[Chem. Pharm. Bull. 28(4)1150—1156(1980)]

Synthesis of [21-^{2}H]- and [22-^{2}H]-Derivatives of 23,24,25,26,27-Pentanorcholesterol and 23,24,25,26,27-Pentanordihydrolanosterol and the Stereochemical Significance of the Deuterium Spin-Lattice Relaxation Times

Yoshihiro Sato, Yoshiko Sonoda,[1a] and Hazime Saitô[1b]

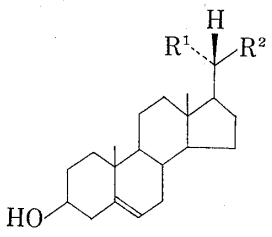
Kyoritsu College of Pharmacy[1a] *and Biophysics Division, National Cancer Center Research Institute*[1b]

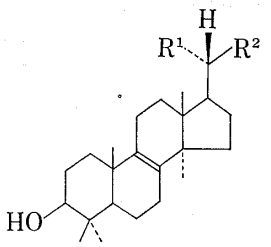
(Received October 4, 1979)

The deuterated and undeuterated 21- and 22-methyl derivatives of the 23,24,25,26,27-pentanor analogs of cholesterol and dihydrolanosterol were synthesized. As starting materials, (20*S*)- and (20*R*)-3β-acetoxybisnorchol-5-en-22-oic acid and (20*S*)- and (20*R*)-3β-acetoxy-23,24,25,26,27-pentanorlanost-8-en-22-oic acid were used. The deuterium relaxation times of the two groups of compounds were measured and the relationship between the T_1 values and the structures was examined.

Keywords——23,24,25,26,27-pentanorcholesterol; 23,24,25,26,27-pentanordihydrolanosterol; deuterated compounds; deuterium relaxation time; ^{2}H NMR

During the course of our studies on cholesterol biosynthesis, it was necessary to synthesize the pentanor side chain analogs of cholesterol and lanosterol. In this paper we report the synthesis of some deuterated analogs at the 21- or 22-methyl group of 23,24,25,26,27-pentanorcholesterol (3β-hydroxybisnorchol-5-ene) and 23,24,25,26,27-pentanordihydrolanosterol, together with the results of deuterium relaxation time measurements. The latter measurements are expected to cast light on the stereochemistry of the isopropyl side chain, since the deuterium relaxation time is determined by anisotropic intramolecular rotation.[2,3]





I: $R^1=CH_3$, $R^2=CH_3$
IIa: $R^1=CH_3$, $R^2=CH_2D$
IIb: $R^1=CH_2D$, $R^2=CH_3$
IIIa: $R^1=CH_3$, $R^2=CD_3$
IIIb: $R^1=CD_3$, $R^2=CH_3$

IV: $R^1=CH_3$, $R^2=CH_3$
Va: $R^1=CH_3$, $R^2=CH_2D$
Vb: $R^1=CH_2D$, $R^2=CH_3$

Chart 1

First, the synthesis of 22- and 21-deuterated compounds (IIa and IIb) of 3β-hydroxybisnorchol-5-ene was carried out. As a first step, in order to obtain the 20-iso compounds of cholesterol analogs, isomerization of (20*S*)-3β-acetoxybisnorchol-5-en-22-al (VIa) was per-

1) Location: *a) Shibakoen 1-chome, Minato-ku, Tokyo 105, Japan; b) Tsukiji 5-chome, Chuo-ku, Tokyo 104, Japan.*
2) H.H. Mantsch, H. Saitô, and I.C.P. Smith, "Progress in Nuclear Magnetic Resonance Spectroscopy," Vol. 11, ed. by J.W. Emsley, J. Feeney, and L.H. Sutcliffe, Pergamon Press, Oxford, 1977, pp. 211—272.
3) H.H. Mantsch, H. Saitô, L.C. Leitch, and I.C.P. Smith, *J. Am. Chem. Soc.*, **96**, 256 (1974).

formed according to the procedure of Barton *et al.*[4] to afford an equilibrium mixture of the epimers at C-20. The benzene solution of VIa was adsorbed on an alumina column overnight and eluted with benzene to give the products in an *S*: *R* ratio of 1: 2, as determined from the ^{1}H nuclear magnetic resonance (NMR) spectrum. Since the epimers could not be separated by silica gel or Sephadex LH-20 column chromatography, the mixture was oxidized with chromium trioxide in acetic acid. In studies of 3-oxobisnorchol-4-enic acid, Herr *et al.*[5] have shown that epimerization at C-20 occurs in the oxidation of the corresponding 20-isoaldehyde. The ^{1}H NMR spectra of the products showed that VIIa and VIIb are formed in a ratio of 1: 1. Upon column chromatography on Sephadex LH-20, the 20*R*-carboxylic acid (VIIb)[6] was eluted first with chloroform, followed by the 20*S*-epimer; this was confirmed by ^{1}H NMR spectroscopy using the 21- and 22-methyl signals at 1.22 and 1.14 ppm as indicators for VIIa and VIIb, respectively. After treatment of the carboxylic acids (VIIa and VIIb) with ethyl chloroformate and triethylamine in tetrahydrofuran, reduction of the products with sodium borohydride afforded the corresponding hydroxy compound,[7] and subsequent phosphoryl chloride treatment in pyridine gave the corresponding chloro compounds (VIIIa and VIIIb). Upon lithium aluminum hydride reduction, each chloro compound afforded the same product, 3β-hydroxybisnorchol-5-ene (I),[8,9] mp 137—138°. The ^{1}H NMR spectrum of I showed two doublets at 0.93 and 0.84 ppm, indicating a chemical shift difference between the 21- and 22-methyl groups.[10] When lithium aluminum deuteride instead of lithium aluminum hydride was used as the reducing reagent in the last step of the reactions, VIIIa and VIIIb afforded the corresponding deuterated compounds, IIa and IIb, respectively, as shown in Chart 2. It was

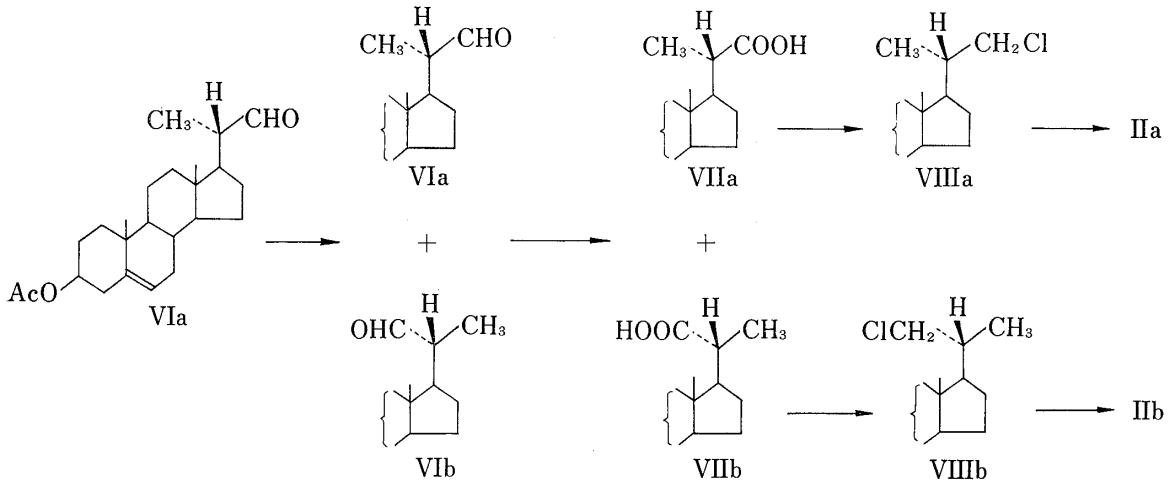


Chart 2

proved by mass spectroscopy that one deuterium atom is incorporated into both IIa and IIb in about 98% yield. Accordingly, the structure of IIa is determined as (20*R*)-[22-^{2}H$_1$]-3β-hydroxybisnorchol-5-ene and that of IIb is (20*S*)-[21-^{2}H$_1$]-3β-hydroxybisnorchol-5-ene. In the reactions in which both sodium borodeuteride and lithium aluminum deuteride are used as reducing reagents in each step, the 22-deuterated compound (IIIa) and 21-deuterated

4) D.H.R. Barton, T. Shioiri, and D.A. Widdowson, *J. Chem. Soc.*, (*C*), **1971**, 1968.
5) M.E. Herr and F.W. Heyl, *J. Am. Chem. Soc.*, **74**, 3627 (1952) and references cited therein.
6) R. Hayatsu, *Chem. Pharm. Bull.*, **5**, 452 (1957).
7) M. Ishiguro and N. Ikekawa, *Chem. Pharm. Bull.*, **23**, 2860 (1975).
8) J.P. Dusza and W. Bergman, *J. Org. Chem.*, **25**, 79 (1960).
9) A.D. Tait, *Biochem. J.*, **128**, 467 (1972); *idem*, *Steroids*, **22**, 239 (1973); *idem*, *ibid.*, **22**, 609 (1973).
10) After completion of this study, we were informed that the same observation regarding the chemical shifts has been obtained by Dr. Ikekawa's group: M. Morisaki, M. Shibata, C. Dugue, N. Imamura, and N. Ikekawa, *Chem. Pharm. Bull.*, **28**, 606 (1980).

compound (IIIb) were obtained; both gave deuterium contents of $^2H_3$ 74%, $^2H_2$ 22%, $^2H_1$ 3% and $^2H_0$ *ca.* 1%. The ^1H NMR signals at 0.84 and 0.93 ppm, which correspond to the chemical shifts of the deuterated 22- and 21-methyl groups, respectively, disappeared. The ^2H chemical shifts of IIIa and IIIb were 0.86 and 0.94 ppm, respectively. The ^2H NMR chemical shifts, expressed in ppm, are essentially the same as those of the analogous ^1H compound.[2,11] In proving this, the above values were found to coincide with the ^1H chemical shifts of I.

The second stage of the present studies was the synthesis of the same type of compounds in the lanosterol series; they were 23,24,25,26,27-pentanordihydrolanosterol (IV) and its 22- and 21-deuterated compounds (Va and Vb). A solution of 3β-acetoxy-23,24,25,26,27-pentanorlanost-8-en-22-al (IX)[12,13] was reduced with sodium borohydride and the resulting alcohol was chlorinated with phosphoryl chloride in pyridine to give the 22-chloro compound (XIa). The latter compound was also obtained from Xa[13] by the reaction described for VIIa. Lithium aluminum hydride reduction of XIa gave 23,24,25,26,27-pentanordihydrolanosterol (IV),[14] mp 190—191° (lit. 191—191.5°). The mass spectrum of IV showed a molecular ion at *m/e* 358 and two prominent fragments at *m/e* 343 (M^+-CH_3) and 325 ($M^+-CH_3-H_2O$). In the ^1H NMR spectrum of IV, the signals of 18-CH_3 (0.70 ppm), 32-CH_3 (0.82 ppm), 19-CH_3 (1.00 ppm) and 3α-H (3.28 ppm, multiplet) were assigned. On the other hand, reduction of XIa with lithium aluminum deuteride afforded the 22-deuterated compound (Va), in which the deuterium contents were determined to be $^2H_1$ 94% and $^2H_0$ 6% from the mass spectrum. Consequently, the structure of Va is (20*R*)-[22-$^2H_1$]-23,24,25,26,27-pentanordihydrolanosterol. Synthesis of the 21-deuterated compound was attempted starting from the aldehyde (IX). The treatment of IX on an alumina column under the conditions described above, however,

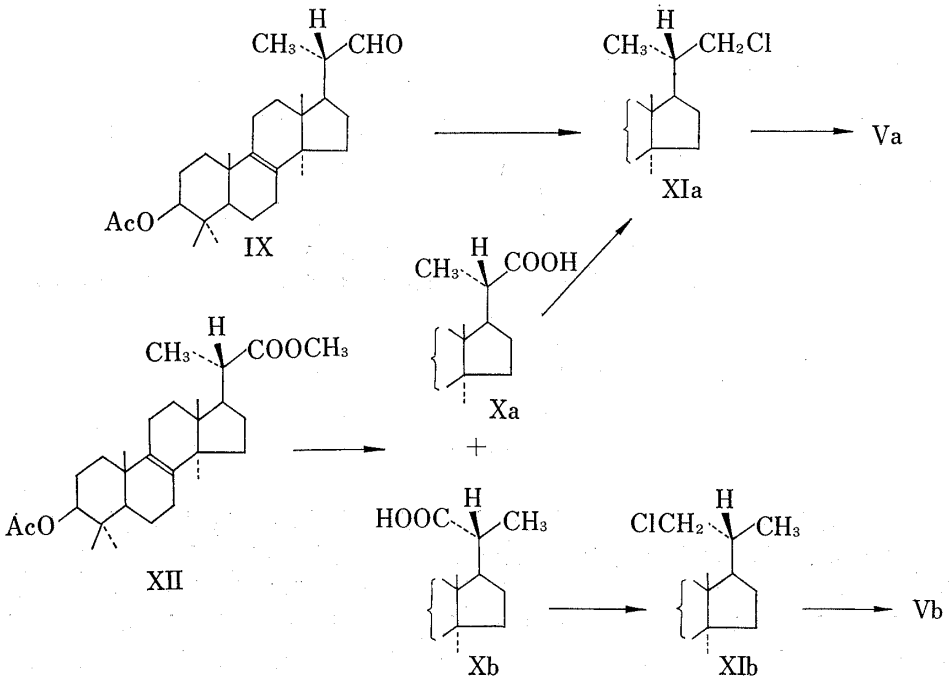
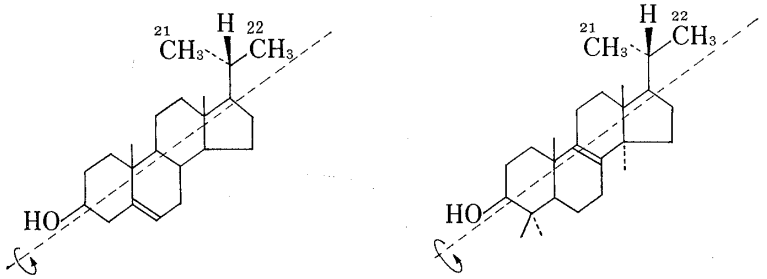


Chart 3

11) P. Diehl and Th. Leipert, *Helv. Chim. Acta*, **47**, 545 (1965); P. Diehl, "Nuclear Magnetic Resonance Spectroscopy of Nuclei Other than Protons," ed. by T. Axenrod and G.A. Webb, Wiley-Interscience, New York, 1975, p. 257.
12) L.H. Briggs, J.P. Bartley, and P.S. Rutledge, *J. Chem. Soc. Perkin I*, **1973**, 806.
13) G. Habermehl and G. Volkwein, *Ann. Chem.*, **742**, 145 (1970).
14) R.J. Anderson, R.P. Hanzlik, K.B. Sharpless, E.E. van Tamelen, and R.B. Clayton, *J. Chem. Soc. Chem. Commun.*, **1969**, 53; E.E. van Tamelen and J.H. Freed, *J. Am. Chem. Soc.*, **92**, 7206 (1970); S. Iwasaki, *Helv. Chim. Acta*, **59**, 2753 (1976).

resulted in recovery of the starting material. Accordingly, as an alternative, the methyl ester (XII) of 3β-acetoxy-23,24,25,26,27-pentanorlanost-8-en-22-oic acid (Xa)[13] was subjected to isomerization at C-20 according to the procedure of Hayatsu.[6] A solution of XII in diethylene glycol containing 12.5% potassium hydroxide was refluxed, and after acetylation of the products, column chromatography on Sephadex LH-20 afforded the 20*R*-carboxylic acid (Xb) and 20*S*-carboxylic acid (Xa) in 28 and 32% yields, respectively. The isomers, 20*R* and 20*S*, were distinguished by ^{1}H NMR, which showed the 21- and 22-methyl signals at 1.22 and 1.17 ppm for Xa and Xb, respectively. Xb thus obtained was transformed to the chloro compound and then into the deuterated compound (Vb) by hydrogenation with lithium aluminum deuteride as shown in Chart 3. The MS fragmentation pattern of Vb was the same as that of Va and the deuterium contents were $^2H_1$ 85% and $^2H_0$ 15%. However, ^{1}H NMR spectroscopy of the chloroform solutions of IV, Va and Vb failed to distinguish the chemical shifts of the 21- and 22-methyl groups, which resonate in almost the same region as other methyl and methylene groups. The ^{2}H NMR spectra of Va and Vb showed signals at 0.90 ppm without any difference between the deuterated 21- and 22-methyl groups. The lanthanide shift reagent,[15] Eu(dpm)$_3$, was employed to resolve the ^{1}H signals of the 21- and 22-methyl groups. A 0.1 M solution of IV in deuteriochloroform was treated serially with 0.25, 0.50, 0.75, and 1.0 molar equivalents of Eu(dpm)$_3$. Signal separation of the 21- and 22-methyl groups was obtained with downfield shifts in the presence of 1.0 molar equivalent of Eu(dpm)$_3$; the signals appeared at 0.99 and 1.07 ppm as two doublets. Accordingly, the ^{1}H NMR spectra of 0.1 M solutions of Va and Vb in deuteriochloroform were measured in the presence of 1.0 molar equivalent of Eu(dpm)$_3$. The ^{1}H signal decreased at 0.99 and 1.07 ppm for Va and Vb, respectively. Consequently, it can be concluded that in the presence of 1.0 molar equivalent of Eu(dpm)$_3$, the 21- and 22-methyl signals of IV appear at 1.07 and 0.99 ppm, respectively, with fairly good separation.



IIa: 22-CH_2D 0.14 sec
IIb: 21-CH_2D 0.19 sec

Va: 22-CH_2D 0.15 sec
Vb: 21-CH_2D 0.16 sec

Fig. 1. The Deuterium Spin-lattice Relaxation Times of the [21-$^2H_1$]- and [22-$^2H_1$]-Derivatives of 23,24,25,26,27-Pentanorcholesterol and 23,24,25,26,27-Pentanordihydrolanosterol

Generally, the ^{2}H spin-lattice relaxation time (T_1) is greatly affected by the anisotropic tumbling of the molecule, which is usually a rigid, rod-like molecule. The relaxation behavior of ^{2}H is dominated by a quadrupolar mechanism and therefore is indicative of the molecular dynamics (internal and/or overall motion) at the position of substitution. This makes interpretation of the relaxation data much simpler for ^{2}H than for ^{1}H or ^{13}C.[3,16] We examined the ^{2}H T_1's of IIa and IIb, as shown in Figure 1. In view of the molecular geometry, it may be possible to treat the molecular tumbling motion in solution as that of a symmetrical top,

15) A.F. Cockerill, G.L.O. Davies, R.C. Harden, and D.M. Rackham, *Chem. Rev.*, **73**, No. 6, 553 (1973).
16) I.C.P. Smith, *Aldrichimica Acta*, **10**, 35 (1977).

the principal axis being the dotted line across the steroid skeleton. Thus, the C-^{2}H vector of the deuterated 22-methyl group of IIa would be close to the principal axis compared with that of the deuterated 21-methyl group of IIb. The T_1 value of the deuterated 21-methyl group should be longer than that of the 22-methyl group, since the T_1 value of the latter is mainly influenced by the rather longer correlation time for the motion around the axis perpendicular to the principal axis. It should be emphasized here that the longer T_1 value is caused by a shorter correlation time, while the shorter T_1 value is caused by a longer correlation time. This expectation is consistent with the experimental results, giving T_1 values of 0.14 and 0.19 sec for IIa and IIb, respectively. Undoubtedly, this result arises because the rate of internal rotation around the C-17—C-20 bond is much slower than $1/T_1$ (~5 sec). Therefore the methyl groups in question have restricted orientation, in agreement with the view of Nes *et al.*[17] rather than the arguments of Gut *et al.*[18] on the stereochemistry of sterols at C-20.

On the other hand, we found that there appears to be no significant difference in the T_1 values of methyl groups between Va and Vb (0.15 and 0.16 sec, respectively). As indicated by CPK models, the molecular shapes of Va and Vb exhibit a "swelling," mainly due to the presence of three more methyl groups at the 4, 4 and 14 positions. Therefore, it is likely that the similarity of 21- and 22-^{2}H T_1 values arises from the rather isotropic nature of the tumbling motion, in contrast to that of IIa and IIb.

The present discussion should provide a useful basis for considering the relationship between stereochemistry and anisotropic motion in the field of steroid chemistry.

Experimental

All melting points were obtained on a micro-melting point apparatus, type MM2 (Shimadzu Seisakusho Ltd.), and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL JNM-100 NMR spectrometer at 100 MHz, using tetramethylsilane as an internal standard in deuteriochloroform. Abbreviations used: s=singlet, d=doublet, m=multiplet, bd=broad doublet, bs=broad singlet. ^{2}H NMR spectra were recorded with a JEOL PFT-100/EC-100 pulsed Fourier transform spectrometer operating at 15.28 MHz with proton-noise decoupling. A 90° pulse of 28 μs duration was employed with repetition time of 2.2 s, a spectral width of 500 Hz and 2K data points. All samples in chloroform solution were contained in 10 mm o.d. sample tubes. Field-frequency control was performed on the internal ^{19}F signal of C_6F_6, which was added in amounts of a few drops to the chloroform solution. Spin-lattice relaxation times (T_1's) were obtained using the pulse sequence of 180°—*t*—90°. Mass spectra (MS) were determined on a JEOL JMS D-100 spectrometer. Column chromatography was performed with Kanto Kagaku silica gel (100 mesh), Sephadex LH-20 (Pharmacia Fine Chemical), or aluminium oxide 90 (E. Merck, Darmstadt). The isotopic purities of $LiAl^2H_4$ and NaB^2H_4 were over 98% (E. Merck, Darmstadt).

"The usual work-up" refers to dilution with water, extraction with methylene chloride, washing to neutrality, drying over anhydrous sodium sulfate, filtration, and evaporation under reduced pressure.

Isomerization of (20*S*)-3β-Acetoxybisnorchol-5-en-22-al (VIa)——A solution of VIa (2 g) in benzene was adsorbed on aluminium oxide (50 g) and allowed to stand overnight at room temperature. Elution with benzene gave the 20*R*-aldehyde (VIb) and 20*S*-aldehyde (VIa) as a 2:1 mixture. 20*S*-Aldehyde: ^{1}H NMR δ (ppm); 0.72 (18-CH_3), 1.14 (21-CH_3, d, J=6.5 Hz), 9.58 (22-CHO, d, J=3 Hz). 20*R*-Aldehyde (VIb): ^{1}H NMR δ (ppm); 0.69 (18-CH_3), 1.05 (22-CH_3, d, J=6.5 Hz), 9.55 (21-CHO, d, J=5 Hz).

Oxidation of Aldehyde Mixture (VIa and VIb)——A solution of CrO_3 (30 mg) in acetic acid (5 ml) and water (0.2 ml) was added dropwise to a stirred solution of aldehyde mixture (VIa: VIb; 1:2) (100 mg) in benzene (10 ml) and acetic acid (10 ml) and the mixture was stirred at 0—4° for 2 hr. After usual work-up, the crude green-brown oil was column-chromatographed on Sephadex LH-20 (20 g); elution with chloroform gave the 20*R*-carboxylic acid (VIIb) (37 mg) in fractions 2—3 and the 20*S*-carboxylic acid (VIIa) (34 mg) in fractions 5—7. Recrystallization of the residue from fractions 2—3 from petroleum ether–methanol gave VIIb as colorless needles, mp 203—204° (lit.[6] mp 197—198°). *Anal.* Calcd for $C_{24}H_{36}O_4$: C, 74.19; H, 9.34. Found: C, 73.73; H, 9.08. MS *m/e*: 328 (M^+—AcOH, base peak), 313 (M^+—AcOH—CH_3). ^{1}H NMR δ (ppm): 0.70 (3H, s, 18-CH_3), 0.98 (3H, s, 19-CH_3), 1.14 (3H, d, J=6.5 Hz, 21-CH_3), 2.00 (3H, s, 3β-$OCOCH_3$), 4.60 (1H, m, 3α-H), 5.36 (1H, bd, 6-H).

3β-Acetoxy-22-chlorobisnorchol-5-ene (VIIIa)——A solution of 388 mg (1 mmol) of (20*S*)-3β-acetoxybisnorchol-5-en-22-oic acid (VIIa) in tetrahydrofuran (THF) (5 ml) was stirred with ethyl chloroformate

17) W.R. Nes, T.E. Varkey, and K. Krevitz, *J. Am. Chem. Soc.*, **99**, 260 (1977) and references cited therein.
18) E.N. Trachtenberg, C. Byon, and M. Gut, *J. Am. Chem. Soc.*, **99**, 6145 (1977).

(130 mg, 1.2 mmol) and triethylamine (120 mg, 1.2 mmol) at 0° for 20 min. To this was added a solution of $NaBH_4$ (380 mg, 10 mmol) in water (2 ml) at 0°, and the mixture was stirred at 0° for 2 hr. The usual work-up afforded the crystalline alcohol. To this were added $POCl_3$ (6 ml) and pyridine (50 ml), and the mixture was refluxed for 12 hr. After usual work-up, the brown residue was column-chromatographed on silica gel. Elution with benzene and recrystallization from MeOH gave VIIIa (295 mg) as colorless needles, mp 147—148°. *Anal.* Calcd for $C_{24}H_{37}ClO_2$: C, 73.34; H, 9.47. Found: C, 73.24; H, 9.54. MS *m/e*: 332 (M^+-AcOH, base peak), 317 ($M^+-AcOH-CH_3$). ^1H NMR δ (ppm): 0.70 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 1.10 (3H, d, J=6.5 Hz, 21-CH_3), 2.01 (3H, s, 3β-$OCOCH_3$), 3.30—3.70 (2H, two quartets, 22-CH_2), 4.56 (1H, m, 3α-H), 5.36 (1H, bd, 6-H).

3β-Acetoxy-21-chlorobisnorchol-5-ene (VIIIb)——Ethyl chloroformate and triethylamine were added to a THF solution of the 20*R*-carboxylic acid (VIIb), and after 20 min, $NaBH_4$ was added. The mixture was stirred for 2 hr and treated with $POCl_3$ in pyridine as described for the preparation of the 22-chloro compound (VIIIa). Recrystallization of the product from MeOH gave VIIIb as colorless needles, mp 155—156°. *Anal.* Calcd for $C_{24}H_{37}ClO_2$: C, 73.34; H, 9.47. Found: C, 73.80; H, 9.80. MS *m/e*: 332 (M^+-AcOH, base peak), 317 ($M^+-AcOH-CH_3$). ^1H NMR δ (ppm): 0.70 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 1.02 (3H, d, J=6.5 Hz, 22-CH_3), 2.01 (3H, s, 3β-$OCOCH_3$), 3.36—3.80 (2H, two quartets, 21-CH_2), 4.56 (1H, m, 3α-H), 5.36 (1H, bd, 6-H).

3β-Hydroxybisnorchol-5-ene (I)——$LiAlH_4$ (300 mg) was added to a solution of the 22-chloro compound (VIIIa) or the 21-chloro compound (VIIIb) (250 mg) in anhydrous dioxane (20 ml) and the mixture was refluxed for 12 hr. After usual work-up, the residue was column-chromatographed on silica gel. Elution with methylene chloride and recrystallization from MeOH gave I (62 mg) as colorless needles, mp 137—138°. *Anal.* Calcd for $C_{22}H_{36}O$: C, 83.48; H, 11.47. Found: C, 83.70; H, 11.57. MS *m/e*: 316 (M^+, base peak), 301 (M^+-H_2O), 283 ($M^+-CH_3-H_2O$), 231, 205. ^1H NMR δ (ppm): 0.68 (3H, s, 18-CH_3), 0.84 (3H, d, J=6.5 Hz, 22-CH_3), 0.93 (3H, d, J=6.5 Hz, 21-CH_3), 1.00 (3H, s, 19-CH_3), 3.50 (1H, m, 3α-H), 5.37 (1H, bd, 6-H).

[22-$^2H_1$]-3β-Hydroxybisnorchol-5-ene (IIa)——IIa was prepared from 100 mg of the 22-chloro compound (VIIIa) by the methods described above, except for the use of $LiAl^2H_4$ instead of $LiAlH_4$. After chromatography, the product was recrystallized from MeOH to give IIa (23 mg) as colorless needles, mp 137—138°. MS *m/e*: 317 (M^+ for $C_{22}H_{35}{}^2HO$; $^2H_1$ 98%, $^2H_0$ 2%), 302 (M^+-CH_3), 299 (M^+-H_2O), 284 ($M^+-CH_3-H_2O$), 232, 206. The ^1H NMR spectrum was identical with that of I, except for some decrease of the signal at 0.84 ppm. ^2H NMR δ (ppm): 0.86 (22-$CH_2{}^2H$, 8 mg, 200 transients). ^2H T_1: 0.14 sec.

[21-$^2H_1$]-3β-Hydroxybisnorchol-5-ene (IIb)——IIb was prepared from 100 mg of the 21-chloro compound (VIIIb) by the methods described above, except for the use of $LiAl^2H_4$ instead of $LiAlH_4$. After chromatography, the product was recrystallized from MeOH to give IIb (19 mg) as colorless needles, mp 137—138°. The mass spectrum was the same as that of IIa. The ^1H NMR spectrum was identical with that of I, except for some decrease of the signal at 0.93 ppm. ^2H NMR δ (ppm): 0.94 (21-$CH_2{}^2H$, 5 mg, 300 transients). ^2H T_1: 0.19 sec.

[22-$^2H_3$]-3β-Hydroxybisnorchol-5-ene (IIIa)——The synthetic procedure was essentially as described above for the preparation of I, except that NaB^2H_4 and $LiAl^2H_4$ were used instead of $NaBH_4$ and $LiAlH_4$, respectively. Starting from 388 mg (1 mmol) of the 20*S*-carboxylic acid (VIIa), 65 mg of IIIa was obtained, mp 137—138°. MS *m/e*: 319 (M^+ for $C_{22}H_{33}{}^2H_3O$); $^2H_3$ 74, $^2H_2$ 22, $^2H_1$ 3 and $^2H_0$ 1%. The ^1H NMR spectrum was identical with that of I, except for the disappearance of the signal at 0.84 ppm. ^2H NMR δ (ppm): 0.86 (22-C^2H_3, 5 mg, 980 transients).

[21-$^2H_3$]-3β-Hydroxybisnorchol-5-ene (IIIb)——The synthetic procedure was essentially as described above for the preparation of I, except that NaB^2H_4 and $LiAl^2H_4$ were used instead of $NaBH_4$ and $LiAlH_4$, respectively. Starting from 388 mg (1 mmol) of the 20*R*-carboxylic acid (VIIb), 49 mg of IIIb was obtained, mp 137—138°. The mass spectrum was the same as that described above. The ^1H NMR spectrum was identical with that of I, except for the disappearance of the signal at 0.93 ppm. ^2H NMR δ (ppm): 0.94 (21-C^2H_3, 5 mg, 1114 transients).

3β-Acetoxy-22-chloro-23,24,25,26,27-pentanorlanost-8-ene (XIa)——a) A solution of the 22-aldehyde (IX) (100 mg) in MeOH (100 ml) was treated with $NaBH_4$ (11 mg) and the mixture was stirred at room temperature for 12 hr. After usual work-up, the crude product (22-alcohol), without further purification, was dissolved in pyridine (25 ml). To this was added $POCl_3$ (3 ml) and the mixture was refluxed for 12 hr. After usual work-up, the brown residue was column-chromatographed on silica gel. Elution with benzene and recrystallization from MeOH gave XIa (55 mg) as colorless needles, mp 195°.

b) Ethyl chloroformate (130 mg, 1.2 mmol) and triethylamine (120 mg, 1.2 mmol) were added to a solution of (20*S*)-3β-acetoxy-23,24,25,26,27-pentanorlanost-8-en-22-oic acid (Xa) (430 mg, 1 mmol) in THF (5 ml) at 0° and the mixture was stirred at 0° for 20 min. To this was added a solution of $NaBH_4$ (380 mg, 10 mmol) in water (2 ml) at 0°, and the solution was stirred at 0° for 2 hr. The usual work-up of the reaction mixture afforded the crystalline alcohol. A solution of the alcohol in pyridine (50 ml) was treated with $POCl_3$ (6 ml), and the solution was refluxed for 12 hr. After usual work-up, the residue was column-chromatographed on silica gel. Elution with benzene and recrystallization from MeOH gave XIa (283 mg) as colorless needles, mp 195°. *Anal.* Calcd for $C_{27}H_{43}ClO_2$: C, 74.53; H, 9.96. Found: C, 74.33; H, 9.60. MS *m/e*:

434 (M^+ for ^{35}Cl-compound), 419 (M^+-CH_3), 359 (M^+-CH_3-AcOH, base peak). ^1H NMR δ (ppm): 0.72 (3H, s, 18-CH_3), 0.89 (9H, bs, 30-, 31- and 32-CH_3), 1.00 (3H, s, 19-CH_3), 1.06 (3H, d, J=6.5 Hz, 21-CH_3), 2.02 (3H, s, 3β-$OCOCH_3$), 3.32—3.72 (2H, two quartets, 22-CH_2), 4.52 (1H, m, 3α-H).

23,24,25,26,27-Pentanordihydrolanosterol (IV)——A solution of the 22-chloro compound (XIa) (50 mg) in anhydrous dioxane (10 ml) was treated with $LiAlH_4$ (100 mg) and the mixture was refluxed for 12 hr. After usual work-up, the residue was column-chromatographed on silica gel. Elution with methylene chloride and recrystallization from MeOH gave IV (25 mg) as colorless needles, mp 190—191°. *Anal.* Calcd for $C_{25}H_{42}O$: C, 83.73; H, 11.81. Found: C, 83.34; H, 11.46. MS *m/e*: 358 (M^+), 343 (M^+-CH_3, base peak), 325 ($M^+-CH_3-H_2O$). ^1H NMR δ (ppm): 0.70 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 3.28 (1H, m, 3α-H).

[22-$^2H_1$]-23,24,25,26,27-Pentanordihydrolanosterol (Va)——Starting from 50 mg of the 22-chloro compound (XIa), Va was prepared in the manner described above, except that $LiAl^2H_4$ was used instead of $LiAlH_4$. The product was recrystallized from MeOH to give Va as colorless needles, mp 190—191°. MS *m/e*: 359 (M^+ for $C_{25}H_{41}{}^2HO$); $^2H_1$ 94%, $^2H_0$ 6%. ^1H NMR δ (ppm): 0.70 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 3.28 (1H, m, 3α-H). ^2H NMR δ (ppm): 0.90 (22-$CH_2{}^2H$, 4 mg, 748 transients). ^2H T_1: 0.15 sec.

(20*R*)-3β-Acetoxy-23,24,25,26,27-pentanorlanost-8-en-22-oic Acid (Xb)——The (20*S*)-acid (Xa) (860 mg) was treated with a solution of diazomethane in ether to give the methyl ester (XII). KOH (2.5 g) was added to a solution of XII (600 mg) in diethylene glycol (20 ml), and the mixture was heated at 190° for 6 hr. After cooling, the mixture was acidified with 10% HCl. After usual work-up of the mixture, the residue was treated with an Ac_2O–pyridine mixture and the acetylation product was column-chromatographed on Sephadex LH-20. Elution with chloroform gave Xb (140 mg) in fractions 6—11 and Xa (165 mg) in fractions 13—16. Recrystallization of Xb from MeOH gave colorless needles, mp 195—197°. *Anal.* Calcd for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.55; H, 9.90. MS *m/e*: 430 (M^+), 415 (M^+-CH_3), 355 (M^+-CH_3-AcOH, base peak). ^1H NMR δ (ppm): 0.74 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 1.17 (3H, d, J=6.5 Hz, 21-CH_3), 2.02 (3H, s, 3β-$OCOCH_3$).

3β-Acetoxy-21-chloro-23,24,25,26,27-pentanorlanost-8-ene (XIb)——The 20*R*-carboxylic acid Xb (50 mg) was reduced with $NaBH_4$ in the presence of ethyl chloroformate and triethylamine, and the product was treated with $POCl_3$–pyridine mixture as described for the preparation of the 22-chloro compound (XIa). Recrystallization of the product from MeOH gave XIb as colorless needles, mp 170—171°. *Anal.* Calcd for $C_{27}H_{43}ClO_2$: C, 74.53; H, 9.96. Found: C, 74.78; H, 10.08. MS *m/e*: 434 (M^+ for ^{35}Cl-compound), 419 (M^+-CH_3), 359 (M^+-CH_3-AcOH). ^1H NMR δ (ppm): 0.72 (3H, s, 18-CH_3), 0.89 (9H, bs, 30-, 31- and 32-CH_3), 1.00 (3H, s, 19-CH_3), 1.02 (3H, d, J=6.5 Hz, 22-CH_3), 2.02 (3H, s, 3β-$OCOCH_3$), 3.32—3.80 (2H, two quartets, 21-CH_2), 4.52 (1H, m, 3α-H).

[21-$^2H_1$]-23,24,25,26,27-Pentanordihydrolanosterol (Vb)——Compound XIb (10 mg) was reduced with $LiAl^2H_4$ and the product was worked up as described for the preparation of the [22-$^2H_1$]-compound (Va). mp 190—191°. MS *m/e*: 359 (M^+ for $C_{25}H_{41}{}^2HO$); $^2H_1$ 85%, $^2H_0$ 15%. ^1H NMR δ (ppm): 0.70 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 3.28 (1H, m, 3α-H). ^2H NMR δ (ppm): 0.90 (21-$CH_2{}^2H$, 3 mg, 1263 transients). ^2H T_1: 0.16 sec.