Synthesis of 21-Hydroxycholesterol and 25-Hydroxycholesterol from 3β-Hydroxyandrost-5-en-17-one. A Method for the Stereospecific Construction of Sterol Side-chains [1]

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Alkylation of lithium derivatives of the ethyl and methyl esters of 3β-tetrahydropyran-2-yloxypregn-5-en-21-oic acid (3a) and (3c) with 1-bromo-4-methylpentane gave corresponding derivatives of cholest-5-en-21-oic acid, which were converted into 21-hydroxycholesterol (5e) by reduction of the alkoxycarbonyl groups and acid hydrolysis of the ether group. Alkylation of lithium derivatives of esters (3a) and (3c) with 2-(3-bromopropyl)-2-methyl-1,3-dioxolan (8) followed by conversion of the alkoxycarbonyl groups into a methyl group and removal of the protecting groups gave 3β-hydroxy-27-norcholest-5-en-25-one (7a) which was converted into 25-hydroxy-cholesterol (7c). Alkylation of the lithium derivative of the ester (3c) with methyl iodide gave methyl (20R)-3β-hydroxy-24,25-bisnorchol-5-en-22-oate (4b).

THE efficient preparation of 25-hydroxycholesterol and other sterols hydroxylated in the side-chain has recently attracted considerable attention. The synthesis of sterols starting from readily accessible 17- and 20-ketones involves the formation of the epimer having the required configuration at C-20. The synthesis involving Δ-17(20) and Δ-20(21) intermediates † (e.g. those
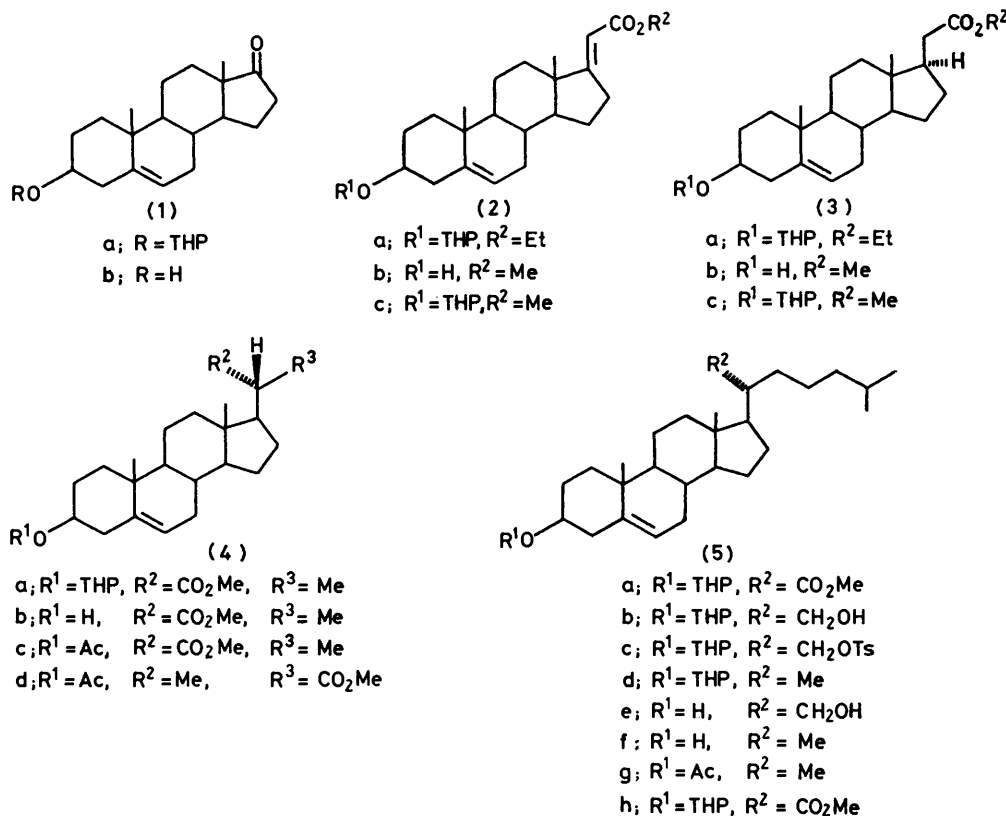
obtained by dehydrogenation of the corresponding 20-alcohols or by the Wittig reaction) appear to be non-stereospecific since the catalytic hydrogenation of the double-bonds gives either the unnatural ‡ C-20 epimer or a mixture of epimers.[2,3] Similarly the catalytic hydro-

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† Only the *E*-isomer has been studied.

‡ Natural configuration refers to that of cholestane *i.e.* (20R) for compounds having no additional substituent at C-21 and C-22 or compounds having an oxygen substituent at C-21, but (20S) for compounds having an oxygen substituent at C-22.

[1] J. Wicha and K. Bal, *J.C.S. Chem. Comm.*, 1975, 968.

[2] For hydrogenation of the 17,20 double bond see (a) N. K. Chaudhuri, R. Nickolson, J. G. Williams, and M. Gut, *J. Org. Chem.*, 1969, **34**, 3767; (b) A. Scetti, E. Castagino, and G. Piancatelli, *Gazzetta*, 1974, **104**, 437.

[3] For hydrogenation of the 20,21 double bond see F. Sondheimer and R. Mechoulam, *J. Amer. Chem. Soc.*, 1958, **80**, 3087; G. Nair and E. Mosettig, *J. Org. Chem.*, 1962, **27**, 4659; J. J. Schneider, *Tetrahedron*, 1972, **28**, 2717.

genation of $\Delta^{20(22)}$ compounds generally gives[4] a 1 : 1 mixture of C-20 epimers although some workers[5] reported selective formation of the (20R)-epimer * ('natural'). The use of the 'natural' (20S)-23,24-dinorcholane-22-carbaldehyde derivatives as the starting materials presents difficulties in that epimerisation at the chiral centre may occur. Several reports concerned with epimerisation at C-20 of 22-aldehydes,[7] 22-ketones,[8] and 22-carboxylic acids or their esters [8b,9] suggests that the 'unnatural' epimer is thermodynamically more stable and preponderates in an equilibrium mixture under normal conditions. It is noteworthy that in some recent publications greater stability of the 'natural' isomers of 22-oxocholesterol derivatives is

by alkylation at the C_{20} methylene group, and in this case the required epimer (20R) of the compound formed is thermodynamically more stable and for this reason it can be obtained, when necessary, by equilibration of the direct alkylation product. The relative stabilities of the epimers of the alkylation product can be estimated on the basis of their structural similarity to eburicoic acid, which has been investigated from this point of view,[10] and on the basis of the results of studies on the stability of epimers of bisnorcholanic acid.[9] Furthermore, analysis of the molecular model of pregnan-21-oic acid suggested that alkylation in position 20 should lead predominantly to compounds having the natural configuration at C-20. Having obtained the side chain of

(1)

a; R = THP
b; R = H

(2)

a; R^1 = THP, R^2 = Et
b; R^1 = H, R^2 = Me
c; R^1 = THP, R^2 = Me

(3)

a; R^1 = THP, R^2 = Et
b; R^1 = H, R^2 = Me
c; R^1 = THP, R^2 = Me

(4)

a; R^1 = THP, R^2 = CO$_2$Me, R^3 = Me
b; R^1 = H, R^2 = CO$_2$Me, R^3 = Me
c; R^1 = Ac, R^2 = CO$_2$Me, R^3 = Me
d; R^1 = Ac, R^2 = Me, R^3 = CO$_2$Me

(5)

a; R^1 = THP, R^2 = CO$_2$Me
b; R^1 = THP, R^2 = CH$_2$OH
c; R^1 = THP, R^2 = CH$_2$OTs
d; R^1 = THP, R^2 = Me
e; R^1 = H, R^2 = CH$_2$OH
f; R^1 = H, R^2 = Me
g; R^1 = Ac, R^2 = Me
h; R^1 = THP, R^2 = CO$_2$Me

postulated on the basis of erronous interpretation [2a] of previously published experimental data.[8a]

It seemed to us that the above stereochemical complications could be conveniently eliminated by using derivatives of pregnan-21-oic acid as intermediate compounds in sterol synthesis. A suitable precursor of the C_{22}–C_{27} moiety could be added to these compounds

required configuration at the C-20 carbon atom by alkylation or alkylation and epimerisation, the synthesis could be terminated by reduction of the ester group and transformations of the C-22—C-27 fragment. This theoretical scheme was checked by the preparation of the ester of (20R)-3β-hydroxy-23,24-dinorchol-5-en-22-oic acid (4b), the hitherto unknown 21-hydroxycholesterol

* It was noted [6] that stereoselective hydrogenation of the 20,22 double bond is in marked contrast with the steric course of electrophilic reactions at this position.

[4] (a) Hydrogenation of the Z-isomer; W. R. Nes, T. E. Varkey, and K. Krevitz, *J. Amer. Chem. Soc.*, 1977, **99**, 260; hydrogenation of the E- and Z-isomers; T. A. Narvid, K. E. Cooney, and M. R. Uskokovič, *Helv. Chim. Acta*, 1974, **57**, 771.

[5] (a) J. P. Schmit, M. Piraux, and J. F. Pilette, *J. Org. Chem.*, 1975, **40**, 1586; (b) T. C. McMorris and S. R. Schow, *J. Org. Chem.*, 1976, **41**, 3759.

[6] K. Bannai, M. Morisaki, and N. Ikekawa, *J.C.S. Perkin I*, 1976, 2116.

[7] (a) D. H. R. Barton, T. Shioiri, and D. A. Widdowson, *Chem. Comm.*, 1970, 939; (b) M. E. Herr and F. W. Heyl, *J. Amer. Chem. Soc.*, 1952, **74**, 3627.

[8] (a) W. Cole and P. J. Julian, *J. Amer. Chem. Soc.*, 1945, **67**, 1369; (b) R. Hayatsu, *Pharm. Bull (Japan)*, 1957, **5**, 452.

[9] Y. Mazur and F. Sondheimer, *Experientia*, 1960, **16**, 181.

[10] A. Bowers, T. G. Halsall, and G. C. Sayer, *J. Chem. Soc.*, 1954, 3070.

(5e) and 25-hydroxycholesterol (7c) from 3β-hydroxy-androst-5-en-17-one (1b).

The reaction of ketone (1a) [11] with a salt prepared from triethyl phosphonoacetate and sodium ethoxide in ethanolic solution [12] gave the ethyl ester of (E)-3β-tetrahydropyranyloxypregna-5,17(20)-dien-21-oic acid (2a) in 85% yield. A similar treatment of ketone (1b) with a salt prepared from trimethyl phosphonoacetate and sodium methoxide in methanol gave the corresponding methyl ester (2b), but in this case the yield was considerably lower. Hydrogenation of compounds (2a) and (2b) in the presence of a platinum catalyst in ethanol gave the expected [13] selective and stereospecific saturation of the 17,20 bond resulting in the formation of compounds (3a) and (3b) respectively. Treatment of the 3-hydroxy-compound (3b) with dihydropyran in the presence of an acidic catalyst gave the 3-tetrahydropyranyloxy-compound (3c). Compounds (3a) and (3c) are the desired steroidal components of the alkylation reaction. In further reactions both the ethyl ester (3a) and the methyl ester (3c) were used. The overall yields of the ethyl ester were higher but the alkylation products of the methyl ester were more suitable for stereochemical studies.

The alkylation of ester (3c) with methyl iodide was carried out by the general method described by Cregge et al.[14] A solution of the ester in tetrahydrofuran (THF) was treated at −30 °C with a slight excess of lithium di-isopropylamide (LDA) and the resulting lithium derivative of the ester was slowly treated at −78 °C with a solution of methyl iodide in hexamethylphosphorotriamide. The reaction mixture was set aside for several hours at −78 °C, then diluted with a large amount of ether and rapidly washed with water. The only product of the reaction was crystalline (4a) which after acid hydrolysis carried out under mild conditions gave the hydroxy-compound (4b). The last compound was converted into the acetate (4c). Compounds (4a), (4b), and (4c) were chromatographically homogeneous and had satisfactory analyses. Their [1]H n.m.r. spectra contained sharp signals; of the greatest diagnostic value were the doublets of the C-21 methyl group protons, occurring at δ 1.13 (4a), 1.15 (4b), and 1.11 (4c). The acetate (4c) had the same m.p. and optical rotation as those of the (20R) isomer [8b] obtained by epimerisation of the 'natural' acid and different from those [15] of the (20S)-isomer. Direct comparison of the alkylation product of (4c) and a sample of (4d) having the configuration (20S) confirmed that these compounds are epimers at C-20. In their n.m.r. spectra (obtained in deuteriochloroform) all the signals were superimposable with the exception of doublets corresponding to the C-21 methyl group protons which in the case of (20R)-(4c) occur at

δ 1.11 and in the case of (20S)-(4d) at 1.21. An analogous difference in the chemical shift of C-21 protons was observed for C-20 epimers of 20-hydroxycholesterol,[16] cholesterol,[2a] and several 25-substituted cholestane derivatives.[4b] The n.m.r. spectra of artificially prepared mixtures of compounds (20R)-(4c) and (20S)-(4d) showed that the presence of the two components can be detected when the ratio of the major component to the minor one is not more than 9 : 1. Analysis of the n.m.r. spectrum of crude (4a) and its crude transformation products (4b) and (4c) showed that alkylation of ester (3b) with methyl iodide leads to the formation of >90% of the (20R)-epimer since the presence of the (20S)-epimer was not detected. These results showed the viability of the proposed plan for formation of the side-chain.

The alkylation of the ester (3b) with 1-bromo-4-methylpentane (isohexyl bromide) under similar conditions gave the methyl ester of (20S)-3β-tetrahydropyranyloxycholest-5-en-21-oic acid (5a). The best yields of (5a) (80—85%) were obtained when the reaction of the lithium derivative of the ester with isohexyl bromide was started at ca. −70 °C and then gradually raised to room temperature. Reduction of (5a) with LiAlH₄ gave the 21-hydroxy-derivative (5b) which on acid hydrolysis gave 21-hydroxycholesterol (5e). Compounds (5b) and (5e) were chromatographically homogeneous with sharp [1]H n.m.r. signals. In order to prove the stereochemistry of 21-hydroxycholesterol (5e) and to show its epimeric purity additional experiments were carried out. The alcohol (5b) was esterified with toluene-p-sulphonyl chloride and the resulting ester (5c) was reduced with LiAlH₄. The product (5d) was transformed in the usual way into cholesterol (5f) and cholesteryl acetate (5g). All the reactions in this sequence starting with the alkylation of the ester (3b) were carried out without purification of the intermediate compounds and under such conditions that the composition of the eventual mixtures remained unchanged. Chromatographic and spectroscopic comparison of the resulting cholesterol and its acetate with authentic samples confirmed the identity of these compounds.

In the synthesis of 25-hydroxycholesterol (7c) the precursor of the side chain was 2-(3-bromopropyl)-2-methyl-1,3-dioxolan (8) which was readily obtainable from acetylbutyrolactone.[17] Compound (8) contains a masked oxo-group and can be useful in syntheses of side chains containing functional groups but it has not previously been used in syntheses of sterols. The alkylation of esters (3a) and (3b) with bromo-compound (8) was carried out under conditions similar to those used in the synthesis of 21-hydroxycholesterol. The resulting compounds (6a) and (6b) were obtained in 80—85% yield.

[11] H. M. E. Cardwell, J. W. Cornforth, S. R. Duff, H. Holtermann, and R. Robinson, J. Chem. Soc., 1953, 361.

[12] J. Wicha, K. Bal, and S. Piekut, Synth. Comm., 1977, 7, 215.

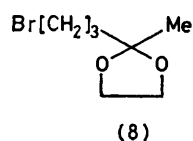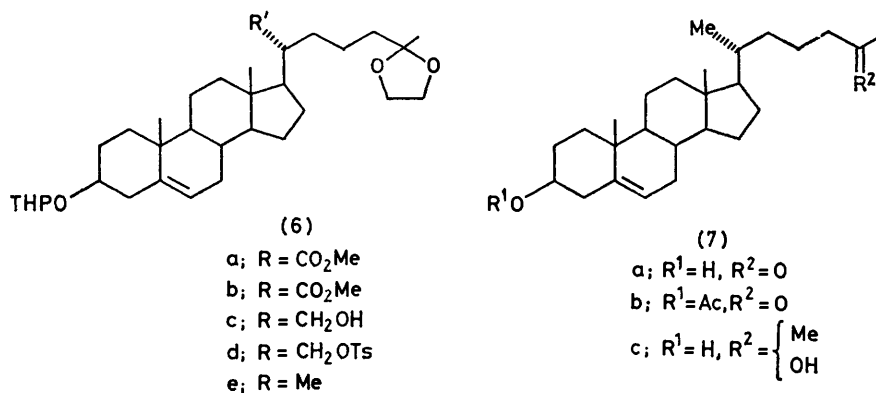[13] Pl. A. Plattner and W. Schreck, Helv. Chim. Acta, 1939, 22, 1178.

[14] R. J. Cregge, J. L. Herrmann, C. S. Lee, J. E. Richman, and R. H. Schlessinger, Tetrahedron Letters, 1973, 2425.

[15] F. C. Chang, R. T. Blickenstaff, A. Feldstein, J. R. Gray, G. S. McCaleb, and D. H. Sprunt, J. Amer. Chem. Soc., 1957, 79, 2161 and references cited therein.

[16] A. Mijares, D. J. Cargill, J. A. Glasel, and S. Lieberman, J. Org. Chem., 1967, 32, 810.

[17] Org. Synth., 1963, Coll. Vol. 4, 278; C. A. Grob and R. Mosch, Helv. Chim. Acta 1959, 42, 728.

Esters (6a) and (6b) were reduced with $LiAlH_4$ to the 21-alcohol (6c) which was converted into its toluene-sulphonate (6d). This was reduced with $LiAlH_4$ to

estimated that the alkylation of ester (3a) with methyl iodide is at least 90% stereospecific but the formation of the epimeric product having the configuration (20S) was

(6)

a; R = CO$_2$Me
b; R = CO$_2$Me
c; R = CH$_2$OH
d; R = CH$_2$OTs
e; R = Me

(7)

a; R^1= H, R^2= O
b; R^1=Ac,R^2 = O
c; R^1= H, R^2 = $\begin{cases} Me \\ OH \end{cases}$

Br[CH$_2$]$_3$ Me

(8)

compound (6e) which was hydrolysed under acid conditions of 3β-hydroxy-27-norcholest-5-en-25-one (7a). Both (7a) and its acetate (7b) had physical constants agreeing with those quoted in the literature.[18] Direct comparison of compound (7b) with an authentic sample of 3β-acetoxy-27-norcholest-5-en-25-one confirmed the structure of the product of this synthesis.

The treatment of the acetoxy-ketone (7b) with methyl-magnesium iodide in ether gave 25-hydroxycholesterol

not observed in any of the alkylation reactions. In the alkylations the equivalent amount or a slight excess of the base was used and the reaction was carried out under oxygen-free conditions, in which the equilibration of lithium salt with alkylation product is unlikely.[20] For this reason it can be assumed that at least the majority of the alkylation products are direct reaction products from the lithium derivative of the starting ester.

The consideration of the structure of the substrate and

(a)   (b)   (c)
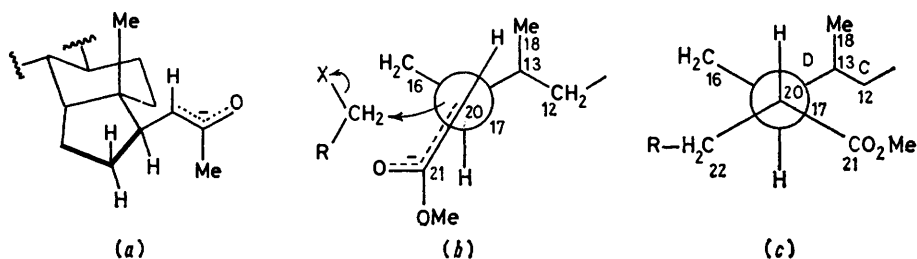
FIGURE

(7c) having the expected [19] physical and spectroscopic constants. This synthesis was repeated without isolation and purification of the intermediate compounds from the ester (3a) to the final product (7c). In this case the overall yield of 25-hydroxycholesterol (7c) was 47% calculated for the starting 3β-tetrahydropyran-2-yloxy-androst-5-en-17-one (1a) and 61% calculated for the ester of pregnenic acid (3a).

All the alkylations of esters (3a) and (3b) gave good yields of compounds having configuration (20R). On the basis of analysis of the ^1H n.m.r. spectra it was

the reaction products makes it possible to draw the following conclusions. In the lithium derivatives of the esters (3a) or (3c) the conjugation of the electron pair on the C-20 carbon atom with electrons of the carbonyl group causes flattening of this fragment of the molecule. In the stable conformation of the anion the C-21 carbon atom and its substituents are situated between the small (H) and the medium (C-16) substituents of the C-17 carbon atom, whereas the hydrogen atom at C-20 is situated between the medium (C-16) and the large (C-13) substituents as shown in the Figure. The approach of alkyl halide (R–CH$_2$–X) to the anion having this conformation from the least screened side [i.e. from the side

[18] L. Ružička and W. H. Fischer, Helv. Chim. Acta, 1937, 20, 1291.
[19] J. J. Partridge, S. Faber, and M. R. Uskoković, Helv. Chim. Acta, 1974, 57, 764.

[20] H. O. House, ' Modern Synthetic Reactions,' Benjamin, 1972, p. 564.

of the C-16 carbon atom shown by an arrow in the Figure, (*b*)] leads to the formation of the epimer having the configuration (20*R*). On the other hand the consideration of the relative stabilities of the possible alkylation products leads to the conclusion that epimer (20*R*) [Figure, (*c*)] is more stable since the side chain can have the conformation in which the small substituent at C-20 (H) is directed towards the most crowded part of the molecule (the angular C-18 methyl group), the medium substituent (the alkoxycarbonyl group) is directed towards ring C and the largest substituent (the alkyl group) is directed towards the least crowded part of the molecule (the C-16 carbon atom). The above analysis shows that the formation of products having the configuration (20*R*) in the reactions of alkylation of esters (3a) and (3b) is preferred as a result of both the ease of access of the reagent to the anion having the stable conformation and the thermodynamic stability of the product. The convergence of the directions of action of the kinetic and the thermodynamic effects is probably the cause of the high stereospecificity of the alkylation reaction.

EXPERIMENTAL

All melting points were determined with a Kofler hot-stage apparatus. Optical rotations were determined with a Perkin-Elmer 141 polarimeter for *ca.* 1% solutions in $CHCl_3$ unless otherwise stated. Spectra were measured using a Unicam SP700 (u.v., EtOH solutions), a Unicam SP 200 (i.r.) and JEOL 100 MHz or Varian 60 MHz spectrometers (¹H n.m.r., $CDCl_3$ solutions). Mass spectra were obtained with an LKB 9000A spectrometer at 70 eV ionization potential. Extracts were dried over Na_2SO_4; solvents were distilled off on a rotary evaporator. The yields reported correspond to chromatographically (t.l.c.) pure compounds. Light petroleum had b.p. 70—80 °C.

Reaction of 3β-*Tetrahydropyran-2-yloxyandrost-5-en-17-one* (1a) *with Triethyl Phosphonoacetate and Sodium Ethoxide.*—A solution of the ketone (1a) (3.74 g, 10 mmol) and triethyl phosphonoacetate (6.88 g, 30 mmol) in anhydrous ethanol (50 ml) under argon was treated slowly dropwise with stirring at 35—40 °C with a solution of sodium ethoxide [from sodium (0.8 g, 30 mg atom) in ethanol (25 ml)]. The mixture was refluxed for 14 h and after cooling it was concentrated under reduced pressure, diluted with water, and the product was extracted with ether. The solvent was evaporated off and the residue was recrystallised from Me_2-CO—H_2O to give (2a) (3.75 g, 85%), m.p. 121—124 °C, $\lambda_{max.}$ 223 nm (ε 18 200); $\nu_{max.}$ ($CHCl_3$) 1 700 and 1 650 cm^{-1}; ¹H n.m.r. δ 5.54 (t, *J* 2 Hz, 1 H, 20-H), 5.36 (m, 1 H, 6-H), 4.73 (m, 1 H, 2-THP-H), 4.13 (q, *J* 7 Hz, 2 H, OCH_2CH_3), 3.90 (m, 1 H, 3-H), 3.51 (m, 2 H, 6-THP-H), 2.84 (m, 2 H, 16-H), 1.36 (t, *J* 7 Hz, 3 H, OCH_2CH_3), 1.14 (s, 3 H, 18-H), and 0.94 (s, 3 H, 19-H) (Found: C, 76.1; H, 9.6. Calc. for $C_{28}H_{42}O_4$: C, 76.0; H, 9.6%).

Reaction of 3β-*Hydroxyandrost-5-en-17-one* (1b) *with Trimethyl Phosphonoacetate and Sodium Methoxide.*—A solution of the ketone (1b) (580 mg, 2 mmol) and trimethyl phosphonoacetate (1.10 g, 6 mmol) in anhydrous methanol (35 ml) was stirred under argon and slowly treated dropwise at room temperature with a solution of sodium methoxide [from sodium (0.14 g, 6 mg atom) in methanol (5 ml)]. The

mixture was refluxed for 3 h and was then cooled. Trimethyl phosphonoacetate (1.1 g) and a solution of sodium methoxide [from sodium (0.14 g) in methanol (5 ml)] were added and the mixture was refluxed for 10 h and then worked up as in the preceding section. The crude product was chromatographed on a SiO_2 column (light petroleum-ethyl acetate, 95 : 5) to give starting material (0.25 g, 45%) and compound (2b) (0.28 g, 41%), m.p. 185—188 °C, $[\alpha]_D^{20} = -73°$ (dioxan, *c* = 1.0 g dm^{-3}) (lit.,[13] m.p. 188—189 °C, $[\alpha]_D = -73°$).

Preparation of the Methyl Ester of 3β-*Hydroxypregn-5-en-21-oic Acid* (3b).—A solution of (2b) (0.26 g) in ethanol (50 ml) was shaken under hydrogen with pre-reduced platinum oxide (0.1 g). After the absorption of 1 equivalent of hydrogen the reaction was stopped and the catalyst was removed by filtration through Celite. Concentration of the filtrate gave compound (3b), identical with a sample prepared by another method,[21] m.p. 127—130 °C (Me_2CO–H_2O); $[\alpha]_D^{18} = -60°$ (lit.,[13] m.p. 132—133 °C, $[\alpha]_D = -63.5°$).

Preparation of the Methyl Ester of 3β-*Tetrahydropyran-2-yloxy-pregn-5-en-21-oic Acid* (3c).—A suspension of alcohol (3b) (6.5 g) in dry ether (50 ml) containing dihydropyran (5 ml) and toluene-*p*-sulphonic acid monohydrate (0.02 g) was stirred at room temperature for 16 h. The resulting clear solution was diluted with ethyl acetate (50 ml) and washed successively with cold 2% NaOH solution and water. After evaporating off the solvent, the remaining colourless oil was crystallised from methanol and gave (3c) (7.4 g, 92%), m.p. 90—100 °C; $[\alpha]_D^{16} = -43°$; $\nu_{max.}$ ($CHCl_3$) 1 730 cm^{-1}; ¹H n.m.r. (60 MHz) δ 5.34 (m, 1 H, 6-H), 4.73 (m, 1 H, 2-THP-*H*), 3.65 (s, 3 H, OCH_3), 1.00 (s, 3 H, 19-H), and 0.60 (s, 18-H) (Found: C, 75.5; H, 10.0. Calc. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8%).

Preparation of the Ethyl Ester of 3β-*Tetrahydropyran-2-yloxypregn-5-en-21-oic Acid* (3a).—Hydrogenation of (2a) (2.9 g) in ethanol (130 ml) over pre-reduced platinum oxide (0.25 g) under the conditions used previously gave compound (3a) (2.8 g, 96%), m.p. 110—111 °C, $\nu_{max.}$ ($CHCl_3$) 1 730 and 1 030 cm^{-1}; ¹H n.m.r. δ 5.35 (m, 1 H, 6-H), 4.73 (m, 1 H, 2-THP-H), 4.09 (q, *J* 7 Hz, 2 H, OCH_2CH_3), 3.47 (m, 2 H, 6-THP-H), 1.25 (t, *J* 7 Hz, 3 H, OCH_2CH_3), 1.02 (s, 3 H, 19-H), and 0.62 (s, 3 H, 19-H) (Found: C, 75.8; H, 10.0. Calc. for $C_{28}H_{44}O_4$: C, 75.6; H, 10.0%).

Alkylation of the Esters of 3β-*Tetrahydropyran-2-yloxy-pregn-5-en-21-oic Acid,* (3a) *and* (3c).—The alkylations were carried out with magnetic stirring under argon with exclusion of oxygen and moisture. The solvents were distilled immediately before use (THF over $LiAlH_4$ and HMPTA over molecular sieves).

(*a*) *Alkylation of the ester* (3c) *with methyl iodide.* THF (10 ml) and di-isopropylamine (0.91 g, 9.0 mmol) were placed in a reactor fitted with a magnetic stirrer, a thermometer, a dropping funnel with pressure equilibrium, and an argon inlet. A solution of the methyl ester (3c) (2.58 g, 6 mmol) in THF (15 ml) was placed in the dropping funnel. The contents of the reactor were cooled to −30 °C and a 2.5M solution of n-butyl-lithium in n-hexane (3.6 ml, 9 mmol) was added by means of a syringe. After 20 min the contents of the reactor were cooled to *ca.* −70 °C and the ester (3c) was added dropwise, maintaining the temperature below −70 °C. After *ca.* 40 min a mixture of methyl iodide (2.25 g, 18 mmol) and HMPTA (4.5 ml) was added by

[21] J. Wicha and K. Bal, *Steroids,* 1977, **30**, 363.

means of a syringe. The reaction mixture was kept at $-70\ ^\circ$C for 5 h, then diluted with ether (300 ml) and washed with water. The organic layer was dried and the solvent evaporated off. Several crystallisations from acetone of the crude product gave compound (4a) (2.40 g, 91%), m.p. 160—165 $^\circ$C, $\nu_{max.}$ (KBr) 1 730, 1 070, and 1 045 cm^{-1}; ^{1}H n.m.r. δ 5.33 (m, 1 H, 6-H), 4.71 (m, 1 H, 2-THP-H), 3.65 (s, 3 H, OCH$_3$), 3.50 (m, 2 H, 6-THP-H), 1.10 (d, J 7 Hz, 3 H, 21-H), 1.01 (s, 3 H, 19-H), and 0.70 (s, 3 H, 18-H); m/e 360 (M — C$_5$H$_8$O]$^+$), 342, and 85 ([C$_5$H$_9$O]$^+$, 100%).

(b) *Alkylation of ester* (3c) *with isohexyl bromide.* A 2.6M solution of n-butyl-lithium in hexane (3.45 ml, 9 mmol) was added to a solution of di-isopropylamine (0.91 g, 9 mmol) in THF (5 ml) cooled to $-30\ ^\circ$C for *ca.* 30 min. A solution of the ester (3c) (2.58 g, 6 mmol) in THF (20 ml) was then added dropwise. After 1 h, the temperature of the reaction mixture was lowered to $-70\ ^\circ$C and a solution of isohexyl bromide (2.97 g, 19 mmol) in HMPTA (5.92 g) was slowly added. The mixture was stirred for 3 h and its temperature was then allowed to rise to 0 $^\circ$C during *ca.* 2 h. The reaction was terminated by addition of ether (150 ml) and the product was isolated as in (a) to give compound (5a) (2.60 g, 86%), m.p. 163—167 $^\circ$C (methanol); $\nu_{max.}$ (CHCl$_3$) 1 720 and 1 030 cm^{-1}.

(c) *Alkylation of the ester* (3a) *with isohexyl bromide.* A 2.0M solution of n-butyl-lithium in hexane (8.45 ml, 16.9 mmol) was added to a solution of di-isopropylamine (1.7 g, 16.9 mmol) in THF (10 ml) cooled to $-30\ ^\circ$C. After 20 min a solution of the ester (3a) (5.0 h, 11.2 mmol) in THF (40 ml) was added dropwise. After 1 h the temperature of the reaction mixture was lowered to $-70\ ^\circ$C and a solution of isohexyl bromide (4.7 g, 33 mmol) in HMPTA (10.4 ml, 60 mmol) was slowly added. The mixture was stirred at $-70\ ^\circ$C for 2 h and then its temperature was allowed to rise to room temperature during *ca.* 3 h. The reaction was terminated by adding ether (300 ml) and the product was isolated as in (a) to give (5 h) (5.1 g, 85%) m.p. 158—159 $^\circ$C (acetone); $\nu_{max.}$ (CHCl$_3$) 1 720 and 1 030 cm^{-1}; ^{1}H n.m.r. δ 5.34 (m, 1 H, 6-H), 4.72 (m, 1 H, 2-THP-H), 4.11 (q, J 7 Hz, 2 H, OCH$_2$CH$_3$), 3.90 (m, 1 H, 3-H), 3.49 (m, 2 H, 6-THP-H), 1.32 (t, J 7 Hz, 3 H, OCH$_2$CH$_3$), 1.05 (s, 3 H, 19-H), 0.89 (d, J 7 Hz, 6 H, 26-, 27-H), and 0.74 (s, 3 H, 18-H).

(d) *Alkylation of the ester* (3c) *with* 2-(3-bromopropyl)-2-methyl-1,3-dioxolan (8). A 1.8M solution of n-butyl-lithium in hexane (10 ml, 18 mmol) was added to a solution of di-isopropylamine (1.82 g, 18 mmol) in THF (10 ml) cooled to $-30\ ^\circ$C. After *ca.* 30 min a solution of compound (3b) (2.58 g, 6 mmol) in THF (20 ml) was added dropwise. After *ca.* 1 h the temperature of the reaction mixture was lowered to $-70\ ^\circ$C and a solution of bromodioxolan (8) (3.23 g, 16 mmol) in HMPTA (5 ml) was slowly added. The mixture was stirred at $-70\ ^\circ$C for 5 h and then it was warmed to about 20 $^\circ$C during *ca.* 2 h. The reaction was terminated by addition of ether (150 ml) and the product was isolated as in (a) to give (6a) (2.92 g, 84%), m.p. 159—165 $^\circ$C (methanol); $\nu_{max.}$ (KBr) 1 730, 1 070, and 1 045 cm^{-1}; ^{1}H n.m.r. δ 5.36 (m, 1 H, 6-H), 4.72 (m, 1 H, 2-THP-H), 3.82 (s, 4 H, O-CH$_2$-CH$_2$-O), 3.87 (m, 1 H, 3-H), 3.55 (m, 2 H, 6-THP-H), 3.65 (s, 3 H, OCH$_3$), 1.28 (s, 3 H, 26-H), 1.00 (s, 3 H, 19-H), and 0.70 (s, 3 H, 18-H); m/e 474 ([M — C$_5$H$_8$O]$^+$), 456 [M — C$_5$H$_8$O — H$_2$O]$^+$), and 85 ([C$_5$H$_9$O]$^+$).

(e) *Alkylation of the ester* (3a) *with* 2-(3-bromopropyl)-2-

*methyl-*1,3-*dioxolan* (8). A 2.0M solution of n-butyl-lithium in hexane (3.38 ml, 6.75 mmol) was added to a solution of di-isopropylamine (0.46 g, 6.75 mmol) in THF (5 ml) cooled to $-30\ ^\circ$C. After 20 min a solution of the ester (3a) (2.0 g, 4.5 mmol) in THF (15 ml) was added dropwise. After 1 h the temperature of the reaction mixture was lowered to $-70\ ^\circ$C and a solution of bromodioxolan (1.9 g, 9 mmol) in HMPTA (3.5 ml, 20 mmol) was slowly added. The temperature was allowed to rise to room temperature during 2.5 h. The reaction was terminated by adding ether (150 ml) and the product was isolated as in (a) to give (6b) (2.3 g, 91%), m.p. 181—186 $^\circ$C (acetone); $\nu_{max.}$ (KBr) 1 730, 1 070, and 1 050 cm^{-1}; ^{1}H n.m.r., δ 5.37 (m, 1 H, 6-H), 4.73 (m, 1 H, 2-THP-H), 3.88 (m, 1 H, 3-H); 3.81 (s, 4 H, O-CH$_2$-CH$_2$-O), 3.53 (m, 2 H, 6-THP-H), 1.33 (t, J 7 Hz, 3 H, OCH$_2$CH$_3$), 1.27 (s, 3 H, 26-H), 1.01 (s, 3 H, 19-H), and 0.72 (s, 3 H, 18-H).

Preparation of the Methyl Ester of (20R)-3β-*Hydroxy-*23,24-*dinorchol-*5-en-21-oic *Acid* (4b).—A solution of the pyranyloxy-ester (4a) (0.25 g) and toluene-p-sulphonic acid monohydrate (0.05 g) in acetone (30 ml) was kept at room temperature for 16 h. Work-up with ethyl acetate and crystallisation from acetone gave (4b) (0.18 g, 88%), m.p. 169—175 $^\circ$C, $[\alpha]_D^{24} = -62^\circ$; $\nu_{max.}$ (KBr) 3 550, 1 715, and 1 070 cm^{-1}; ^{1}H n.m.r., δ 5.32 (m, 1 H, 6-H), 3.67 (s, 3 H, OCH$_3$), 3.53 (m, 1 H, 3-H), 1.15 (d, J 7 Hz, 3 H, 21-H), and 0.76 (s, 3 H, 18-H); m/e 360 (M^+, 100%), 345, 342 (68%), 327 (for C$_{23}$H$_{36}$O$_3$ calc. $M^+ = 360$).

Preparation of the Methyl Ester of (20R)-3β-*Acetoxy-*23,24-*dinorchol-*5-en-21-oic *Acid* (4c).—The hydroxy-ester (4b) was treated with a mixture of acetic anhydride and pyridine at room temperature. Work-up gave the ester (4c), m.p. 141—143 $^\circ$C (methanol), $[\alpha]_D^{30} = -55^\circ$, $\nu_{max.}$ (KBr) 1 730 and 1 240 cm^{-1}; ^{1}H n.m.r., δ 5.35 (m, 1 H, 6-H), 4.55 (m, 1 H, 6-H), 3.65 (s, 3 H, OCH$_3$), 2.01 (s, 3 H, CH$_3$COO), 1.11 (d, J 7 Hz, 21-H), 1.02 (s, 3 H, 19-H), 0.71 (s, 3 H, 18-H); m/e 342 ([M — CH$_3$COOH]$^+$, 100%) and 327 (lit.,[8] m.p. 142—143 $^\circ$C; $[\alpha]_D = -53.6^\circ$).

The n.m.r. spectrum of the epimeric ester (4d) was identical with that of (4c) except for the signal of the C-21 methylene group protons, which was situated at δ 1.21 (d, J 7 Hz).

Preparation of 21-*Hydroxycholesterol* (5e) *from the Ester* (5a).—A mixture of ester (5a) (0.60 g, 1.2 mmol), LiAlH$_4$ (1.2 g), and dry ether (80 ml) was refluxed for 2 h, then cooled and the excess of the reagent decomposed with a saturated sodium sulphate solution. The inorganic precipitate was separated and washed with ether. The ether solutions were combined and evaporated to give (5b) (0.55 g, 96%), m.p. 157—161 $^\circ$C (Found: C, 79.2; H, 11.2; Calc. for C$_{32}$H$_{54}$O$_3$: C, 79.0; H, 11.2%), $\nu_{max.}$ (KBr) 3 500 cm^{-1} (no carbonyl-group absorption); ^{1}H n.m.r., δ 5.37 (m, 1 H, 6-H), 4.69 (m, 1 H, 2-THP-H), 3.85 (m, 1 H, 3-H), 3.67 (m, 2 H, 21-H), 3.51 (m, 2 H, 6-THP-H), 1.01 (s, 3 H, 19-H), 0.86 (d, J 6.5 Hz, 6 H, 26-, 27-H), and 0.69 (s, 3 H, 18-H). In the same way ester (5h) was reduced to alcohol (5b).

Crude (5b) (0.08 g, 0.16 mmol) was dissolved in acetone (30 ml) containing toluene-p-sulphonic acid (0.1 g) and the solution was kept at room temperature for 16 h. The product was extracted with ether to give (5e) (50 mg, 75%) m.p. 154—157 $^\circ$C (acetone), (Found: C, 80.4; H, 11.5. Calc. for C$_{27}$H$_{46}$O$_2$: C, 80.5; H, 11.5%) $[\alpha]_D^{18} = -31^\circ$, $\nu_{max.}$ (CHCl$_3$) 3 650 and 1 040 cm^{-1}; ^{1}H n.m.r., δ 5.36 (m, 1 H, 6-H), 3.69 (m, 2 H, 21-H), 3.54 (m, 1 H, 3-H), 1.01

(s, 3 H, 19–H), 0.87 (d, J 7 Hz, 6 H, 26, 27–H), and 0.70 (s, 3 H, 18–H); m/e 402 (M^+, 98.6%), 384 ($[M - H_2O]^+$, 0.63%), 212 (100%, $[M - \{\text{side chain} + \text{ring D} + H\}]^+$).

Preparation of Cholesterol (5f) *from* 3β-*Tetrahydropyran*-2-*yloxycholest*-5-*en*-21-*ol* (5b).—Toluene-*p*-sulphonyl chloride (0.5 g) was added to a solution of the alcohol (5b) (0.55 g) in dry pyridine (10 ml) and the mixture was set aside at room temperature for 16 h. It was the poured into saturated aqueous sodium hydrogen carbonate solution. The mixture was then extracted with ethyl acetate, the extract washed several times with water and then dried. The solvent was evaporated off at room temperature, first at the water pump and then at the oil pump. The product was t.l.c.-pure tosylate (5c) (solid, 0.70 g). It was dissolved in dry ether (50 ml) and was treated with LiALH₄ (1.1 g). The reaction mixture was refluxed for 10 h and after cooling it was treated as in the preceding experiment to give first crude (5d) and then crude cholesterol (5f). After purification of (5f) by filtration through a SiO₂ column and crystallisation, the product (0.14 g, 89%), m.p. 144—146°C (ethanol), $[\alpha]_D^{24} = -39°$, was identical with an authentic sample (i.r., ¹H n.m.r.).

Acetylation of the crude (5f) gave cholesteryl acetate (5g) (83%) identical with an authentic sample.

Preparation of 25-*Ethylenedioxy*-3β-*tetrahydropyran*-2-*yloxy*-27-*norcholest*-5-*en*-21-*ol* (6c).—A mixture of ester (6a) (1.00 g), LiAlH₄ (2.50 g), and dry ether (120 ml) was refluxed for 2.5 h. Work-up as previously gave (6c) (0.88 g, 93%), $\nu_{max.}$ (Nujol) 3 450, 1 140, and 1 035 cm⁻¹; ¹H n.m.r., δ 5.35 (m, 1 H, 6–H), 4.70 (m, 1 H, 2-THP-H), 3.92 (s, 4 H, O–CH₂–CH₂–O), 1.01 (s, 3 H, 19–H), and 0.70 (s, 3 H, 18–H).

In the same way ester (6b) was reduced to alcohol (6c).

Preparation of 3β-*Hydroxy*-27-*norcholest*-5-*en*-25-*one* (7a).—Toluene-*p*-sulphonyl chloride (0.5 g) was added to a solution of alcohol (6c) (0.73 g) in dry pyridine (10 ml). The mixture was set aside at room temperature for 2 d and then worked up as described previously to give tosylate (6d) (0.9 g, 96%), $\nu_{max.}$ (Nujol) 1 600, 1 180, and 1 035 cm⁻¹, ¹H n.m.r., δ 7.78 (d, J 8.5 Hz, 2 H), 7.33 (d, J 8.5 Hz, 2 H) (aromatic protons), 5.34 (m, 1 H, 6–H), 4.71 (m, 1 H, 2-THP-H), 3.89 (s, 4 H, O–CH₂–CH₂–O), 2.42 (s, 3 H, CH_3–C₆H₄SO₂), 1.22 (s, 3 H, 26–H), 0.99 (s, 3 H, 19–H), and 0.61 (s, 3 H, 18–H).

The tosylate (6d) (0.4 g) was dissolved in dry ether (80 ml) and was treated with LiAlH₄ (1.2 g). The reaction mixture was refluxed for 6.5 h and after cooling was worked

up as described previously to give (6e) (0.28 g, 90%), $\nu_{max.}$ (Nujol) 1 060 and 1 035 cm⁻¹; ¹H n.m.r., δ 5.30 (m, 1 H, 6–H), 4.68 (m, 1 H, 2-THP-H), 3.92 (s, 4 H, O–CH₂–CH₂–O), 1.33 (s, 3 H, 26–H), 1.09 (s, 3 H, 19–H), and 0.71 (s, 3 H, 18–H); m/e 499 ($[M - CH_3]^+$).

The compound (6e) (0.25 g) was dissolved in acetone (50 ml) containing toluene-*p*-sulphonic acid monohydrate (0.05 g) and the solution was kept at room temperature for 16 h. Work-up gave 25-oxo-27-norcholesterol (7a) (0.18 g, 98%). An analytical sample was prepared by crystallisation from ethanol and drying in high vacuum at 110 °C for several hours, m.p. 113 °C, (lit.,¹⁸ m.p. 114 °C) (Found: C, 80.41; H, 10.95. Calc. for C₂₆H₄₂O₂: C, 80.77; H, 10.95%) $\nu_{max.}$ (KBr) 3 500, 1 710, and 1 060 cm⁻¹, ¹H n.m.r., δ 5.31 (m, 1 H, 6–H), 3.53 (m, 1 H, 3–H), 2.11 (s, 3 H, CH₃CO), 1.01 (s, 3 H, 19–H), 0.94 (d, J 6 Hz, 3 H, 21–H), and 0.68 (s, 3 H, 18–H); m/e 386 (M^+), 371 ($[M - CH_3]^+$), 368 $[M - H_2O]^+$), and 353 ($[M - H_2O - CH_3]^+$).

Preparation of 3β-*Acetoxy*-27-*norcholest*-5-*en*-25-*one* (7b).—The hydroxy-ketone (7a) (0.25 g) was treated with acetic anhydride (5 ml) and pyridine (5 ml) at room temperature for 16 h. Work-up gave (7b) (0.27 g, 97%), m.p. 138—139.5 °C (ethanol), $[\alpha]_D^{25} = -45.3°$ (lit.,⁴ m.p. 139—140 °C, $[\alpha]_D^{25} = -45.3°$), identical in all respects with an authentic sample (i.r., n.m.r., no depression in mixed m.p.).

Preparation of 25-*Hydroxycholesterol* (7c).—To a solution of ketone (7b) (0.08 g) in dry ether (80 ml) under argon was added dropwise a 1.66M ethereal solution of methylmagnesium iodide (3 ml) with stirring. The resulting mixture was refluxed for 2 h, then cooled and the excess of reagent was decomposed with saturated ammonium chloride solution. The organic layer was diluted with ether (100 ml) and washed with water. Removal of solvent gave (7c) (0.065 g, 87%), m.p. 172—174 °C (methanol) (lit.,⁴ᵇ m.p. 177—180 °C; lit.,²² m.p. 172—174 °C); $\nu_{max.}$ (KBr) 3 300 and 1 050 cm⁻¹; ¹H n.m.r., δ 5.30 (m, 1 H, 6–H), 3.50 (m, 1 H, 3–H), 1.22 (s, 6 H, 26–, 27–H), 1.02 (s, 3 H, 18–H), 0.95 (d, J 7 Hz, 3 H, 21–H), and 0.70 (s, 3 H, 18–H): m/e 402 (M^+, 51%), 387, 384 (100%), 369, 366, and 351.

²² J. P. Moreau, D. J. Aberhart, and E. Caspi, *J. Org. Chem.*, 1974, **39**, 2018.