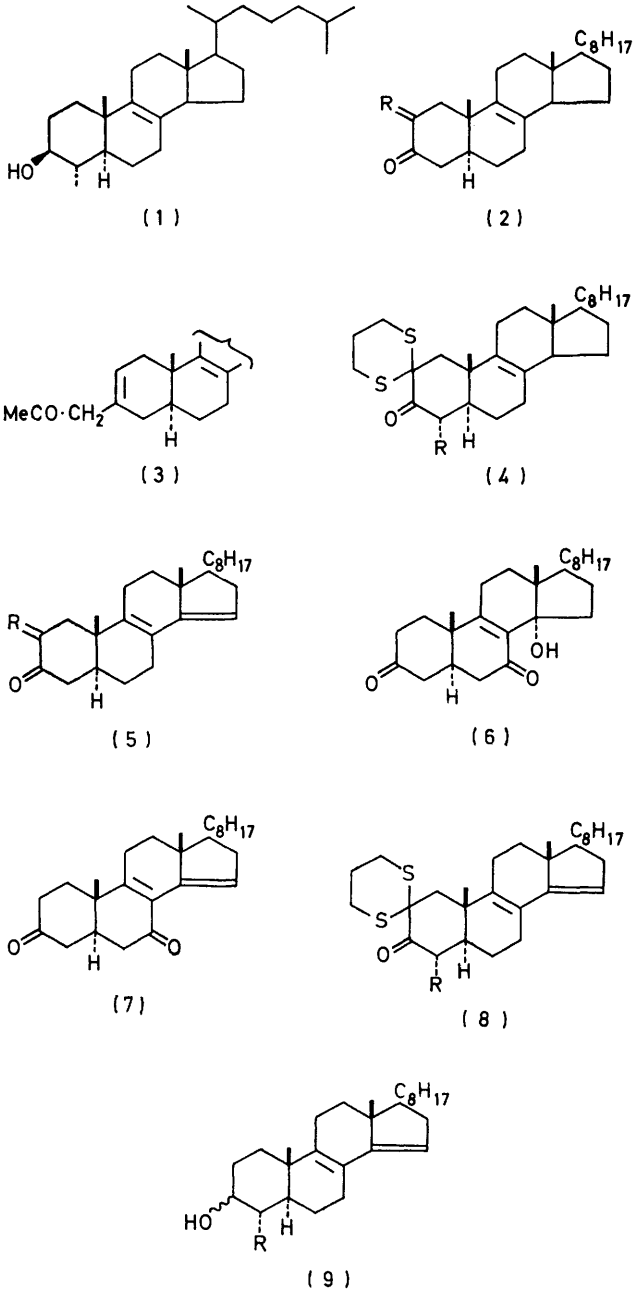
Unsaturated Steroids. Part 5.[1] Synthesis of 4α-Methylcholest-8(9)-en-3β-ol

By **Peter J. Hylands, John M. Midgley, Christopher Smith, Adrian F. A. Wallis, and W. Basil Whalley,*** The School of Pharmacy, The University, London WC1N 1AX

4α-Methylcholest-8(9)-en-3β-ol (1) has been synthesised from 5α-cholest-8-en-3-one by way of 2,2-trimethylenedithio-5α-cholest-8-en-3-one (4; R = H) and the corresponding 4α-methyl derivative (4; R = Me). Alternatively 5α-cholesta-8,14-dien-3-one (5; R = H_2) was converted into the 2,2-trimethylenedithio-derivative (8; R = H), which was monomethylated at C-4. Removal from this 4-methyl compound of the dithio-substituent and subsequent hydrogenation of the 14(15)-double bond gave 4α-methylcholest-8(9)-en-3β- (and 3α-) ol.

In 1960, Kandutsch and Russell [2] reported the isolation of a steroid which they designated B_2 from a transplantable, preputial gland tumour in certain strains of mice. On the basis of analytical evidence they formulated B_2 as 4α-methylcholest-8-en-3β-ol (1). The structures of other steroids (*e.g.* ref. 3) have been based on the validity of this conclusion, which we have confirmed by the synthesis of B_2 from 5α-cholesta-8,14-dien-3β-ol using two methods.

In the first, hydrogenation of 5α-cholesta-8,14-dien-3β-yl acetate,[4] by a modification of the published methods,[5] which in our hands were difficult to reproduce, gave 5α-cholest-8-en-3β-yl acetate. The corresponding alcohol was oxidised (Oppenauer) to the ketone (2; R = H_2), accompanied by a minor amount of a second product formulated as (3) (or much less likely the corresponding $\Delta^{3,4}$-isomer), on the basis of elemental analysis and the n.m.r. spectrum [τ 4.49br (vinylic H-2), 6.96 (s, CO·CH_2·C), and 7.80 (s, CH_3·CO·CH_2)]. The ketone (2; R = H_2) was converted by way of the 2-hydroxymethylene derivative (2; R = CH·OH) into 2,2-trimethylenedithio-5α-cholest-8-en-3-one (4; R = H). Methylation of this dithio-derivative furnished the 4α-methyl compound (4; R = Me), from which the blocking group was removed by reduction with Raney nickel, to yield 4α-methyl-5α-cholest-8-en-3β-ol (1), mixed with a minor amount of the 3α-isomer. Differentiation of the isomeric alcohols was based upon a self-consistent set of physical properties, so that, *inter alia*, (*a*) the minor isomer faster moving on chromatography [6] was regarded as the axial (3α-) alcohol; (*b*) in accord with general principles, this 3α-ol had $\nu_{max.}$ 3 440 cm^{-1} whereas the more slowly running, equatorial (3β-) alcohol, had $\nu_{max.}$ 3 300 cm^{-1} (broad band); and (*c*) the C-3 proton in the 3α-ol had a sharp n.m.r. signal at τ 6.2, whereas the corresponding proton in the 3β-ol exhibited a broad signal at τ 6.6—7.1.[7] These assignments were confirmed by oxidation of the 3α-ol to 4α-methyl-5α-cholest-8-en-3-one, which was reduced with sodium borohydride to the 3β-ol. The 3β-ol and its acetate were identical with specimens isolated from natural sources.[2]



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The second pathway to (1) avoided the difficult hydrogenation of 5α-cholesta-8,14-dien-3β-yl acetate. Thus, oxidation (Oppenauer) of 5α-cholesta-8,14-dien-3β-ol readily gave the ketone (5; R = H_2); although Jones oxidation furnished the same ketone (5; R = H_2) it was accompanied by substantial amounts of a second ketone provisionally formulated as 14α-hydroxy-5α-cholest-8-ene-3,7-dione (6). Thus the elemental analysis and mass spectrum were in accord with the molecular formula, $C_{27}H_{42}O_3$. The n.m.r. spectrum, which was devoid of the C-15 vinylic proton signal, contained a broad peak at τ 6.05 (4 H, 2 t, *J* 15 Hz) and another broad peak at τ 7.40—8.00 (7 H α to carbonyl and/or double-bond functions). A signal at τ 8.04 (1 H, s) was removed by deuterium oxide; signals attributable to a methine proton were absent. Additionally (6) was stable to Jones oxidation and had $\nu_{max.}$ (sharp) 3 450 (tertiary alcohol), 1 708 (six-membered ring C=O), and 1 689 cm^{-1} (αβ-unsaturated six-membered ring C=O); the u.v. spectrum showed $\lambda_{max.}$ 260 nm (log ε 4.71) (αβ-unsaturated ketone).

Treatment of this diketone with phosphoryl chloride–pyridine gave a product devoid of hydroxy-groups, of molecular formula $C_{27}H_{40}O_2$ (elemental analysis and mass spectrum), and having $\nu_{max.}$ 1 708 (six-membered ring C=O) and 1 690 cm^{-1} (αβ-unsaturated six-membered C=O), and $\lambda_{max.}$ 242 (log ε 4.04). The n.m.r. spectrum was devoid of signals attributable to protons strongly deshielded by a carbonyl group, but exhibited a multiplet (1 H) at τ 4.48. It thus appeared that the dehydration had formed a diene in which the second double bond was not in complete conjugation with the original αβ-unsaturated ketone. These combined data strongly suggest that the oxidation product has formula (6) and the dehydrated compound formula (7) (*cf.* refs. 4 and 8).

The 5α-cholesta-8,14-dien-3-one (5; R = H) was converted successively by way of the 2-hydroxymethylene ketone (5; R = CHOH) and the 2,2-trimethylenedithio-5α-cholesta-8,14-dien-3-one (8; R = H) into the 4α-methyl derivative (8; R = Me). Reductive desulphurisation of (8; R = Me) gave a mixture (separable by chromatography) of 4α-methyl-5α-cholesta-8,14-dien-3α- and -3β-ols (9), together with 4α-methyl-5α-cholest-8-en-3β-ol (1).

Reduction with Raney nickel of the separated diastereoisomeric alcohols (9) gave the corresponding 4α-methyl-5α-cholest-8-en-3α- and -3β-ols, identical with the specimens prepared previously.

EXPERIMENTAL

Specific rotations were determined for solutions in chloroform. Light petroleum used had b.p. 60—80 °C.

Synthesis of 4α-Methyl-5α-cholest-8-en-3β- (1) *and -3α-ol.*—*Method A.* Hydrogenation of 5α-cholest-8,14-dien-3β-yl acetate was performed by the following modification of the literature method.[5] A solution of the acetate (1 g) in ethanol (100 ml) containing W-4 Raney nickel (2 g) was shaken in hydrogen during 90 min (uptake 60 ml, 1 mol. equiv.). Purification by the Weissberger technique [9] gave 5α-cholest-8-en-3β-yl acetate (0.4 g) in plates, m.p. 123.5—125° (from ethyl acetate–methanol), $[\alpha]_D^{25}$ +52° (*c* 1.80) {lit.,[5] m.p. 125—126°, $[\alpha]_D^{20}$ +55° (*c* 1.08)}.

Oxidation of the alcohol (1.1 g) from this acetate by the Oppenauer procedure gave, after chromatography from light petroleum on deactivated alumina, followed by elution with light petroleum–ethyl acetate (99 : 1), (i) 3-(2-*oxopropyl*)-5α-*cholesta*-2,8-*diene* (3) (0.13 g), in plates, m.p. 117—118° (from aqueous acetone) (Found: C, 85.0; H, 11.1. $C_{30}H_{48}O$ requires C, 84.8; H, 11.4%), $[\alpha]_D^{25}$ +131° (*c* 1.68); followed by (ii) 5α-cholest-8-en-3-one (0.7 g) in needles, m.p. 123—124.5° (from aqueous acetone), $[\alpha]_D^{25}$ +68° (*c* 1.20) {lit.,[10] m.p. 124—125°, $[\alpha]_D$ +66.4° (*c* 0.99)}.

Crystallisation of this ketone from methanol resulted in quantitative conversion into 3,3-*dimethoxy*-5α-*cholest*-8-*ene*, which formed plates, m.p. 90—93°, $[\alpha]_D^{25}$ +42.0° (*c* 2.90) [Found: C, 81.0; H, 11.9; OMe, 14.9. $C_{27}H_{44}(OMe)_2$ requires C, 80.9; H, 11.7; OMe, 14.2%]. The corresponding ketone was regenerated (quantitatively) from this acetal when a solution in methanol–water (8 : 1) was added to an excess of acetic acid and the product isolated 2 h later.

2,2-*Trimethylenedithio*-5α-*cholest*-8-*en*-3-*one* (4; R = H).—Prepared during 60 h from 5α-cholest-8-en-3-one (3 g) in benzene (25 ml) containing sodium hydride (0.5 g) and methyl formate (4 ml) (stirred under nitrogen), 2-hydroxymethylene-5α-cholest-8-en-3-one (2.8 g) formed an amorphous yellow solid, m.p. 155—157° (from ether), and showed one spot on t.l.c. A solution of this hydroxymethylene derivative (2 g) in ethanol (200 ml) containing sodium acetate (4.6 g) and *SS*-trimethylene bis(toluene-*p*-thiosulphonate) (2 g) was refluxed under nitrogen for 8 h. After isolation the product was purified by chromatography on deactivated alumina from light petroleum to yield 2,2-*trimethylenedithio*-5α-*cholest*-8-*en*-3-*one* (1.8 g) in plates, m.p. 159—160° (from ethyl acetate–methanol), $[\alpha]_D^{25}$ +107° (*c* 0.95) (Found: C, 73.5; H, 9.6. $C_{30}H_{48}OS_2$ requires C, 73.7; H, 9.9%).

A mixture of this trimethylenedithio-compound (1 g) with 5% potassium t-butoxide in t-butyl alcohol (15 ml) and benzene (20 ml) was stirred under nitrogen until dissolution was complete.[11] Methyl iodide (3 ml) was then added and after 2 h further the product was isolated and purified by chromatography from light petroleum on alumina to yield 4α-*methyl*-2,2-*trimethylenedithio*-5α-*cholest*-8-*en*-3-*one* (0.9 g) in needles, m.p. 125° (from ethyl acetate–methanol), $[\alpha]_D^{25}$ +80° (*c* 0.65) (Found: C, 73.8; H, 10.1. $C_{31}H_{50}OS_2$ requires C, 74.0; H, 10.0%).

4α-*Methyl*-5α-*cholest*-8-*en*-3α- *and* -3β-*ol.*—A solution of the foregoing 2,2-trimethylenedithio-derivative (2.4 g) in ethanol (300 ml) was shaken in hydrogen with Raney nickel (W-4) for 4 h. After isolation the product was purified by chromatography from light petroleum on deactivated alumina [light petroleum–ethyl acetate (49 : 1) as eluant] to yield (i) 4α-*methyl*-5α-*cholest*-8-*en*-3α-*ol* (0.21 g) as needles, m.p. 107° (from methanol), $[\alpha]_D^{25}$ +42° (*c* 0.70) (Found: C, 84.1; H, 11.9. $C_{28}H_{48}O$ requires C, 83.9; H, 12.1%); and (ii) 4α-methyl-5α-cholest-8-en-3β-ol (1.1 g) in needles, m.p. 136—137° (from methanol), $[\alpha]_D^{25}$ +58° (*c*

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1.20) (Found: C, 83.4; H, 12.0. Calc. for $C_{28}H_{48}O$: C, 83.9; H, 12.1%).

Synthesis of 4α-*Methyl*-5α-*cholest*-8-*en*-3β- *and* -3α-*ol*.—*Method B.* 5α-*Cholesta*-8,14-*dien*-3-*one*. (*a*) Jones reagent was added to a solution of 5α-cholesta-8,14-dien-3β-ol (1 g) in acetone (50 ml) until present in an excess, which was discharged with methanol (5 ml). The product was purified from benzene on alumina (elution with light petroleum) to yield 5α-*cholesta*-8,14-*dien*-3-*one* (0.16 g) in needles, m.p. 133.5° (from aqueous acetone), $[\alpha]_D$ +15° (*c* 0.95) (Found: C, 84.7; H, 10.6. $C_{27}H_{42}O$ requires C, 84.8; H, 11.1%). Further elution with ether–light petroleum (8 : 92) gave 14α-*hydroxy*-5α-*cholest*-8-*ene*-3,7-*dione* (0.68 g) in needles, m.p. 204—205° (decomp.) (from aqueous acetone), $[\alpha]_D$ +73° (*c* 1.0) (Found: C, 77.6; H, 9.9%; M^+, 414. $C_{27}H_{42}O_3$ requires C, 78.2; H, 10.2%; *M*, 414).

(*b*) Oxidation of 5α-cholesta-8,14-dien-3β-ol (1 g) dissolved in pyridine (30 ml) with chromium trioxide (0.4 g) in pyridine (12 ml) during 12 h gave 14α-hydroxy-5α-cholest-8-ene-3,7-dione (0.91 g). Dehydration of this dione (0.18 g) dissolved in pyridine (15 ml) with phosphoryl chloride (25 mg) in pyridine (1 ml) at room temperature during 24 h gave 5α-*cholesta*-8,14-*diene*-3,7-*dione* (47 mg) in needles, m.p. 172—175° (from aqueous acetone), $[\alpha]_D$ +17° (*c* 0.5) (Found: C, 79.9; H, 10.1%; M^+, 396. $C_{27}H_{40}O_2$ requires C, 81.8; H, 10.2%; *M*, 396).

(*c*) A solution of 5α-cholesta-8,14-dien-3β-ol (10 g) in cyclohexanone (150 ml) was added to a boiling solution of aluminium t-butoxide (11 g) in xylene (200 ml). After refluxing for 7 h, the product was isolated and purified by chromatography from ether on alumina [elution with ether–light petroleum (3 : 7)] to yield 5α-cholesta-8,14-dien-3-one (6.4 g) in needles, m.p. 132—133.5° (from aqueous acetone), $[\alpha]_D$ +15° (*c* 0.9) (Found: C, 84.7; H, 10.6. Calc. for $C_{27}H_{42}O$: C, 84.7; H, 11.1%). When crystallised from methanol this ketone was converted quantitatively into 3,3-*dimethoxy*-5α-*cholesta*-8,14-*diene* which formed needles, m.p. 105—107°, $[\alpha]_D$ +9° (*c* 1.0) (Found: C, 80.9; H, 11.9. $C_{29}H_{48}O_2$ requires C, 81.3; H, 11.3%). The parent ketone was regenerated quantitatively when a solution of this acetal in acetic acid–water–methanol (4 : 6 : 90) was kept at room temperature for 4 h.

2,2-*Trimethylenedithio*-5α-*cholesta*-8,14-*dien*-3-*one* (8; R = H).—Prepared by condensation of 5α-cholesta-8,14-dien-3-one (2 g) with methyl formate (5 ml) in benzene (50 ml) containing sodium hydride (0.5 g) during 4 h, under nitrogen, 2-*hydroxymethylene*-5α-*cholesta*-8,14-*dien*-3-*one* (1.94 g) formed yellow prisms, m.p. 134—136° (from ether), $[\alpha]_D$ +3° (*c* 1.0) (Found: C, 81.4; H, 10.3. $C_{28}H_{42}O_2$ requires C, 81.9; H, 10.3%).

Condensation of this hydroxymethylene derivative (0.5 g) with *SS*-trimethylene bis(toluene-*p*-thiosulphonate) (0.5 g) occurred in boiling ethanol (50 ml) containing sodium acetate (1.5 g) during 8 h to yield 2,2-*trimethylenedithio*-5α-*cholesta*-8,14-*dien*-3-*one* (0.4 g) which formed plates, m.p. 131.5° (from aqueous acetone), $[\alpha]_D$ +95° (*c* 1.0) (Found: C, 74.0; H, 9.6%; M^+, 486. $C_{30}H_{46}OS_2$ requires C, 74.0; H, 9.5%; *M*, 486).

4α-*Methyl*-5α-*cholest*-8-*en*-3β- *and* -3α-*ol*.—A solution of methyl iodide (0.7 ml, 1.1 mol. equiv.) in benzene (6 ml) was added to a stirred solution of 2,2-trimethylenedithio-5α-cholesta-8,14-dien-3-one (0.5 g) in benzene (20 ml) containing potassium t-butoxide (0.55 g) under nitrogen. After 2 h the product was isolated and purified by chromatography from light petroleum, on alumina (elution with the same solvent) to yield 4α-*methyl*-2,2-*trimethylenedithio*-5α-*cholesta*-8,14-*dien*-3-*one* (0.28 g) in needles, m.p. 119—120.5° [from methanol–ethyl acetate (4 : 1)], $[\alpha]_D$ +67° (*c* 0.45) (Found: C, 74.2; H, 10.0%; M^+, 500. $C_{31}H_{48}OS_2$ requires C, 74.4; H, 9.6%; *M*, 500).

A solution of this ketone (0.8 g) in ethanol (150 ml) containing Raney nickel (W-2; 5 g) was shaken in hydrogen during 4 h at 60 °C. The crude product was purified on deactivated alumina from light petroleum to yield 4α-*methyl*-5α-*cholesta*-8,14-*dien*-3α-*ol* (74 mg), which formed needles, m.p. 94—97° (from aqueous acetone), $[\alpha]_D$ +37° (*c* 1.0) (Found: C, 83.8; H, 10.8. $C_{28}H_{46}O$ requires C, 84.4; H, 11.6%). Continued elution with benzene–light petroleum (1 : 9) gave 4α-*methyl*-5α-*cholesta*-8,14-*dien*-3β-*ol* (0.31 g) in needles, m.p. 120—122° (from methanol), $[\alpha]_D$ +21° (*c* 0.45) (Found: C, 84.2; H, 10.8. $C_{28}H_{46}O$ requires C, 84.4; H, 11.6%).

The mother liquors from this crystallisation were evaporated and the residual oil was separated by t.l.c. on silica impregnated with 5% silver nitrate (elution with light petroleum containing 2% of ethyl acetate) to yield 4α-methyl-5α-cholest-8-en-3β-ol (30 mg) in needles, m.p. 134—136° (from methanol), $[\alpha]_D$ +55°, identical (m.p., mixed m.p., i.r., u.v., and n.m.r.) with the specimen prepared by Method A.

Reduction of 4α-*Methyl*-5α-*cholesta*-8,14-*dien*-3β- *and* -3α-*ol*.—(*a*) A solution of 4α-methyl-5α-cholesta-8,14-dien-3α-ol (170 mg) in ethanol (100 ml) containing Raney nickel (W-2; 1 g) was shaken in hydrogen until 95 ml (1 mol. equiv.) had been absorbed (40 min). The products were separated by the Weissberger[9] technique to yield 4α-methyl-5α-cholest-8-en-3α-ol (65 mg) in plates, m.p. 105—107°, $[\alpha]_D$ +42° (*c* 0.64), identical (m.p., mixed m.p., i.r., and g.l.c.) with the specimen prepared previously.

(*b*) Reduction of 4α-methyl-5α-cholesta-8,14-dien-3β-ol (0.2 g) in a similar manner gave 4α-methyl-5α-cholest-8-en-3β-ol (75 mg) in needles, m.p. 133—134°, $[\alpha]_D$ +55°, identical (m.p., mixed m.p., i.r., and g.l.c.) with an authentic specimen. The acetate was identical (m.p., mixed m.p., and mass spectrum) with the acetate of the natural sterol.

Conversion of 4α-*Methyl*-5α-*cholest*-8-*en*-3α-*ol into the* 3β-*Ol*.—A solution of 4α-methyl-5α-cholest-8-en-3α-ol (180 mg) in cyclohexanone (14 ml) and xylene (20 ml) containing potassium t-butoxide (240 mg) was refluxed for 60 h under nitrogen. The crude 4α-methyl-5α-cholest-8-en-3-one thus obtained was dissolved in methanol (40 ml) and reduced during 2 h with sodium borohydride (0.1 g). Purification of the product from methanol gave 4α-methyl-5α-cholest-8-en-3β-ol (120 mg), identical with an authentic specimen.

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