The Synthesis of some Cholesterol Derivatives as Probes for Mechanisms of Cholesterol Metabolism

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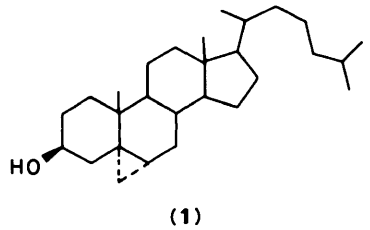
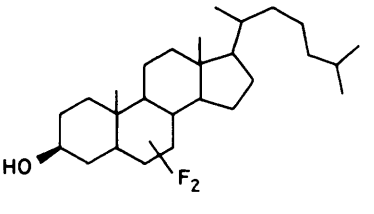
The syntheses of a series of cyclopropa[5,6]cholestanes, difluorocholestanes, and a ring B azacholestenone are described. Cyclopropa[5,6]cholestane-3,7-diols and their oxo derivatives were prepared from 3β-acetoxy-5α-cholest-5-en-7-one and a new route to 5α,6α-cyclopropa[5,6]-cholestan-3β-ol was developed. 7,7-and 6,6-Difluorocholestan-3-ols were obtained from fluorination of the acetoxy ketone precursors with sulphur tetrafluoride. 3β-Hydroxy-6-azacholest-4-en-7-one was prepared *via* the 3β-acetate by ozonolysis and ammonolysis of 3β-acetoxy-5α-cholest-4-en-7-one. The products have been used to study the mechanism of oxidation of cholest-5-en-3β-ol by its 7α-hydroxylase.

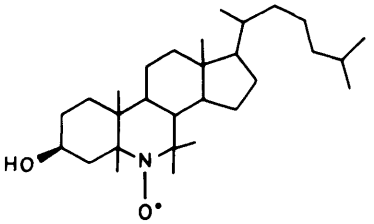
Cholest-5-en-5β-ol (cholesterol) 7α-hydroxylase is an enzyme of the cytochrome P-450 class and is of interest both in terms of the mechanism of hydroxylation and because it is the rate limiting enzyme in the oxidation of cholesterol to bile acids.[1] We designed a series of cyclopropa, fluoro, and aza steroids to act as probes of the mechanism of this oxidation and in addition to have potential in studying wider aspects of cholesterol metabolism.[2] Substrates containing a cyclopropane ring, under carefully controlled circumstances,[3–7] have found use in probing for radical intermediates in enzyme-catalysed reactions and their models, although their limitations have been recognised.[3–5,8,9] Since, so far, such derivatives have not been used to study the chemistry of a major steroid metabolising system, we decided to synthesize 5α,6α-cyclopropa[5,6]cholest-3β-ol (**1**)[10] by a new route that would also afford a series of 7-oxidised derivatives. To complement this probe, we chose to prepare the fluoro steroids (**2**) which, in addition to modifying the electronic characteristics of the oxidation site in ring B, would also be useful as more general probes of steroid metabolism and membrane properties through ^{19}F n.m.r. spectroscopy.[11] Finally, we attempted the synthesis of a spin probe (**3**).



(**1**)

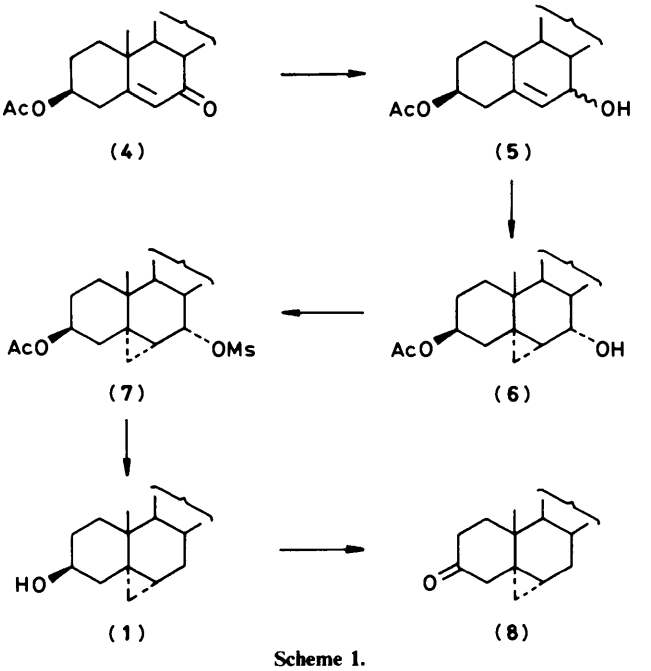


(**2**)



(**3**)

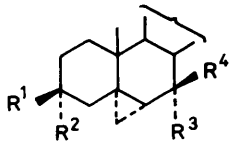
Synthesis of Cyclopropa-steroids.—5α,6α-Cyclopropa[5,6]-cholestan-3β-ol (**1**) has been synthesized by Templeton and Wie[10] from *epi*-cholesterol. However, since we wished to be able to identify oxidation products of (**1**) produced by cholesterol 7α-hydroxylase, a synthetic route that led to 7-oxidised derivatives was required. Accordingly (Scheme 1) 3β-acetoxycholest-5-en-7-one (**4**)[12] was reduced with sodium borohydride to afford a mixture of the epimeric 7-ols (**5**).[13] The mixture was cyclopropanated by the Simmons-Smith technique following Templeton and Wie[10] to afford the acetoxycyclopropasterol (**6**) in good yield. The ^1H n.m.r. spectrum of this product showed the 7-H resonance (δ 3.62) as a double doublet (*J* 5 and 8 Hz) which is consistent with the α-cyclopropa-α-ol structure shown for (**6**).



Scheme 1.

This result was confirmed by spectra of derivatives and represents the expected course of reaction.[10] The cyclopropasterol (**6**) was mesylated to afford (**7**) and the product, without extensive purification, was reduced with lithium aluminium hydride to afford 5α,6α-cyclopropa[5,6]cholestan-3β-ol (**1**). Unfortunately, the final reduction was not clean, mass spectroscopic analysis suggesting that a dihydro derivative had formed. The product was extensively purified by preparative thick-plate chromatography on silica gel. Although a crystalline product was not obtained, the sample was chromatographically homogeneous and showed spectroscopic properties in agreement with those previously described.[10] A sample was oxidised with Jones reagent to the corresponding 3-one[10] and this material (**8**) was reduced with tritiated sodium borohydride to afford a labelled sample of (**1**) for biochemical experiments.[14]

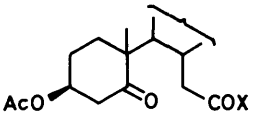
To provide reference samples to assist in the characterisation of enzymic oxidation products of (**1**), the first prepared cyclopropasteroid (**6**) was subjected to a series of hydrolysis (methanolic potassium hydroxide) and oxidation reactions (Jones reagent) leading to compounds (**9**—**12**).



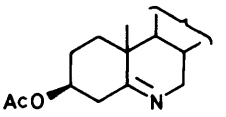
	R^1	R^2	R^3	R^4
(**6**)	AcO	H	HO	H
(**9**)	HO	H	HO	H
(**10**)	O		O	
(**11**)	AcO	H	O	
(**12**)	HO	H	O	

Fluorosterols.—Diethylaminosilylsulphur trifluoride and sulphur tetrafluoride are regarded as general reagents for the conversion of cycloalkyl ketones into the corresponding gem-difluorides.[15] To obtain a close ring B fluorinated analogue of cholesterol, we attempted the fluorination of the enone (**4**) with the milder of the two reagents diethylaminosilylsulphur trifluoride. We were unable to obtain clean fluorination under many reaction conditions. However, fluorination (Scheme 2) of the saturated ketone (**13**),[16] obtained by hydrogenation of (**4**), with sulphur tetrafluoride proceeded cleanly and in good yield to afford the acetoxydifluorocholestane (**14**) which was hydrolysed to give the required sterol (**15**). The 6,6-difluoro (**18**) analogue was obtained similarly *via* its acetate (**17**) from the 3-acetoxy ketone (**16**) which was obtained by oxidation of cholesteryl acetate.[17]

Aza Steroids.—Our initial strategy for the synthesis of spin probe molecules (**3**) was to prepare the aza steroid (**22**) following Lettre and Knopf[18]. Accordingly, cholesteryl acetate was oxidised to the seco oxo acid (**19**)[12] which was converted as planned into the acid chloride (**20**) and azide (**21**). We were unable to convert the azide (**21**) into the required aza steroid (**22**) under the published conditions or many variations. We



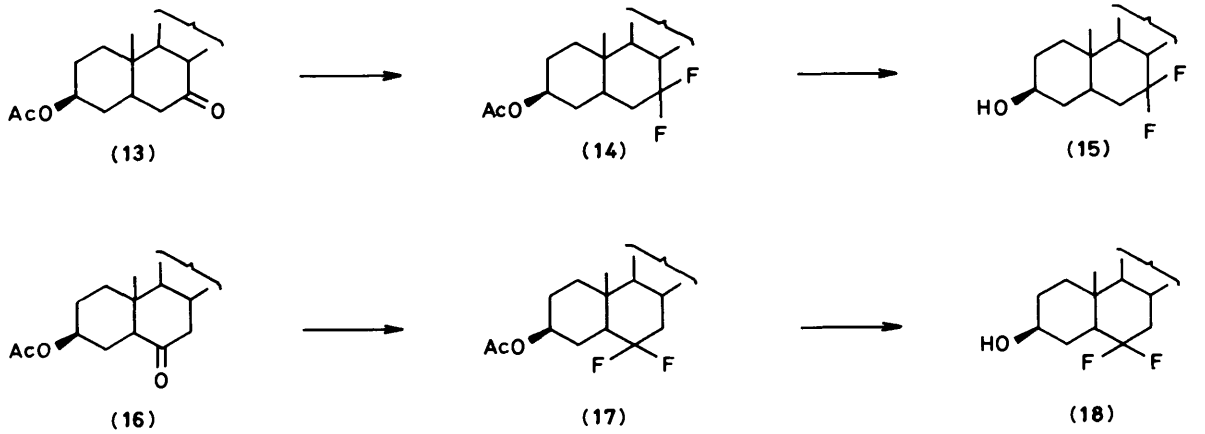
(**19**) X = OH
(**20**) X = Cl
(**21**) X = N_3



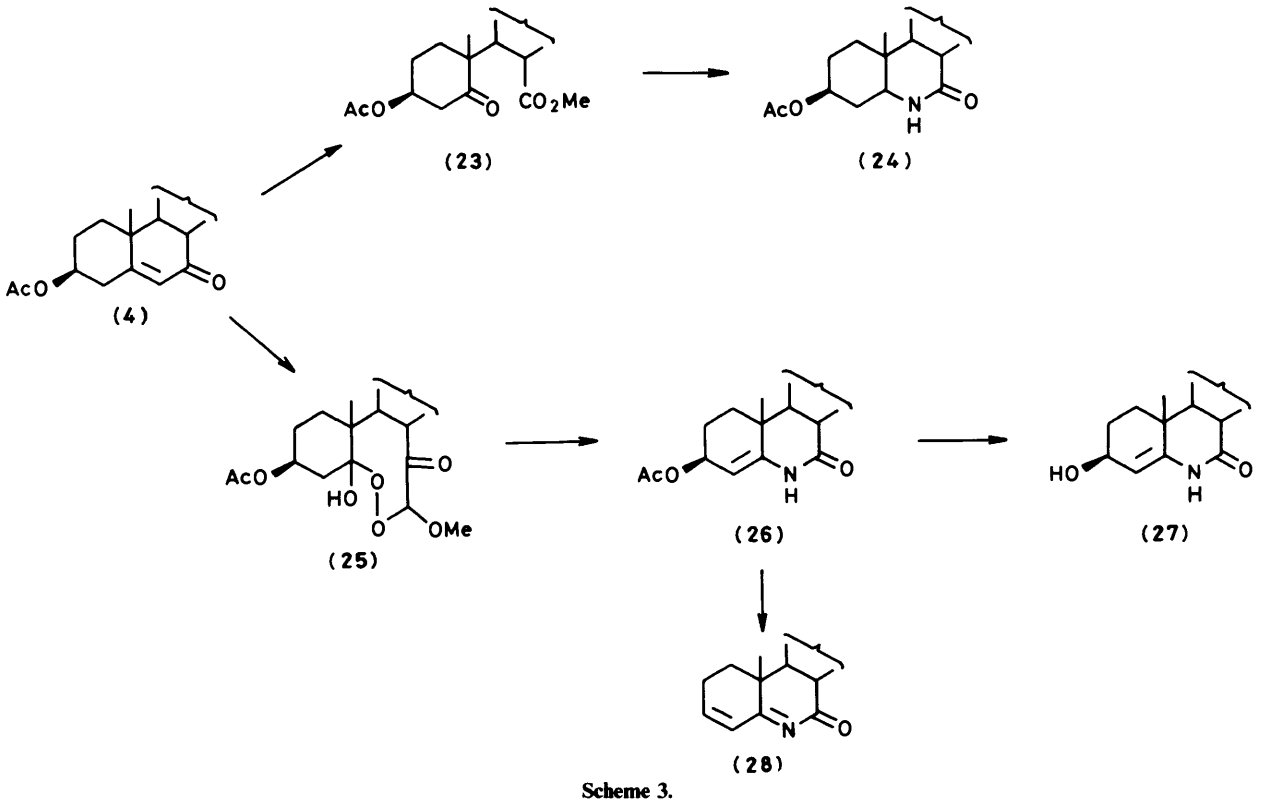
(**22**)

therefore obviated the need for azide rearrangement and subsequent decarboxylation by ozonising the enone (**4**), thereby removing the surplus carbon atom (Scheme 3). Such a strategy for the incorporation of nitrogen into rings A[19,20] and D[21,22] has been successful. Ozonolysis of (**4**)[23] in methanolic solution led to the oxo ester (**23**). It proved impossible to cyclise ring B with ammonia or hydroxyl amine because of facile elimination of the β-acetate. However if the primary ozonolysis product (**25**) was treated with ammonia at −78 °C, the aza steroid (**26**) was obtained in good yield. Similar conversions have been achieved in aromatic heterocyclic compounds.[24] Under very mild conditions, the acetoxy aza steroid (**26**) was hydrolysed to afford the aza sterol (**27**), the enzyme chemistry of which is being studied. More vigorous hydrolysis led to elimination of the 3β-acetate to form the aza dienone (**28**).

We have so far been unable to transform the accessible aza steroid (**26**) into compounds closer to the target spin probe. Although it is attractively functionalised, the ready elimination of acetate impedes reactions with nucleophilic reagents and its enamine character complicates reactions with electrophilic reagents. We have made many attempts to circumvent these difficulties. For example the analogous series of reactions (Scheme 3) starting from the tetrahydropyranol ether in place of the acetate failed to yield a clean aza steroid. The acetate (**26**) was also resistant to catalytic reduction and to formation of imino esters. Much more work is required to capitalise upon the facile new entry into ring B aza steroids provided by the reactions in Scheme 3.



Scheme 2.



Scheme 3.

Experimental

^{1}H N.m.r. spectra were determined using (a) Perkin Elmer R spectrometer at 90 MHz or, (b) Bruker WH-250 spectrometer at 250 MHz with tetramethylsilane as internal standard. Mass spectra were obtained using an AEI-Kratos MS 902 spectrometer progammed for high resolution spectroscopy by Dr. P. Bladon. M.p.s were determined in capillary tubes using an Electrothermal apparatus and are uncorrected.

The following compounds were prepared by published procedures: 3β-Acetoxycholest-5-en-7-one (**4**),[12] 3β-acetoxy-5α-cholestan-7-one (**13**),[16] 3β-acetoxy-5α-cholestan-6-one (**16**),[17] 3β-acetoxy-5-oxo-5,6-secocholestane-6-carboxylic acid (**19**),[12] and 3β-acetoxycholest-5-en-7-ol (**5**).[13]

3β-*Acetoxy*-5α,6α-*cyclopropa*[5,6]*cholestan*-7α-*ol* (**6**).[10]—Copper acetate dihydrate (0.05 g, 0.28 mmol) was dissolved in hot glacial acetic acid (2 ml) at 130 °C and zinc dust (0.98 g, 0.015 mol) added in one portion. Acetic acid (2 ml) was added and the mixture stirred for 5 min after which it was allowed to cool and settle. The supernatant acetic acid was pipetted off and the residue washed with dry diethyl ether (3 × 10 ml) to remove acetic acid.

The copper–zinc couple so prepared was suspended in dry ether (15 ml) and a solution of dry di-iodomethane (1.2 ml, 10 mmol) in dry ether (10 ml) added causing the solution to boil. After 20 min at reflux, a solution of 3β-acetoxycholest-5-en-7-ol (**5**) (0.5 g, 1.1 mmol) in dry ether (20 ml) was added dropwise with stirring and the resulting mixture heated under reflux for 1.5 h.

The cooled reaction mixture was poured into saturated aqueous sodium borate and the ether layer separated. The aqueous phase was extracted with ether (3 × 20 ml) and the combined ethereal extracts washed successively with aqueous sodium carbonate (10 ml), water (3 × 10 ml), and evaporated to dryness to give a viscous oil (400 mg) which did not crystallise, but was chromatographically and spectroscopically homogeneous (Found: M^+, *m/z* 458.3760. $C_{30}H_{50}O_3$ requires 458.3760) δ_H (CD_3OD)[b] 3.62 (1 H, dd, *J* 5 and 8 Hz, 7-H), 1.98 (3 H, s, $COCH_3$), 0.85 (1 H, m), 0.55 (1 H, t, *J* 5 Hz), and 0.10 (1 H, dd, *J* 5 and 8 Hz, 6-H); $\nu_{max.}$(film) 1 720, 3 040 (cyclopropa CH) and 3 500 cm^{-1}.

3β-*Acetoxy*-5α,6α-*cyclopropa*[5,6]*cholestan*-7α-*yl Methanesulphonate* (**7**).—To a solution of the preceding alcohol (3.94 g) in dry dichloromethane (30 ml) at 0 °C, triethylamine (1.31 g) and methanesulphonyl chloride (1.08 g) were added successively dropwise. The mixture was stirred at 0 °C for 3 h after which it was diluted with cold water (50 ml), the layers mixed, and then separated. The organic layer was washed with cold hydrochloric acid (2M; 2 × 20 ml), cold brine (5%; 20 ml), and saturated aqueous sodium hydrogen carbonate (20 ml) and then dried (Na_2SO_4). Evaporation of the solvent under reduced pressure afforded an oil (3.65 g) which was used directly for the following stage; $\nu_{max.}$(film) 1 730 (C=O), 1 245, 1 175, and 1 025 cm^{-1} (SO_2).

5α,6α-*Cyclopropa*[5,6]*cholestan*-3β-*ol* (**1**).—The product (**7**) from the preceding reaction was dissolved in dry diethyl ether (50 ml) and added dropwise to a suspension of lithium aluminium hydride (0.98 g) in dry diethyl ether (50 ml). An exothermic reaction ensued during addition and the mixture was heated under reflux for a further 1.5 h when addition was complete. Aqueous sodium hydroxide (2M; 50 ml) was then added followed by water (50 ml). The ether layer was separated and the aqueous layer extracted with ether (4 × 50 ml). The combined ether extracts were washed with water (2 × 25 ml), dried (Na_2SO_4), and evaporated to dryness to afford the crude product (2 g). The product was purified from two faster running impurities by chromatography on neutral alumina (70 g), eluting with light petroleum (b.p. 60—80 °C)–ethyl acetate (4:1, v/v) to remove the impurities, and then (3:1, v/v) to give the product alcohol (1.67 g) as a clear oil. Further purification of

samples was achieved by chromatography on silica plates (20 × 20 cm) eluting with dichloromethane. Samples homogeneous by t.l.c. and g.l.c. were obtained but the products failed to crystallise (Found: M^+, m/z 398.3563. $C_{28}H_{46}O$ requires 398.3548); $\delta_H(CDCl_3)$ 3.90 (1 H, m, 3-H), 0.32 (1 H, t, J 4.5 Hz), and −0.05 (1 H, dd, J 4.5 and 8 Hz).

5α,6α-*Cyclopropa*[5,6]*cholestane*-3β,7α-*diol* (**9**).—3β-Acetoxy-5α,6α-cyclopropa[5,6]cholestan-7α-ol (**6**) (400 mg) was dissolved in 5% (w/v) potassium hydroxide in methanol (25 ml) and the solution heated under reflux for 1 h. Methanol was evaporated under reduced pressure and water (15 ml) was added. The required *diol* crystallised readily and was recrystallised from aqueous methanol, m.p. 173—174 °C (90% yield) (Found: C, 80.2; H, 11.6%; M^+, 416.3635. $C_{28}H_{44}O_2$ requires C, 80.7; H, 11.4%, M^+, m/z 416.3654), $\delta_H(CDCl_3)$[b] 3.90 (1 H, m, 3-H), 3.65 (1 H, dd J 4.8 and 8.3 Hz, 7-H), 0.75 (1 H, m), 0.48 (1 H, t, J 4.5 Hz), and 0.10 (1 H, dd, J 4.5 and 8.3 Hz, 6-H); $\nu_{max.}$(KCl) 3 050 and 3 500 cm^{-1}.

5α,6α-*Cyclopropa*[5,6]*cholestane*-3,7-*dione* (**10**).—The preceding diol (300 mg) was dissolved in acetone (2 ml) and dichloromethane (3 ml) and the solution was cooled to 0 °C. A solution (1 ml) of chromium trioxide (3.43 g) in water (10 ml) and concentrated sulphuric acid (2.9 ml) (Jones' reagent) was added dropwise with stirring. After 1 h reaction at 0 °C, the solution was poured into water (50 ml) and the product extracted with ether (3 × 20 ml). The combined ether extracts were washed with water (20 ml), dried (Na_2SO_4), and evaporated under reduced pressure. The required *dione* was recrystallised from aqueous methanol and aqueous acetone and had m.p. 115—118 °C (60% yield) (Found: C, 80.7; H, 10.6%; M^+, m/z 413.3374. $C_{28}H_{40}O_2$ requires C, 81.5; H, 10.8%; M^+, 413.3341); $\delta_H(CDCl_3)$[b] 2.81 and 2.74 (2 H, d J 14 Hz, 5-H).

3β-*Acetoxy*-5α,6α-*cyclopropa*[5,6]*cholestan*-7-*one* (**11**).—This compound was prepared in 60% yield from the corresponding alcohol (**6**) by the method described for the preceding compound (**10**). The required *acetoxy ketone* (**11**) was recrystallised from methanol and had m.p. 110—112 °C (Found: C, 79.2; H, 10.7%; M, m/z 456.3603. $C_{30}H_{44}O_3$ requires C, 78.9; H, 10.4%; M^+, 456.3602); $\delta_H(CDCl_3)$[b] 4.95 (1 H, m, 3-H), 2.01 (3 H, s, CH_3CO), and 1.75 (1 H, dd, J 4.5 and 8.3 Hz); $\nu_{max.}$(KCl) 1 725 and 167.5 cm^{-1}.

3β-*Hydroxy*-5α,6α-*cyclopropa*[5,6]*cholestan*-7-*one* (**12**).—This compound was prepared by hydrolysis with 5% (w/v) potassium hydroxide in methanol as described for (**9**) above. The required *hydroxy ketone* (**12**) was recrystallised from methanol and had m.p. 140—142 °C (Found: C, 79.6; H, 11.0%; M, m/z 414.3536 $C_{28}H_{42}O_2$ requires C, 80.1; H, 11.2%; M, 414.3536); $\delta_H(CDCl_3)$[b] 3.97 (1 H, m, 3-H); $\nu_{max.}$(KCl) 3 500 and 1 680 cm^{-1}.

3β-*Acetoxy*-6,6-*difluoro*-5α-*cholestane* (**17**).—3β-Acetoxy-5α-cholestan-6-one (**16**) (2 g) was dissolved in dichloromethane (15 ml) and the solution placed in a stainless steel pressure vessel. Water (one drop) was added and the vessel attached to a vacuum line *via* a threaded seal and screw valve *via* a male cone to a vacuum line to which a cylinder of sulphur tetrafluoride was attached. Sulphur tetrafluoride (6 ml) was transferred *via* a preliminary calibrated glass trap to the reaction vessel which was then sealed and removed from the vacuum line. The vessel was shaken mechanically for 18 h at room temperature. The vessel was then vented and the contents poured into saturated aqueous sodium hydrogen carbonate (50 ml) and the vessel rinsed with a further portion of the same solution. The aqueous solutions were extracted with dichloromethane (4 × 50 ml), the combined organic extracts washed further with aqueous sodium hydrogen carbonate and water, and then dried (Na_2SO_4). Evaporation of the solution yielded a yellow solid which was purified by chromatography on silica gel (100 g). Chloroform eluted sulphur (0.2 g), 5% methanol in chloroform eluted the required *difluoro steroid* (1.5 g), and 10% methanol in chloroform eluted a trace of starting ketone. The product was recrystallised from propan-2-ol and had m.p. 91—92 °C (Found: C, 74.6; H, 10.5; F, 7.9. $C_{29}H_{46}F_2O_2$ requires C, 74.6; H, 10.4; F, 8.1%; $\delta_H(CDCl_3)$[b] 4.70 (1 H, m, 3-H), 2.20—2.0 and 1.85 (3 H, 3 m, 5-, 7-H) and 2.04 (3 H, s, CH_3CO); $\nu_{max.}$(KCl) 1 735 (CH_3CO).

3β-*Acetoxy*-7,7-*difluoro*-5α-*cholestane* (**14**).—This compound was prepared similarly from 3β-acetoxycholestan-7-one (**13**) and had m.p. 74—75 °C (Found: C, 75.4; H, 10.7; F, 8.2. ($C_{29}H_{46}F_2O_2$ requires C, 74.6; H, 10.4; F, 8.1%); δ_H ($CDCl_3$)[b] 4.70 (1 H, m, 3-H), 2.02 (3 H, s, CH_3CO), 2.05—1.95 and 1.90—1.70 (3 H, m, 6,8-H). $\nu_{max.}$(film) 1 735 (CH_3CO).

6,6-*Difluoro*-5α-*cholestan*-3β-*ol* (**18**).—3β-Acetoxy-6,6-difluoro-5α-cholestane (**17**) was hydrolysed as described for previous acetates above [*e.g.* (**9**)]. The required *difluoro alcohol* was recrystallised from methanol and had m.p. 124—127 °C (Found: C, 75.9; H, 11.1; F, 8.60. $C_{27}H_{44}F_2O$ requires C, 76.4; H, 10.9; and F, 8.95%); $\delta_H(CDCl_3)$[b] 3.60 (1 H, m, 3-H), and 2.15, 2.05, 1.82 (3 H, 3m, 5-, and 7-H).

7,7-*Difluoro*-5α-*cholestan*-3β-*ol* (**15**).—This compound was similarly prepared from the corresponding ketone (**14**) and had m.p. 113—115 °C (Found: C, 76.6; H, 11.3; F, 8.2. $C_{27}H_{44}F_2O$ requires C, 76.4; H, 10.9; F, 8.9%). $\delta_H(CDCl_3)$[b] 3.63 (1 H, m, 3-H), and 2.00, 1.80—1.9 (3 H, 2m, 6-, and 8-H).

3β-*Acetoxy*-6-*azacholest*-4-*en*-7-*one* (**26**).—A solution of 3β-acetoxycholest-5-en-7-one (**4**) (5 g) in dry dichloromethane (90 ml) and dry methanol (33 ml) was ozonolysed at −78 °C until all the starting material had been consumed. The solvent was then removed under reduced pressure and the residual white powder immediately redissolved in a mixture of dichloromethane (100 ml), dioxane (50 ml), and liquid ammonia (100 ml) at −78 °C. The mixture was stirred at this temperature for 3 h and allowed to warm to room temperature. Dry ammonia gas was bubbled through the solution with stirring for 6 h. The solution was then evaporated to dryness, the residue redissolved in diethyl ether, and the ether solution washed successively with 5% aqueous sodium carbonate, water, and brine, dried (Na_2SO_4), and evaporated to dryness to yield the *aza steroid* (3.6 g, 72%), m.p. 158—159 °C; (Found: M^+, m/z 443.3401. $C_{28}H_{45}NO_3$ requires M^+, 443.3399); $\delta_H(CCl_4)$[a] 5.60 (1 H, s, 4-H), 4.61 (1 H, m, 3-H), 4.52 (1 H, m, NH), and 1.94 (3 H, s, CH_3CO); ν_{max}(CCl_4) 3 340, 1 730, and 1 680 cm^{-1}.

3β-*Hydroxy*-6-*azacholest*-4-*en*-7-*one* (**27**).—A solution of the above aza steroid (0.443 g, 2 mmol) in methanol (100 ml) was mixed with a solution of potassium hydrogen carbonate (2.0 g) in water (120 ml) and the solution heated under reflux for 3 h. After cooling, the solution was acidified and the precipitated product filtered off. Recrystallisation from methanol afforded the required *aza steroid* (**27**), m.p. 163—164 °C (Found: M^+, m/z 401.3358. $C_{26}H_{43}NO_2$ requires 401.3294); $\delta_H(CCl_4)$[a] 5.55 (1 H, 4-H) and 3.60—3.20 (3 H, br m, NH, OH, 3-H).

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References

1 K. M. Botham in 'Cholesterol 7α-hydroxylase,' Raven Press, 1986, in press.
2 K. E. Suckling and E. F. Stange, *J. Liquid Res.*, 1985, **26**, 647; R. P. Ford, K. M. Botham, K. E. Suckling, and G. S. Boyd, *FEBS Lett*, 1985, **179**, 177.
3 I. MacInnes, D. C. Nonhebel, S. T. Orszulik, and C. J. Suckling, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2777.
4 D. Laurie, E. Lucas, D. C. Nonhebel, C. J. Suckling, and J. C. Walton, *Tetrahedron*, 1986, **42**, 1035.
5 R. J. Breckenridge and C. J. Suckling, *Tetrahedron*, 1986, **42**, 5665.
6 H. Dalton, B. T. Golding, B. W. Waters, R. Higgins, and J. A. Taylor, *J. Chem. Soc., Chem. Commun.*, 1981, 482.
7 J. T. Groves, W. J. Kruger, Jr., and R. C. Haushalter, *J. Am. Chem. Soc.*, 1980, **102**, 6375.
8 S. K. Chung and S.-V. Park, *J. Org. Chem.*, 1982, **47**, 3197.
9 B. Sherry and R. H. Abeles, *Biochemistry*, 1985, **24**, 2594.
10 J. F. Templeton and C. W. Wie, *Can. J. Chem.*, 1971, **49**, 3636.
11 S. R. Dowd, V. Simplaceanu, and C. Ho, *Biochemistry*, 1984, **23**, 6142.
12 F. Sorm, *Collect. Czech. Chem. Commun.*, 1947, **12**, 437.
13 C. W. Shoppee and B. C. Newman, *J. Chem. Soc. C*, 1968, 981.
14 K. E. Suckling, J. Haughton, S. Beddows, and C. J. Suckling, *Tetrahedron Lett.*, 1986, **27**, 4655.
15 W. C. Smith, *Angew. Chem., Int. Ed. Engl.*, 1962, **1**, 647.
16 T. Barr, I. M. Heilbron, E. R. H. Jones, and F. S. Spring, *J. Chem. Soc.*, 1938, 334.
17 F. S. Spring, I. M. Heilbron, M. Jackson, and E. R. H. Jones, *J. Chem. Soc.*, 1938, 102.
18 H. Lettre and L. Knof, *Chem. Ber.*, 1960, **93**, 2860.
19 M. Uskokovic and M. Gut, *J. Org. Chem.*, 1961, **26**, 1943.
20 M. Uskokovic and M. Gut, *Helv. Chim. Acta*, 1959, **42**, 2258.
21 N. J. Doorenbos, C. L. Huang, C. R. Tamorria, and M. T. Wu, *J. Org. Chem.*, 1961, **26**, 2546.
22 N. J. Doorenbos and C. L. Huang, *J. Org. Chem.*, 1961, **26**, 4548.
23 N. W. Atwater, *J. Am. Chem. Soc.*, 1961, **83**, 3031.
24 M. I. Fremery and E. L. Fiebos, *J. Org. Chem.*, 1964, **29**, 2240.

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