Experiments towards a Synthesis of Antheridiol: a Synthesis of Biologically Active Material

By **Gerald A. Smith** and **Dudley H. Williams,*** University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW

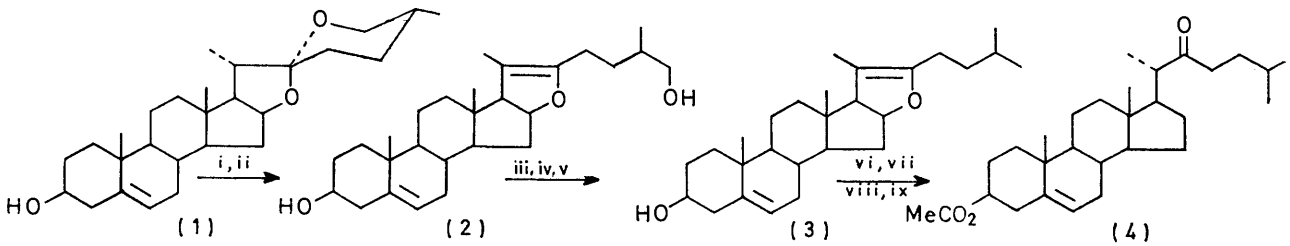
A conversion of diosgenin into a product exhibiting the biological activity of antheridiol (but at a lower level) is described. The product is either an active stereoisomer of antheridiol, or, more probably, a stereoisomer contaminated with antheridiol. In the course of the synthesis novel conditions were used for (i) the reduction of a hindered ketone in the presence of ester and lactone functions and (ii) an oxidative demethoxycarbonylation reaction to introduce a double bond.

In a preliminary communication,[1] we described the conversion of diosgenin acetate (1) into (20*S*)-3β-acetoxycholest-5-en-22-one (4) (and the 20*R*-isomer) in 25% overall yield. This work provided us with a cheap source of compound (4),† which was regarded as a suitable intermediate for the synthesis of antheridiol (5), the substance secreted by the female of the species of aquatic fungus *Achlya bisexualis* to activate the formation of the male sex organs.[4] Two previous syntheses of antheridiol, the first steroidal sex hormone to be recognized in the plant kingdom, have been described.[5,6] The present paper reports the conversion of (20*S*)-3β-acetoxycholest-5-en-22-one (4) into a product exhibiting the biological activity of antheridiol (5), although at a reduced level (probably associated with stereochemical problems at C-22 and, possibly, C-23), and provides experimental details of the whole sequence from diosgenin acetate (1).

† The economics of producing (20*S*)-3β-acetoxycholest-5-en-22-one (4) are dependent on (among other things) the scale involved, the number of steps in the route, and the relative cost of possible starting materials. The yields of compound (4) from (1), or of (4) from stigmasterol *via* oxidative cleavage of the side chain [2] followed by addition of an isopentyl group at C-22 (*via* the Cole and Julian procedure [3]), are comparable in our hands although the route from stigmasterol involves fewer steps. At the time of writing, diosgenin is considerably cheaper than stigmasterol as a research chemical in the U.K., whereas the reverse situation holds in the U.S. Nevertheless, the world market price for bulk diosgenin is currently considerably less than that for stigmasterol.

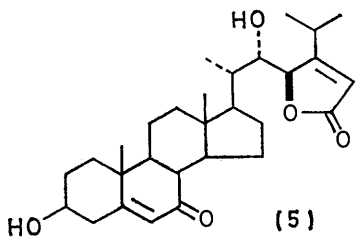
[1] G. A. Smith and D. H. Williams, *Chem. Comm.*, 1971, 402.
[2] M. Fryberg, A. C. Oehlschlager, and A. M. Unrau, *Chem. Comm.*, 1971, 1194.
[3] W. Cole and P. L. Julian, *J. Amer. Chem. Soc.*, 1945, **67**, 1369.
[4] G. P. Arsenault, K. Biemann, A. W. Barksdale, and T. C. McMorris, *J. Amer. Chem. Soc.*, 1968, **90**, 5635; see also A. W. Barksdale, *Science*, 1969, **166**, 831.
[5] J. A. Edwards, J. S. Mills, J. Sundeen, and J. H. Fried, *J. Amer. Chem. Soc.*, 1969, **91**, 1248.
[6] J. A. Edwards, J. Sundeen, W. Salmond, T. Iwadare, and J. H. Fried, *Tetrahedron Letters*, 1972, 791.

The strategy of our synthesis was to generate the Δ^{23}-22-ketone from the 22-ketone, and to introduce the two carbon atoms of the butenolide ring by Michael addition to this chromophore. Bromination of compound (4) with 2 mol. equiv. of bromine and a trace of hydrobromic acid in acetic acid was specific and gave (20*S*)-3β-acetoxy-5α,6β,23ξ-tribromocholestan-22-one, which was



Reagents: i, AcCl–pyridine; ii, $NaHCO_3$–MeOH; iii, TsCl–pyridine; iv, heat in H_2O–Me_2CO; v, $LiAlH_4$; vi, HBr–LiBr; vii, Zn–Cu; viii, NaOMe–MeOH; ix, Ac_2O–pyridine.

debrominated with sodium iodide in acetone [7] to give (20*S*)-3β-acetoxy-23ξ-bromocholest-5-en-22-one (6). This product was characterized by a well defined n.m.r. triplet (due to the 23-H) at δ 4·37 p.p.m. (splitting 8 Hz). Dehydrobromination of the 23-bromide (6) with calcium carbonate and dimethylformamide proceeded smoothly



(5)

above 130° to give (20*S*)-3β-acetoxycholesta-5,23-dien-22-one (7) in 90% yield from the ketone (4).

The enone (7) was treated with the Reformatsky reagent prepared from ethyl α-bromoacetate. Reformatsky reagents are known to undergo Michael addition with enones,[8] but the product obtained in low yield after prolonged reaction was identified as the ester arising from 1,2-addition of the reagent to the 22-ketone. In attempts to add the anion of diethyl malonate to C-24 of the enone system, involving use of a wide range of solvents and bases (*e.g.* sodium ethoxide in ethanol, sodium hydride in tetrahydrofuran), yields of the desired product were either low or negligible. Nysted and Burtner found similar problems in the condensation of a 17-nitroandrostane with methyl acrylate, but overcame them by using tetramethylguanidine as a base in neat methyl acrylate.[9] The substrate and addendum are reversed with respect to the steroid skeleton in the present case; nevertheless, a similar system with tetramethylguanidine as base and dimethyl malonate as solvent was tried, and an almost quantitative yield of methyl 3β-acetoxy-28-methoxycarbonyl-22-oxo-24ξ-stigmast-5-en-29-oate (8) was obtained. The product was a mixture of C-24 epimers; crystallisation from methanol gave a pure isomer in 33% yield.*

Hydrolysis of the crystalline malonate adduct (8) (stereochemically pure at C-24) with potassium carbonate in aqueous methanol gave the monomethyl ester (9) in essentially quantitative yield. This product, in acetic acid containing a trace of hydrogen bromide, was treated with 2 mol. equiv. of bromine. As soon as all the bromine had reacted, the solution was diluted with water so as to avoid reacetylation of the 3β-hydroxy-group. The Δ^5-system was regenerated in the usual manner (sodium iodide–acetone) to give the 23-bromo-derivative (10). In earlier experiments, the bromination had been carried out directly on the malonate adduct (8), and two products had been isolated by preparative t.l.c.; the n.m.r. spectra of these materials had indicated the major product (60%) to be the 23-bromo-derivative (doublet at δ 4·96 p.p.m., *J* 7·5 Hz, due to 23-H) and the minor product (30%) to be the 28-bromo-derivative (this showed no extra low-field signals in the n.m.r. spectrum, but showed non-equivalent methoxy-resonances). However, bromination of compound (9) appeared to be more selective, occurring at C-23. The required γ-lactone system (11) was obtained from compound (10) by treatment with sodium hydrogen carbonate in methanol. The conversion of stereochemically pure, crystalline compound (8) into a γ-lactone (11) which was pure by t.l.c. could be carried out in yields in excess of 90%.

We next wished to reduce the 22-carbonyl group of compound (11); this reaction had to be carried out selectively in the presence of the γ-lactone and methyl ester functions. Aluminium isopropoxide, the tetrapyridyl and tri-t-butoxy-derivatives of lithium aluminium hydride,[10] and sodium borohydride were tried without success. Eventually, the keto-lactone (11) was converted into the 3β-tetrahydropyranyl ether, and was treated with lithium borohydride in the presence of methyl acetate as solvent (so that competitive reduction of the ester solvent would avoid reduction of the ester

* The 20*R*-isomer of compound (4) has also been converted into (20*R*)-(8) which has an n.m.r. spectrum distinct from that of (8); this indicates retention of configuration at C-20 during the sequence.

[7] R. P. Holysz, *J. Amer. Chem. Soc.*, 1953, **75**, 4432.

[8] C. Gandolfi, G. Doria, M. Amendola, and E. Dradi, *Tetrahedron Letters*, 1970, **45**, 3923 and references cited therein.

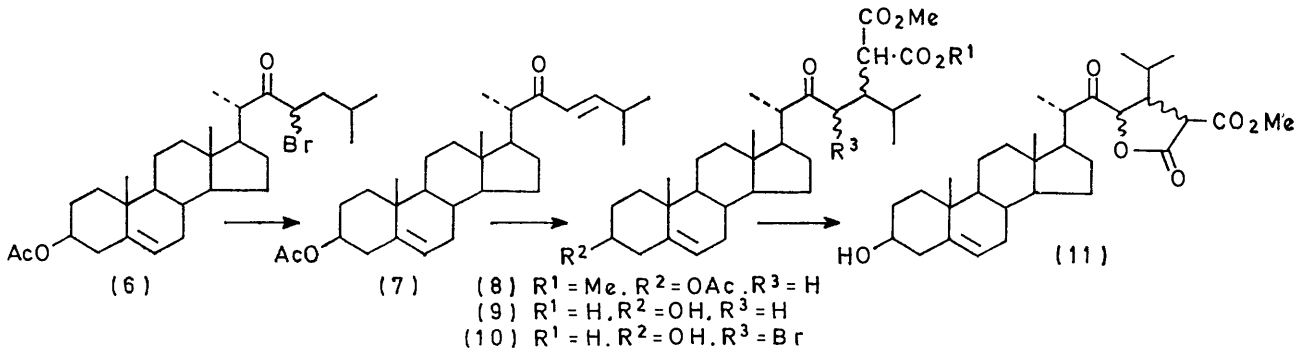
[9] L. N. Nysted and P. R. Burtner, *J. Org. Chem.*, 1962, **27**, 3175.

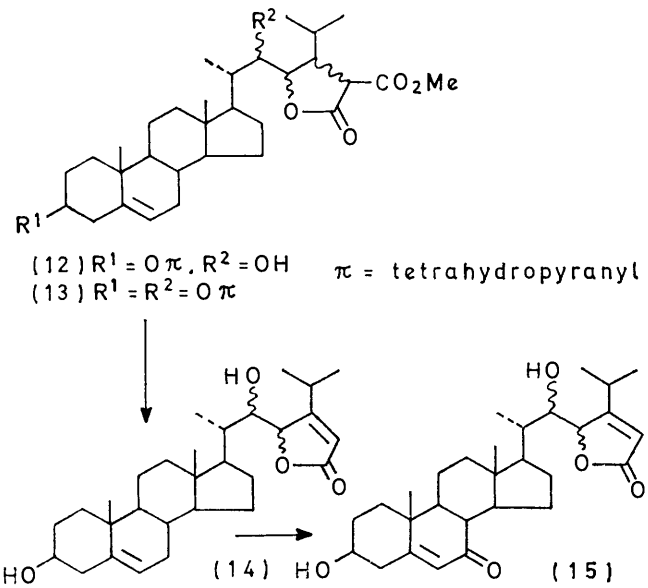
[10] L. F. Fieser and M. Fieser, ' Reagents for Organic Synthesis,' Wiley, New York, 1967.

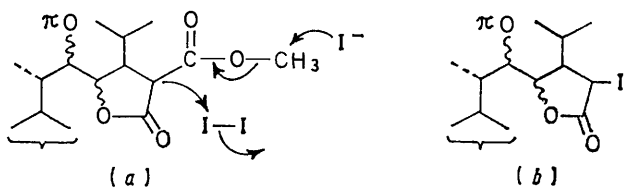
functions of the steroid system), and a high yield of a 22-ξ-hydroxy-compound (12) was obtained.* The product (12) was isolated from the reaction mixture by hydrolysis with dilute acetic acid and extraction with ether, and converted directly into the 3β,22ξ-bistetrahydropyranyl ether (13).

All conventional methods which were used in an attempt to replace the C-28 methoxycarbonyl group by a halogen atom (or to introduce directly the 24,28-double bond) failed (*e.g.* Hunsdiecker reaction [11] or Barton oxidative decarboxylation [12]). The 3β,22ξ-bistetrahydropyranyl ether (13) was therefore heated under reflux in dimethylformamide solution with calcium carbonate, lithium iodide, and iodine. The intention was to demethylate the methyl ester *via* a nucleophilic attack of iodide ion, and to oxidise the C-28 carbanion (generated *in situ* following decarboxylation) with iodine [see (*a*) ⟶ (*b*)]. This procedure worked well in practice and resulted in a 40% yield of the αβ-unsaturated γ-lactone (14), the elimination of hydrogen iodide and loss of the pyranyl ether groups having occurred in the refluxing dimethylformamide. The desired product (14) was contaminated with an equal quantity of the saturated γ-lactone [24,28-dihydro-(14)] from which it could be separated by crystallization; this contaminant arises from a competing protonation of the C-28 carbanion.

We have modified the conditions used by Syntex [5,13] for the photo-oxidation of 7-deoxyantheridiol to antheridiol, on finding that cholesterol can be converted into the Δ^6-5-hydroperoxide in >95% yield on carrying out the photo-oxidation in dimethylformamide as solvent with Methylene Blue as the photo-sensitizer. The conversion (14) ⟶ (15) was carried out in approximately 28% yield by use of these conditions, followed by rearrangement of the 5α-hydroperoxide to the Δ^5-7-ketone system with copper(II) acetate in pyridine.[14] Our product (15) had m.p. 250—254°, which is essentially the same as that described for antheridiol (m.p. 250—255°).[4] The n.m.r spectrum of the synthetic material is almost identical with that of antheridiol, except for the signal arising from the C-21 methyl protons (centred at δ 1·08 p.p.m. in the spectrum of the synthetic product, but appearing at δ ⩾ 1·12 p.p.m. in that of authentic antheridiol and not clearly resolved from the signals associated with other methyl protons). The synthetic product was tested for biological activity by Dr. A. Musgrave of the Developmental Botany Unit, Cambridge, who found that it produced antheridial branching of the male *Achlya bisexualis* at a concentration of 2×10^{-12} g ml^{-1} which is comparable to the activity of antheridiol (10^{-11} g ml^{-1} for







* It is essential in these experiments to use *soluble* lithium borohydride. Lithium borohydride occurs in two forms with different solubilities (' Handbook of Chemistry and Physics,' ed. Weast, 51st edn., 1970—1971).

[11] J. R. Dice and J. N. Bowden, *J. Amer. Chem. Soc.*, 1949, **71**, 3107.

[12] D. H. R. Barton and E. P. Serebryakov, *Proc. Chem. Soc.*, 1962, 309.

[13] A. Nickon and J. D. Bagli, *J. Amer. Chem. Soc.*, 1961, **83**, 1498.

[14] G. O. Schenk, O. A. Neumuller, and W. Eisfield, *Angew. Chem.*, 1958, **70**, 595.

branching). However, it was obviously desirable that the synthetic material be tested under conditions as close as possible to those used for authentic antheridiol. Such tests were arranged and carried out by Dr. T. C. McMorris and Dr. A. Barksdale at the New York Botanical Gardens. In these experiments, our crystalline synthetic product induced branching at 2×10^{-10} g ml^{-1} and delimitation of antheridia at 2×10^{-8} g ml^{-1}; the product from the mother liquors of our crystallization (see Experimental section) induced branching at 10^{-10} g ml^{-1} and delimitation of antheridia at 2×10^{-9} g ml^{-1}. (The corresponding figures for authentic antheridiol in the same hands are 10^{-11} and 10^{-10} g ml^{-1}, respectively.) Thus our synthetic product has about one tenth of the activity of the natural hormone. Since this work was carried out, antheridiol has been shown to have the 22*S*,23*R*-stereochemistry.[6] On the basis of the chemical shift of the C-21 methyl resonance (see before), the major product of our synthesis is likely to have the opposite stereochemistry at C-22 to that in the natural hormone. Thus our crystalline synthetic product is either a pure epimer of antheridiol (which exhibits a lower level of biological activity) or it is an epimer contaminated with *ca.* 5% of antheridiol. The latter explanation is consistent with the fact that the mother liquors contain material of higher biological activity.

Efforts to invert the stereochemistry at C-22 during the course of the synthesis, although not exhaustive, have not been successful. Thus the experiments reported complete a 16-stage synthesis of highly active material from the readily available steroid diosgenin, but not a synthesis of pure antheridiol.

EXPERIMENTAL

N.m.r. spectra were recorded on a Varian HA 100 MHz instrument for solutions in deuteriochloroform unless otherwise stated; resonance positions are given on the δ scale (p.p.m.) relative to internal tetramethylsilane. I.r. spectra were taken for Nujol mulls. U.v. spectra were determined for ethanolic solutions. Mass spectra were obtained with A.E.I. MS 9 and MS 12 spectrometers, by use of a direct inlet system, an ionizing voltage of 70 eV, an accelerating voltage of 8 kV, and a source temperature of *ca.* 180°. Silica GF_{254} adsorbent was used for t.l.c.

Pseudodiosgenin (2).—Diosgenin acetate (110 g) was heated under reflux, with stirring, for 45 min, in a mixture of pyridine (22·0 ml), acetyl chloride (17·1 ml), methylamine hydrochloride (16·2 g), and acetic anhydride (110 ml) in ethylene glycol diacetate (550 ml). The resulting pale yellow solution was cooled, poured into water (11·0 l), and stirred to destroy the acetic anhydride. The solid was filtered off and washed with water to give crude pseudodiosgenin diacetate. The wet diacetate was hydrolysed by heating under reflux for 12 h in methanol (3·0 l) containing saturated sodium hydrogen carbonate solution (1·0 l). Dilution of the mixture to 15·0 l with water and isolation of the solid product by filtration gave a gel, which on drying gave pseudodiosgenin as a white powder (98·0 g, 98%), m.p. 150—165° (lit.,[15] 163—168°); δ (C_5D_5N) 5·8 (m, OH), 5·35 (d, *J* 5 Hz, 6-H), 4·77 (m, 16-H), 3·7 (m, 26-H, 3-H), 1·63 (s, 21-H), 1·06 (d, *J* 7 Hz, 27-H), 1·04 (s, 19-H), and 0·76 (s, 18-H).

Pseudodiosgenin 3β,26-*Ditoluene*-p-*sulphonate*.—Pseudodiosgenin (98·0 g) was added during 30 min to a stirred solution of toluene-*p*-sulphonyl chloride (200 g) in pyridine (500 ml) with the temperature maintained below 5°. The mixture was stirred for 4 h to complete the reaction, then poured slowly into a solution of sodium hydrogen carbonate (160 g) in water (2·0 l) under ether (1·0 l). The ethereal phase was separated and combined with one further extract, dried ($MgSO_4$), and evaporated under vacuum below 50°, to remove ether and pyridine. Trituration of the red oil with di-isopropyl ether and filtration gave pseudodiosgenin ditoluene-*p*-sulphonate (145·0 g, 85%), m.p. 80—83° (lit.,[16] 85—86°).

Pseudodiosgenin 26-*Toluene*-p-*sulphonate*.—The 3β,26-ditosylate (145·0 g) was dissolved in boiling acetone (3·0 l) containing water (1·0 l) and heated under reflux while water (1·0 l) was added during 1 h. The resulting suspension of product was heated under reflux for a further 2 h to complete the hydrolysis and then left to cool for 12 h. The solid was filtered off, washed with water, and dried to give pseudodiosgenin 26-toluene-*p*-sulphonate as a white crystalline solid (89·0 g, 77%), m.p. 156—157° (lit.,[16] 157—159°).

26-*Deoxypseudodiosgenin* (3).—Pseudodiosgenin 26-toluene-*p*-sulphonate (50·0 g) was added to a stirred solution of lithium aluminium hydride (5·0 g) in tetrahydrofuran (500 ml), and the resulting mixture was stirred for 12 h. The excess of hydride was destroyed with water. Dilution of the resulting solution with 3N-hydrochloric acid (500 ml) and water (4·0 l) gave a white solid, which was filtered off and dried to give 26-*deoxypseudodiosgenin* (35·0 g, 100%), m.p. 133—139° [m.p. 142—143° (from acetone)], $[\alpha]_D$ −53° ($CHCl_3$); $\nu_{max.}$ 1695 (vinyl ether) and 1050 cm^{-1}; δ 5·35 (d, *J* 4 Hz, 6-H), 4·74 (m, 16-H), 3·5 (m, 3-H), 1·59 (s, 21-H), 1·04 (s, 19-H), 0·90 (d, *J* 6 Hz, 26-, 27-H), and 0·72 (s, 18-H); M^+ at *m/e* 398 (Found: C, 79·5; H, 10·7. $C_{27}H_{42}O_2,0{\cdot}5H_2O$ requires C, 79·5; H, 10·6%).

3β-*Hydroxy*-20ξ-*cholest*-5-*en*-22-*one*.— 26-Deoxypseudodiosgenin (10 g) was dissolved in methylene chloride (250 ml) and treated with a cooled mixture of water (100 ml), lithium bromide (100 g), and 48% hydrobromic acid (100 ml), with stirring for 6 h. The layers were separated. The aqueous layer was extracted with methylene chloride (100 ml) and then put aside for further use. The organic phases were washed consecutively with water (50 ml) and 5% sodium hydrogen carbonate (100 ml), and then combined, dried, and evaporated at room temperature under vacuum to give an amorphous solid, crude (20*R*)-16α-bromo-3β-hydroxycholest-5-en-22-one; $\nu_{max.}$ 3400 and 1705 cm^{-1}; δ 5·35 (d, *J* 5 Hz, 6-H), 4·0 (m, 16-H), 3·5 (m, 3-H), 1·0 (d, *J* 6 Hz, 21-H), 0·92 (s, 19-H), 0·9 (d, *J* 6 Hz, 26-, 27-H), and 0·66 (s, 18-H).

The product was treated immediately with a zinc–copper couple [prepared from zinc (120 g) and copper sulphate (50 g)] in 90% ethanol (500 ml) for 1 h. The couple was filtered off, washed with absolute ethanol, and stored under absolute ethanol for further use. The aqueous ethanolic solution was evaporated to dryness; the product was dissolved in methylene chloride (250 ml) and the solution was washed with water (20 ml) and dried ($MgSO_4$). This solution was recycled twice through the hydrobromic acid and zinc–copper couple treatments with the same reagents.

[15] F. C. Uhle, *J. Amer. Chem. Soc.*, 1961, **83**, 1460.
[16] F. C. Uhle, *J. Org. Chem.*, 1962, **27**, 2797.

The final methylene chloride solution was evaporated to dryness to give an amorphous solid (9·9 g, 98%), containing 95% (by t.l.c.) of a mixture of (20*R*)- and (20*S*)-22-oxocholesterols. The ratio 20*R* : 20*S* was determined as *ca.* 6 : 1 from the intensity ratio of the C-18 proton resonances in the n.m.r. spectrum (δ 0·73 and 0·79, respectively).

(20*R*)-3β-*Acetoxycholest-5-en-22-one*.—The 6 : 1 mixture of 22-oxocholesterols (1 g) was dissolved in a mixture of anhydrous pyridine (10 ml) and acetic anhydride (2 ml) at *ca.* 50° and set aside at room temperature for 12 h. The mixture was added to water (100 ml) and the precipitated solid was filtered off and washed with water. The product crystallised from ethanol as a waxy solid, giving (20*R*)-3β-acetoxycholest-5-en-22-one (0·45 g), contaminated with 10% of the 20*S*-isomer, m.p. 114—124°; $[\alpha]_D$ −62° ($CHCl_3$); $\nu_{max.}$ 1730, 1705, 1245, 1040, and 800 cm^{-1}; δ 5·35 (d, *J* 5 Hz, 6-H), 4·6 (m, 3-H), 2·0 (s, acetate), 1·0 (d, *J* 6 Hz, 21-H), 0·96 (s, 19-H), 0·86 (d, *J* 6 Hz, 26-, 27-H), and 0·73 (s, 18-H); M^+ at *m/e* 442. Repeated crystallisation of this material failed to give a pure sample of the 20*R*-ketone.

(20S)-3β-*Acetoxycholest-5-en-22-one* (4).—A solution of the 6 : 1 mixture of 22-oxocholesterols (5·0 g) and sodium methoxide (12 g) in AnalaR methanol (100 ml) was heated under reflux for 24 h. The product was poured into 3N-hydrochloric acid (500 ml) and the solid filtered off and dried (5·0 g). The product was acetylated in the same manner as the 20*R*-isomer and recrystallised from ethanol six times to give the pure (20*S*)-acetate (4) (410 mg, 8·2%), m.p. and mixed m.p. 152° (lit.,[3] 152°); $[\alpha]_D$ −59° ($CHCl_3$) (lit.,[3] −63°); $\nu_{max.}$ 1730, 1705, 1245, and 1040 cm^{-1}; δ 5·25 (d, *J* 5 Hz, 6-H), 4·6 (m, 3-H), 1·99 (s, acetate), 1·07 (d, *J* 6 Hz, 21-H), 0·99 (s, 19-H), 0·86 (d, *J* 6 Hz, 26-, 27-H), and 0·78 (s, 18-H); M^+ at *m/e* 442.

(20*S*)-3β-*Acetoxy-23-bromocholest-5-en-22-one* (6).— The 3β-acetate (4) (1·0 g) in acetic acid (20 ml) containing 50% hydrogen bromide in acetic acid (0·1 ml) was treated with bromine (0·24 ml, 0·73 g). Reaction was immediate. After 10 min, the mixture was poured into an excess of saturated sodium hydrogen carbonate solution (300 ml) and extracted with ether. The extract was dried ($MgSO_4$) and evaporated to give the 5α,6β-dibromo-derivative of the title compound (6) (1·53 g, 98%). Crystallisation from ether gave a sample with m.p. 123—125°; $[\alpha]_D$ −9° ($CHCl_3$). This tribromide was dissolved in acetone (20 ml) containing dry sodium iodide (2·0 g) and stirred for 30 min. The acetone was evaporated off *in vacuo* and the product partitioned between ether and dilute sodium thiosulphate solution. The ethereal layer was separated, dried and evaporated to give *compound* (6) (1·1 g, 96%) as a pale yellow solid. Crystallisation from ether gave a sample with m.p. 133—135°; $[\alpha]_D$ +9° ($CHCl_3$); $\nu_{max.}$ 1730, 1270, 1240, and 1040 cm^{-1}; δ 5·35 (d, *J* 5 Hz, 6-H), 4·6 (m, 3-H), 4·37 (t, *J* 8 Hz, 23-H), 2·85 (m, 21-H), 2·0 (s, 3-acetate), 1·27 (d, *J* 6 Hz, 21-H), 1·03 (s, 19-H), 0·93 (m, 26-, 37-H), and 0·74 (s, 18-H); M^+ at *m/e* 520, 522. A sample recrystallised from methanol was analysed (Found: C, 65·9; H, 8·6; Br, 14·3. Calc. for $C_{29}H_{45}BrO_3,0{\cdot}5CH_3OH$ requires C, 65·9; H, 8·8; Br, 14·8%).

(20*S*)-3β-*Acetoxycholesta-5,23-dien-22-one* (7).—The 23-bromide (6) (1·1 g) was heated at 130° with light calcium carbonate (2·0 g) and lithium bromide (1·0 g) in dimethylformamide (10 ml) for 1·5 h. The resulting slurry was cooled and poured into iced dilute hydrochloric acid, with care to avoid frothing. The resulting solid was filtered off and dried to give the conjugated enone (7) as an off-white powder (850 mg). This material, homogeneous on t.l.c. would not crystallise except from boiling ethanol. Multiple 'recrystallisation' from aqueous ethanol gave a pure *sample*, m.p. 143—145°; $[\alpha]_D$ −65° ($CHCl_3$); $\lambda_{max.}$ 228 nm; $\nu_{max.}$ 3400 (H_2O), 1725, 1690, 1660, and 1625 cm^{-1}; δ 6·83 (d, *J* 16 Hz, d, *J* 6 Hz, 24-H), 6·08 (d, *J* 16 Hz, 23-H), 5·35 (d, *J* 5 Hz, 6-H), 4·6 (m, 3-H), 1·97 (s, acetate), 1·07 (d, *J* 6 Hz, 21-H), 1·01 (d, *J* 7 Hz, 26-, 27-H), 0·97 (s, 19-H), and 0·68 (s, 18-H); low abundance M^+ ion at *m/e* 440 and base peak at *m/e* 380 (M^+ −AcOH) (Found: C, 77·8; H, 10·1. $C_{29}H_{44}O_3,0{\cdot}5H_2O$ requires C, 77·5; H, 10·0%).

Methyl 3β-*Acetoxy-28ξ-methoxycarbonyl-22-oxostigmast-5-en-29-oate* (8).—The 5,23-dien-22-one (7) (7·4 g) dissolved in tetrahydrofuran (10 ml) was added to a solution of tetramethylguanidine (4 ml) in dimethyl malonate (16 ml). The mixture was left at room temperature for 2 days. In order to avoid the troublesome evaporation of dimethyl malonate, the mixture was poured into a large volume of water (1·2 l) and the solid was filtered off through Hyflo. The last traces of dimethyl malonate were removed by washing with water. Extraction of the filtration pad with ether and evaporation of the dried solution gave a yellow oil (7·9 g, 83%) containing only traces of impurity (t.l.c.). Crystallisation from methanol gave a single pure isomer of the *adduct* (8) (3·1 g, 33%), m.p. 143—144°; $[\alpha]_D$ −31° ($CHCl_3$); $\nu_{max.}$ 1720br, 1230, and 1020 cm^{-1}; δ 5·35 (d, *J* 5 Hz, 6-H), 4·6 (m, 3-H), 3·67 (s, OMe), 1·99 (s, OAc), 1·07 (d, *J* 7 Hz), 21-H), 1·00 (s, 19-H), 0·87 and 0·81 (d, *J* 6 Hz, 26-, 27-H), and 0·67 (s, 18-H); M^+ at *m/e* 572 (Found: C, 71·3; H, 9·0. $C_{24}H_{52}O_7$ requires C, 71·3; H, 9·2%).

The mother liquors were hydrolysed by heating under reflux for 30 min with an equal weight (4·8 g) of potassium carbonate in aqueous methanol. A solution of the potassium salt in water was washed with ether, acidified with 2N-sulphuric acid, and extracted with ethyl acetate. The extract was treated with an excess of diazomethane, dried ($MgSO_4$), and evaporated. Acetylation (acetic anhydride–pyridine) of the product gave a non-crystallisable oil (3·5 g, 37%). This oil contained *ca.* 65% of the crystalline isomer (n.m.r. spectrum).

3β-*Hydroxy-28ξ-methoxycarbonyl-22-oxostigmast-5-en-29,23ξ-olactone* (11).—The malonate adduct (8) (1·0 g) was heated under reflux with potassium carbonate (2·0 g) in a mixture of methanol (50 ml) and water (30 ml) for 60 min. The mixture was cooled and acidified with dilute hydrochloric acid (100 ml). The free acid (9) was extracted into chloroform (3 × 100 ml); the solution was dried ($MgSO_4$) and evaporated below room temperature. The resulting gum was stirred with 1% hydrogen bromide in acetic acid (6 ml) and treated with 1·8% v/v bromine in acetic acid (10 ml). As soon as the bromine colour had gone (*ca.* 5 min) the solution was diluted rapidly with water (100 ml); the tribromo-ketone was filtered off and air-dried (1·2 g).

The off-white solid was dissolved in acetone (20 ml) and treated with sodium iodide (2·0 g) for 15 min. Saturated sodium hydrogen carbonate solution (1·9 ml) was added, followed by methanol (20 ml) and water (20 ml), and the solution was heated under reflux for 30 min with stirring. The suspension obtained was cooled, treated with an excess of sodium hydrogen sulphite, and diluted to 150 ml with water. *Compound* (11) was filtered off, washed with water, and dried (850 mg, 96%). Recrystallisation from methanol gave a sample of m.p. 208—212°; $[\alpha]_D$ +47° ($CHCl_3$); $\nu_{max.}$ 3400, 1775, 1790 (lactone), 1735 (ester), and 1715 (ketone) cm^{-1}; δ 5·35 (d, *J* 5 Hz, 6-H), 5·09 (d, *J* 8 Hz, 23-H), 3·80 (s, OMe), 5·63 (d, *J* 10 Hz, 28-H), 2·6 (m, 20-, 24-H),

1·14 (d, *J* 7 Hz, 26-H), 1·0 (d, *J* 7 Hz, 27-H), 1·0 (s, 19-H), 0·82 (d, *J* 7 Hz, 21-H), and 0·72 (s, 18-H); M^+ at *m/e* 514 (Found: C, 69·6; H, 8·7. $C_{31}H_{46}O_6,H_2O$ requires C, 69·9; H, 9·0%).

28ξ-*Methoxycarbonyl*-3β,22ξ-*bistetrahydropyranyloxystigmast*-5-*en*-29,23ξ-*olactone* (13).—The keto-lactone (9) (300 mg) was dissolved in tetrahydrofuran (3 ml) containing dihydropyran (0·3 ml) and treated with anhydrous toluene-*p*-sulphonic acid (30 mg) at room temperature. After 30 min the mixture was diluted with methyl acetate (10 ml), cooled to 0°C, and treated with lithium borohydride (300 mg). The mixture was stirred with efficient cooling at 0° for 2 h. The excess of borohydride was destroyed by addition of dilute acetic acid, and the product was extracted into ether, washed with sodium carbonate, dried, and evaporated to give crude 22ξ-hydroxy-28ξ-methoxycarbonyl-3β-tetrahydropyranyloxystigmast-5-en-29,23ξ-olactone (12) (450 mg). The monoether was dissolved in tetrahydrofuran (3 ml) containing dihydropyran (0·3 ml) and treated with toluene-*p*-sulphonic acid (30 mg) at room temperature for 30 min. The crude product (650 mg) was isolated by partitioning between ether and sodium carbonate solution. *Compound* (13) (260 mg, 65%) was isolated as an amorphous solid by preparative t.l.c. on silica gel (developed with 4 : 1 petroleum–acetone); $\nu_{max.}$ 1765 cm^{-1} (lactone); δ 5·35 (d, *J* 5 Hz, 6-H), 4·5—4·9 (m, 23-H and tetrahydropyranyl), 3·8 (s, OMe), 3·2—4·0 (m, 3-H, 22-H, 28-H, and tetrahydropyranyl), 1·0 (s, 19-H), and 0·7 (s, 18-H).

7-*Deoxy*-22ξ,23ξ-*antheridiol* (14).—To the bistetrahydropyranyl ether (13) (250 mg) in rigorously dried dimethylformamide (10 ml) were added dry calcium carbonate (1·0 g), lithium iodide (1·2 g), and iodine (1·2 g). The resulting suspension was stirred and heated at 150° for 2 h. T.l.c. (2 : 1 petroleum–acetone) indicated that the reaction was complete and that the tetrahydropropyranyl ether groups had been cleaved.

The mixture was poured into dilute hydrochloric acid and extracted with ether. The ethereal layer was washed with water and sodium hydrogen carbonate solution containing sodium hydrogen sulphite, dried, and evaporated to give a yellow gum. The required butenolide (14) was separated as a crude product (140 mg) by thick-layer chromatography (two runs in 2·5 : 1 petroleum–acetone). The n.m.r. spectrum indicated contamination with *ca.* 50% of the 24,28-dihydro-compound. Crystallisation from di-isopropyl ether gave *compound* (14), m.p. 209—211°; $[\alpha]_D$ −67° (3 : 1 $CHCl_3$–MeOH); $\nu_{max.}$ 3350 (OH), 1750 (lactone), and 1620 (vinyl) cm^{-1}; $\lambda_{max.}$ (95% EtOH) 223 nm [the 3β-acetate had $\lambda_{max.}$ (cyclohexane) 217 nm]; δ (6 : 1 $CDCl_3$–CD_3OD) 5·75 (t, *J* 1 Hz, 28-H), 5·34 (d, *J* 5 Hz, 6-H), 4·90 (d, *J* 8·5 Hz, d, *J* 1 Hz, 23-H), 3·57 (d, *J* 8·5 Hz, 22-H), 2·95 (m, 25-H), 1·22 (d, *J* 7 Hz, 26-H), 1·17 (d, *J* 7 Hz, 27-H), 1·03 (d, *J* 7 Hz, 21-H), 1·01 (s, 19-H), and 0·70 (s, 18-H); M^+ at *m/e* 456. Microanalysis indicated that the substance crystallizes as a 1 : 1 solvate with di-isopropyl ether (Found: C, 75·0; H, 10·4. $C_{29}H_{44}O_4,C_6H_{14}O$ requires C, 75·2; H, 10·5%).

22ξ,23ξ-*Antheridiol* (15).—The 1 : 1 mixture (140 mg) of 22ξ,23ξ-deoxyantheridiol and the dihydro-compound was dissolved in dimethylformamide (50 ml) containing Methylene Blue (50 mg) and oxygenated for 20 h in a Pyrex cell containing a 14 W, 50 cm fluorescent lamp through its axis. T.l.c. (10% MeOH–$CHCl_3$) indicated that *ca.* 60% reaction had occurred. The solvent was evaporated off under vacuum below 50° and the resulting gum suspended in pyridine (5 ml) and shaken with copper(II) acetate (140 mg) for 3 h. The resulting mixture was partitioned between ethyl acetate and dilute phosphoric acid; the organic phase was then washed with water and dilute sodium hydrogen carbonate solution, dried, and evaporated. Thick-plate chromatography in 5% methanol–chloroform gave 22ξ,23ξ-antheridiol (15) (*ca.* 20 mg). Crystallisation from methanol gave a chromatographically homogeneous and crystalline product, m.p. 250—254° (lit.,[4] for authentic antheridiol, 250—255°); $[\alpha]_D$ −95° ± 5° (3 : 1 $CHCl_3$–MeOH); $\nu_{max.}$ 3340 (OH), 1750 (lactone), 1640 (ketone), and 1620 (vinyl) cm^{-1}; $\lambda_{max.}$ (EtOH) 225 nm (ε 14,000); δ (6 : 1 $CDCl_3$–CD_3OD; prior to crystallisation) 5·76 (t, *J* 1 Hz, 28-H), 5·68 (s, 6-H), 4·94 (d, *J* 8·5 Hz, d, *J* 1 Hz, 23-H), 3·7 (m, 3-H), 3·59 (d, *J* 8·5 Hz, 22-H), 3·0 (m, 25-H), 1·21 (s, 19-H), 1·05 (d, *J* 7 Hz, 21-H), and 0·72 (s, 18-H); *m/e* 470 (M^+, 1%), 452 ($M^+ - H_2O$, 7%), 345 ($M^+ - C_7H_9O_2$, 70%), and 344 ($M^+ - C_7H_{10}O_2$, 100%) [lit.,[17] $\nu_{max.}$ 3400, 1740, 1670, and 1625 cm^{-1}; δ 5·76 (t, *J* 1 Hz, 28-H), 5·68 (s, 6-H), 4·94 (d, *J* 8·5 Hz, d, *J* 1 Hz, 23-H), 3·60 (d, *J* 8·5 Hz, 22-H), 3·0 (m, 25-H), 1·2 (s, 19-H), and 0·7 (s, 18-H)].

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[17] Dr. T. C. McMorris, personal communication.