ ($1H_s$; C_3), and 4.16τ ($1H_s$; C_6).

Preparation of N-Salicylidene Derivative of 4,4-Dimethylcholest-5-en-3α-ylamine

Salicylaldehyde (0.06 ml.) was added to 4,4-dimethylcholest-5-en-3α-ylamine (50 mg.) in methanol (1 ml.), the mixture was shaken, diluted with water and extrated with ether. Excess of the aldehyde was removed from the ether solution by extraction with 2% sodium hydroxide solution, and the etheral solution was then washed with water until the washings were neutral to litmus, dried, and evaporated to dryness. Crystallization of the product from light petroleum and methanol furnished greenish yellow plates of N-salicylidene derivative of 4,4-dimethylcholest-5-en-3α-ylamine, m.p. 117-118° (36 mg., 59%).

Found : C=83.31%; H=10.52%; N=2.91%

Calc. for $C_{36}H_{55}NO$: C=83.49%; H=10.70%; N= 2.70%

Strong absorption in the spectrum of this compound (Fig.5, p. 85) is observed at 3400 cm^{-1} (OH stretching), 3040 cm^{-1}(phenyl

CH stretching), 2920, 2858 and 1450 cm^{-1} (stretching and bending vibrations of methylene), 1630 cm^{-1} (C=N stretching), 1575 and 1490 cm^{-1} (phenyl C=C stretching), 1378 cm^{-1} (C-CH_3 bending vibrations), 1365 cm^{-1} (gem-dimethyl), 1277 and 1190 cm^{-1} (C-O stretching vibrations of phenolic OH), 835 and 790 cm^{-1} (stretching and bending vibrations of CH of a trisubstituted alkene), and 735 cm^{-1} (four adjacent hydrogen of benzene ring).

Its circular dichroism curve as determined in ethanol (C=0.019) had $\Delta\epsilon_{318} = -18.36\times10^{-4}$; $\Gamma = 7$ mμ, and in dioxane (C=0.017) had $\Delta\epsilon_{318} = -38.02\times10^{-4}$; $\Gamma = 3$ and $\Delta\epsilon_{311} = -40.74\times10^{-4}$; $\Gamma = 10$ mμ (Fig.17, P. 97).

The UV spectrum of this compound (Fig.21, P.101) shows maximum absorption at 215, 256, shoulder 262, and 320 mμ (ϵ = 6787, 3555, 3232, and 1252 respectively).

Preparation of N-Salicylidene Derivative of 4,4-Dimethylcholest-5-en-3β-ylamine

This compound was prepared from 4,4-dimethylcholest-5-en-3β-ylamine in a similar way to that reported for the 3α-epimer. Crystallization several times from light petroleum and methanol furnished N-salicylidene derivative, as greenish-yellow fine needles, m.p. 164-165°. The yield was 42 mg. (69%).

Found : C=83.40%; H=10.75%; N=2.89%

Calc. for $C_{36}H_{55}NO$: C=83.49%; H=10.70%; N=2.70%

The IR spectrum of this compound (Fig.6, P.86) has absorption peaks at 3400 cm^{-1} (OH stretching); 3030 cm^{-1} (aromatic CH

stretching) 2920,2858 and 1450 cm^{-1} (CH_2 stretching and bending vibrations); 1625 cm^{-1} (C=N stretching); 1572 and 1490 cm^{-1} (phenyl C=C stretching); 1378 cm^{-1} ($C-CH_3$ deformation vibrations); 1364 cm^{-1} (gem-dimethyl); 1275, 1250 and 1200 cm^{-1} (C-O stretching); 835 and 790 cm^{-1} (stretching and bending vibrations of CH of a trisubstituted alkene); and 738 cm^{-1} (four adjacent hydrogen on benzene ring).

The circular dichroism curve of this compound as determined in ethanol (C=0.014) had $\Delta\epsilon_{213} = +21.25\times10^{-4}$; Γ =14 mμ, $\Delta\epsilon_{318} = +19.62\times10^{-4}$; Γ =10 mμ, and in dioxane (C=0.012) had $\Delta\epsilon_{323} = +29.36\times10^{-4}$; Γ =6 mμ, $\Delta\epsilon_{310} = +35.23\times10^{-4}$; Γ =20 mμ (Fig. 18, P. 98).

Its UV spectrum (Fig. 22, P. 102) had $\lambda_{max.}$ 215, 256, shoulder 262, and 320 mμ (ϵ =5808, 3769, 3223, and 1308 respectively).

Reduction of 4,4-Dimethylcholest-5-en-3-one (102)

To a stirred suspension of lithium aluminum hydride (1.52 g.) in ether (144 ml.), dried over lithium aluminum hydride, was added 4,4-dimethylcholest-5-en-3-one (8 g.) in dry ether (700 ml.), and the mixture was worked up as described for the reduction of 4,4-dimethylcholest-5-en-3-one oxime by lithium aluminum hydride. Crystallization of the product from ether and methanol afforded 4,4-dimethylcholest-5-en-3β-ol as needles, m.p. 144-146°. Woodward et al. (102) have reported m.p. 144-146°.

This alcohol showed strong absorption at 3340 cm^{-1} (OH stretching), 795 and 840 cm^{-1} (CH stretching and bending of a trisubstituted alkene) in the infra-red region (Fig. 7, P. 87).

Reaction of 4,4-dimethylcholest-5-en-3β-ol with Phosphorus Oxychloride

4,4-Dimethylcholest-5-en-3β-ol (500 mg.) in hexane (50 ml.) was shaken with phosphorus oxychloride (500 mg.) for half an hour. After pouring into water (50 ml.), the mixture was extracted with ether. Three water washes rendered the ethereal layer neutral. After drying, evaporation yielded an oily residue (400 mg.) which was applied to thin-layers of silica gel impregnated with 10% silver nitrate solution. The chromatogram was developed with hexane, sprayed with concentrated sulphuric acid, and heated in an oven at 120-130°. The R_F values of the spots obtained indicated that the reaction products were hydrocarbons.

Reaction of Lanosterol with Thionyl Chloride

Commercial lanosterol (500 mg.) in pyridine (1 ml.) was treated with thionyl chloride (0.15 ml.). The reaction mixture was refluxed for one hour, cooled, poured into water and then extracted with ether. The ethereal solution after being dried, showed no difference from lanosterol on thin-layer chromatography on silica gel.

Reaction of Lanosteryl Methyl Ether with Hydrobromic acid

Hydrobromic acid (2 ml.) was added to a solution of lanosteryl methyl ether (100 mg.) in benzene, and the mixture was refluxed for 2 hours. The major product of this reaction had the same R_F value as 3-isopropylidene-A-nor-lanosta-8, 24-diene when

chromatographed on thin-layers of silica gel using chloroform : light petroleum (1:2) as developing solvent.

Tosylation of 4,4-Dimethylcholest-5-en-3β-ol (48)

4,4-Dimethylcholest-5-en-3β-ol (5 g.) in pyridine (40 ml.) was treated with toluene-p-sulphonyl chloride (5 g.). After standing at room temperature for one day, ice was added and the mixture set aside for one hour. The product was extracted with ether several times. The ether extracts were washed with cold dilute hydrochloric acid solution (2 N.), water, sodium bicarbonate solution (2 N.), and to neutrality with water, dried and evaporated in vacuo. The solid residue (7 g.) gave, after crystallization from pentane, 4,4-dimethylcholest-5-en-3β-yl toluene-p-sulphonate, as needles, m.p. 92-95° dec. This compound have been reported (48) to have m.p. 92-95° dec.

Tosylation of Lanosterol (48)

Lanosta-8, 24-dienyl toluene-p-sulphonate was prepared from lanosterol in a similar way to that described for 4,4-dimethylcholest-5-en-3β-yl toluene-p-sulphonate. It crystallized from pentane at 0° as needles, m.p. 116-122° (85%). Haddad and Summers (48) have reported it has m.p. 119-124°.

Reaction of Lanosteryl Tosylate with Sodium Cyanide

Sodium cyanide (1.5 mole, 30 mg.) was added to a solution of lanosteryl tosylate (200 mg.) in dimethylsulphoxide (15 ml.) and the mixture was heated under reflux for 3 hours. The mixture

was set aside at room temperature, diluted with water, and extracted with ether. The ether layer was washed with water, dried, and evaporated to dryness. This gave an oil (150 mg.) which was chromatographed on a column of alumina (10 g.). Elution with light petroleum gave an oil (100 mg.) which had a R_F value identical to 3-isopropylidene-A-nor-lanosta-8, 24-diene on thin-layer chromatography of silica gel.

Reaction of Lanosteryl Tosylate with Diethylsodiomalonate

To a solution of diethylsodiomalonate, prepared by refluxing toluene (2 ml.), sodium (50 mg.) and diethyl malonate (0.5 ml.) until all the sodium had dissolved, was added a solution of lanosteryl tosylate (500 mg.) in toluene (2 ml.). Refluxing was continued for 10 hours; the solution was cooled, the precipitated sodium toluene-p-sulphonate was filtered off, washed with toluene, and the combined toluene extracts were evaporated to dryness. The oil obtained was dissolved in ether and washed with water, and the solution was dried and evaporated. The oil was applied as a spot to a thin-layer of silica gel, and the chromatogram was developed with chloroform : light petroleum (1:2). In this way the major component of the reaction was shown to be 3-isopropylidene-A-nor-lanosta-8, 24-diene.

Reaction of 4,4-Dimethylcholest-5-en-3-one with Dimethylsulphonium Methylide

A solution of methylsulphinyl carbanion was prepared (29). Sodium hydride (240 mg.) was placed in three-necked round

bottomed flask (250 ml.) and washed several times with light petroleum by swirling and decanting the liquid portion in order to remove the mineral oil. The flask was immediately fitted with a mechanical stirrer, a reflux condenser, and a rubber stopper. A three way stopcock connected to the top of the reflux condenser was connected to a water aspirator and a source of dry nitrogen. The system was evacuated until light petroleum was completely removed from the sodium hydride. The system was placed under nitrogen by evacuating and filling with nitrogen several times. The aspirator hose was removed and this arm of the stopcock was then connected to mercury-sealed U-tube to which the system was opened. Dimethylsulphoxide (5 ml.), distilled over calcium hydride, b.p. 64° (4 m.m.), was introduced slowly by hypodermic syringe. The stirrer was started, and hydrogen evolved, which ceased after 20 minutes and afforded a milky-white reaction mixture. The solution was cooled to room temperature, diluted with tetrahydrofuran (5 ml.), dried over lithium aluminum hydride, to prevent freezing, and then cooled in salt-ice bath. With stirring, a solution of trimethylsulphonium iodide (1.1 g.)(29) in dimethylsulphoxide (4 ml.) was added over a period of about 3 minutes; the temperature was controlled below 5°. The mixture was stirred for one minute longer after the addition of the salt was complete. The ketone (1.5 g.) in tetrahydrofuran (7.5 ml.), distilled over lithium aluminum hydride, was added by hypodermic syringe. Stirring was continued at salt-ice temperature for several

minutes and then for 60 minutes with the bath removed. The reaction mixture was diluted with water (100 ml.), and the product was extracted with pentane, washed with water, and dried. Thin-layer chromatography on silica gel revealed the presence of only starting material.

Hydrogenation of 4,4-Dimethylcholest-5-en-3-one (25)

4,4-Dimethylcholest-5-en-3-one (1 g.) and Adam's catalyst (1 g.) in glacial acetic acid (20 ml.) containing one drop of 60% perchloric acid at 50-60° and atmospheric pressure were stirred under an atmosphere of hydrogen for 7 hours. The solution was filtered and evaporated to dryness to afford a viscous mass (1 g.). The total hydrogenation product was hydrolysed with 3% methanolic potassium hydroxide solution (20 ml.) under reflux on a water bath for two hours, water (50 ml.) was added and the aqueous suspension was extracted several times with ether. The combined ether extracts were washed with water until the wash solution was neutral, and dried. Evaporation of the ether solution furnished a non-crystalline product (900 mg.) which was chromatographed on aluminum oxide (100 g.), deactivated with 10% aqueous acetic acid. Elution of the column with light petroleum gave the fractions shown in Table XVIII.

4,4-Dimethylcholestan-3β-ol crystallized from methylene dichloride and methanol as needles, m.p. 156-158°. Chaudhry et al. (25) have reported has m.p. 157-158°.

Table XVIII

Eluant	Number of 200 ml. fractions collected	Compound (as revealed by thin-layer chromatography)	Weight (g.)	M.P. (°C)
Light petroleum	10	Unidentify liquid smear	0.2941	-
Light petroleum	2	Mixture of unidentify liquid smear and 4,4-dimethyl-cholestan-3β-ol	0.1013	-
Light petroleum	5	4,4-Dimethylcholestan-3β-ol	0.7094	155-158°

Oxidation of 4,4-Dimethylcholestan-3β-ol (25)

A cold solution of chromic acid (8 N.)(21), prepared by dissolving sodium dichromate dihydrate (26.7 g.) in concentrated sulphuric acid (23 ml.) and water (40 ml.), and making up the solution to 1 litre with water, was added dropwise from a micro-burette to 4,4-dimethylcholestan-3β-ol (700 mg.) in acetone (distilled over potassium permanganate) until a orange brown colour persisted. The precipitated chromic sulphate was destroyed by addition of water (50 ml.), and the resulting solution was extracted with ether. The ethereal solution, which was washed successively with 5% sodium bicarbonate solution and water, gave a solid (700 mg.), m.p. 98-100° on evaporation. Chromatography of this compound on aluminum oxide with light petroleum is eluant afforded 4,4-dimethylcholestan-3-one (600 mg.) which crystallized from methylene dichloride and methanol as needles (600 mg.), m.p. 102-103°. Chaudhry et al. (25) have reported it has m.p. 103-104°.

Maximum absorption in the IR spectrum of this compound (Fig. 8, P.88) at 1710 cm^{-1} is due to C=O stretching of cyclohexanone.

The UV spectrum of this compound is transparant above 200 mμ.

Preparation of 4,4-Dimethylcholestan-3-one Oxime

The oxime was prepared in a similar manner to that described for 4,4-dimethylcholest-5-en-3-one oxime. Crystallization of 4,4-dimethylcholestan-3-one oxime from acetone and methanol afforded needles, m.p. 208-209°, as quantitative yield. Djerassi et al. (76) have reported that 4,4-dimethylcholestan-3-one oxime m.p. 205-206°.

This compound shows IR absorption peak at 3260 cm^{-1} (NH stretching)(Fig.9, P. 89).

Reduction of 4,4-Dimethylcholestan-3-one Oxime

Reduction of 4,4-dimethylcholestan-3-one oxime (600 mg.) with sodium (7 g.) and butanol-1 (200 ml.) was carried on in a similar way to that described for 4,4-dimethylcholest-5-en-3-one oxime. The reaction product (670 mg.) in light petroleum was adsorbed on to a column of aluminum oxide (70 g.), deactivated with water, and the column was eluted with mixtures of ether : light petroleum to give the fractions listed in Table XIX.

Preparation of 4,4-Dimethylcholestan-3β-ylamine Hydrochloride

4,4-Dimethylcholestan-3β-ylamine hydrochloride (250 mg.; 58%) was prepared by the procedure described for the 4,4-dimethyl-

Table XIX

Eluant	Number of 200 ml. fractions collected	Compound (as revealed by thin-layer chromatography)	Weight (g.)	M.P. (°C)
Light petroleum	3	Oxime plus traces of two epimeric 4,4-dimethylcholestan-3-ylamine and two other spots	0.2002	-
10% Ether in light petroleum	4			
20% Ether in light petroleum	4			
20% Ether in light petroleum	2	Mixture of two epimeric 4,4-dimethylcholestan-3-ylamine	0.1011	-
30% Ether in light petroleum	1			
30% Ether in light petroleum	2	4,4-Dimethylcholestan-3β-ylamine	0.4024	114-116°
40% Ether in light petroleum	3			

cholest-5-en-3α-ylamine hydrochloride. Crystallization of the hydrochloride from methanol and acetone furnished needles, m.p. 285°dec. $[\alpha]_D$ = +8.2° (C=0.624; $CHCl_3$).

Found : C=76.94%; H=11.99%; N=3.11% Cl=7.95%

Calc. for $C_{29}H_{54}NCl$: C=77.02%; H=12.04%; H=3.10% Cl=7.84%

The IR spectrum of this amine hydrochloride (Fig. 10, P. 90) exhibits absorption peaks at 2940, 2870, 1475 and 1450 cm^{-1} (stretching and bending vibration of methylene), 1620, 1580, and 1520 cm^{-1} ($C-NH_3^+$ stretching and bending vibrations), 1385 cm^{-1} (methyl bending), and 1375 cm^{-1} (gem-dimethyl).

Hydrogenation of 4,4-Dimethylcholest-5-en-3β-ylamine Hydrochloride

4,4-Dimethylcholest-5-en-3β-ylamine hydrochloride (200 mg.) and 10% palladium charcoal catalyst (1 g.) in absolute ethanol (15 ml.) at atmospheric pressure and 80-82° were stirred under an atmosphere of hydrogen for 14 hours. The palladium charcoal was filtered off and the solution, on evaporation, gave a crystalline product (100 mg.). Crystallization of the product from 95% ethanol and acetone gave 4,4-dimethylcholestan-3β-ylamine hydrochloride, as needles, m.p. 284° dec., $[\alpha]_D$ = +5° (C=0.44; $CHCl_3$).

Found : C=77.09%; H=11.88%; N=3.13%; Cl=7.90%

Calc. for $C_{29}H_{54}NCl$: C=77.02%; H=12.04%; N=3.10% Cl=7.84%

The IR spectrum of this compound is shown in (Fig. 11, P. 91). The absorption peaks at 1620, 1580 and 1520 cm^{-1} are due to $C-NH_3^+$ stretching and deformation vibrations.

Reaction of 4,4-Dimethylcholest-5-en-3α-ylamine Hydrochloride with Hydrogen in the Presence of Raney Nickel Catalyst (W_6)

4,4-Dimethylcholest-5-en-3α-ylamine hydrochloride (190 mg.) and Raney nickel catalyst (W_6, 1 g.)(16) in absolute ethanol (13 ml.) at atmospheric pressure and 80-82° were stirred for 34 hours. The hydrogenation mixture was filtered to remove Raney nickel. The solution after being concentrated to 2 ml. gave prism shiny crystals, m.p. 64-66° when cooled at 15°. Crystallization of this solid from 95% ethanol afforded shiny prisms (75 mg.), m.p. 64-66° $\lambda_{max.}$ 208 mμ (ε =1764).

Acetylation of 4,4-Dimethylcholest-5-en-3β-ol

Acetic anhydride (1 ml.) was added to 4,4-dimethylcholest-5-en-3β-ol (400 mg.) in pyridine (4 ml.) and the mixture was refluxed for 2 hours. After cooling, water (10 ml.) was added and the acetylated material was filtered off and washed with water until the washings were free from pyridine. After being dried in a desiccator, the acetylated compound (450 mg.) was crystallized from ether and methanol to give 4,4-dimethylcholest-5-en-3β-ylacetate, as needles, m.p. 135-136.5°. Woodward et al. (102) have reported that 4,4-dimethylcholest-5-en-3β-ol has m.p. 136-137°.

Its IR analysis (Fig.13, P.93) showed absorption at 1740 cm^{-1} (C=O stretching of acetate) 1240 cm^{-1} (C-O-C stretching of acetate), 800 and 830 cm^{-1} (CH stretching and bending of a trisubstituted alkene).

Isomerization of 4,4-Dimethylcholest-5-en-3β-ylacetate

A solution of 4,4-dimethylcholest-5-en-3β-ylacetate (200 mg.) in benzene (2 ml.) was treated with acetic acid (10 ml.) and sulphuric acid (0.7 ml., d. 1.84). The colorless solution turned yellow within 15 minutes, became brown within one day and finally turned dark brown. The mixture was allowed to stand at room temperature for an additional 4 days, then poured into water (50 ml.) and the organic material extracted with ether. The ethereal solution was washed with 5% sodium bicarbonate solution and with water; it was dried and the solvent removed by evaporation. A non-crystalline residue was obtained which was chromatographed on alumina (5 g.) using light petroleum (500 ml.) as eluant. This gave a mixture of the expected isomerized material as revealed by thin-layer chromatography on alumina, using ether : light petroleum (1:9) as eluting solvent. The R_F values for the products were 0.93 and 0.97, and for the starting material 0.47.

This mixture exhibited absorption peaks in the IR spectrum (Fig.14, P. 94) at 1732 cm^{-1} (C=O stretching of acetate), 1285 and 1260 cm^{-1} (C-O-C stretching of acetate).