Synthesis of Putative Precursors of Ecdysone. Part 3.[1] Synthesis of 3β,14α,25-Trihydroxy-5β-cholest-7-en-6-one

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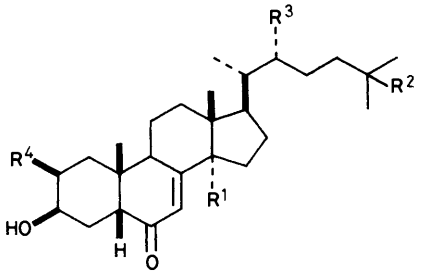
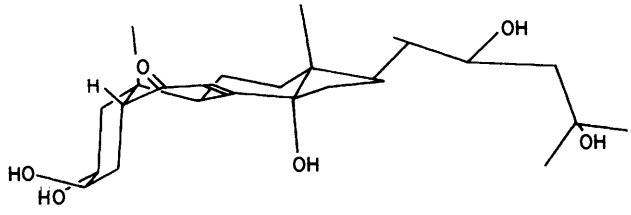
We describe here the first synthesis of 2,22-dideoxyecdysone (**3**) (3β,14α,25-trihydroxy-5β-cholest-7-en-6-one), a putative precursor in the biosynthesis of ecdysone (**1**). All the intermediates synthesized have a *cis* junction of the A/B rings (5β-H configuration).

Ecdysone (**1**) is one of the most important moulting hormones present in insects and in other arthropods. This polyhydroxylated steroid is synthesized in the endocrine glands (*e.g.* prothoracic glands in Orthoptera) during the post-embryonic development. In 1977 we showed the presence of ecdysone in the ovaries of adult females of *Locusta migratoria*, during vitellogenesis.[2] Besides ecdysone, we have reported[3] the occurrence of four other less polar ecdysteroids *viz.*, 2-deoxyecdysone (**2**), 2,22-dideoxyecdysone (**3**), 2,22,25-trideoxyecdysone (**4**), and 2,14,22,25-tetradeoxyecdysone (**5**).



(**1**)	OH	OH	OH	OH
(**2**)	OH	OH	OH	H
(**3**)	OH	OH	H	H
(**4**)	OH	H	H	H
(**5**)	H	H	H	H



Their structures were assigned by interpretation of their mass spectra.[3] Later, the synthesis of three of them [(**2**), (**4**), and (**5**)] confirmed our earlier structural interpretations,[4] but neither the complete structural assignment nor the synthesis of 2,22-dideoxyecdysone (**3**) has been reported. This compound, like the others, is extremely useful for biological studies on the biosynthesis of ecdysone; hence we planned to synthesize it and here we describe our work.

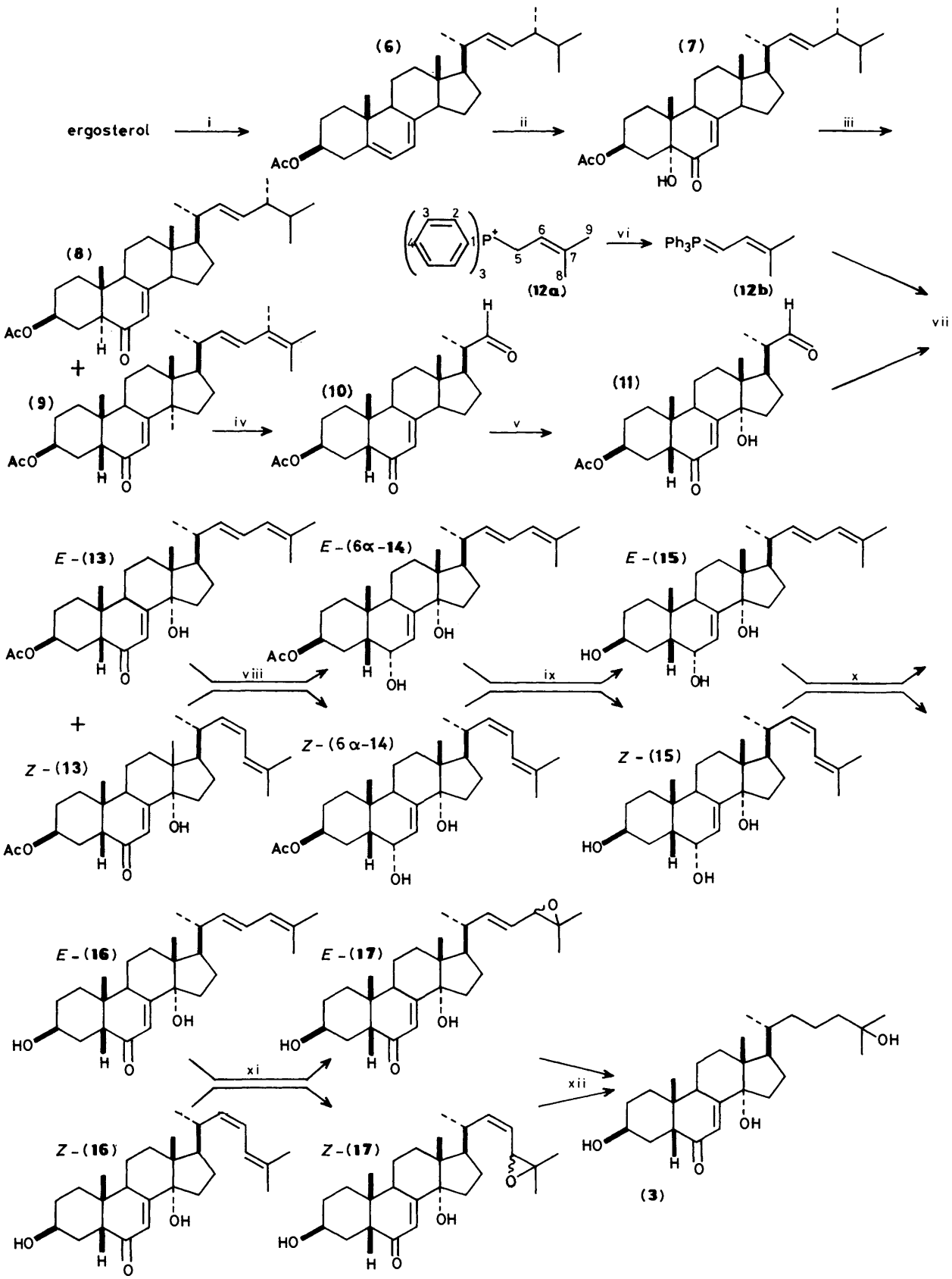
The instability of the A/B *cis* ring junction (5β-H configuration) caused by the absence of a 2β-hydroxy group in this target molecule, led us to develop a novel route allowing the synthesis of molecules with 5β-H stereochemistry.

2,22-Dideoxyecdysone (**3**) belongs to the 5β-2-deoxyecdysteroid series, in which the A/B rings are *cis* fused. In ecdysteroids containing a 2β-hydroxy group, the 5β-epimer is the more stable one, because of steric interaction between the 2β-hydroxy group and the 10-methyl.[5] However, in the case of 2-deoxyecdysteroids, where this steric interaction is absent, the 5α-epimer is the more stable.[6,7] The C-5 proton, α to an unsaturated ketone, can be easily removed and in equilibration conditions there is isomerisation to the more stable compound: that is, to the 5α isomer (A/B *trans* fused) in the case of 2-deoxyecdysteroids,[6,7] and to the 5β one (A/B *cis* fused) in the case of ecdysteroids like ecdysone.[8]

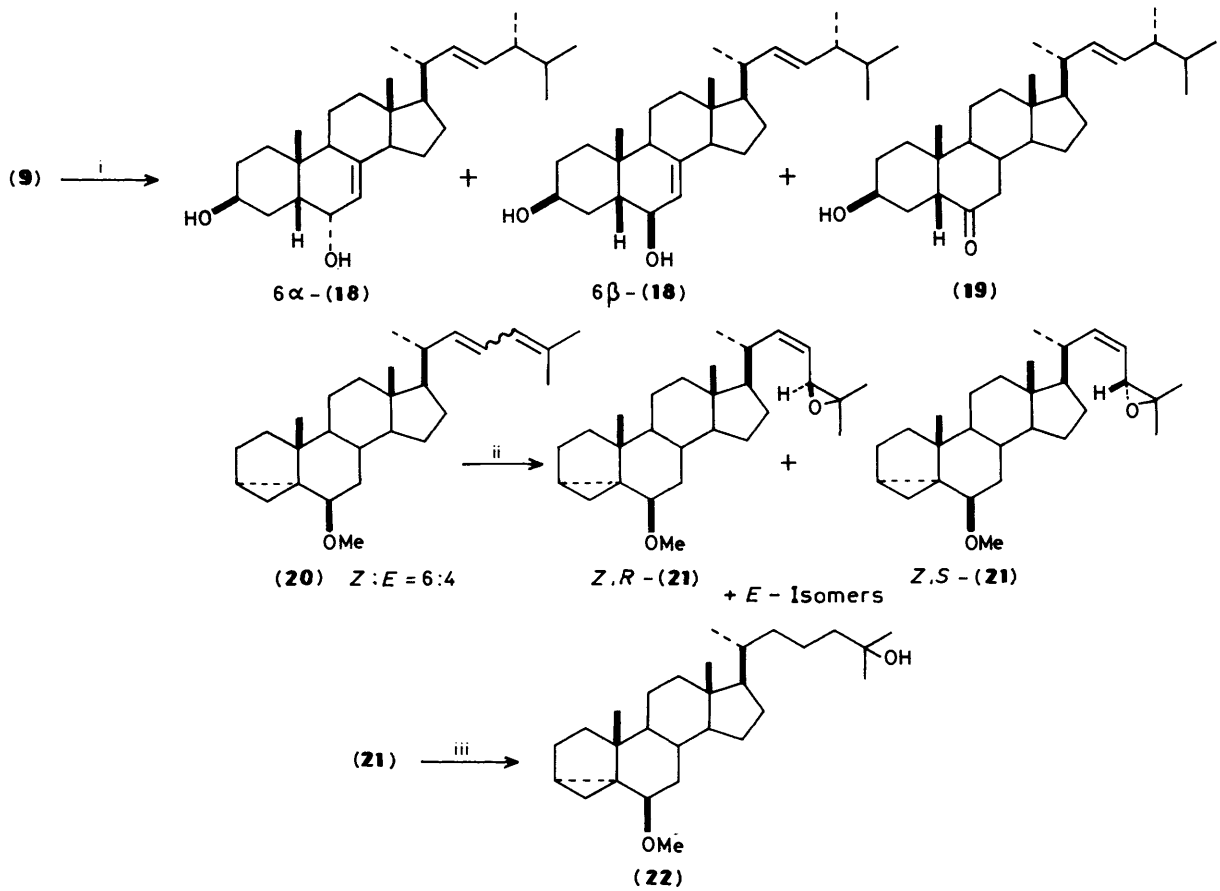
The previously reported syntheses (*e.g.* ref. 5—7, 9) of 5β-2-deoxyecdysteroids starting from Δ^5 sterols involved the use of 5α-2-deoxysteroids as intermediates. Equilibration of the 5α hydrogen at the end of the synthesis resulted in a mixture in which the 5α epimer predominates; the tedious separation of the two C-5 isomers gave a very low yield of the desired 5β-ecdysteroid (AB-*cis*:AB-*trans* < 1:4 under equilibration conditions[9]). To overcome these problems in the present synthesis, we chose our starting material with the 5β configuration, which was transferred to the target molecule through a series of reactions. A logical starting material for this purpose is 3β-acetoxy-5β-ergosta-7,22-dien-6-one (**9**).[1] This compound allows the introduction of the required side-chain after cleavage of the 22(23) double bond, and the 14α-hydroxy group can be introduced by allylic oxidation.

The overall synthesis of (**3**) is depicted in Scheme 1. The 5β-H ketone (**9**) was easily obtained in two steps from ergosterol 3β-acetate (**6**); (**6**) was oxidised by chromium trioxide to give the Buravoy ketone (**7**)[9] and the reduction of the 5α-hydroxy group with zinc–acetic acid led to two isomers (**8**) and (**9**), which are easily separated.[1,10] Treatment of (**9**) with SeO_2 resulted in only 10% of C-14 hydroxylation after several hours; the allylic positions of the side chain seem to compete with the C-14 one. In contrast, compound (**8**) reacts in a few minutes under the same conditions, to give the 14α-hydroxy derivative in good yield.[9] This difference of reactivity between compounds (**8**) and (**9**) can be attributed to the fact that, in A/B *trans* fused steroids [*e.g.* (**8**)], the molecule is flat, making the C-14α position readily accessible, whereas in the case of the A/B *cis* junction [*e.g.* (**9**)], the structure is bent, rendering the C-14α position very hindered. To overcome the competition of the side-chain, the C-22 double bond of (**9**) was first cleaved to the aldehyde (**10**) by mild ozonolysis.[1] Oxidation of (**10**) with selenium dioxide in dioxane at 110 °C* for 10 min gave (**11**) (78% yield). Neither epimerisation at the C-20(*S*) and C-5(β) centres, nor oxidation

* By comparison, it is interesting to note that the A/B *trans* isomer of (**10**) reacts at 80 °C in *ca.* 5 min.



Scheme 1. *Reagents and conditions:* i, Ac_2O, 120 °C, 10 min; ii, CrO_3, AcOH–H_2O, exothermic, 2 h; iii, Zn, AcOH, 90 °C, 6 h; iv, (1) O_3, CH_2Cl_2–Py (1%), −78 °C, 1.5 h, (2) Me_2S, −78 °C; 1 h; v, SeO_2, dioxane, 110 °C, 6 min; vi, BuLi (0.9 equiv.), THF, −78 °C, 0.5 h; vii, ylide (**12b**), THF, −30 °C, 1 h; viii, $NaBH_4$–$CeCl_3$, THF–EtOH, −20 °C, 1 h; ix, K_2CO_3, MeOH–H_2O, reflux, 6 h; x, MnO_2–$MgSO_4$, $CHCl_3$, room temp., 8 h; xi, *p*-nitroperbenzoic acid–NaF–KF, CH_2Cl_2, room temp., 10 min; xii, (1) H_2 or $^2H_2$, Pt–C (5%), dioxane, 80 °C, 35 min, (2) H_2 or $^2H_2$, Pd–C (5%), dioxane, 35 °C, 30 min



Scheme 2. *Reagents and conditions:* i, $LiAlH_4$, THF, room temp., 0.5 h; ii, *p*-nitroperbenzoic acid (1.1 equiv.)–NaF (1.5 equiv.)–KF (0.5 equiv.), CH_2Cl_2, room temp., 0.5 h (isomers are separated on 'Et_2NMe deactivated' SiO_2); iii, H_2, Pt–C (5%), CH_2Cl_2, room temp., 5 h

of the aldehyde occurred, thanks to the short reaction time (by t.l.c. and ^1H n.m.r. analysis*). The introduction of a conjugated diene from this aldehyde was accomplished by a Wittig reaction,[12] providing the two isomers *Z*-(**13**) and *E*-(**13**), without ylide-induced epimerisation at C-20† or at C-5 (67% yield). Isomers *Z*-(**13**) and *E*-(**13**) were separated by chromatography in the ratio *Z*-(**13**):*E*-(**13**) = 3:2. The poor selectivity of this reaction was not a handicap in our strategy, because the diene was finally hydrogenated.‡ However, both isomers were converted separately into the target molecule to allow a complete characterisation of the key intermediates.

Attempts to deprotect the acetate group of (**13**) under normal basic conditions without epimerisation at C-5 were unsuccessful (*e.g.* refs. 1, 5, 6, 9, and 15). Treatment of (**13**) with neutral potassium cyanide salt in ethanol[16] was also unsuccessful. We thus chose to deactivate the C-5 position by 1,2-reduction of the conjugated ketone at C-6. Treatment of (**9**) [analogue of (**13**)] with lithium aluminium hydride in tetrahydrofuran allowed the reduction of the conjugated system as well as the concomitant deprotection of the 3β-alcohol (Scheme 2). Nevertheless, this reaction gave a mixture of the 1,2-reduction product (**18**) [49% yield; a 4:3 mixture of the two isomers at C-6, 6α-(OH) and 6β-(OH) was separated by chromatography]§ as well as the 1,4-reduction compound (**19**) (28% yield). Increase of the yield of the 1,2-reduction by use of ether[18] was not possible because of the insolubility of our compounds in this solvent. Treatment of (**9**) with sodium borohydride–cerium chloride[19] resulted in the selective 1,2-reduction of the conjugated ketone quantitatively, without deprotection of the 3β-alcohol. In this way, the regiospecific and stereoselective reduction of (**13**) by introduction of a hydride mainly at the 6β position gave compound (**14**) (97% yield; 6β-H:6α-H = 6α-OH:6β-OH > 9:1, by ^1H n.m.r.). The two C-6 isomers 6α-(**14**) and 6β-(**14**) were isolated and characterised [*e.g.* δ_H 4.6 (6β-H_a), 3.8 (6α-H_e)]. The acetate at C-3 was then hydrolysed with potassium carbonate–methanol [to give (**15**), 91% yield] and the ketone at C-6 was regenerated by oxidation of the allylic alcohol with manganese dioxide [to give (**16**), 83% yield].

The final step in the synthesis of (**3**) involves modification of the side-chain of (**16**). The 25-hydroxy group can be obtained by the regiospecific introduction of a 24(25) epoxide,[20] followed by

* The two possible isomers at C-20 can be differentiated by ^1H n.m.r. (ref. 11*a*). (20*S*) aldehyde: δ_H 0.77 (18-H) and 1.13 (21-H); (20*R*) aldehyde: δ_H 0.73 (18-H) and 0.99 (21-H). For the ^1H n.m.r. of the two isomers 5α and 5β see refs. 6 and 11*b*.

† The retention of the (20*S*) configuration was established by a ^1H and ^{13}C n.m.r. study of *Z*-(**13**) and *E*-(**13**). This result is in agreement with the literature where it has been established that the natural configuration at C-20 is retained in this reaction (ref. 13).

‡ The conversion of the *Z* isomer into the *E* one with iodine (ref. 14) was not employed because of the poor yield of the reaction in our case. This reaction, when accomplished on the mixture *Z*- and *E*-(**13**) (ratio *Z*:*E* = 3:2), afforded *Z*- and *E*-(**13**) with a 60% yield in the reversed ratio *Z*:*E* = 3:7, namely a 50% conversion of *Z* isomer into *E* (see Experimental section). Separation and recycling of the *Z* isomer may have led to a complete conversion.

§ For each isomer, the C-6 stereochemistry was established by ^1H and ^{13}C n.m.r. spectroscopy. Our interpretation is corroborated by literature (ref. 17).

regiospecific reductive cleavage at C-24. To achieve this key transformation, we chose the 3,5-cyclo analogue (**20**) as a model compound (Scheme 2). Because of the instability of the allylic epoxide, we used sodium fluoride–potassium fluoride with *p*-nitroperbenzoic acid[21] for the synthesis of the epoxide and 2% triethylamine deactivated silica gel for the purification. The 24(25) double-bond of the side-chain of the diene (**20**) was thus oxidized by 1.1 equiv. of peracid to give 88% of (**21**). From this mixture of four isomers, the two isomers (*R*)-*Z*-(**21**) and (*S*)-*Z*-(**21**) have been separated and characterised (but the absolute configuration of the epoxide was not resolved). Having established the conditions for the reaction and the purification, the epoxidation of (**16**) was carried out to give (**17**) in 78% yield. Separately, *Z*-(**17**) was obtained in a similar way directly from *Z*-(**13**) in a four-step procedure without intermediate purification (69% yield over four steps). In the same way, compound *E*-(**17**) was separately obtained from *E*-(**13**) (67% yield over four steps).

Our preliminary investigation of the hydrogenation of the allylic epoxide was run on the model compound (**21**) [analogue of (**17**)] with 5% Pt–C in dichloromethane,[20] but the reaction was too slow, resulting in only 43% yield after a 5 h reaction period. Moreover, the *Z*-(**21**) isomer seemed to react preferentially under these conditions. Use of 5% Pd–C instead of 5% Pt–C for the hydrogenation of the allylic epoxide resulted in a mixture of products. Hence, the one pot-reduction of both the epoxide function and the 22(23) double-bond of *Z*-(**17**) * was carried out in two stages. The epoxide of compound *Z*-(**17**) was first regiospecifically reduced with 5% platinum-on-charcoal at 80 °C in dioxane for 0.5 h. The hydrogenation of the 22(23) double bond was then completed by addition of 5% palladium on charcoal at room temperature for a further 0.5 h. 2,22-Dideoxyecdysone (**3**; R = H) was obtained quantitatively and the same conditions afforded the deuteriated 2,22-dideoxyecdysone (**3'**; R = D) with deuterium gas.

In conclusion, we have shown a general strategy for the synthesis of an original series of steroids which have no 2-hydroxy group, are A/B *cis* fused, and contain a conjugated ketone at C-6. We have used ^{13}C n.m.r. spectroscopy for an extensive structural analysis of these compounds.

The mass spectra of natural[3],† and synthetic 2,22-dideoxyecdysone are very similar. Furthermore, we have analysed both compounds by reverse phase h.p.l.c.[22] The two compounds have the same retention time and, when co-injected, they are co-eluted in a single peak. The identity of the natural product is thus established 2,22-dideoxyecdysone, corroborating our first interpretation. This observation has led us to consider the synthesis of [^{3}H]-labelled 2,22-dideoxyecdysone for biological studies. Using tritium gas instead of hydrogen for the final hydrogenation step has proven unsuccessful after many experiments. Such differences between hydrogenation and tritiation have been recorded for heterogenous catalysis[23] and the present results are not surprising, considering the drastic conditions used for hydrogenation. Since experimental investigations are tedious with tritium, we are developing a new strategy for the synthesis of labelled 2,22-dideoxyecdysone.[24]

Experimental

M.p.s were measured on a Reichert hot-stage microscope and are uncorrected and $(\alpha)_D$ values were measured on a Perkin-Elmer 141 polarimeter. I.r. spectra were recorded in KBr on a Perkin-Elmer spectrometer and a Pye Unicam SP3-300S infrared spectrophotometer Philips. U.v. Spectra were measured on a Kontron Uvikon 810 u.v.–vis. spectrophotometer. ^1H, ^2H, and ^{13}C F.t. n.m.r. spectra were recorded on a Bruker SY (^1H, 200 MHz; ^{13}C, 50 MHz) and a Bruker AM (^1H, 400 MHz; ^2H, 61.4 MHz; ^{13}C, 100 MHz) apparatus in $CDCl_3$, CD_2Cl_2, or CD_3OD for ^1H and ^{13}C n.m.r. and in CH_3OH for ^2H n.m.r., using a 5-mm spinning tube. Chemical shifts are given in p.p.m. downfield from internal tetramethylsilane. δ_C Signals were assigned for totally ^1H decoupled spectra and confirmed by analyses of the signal multiplicities determined by the d.e.p.t. (distortionless enhancement by polarisation transfer) spectra, by comparison with data of model compounds. Mass spectra (m.s.) were measured at 70 eV on a Thomson THN 208 instrument by direct inlet for unmodified compounds, and on a LKB 9000 S apparatus coupled to a g.l.c. (OV-1 column) for $SiMe_3$ derivatives. High-resolution m.s. measurements have been done on the Thomson THN 208 by the 'peak-matching method'. T.l.c. were run on pre-coated plates of silica gel 60F254 (Merck), and silica gel (200—63 μm, 40—63 μm, or 15 μm, Merck) was used for column chromatography. Microanalyses were performed by the Strasbourg Division of the Service Central de Microanalyses of the C.N.R.S.

We have already described the synthesis of the Buravoy ketone (**7**)[9] as well as that of the 5β-isomers (**9**)[1] and (**10**).[1]

3β,14α,25-*Trihydroxy*-5β-*cholest*-7-*en*-6-*one* (**3**; R = H).—The epoxide *Z*-(**17**) (11.6 mg, 27.1 μmol) dissolved in dry dichloromethane (300 μl) and freshly distilled dioxane (3 ml) was hydrogenated in the presence of platinum-on-charcoal (5% Pt–C; 10 mg) at 80 °C during 0.5 h. The temperature was then decreased to 35 °C and palladium-on-charcoal (5% Pd–C; 10 mg) was added and the hydrogenation continued for a further 1 h at this temperature. The black solution was then filtered through a millipore membrane (0.5 μm; solvent resistent) and the solvent removed to yield the t.l.c.-pure compound (**3**; R = H) (11.5 mg). This product was further purified by chromatography on a silica gel column (0.063—0.200 mm; which had been previously washed with toluene–acetone–Et_2MeN, 8:2:0.1) to afford, after elution with toluene–acetone–Et_2MeN 6:4:0.1, the 2,22-dideoxyecdysone (**3**; R = H) (10.4 mg, 24.1 μmol, 89%) {t.l.c. toluene–acetone–Et_2MeN, 6:4:0.1, R_F[*Z*-(**17**)] = R_F[*E*-(**17**)] = 0.5, R_F[(**3**)] = 0.3}, m.p. 262—263 °C (from AcOEt–hexane–MeOH), $[\alpha]_D^{21}$ +63° (*c* 5.9 in MeOH) (Found: C, 73.8; H, 10.5%; M^+ 432.3238 ± 0.0017. $C_{27}H_{44}O_4$·0.5MeOH requires C, 73.66; H, 10.27%; *M*, 432.3239); $\lambda_{max.}$(MeCN) 241 nm (ε 11 500 dm^3 mol^{-1} cm^{-1}); $\nu_{max.}$ 3 600m, 2 970s, 2 940s, 2 880m, 1 660vs, 1 600w, 1 440—1 470m, 1 380m, 1 150w, and 1 090w cm^{-1}; ^1H n.m.r. results given in Table 1 and the Figure, and ^{13}C n.m.r. results given in Table 2; *m/z* 432 (M^+, $C_{27}H_{44}O_4$, 10%), 414 (21), 404 (29), 399 (29), 396 (10), 386 (90), 381 (10), 372 (6), 353 (13), 341 (4), 325 (3), 323 (2), 315 (9), 285 (11), 234 (49), 233 (100), 215 (16), 207 (15), and 194 (11).

G.l.c.–m.s. ($SiMe_3$-*derivatization*). (*a*) Trisilylated compound. *M/z* 648 (M^+, $C_{36}H_{68}O_4Si_3$. 22%), 633 (21), 620 (39), 558 (23), 543 (17), 468 (20), 453 (21), 377 (13), and 131 (100); isotopic peaks in the molecular area, 649 [$(M + 1)^+$, 11%], 648 (M^+, 22%) {[$(M + 1)/(M)$ + $(M + 1)$] = 33% and [$(M)/(M)$ + $(M + 1)$] = 67%} (*b*) Disilylated compound. *M/z* 576 (M^+, $C_{33}H_{60}O_4Si_2$, 8%), 561 (13), 543 (5), 518 (10), 486 (14), 471 (5), 468 (9), 458 (49), 453 (11), 396 (2), 381 (7), 368 (40), 305 (21), 215 (14), and 131 (100).

Comparison with the g.l.c.–m.s. ($SiMe_3$-*derivatization*) *of the natural* 2,22-*dideoxyecdysone*.[3] (*a*) Trisilylated compound. *M/z* 648 (M^+, $C_{36}H_{68}O_4Si_3$, 16%), 633 (5), 558 (19), 543 (6), 530 (10), 468 (16), 448 (18), 147 (32), and 131 (500). (*b*) Disilylated

* Because the *Z* isomer seems to be more appropriate, we used *Z*-(**17**) for this reaction, but *E*-(**17**) can also be used, though it is less reactive towards the opening of the epoxide.

† Natural 2,22-dideoxyecdysone was extracted from the ovaries of adult female of *Locusta migratoria* in ng quantities.[3] H.p.l.c.-pure samples of the natural compound were furnished by C. Kappler (Laboratoire de Biologie Générale, 12 rue de l'Université, 67000 Strasbourg, France).

compound. *M/z* 576 (M^+, $C_{33}H_{60}O_4Si_2$, 33%), 561 (62), 486 (100), 471 (31), 396 (38), 381 (19), 147 (67), and 131 (76).

H.p.l.c.-techniques. Natural and synthetic 2,22-dideoxyecdysone have been analysed by C_{18} reverse-phase h.p.l.c.; elution was performed with a gradient from 0 to 100% of methanol in water over 30 min. The retention time in both cases was 40 mn. When co-injected, the two compounds are co-eluted in a single peak.

[$^2H_3$-22,23,24]-3β,14α,25-*Trihydroxy*-5β-*cholest*-7-*en*-6-*one* (**3′**; R = D).—Compound *Z*-(**17**) (2.8 mg, 6.5 μmol) has been deuteriated in the same way as (**3**; R = H) to yield quantitatively the t.l.c.-pure deuteriated compound (**3′**; R = D), that has not been chromatographed [t.l.c. parameters identical with those of (**3**; R = H), see before], ^1H n.m.r. spectrum identical with that of (**3**; R = H); δ_D(60 MHz, solvent CH_3OH) 0.88 (m, $w_{\frac{1}{2}}$ 6 Hz, 22-H), 1.19 (m, $w_{\frac{1}{2}}$ 15 Hz, 23-H), 1.44 (m, $w_{\frac{1}{2}}$ 12 Hz, 24-H), and 5.35 (solvent); *m/z* 435 (M^+, $C_{27}H_{41}D_3O_4$, 8%), 434 (9), 433 (11), 432 (9), 417 (31), 416 (31), 415 (31), 414 (24), 407 (13), 406 (22), 405 (31), 404 (28), 402 (26), 401 (34), 400 (42), 399 (42), 389 (69), 388 (9), 387 (100), and 386 (75).

G.l.c.–m.s. (SiMe$_3$-*derivatization*). *M/z* 651 (M^+, $C_{36}H_{65}D_3O_4Si_3$, 11%), 636 (13), 623 (14), 561 (10), 546 (10), 456 (8), 377 (17), and 131 (100); isotopic peaks in the molecular area, 651 (M^+, $C_{36}H_{65}D_3O_4Si_3$, 11%), 650 [$(M-1)^+$, $C_{36}H_{66}D_2O_4Si_3$, 13%], 649 [$(M-2)^+$, $C_{36}H_{67}D_1O_4Si_3$], 21%), and 648 [$(M-3)^+$, $C_{36}H_{68}O_4Si_3$, 7%]. Corrected intensities by comparison with the natural isotopic patent of '(**3**; R = H) – $(SiMe_3)_3$': if $[(M)+(M-1)+(M-2)+(M-3)] = 100\%$, then the corrected intensity for each peak is: $(M) = 15\%$ (=% of trideuteriated compound), $(M-1) = 8\%$ (=% of dideuteriated compound), $(M-2) = 54\%$ (=% of monodeuteriated compound), and $(M-3) = 23\%$ =% of unlabelled compound).

(20S)-3β-*Acetoxy*-14α-*hydroxy*-6-*oxo*-5β-*pregn*-7-*ene*-20-*carbaldehyde* (**11**).—Powdered SeO_2 (1.75 g, 16 mmol) was added to a solution of aldehyde (**10**) (582 mg, 1.51 mmol) in freshly distilled dioxane (40 ml), and the flask was heated to 120 °C for 6 min. The solution was then frozen at 0 °C, and allowed to warm to room temperature. The solution was filtered through Celite, the filtrate evaporated, and the crude mixture adsorbed on a small amount of SiO_2 (0.063—0.200 mm); it was then chromatographed on SiO_2 (0.040—0.063 mm; eluant hexane–AcOEt 65:35) {t.l.c. hexane–AcOEt 1:1, R_F[5β-(**10**)] = 0.50, R_F[5α-(**10**)] = 0.60, R_F[5β-(**11**)] = 0.30, and R_F[5α-(**11**)] = 0.35}. After purification, compound (**11**) was obtained in 78% yield (473 mg, 1.18 mmol), m.p. 198—200 °C (from MeOH), $[\alpha]_D^{20}$ +90° (*c* 10.1 in CH_2Cl_2) (Found: C, 68.95; H, 8.65; M^+, 402.2404 ± 0.0012. $C_{24}H_{34}O_5$·MeOH requires C, 69.12; H, 8.76%; *M*, 402.2406); $\lambda_{max.}$(MeCN) 239 nm (ε 12 000 dm^3 mol^{-1} cm^{-1}); $\nu_{max.}$ 3 570m, 3 520—3 300m, 3 020m, 2 970s, 2 940s, 2 860m, 2 690w, 1 730vs, 1 660s, 1 620m, 1 440m, 1 370m, 1 350m, 1 230s, 1 140m, 1 020m, 930m, and 860m cm^{-1}; ^1H n.m.r. results given in Table 1 and the Figure, and ^{13}C n.m.r. results given in Table 2; *m/z* 402 (M^+, $C_{24}H_{34}O_5$, 29%), 374 (11), 356 (6), 346 (44), 342 (19), 314 (10), 299 (8), 286 (100), 276 (57), 216 (56), and 215 (39).

(2-*Methylbut*-2-*enyl*)*triphenylphosphonium Bromide* (**12a**).[12]—4-Bromo-2-methylbut-2-ene (10 g, 67 mmol) and triphenylphosphine (16 g, 61 mmol) were dissolved in freshly distilled toluene (20 ml) and the solution was stirred overnight at room temperature. The resulting precipitate was filtered off, washed with toluene and hexane, and dried *in vacuo* at 65 °C overnight to afford the Wittig salt (**12a**) (23.3 g, 57 mmol, 93% yield), m.p. 236—237 °C (from toluene) (Found: C, 66.9; H, 5.85; Br, 18.95; P, 7.25. Calc. for $C_{23}H_{24}BrP$: C, 66.99; H, 5.83; Br, 19.42; P, 7.52%); $\nu_{max.}$ 3 000s, 2 930s, 2 840s, 2 760m, (2 510, 2 400, 2 300, 2 200)w, 1 990—1 950w, 1 910—1 880w, 1 800w, 1 860w, 1 650m, 1 570m, 1 470m, 1 420s, 1 370m, 1 360m, 1 100s, 980s, and 830m cm^{-1}; δ_H(400 MHz; solvent $CDCl_3$; standard Me_4Si) 1.28 (3 H, d, $J_{P,H}$ 4 Hz, 9-H), 1.66 (3 H, d, $J_{P,H}$ 6 Hz, 8-H), 4.63 (2 H, dd, $J_{P,H}$ 14.5 Hz, and $J_{5,6}$ 7.5 Hz, 5-H), 5.12 (1 H, dt, $J_{P,H}$ 12 Hz and $J_{6,5}$ 7 Hz, 6-H), and 7.65—7.90 (15 H, complex, ArH), 7.27 ($CHCl_3$); δ_C(100 MHz; solvent $CDCl_3$; standard Me_4Si) 18.0 (C-8), 24.0 (d, $J_{P,C}$ 50 Hz, C-5), 25.3 (C-9), 107.6 (d, $J_{P,C}$ 9 Hz, C-6), 117.6 (d, $J_{P,C}$ 85 Hz, C-1), 129.8 (d, $J_{P,C}$ 13 Hz, C-3), 133.2 (d, $J_{P,C}$ 10 Hz, C-2), 134.4 (d, $J_{P,C}$ 2.5 Hz, C-4), and 143.0 (d, $J_{P,C}$ 13 Hz, C-7); *m/z* 331 (M – Br^-, $C_{23}H_{24}P^+$, 1%), 262 (100, PPh_3), and 183 (55).

(22*Z*)-3β-*Acetoxy*-14α-*hydroxy*-5β-*cholesta*-7,22,24-*trien*-6-*one* *Z*-(**13**) *and* (22*E*)-3β-*Acetoxy*-14α-*hydroxy*-5β-*cholesta*-7,22,24-*trien*-6-*one* *E*-(**13**).—Phosphonium bromide (**12a**) (1.4 g, 3.4 mmol) was suspended in tetrahydrofuran (freshly distilled over $LiAlH_4$), and butyl-lithium (3 mmol; hexane solution) was then added dropwise at −78 °C under argon. Strong red coloration indicated the formation of the ylide (**12b**). A solution of (**11**) [175 mg, 0.436 mmol in tetrahydrofuran (3 ml)] was added dropwise to the solution of the ylide at −78 °C, and the combined mixture was then carefully allowed to reach room temperature. After 1 h, the reaction was interrupted by addition of acetic acid (150 μl, 2.56 mmol) in order to neutralise the excess of ylide. Care was taken to avoid conditions that may epimerise the unstable 5β-H configuration. The white suspension was then treated with $NaHCO_3$ (300 mg) for 15 min with vigorous stirring, after which the crude mixture was cooled to −78 °C and filtered at this temperature through Celite. The filtrate was washed with cold (0 °C) tetrahydrofuran. After removal of the solvent, the mixture was dissolved in dichloromethane, adsorbed on a small amount of SiO_2 (0.063—0.200 mm) and chromatographed on SiO_2 (0.040—0.063 mm). Elution with a gradient of eluants of hexane–AcOEt 8:2 ⟶ 3:1, gave successively *E*-(**13**) (33 mg), then a mixture of *Z*- and *E*-(**13**) isomers (55 mg; *Z*:*E* = 6:4 by ^1H n.m.r.) and finally *Z*-(**13**) (45 mg). The overall yield for compound *Z*- and *E*-(**13**) is 67% (133 mg, 0.293 mmol; *Z*:*E* = 6:4) {t.l.c. hexane–AcOEt 6:4, R_F[(**11**)] = 0.2, R_F[*Z*-(**13**)] = 0.40, R_F[*E*-(**13**)] = 0.45}.

Z-(**13**): m.p. 172—174 °C (from AcOEt–hexane–CH_2Cl_2), $[\alpha]_D^{20}$ +55 °C (*c* 12 in CH_2Cl_2) (Found: C, 76.9; H, 9.45%; M^+, 454.3089 ± 0.0017. $C_{29}H_{42}O_4$ requires C, 76.65; H, 9.25%; *M*, 454.3083); $\lambda_{max.}$(MeCN) 236 nm (ε 32 000 dm^3 mol^{-1} cm^{-1}); $\nu_{max.}$ 3 580w, 3 040w, 2 960s, 2 860m, 1 730s, 1 660s, 1 440m, 1 370m, 1 230s, 1 150m, and 1 020m cm^{-1}; ^1H n.m.r. results given in Table 1 and the Figure, and ^{13}C n.m.r. results given in Table 2; *m/z* 454 (M^+, $C_{29}H_{42}O_4$, 11%), 436 (3), 421 (2), 394 (1), 373 (27), 367 (1), 361 (1), 355 (2), 344 (3), 327 (8), 313 (53), 295 (6), 109 (100), and 82 (57).

E-(**13**): m.p. 153—155 °C (from hexane–AcOEt–CH_2Cl_2), $[\alpha]_D^{20}$ +69° (*c* 8.3 in CH_2Cl_2) (Found: C, 76.85; H, 9.3%; M^+, 454.3089 ± 0.0017. $C_{29}H_{42}O_4$ requires C, 76.65; H, 9.25; *M*, 454.3083); $\lambda_{max.}$(MeCN) 235 nm (ε 31 000 dm^3 mol^{-1} cm^{-1}); $\nu_{max.}$ 3 580w, 3 040w, 2 960s, 2 860m, 1 730s, 1 660s, 1 440m, 1 370m, 1 230s, 1 150m, and 1 020m cm^{-1}; ^1H n.m.r. results given in Table 1 and the Figure, and ^{13}C n.m.r. results in Table 2; *m/z* 454 (M^+, $C_{29}H_{42}O_4$, 14%), 436 (4), 421 (2), 394 (4), 373 (37), 367 (1), 361 (1), 355 (2), 344 (3), 327 (7), 313 (54), 295 (5), 109 (100), and 82 (48).

Isomerisation of Z-(**13**) *to E*-(**13**).—A catalytic quantity of iodine[14] [0.1 equiv., 10 μmol; *i.e.* 0.2 ml of a solution of I_2 (13 mg, 50 μmol) in dichloromethane (1 ml)] was added to a mixture of *Z*-(**13**) and *E*-(**13**) (*Z*:*E* = 6:4) (40 mg, 88 μmol) in dichloromethane (5 ml). The solution was stirred for 24 h at room temperature, protected from light by aluminium foil, and then treated with aqueous thiosulphate ($Na_2S_2O_3$) to destroy

Table 1. ^{1}H N.m.r. chemical shifts. δ_H(400 MHz; standard Me_4Si); solv. = solvent, δ_H 5.35 (CHD_2OD), 3.34 ($CHDCl_2$), 7.27 ($CHCl_3$). * interchangeable assignments. For compound 6α-(**18**) and 6β-(**18**): δ_H 2.05 (14- and 17-H). For compound *Z*- and *E*-(**15**): δ_H 2.21 (17-H). For compound (**11**): δ_H 9.61 (d, $J_{22,20}$ 2.7 Hz, 22-H). Coupling constants are given in the Figure

					6α-(**14**)		6β-(**14**)		(**15**)				*Z*-(**17**)		*E*-(**17**)					*Z*-(**21**)	*Z*-(**21**)	
	(**3**)	(**11**)	*Z*-(**13**)	*E*-(**13**)	*Z*	*E*	*Z*	*E*	*Z*	*E*	*Z*-(**16**)	*E*-(**16**)	*R**	*S**	*R**	*S**	6α-(**18**)	6β-(**18**)	(**19**)	*R**	*S**	(**22**)
3-H	4.04	5.03	5.04	5.04	5.14		5.06		4.19		4.01	4.01	4.05		4.08		4.15	4.07	3.89			
4-H																				0.43	0.43	0.43
4-H																				0.65	0.65	0.65
5-H	2.41	2.38	2.34	2.34					2.07		2.44	2.44	2.45		2.48		2.00	2.01				
6-H					4.61		3.75		4.63								4.61	3.78		2.77	2.77	2.78
7-H	5.83	5.85	5.83	5.83	5.41		5.67		5.42		5.82	5.82	5.83		5.86		5.07	5.09				
9-H	3.10	3.15	3.13	3.13					2.73		3.22	3.22	3.09		3.07		2.05	2.06				
18-H	0.72	0.75	0.77	0.73	0.74	0.71	0.77	0.74	0.75	0.71	0.80	0.76	0.72		0.70		0.56	0.59	0.83	0.74	0.79	0.72
19-H	1.00	1.01	1.01	1.00	0.97	0.96	1.09		0.96	0.95	1.00	1.00	0.98		1.00		0.93	1.01	1.18	1.03	1.03	1.03
20-H		2.38	2.71		2.73		2.64		2.67		2.75		2.67		2.17		2.05	2.06	2.06	2.61	2.57	
21-H	0.97	1.15	1.02	1.07	1.00	1.04	1.12	1.14	0.98	1.02	1.03	1.08	1.03	1.05	1.07	1.06	1.02	1.06	0.98	1.05	1.03	0.93
22-H		9.61	5.19	5.44	5.18	5.45	5.16	5.44	5.18	5.45	5.17	5.42	5.13		5.76		5.25	5.25	5.23	5.07	5.07	
23-H			6.12	6.21	6.04	6.17	6.05	6.17	6.05	6.17	6.12	6.22	5.56	5.61	5.27	5.29	5.14	5.14	5.12	5.51	5.55	
24-H			6.15	5.78	6.08	5.77	6.09	5.77	6.09	5.77	6.08	5.76	3.43		3.16					3.45	3.45	
26-H	1.21		1.77	1.76	1.76	1.76	1.76	1.76	1.75	1.76	1.77	1.76	1.27	1.28	1.26	1.28	0.84	0.84	0.84	1.27	1.29	1.22
27-H	1.21		1.83	1.77	1.82	1.76	1.83	1.76	1.82	1.76	1.83	1.76	1.37	1.37	1.35	1.35	0.82	0.82	0.82	1.37	1.38	1.22
28-H																	0.92	0.92	0.92			
1′-H																				3.33	3.33	3.33
2′-H		2.07	2.07	2.06	2.06		2.08															
Solv.	5.35	5.35	5.35	5.35	7.27		7.27		7.27		3.34	3.34	7.27		7.27		7.26	7.27	7.27	7.27	7.27	7.27

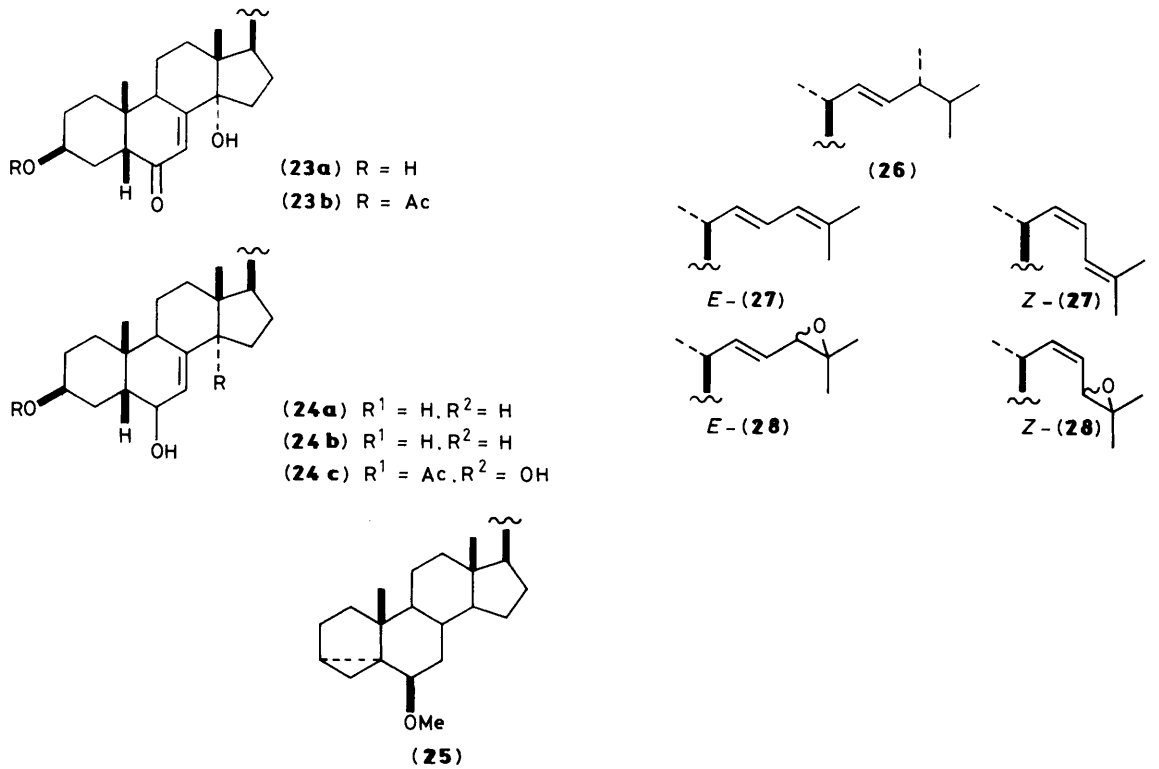


Figure. ^{1}H N.m.r. coupling constants relative to the typical fragments (**23**)—(**28**). These structures are representative of the compounds described in Table 1. δ_H (400 MHz; same solvents as in Table 1; standard Me_4Si), *a* = axial, *e* = equatorial referring to the A-ring for 3-H and 5-H, and to the B-ring for 6-H.

(**23a**) and (**23b**): δ_H 2.34 [dd, $J_{5a,4a}$ 12 Hz, $J_{5a,4e}$ 4 Hz, for compound *E*-(**13**) $J_{5a,4e} = J_{5a,4a} = 8.5$ Hz, 5(*a*)-H (R = Ac)], 2.45 [dd, $J_{5a,4a}$ 12 Hz, $J_{5a,4e}$ 4 Hz, 5(*a*)-H (R = H)], 3.22 [dt, $J_{9a,11}$ 10 Hz, $J_{9a,7}$ 3 Hz, this multiplet is generally not resolved and $w_{\frac{1}{2}}$ 20—25 Hz, 9(*a*)-H], 4.05 [m, $w_{\frac{1}{2}}$ 15 Hz, 3(*e*)-H (R = H)], 5.03 [m, $w_{\frac{1}{2}}$ 10 Hz, 3(*e*)-H (R = Ac)], and 5.85 (d, $J_{7,9a}$ 2.5 Hz, 7-H).

(**24a**), (**24b**), and (**24c**): δ_H 2.8 [m, $w_{\frac{1}{2}}$ 20 Hz, 9(*a*)-H (R^2 = OH)], 4.1 [m, $w_{\frac{1}{2}}$ 7 Hz, 3(*e*)-H (R^1 = H)], 5.14 (m, $w_{\frac{1}{2}}$ 8—9 Hz, 3(*e*)-H (R^1 = Ac)].

6α(e)-(OH) *isomer*: 4.6 [m, $w_{\frac{1}{2}}$ 11 Hz, 6β-(*a*)-H], 5.07 [m, $w_{\frac{1}{2}}$ 6 Hz, 7-H (R^2 = H)], 5.42 [m, $w_{\frac{1}{2}}$ 6 Hz, 7-H (R^2 = OH)]. 6β(a)-(OH) *isomer*: 3.78 [m, $w_{\frac{1}{2}}$ 15 Hz, 6α(*e*)-H], 5.09 [m, $w_{\frac{1}{2}}$ 10 Hz, 7-H (R^2 = H)], and 5.67 [m, $w_{\frac{1}{2}}$ 10 Hz, 7-H (R^2 = OH)].

(**25**): δ_H 0.43 (dd, $J_{4\alpha,3\alpha}$ 8 Hz, $J_{4\alpha,4\beta}$ 5 Hz, 4α-H), 0.65 (dd, $J_{4\alpha,4\beta}$ 5 Hz, $J_{4\beta,4\alpha}$ 5 Hz, 4β-H), 2.77 (dd, $J_{6,7\alpha} = J_{6,7\beta} = 2.7$ Hz, 6α-H).

(**26**): δ_H 5.14 (dd, AB part of an ABXY 22,23,20,24-H which looks like two dd, $J_{23,22}$ 15 Hz, $J_{23,24}$ 7 Hz, 23-H), and 5.25 (dd, AB part of an ABXY system which looks like two dd, $J_{22,20}$ 7.5 Hz, $J_{22,23}$ 15 Hz, 22-H).

E-(**27**): δ_H 1.02 (d, $J_{20,21}$ 6.5 Hz, 21-H), 5.45 (dd, $J_{22,20}$ 8.5 Hz, $J_{22,23}$ 15 Hz, 22-H), 5.77 (d, $J_{24,23}$ 11 Hz, 24-H), and 6.21 (dd, $J_{23,24}$ 11 Hz, $J_{23,22}$ 15 Hz, 23-H).

Z-(**27**): δ_H 1.02 (d, $J_{20,21}$ 6.5 Hz, 21-H), 5.19 (m, X part of an ABX system, $w_{\frac{1}{2}}$ 20 Hz, 22-H), 6.12 [m, AB part of an ABX system where ν_{24}–ν_{23} 5 Hz, and $J_{23,24}$(apparent) 7 Hz, 23-H], and 6.15 [m, AB part of an ABX system, $J_{23,24}$(apparent) 7 Hz, 24-H].

E-(**28**): δ_H 1.08 (d, *J* 6.5 Hz, 21-H), 2.24 (m, $J_{20,22}$ 9 Hz, 20-H), 3.14 (d, $J_{24,23}$ 8 Hz, 24-H), 5.32 (dd, $J_{23,22}$ 15 Hz, $J_{23,24}$ 8 Hz, 23-H), and 5.80 (dd, $J_{22,20}$ 9 Hz, $J_{22,23}$ 15 Hz, 22-H).

Z-(**28**): δ_H 1.07 (d, *J* 6.5 Hz, 21-H), 2.60 (m, $J_{20,22}$ 8 Hz, 20-H), 3.45 (d, $J_{24,22}$ 2 Hz, $J_{24,23}$ 8 Hz, 24-H), 5.15 (dd, $J_{22,20}$ 8 Hz, $J_{22,23}$ 11 Hz, $J_{22,24}$ 2 Hz, 22-H), 5.64 (dd, $J_{23,20}$ 1 Hz, $J_{23,22}$ 11 Hz, $J_{23,24}$ 8 Hz, 23-H)

Table 2. ^{13}C N.m.r. chemical shifts. δ_C(100 MHz; standard Me_4Si); Solv. = solvent: δ_C 49.9 (CD_3OD), 53.6 (CD_2Cl_2), 76.9 ($CDCl_3$). * (or †'‡): interchangeable assignments. The assignments were based upon: (1) shielding data; (2) off-resonance decoupled spectra; (3) selective proton decoupled spectra (distortionless enhancement by polarisation transfer: d.e.p.t.-technique); and (4) by comparison with the spectra of closely related ecdysteroids[a–c] and steroids.[d]

References. (*a*) W. B. Smith, *Org. Magn. Reson.*, 1977, **9**, 644; (*b*) Refs. 1, 6, 9. (*c*) See ref. 15. (*d*) J. W. Blunt, J. B. Stothers, *Org. Magn. Reson.*, 1977, **9**, 439

					6α-(**14**)		(**15**)				*Z*-(**17**)		*E*-(**17**)					*Z*-(**21**)	*Z*-(**21**)	
	(**3**)	(**11**)	*Z*-(**13**)	*E*-(**13**)	*Z*	*E*	*Z*	*E*	*Z*-(**16**)	*E*-(**16**)	*R**	*S**	*R**	*S**	6α-(**18**)	6β-(**18**)	(**19**)	*R**	*S**	(**22**)
1	26.8	25.4‡	25.5†	25.5*	24.2‡		26.6†		28.3	28.7	26.3		26.5		28.1*	28.5‡	28.1‡	33.2	33.2	33.2
2	29.9*	25.5‡	26.5†	26.7*	25.2‡		28.0‡		30.0‡	30.0*	28.7‡		28.7		26.9*	33.8‡	30.4‡	24.8	24.8	24.8
3	65.0	67.8	67.9	67.9	69.2		65.8		66.4	66.4	64.5		64.5		66.8	66.3	66.1	21.3	21.4	21.4
4	31.9*	29.7*	29.5‡	29.5	26.9‡		26.9†		34.2‡	34.2*	32.4*		32.4‡		30.0‡	33.4‡	33.2‡	12.9	12.9	12.9
5	50.8‡	51.6†	50.8*	50.8†	31.1		31.0		53.2	53.2†	50.2†		49.8*		36.2	43.2	51.0†	43.3	43.3	43.3
6	203.1	202.1	202.4	202.4	66.6		66.5		207.3	207.3	203.6		203.7		65.7	66.0	112.3	82.2	82.2	82.3
7	121.4	121.1	120.5	121.3	121.7		121.8		124.3	122.7	121.1		121.2		120.0	118.1	40.4	34.9	34.8	34.9
8	164.6	163.6	164.7	164.6	142.7		142.7		169.2	169.2	164.7		164.6		140.5	142.2	35.4	30.3	30.3	30.4
9	34.4	33.8	34.2	34.1	42.4		41.6		36.3	36.3	36.4		36.4		41.7	43.9	48.8	47.9	47.9	47.9
10		36.4	36.5	36.6	35.3		35.6		38.5	38.5	39.7		40.2			35.3		35.1	35.1	35.2
11	21.1	20.7	21.3	21.3	20.1		20.1		23.0	23.0	26.2		26.5		21.5†	21.2†	19.2	22.6	22.6	22.7
12	32.6*	29.4*	29.5‡	29.5	30.6†		30.0‡		30.8‡	30.8*	27.9‡		28.0†		39.6	39.6	39.6	40.0	40.1	40.2
13	46.3	46.2		46.0	46.4		46.4				47.3		45.9		43.8	41.9		42.7	42.6	42.7
14	85.6	84.5	85.4	85.6	85.7		85.8		86.7	86.8	85.2		85.2		55.9	56.0	52.7†	56.4	56.4	56.4
15	31.3*	32.2*	31.9‡	31.9‡	32.1†	32.0†	32.1‡		33.2‡	33.1*	31.5*		31.6*		22.6†	22.6†	22.6	24.0	24.0	24.1
16	28.5*	30.8*	31.1‡	31.0‡	31.3†	31.4†	31.4‡	31.3‡	32.7‡	32.7*	30.9*		30.8		27.9‡	28.0‡	29.7‡	28.2	28.1	28.2
17	50.8‡	45.9†	50.5*	50.5†	50.6	50.3	50.6	50.3	52.9	52.7†	49.9†	50.5†	48.9*	49.7*	54.9	55.2	56.9	60.2	59.8	56.1
18	15.9	16.0	16.2	16.1	16.5	16.4	16.5	16.4	17.7	17.5	16.06	16.14	15.9	15.99	12.0	12.1	14.3	12.4	12.5	12.1
19	23.9	23.6	23.8	23.8	24.8		24.9		25.3	25.3	23.7		23.8		25.1	33.0	23.4	19.2	19.2	19.2
20	35.8	49.1	34.2	40.1	34.2	40.1	34.2	40.2	36.1	42.1	34.6	34.7	39.67	39.71	40.2	40.3	39.7	35.0	35.0	35.6
21	18.8	13.5	17.9	18.1	18.1	18.0	18.1	18.0	18.9	19.1	20.5	20.7	20.2	20.04	21.0	21.0	20.4	20.3	20.6	18.5
22	36.8	204.2	135.9	138.0	136.0	138.2	136.1	138.3	137.6	139.9	122.1	122.2	123.1		135.4	135.4	135.2	121.7	121.5	36.3
23	21.1		122.3	125.3	120.4	125.1	120.4	125.1	122.6	127.4	142.5	143.1	142.8		131.9	131.9	132.0	143.1	143.5	20.7
24	44.6		121.3	124.8	121.8	125.4	121.8	125.4	122.6	126.9	59.8	60.1	64.1	64.2	42.7	42.7	42.7	59.6	59.9	44.3
25	70.9		135.1	133.0			135.7		136.5	134.1	64.2				33.0	33.0	32.9	64.0		71.2
26	29.4		20.8	20.8	20.8	20.8	20.8	20.8	22.3	22.4	24.5		24.5		19.8	19.8	19.8	24.6	24.6	29.1
27	29.2		26.2	25.7	26.5	25.8	26.3	25.8	27.3	26.8	19.1		18.8		19.5	19.5	19.5	19.1	19.2	29.2
28															17.5	17.5	17.4			
1′		170.1	170.4	170.4	170.5													55.6	55.9	56.1
2′		21.0	21.3	21.3	21.4															
Solv.	53.6	53.3	53.6	53.6	76.9		76.9		49.9	49.9	76.89		76.90		76.9	76.9	76.9	76.9	76.9	76.9

the I_2. The mixture was extracted with dichloromethane and the extract dried (Na_2SO_4), filtered, and evaporated. Silica gel chromatography (0.040—0.063 mm) using hexane–AcOEt (8:2) as eluant afforded a mixture of *Z*-(**13**) and *E*-(**13**) (23 mg, 51 μmol, 58%; *Z*:*E* = 3:7 by ^{1}H n.m.r. referring to 13-Me) {t.l.c. hexane–AcOEt 6:4, R_F[*Z*-(**13**)] = 0.40, R_F[*E*-(**13**)] = 0.45}.

(22Z)- *and* (22E)-3β-*Acetoxy*-5β-*cholesta*-7,22,24-*triene*-6α,14α-*diol*, 6α-(**14**).—Compound *Z*- and *E*-(**13**) (*Z*:*E* = 6:4) (55 mg, 0.12 mmol) was dissolved in a mixture of dry dichloromethane (1 ml) and dry tetrahydrofuran (2 ml). After addition of methanol (3 ml), a 0.4M solution of $CeCl_3 \cdot 7H_2O$ in EtOH–tetrahydrofuran (1:1; 625 μl, 0.25 mmol) was added followed by $NaBH_4$ (10 mg, 0.26 mmol) at −30 °C; the solution was then allowed to reach room temperature slowly. After 1 h, the reaction was complete, and chloroform (5 ml) was added to the mixture, which was then filtered through Celite. Concentration of the filtrate gave the diol (**14**) quantitatively (53 mg, 97% yield) as an isomeric mixture (6α-OH:6β-OH > 9:1 by ^{1}H n.m.r.). A sample (16 mg) was chromatographed on analytical t.l.c. plates, to give each isomer [0.9 mg of 6β-(**14**) (6β-OH); 12.3 mg of 6α-(**14**) (6α-OH)] {t.l.c. hexane–AcOEt 1:1, R_F[*Z*-(**13**)] = 0.55, R_F[*E*-(**13**)] = 0.60, R_F[*Z*-(6α-(**14**)] = 0.25, R_F[*E*-(6α-**14**)] = 0.30, R_F[*Z*-(6β-**14**)] = 0.40, R_F[*E*-(6β-**14**)] = 0.45}. Both compounds 6α-(**14**) and 6β-(**14**) were oils, which resisted all attempts at crystallisation.

6α-(**14**): (Found: M^+, 456.3232 ± 0.0012. $C_{29}H_{44}O_4$ requires *M*, 456.3239); $\lambda_{max.}$(MeCN) 235 nm (ε 25 000 dm^3 mol^{-1} cm^{-1}); $\nu_{max.}$ 3 650—3 600w, 3 000s, 2 950s, 2 910s, 2 850m, 1 720s, 1 650w, 1 600w, 1 510w, 1 450m, 1 370m, 1 270—1 200vs, 1 150m, 1 020m, and 900m cm^{-1}; ^{1}H n.m.r. results given in Table 1 and the Figure, and ^{13}C n.m.r. results given in Table 2; *m*/*z* 456 (M^+, $C_{29}H_{44}O_4$, 5%), 438 (21), 423 (2), 420 (2), 396 (3), 378 (11), 374 (2), 373 (2), 363 (5), 356 (13), 341 (21), 329 (3), 314 (7), 313 (5), 297 (39), 296 (24), 281 (11), 269 (30), and 109 (100).

6β-(**14**): (Found: M^+, 456.3232 ± 0.0012. $C_{29}H_{44}O_4$ requires *M*, 456.3239); ^{1}H n.m.r. results given in Table 1 and the Figure; *m*/*z* 456 (M^+, $C_{29}H_{44}O_4$, 8%), 438 (11), 423 (3), 420 (4), 396 (4), 378 (6), 374 (3), 373 (4), 363 (3), 356 (7), 341 (12), 329 (5), 314 (7), 313 (7), 297 (16), 296 (13), 281 (7), 269 (15), and 109 (100).

(22Z)- *and* (22E)-5β-*Cholesta*-7,22,24-*triene*-3β,6α,14α-*triol* (**15**).—The diol 6α-(**14**) (40 mg, 88 μmol) was dissolved in a mixture of dichloromethane (1 ml) and tetrahydrofuran (1 ml), and methanol (3 ml) was added followed by 2M aqueous K_2CO_3 (0.5 ml, 1 mmol). The resulting heterogenous solution was refluxed for 3 h and became homogenous during this period. Anhydrous Na_2SO_4 (2 g) was added to remove water after which the solution was filtered through Celite, the latter then being washed with chloroform–tetrahydrofuran. The filtrate was neutralised with acetic acid (65 μl, 1 mmol), which was again neutralised with $NaHCO_3$ (50 mg), and filtered through Celite. Evaporation of the filtrate gave the t.l.c.-pure triol *Z*- and *E*-(**15**) (35 mg, 85 μmol, 96%). A sample (15%) of this mixture was chromatographed on analytical t.l.c. plates and eluted with hexane–AcOEt–MeOH (5:4:1). The u.v.-detectable product was desorbed by $CHCl_3$–tetrahydrofuran–MeOH (1:1:0.2), and the resulting suspension filtered through a millipore membrane (solvent resistent, 0.5 μm). From this chromatography, the triol *Z*- and *E*-(**15**) (5.0 mg, 12 μmol, 91%) was obtained {t.l.c. hexane–AcOEt–MeOH, 6:4:1, R_F[*E*-(**14**)] = 0.70, R_F[*Z*-(**14**)] = 0.65, R_F[*E*-(**15**)] = 0.30, R_F[*Z*-(**15**)] = 0.25}. This unstable triol was an oil, which resisted all attempts at crystallization (Found: M^+, 414.3152 ± 0.0018. $C_{27}H_{42}O_3$ requires *M*, 414.3134); $\lambda_{max.}$(MeCN) 235 nm (ε 24 000 dm^3 mol^{-1} cm^{-1}); $\nu_{max.}$ 3 680w, 3 600w, 3 000s, 2 950s, 2 920s, 2 860m, 1 700w, 1 650w, 1 600w, 1 510w, 1 450—1 430m, 1 370m, 1 230—1 200s, 1 150w, and 1 030m, cm^{-1}; ^{1}H n.m.r. results given in Table 1 and the Figure, and ^{13}C n.m.r. results given in Table 2; *m*/*z* 414 (M^+, $C_{27}H_{42}O_3$, 11%), 396 (49), 381 (26), 378 (5), 363 (3), 353 (2), 345 (3), 331 (15), 315 (22), 314 (22), 299 (43), 287 (31), 285 (16), 281 (9), 269 (18), 261 (15), 247 (24), and 109 (100).

(22Z)- *and* (22E)-3β,14α-*Dihydroxy*-5β-*cholesta*-7,22,24-*trien*-6-*ones*, Z-(**16**) *and* E-(**16**).—Anhydrous Na_2SO_4 (100 mg) was added to a solution of the triol *Z*- and *E*-(**15**) (29 mg, 70 mmol; *Z*:*E* = 6:4) in anhydrous chloroform (2 ml). After addition of MnO_2 (Merck activated; 30 mg, 0.3 mmol), the mixture was stirred for 4 h at room temperature, and filtered through Celite. The crude yellow product was chromatographed on SiO_2 (0.040—0.063 mm) and eluted with hexane–AcOEt–MeOH (6:4:0.2) to give the diol *Z*- and *E*-(**16**) (24 mg, 58 mmol, 83%) {t.l.c. hexane–AcOEt–MeOH, 6:4:1, R_F[*Z*-(**15**)] = 0.25, R_F[*E*-(**15**)] = 0.30, R_F[*Z*-(**16**)] = 0.45, R_F[*E*-(**16**)] = 0.50}.

Z- and *E*-(**16**): (Found: M^+, 412.2972 ± 0.0017. Calc. for $C_{27}H_{40}O_3$: *M*, 412.2977); $\nu_{max.}$ 3 600—3 200s, 2 960s, 2 920s, 1 650s, 1 440m, 1 380m, 1 160w, 1 140w, 1 040m, 990w, and 960w cm^{-1}; *m*/*z* 412 (M^+, $C_{27}H_{40}O_3$, 16%), 394 (5), 383 (10), 379 (4), 331 (68), 313 (8), 302 (8), 297 (8), 285 (15), 269 (8), 234 (23), and 109 (100).

Z-(**16**)[6]: m.p. 204—206 °C (from MeOH), $[\alpha]_D^{20}$ +31° (*c* 2.7 in MeOH) (Found: C, 75.65; H, 9.8%; M^+, 412. Calc. for $C_{27}H_{40}O_3$·MeOH: C, 75.68; H, 9.91%; *M*, 412); $\lambda_{max.}$(MeCN) 239 nm (ε 31 000 dm^3 mol^{-1} cm^{-1}); ^{1}H n.m.r. results given in Table 1 and the Figure, and ^{13}C n.m.r. results given in Table 2; *m*/*z* 412 (M^+, $C_{27}H_{40}O_3$, 10%), 394 (4), 383 (1), 379 (2), 331 (100), 313 (6), 302 (4), 297 (7), 285 (10), 269 (5), 234 (9), and 109 (81).

E-(**16**)[6]: $[\alpha]_D^{20}$ +66° (*c* 5.5 in MeOH) (Found: C, 73.45; H, 9.65%; M^+, 412. Calc. for $C_{27}H_{40}O_3$·2MeOH: C, 73.10; H, 10.08%; *M*, 412); $\lambda_{max.}$(MeCN) 238 nm (ε 33 000 dm^3 cm^{-1} mol^{-1}); ^{1}H n.m.r. results given in Table 1 and the Figure, and ^{13}C n.m.r. results given in Table 2; *m*/*z* 412 (M^+, $C_{27}H_{40}O_3$, 13%), 394 (4), 383 (1), 379 (2), 331 (91), 313 (5), 302 (5), 297 (7), 285 (11), 269 (6), 234 (8), and 109 (100).

(24R/24S; 22Z/22E)-24,25-*Epoxy*-3β,14α-*dihydroxy*-5β-*cholesta*-7,22-*dien*-6-*one* Z- *and* E-(**17**).—A mixture of NaF (anhydrous; 3 mg, 71 μmol, 2 equiv.), KF (anhydrous; 2 mg, 34 μmol, 1 equiv.), and *p*-nitroperbenzoic acid (Aldrich tech. 85%; 10 mg, 47 μmol of peracid, 1.3 equiv.) was added at room temperature.[21] to compound *Z*- and *E*-(**16**) (14.9 mg, 36.2 μmol; *Z*:*E* = 6:4) dissolved in dichloromethane (2 ml). After 15 min, peracid (3 mg) was added, and the reaction was complete after 10 min. The mixture was filtered through Celite and the latter washed with chloroform. The combined filtrate and washings were concentrated and the residue, dissolved in a minimum of chloroform containing 2% of Et_2NMe (*N*,*N*-diethylmethylamine), was chromatographed on a SiO_2 column (0.040—0.063 mm) that has been previously washed with toluene–acetone–$EtNMe_2$ (9:1:0.2). After elution with toluene–acetone–$EtNMe_2$ (8:2:0.2), the epoxide *Z*- and *E*-(**17**) was obtained (12.1 mg, 28.3 μmol, 78%; oil) {t.l.c.; toluene–acetone–$EtNMe_2$, 7:3:0.2, R_F[*E*-(**16**)] = R_F[*Z*-(**16**)] = 0.40, R_F[*E*-(**17**)] = R_F[*Z*-(**17**)] = 0.30} (Found: M^+, 428.2918 ± 0.0017. $C_{27}H_{40}O_4$ requires *M*, 428.2926); *m*/*z* 428 (M^+, $C_{27}H_{40}O_4$, 71%) 416 (43), 410 (13), 400 (9), 395 (23), 285 (36), 234 (93), and 233 (100). For extensive structural analysis of compound (**17**), see the experimental part of the pure compounds *Z*-(**17**) and *E*-(**17**).

Direct Conversion of Z-(**13**) *into* (24R/24S; 22Z/22E)-24,25-*Epoxy*-3β,14α-*dihydroxy*-5β-*cholesta*-7,22-*dien*-6-*one* Z-(**17**).—$CeCl_3 \cdot 7H_2O$ (0.4M solution of $CeCl_3 \cdot 7H_2O$ in EtOH–THF, 1:1; 0.17 mmol, 430 μl) was added to the ketone *Z*-(**13**) (38.7 mg, 85.2 μmol) dissolved in dry $CHCl_3$ (2 ml) and dry

MeOH (3 ml) at −20 °C. After addition of $NaBH_4$ (15 mg, 0.39 mmol), the temperature was allowed to come slowly to +5 °C. When, after 1 h, the reduction of *Z*-(**13**) to the diol *Z*-(**14**) was complete, MeOH (3 ml) was added to the mixture which was then heated to reflux; a solution of K_2CO_3 (2M aqueous K_2CO_3; 0.5 ml, 1 mmol) was then added and, if necessary, more water (1 ml) in order to achieve homogeneity. The resulting mixture was stirred under reflux for 8 h after which it was cooled and anhydrous sodium sulphate (2 g) added to it. After being stirred for a few minutes, the solution was filtered through Celite and the latter washed with MeOH–$CHCl_3$; the combined filtrate and washings were concentrated, after addition of a little toluene, in order to remove the remaining water, to give the crude triol *Z*-(**15**) (34 mg, 82 μmol, 96%). This was dissolved in $CHCl_3$ (2 ml) and anhydrous $MgSO_4$ (100 mg) added to the solution which was then stirred for a few minutes; MnO_2 (Merck activated; 50 mg) was then added at room temperature. After 8 h, the resulting yellow solution was filtered through Celite and the oxide washed with $CHCl_3$–THF. The combined filtrate and washings upon work-up afforded the ketone *Z*-(**16**) (33 mg, 80 μmol, 93%) without the need for chromatography. The relatively pure compound *Z*-(**16**) (0.080 mmol) was dissolved in dry CH_2Cl_2 (2 ml), and a mixture of KF (anhydrous; 5.0 mg, 0.086 mmol), NaF (anhydrous; 10 mg, 0.24 mmol), and *p*-nitroperbenzoic acid (Aldrich tech. 85%; 22 mg, 0.10 mmol of peracid, 1.25 equiv.) was added. After 15 min, further peracid (5 mg) was added, and after 10 min the reaction was complete. The acid (0.15 mmol) was then neutralised with Et_2NMe (25 μl, 0.21 mmol) [note that no $EtNMe_2$ (dimethylethylamine) should be used in the presence of CH_2Cl_2 or even $CHCl_3$].

The epoxide solution was then filtered through Celite and the latter washed with $CHCl_3$–THF. Concentration of the combined filtrate and washings gave crude compound *Z*-(**17**) which was dissolved in a minimum of $CHCl_3$ containing 5% Et_2NMe and chromatographed on a silica gel column (0.040—0.063 mm) previously washed with toluene–acetone–Et_2NMe (9:1:0.2). After elution with toluene–acetone–Et_2NMe (8:2:0.2), the racemic epoxide *Z*-(**17**) was obtained (25.2 mg, 58.9 μmol) as an oil which resisted all attempts at crystallisation. The overall yield of *Z*-(**17**) from *Z*-(**13**) was 69%, which gives a typical average yield of 91% for each of the four steps which have been used to obtain the epoxide *Z*-(**17**) (for the t.l.c. characteristics, see the synthesis of each isolated intermediate) (Found: M^+, 428.2932 ± 0.0012. $C_{27}H_{40}O_4$ requires *M*, 428.2926); $\lambda_{max.}$(MeCN) 236 nm (ε 12 000 dm^3 mol^{-1} cm^{-1}); $\nu_{max.}$ 3 600m, 3 550—3 250m, 3 000s, 2 960s, 2 930s, 2 860m, 1 650vs, 1 450m, 1 380m, 1 250—1 200m, and 1 050m cm^{-1}; ^1H n.m.r. results given in Table 1 and the Figure, and ^{13}C n.m.r. results given in Table 2; *m/z* 428 (M^+, $C_{27}H_{40}O_4$, 65%), 416 (7), 410 (12), 400 (5), 395 (23), 392 (7), 385 (7), 353 (10), 340 (9), 337 (8), 331 (13), 330 (10), 302 (12), 297 (8), 285 (30), 283 (18), 269 (9), 234 (35), 233 (30), and 97 (100).

Direct Conversion of E-(**13**) *into* (24R/24S; 22E)-3β,14α-*Dihydroxy*-5β-*cholesta*-7,22-*dien*-6-*one* E-(**17**).—The method used was identical with that for *Z*-(**17**). Compound *E*-(**13**) (25.9 mg, 57.0 μmol) afforded, the epoxide *E*-(**17**) (16.3 mg, 38.1 μmol) as an oil, which resisted all attempts at crystallisation. This gives an overall yield of 67% starting from *E*-(**13**), and an average yield of 90% for each of the four steps (for the t.l.c. parameters see the synthesis of the isolated intermediates) (Found: M^+, 428.2946 ± 0.0017. $C_{27}H_{40}O_4$ requires *M*, 428.2926); $\lambda_{max.}$(MeCN) 236 nm (ε 12 000 dm^3 mol^{-1} cm^{-1}); $\nu_{max.}$ 3 600m, 3 550—3 300m, 3 000s, 2 960s, 2 940s, 2 880m, 1 660vs, 1 450m, 1 380m, 1 230—1 200m, 1 040m, and 1 010m, cm^{-1}; ^1H n.m.r. results given in Table 1 and the Figure, and ^{13}C n.m.r. results given in Table 2; *m/z* 428 (M^+, $C_{27}H_{40}O_4$, 33%), 416 (5), 412 (11), 410 (14), 400 (7), 395 (14), 353 (7), 340 (7), 331 (12), 330 (9), 302 (11), 297 (7), 285 (18), 284 (12), 269 (12), 234 (23), 233 (26), and 97 (100).

(22E)-5β-*Ergosta*-7,22-*diene*-3β,6α-*diol* 6α-(**18**) *and* -3β,6β-*diol* 6β-(**18**).—$LiAlH_4$ (150 mg. 3.9 mmol) was added at room temperature to (22*E*)-3β-acetoxy-5β-ergosta-7,22-dien-6-one (**9**) (1 g, 2.2 mmol) dissolved in dry tetrahydrofuran (40 ml). The reaction was complete after 0.5 h when saturated aqueous potassium sodium L-(+)-tartrate was added until initial appearance of a white precipitate. The mixture was filtered and concentrated and the product, dissolved in $CHCl_3$, was washed with brine. T.l.c. analysis showed three products resulting from the non-stereospecific 1,2-reduction of the C-6 ketone: compounds 6α-(**18**) (6α-OH) and 6β-(**18**) (6β-OH), and from the 1,4-reduction of the conjugated system, compound (**19**). SiO_2-chromatography (0.040—0.063 mm) (hexane–AcOEt; 1 : 1) of this mixture afforded the ketone (**19**) (267 mg, 0.64 mmol, 28%; R_F = 0.7), the alcohol 6β-(**18**) (142 mg, 0.34 mmol, 16%; R_F = 0.3), the alcohol 6α-(**18**) (192 mg, 0.46 mmol, 21%; R_F = 0.2) and an isomeric mixture of 6α- and 6β-(**18**) (112 mg, 0.27 mmol, 12%; 6α-OH:6β-OH = 4:3 according to ^1H n.m.r. of 10-Me). The overall yield of the C-6 1,2-reduction products (**18**) was 49% (445 mg, 1.1 mmol).

6α-(**18**): m.p. 80—82 °C (from MeOH), $[\alpha]_D^{20}$ +19° (*c* 61 in $CHCl_3$) (Found: C, 79.8; H, 11.05%; M^+, 414. $C_{28}H_{46}O_2$·0.5-MeOH requires C, 79.53; H, 11.16%; *M*, 414); $\nu_{max.}$ 3 500—3 300s, 2 970s, 2 960s, 2 880m, 1 650w, 1 430m, 1 370m, 1 050w, and 1 030m cm^{-1}; ^1H n.m.r. results given in Table 1 and the Figure, and ^{13}C n.m.r. results given in Table 2; *m/z* 414 (M^+, $C_{28}H_{46}O_2$, 100%), 396 (20), 381 (11), 371 (6), 363 (4), 353 (3), 343 (20), 289 (12), and 271 (29).

6β-(**18**): m.p. 76—78 °C (from MeOH), $[\alpha]_D^{21}$ +16° (*c* 11.7 in $CHCl_3$) (Found: C, 77.9; H, 10.9%; M^+, 414. $C_{28}H_{46}O_2$·MeOH requires C, 78.03; H, 11.21% *M*, 414); $\nu_{max.}$ 3 500—3 300s, 2 960s, 2 860s, 1 650w, 1 440m, 1 380m, 1 040m, and 1 250w cm^{-1}; ^1H n.m.r. results given in Table 1 and the Figure, and ^{13}C n.m.r. results given in Table 2; *m/z* 414 (M^+, $C_{28}H_{46}O_2$, 100%), 396 (40), 381 (14), 371 (6), 363 (8), 353 (3), 343 (24), 289 (14), and 271 (50).

(**19**): m.p. 145—147 °C (from MeOH), $[\alpha]_D^{18}$ +19° (*c* 33.4 in $CDCl_3$) (Found: C, 81.05; H, 11.15%; M^+, 414. $C_{28}H_{46}O_2$ requires C, 81.16; H, 11.11%; *M*, 414); $\nu_{max.}$ 3 500m, 3 450—3 300, 2 950s, 2 860s, 1 690s, 1 450m, 1 440m, 1 370m, 1 060m, 970m, 760m, and 740m cm^{-1}; ^1H n.m.r. results given in Table 1 and the Figure, and ^{13}C n.m.r. results given in Table 2; *m/z* 414 (M^+, $C_{28}H_{46}O_2$, 40%), 396 (14), 381 (2), 371 (4), 353 (2), 343 (100), 341 (8), 316 (2), 271 (5), 95 (16), and 85 (32).

(24S; 22Z)-, S-(Z-**21**)-, *and* (24R; 22Z)-24,25-*Epoxy*-6β-*methoxy*-3α,5-*cyclocholest*-22-*ene*-Z-(**21**).—The diene *Z*-(**20**) and *E*-(**20**) [*Z*:*E* = 6:4; 76 mg, 0.19 mmol; prepared in the same way as the diene (**13**)] was dissolved in CH_2Cl_2 (5 ml) and the solution added to a mixture of NaF (13 mg, 0.3 mmol), KF (6 mg, 0.1 mmol), and *p*-nitroperbenzoic acid (Aldrich tech 85%; 45 mg, 0.21 mmol of peracid) in CH_2Cl_2 (5 ml) at room temperature.[21] After 30 min at this temperature the reaction was complete, and the solution was filtered through Celite. From SiO_2-chromatography [elution with hexane–AcOEt 97:3; 0.040—0.063 mm SiO_2 was previously treated with hexane–Et_2NMe (2%)], the two epoxides corresponding to the *Z* isomers were obtained, the *E* ones having not been isolated. This gave successively the epoxides *R*-(*Z*-**21**) (19 mg, 46 μmol, 24%), *R*- and *S*-(*Z*-**21**) (9 mg, 22 μmol, 12%), and *S*-(*Z*-**21**) (13 mg, 32 μmol, 17%) as oils; this gives an overall yield of 53% for the *Z*-allylic epoxide *R*- and *S*-(*Z*-**21**) (41 mg, 0.10 mmol). From the starting material *Z*- and *E*-(**20**) (*Z*:*E* = 6:4), we expect that the reaction had also led to *ca.* 35% of the unisolated isomers *R*-

and *S*-(*E*-**21**), which gives a corrected overall yield of 88% for the epoxidation of the diene *Z*- and *E*-(**20**) {t.l.c. hexane–AcOEt, 9:1, R_F[*Z* + *E*-(**20**)] = 0.8, R_F[(**21**)] = 0.30—0.40}. Both compounds *R*-(*Z*-**21**) and *S*-(*Z*-**21**) were oils, which resisted all attempts at crystallisation.

S-(*Z*-**21**): ^{1}H n.m.r. results given in Table 1 and the Figure, and ^{13}C n.m.r. results given in Table 2 (the *R* or *S* attribution is not absolute and can be permuted); *m/z* 412 (M^+, $C_{28}H_{44}O_2$, 100%), 397 (7), 380 (9), 365 (3), 357 (12), 337 (4), 299 (5), 283 (19), 255 (13), and 253 (27).

R-(*Z*-**21**): ^{1}H n.m.r. results given in Table 1 and the Figure, and ^{13}C n.m.r. results given in Table 2 (the *R* or *S* attribution is not absolute and can be permuted); *m/z* 412 (M^+, $C_{28}H_{44}O_2$, 100%), 397 (10), 380 (15), 357 (18), 337 (7), 299 (7), 283 (25), 255 (25), and 253 (45).

6β-*Methoxy*-3α,5-*cyclocholestan*-25-ol (**22**).—The epoxide (**21**) (19 mg, 46 μmol; mixture of isomers) dissolved in dry CH_2Cl_2 (5 ml) was hydrogenated in the presence of 5% platinum-on-charcoal[20] (5% Pt–C; 15 mg) at room temperature with vigorous stirring for 5 h. Although after this time, only the *Z*-isomers seemed to have been hydrogenated, the reaction was stopped and the solution was filtered through Celite. SiO_2-Chromatography (15 μm; hexane–AcOEt, 9:1) afforded the alcohol (**22**) (9 mg, 22 μmol, 48%). Unchanged starting *E*-isomers (**21**) were not isolated {t.l.c. hexane–AcOEt, 85:15, R_F[*Z*-(**21**)] = 0.60—0.65, R_F[*E*-(**21**)] = 0.55—0.60, R_F(**22**) = 0.3}, m.p. 149—151 °C (from CH_2Cl_2–MeOH); ^{1}H n.m.r. results given in Table 1 and the Figure, and ^{13}C n.m.r. results given in Table 2; *m/z* 416 (M^+, $C_{28}H_{48}O_2$, 100%), 401 (52), 398 (10), 384 (83), 369 (25), 366 (20), 361 (75), and 358 (27).

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