CHAPTER II

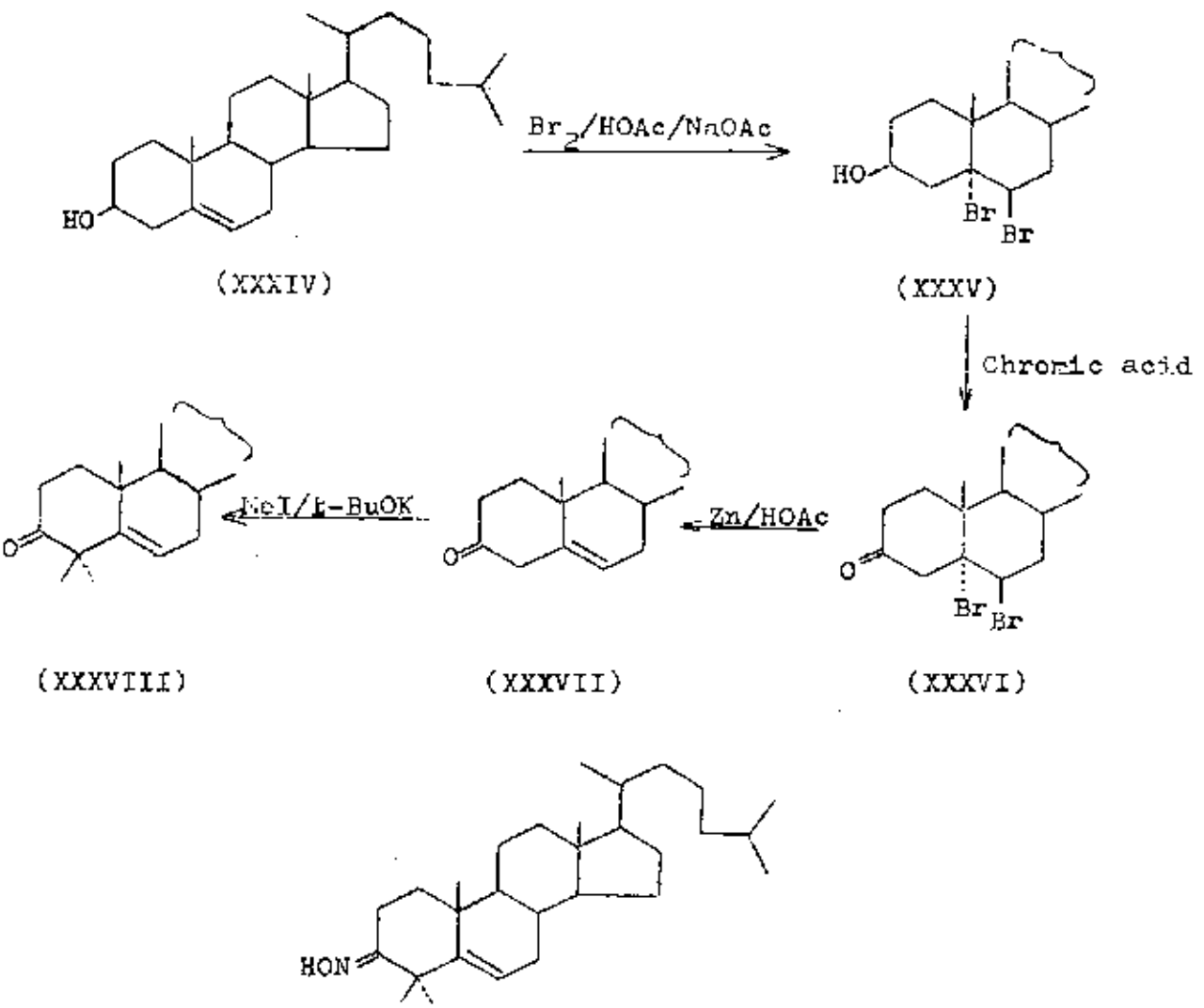
DISCUSSION AND INTERPRETATION

4,4-Dimethylcholest-5-en-3-one (XXXVIII) was synthesized as shown in Scheme I. Cholesterol (XXXIV) in glacial acetic acid was brominated in the presence of sodium acetate to 5α,6β-dibromocholestan-3β-ol (XXXV), m.p. 110.5-115°, which was immediately oxidised by chromic acid to the dibromoketone (XXXVI). Debrominated with zinc dust and acetic acid gave cholest-5-en-3-one (XXXVII), as large colorless prisms, m.p. 117.5-119°. Methylation of the cholest-5-en-3-one (XXXVII) was accomplished by means of the method of Woodward et al. (102) using methyl iodide and potassium t-butoxide; this furnished 4,4-dimethylcholest-5-en-3-one (XXXVIII; 57%), m.p. 170-172°. The IR spectrum of the latter compound showed strong 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 vibration of a trisubstituted alkene), 2940,2860 and 1455 cm^{-1} (methylene stretching and bending vibrations), 1370 cm^{-1} ($C-CH_3$ bending), and 1360 and 1380 cm^{-1} (gem-dimethyl doublet) which is consistant with the structure (XXXVIII).

4,4-Dimethylcholest-5-en-3-one (XXXVIII) was converted to the oxime (XXXIX) by refluxing with hydroxylamine hydrochloride (1.1 mole) and pyridine for 3 hours. Crystallization of the product from a mixture of chloroform and acetone gave 4,4-dimethylcholest-5-en-3-one oxime, $C_{29}H_{49}NO$, as colorless rods, m.p. 227-229° (XXXIX). A broad absorption peak at 3300 cm^{-1} (OH stretching),

and strong absorption peaks at 850 and 785 cm^{-1} (CH stretching and bending vibrations of a trisubstituted alkene) in the IR spectrum of this compound suggested that it was the unsaturated oxime (XXXIX).

Scheme I



(XXXIV) (XXXV) (XXXVIII) (XXXVII) (XXXVI) (XXXIX)

Reduction of the 4,4-dimethylcholest-5-en-3-one oxime (XXXIX) by refluxing with sodium and butanol-1 or with lithium aluminum hydride in dry ether gave a mixture. Thin-layer chromatography of this mixture using chloroform as a solvent and 2′,7′-

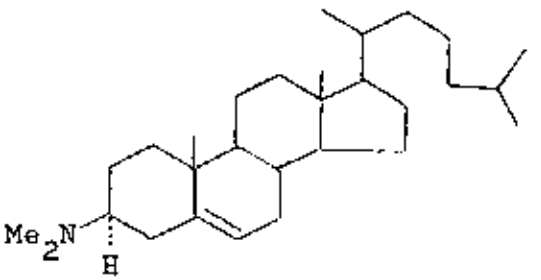
dichlorofluorescein as a detector showed the presence of five spots, one of which corresponded to the oxime (see Table X). Two of the four remaining spots were more intense than the other two spots, and these were attributed to the two 3-amino-4,4-dimethylcholest-5-enes. The structures of the two remaining spots were not determined.

Table X

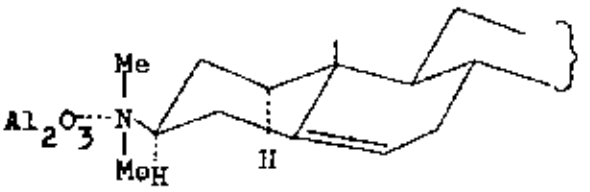
Compound	R_F value
First spot; 4,4-dimethylcholest-5-en-3-one oxime	0.93
Second spot; undetermined compound	0.80
Third spot; undetermined compound	0.70
Fourth spot; 4,4-dimethylcholest-5-en-3α-ylamine	0.20
Fifth spot; 4,4-dimethylcholest-5-en-3β-ylamine	0.13

Lábler and Cerný (66) have found that equatorial steroidal bases of the type (XL) have lower R_F values than the corresponding axial epimers. This has been attributed to the more ready accessibility of the dimethylamino group in the equatorial amines for adsorption; in the axial epimers steric compression prevents such strong adsorption. Thus the strength of intermolecular bridges between the dimethylamino group and alumina is greater in the equatorial amine (XLI) than in the corresponding axial epimers (XLII).

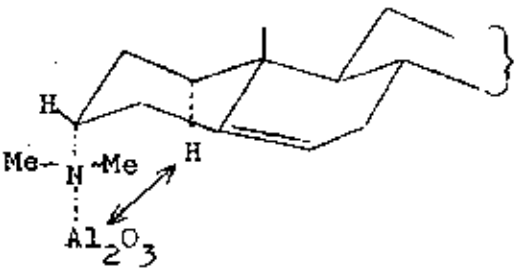
In the case of the 3-amino-4,4-dimethylcholest-5-enes we have concluded, on the basis of Lábler and Cerný's evidence, that



(XL)



(XLI)



(XLII)

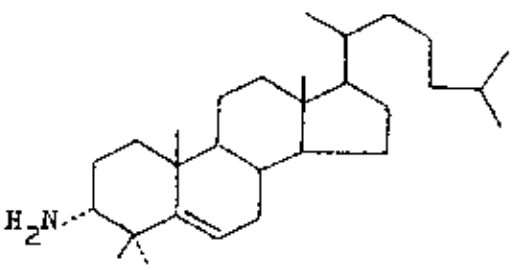
the amine of R_f 0.13 would have an equatorial configuration and that of R_f 0.20 an axial configuration provided that no conformational change in ring A has taken place on the alumina surface.

Isolation of the axial and equatorial amine was accomplished by chromatography of the mixture on a column of aluminum oxide, deactivated with water, using mixtures of ether and light petroleum as eluting solvents; the fractions which were found to be identical by thin-layer chromatography were combined. The axial amine (XLIII), m.p 63-65°, was eluted by ether: light petroleum (1:2) and the equatorial epimer (XLIV), m.p. 90-93°by ether: light petroleum (1:1). The quantities of the two amines obtained from the reduction of 4,4-dimethylcholest-5-en-3-one oxime by lithium aluminum hydride,

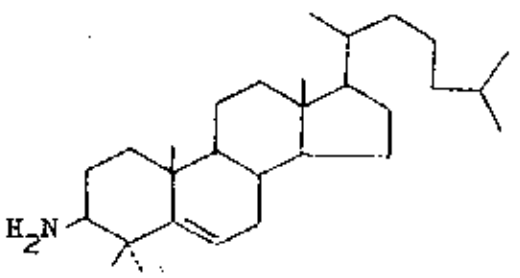
and sodium and butanol-1 are give in Table XI.

Table XI

Compound	Method	
	Sodium and butanol-1	Lithium aluminum hydride
4,4-Dimethylcholest-5-en-3α-ylamine (XLIII)	1.8431 g. (30.26%)	0.3247 g. (3.75%)
4,4-Dimethylcholest-5-en-3β-ylamine (XLIV)	2.4296 g. (40%)	3.0714 g. (35.66%)



(XLIII)



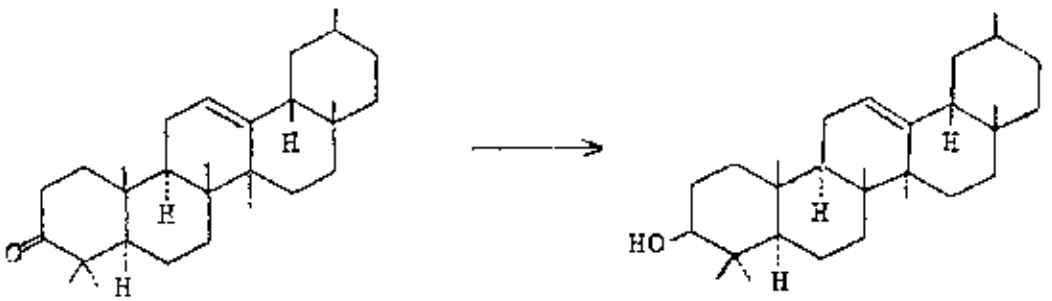
(XLIV)

Bird and Cookson (17) have found that reduction of number of oximinocholestanes gave in each case a mixture of the two epimeric amines; the equatorial amine is the major product formed in sodium and alcohol reductions, and the axial amine in lithium aluminum hydride reductions.

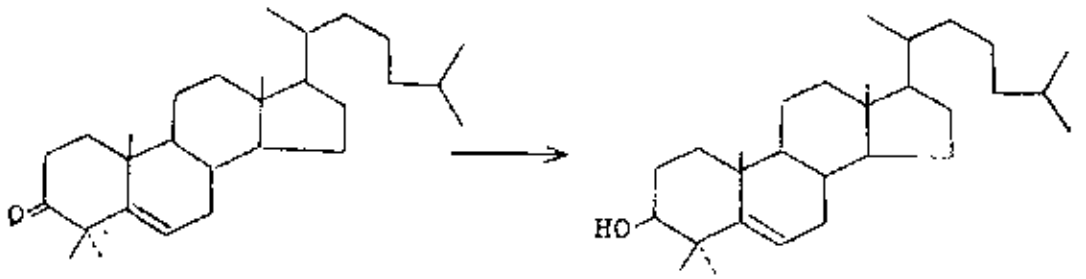
A similar observation is found in the reduction of steroidal ketones. Hindered carbonyl groups afford axial alcohols on metal hydride reduction, and partially hindered carbonyl groups furnish a mixture of axial and equatorial alcohols (6,7,8,10). The formation of axial alcohols from hinderad carbonyls on metal hydride reduction

has been referred to as steric approach control. An example is found the reduction of 11-keto-steroids (XLV) which afford exclusively the axial 11-β alcohols (XLVI)(64). However, in the case of β-amyrenone (XLVII) and 4,4-dimethylcholest-5-en-3-one (XLIX), metal hydride reduction furnishes mainly β-amyrin (XLVIII) (95) and 4,4-dimethylcholest-5-en-3β-ol (L)(102) respectively. Similarly, the ketone (LI) obtained from cafestol is also reduced to the equatorial alcohol (LII)(37). However, lithium aluminum hydride reduction of friedelin (LIII) yields the axial alcohol epifriedelanol (LIV)(30). A satisfactory explanation of the steric course of these reductions has not been given.

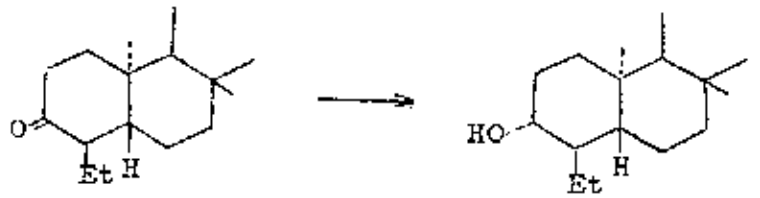
(XLV) (XLVI)



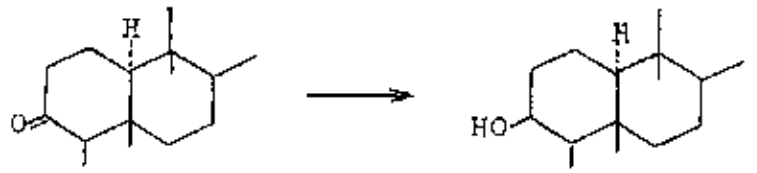
(XLVII) (XLVIII)



(XLIX) (L)



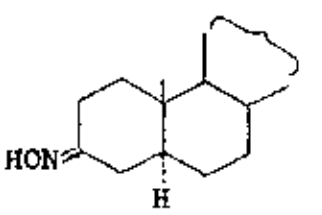
(LI) (LII)



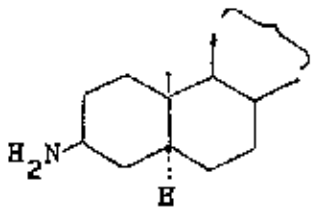
(LIII) (LIV)

Sodium and alcohol reduction of β-amyrenone (XLVII) yields β-amyrin (XLVIII) in which the C-3 hydroxyl group has an equatorial conformation (6). In the case of the C-11 carbonyl group in the steroid and triterpene series, the 11α-hydroxy compound which has an equatorial conformation is formed exclusively (53). It has been observed that in all cases sodium and alcohol reduction of carbonyl compounds yields the thermodynamically more stable epimer; this reaction is said to proceed with thermodynamic control (55). Thus in the case of the sodium and alcohol reduction of steroidal and terpenoidal ketenes, we would expect the equatorial alcohol to be formed preferentially, and this is found to be the case.

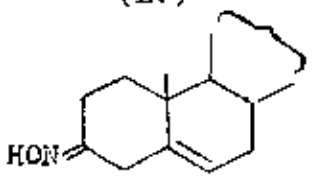
Shoppee et al.(89) have shown that cholestan-3-one oxime (LV) on reduction with sodium and ethanol gives cholestan-3β-ylamine (LVI), but cholestan-3-one oxime (LV) or cholest-5-en-3-one oxime (LVII) on reduction with lithium aluminum hydride yields both epimeric bases. Evans and co-workers (41) as well as Labler and co-workers (61) have also reported that cholestan-3-ketoxime (LV) yields 3α-aminocholestane (LVIII) with lithium aluminum hydride, but the 3β-epimer (LVI) is obtained by reduction with sodium and alcohol. However, steroid 11-ketoximes can be reduced either to the axial amine with lithium aluminum hydride or to the equatorial one with sodium and alcohol (52). It is therefore evident that the oximes behave as if they were hindered toward lithium aluminum hydride.



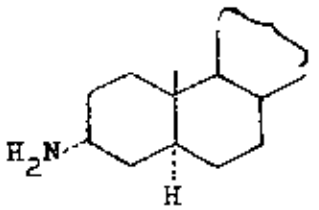
(LV)



(LVI)



(LVII)



(LVIII)

It can be seen from Table XI that the reduction of 4,4-dimethylcholest-5-en-3-one oxime with lithium aluminum hydride or sodium and butanol-1 give 3β-amino-4,4-dimethylcholest-5-ene as

the major product, and that in the metal hydride reduction the ratio of 3β-:3α-amines is considerable greater than in the corresponding sodium and butanol-1 reduction. The product of the lithium aluminum hydride reduction is different from the amine which would have been predicted from the work on the metal hydride reductions of steroidal oximes. In addition, the high yield of the 3β-amine suggests that a stereochemical factor is responsible for the course of the reduction.

Gorodetsky et al. (47) have determined the conformation of ring A in Δ^5-3-keto steroids by means of ultraviolet absorption spectroscopy, circular dichroism and optical rotatory dispersion data, and nuclear magnetic resonance measurements. They have shown that ring A has a boat conformation, and they attribute this to a 1,3-diaxial interaction of the 4β-and 10-methyl group in the chair form (LIX) which is relieved when ring A adopts the boat form (LX).

(LIX)

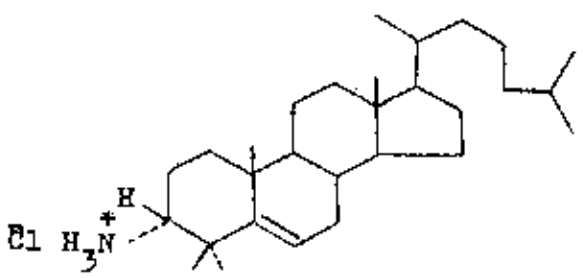
(LX)

Dauben and his co-workers (34) have accounted for the high yield of axial alcohols in metal hydride reductions of hindered ketones by suggesting that the bulky hydride ion will attack the carbonyl carbon atom from the least hindered equatorial face. If one assumes that 4,4-dimethylcholest-5-en-3-one oxime will possess,

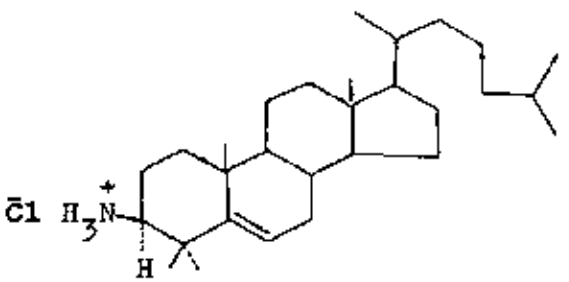
like the parent ketone, a boat conformation, then it is possible to account for the high yield of equatorial amine in the lithium aluminum hydride reduction of this oxime by means of this theory. The alumino hydride ion will attack C_3 from the more readily accessible α-face as shown in (LX), thus leading to the equatorial amine.

Since there was still an element of uncertainty in the configurations of the two amines, we decided to established their configurations by means of nuclear magnetic resonance spectroscopy and circular dichroism.

The two epimeric amines (XLIII and XLIV) were converted into the corresponding hydrochlorides (LXI and LXII); 4,4-dimethylcholest-5-en-3α-ylamine hydrochloride (LXI), $C_{29}H_{52}NCl$, crystallized as colorless plates from absolute ethanol, m.p. 255° dec., $[\alpha]_D = -51.43°$ (C=0.63; $CHCl_3$), $\lambda_{max.}$ 208 mμ (ϵ = 1652), and 4,4-dimethylcholest-5-en-3β-ylamine hydrochloride (LXII), $C_{29}H_{52}NCl$, crystallized as needles from methanol, m.p. 265°dec., $[\alpha]_D = -52.64°$ (C=0.42; $CHCl_3$), $\lambda_{max.}$ 207 mμ (ϵ =1358).



(LXI)



(LXII)

The IR spectra of these two compounds showed the presence of an NH_3^+ grouping; the axial amine hydrochloride showed strong absorption at 1600 and 1500 cm^{-1}, and the equatorial epimer 1590, 1565, and 1510 cm^{-1}.

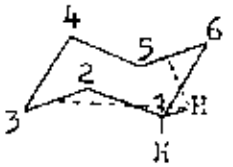
The NMR spectra (Table XII) of the amine hydrochlorides showed a one proton signal in the 6-7 δ region. This signal is due to the proton attached to C-3 and can be used for assigning configuration.

Table XII

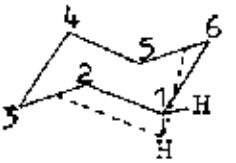
Compound	C_3	C_{26}, C_{27}	C_{21}	C_{18}	C_{19}	C_{30}	C_{31}	C_6
3α-$NH_3^+Cl^-$ (LXI)	6.49	9.09 (J=6cps)	9.03 (J=7cps)	9.21	8.58	8.71	8.71	4.19
3β-$NH_3^+Cl^-$ (LXII)	6.72	9.08 (J=6cps)	9.03 (J=8cps)	9.21	8.68	8.68	8.78	4.16

It has been found in steroids that protons attached to the same carbon atom as a hydroxyl function are deshielded and usually appear in the δ=3.5-4.5 ppm region.(14a). A general rule (14b) which has been established for a wide variety for six-membered ring systems states that axial ring protons absorb at higher field than do their epimeric equatorial counterparts. These chemical shifts (δ_{ae}) are associated with anisotropies of the magnetic susceptibilities of the carbon-carbon single bonds bearing a 2-3 relationship to the absorbing protons. An equatorial proton (LXIII) is deshielded as a result of the anisotropies of the C_2- C_3 and C_5- C_6 bonds,

whereas an axial one (LXIV) is shielded by that of C_2- C_3 and C_5- C_6 bonds.



(LXIII)

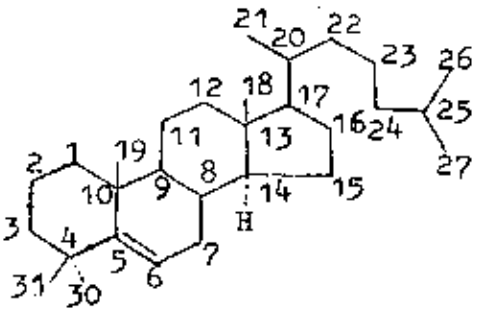


(LXIV)

A similar observation should be found in the case of cyclohexylamines. Thus an equatorial proton attached to a carbon atom bearing an amino grouping should absorb at lower field than a corresponding axial proton. The C-3 protons in (LXI) and (LXII) resonate at 6.49τ and 6.72τ respectively. We can conclude from the above evidence that the compound having a resonance signal at lower field will have an equatorial proton, and thus will correspond to the axial amine hydrochloride. The compound with a resonance signal at higher field will have an axial proton, and so will possess an equatorial ammonium grouping.

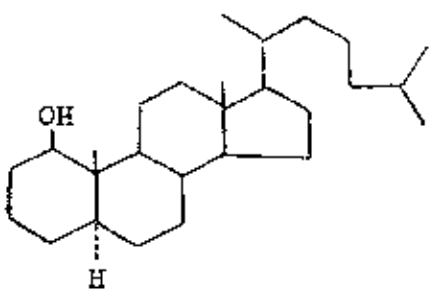
In the NMR spectra of the amine hydrochlorides, the C-3 proton is seen as a broad multiplet. This corresponds to the X portion of an ABX system; spin-spin coupling with the C_2 protons produces the multiplicity, and quadrupole interaction with the nitrogen causes broadening of the signals (58). The half-intensity widths of these signals, which are ~7 cps for (LXI) and 30 cps (LXII), suggest that ring A has a chair conformation (68).

A number of characteristic signals are observed in the NMR spectra of both compounds; these are due to the C-CH_3 protons.



A six proton doublet at 9.09 τ (J = 6 cps) and a three proton doublet at 9.03 τ (J = 7 cps) in (LXI) are virtually unaffected by change in the conformation of the 3-ammonium grouping (eg. LXII). These signals can be assigned to C_{26}, C_{27}, and C_{21} protons respectively. Hemmert et al. (50) have recently reported the NMR spectra of a number of substituted 4,4-dimethylcholest-5-enes in deuteriochloroform; they have found that the C_{26} and C_{27} protons in these molecules resonate at 9.13 τ (J = 6 cps). The slight shift to lower field of these protons which is observed in our compounds is probably due to change of solvent (14d).

The C_{21} protons of cholestan-10α-ol (LXV)(14c) have been observed at 9.07 τ (J = 6 cps) which is at a higher field than these protons in (LXI) and (LXII). This is possible caused by the solvent induced shift of the proton resonance (14d).



(LXV)

The tertiary methyl protons of (LXI) and (LXII), which are attached to C_{18}, C_{19}, C_{30}, and C_{31}, are shown in Table XII. Only the tertiary methyl protons attached to C_{18} are unaffected by a change in the conformation of the ammonium group, and this is to be expected as these protons are a long way from the ammonium group. Hemmert et al. (50) have noted (Table XIII) that the C_{18} protons of 4,4-dimethylcholest-5-enes are unaffected by change of conformation of a 3-hydroxy or acetoxy group.

Table XIII

Substituent	C_{18}	C_{19}	$C_{30}(4\alpha)$	$C_{31}(4\beta)$
3α-OH	9.33	8.87	8.92	8.87
3β-OH	9.33	8.93	8.88	8.96
3α-OAc	9.33	8.92	9.00	8.83
3β-OAc	9.33	8.90	8.98	8.90

The remaining tertiary methyl protons resonate at 8.58 τ (3H) and 8.71τ (6H) in (LXI) and 8.68τ (6H) and 8.78τ (3H) in (LXII). Our next task was to attempt to determine which of these signals belong to specific protons.

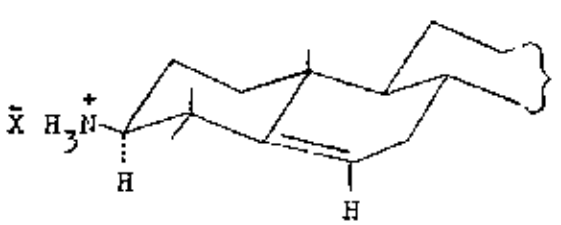
Hemmert et al. (50) have assigned signals to the tertiary methyl protons of C_{19}, C_{30}, and C_{31} in the epimeric 3-hydroxy and 3-acetoxy-4,4-dimethylcholest-5-enes. The chemical shifts of these protons, determined in deuteriochloroform or a mixture of deuteriochloroform and carbon-tetrachloride, are shown in Table XIII.

The C_{30} and C_{31} proton signals are affected by change of conformation of the 3-hydroxy or acetoxy group; the C_{30} proton signal is slightly deshielded on changing from an axial to an equatorial conformation, whereas the C_{31}-proton signals is shielded as a result of this type of conformational change. The effect of conformational change at C_3 in the hydroxy and acetoxy compounds affects the C_{19} proton signal in different ways; in the hydroxy compounds shielding occurs on changing from axial to equatorial whereas deshielding occurs in the acetoxy compounds as a result of a similar conformational change.

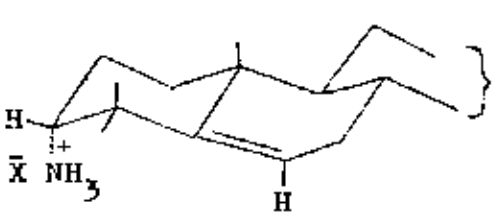
We have attempted to assign signals to the C_{19}, C_{30}, and C_{31} protons in our two ammonium chlorides; the results are shown in Table XII. We would expect on the basis of the results of Hommert et al. (50) that a change in conformation of the ammonium group should produce a chemical shift in the C_{19}, C_{30}, and C_{31} protons. However, we would not expect the signals to occur in the same region of the spectra in our compounds as their spectra have been determined in a different solvent and our compounds contain a 3-ammonium group. Comparision of the chemical shift values of the hydroxy and acetoxy compounds with those of the ammonium chlorides show that the chemical shift variation in the ammonium salts is of a larger magnitude than those of the alcohols or acetates. This could be associated with salt formation.

We have assigned the three proton signal at 8.58τ in the α-ammonium chloride (LXI) to the C_{19} protons; this signal will undergo on upfield shift on changing the conformation of the

ammonium group to β and we suggest that these protons will absorb at 8.68 τ in (LXII). We would expect a considerable change in the shielding of the C_{19} proton to occur in changing from compound (LXVI) to (LXVII); the magnetic field associated with the ammonium group will affect the C_{19} protons less when it has an axial rather than equatorial conformation and this will result in a deshielding of these protons. This fact has been taken into account in our assignment. We would anticipate also that by changing the conformation of the ammonium grouping from axial to equatorial, the C_{30} tertiary methyl protons would be slightly deshielded, and the C_{31} protons shielded. This can be seen from a study of the two conformation (LXVI) and (LXVII); changing the conformation of the ammonium grouping from axial to equatorial should only slightly affect the equatorial C_{30} methyl protons, whereas this conformational change will considerable affect the axial C_{31} protons. We have suggested, therefore, that the C_{30} protons will undergo a downfield shift of 0.03 ppm and the C_{31} protons a upfield shift of 0.07 ppm on changing the conformation of the ammonium group from axial to equatorial.



(LXVI) X = Cl or CF_3CO_2



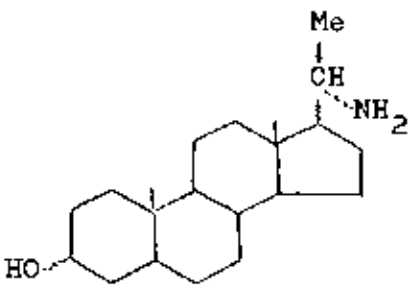
(LXVII) X = Cl or CF_3CO_2

A one proton multiplet is observed in the 4.10-4.20τ region of the spectra of both amine hydrochlorides; in (LXI) the signal occurs at 4.19τ and in (LXII) at 4.16τ. This signal can be attributed to the C_6 olefinic proton, the multiplicity is due to vicinal coupling to the C_7 protons. The NMR spectra of a number of Δ^5-unconjugated steroids have been determined; in these compounds the olefinic C_6 proton signal was found at an average value of 4.4 ± 0.1τ (87). The chemical shift to lower field of these protons which is observed in our molecules is probably due to change of solvent. The olefinic C_6 proton signal in (LXI) is shifted upfield by 0.03 ppm from that of (LXII); this is due to the shielding effect of the ammonium grouping which is closer to the vinylic proton in (LXVII) than (LXVI).

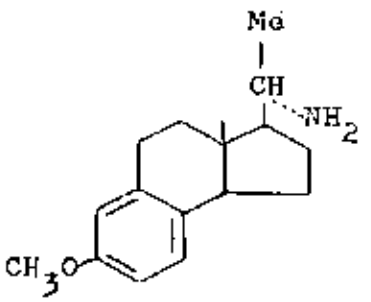
Optically active amines do not show a Cotton effect in the measurable range of the circular dichrograph and so they cannot be used for assignment of configurations. A number of substituted amines have been studied and several of these have been found to give Cotton effects. These are alkyl dithiocarbamates, N-salicylidenes, nitrosamines, thiohydantoins and N-thionocarbethoxy derivatives (98). The alkyl dithiocarbamates, thiohydantoins, and N-thionocarbethoxy derivatives have been used extensively in the determination of the configuration of α-amino acids, and nitrosamines for configurational assignments of acetylated amines.

The imino-ortho-hydroxybenzyl chromophore, which occurs in salicylidene derivatives, is useful for determining configuration of amino groups. Its region of circular dichroic absorption lies

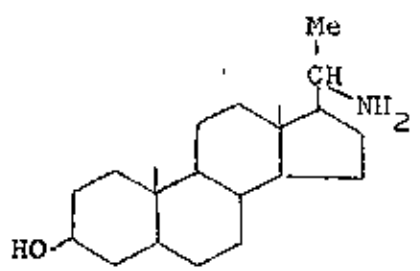
at about 320 mμ (98). Bertin and Legrand (12) have determined the relative configuration of 20α-amino steroids (LXVIII,LXIX, LXX), and 20β-amino steroids (LXXI,LXXII,LXXIII) by means of the sign of the Cotton effect of their salicylidene derivatives. The 17β-amino steroids (LXXIV,LXXV), 17α-amino steroids (LXXVI), and 3α-amino-20α-hydroxyl-5β-pregnane (LXXVII) have also had their configurations determined in this way. It has been found that the Cotton effect is negative for all the compounds possessing an R-configuration, and positive for those with an S-configuration.



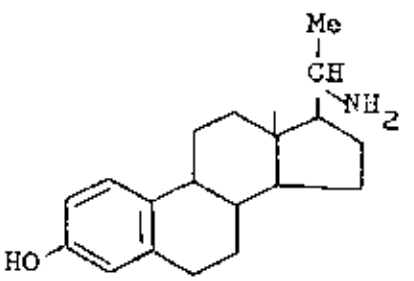
(LXVIII) 3β-OH; 5α
(LXIX) 3α-OH; 5β



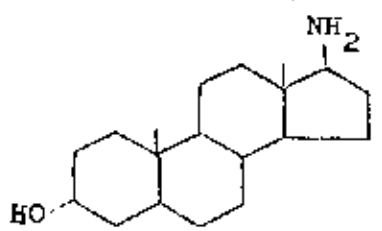
(LXX)



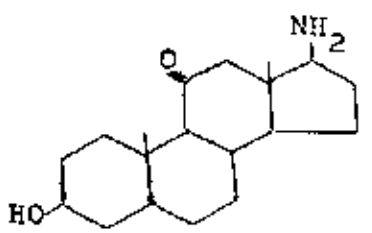
(LXXI) 3β-OH; 5α
(LXXII) 3α-OH; 5β



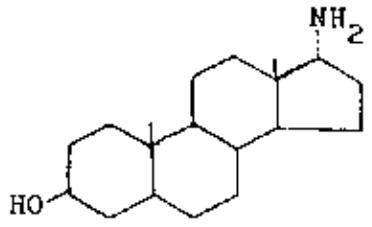
(LXXIII)



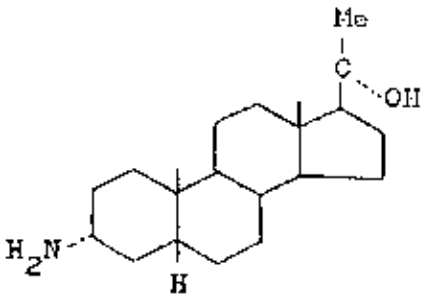
(LXXIV)



(LXXV)

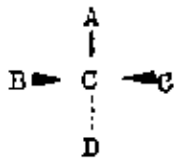


(LXXVI)



(LXXVII)

The Cahn-Ingold-Prelog rule (98) has been introduced to designate the absolute configuration of molecules. For the application of this rule, the molecule is viewed from the side which is remote from the atom of least priority. The sequence of the other three groups is then considered. If the priority of these three groups decreases in a clockwise manner the molecule is said to have an *R*-configuration (rectus = right); whereas if it decreases in an anticlockwise direction the molecule is said to be of the S-type (sinister = left). An illustration of the rule is shown for (LXXVIII); D is considered to be the group of lowest priority. When the molecule is viewed from above, the three groups A,B, and C taken up a anticlockwise arrangement. If A is the largest and C the smallest of the three groups, then the molecule has an *S*-configuration.



(LXXVIII)

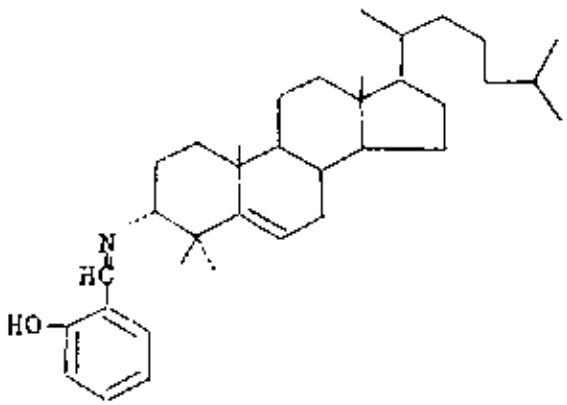
It is important to note that the rule is not perfect for all molecules, as it gives priority to the hetero-atoms, and group

containing these are not necessarily the bulkiert ones.

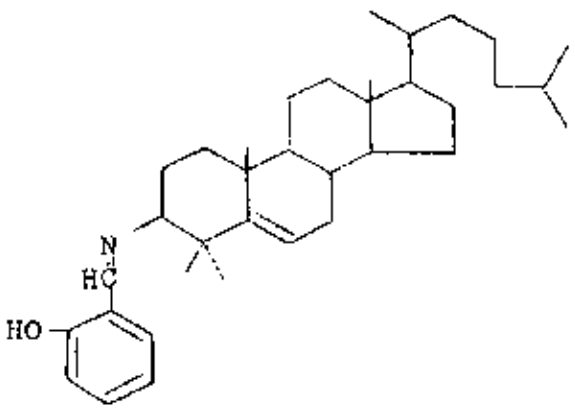
N-Salicylidene derivatives of 3-amino-4,4-dimethylcholest-5-ene epimers (LXXIX,LXXX) were prepared by treating each of 3-amino-4,4-dimethylcholest-5-enes (XLIII,XLIV) in methanol with salicylaldehyde. The N-salicylidene derivative of 4,4-dimethyl-cholest-5-en-3α-ylamine (LXXIX), crystallized from light petroleum and methanol as greenish yellow plates, m.p. 117-118°, $\lambda_{max.}$ 215,256, shoulder 262, and 320 mμ (ϵ = 6787, 3555,3232, and 1252 respectively); that of the corresponding equatorial amine (LXXX), crystallized from light petroleum and methanol as greenish yellow fine needles, m.p. 164-165°, $\lambda_{max.}$ 215,256, shoulder 262, and 320 mμ (ϵ =5808,3769,3223, and 1308 respectively).

Strong absorption peaks in the IR spectra of the axial N-salicylideniimino derivative at 3400 cm^{-1}, 3040 cm^{-1}, 1630 cm^{-1}, 1575 and 1490 cm^{-1}, 1277 and 1190 cm^{-1}, 735 cm^{-1} and the equatorial epimeric derivative at 3400 cm^{-1}, 3030 cm^{-1}, 1625 cm^{-1}, 1572 and 1490 cm^{-1}, 1275,1250 and 1200 cm^{-1}, 738 cm^{-1} indicated the presence of OH stretching, aromatic CH stretching, C = N stretching, phenyl C = C stretching, C-O stretching, and four adjacent hydrogen on benzene ring respectively.

The circular dichroism curves of the salicylidenimino derivatives of the epimeric amines were determined in ethanol and dioxane. A negative Cotton effect was observed for the derivative of the axial amine in both solvents at about 320 mμ. For the equatorial epimer, a positive Cotton effect was observed in this region. Application of the Cahn-Prelog-Ingold rule to the two



(LXXIX)



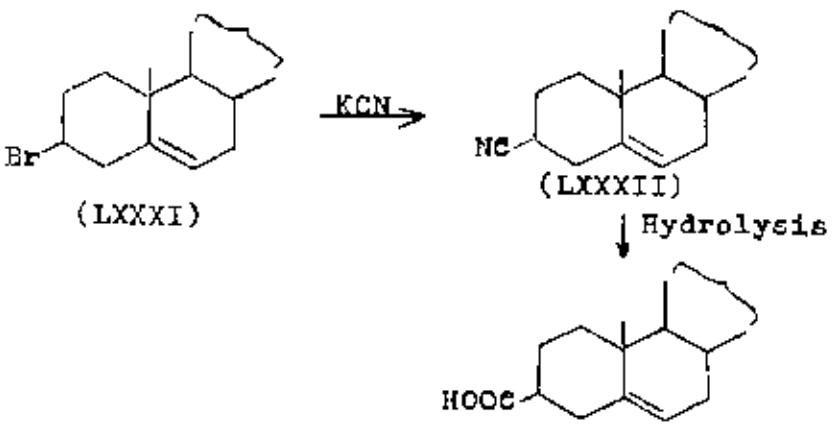
(LXXX)

amines shows that the 3β-salicylidenimino compound (LXXX) has an S-configuration and the 3α-salicylidenimino compound (LXXIX) an R-configuration. The positive Cotton effect exhibited by the 3β-salicylidenimino derivative (LXXX) and the negative Cotton effect exhibited by the 3α-salicylidenimino derivative (LXXIX) are in agreement with the findings of Velluz and conform the configurational assignments based on NMR.

Pierce and Shoppee (78) have determined the configuration of 3β-aminocholest-5-ene (XCI) by chemical methods starting from cholest-5-en-3β-carboxylic acid (LXXXIII). This acid was obtained from cholesteryl bromide (LXXXI) by successive treatment with potassium cyanide and hydrolysis as shown in Scheme II (84).

The 3β-configuration of the carboxy group of this acid was established by means of the reactions shown in Scheme III (84). The acid (LXXXIII) was converted into the ketone (LXXXIV) by successive treatment with thionyl chloride and dimethyl cadmium

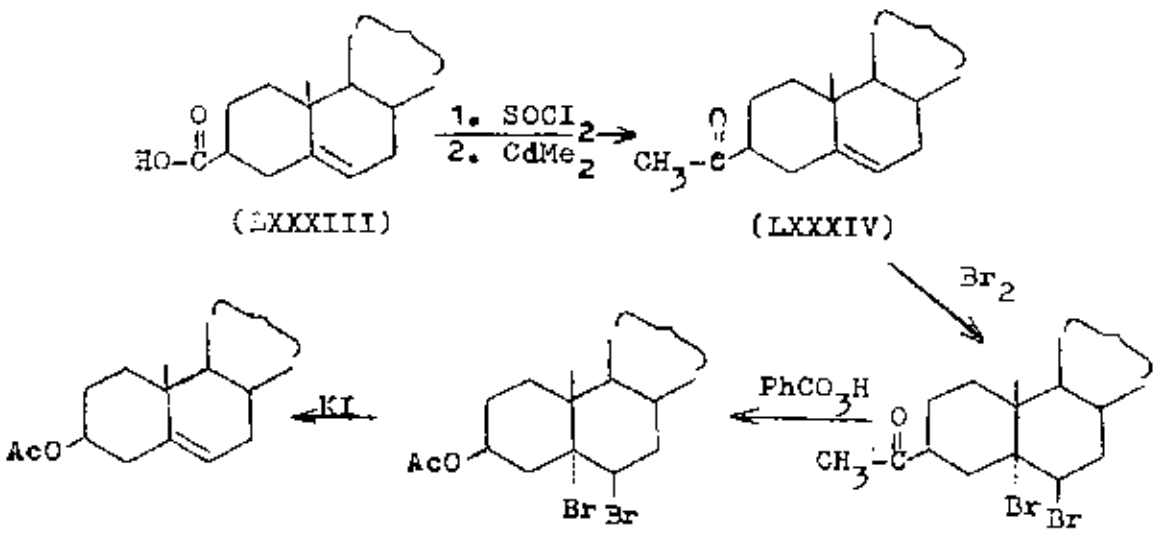
Scheme II



(LXXXI) (LXXXII) (LXXXIII)

which was converted by treatment with bromine into the 5α, 6β-dibromide (LXXXV). Prolonged treatment of this compound with perbenzoic acid in chloroform at 0°and debromination with potassium iodide-acetone gave cholesteryl acetate (LXXXVII).

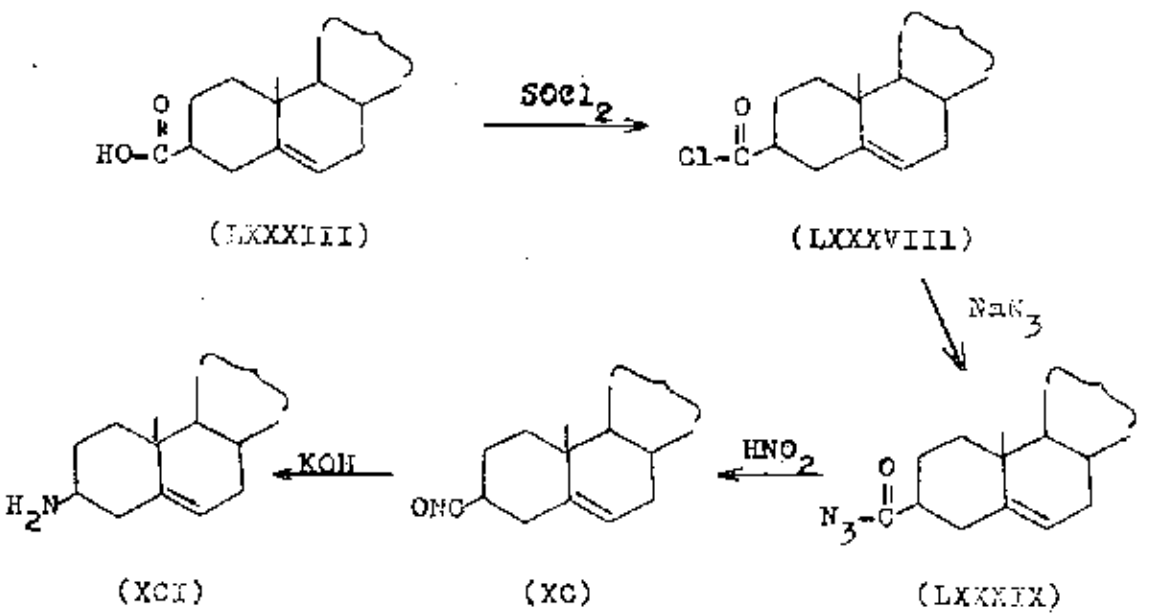
Scheme III



(LXXXIII) (LXXXIV) (LXXXV) (LXXXVI) (LXXXVII)

Only one step of this reaction sequence involves the cleavage of the C_3-CO_2H bond. This occurs in the Baeyer-Villiger oxidation of the acetyl group in (LXXXV) to the acetoxy group in (LXXXVI), and since this rearrangement has been found to proceed with retention of configuration (71) a 3β-configuration can be assigned to the carboxy grouping in the starting compound.

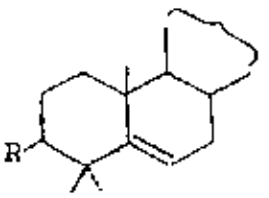
Cholest-5-en-3β-carboxylic acid has been converted into the azide (LXXXIX) by way of the chloride (LXXXVIII). Treatment of the azido with nitrous acid gave the isocyanate (XC) by a Curtius rearrangement, and this was hydrolysed by alkali to 3β-aminocholest-5-ene (XCI). The configuration of this base follows from the known configuration of the acid (LXXXIII); the preservation of configuration of the migrating group during the Curtius rearrangement has been established by Kenyon et al. (23,59).



(LXXXIII) (LXXXVIII)

(XCI) (XC) (LXXXIX)

We have attempted to prepare 3β-carboxy-4,4-dimethylcholest-5-ene (XCII; R = COOH) as the first step towards establishing the configurations of our two amines by a similar route to that used by Shoppee (84) for 3β-aminocholest-5-ene (XLIII,XLIV).



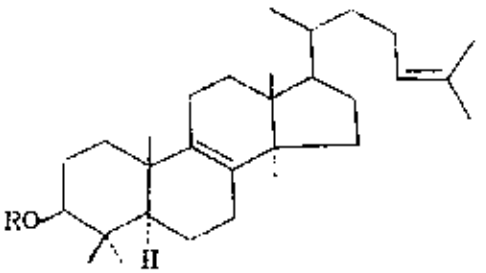
(XCII)

4,4-Dimethylcholesteryl halides (XCII; R = Cl,Br, or I) would be useful starting materials for the preparation of the acid. Cholesteryl chloride has been prepared from cholesterol by treatment with phosphorus pentachloride (65,67,79), or with thionyl chloride in the presence of pyridine (35).

Haddad and Summers (48) have found that treatment of 4,4-dimethylcholesterol with phosphorus pentachloride yielded the hydrocarbon (XXX). Thus elimination of water occurs in this compound rather than substitution as is found in the cholesteryl series.

Since the reactions of 4,4-dimethylsteroids are similar with respect to substitution and elimination, we decided to use the readily available commercial lanosterol (XCIII; R = H) for exploratory investigations into the preparation of 3β-substituted-4,4-dimethylsteroids.

Treatment of lanosterol in pyridine with thionyl chloride



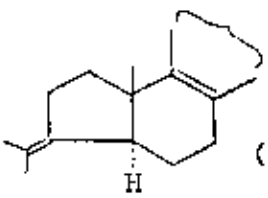
(XCIII)

afforded only the starting material as shown by thin-layer chromatography. The above reactions suggest that the direct route from the alcohol to the 3β-halogeno-4,4-dimethylcholest-5-ene is not possible.

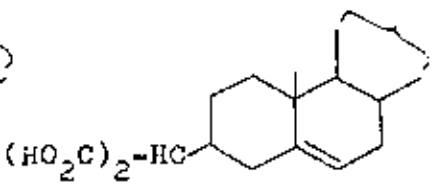
We decided next to attempt the preparation of lanosteryl bromide from lanosteryl methyl ether (XCIII; R = Me) by treatment with hydrobromic acid ; this method has been used successfully in the preparation of cholesteryl halides (13). Treatment of lanosteryl methyl ether with hydrobromic acid yielded a mixture which was shown by thin-layer chromatography to be composed mainly of 3-isopropylidene-A-nor-lanosta-8, 24-diene (XCIV).

Since all our attempts to prepare lanosteryl halides had resulted in failure, we decided to try direct insertion of a suitable substituent (eg. CN, or $CH(CO_2Et)_2$) into the 3-position. Cholesteryl cyanide (LXXXII) and cholesteryl malonic acid (XCV) have been prepared by treatment of cholesteryl tosylate (XCVI) with sodium cyanide (4) or diethylsodiomalonate (94). We have found that treatment of lanosteryl tosylate (XCIII; R = Ts) with sodium cyanide in refluxing dimethylsulphoxide, a solvent which

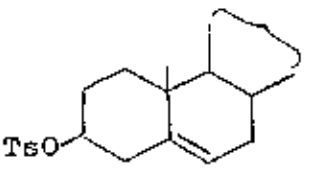
is known to accelerated substitution reaction (24,70,75), afforded 3-isopropylidene-A-nor-lanosta-8, 24-diene as shown by thin-layer chromatography. Similarly, treatment of lanosteryl tosylate with diethylsodiomalonate yielded 3-isopropylidene-A-nor-lanosta-8, 24-diene as the major product.



(XCIV)



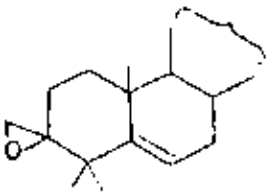
(XCV)



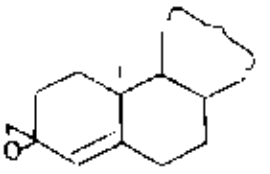
(XCVI)

Since all our attempts to prepare 3-substituted 4,4-dimethyl-steroids had resulted in elimination and rearrangement rather than substitution, we decided to attempt to prepare 4,4-dimethylcholest-5-en-3β-carboxylic acid by means of the epoxide (XCVII). Corey and Chaykovsky (29) have recently found that ketones react with dimethylsulphonium methylide to give epoxides of the type (XCVIII). Thus cholest-4-en-3-one affords (XCVIII) on treatment with dimethylsulphonium methylide. Epoxides are known to yield aldehydes or ketones on treatment with boron trifluoride etherate. For example, Henbest and Wrigley (51) have used this reaction for the preparation of the aldehyde (C) from the epoxide (XCIX). This aldehyde was converted to the corresponding acid by treatment with chromic acid. Such a reaction sequence would seem an attractive route to 3-carboxy-4,4-dimethylcholest-5-ene.

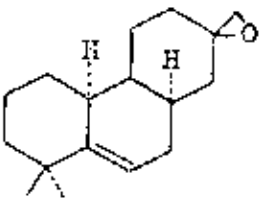
We have found that treatment of 4,4-dimethylcholest-5-en-3-one (XXXVIII) with dimethylsulphonium methylide yielded only the



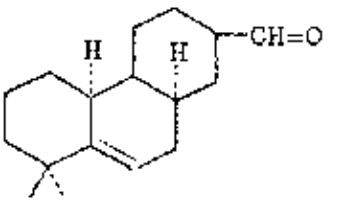
(XCVII)



(XCVIII)



(XCIX)



(C)

starting material. We were unable to successfully perform this transformation under the conditions described by Corey and Chaykovesky (29), and we consider that this may possibly be due to the difficulty of excluding moisture from the reaction flask.

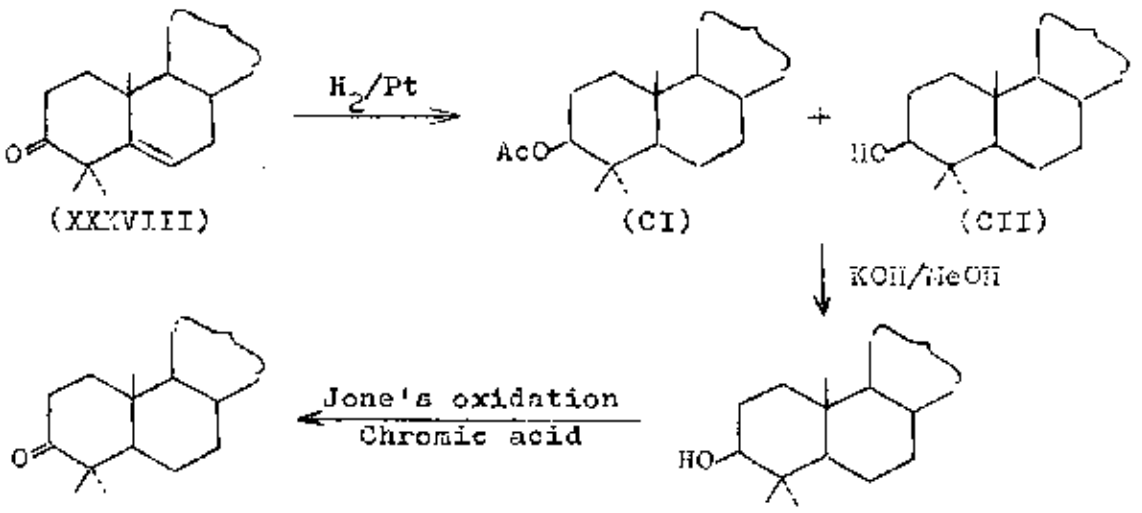
We decided at this stage to abandon this approach to the configuration of our amines as the results of nuclear magnetic resonance and circular dichroism became available.

We next turned our attention to the preparation of the 3-amino-4,4-dimethylcholestanes. 4,4-Dimethylcholestan-3-one (CIII) was synthesized by the method of Chaudhry et al. (25) as shown in Scheme IV. 4,4-Dimethylcholest-5-en-3-one (XXXVIII) in glacial acetic acid containing one drop of 60% perchloric acid was hydrogenated in the presence of Adam's catalyst at atmospheric pressure and 50-60°. This gave, after hydrolysis, a mixture of 4,4-dimethylcholestanylacetate (CI) and 4,4-dimethylcholestan-3β-ol

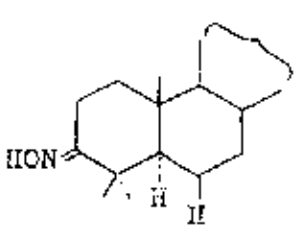
(CII), which was hydrolysed with 3% methanolic potassium hydroxide. 4,4-Dimethylcholestan-3β-ol (CII) crystallized from methylene dichloride and methanol as needles, m.p. 156-158°. Oxidation of this saturated alcohol with chromic acid furnished, after chromatography of the product on alumina using light petroleum as eluant, 4,4-dimethylcholestan-3-one (CIII), m.p. 102-103°.

The IR spectrum of this ketone showed strong carbonyl stretching at 1710 cm^{-1}. The absence of bands at 790 cm^{-1} in the IR spectrum and no absorption maximum above 200 mμ in the UV spectrum indicated that the double bond had been reduced.

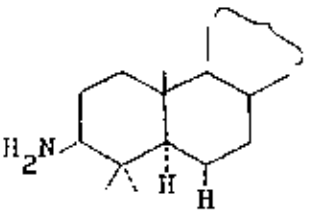
Scheme IV



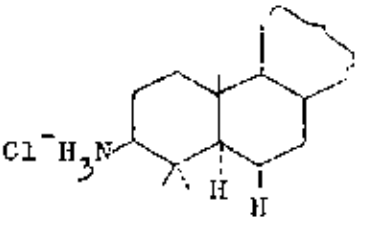
Treatment of the ketone (CIII) in pyridine with hydroxylamine hydrochloride afforded 4,4-dimethylcholestan-3-one oxime (CIV), m.p. 208-209°. A broad maximum at 3260 cm^{-1} showed the presence of the oximino group.



(CIV)



(CV)



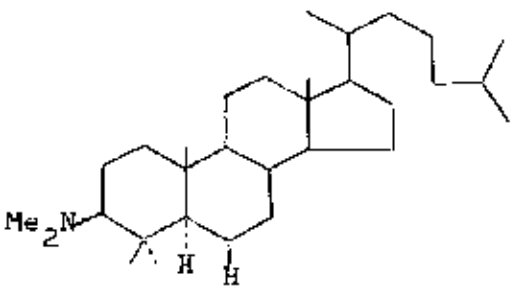
(CVI)

Reduction of this oxime with sodium and butanol-1 afforded a mixture, from which the 3β-amino derivative (CV), the major product, was isolated by column chromatography on deactivated alumina, using a mixture of ether:light petroleum (1:2) as eluant. The 4,4-dimethylcholestan-3β-ylamine was converted to 4,4-dimethylcholestan-3β-ylamine hydrochloride (CVI), $C_{29}H_{54}NCl$, which crystallized from methanol and acetone as needles, m.p 285°dec., $[\alpha]_D$ = +8.2° (C=0.624; $CHCl_3$). Its IR spectrum showed absorption peaks at 2940, 2870, 1475 and 1450 cm^{-1} (stretching and bending vibrations 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).

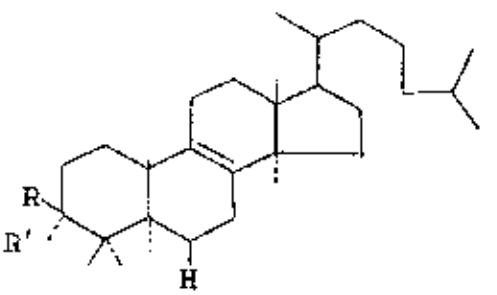
We have assigned a 3β-configuration to this amine on the basis of our findings on the reduction of 3-oximino-4,4-dimethyl-cholest-5-ene; this assignment will be confirmed by means of NMR in the near future.

Djerassi et al. (76) have described the preparation of 3β-dimethylamino-4,4-dimethyl-5α-cholestane (CVII) by reduction of the oxime (CIV) with lithium aluminum hydride in ether followed by methylation (38). They were unable to assign a configuration

to this amine (CVII), but we would suggest a 3β-configuration for this compound by analogy with our findings on the reduction of 4,4-dimethylcholest-5-en-3-one oxime. This assignment is supported by the findings of McKenna (68) that the reduction of lanost-8-en-3-one oxime (CVIII; R, R′= NOH) with lithium aluminum hydride affords 3β-amino-lanost-8-ene (CVIII; R = NH_2, R′= H) as the major product.



(CVII)



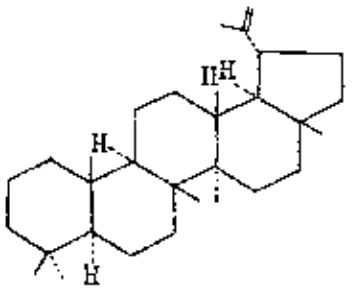
(CVIII)

We have also prepared 4,4-dimethylcholestan-3β-ylamine hydrochloride (CVI) by hydrogenation of 4,4-dimethylcholest-5-en-3β-ylamine hydrochloride (LXII) in absolute ethanol at atmospheric pressure and 80-82° using a 10% palladium charcoal catalyst. The amine hydrochloride, $C_{29}H_{54}NCl$, obtained in this way had m.p. 284° dec., $[\alpha]_D$ = +5 (C=0.44; $CHCl_3$).

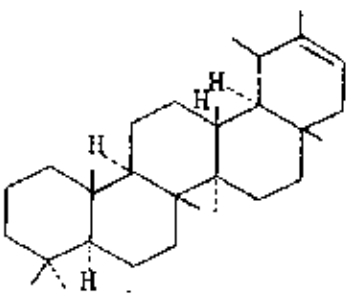
In contrast, 4,4-dimethylcholest-5-en-3α-ylamine hydrochloride (LXI) resisted hydrogenation at atmospheric pressure and 80-82° on a 10% palladium charcoal catalyst. However, when 4,4-dimethylcholest-5-en-3α-ylamine hydrochloride in ethanol was treated with hydrogen in the presence of Raney nickel catalyst (W_6) at atmospheric pressure and 80-82°. It afforded a compound which crystallized from ethanol as shiny prisms, m.p. 64-66°. The UV

spectrum of this compound showed $\lambda_{max.}$ 208 mμ (ϵ =1764) which indicated that it still contained a double bond. Thin-layer chromatography showed it had lower R_F value than 4,4-dimethylcholestan-3-one oxime but a higher R_F value than 3α-amino-4,4-dimethylcholest-5-ene (XLIII). This suggests that this compound is an isomer of 3α-amino-4,4-dimethylcholest-5-ene, and could be formed by a carbonium ion rearrangement.

Ames et al. (1) have reported that treatment of α-lupene (CIX) in benzene with a mixture of acetic acid and sulphuric acid at room temperature yields lupene-1 (CX).



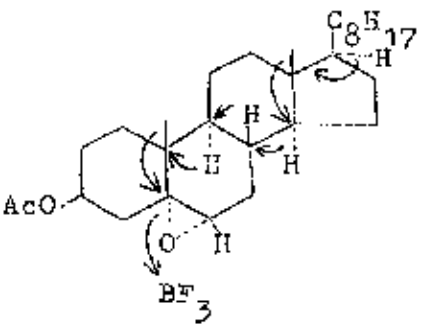
(CIX)



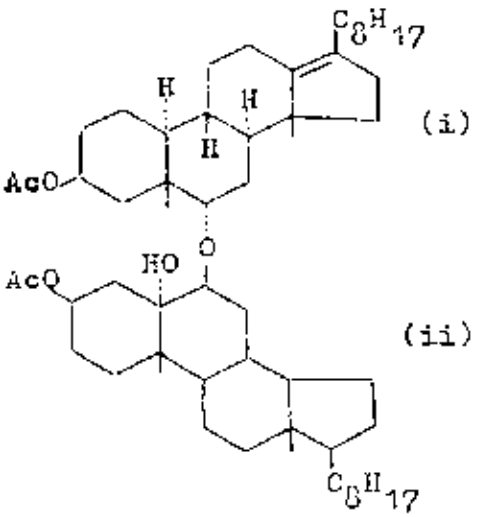
(CX)

We have subjected 3β-acetoxy-4,4-dimethylcholest-5-ene (XCII; R = OAc) to acid rearrangement under similar conditions to those described for α-lupene by Ames. This furnished a viscous mass which was shown by thin-layer chromatography to be composed of two compounds which have higher R_F values than 3β-acetoxy-4,4-dimethylcholest-5-ene. Strong absorption in the IR spectrum of this product at 1732 cm^{-1} (C=Ostretching of acetate), 1285 and 1260 cm^{-1} (C-O-C stretching of acetate) showed that the reaction products were acetates.

Blunt et al. (19) have studied a reaction of 3β-acetoxy-5α, 6α-epoxy-5α-cholestane (CXI) in benzene with boron trifluoride etherate. They have found that the ether (CXII) was obtained. This ether(CXII) is considered to be formed by nucleophillic attack by the epoxide oxygen of one epoxide molecule (i) to give normal diaxial opening of a 5α,6α-epoxide in molecule (ii). The C-5-O cleavage in molecule (i) underwent a number of 1,2 shifts as shown in (CXI), and the loss of the hydrogen atom from C-17, to form the $\Delta^{13(17)}$bond and inversion at each ring junction. This rearrangement is known as a "back-bone" rearrangement, and similar rearrangements have been observed in triterpenoid chemistry.



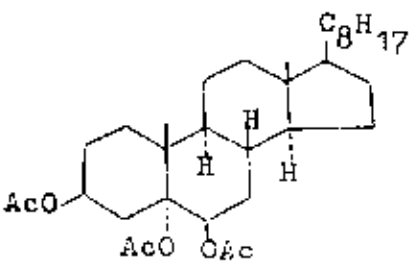
(CXI)



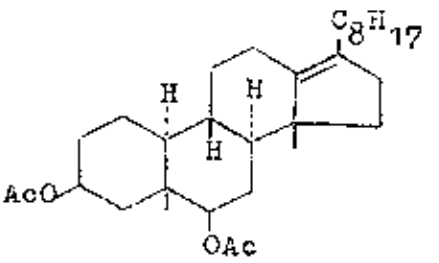
(CXII)

They (20) also reinvestigated the reaction of the triacetate (CXIII) with acetic anhydride in the presence of a variety of acidic catalyats at 80° or 140° which had been previously investigated by

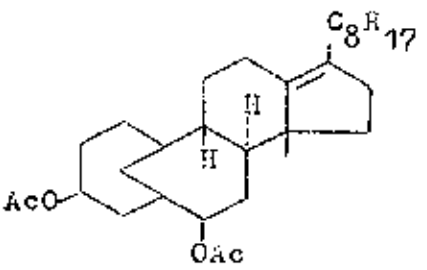
Snatzke and Fehlhaber (96). They (20) have assigned the structure (CXIV) to the unsaturated diacetate obtained in this reaction instead of (CXV) which was reported by Snatzke and Fehlhaber (96). Blunt et al. (20) have shown that the skeleton of this unsaturated diacetate (CXIV) has undergone a "back-bone" rearrangement. This assigned structure is supported by UV and NMR spectra, and conversion into a known compound.



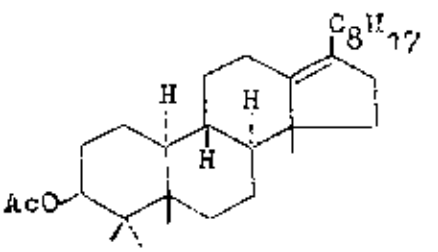
(CXIII)



(CXIV)



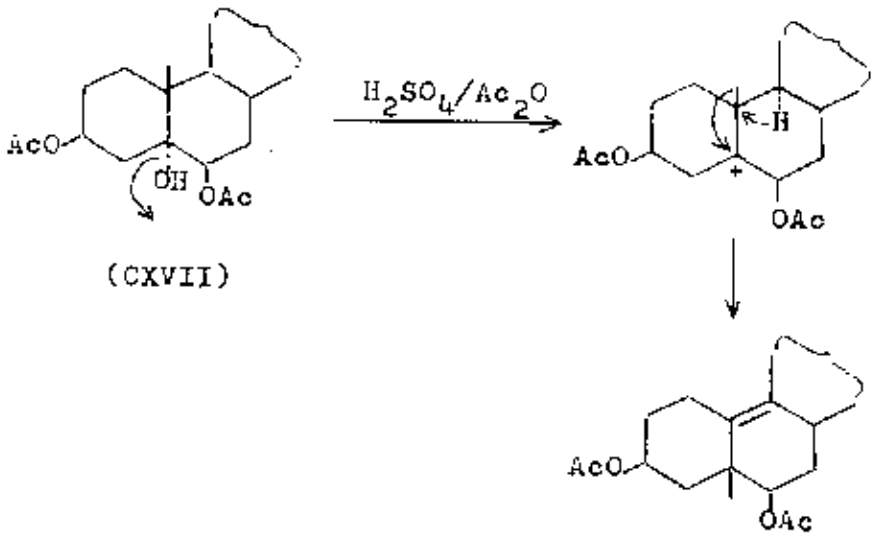
(CXV)



(CXVI)

Thus one of the possible products of acid isomerization of 3β-acetoxy-4,4-dimethylcholest-5-ene is (CXVI); this would involve a "back-bone" rearrangement of the type described by Blunt.

Westphalen et al. (18,39,63,100) had found that treatment of cholestan-3β ,5α ,6β-triol-3,6-diacetate (CXVII) with acetic anhydride and a little sulphuric acid affords a dehydration



(CXVII)

(CXVIII)

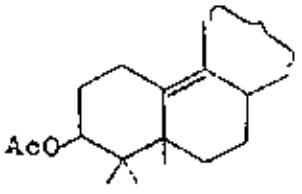
product which is known as Westphalen's diacetate. This compound has been shown to have the structure (CXVIII).

Petrow (77) has also found that the triol diacetate (CXVII) is dehydrated by potassium bisulphate in acetic anhydride to Westphalen's diacetate (CXVIII).

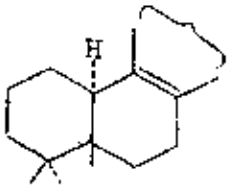
The formation of the product is readily explained in terms of a carbonium ion rearrangement; the driving force being the release of steric compression in ring B due to the 1,3-diaxial interaction of the 6β-acetoxy group and the C_{10}-methyl group.

These reactions suggest (CXIX) as a possible product of the acid isomerization of 3β-acetoxy-4,4-dimethylcholest-5-ene, although structures such as(CXX) and (CXXI) cannot be exclude. Confirmation of the structures of these products will have to await spectroscopic data on the pure compounds. The driving force in this rearrangement is the release of steric compression in Ring A due to 1,3-diaxial non-bonded interaction of the 4β-and C_{10}-methyl

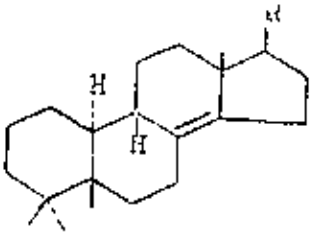
groups.



(CXIX)



(CXX)



(CXXI)