Synthesis of Cholestanes containing an Oxygenated 14α-Methyl Group

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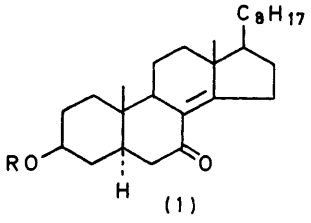
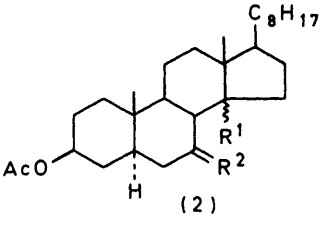
Hydrocyanation of 3β-acetoxy-5α-cholest-8(14)-en-7-one has been achieved by reaction with diethylaluminium cyanide. The resulting 14α-cyano-7-ketone was reduced with sodium borohydride to the 7α-hydroxy-compound, the methanesulphonate of which, when refluxed in collidine, gave 3β-acetoxy-5α-cholest-7-ene-14α-carbonitrile. Reduction of the cyano-group with di-isobutylaluminium hydride afforded 3β-hydroxy-5α-cholest-7-ene-14α-carbaldehyde, which was transformed with lithium aluminium hydride into 14α-hydroxymethyl-5α-cholest-7-en-3β-ol.

32-OXYGENATED derivatives of lanosterol are of interest as probable intermediates in the biosynthesis of cholesterol.[1] Such compounds have been obtained *via* functionalization of the 32-methyl groups of a number of 7α-hydroxylanostane derivatives.[2] However, cholestanes containing an oxygenated 14α-methyl group can also be



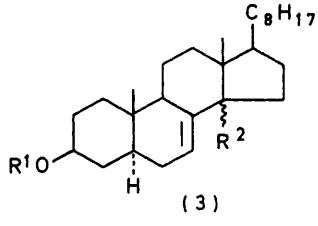
(1)

	R
a;	Ac
b;	OH



(2)

	R^1	R^2
a;	α-CN	:O
b;	α-CN	H, α-OH
c;	α-CH_3	H, α-OH
d;	β-H	:O
e;	α-CN	H,α-O_3SMe
f;	α-CN	:N·NHTs



(3)

	R^1	R^2
a;	Ac	α-CN
b;	Ac	α-H
c;	H	α-CHO
d;	Ac	β-H
e;	H	α-CH_2·OH
f;	Ac	α-CH_2·OAc

utilized in biosynthetic work; such compounds have been obtained in low yield *via* oxygenation of the 14-methyl group of 3β-acetoxy-7α-hydroxy-14α-methyl-5α-cholestane.[3] In connection with our studies on the elimination of the 32-methyl group of lanosterol,[4] we have developed a convenient synthesis of this type of compound *via* addition of an ' extra 'carbon atom to a cholestane derivative.

Treatment of 3β-acetoxy-5α-cholest-8(14)-en-7-one (1a)[5] with diethylaluminium cyanide[6] gave 3β-acetoxy-7-oxo-5α-cholestane-14α-carbonitrile (2a) as the only product, in 86% yield. That the product (2a) had the ' natural ' 8β,14α-configuration was demonstrated as follows. Treatment with methanolic sodium hydroxide and reacetylation † had no effect on the properties of this ' ketone '; therefore C-8 is in the thermodynamically most stable configuration. Molecular models indicate that the 8α,14α- and the 8α,14β-configurations are considerably less stable than the 8β,14α- and 8β,14β-configurations, and even if formed initially the 8α-structure would be expected to rearrange in the presence of base to the more stable epimer of ' natural ' stereochemistry. Therefore if (2a) possesses a 14α-cyano-group, reduction of the 7-ketone with sodium borohydride would be expected to proceed predominantly by β-attack and so produce relatively more of the 7α-alcohol than does ' natural ' 3β-hydroxy-5α-cholestan-7-one (73% α, 27% β).[7] In fact only one alcohol (as shown by g.l.c., t.l.c., n.m.r., *etc.*) was obtained in crystalline form, although chromatographic analysis indicated that a small amount of another, more polar epimer (*ca.* 5%) was present in the mother liquors. The axial configuration of the hydroxy group in the product 3β-acetoxy-7α-hydroxy-5α-cholestane-14α-carbonitrile (2b) was evident from the relative difficulty of acetylation[8] and from the n.m.r. spectrum. A peak at δ 3.92, identical in shape with the peak at δ 3.95 in the spectrum of 3β-acetoxy-7α-hydroxy-14α-methyl-5α-cholestane (2c),[3] was assigned to the 7β-proton.[3] A 7α-proton would be strongly coupled and should give rise to a broad multiplet.

The position of the signal for the C-18 protons in the

† The nitrile function was smoothly eliminated with potassium t-butoxide in t-butyl alcohol to give 3β-hydroxy-5α-cholest-8(14)-en-7-one (1b).

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[2] P. L. Batten, T. J. Bentley, R. B. Boar, R. W. Draper, J. F. McGhie, and D. H. R. Barton, *J.C.S. Perkin I*, 1972, 739, and references cited therein.

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[5] M. Anastasia, A. Fiecchi, and A. Scala, *J.C.S. Perkin I*, 1976, 378.

[6] W. Nagata, M. Yoshioka, and T. Teresawa, *J. Amer. Chem. Soc.*, 1972, **94**, 4672, and references cited therein.

[7] W. G. Dauben, E. J. Blanz, jun., J. Jiu, and R. A. Micheli, *J. Amer. Chem. Soc.*, 1956, **78**, 3752.

[8] J. Fried, J. W. Brown, and L. Borkenhagen, *Tetrahedron Letters*, 1965, 2499.

n.m.r. spectrum of (2a) strongly suggested the 8β,14α-configuration. It has been reported [9] that the cyano-group of a steroidal 14β-cyano-ketone causes a downfield shift in the C-18 proton resonance in comparison with the 14β-H ketone. The C-18 proton signal of 3β-acetoxy-5α,14β-cholestan-7-one (2d) [5] occurs at δ 0.96, whereas the observed value (δ 0.8) for same protons in (2a) corresponds to an upfield shift. Therefore it is reasonable to exclude the 8β,14β-configuration for (2a).

Attempted dehydration of 3β-acetoxy-7α-hydroxy-5α-cholestane-14α-carbonitrile (2b) to 3β-acetoxy-5α-cholest-7-ene-14α-carbonitrile (3a) with thionyl chloride or phosphoryl chloride in pyridine was unsuccessful. However, compound (3a) was obtained in high yield by treating compound (2b) with methanesulphonyl chloride in pyridine to furnish the mesyloxy-nitrile (2e), followed by heating in collidine for 16 h. In an alternative approach, the cyano-ketone (2a) was transformed into the tosylhydrazone (2f), which afforded compound (3a) on treatment with lithium hydride in toluene.[10] N.m.r. and mass spectra (M^+ 453) confirmed structure (3a). The C-18 proton signal occured at δ 0.64, downfield with respect to that in the 14α-unsubstituted compound (3b) (δ 0.533).[11] Attempts to isomerise (3a) to 3β-acetoxy-5α-cholest-8-ene-14α-carbonitrile under a variety of acidic conditions (*cf.* ref. 12) were unsuccessful.

Various types of cyanide have been successfully treated with di-isobutylaluminium hydride to give aldehydes.[13] Accordingly, treatment of compound (3a) with this reagent, followed by acidic hydrolysis, afforded 3β-hydroxy-5α-cholest-7-ene-14α-carbaldehyde (3c) in 85% yield. This method represents an improvement with respect to reduction with lithium aluminium hydride.[2] The mass spectrum of (3c) showed peaks at *m/e* 414 (M^+) and 385 (corresponding to loss of the 14α-formyl group). The n.m.r. spectrum showed a singlet for the aldehydic proton (δ 9.5) and a multiplet at δ 5.4 for the C-7 vinylic proton.

Compound (3c) was decarbonylated by refluxing in benzene with chlorotris(triphenylphosphine)rhodium for 12 h, and the products were acetylated. Aldehyde decarbonylation is known to proceed with retention of configuration.[14] In our case 3β-acetoxy-5α-cholest-7-ene (3b) or its 14β-epimer (3d) [5] would be obtained. Indeed compound (3b) was obtained, in low yield, with minor amounts of 3β-acetoxy-5α-cholest-8(14)-ene. This supports the stereochemical assignment of all 14-functionalized compounds so far described.

Reduction of the aldehyde (3c) with lithium aluminium hydride gave 14α-hydroxymethyl-5α-cholest-7-en-3β-ol (3e). The acetate (3f) was identical with the compound described by Knight *et al.*[3] thus confirming the configurational assignments of the C-14 substituents.

Adoption of this type of sequence for the synthesis of ring D-functionalized steroids will be considered in future publications. Biosynthetic experiments complementary to the foregoing work are in progress.

EXPERIMENTAL

I.r. spectra were taken for solutions in $CHCl_3$; n.m.r. data relate to 10% solutions in $CDCl_3$ with tetramethylsilane as internal standard, and $[\alpha]_D$ values to 1% solutions in $CHCl_3$. Mass spectra were obtained with an LKB 9000 gas chromatograph–mass spectrometer either by g.l.c. (2 m silanized glass column of 3% SE 30 on GasChrom Q, operating at 240–260 °C) or by direct insertion (d.i.). Column chromatography was carried out with hexane–benzene or benzene-ether on silica gel G–Celite (50 : 50 v/v). Reactions and column chromatographic fractions were monitored by t.l.c., g.l.c., and (when necessary) g.l.c.–mass spectrometry.

Reaction of 3β-*Acetoxy*-5α-*cholest*-8(14)-*en*-7-*one* (1a) *with Diethylaluminium Cyanide.*—The enone (1a) (840 mg) in benzene (4 ml) was treated with diethylaluminium cyanide (16 ml of 0.7M-solution in benzene) at 0 °C for 1 h. The mixture was poured dropwise onto a solution of sodium potassium tartrate and extracted with dichloromethane. Crystallization of the crude product from diethyl ether gave 3β-*acetoxy*-7-*oxo*-5α-*cholestane*-14α-*carbonitrile* (2a) (766 mg), m.p. 178—180 °C; $[\alpha]_D^{20}$ 6.1; $\nu_{max.}$ 2 240, 1 735, and 1 720 cm^{-1}; δ 1.09 (3 H, s, 10β-Me), 0.8 (3 H, s, 13β-Me); M^+ (g.l.c.) 469 (Found: C, 76.55; H, 10.2; N, 2.9. $C_{30}H_{47}NO_3$ requires C, 76.7; H, 10.1; N, 3.0%).

3β-*Hydroxy*-5α-*cholest*-8(14)-*en*-7-*one* (1b).—The carbonitrile (2a) (150 mg) dissolved in dry, refluxing t-butyl alcohol (13 ml), was treated with potassium t-butoxide (120 mg) in t-butyl alcohol (2.5 ml). The mixture was refluxed in nitrogen for 1 h, acidified with 2N-hydrochloric acid, and poured into water. Extraction with ether gave a pale yellow solid (125 mg). This crystallised from methanol to give 3β-*hydroxy*-5α-*cholest*-8(14)-*en*-7-*one* (1b)(110 mg), m.p. 126—127°; $[\alpha]_D$ −54°; $\nu_{max.}$ 3 300 and 1 680 cm^{-1} (Found: C, 80.8; H, 11.1. $C_{27}H_{44}O_2$ requires C, 80.95; H, 11.05%).

Reduction of the Ketone (2a).—The ketone (2a) (940 mg) in diethyl ether (15 ml) and methanol (50 ml) was treated with sodium borohydride (850 mg) at 20 °C for 30 min. The usual work-up gave a crude product which was crystallized from diethyl ether to yield 3β-*acetoxy*-7α-*hydroxy*-5α-*cholestane*-14α-*carbonitrile* (2b) (840 mg), m.p. 189 °C; $[\alpha]_D^{20}$ +11°; $\nu_{max.}$ 3 600, 2 220, and 1 730 cm^{-1}; δ 3.92 (1 H, m, 7α-H), 0.82 (3 H, s, 10β-Me), and 0.76 (3 H, s, 13β-Me); M^+ (d.i.) 471 (Found: C. 76.2; H, 10.5; N, 2.85. $C_{30}H_{49}NO_3$ requires C, 76.45; H, 10.2; N, 2.9%).

3β-*Acetoxy*-7α-*methylsulphonyloxy*-5α-*cholestane*-14α-*carbonitrile* (2e).—A solution of the 7α-ol (2b) (600 mg) in dry pyridine (20 ml) was cooled to 0 °C. Redistilled methanesulphonyl chloride (1 ml) was added dropwise with shaking. The solution was allowed to warm to 4 °C and, after a further 2 h, was poured into water. Extraction with diethyl ether and crystallization from methanol gave the *methanesulphonate* (2e) (595 mg), m.p. 175—176 °C (varied with rate of

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[10] L. Caglioti, P. Grasselli, and G. Maina, *Chimica e Industria*, 1963, **43**, 559.
[11] N. S. Baccha and D. H. Williams, ' Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry,' Holden-Day, San Francisco, 1966, p. 18.
[12] R. E. Marker, E. L. Witte, and L. W. Nixon, *J. Amer. Chem. Soc.*, 1937, **59**, 1368; J. F. Cavalla, J. F. McGhie, and M. K. Pradhan, *J. Chem. Soc.*, 1951, 3142.
[13] E. Winterfeldt, *Synthesis*, 1975, 617.
[14] H. M. Walborsky and L. E. Allen, *Tetrahedron Letters*, 1970, 823.

heating); $[\alpha]_D^{21}$ +13°, $\nu_{max.}$ 2 240, 1 730, and 1 335 cm^{-1} (Found: C, 67.95; H, 9.05; N, 2.45. $C_{31}H_{51}NO_5S$ requires C, 67.8; H, 9.3; N, 2.55%).

3β-*Acetoxy-5α-cholest-7-ene-14α-carbonitrile*(3a).—A solution of the methanesulphonate (2e) (960 mg) in dry collidine (30 ml) was refluxed in dry nitrogen for 16 h. The mixture was poured into water and extracted with ether. The combined extracts were washed with 2N-hydrochloric acid, then water, and dried. Evaporation left a residue (950 mg) which was chromatographed on silica gel G–Celite–$AgNO_3$ to give the pure 7-*ene* (3a) (710 mg), m.p. 113—114° (from methanol); $[\alpha]_D^{20}$ +41°; $\nu_{max.}$ 2 225 and 1 735 cm^{-1}; δ 5.4 (1 H, m, 7β-H), 0.82 (3 H, s, 10β-Me), and 0.64 (3 H, s, 13β-Me); M^+ (d.i.) 453 (Found: C, 79.35; H, 10.55; N, 3.2. $C_{30}H_{47}NO_2$ requires C, 79.45; H, 10.15; N, 3.1%).

Caglioti Reduction of the Ketone (2a).—The ketone (2a) (0.46 g), toluene-*p*-sulphonohydrazide (460 mg), methanol (80 ml), and concentrated hydrochloric acid (2 drops) were refluxed for 1 h under nitrogen; the mixture was then evaporated and the product isolated by standard techniques. Recrystallization from methanol afforded crystals of the *tosylhydrazone* (2f) (470 mg), m.p. 160° (decomp.), $\nu_{max.}$ 3 310, 2 225, 1 725, and 1 605 cm^{-1} (Found: C, 70.05; H, 8.5; N, 6.65. $C_{37}H_{55}N_3O_4S$ requires C, 69.7; H, 8.75; N, 6.6%). The tosylhydrazone (500 mg) and toluene (30 ml) were refluxed under nitrogen with sodium hydride (50 mg) for 3 h. The mixture was then filtered into 5% sulphuric acid. Conventional treatment afforded a crude product which was chromatographed. 80% Hexane–benzene eluted compound (3a) (300 mg), m.p. 113—114° (from methanol), identical (m.p., mixed m.p., and n.m.r. and mass spectra) with that described above.

Reduction of the Nitrile (3a).—The nitrile (300 mg) in benzene (15 ml) was cooled to −10 °C and di-isobutylaluminium hydride (0.25 ml) in benzene (2 ml) was added under nitrogen. The mixture was stirred at −10 °C for 30 min, then ethyl acetate (1 ml) was added and the stirring was continued for 1 h. N-Sulphuric acid (15 ml) was added and the mixture was refluxed for 2 h under nitrogen. The product was extracted with benzene, and the organic layer was washed with saturated aqueous sodium hydrogen carbonate and water. Evaporation gave a solid which was crystallised from methanol to yield 3β-*hydroxy-5α-cholest-7-ene-14α-carbaldehyde* (3c) (250 mg), m.p. 125°; $[\alpha]_D^{21}$ +32.5°; $\nu_{max.}$ 3 600, 3 450, 2 715, and 1 710 cm^{-1}; δ 9.5 (1 H, s, CHO), 0.82 (3 H, s, 10β-Me), and 0.74 (3 H, s, 13β-Me); M^+ (d.i.) 414 (Found: C, 81.2; H, 11.25. $C_{28}H_{46}O_2$ requires C, 81.1; H, 11.2%).

Reduction of the Aldehyde (3c).—The aldehyde (160 mg) in anhydrous diethyl ether (15 ml) was treated with lithium aluminium hydride (100 mg), then stirred for 5 h at 25 °C. The excess of hydride was decomposed with ethyl acetate; water was then added and the mixture was worked up in the usual manner to give a product which solidified on cooling in ice. 14α-*Hydroxymethyl-5α-cholest-7-en-3β-ol* (3e) (100 mg) had m.p. 124—125° (from methanol); $[\alpha]_D$ +15°; $\nu_{max.}$ 3 695 and 3 450 cm^{-1}; M^+ 416; δ 5.28 (1 H, m, 7-H), 3.64 (1 H, d, *J* 12, 14α-CH_2), 3.18 (1 H, d, *J* 12, 14α-CH_2), 3.58 (1 H, m, 3α-H), 0.82 (3 H, s, 10β-Me), and 0.72 (3 H, s, 13β-Me) (Found: C, 80.6; H, 11.55. $C_{28}H_{48}O_2$ requires C, 80.7; H, 11.65%). Acetylation with acetic anhydride–pyridine gave the acetate (3f), m.p. 103—105° (from methanol), $[\alpha]_D^{21}$ +18°, identical (m.p.; n.m.r. and mass spectra) with the compound described by Knight[3] (Found: C, 77.1; H, 10.5. $C_{32}H_{52}O_4$ requires C, 76.75; H, 10.45%).

Treatment of the Aldehyde (3c) *with Chlorotris(triphenylphosphine)rhodium.*—A solution of the aldehyde (100 mg) in anhydrous benzene (10 ml) was refluxed in the presence of chlorotris(triphenylphosphine)rhodium (100 mg) for 12 h. The solvent was removed in a stream of nitrogen and the residue was filtered through a pad of silica gel. The product was acetylated and chromatographed to yield a mixture (40 mg) of 3β-acetoxy-5α-cholest-8(14)-ene (30%) and 3β-acetoxy-5α-cholest-7-ene (70%), identified by g.l.c. retention time and mass spectrometry. No 3β-acetoxy-5α,14β-cholest-7-ene was present.[5]

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