Stereocontrolled Synthesis of Withanolide D and Related Compounds

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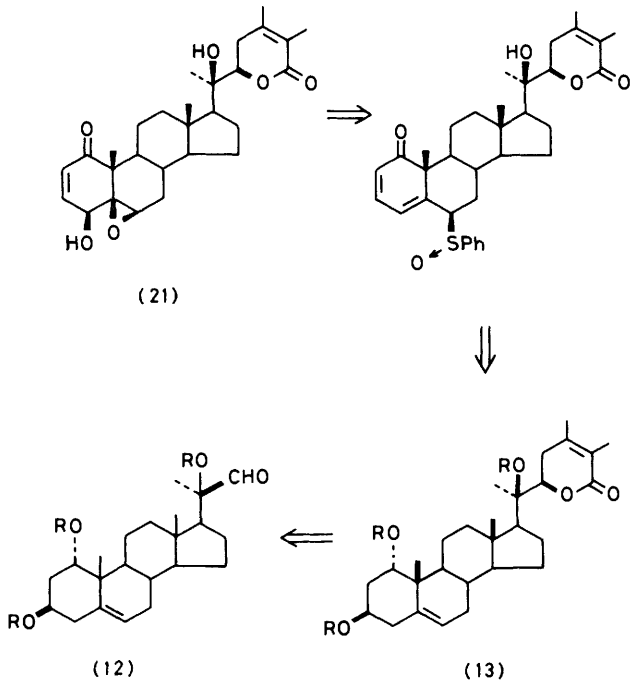
The synthesis of withanolide D, a withanolide having a hydroxy group at the C-20 position, has been accomplished from pregnenolone. The key reactions are based on successful stereochemical control at the C-22 position involving γ-coupling reaction of a lithium enolate with a protected 20-hydroxy-22-aldehyde, and allyl sulphoxide–sulphenate rearrangement of 2,4-dien-1-one 6β-sulphoxide to introduce the 4β-hydroxy-2,5-dien-1-one system. The related natural withanolides, physalolactone B, deacetylphysalolactone B, and 3α,20*R*-dihydroxy-1-oxowitha-5,24-dienolide were also synthesized *via* a common intermediate for withanolide D.

Withanolides,[1] a group of naturally occurring ergostane-type steroids having a δ-lactone in the side chain and a highly oxygenated A/B ring structure, have been isolated from various solanaceous plants belonging to the genera *Withania*, *Acnistus*-(Dunalia), *Physalis*, *Nicandra*, *Datura*, *etc.* Some withanolides possess antitumour [2] and/or antifeedant [3] activities. Recently we have reported the synthesis of jaborosalactones [4] and withaferin A,[5] and here we describe the stereocontrolled synthesis of the withanolides having a C-20 hydroxy group, that is, withanolide D (21),[6] physalolactone B (22),[7] deacetylphysalolactone B (14),[8] and 3β,20*R*-dihydroxy-1-oxowitha-5,24-dienolide (23).[9] Withanolide D (21), (20*R*,22*R*)-4β,20-dihydroxy-5β,6β-epoxy-1-oxowitha-2,24-dienolide, was first isolated in 1968 from *Withania somnifera*, and was reported to have strong antitumour activity.[2,10] The last three withanolides (22), (14), and (23) have recently been isolated from the same genus and are considered to be biogenetic precursors [8] of withanolide D.

Our retrosynthetic analysis of the target molecules typified by withanolide D (21) is illustrated in Scheme 1. In our previous paper,[5] we have described a new efficient method for construction of the 4β-hydroxy-2,5-dien-1-one system, an immediate precursor of withaferin A and related compounds, utilizing the sulphoxide–sulphenate rearrangement of the 2,4-dien-1-one 6β-sulphoxide. Provided that this method was also applicable in the present case, the lactone (13) could be assumed to be an intermediate, because this compound should be accessible by our reported procedure [11] for preparation of the steroidal (20*R*,22*R*)-20-hydroxy-26,22-lactone *via* coupling of a protected 20-hydroxy-22-aldehyde with the lithium enolate generated from the crotonate derivative. This method appeared to be convenient for preparation of the lactone (13). The requisite aldehyde (12) would be synthesized, using the known dithiane method,[12] from the 20-ketone (9) which, in turn, would be easily prepared from commercially available pregnenolone (1) by 1α-hydroxylation according to the method of Barton *et al.*[13]

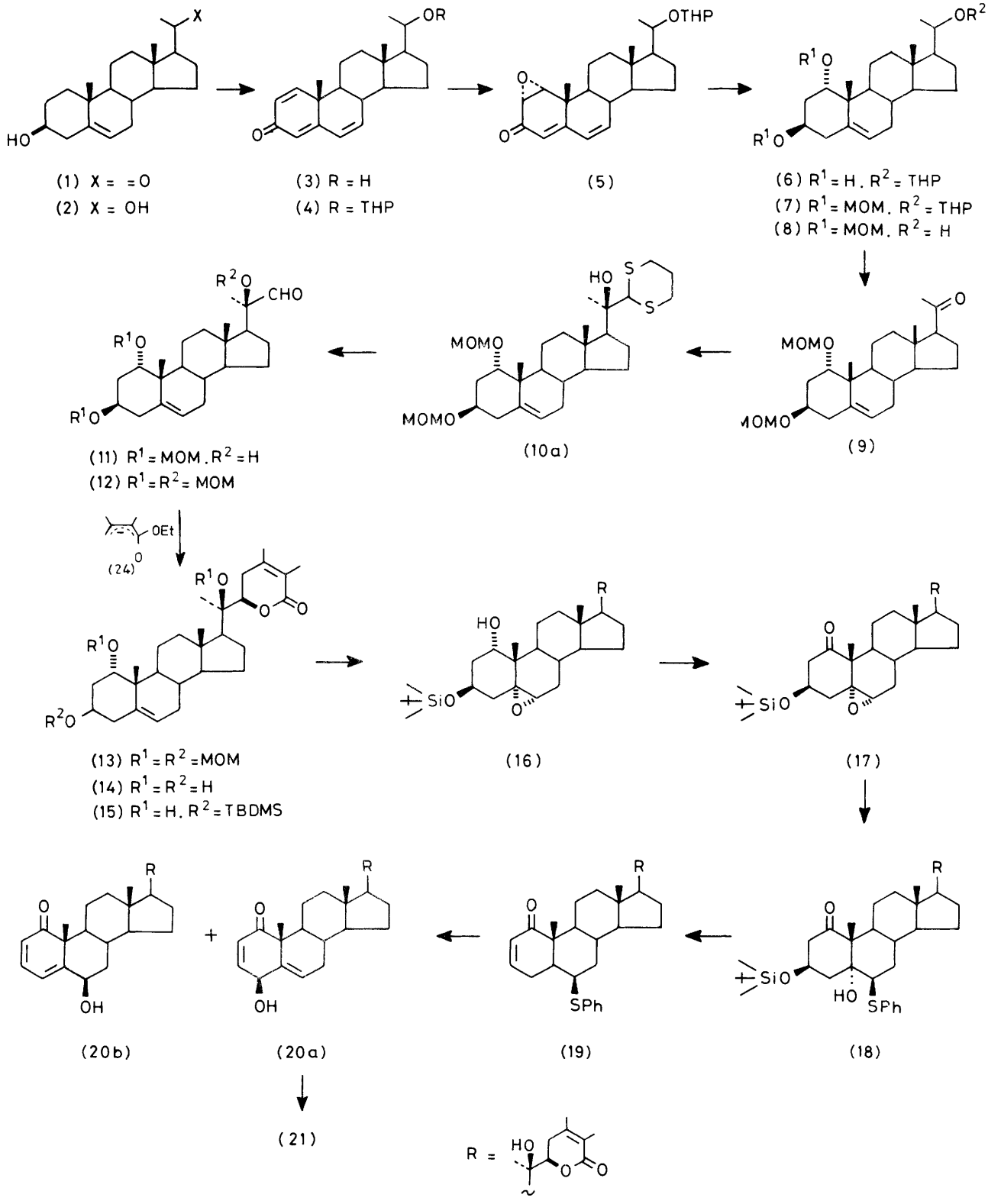
The aldehyde (12) was prepared from pregnenolone (1) in 11 steps as shown in Scheme 2. The diol (2), readily obtained by reduction of (1) with sodium borohydride, was oxidized with dichlorodicyanobenzoquinone (DDQ) [14] to afford the 1,4,6-trien-3-one (3) in 61% yield. After protection of the hydroxy group of (3) as a tetrahydropyranyl (THP) ether, a 1α-hydroxy group was introduced according to a known procedure.[13] The trienone THP ether (4) was epoxidized with alkaline hydrogen peroxide to give the 1α,2α-epoxide (5), which was then reduced with lithium metal–ammonium chloride in liquid ammonia and tetrahydrofuran to give the 1α,3β-diol (6) [66% yield from (3)]. The diol (6) was converted into the bismethoxymethyl (bis-MOM) ether (7) by chloromethyl methyl ether in refluxing dioxane containing diethyl-



Scheme 1.

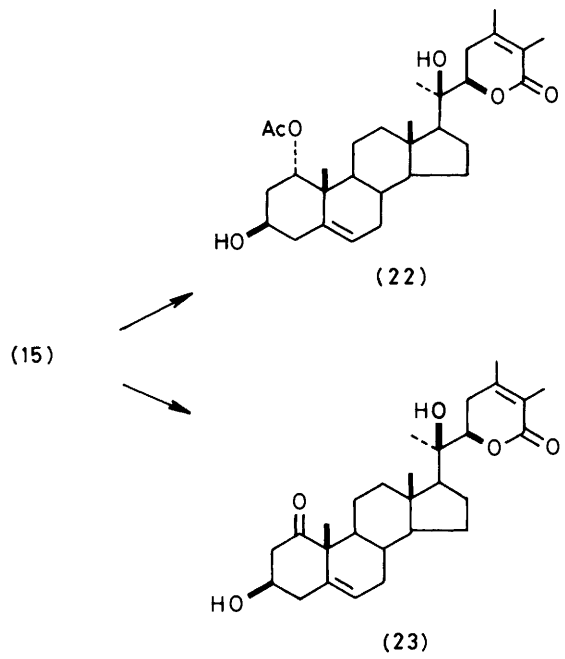
cyclohexylamine and then the THP group was removed to give the 20ξ-hydroxy compound (8). Oxidation of (8) with pyridinium chlorochromate (PCC) followed by coupling with 1,3-dithiane anion afforded a 6 : 1 mixture of the hydroxy dithioacetal epimeric at C-20 (10) in 78% yield. Hydrolysis with mercuric oxide/BF_3–diethyl ether [15] of the less polar major isomer (10a) gave the hydroxy aldehyde (11), the stereochemistry at C-20 of which was inferred from the known stereospecificity [16] in the nucleophilic addition reactions of 20-oxo steroids, and firmly determined as *R* by comparison of the ^{1}H n.m.r. spectrum with those of the reported (20*R*)- and (20*S*)-20-hydroxy-22-al derivatives.[12,17] This type of hydroxy aldehyde (11) is known to be sensitive to basic conditions and to revert easily to the ketone (9).[11,17] To eliminate this undesired reaction, the 20-hydroxy group was protected as the MOM ether under the conditions described above to provide the key intermediate (12) in 69% yield.

For the construction of the side chain moiety, compound (12) was treated with the lithium enolate (26) prepared from ethyl α,β-dimethylcrotonate with LDA in the presence of HMPA at −78 °C to give directly the desired 26,22-lactone



Scheme 2.

(13) in high yield. This result is in contrast with the previous finding [11] that a similar condensation of the 3β-acetoxy-20-methoxymethoxy-22-al with (26) gave the 22-hydroxy-26-ester as a major product, which was transformed into the 26,22-lactone after deprotection of MOM by iodine. The 22*R* stereochemistry of the lactone (13) was deduced from the analogy to our previous coupling reaction [11] and the following physical data. The positive c.d. (Δε, +3.88 at 255 nm) of (13) was similar to that of withanolide D acetate (Δε, +3.72 at 254 nm).[18] The chemical shift (4.24 p.p.m.) and the coupling



Scheme 3.

constant (*J* 12 and 4.2 Hz) at C-22 proton were also in good agreement with those of the reported withanolide D (4.25 p.p.m., *J* 12 and 4 Hz).[19] The stereospecificity observed in this reaction is the same as that in the coupling reaction of the 20-hydroxy-22-aldehyde with organometallic reagents.[16] Therefore, this reaction seems to proceed through the transition state compatible with Cram's cyclic model. Hydrolysis of compound (13) with hydrochloric acid completed the synthesis of deacetylphysalolactone B (14) (m.p. 270—272 °C), which was identical (m.p., ^{1}H n.m.r., and $[\alpha]_D$) with the natural sample.[8]

Attention was now focused on the conversion of the 1,3-dihydroxy system in the A/B ring into the target structure. The transformation of (14) into (19), which is a substrate for the sulphoxide–sulphenate rearrangement, was achieved as outlined in Scheme 2 according to our previous paper.[5] Selective silylation [4] of (14) with t-butyldimethylchlorosilane (TBDMS-Cl), epoxidation with *m*-chloroperbenzoic acid (*m*-CPBA), and pyridinium dichromate (PDC) oxidation yielded the 5α,6α-epoxy-1-one (17) in 55% yield. Regio- and stereospecific ring opening of the epoxide (17) with thiophenol in the presence of Al_2O_3[20] afforded the 5α-hydroxy-6β-phenylthio compound (18) in 75% yield. Heating of (18) at 60 °C in benzene in the presence of toluene-*p*-sulphonic acid hydrate gave the 6β-phenylthio-2,4-dien-1-one (19) in 84% yield. Oxidation of the latter with *m*-CPBA followed by brief treatment of the resulting sulphoxide with an excess of trimethyl phosphite under argon in a dark apparatus afforded the desired 4β-hydroxy-2,5-dien-1-one (20a) in 55% yield, with concomitant formation of the 6β-hydroxy-2,5-dien-1-one (20b) (25% yield). The 4β stereochemistry of the hydroxy group in compound (20a) and 6β-ol in compound (20b) was supported by the signal at 4.60 p.p.m. (d, *J* 4 Hz) for 4α-H and 4.55 p.p.m. (m, $W_{\frac{1}{2}}$ 6 Hz) for 6α-H, respectively, which were consistent with the values of the reference compounds.[21] Epoxidation of (20) with *m*-CPBA proceeded stereoselectively to afford withanolide D (21) in 72% yield, m.p. 252—253 °C (natural, 253—255 °C).[6] On direct comparisons of ^{1}H n.m.r. (400 MHz) and h.p.l.c. (normal phase) the synthetic material (21) was identical with the natural sample of withanolide D.

The remaining two withanolides (22) and (23) were synthesized in a straightforward manner from the intermediate (15). Acetylation and acidic hydrolysis of the latter gave the 1α-acetyl-3β-ol (22) in 68% yield. PDC oxidation and acidic hydrolysis of (15) gave the 1-oxo-3β-ol (23) in 61% yield. Melting points and ^{1}H n.m.r. spectral data of these synthetic samples (22) and (23) were identical with the published data of the natural physalolactone B (22) [7] and 3β,20*R*-dihydroxy-1-oxowitha-5,24-dienolide (23),[9] respectively. Thus, the structures of four 20-hydroxylated withanolides were confirmed by chemical synthesis. The biological activities of those compounds will be reported elsewhere.

Experimental

M.p.s were determined with a hot-stage microscope apparatus. I.r. spectra were recorded with a Hitachi 260-10 spectrometer and n.m.r. spectra were obtained with a Hitachi R-24A, a JEOL PS-100, or a JEOL FX-400 spectrometer with tetramethylsilane as an internal standard. Column chromatography was performed with silica gel (E. Merck silica gel 60). T.l.c. was carried out on pre-coated plates of silica gel (E. Merck). Work-up refers to dilution with water, extraction with an organic solvent, washing to neutrality, drying ($MgSO_4$), filtration, and evaporation under reduced pressure. Ether refers to diethyl ether.

20ξ-*Hydroxypregna*-1,4,6-*trien*-3-*one* (3).—$NaBH_4$ (3 g) was added over 5 min to a solution of pregnenolene (1) (24 g) in methanol (250 ml) and THF (150 ml) at 0 °C. After 40 min the excess of hydride was destroyed by careful addition of water and 1M-HCl and then the resultant mixture was extracted by CH_2Cl_2 and the aqueous layer was washed with CH_2Cl_2. The combined organic fraction was dried and evaporated to dryness to give the diol (2) (28 g), which was used without further purification in the following reaction.

A solution of the diol (12 g) and dichlorodicyanobenzoquinone (DDQ) (28 g) in dioxane (400 ml) was refluxed for 8 h, cooled, filtered, diluted with CH_2Cl_2, and applied to a short column of alumina. Elution with CH_2Cl_2 gave a crude product, which was purified by column chromatography on silica gel [solvent, ethyl acetate–hexane (3 : 7)], to afford the *hydroxy trienone* (3) (7.1 g, 61%), m.p. 131—134 °C (methanol); δ($CDCl_3$) 0.85 (s, 3 H, 13-Me), 1.06 (d, 3 H, *J* 6 Hz, 20-Me), 1.16 (s, 3 H, 10-Me), 3.4—3.9 (m, 1 H, 20-H), 6.0—6.25 (m, 4 H, 2-, 4-, 6-, and 7-H), and 7.20 (d, 1 H, *J* 10 Hz, 1-H) (Found: C, 80.6; H, 9.1. $C_{21}H_{28}O_2$ requires C, 80.73; H, 9.03%).

1α,2α-*Epoxy*-20ξ-*tetrahydropyranyloxypregna*-4,6-*dien*-3-*one* (5).—A solution of the hydroxy trienone (3) (7.14 g), dihydropyran (6 ml), and toluene-*p*-sulphonic acid (5 mg) in dichloromethane (200 ml) was stirred at room temperature for 3 h. Aqueous $NaHCO_3$ was then added to the reaction mixture and separated and the aqueous layer washed with dichloromethane. The combined organic fractions were dried and evaporated to dryness to give the trienone (4), which was used without further purification in the following reaction.

Hydrogen peroxide (30%, 15 ml) was added to a solution of the trienone (7.9 g) in methanol (180 ml) containing 10% methanolic sodium hydroxide (2 ml), and the mixture was stirred at 15 °C overnight. The resulting solution was diluted with ether and dried. Evaporation left a solid, which was crystallised from ethyl acetate–hexane to give the *epoxide* (5) (7.8 g, 83%), m.p. 146—150 °C; δ($CDCl_3$) 0.78 and 0.87 (each s, each 3 H, 13-Me), 1.06 and 1.22 (each d, each 3 H, *J* 6 Hz,

20-Me), 1.17 (s, 3 H, 10-Me), 3.39 (dd, 1 H, *J* 4.8 and 2.2 Hz, 2-H), 3.56 (d, 1 H, *J* 4.8 Hz, 1-H), 4.5—4.7 (m, 1 H, acetal H), 5.55 (br s, 1 H, 4-H), and 6.01 (s, 2 H, 6- and 7-H) (Found: C, 75.5; H, 8.8. $C_{26}H_{36}O_4$ requires C, 75.69; H, 8.79%).

1α,3β-*Dihydroxy*-20ξ-*tetrahydropyranyloxypregn*-5-*ene* (6).—A four-necked flask was fitted with a sealed mechanical stirrer, a dropping funnel, a cold-finger filled with solid CO_2, and an inlet connected to an anhydrous ammonia source. Argon was swept through the system for 10 min, and then ammonia (500 ml) was trapped in the flask. Lithium wire (7.5 g) was cut into short pieces and added. After stirring for 1 h, the epoxide (5) (5 g) in THF (500 ml) was added dropwise during 30 min. The cooling bath was removed and the mixture was allowed to warm to −40 °C for 20 min. The flask was dipped in a cooling bath and anhydrous ammonium chloride (75 g) was added during 2 h; the mixture turned white and pasty. Most of the ammonia was removed in a stream of argon and the residue was diluted with ether, washed with brine, and dried, Evaporation left a white solid, which was chromatographed on silica gel. Elution with ethyl acetate–hexane (2 : 1) afforded the *diol* (6) (3.85 g, 76%), m.p. 165—168 °C (ethyl acetate–hexane); δ($CDCl_3$) 0.76 and 0.88 (each s, each 3 H, 13-Me), 1.04 (s, 3 H, 10-Me), 1.06 and 1.20 (each d, each 3 H, *J* 6 Hz, 20-Me), 3.55—4.1 (m, 1 H, 20-H), 3.86 (br s, 1 H, 1-H), 3.6—4.0 (m, 1 H, 3-H), and 5.60 (m, 1 H, 6-H) (Found: C, 74.4; H, 10.0. $C_{26}H^+{}_2O_4$ requires C, 74.60; H, 10.11%).

1α,3β-*Bismethoxymethoxy*-20ξ-*hydroxypregn*-5-*ene* (8).—*N*,*N*-Diethylcyclohexylamine (12 ml) and chloromethyl methyl ether (4.8 ml) were added to a solution of the diol (6) (3.6 g) in dioxane (20 ml) and then the mixture was refluxed for 6 h. After cooling, the reaction mixture was poured into 2M-HCl (25 ml) in methanol (100 ml) and stirred at room temperature for 2 h. The t.l.c. behaviour of the resulting solution revealed complete hydrolysis of the tetrahydropyranyl ether. Work-up (ether extraction and evaporation of ether) left an oil, which was chromatographed on silica gel [solvent, ethyl acetate–hexane (3 : 7)] to afford the *bis-MOM ether* (8) (2.1 g, 60%), m.p. 135—140 °C (methanol); δ($CDCl_3$) 0.76 (s, 3 H, 13-Me), 1.03 (s, 3 H, 10-Me), 1.11 (d, 3 H, *J* 6.5 Hz, 20-Me), 3.22 and 3.36 (each s, each 3 H, OMe), 3.4—3.9 (m, 1 H, 20-H), 4.62 (ABq, 2 H, *J* 7 Hz, Δ_{AB} 12 Hz, 1α-OCH_2OCH_3), 4.64 (s, 2 H, 3β-OCH_2OCH_3) and 5.52 (m, 1 H, 6-H) (Found: C, 71.0; H, 10.3. $C_{25}H_{42}O_5$ requires C, 71.05; H, 10.02%).

1α,3β-*Bismethoxymethoxypregn*-5-*en*-20-*one* (9).—Pyridinium chlorochromate (PCC) (2.1 g) was added to a solution of the alcohol (8) (2.2 g) in the presence of AcONa (160 mg) in dichloromethane (20 ml) at room temperature, and the mixture was stirred for 3 h. The reaction mixture was diluted with anhydrous ether (200 ml) and passed through a column of Florisil (50 g) eluted with ether. Evaporation left an oil, which was purified by column chromatography on silica gel [solvent ethyl acetate–hexane (4 : 6)] to give the 20-*oxo compound* (9) (1.8 g, 80%), m.p. 128—129 °C; δ($CDCl_3$) 0.66 (s, 3 H, 13-Me), 1.03 (s, 3 H, 10-Me), 2.10 (s, 3 H, 20-Me), 3.33 and 3.40 (each s, each 3 H, OMe), 4.64 (ABq, 2 H, *J* 7 Hz, Δ_{AB} 12 Hz, 1α-OCH_2OCH_3), 4.65 (s, 2 H, 3β-OCH_2OCH_3), and 5.50 (m, 1 H, 6-H); $\nu_{max.}$ ($CHCl_3$) 1 700 cm^{-1} (Found: C, 71.1; H, 9.8. $C_{25}H_{40}O_5$ requires C, 71.39; H, 9.59%).

Dithiane Adduct (10a) *and* (10b).—To a solution of 1,3-dithiane (924 mg) in THF (15 ml) was added n-butyl-lithium (5 ml; 1.6 mM solution in hexane) at −5 °C under argon. To the resulting solution was added dropwise a solution of the ketone (9) (1.67 g) in THF (8 ml). The reaction mixture was stirred at −5 °C for 8 h after which work-up (ether for extraction) gave a mixture of the two hydroxy compounds, which was chromatographed on silica gel [solvent, ethyl acetate–hexane (1 : 9)] to give the desired (20R)-20-*hydroxy compound* (10a) (less polar; 1.66 g, 78%), m.p. 154—156 °C (ether); δ($CDCl_3$) 0.90 (s, 3 H, 13-Me), 1.03 (s, 3 H, 10-Me), 1.45 (s, 3 H, 20-Me), 3.34 and 3.40 (each s, each 3 H, OMe), 4.18 (s, 1 H, 22-H), 4.63 (ABq, 2 H, *J* 7 Hz, Δ_{AB} 12 Hz, 1α-OCH_2OCH_3), 4.64 (s, 2 H, 3β-OCH_2OCH_3), and 5.52 (m, 1 H, 6-H) (Found: C, 64.6; H, 8.7; S, 11.6. $C_{29}H_{48}O_5S_2$ requires C, 64.40; H, 8.95; S, 11.86%), and the (20S)-*epimer* (10b) (more polar; 0.28 g, 13%), m.p. 148—150 °C (ether); δ($CDCl_3$) 0.90 (s, 3 H, 13-Me), 1.02 (s, 3 H, 10-Me), 1.32 (s, 3 H, 20-Me), 3.24 and 3.40 (each s, each 3 H, OMe), 4.30 (s, 1 H, 22-H), 4.63 (ABq, 2 H, *J* 7 Hz, Δ_{AB} 12 Hz, 1α-OCH_2OCH_3), 4.64 (s, 2 H, 3β-OCH_2OCH_3), and 5.52 (m, 1 H, 6-H) (Found: C, 64.6; H, 8.65; S, 11.95. $C_{29}H_{48}O_5S_2$ requires C, 64.40; H, 8.95; S, 11.86%).

(20R)-1α,3β-*Bismethoxymethoxy*-20-*formyl*-20-*hydroxypregn*-5-*ene* (11).—A THF (15 ml) solution of (10a) (1.56 g) was added to a suspension of HgO (1.4 g) in the presence of BF_3–ether (0.8 ml) in aqueous THF (50%, 20 ml) and the mixture was stirred under reflux for 30 min. After filtration, the organic fraction was washed with aqueous $NaHCO_3$. Work-up (ether for extraction) gave a viscous oil, which was chromatographed on silica gel [solvent, ethyl acetate–hexane (3 : 7)] to afford the *hydroxy aldehyde* (11) (1.1 g, 85%), m.p. 122—124 °C; δ($CDCl_3$) 0.80 (s, 3 H, 13-Me), 1.03 (s, 3 H, 10-Me), 1.34 (s, 3 H, 20-Me), 3.33 and 3.40 (each s, each 3 H, OMe), 3.6—4.1 (m, 1 H, 3-H), 3.72 (m, 1 H, 1-H), 4.64 (ABq, 2 H, *J* 7 Hz, Δ_{AB} 12 Hz, 1α-OCH_2OCH_3), 4.65 (s, 2 H, 3β-OCH_2OCH_3), 5.52 (m, 1 H, 6-H), and 9.58 (s, 1 H, 22-H); $\nu_{max.}$ ($CHCl_3$) 3 400, 2 700, and 1 724 cm^{-1} (Found: C, 69.1; H, 9.55. $C_{26}H_{42}O_6$ requires C, 69.30; H, 9.39%).

(20R)-20-*Formyl*-1α,3β,20-*trismethoxymethoxypregn*-5-*ene* (12).—*N*,*N*-Diethylcyclohexylamine (2.5 ml) and chloromethyl methyl ether (1 ml) was added to a solution of the hydroxy aldehyde (11) (1.2 g) in dioxane (10 ml) and the mixture was refluxed for 8 h. After cooling, 1M-HCl was added to the mixture which upon work-up (ether for extraction) gave an oil; this was chromatographed on silica gel [solvent, ethyl acetate–hexane (3 : 7)] to afford the *methoxymethyl ether* (12) (0.86 g, 81%), m.p. 118—120 °C (ether–ethyl acetate); δ($CDCl_3$) 0.78 (s, 3 H, 13-Me), 1.01 (s, 3 H, 10-Me), 1.38 (s, 3 H, 20-Me), 3.35, 3.40, and 3.42 (each s, each 3 H, OMe), 3.5—4.0 (m, 1 H, 3-H), 3.70 (m, 1 H, 1-H), 4.64 (ABq, 2 H, *J* 7 Hz, Δ_{AB} 12 Hz, 1α-OCH_2OCH_3), 4.65 (s, 2 H, 3β-OCH_2OCH_3), 4.71 (ABq, 2 H, *J* 7 Hz, Δ_{AB} 15 Hz, 20-OCH_2OCH_3), 5.50 (m, 1 H, 6-H), and 9.70 (s, 1 H, 22-H) (Found: C, 67.7; H, 9.55. $C_{28}H_{46}O_7$ requires C, 67.99; H, 9.37%).

(20R,22R)-1α,3β,20-*Trismethoxymethoxywitha*-5,24-*dienolide* (13).—A solution of ethyl α,β-dimethylcrotonate (26) (101 mg) in THF (1 ml) and HMPA (35 μl) was added to a solution of LDA (2.2 equiv.) in THF (2 ml) at −78 °C under argon and the mixture was stirred for 1 h. To the resulting lithium enolate was added a solution of the aldehyde (12) (166 mg) in THF (3 ml) at −78 °C and the mixture was stirred for 6 h. After warming to room temperature aqueous NH_4Cl was added to the reaction mixture which upon work-up (ether for extraction) gave a crude product; this was chromatographed on silica gel [solvent, ethyl acetate–hexane (3 : 7)] to afford the *lactone* (13) (170 mg, 86%), m.p. 220—222 °C (ethyl acetate–hexane); c.d. 225 nm (Δε, +3.88) (recorded with a JASCO R-20); δ($CDCl_3$) 0.88 (s, 3 H, 13-Me), 1.02 (s, 3 H, 10-Me), 1.40 (s, 3 H, 20-Me), 3.34, 3.36, and 3.40 (each s, each 3 H, OMe), 3.64—3.92 (m, 1 H, 3-H), 3.74 (m,

1 H, 1-H), 4.24 (dd, 1 H, *J* 12 and 4.2 Hz), 4.64 (ABq, 2 H, *J* 8 Hz, Δ_{AB} 9.6 Hz, 1α-OCH_2OCH$_3$), 4.65 (s, 2 H, 3β-OCH_2-OCH$_3$), 4.88 (ABq, 2 H, *J* 7 Hz, Δ_{AB} 9.6 Hz, 20-OCH_2OCH$_3$), and 5.50 (m, 1 H, 6-H); $\nu_{max.}$ ($CHCl_3$) 1 692 cm^{-1} (Found: C, 69.5; H, 9.0. $C_{34}H_{54}O_8$ requires C, 69.12; H, 9.21%).

(20R,22R)-1α,3β,20-*Trihydroxywitha*-5,24-*dienolide* (14).—A solution of the methoxymethyl ether (13) (40 mg) in THF (1 ml) was treated with 6M-HCl (0.2 ml) at room temperature. After being stirred for 3 h, aqueous $NaHCO_3$ was added to the reaction mixture. Work-up (ethyl acetate for extraction) gave a solid, which was recystallized from ethyl acetate to afford the *trihydroxy lactone* (14) (25 mg, 82%), m.p. 270—272 °C; $[\alpha]_D^{22}$ +19.4° (*c*, 0.14) (recorded with a Carl Zeiss LEP A-1 pakarimeter); δ($CDCl_3$) 0.89 (s, 1 H, 13-Me), 1.05 (s, 1 H, 10-Me), 1.28 (s, 3 H, 20-Me), 1.92 and 1.98 (each s, each 3 H, 24- and 25-H), 3.6—4.0 (m, 1 H, 3-H), 3.88 (br s, 1 H, 1-H), 4.22 (dd, 1 H, *J* 12.2 and 4.1 Hz), and 5.62 (m, 1 H, 6-H); $\nu_{max.}$ ($CHCl_3$) 3 440 and 1 690 cm^{-1} (Found: C, 73.15; H, 9.4. $C_{28}H_{42}O_5$ requires C, 73.33; H, 9.23%).

(20R,22R)-3β-*t-Butyldimethylsilyloxy*-1α,20-*dihydroxy-witha*-5,24-*dienolide* (15).—A solution of the triol (14) (36 mg) and imidazole (88 mg) in dimethylformamide (1 ml) was treated with t-butyldimethylchlorosilane (67 mg) at room temperature under argon. After being stirred for 1 h, the reaction mixture was poured into ice–water. Work-up with ether for extraction gave a solid, which was recrystallized from ether–hexane to afford the *silyl ether* (15) (41 mg, 90%), m.p. 229—231 °C; δ($CDCl_3$) 0.05 (s, 3 H, $SiMe_2$), 0.86 (s, 9 H, $SiCMe_3$), 0.88 (s, 3 H, 13-Me), 1.04 (s, 3 H, 10-Me), 1.28 (s, 3 H, 20-Me), 1.88 and 1.96 (each s, each 3 H, 24- and 25-Me), 3.8—4.1 (m, 1 H, 3-H), 3.86 (br s, 1 H, 1-H), 4.18 (dd, 1 H, *J* 13 and 4 Hz, 22-H), and 5.60 (m, 1 H, 6-H) (Found: C, 71.0; H, 10.0. $C_{34}H_{56}O_5Si$ requires C, 71.28; H, 9.85%).

(20R,22R)-3β-*t-Butyldimethylsilyloxy*-1α,20-*dihydroxy*-5β,6β-*epoxywith*-24-*enolide* (16).—To a solution of the silyl ether (15) (72 mg) in chloroform (3 ml) was added *m*-chloroperbenzoic acid (30 mg) in chloroform (1 ml) at −5 °C. The mixture was stirred for 40 min. Work-up with chloroform for extraction gave a solid, which was recrystallized from ethyl acetate–hexane to afford the *epoxide* (16) (57 mg, 76%), m.p. 264—267 °C; δ($CDCl_3$) 0.05 (s, 3 H, $SiMe_2$), 0.84 (s, 3 H, 13-Me), 0.88 (s, 9 H, $SiCMe_3$), 1.08 (s, 3 H, 10-Me), 1.27 (s, 3 H, 20-Me), 1.88 and 1.94 (each s, each 3 H, 24- and 25-Me), 2.80 (d, 1 H, *J* 4.6 Hz, 6-H), 3.82 (m, 1 H, 1-H), 4.04—4.20 (m, 1 H, 1-H), and 4.18 (dd, 1 H, *J* 13 and 4 Hz, 22-H) (Found: C, 69.5; H, 9.3. $C_{34}H_{56}O_6Si$ requires C, 69.35; H, 9.58%).

(20R,22R)-3β-*t-Butyldimethylsilyloxy*-5β,6β-*epoxy*-20-*hydroxy*-1-*oxowith*-24-*enolide* (17).—To a solution of the diol (16) (118 mg) in dimethylformamide (5 ml) was added pyridinium dichromate (1.8 g) at −5 °C under argon. After being stirred for 8 h, water was added to the reaction mixture. Work-up (ether for extraction) gave a solid, which was recrystallized from ether to afford the *ketone* (17) (94 mg, 80%), m.p. 224—226 °C; δ($CDCl_3$) 0.05 (s, 3 H, $SiMe_2$), 0.86 (s, 3 H, 13-Me), 0.88 (s, 9 H, $SiCMe_3$), 1.28 (s, 3 H, 20-Me), 1.43 (s, 3 H, 10-Me), 1.88 and 1.93 (each s, each 3 H, 24- and 25-Me), 2.95 (d, 2 H, *J* 5 Hz, 6-H), 4.05—4.25 (m, 1 H, 3-H), and 4.20 (dd, 1 H, *J* 12 and 4 Hz, 22-H) (Found: C, 69.8; H, 9.1. $C_{34}H_{54}O_6Si$ requires C, 69.58; H, 9.27%).

(20R,22R)-3β-*t-Butyldimethylsilyloxy*-5α,20-*dihydroxy*-6β-*phenylthio*-1-*oxowith*-24-*enolide* (18).—To a suspension of alumina (118 mg; Woelm N-20) in ether (2 ml) was added thiophenol (95 μl) at room temperature under argon. After being stirred for 20 min, the epoxide (17) (36 mg) in ether–THF (2 ml, 1 : 1) was added to the suspension at room temperature and the mixture was stirred for 2 h. After filtration the filtrate was evaporated to dryness to give a crude product, which was chromatographed on silica gel [solvent, ethyl acetate–hexane (3 : 7)] to afford the *phenylthio compound* (18) (32 mg, 75%), m.p. 196—198 °C (ethyl acetate–hexane); δ($CDCl_3$) 0.05 (s, 6 H, $SiMe_2$), 0.88 (s, 9 H, $SiMe_3$), 0.94 (s, 3 H, 13-Me), 1.29 (s, 3 H, 20-Me), 1.48 (s, 3 H, 10-Me), 1.88 and 1.95 (each s, each 3 H, 24- and 25-Me), 3.20 (m, 1 H, $W_{\frac{1}{2}}$ 4.8 Hz, 6-H), 4.0—4.3 (m, 1 H, 3-H), 4.18 (dd, 1 H, *J* 12 and 4.5 Hz, 22-H) and 7.2—7.4 (m, 5 H, Ph) (Found: C, 68.6; H, 8.65; S, 4.58. $C_{40}H_{60}O_6SSi$ requires C, 68.92; H, 8.67; S, 4.59%).

(20R,22R)-20-*Hydroxy*-6β-*phenylthio*-1-*oxowitha*-2,4,24-*trienolide* (19).—A solution of the hydroxy sulphide (18) (128 mg) in benzene (5 ml) was treated with toluene-*p*-sulphonic acid hydrate (TsOH·H_2O; 480 mg) at 60 °C. After being stirred for 1 h, aqueous $NaHCO_3$ was added to the reaction mixture. Work-up (ethyl acetate for extraction) gave a crude product, which was chromatographed on silica gel [solvent, ethyl acetate–hexane (3 : 7)] to afford the *dienone* (19) (84 mg, 84%), m.p. 174—175 °C (ethyl acetate), 0.98 (s, 3 H, 13-Me), 1.26 (s, 3 H, 20-Me), 1.60 (s, 3 H, 10-Me), 1.88 and 1.94 (each s, each 3 H, 24- and 25-Me), 4.14 (m, 1 H, 6-H), 4.19 (dd, 1 H, *J* 12.5 and 4 Hz, 22-H), 5.78 (d, 1 H, *J* 6 Hz, 4-H), 5.87 (d, 1 H, *J* 10 Hz, 2-H), 6.72 (dd, 1 H, *J* 10 and 6 Hz, 3-H) and 7.25—7.45 (m, 5 H, Ph) (Found: C, 74.4; H, 7.9; S, 5.55. $C_{34}H_{42}O_4S$ requires C, 76.69; H, 7.74; S, 5.86%).

(20R,22R)-4β,20-*Dihydroxy*-1-*oxowitha*-2,5,24-*trienolide* (20a) *and* (20R,22R)-6β,20-*Dihydroxy*-1-*oxowitha*-2,4,24-*trienolide* (20b).—To a solution of the sulphide (19) (60 mg) in chloroform (3 ml) was added *m*-chloroperbenzoic acid (17 mg) in chloroform (2 ml) at −78 °C under argon. After being stirred for 10 min, the t.l.c. behaviour of the resulting solution showed the formation of a polar compound. Work-up gave a crude product, which was immediately used without purification in the following reaction.

The crude product (64 mg) in methanol (3 ml) and THF (2 ml) was treated with trimethyl phosphite (130 μl) at room temperature for 10 h in a dark apparatus under argon (it was necessary to avoid light and oxygen). Work-up (ethyl acetate for extraction) gave two products, which were separated by chromatography on silica gel [solvent, ethyl acetate–hexane (2 : 3)] to afford the desired 4β-*hydroxy dienone* (20a) (less polar; 27 mg, 55%), m.p. 197—198 °C; δ($CDCl_3$) 0.92 (s, 3 H, 13-Me), 1.30 (s, 3 H, 20-Me), 1.45 (s, 3 H, 10-Me), 1.90 and 1.95 (each s, each 3 H, 24- and 25-Me), 4.20 (dd, 1 H, *J* 12 and 4 Hz, 22-H), 4.60 (d, 1 H, *J* 4 Hz, 4-H), 5.91 (d, 1 H, *J* 10 Hz, 2-H), 5.93 (m, 1 H, $W_{\frac{1}{2}}$ 5 Hz, 6-H), 6.73 (dd, 1 H, *J* 10 and 4 Hz, 3-H) (Found: C, 73.7; H, 8.65. $C_{28}H_{38}O_5$ requires C, 73.98; H, 8.42%), and the 6β-*hydroxy dienone* (20b) (more polar; 12 mg, 25%), m.p. 221—223 °C; δ($CDCl_3$) 0.95 (s, 3 H, 13-Me), 1.25 (s, 3 H, 20-Me), 1.46 (s, 3 H, 13-Me), 1.92 and 1.95 (each s, each 3 H, 24- and 25-Me), 4.18 (dd, 1 H, *J* 12 and 4 Hz, 22-H), 4.55 (m, 1 H, $W_{\frac{1}{2}}$ 6 Hz, 6-H), 5.98 (d, 1 H, *J* 10 Hz, 2-H), 6.08 (d, 1 H, *J* 6 Hz, 4-H), and 6.87 (dd, 1 H, *J* 10 and 6 Hz, 3-H) (Found: C, 73.7; H, 8.7. $C_{28}H_{38}O_5$ requires C, 73.98; H, 8.42%).

(20R,22R)-4β,20-*Dihydroxy*-5β,6β-*epoxy*-1-*oxowitha*-2,24-*dienolide* (*Withanolide D*) (21).—To a solution of the dienone (20a) (22 mg) in chloroform (2 ml) was added *m*-chloroperbenzoic acid (8.8 mg) in chloroform (1 ml) at room temperature. After being stirred for 6 h, aqueous $NaHCO_3$

was added to the reaction mixture. Work-up gave a crude product, which was chromatographed on silica gel [solvent, ethyl acetate–hexane (1 : 1)] to afford the *epoxide* (21), m.p. 251—253 °C (ethyl acetate); $[\alpha]_D^{20}$ +78° (*c*, 1.48; $CHCl_3$); δ($CDCl_3$) 0.85 (s, 3 H, 13-Me), 1.26 (s, 3 H, 20-Me), 1.40 (s, 3 H, 10-Me), 1.88 and 1.94 (each s, each 3 H, 24- and 25-Me), 3.23 (m, 1 H, $W_{\frac{1}{2}}$ 4 Hz, 6-H), 3.76 (d, 1 H, *J* 5.8 Hz, 4-H), 4.20 (dd, 1 H, *J* 13 4 and 3.4 Hz, 22-H), 6.20 (d, 1 H, *J* 10 Hz, 2-H), and 6.94 (dd, 1 H, *J* 10 and 5.8 Hz, 3-H); $\nu_{max.}$ ($CHCl_3$) 1 694 cm^{-1} [Found: high resolution mass spectrum, *m/z* 470.2615 (M^+, run on a Hitachi M-80 mass spectrometer); C, 71.45; H, 8.0. $C_{28}H_{38}O_6$ requires *m/z* 470.2667 (M^+), C, 71.46; H, 8.14%]; R_t of the synthetic (21) and natural (21) 12.2 min, analysed with a Shimazu LC-3A Liquid Chromatograph; Column, Zorbax SIL (25 cm × 2.1 mm); 1% methanol in dichloromethane; flow rate 0.8 ml/min.

(20R,22R)-1α-*Acetoxy*-3β,20-*dihydroxywitha*-5,24-*dienolide* (*Physalolactone* B) (22).—The diol (15) in pyridine (0.8 ml) was treated with acetic anhydride (1 ml) at room temperature for 4 h. Work-up (ether for extraction) gave a crude product, which was chromatographed on silica gel [solvent, ethyl acetate–hexane (1 : 4)] to afford the acetate. The acetate was then treated with 75% aqueous acetic acid (2 ml) in THF (0.8 ml) at 40 °C for 1 h. Work-up with ethyl acetate for extraction gave a crude product, which was chromatographed on silica gel [solvent, ethyl acetate–hexane (2 : 3)] to afford the *hydroxy acetate* (22) (18 mg, 68%), m.p. 250—252 °C; δ($CDCl_3$) 0.92 (s, 3 H, 13-Me), 1.08 (s, 3 H, 10-Me), 1.28 (s, 3 H, 20-Me), 1.90 and 1.95 (each s, each 3 H, 24- and 25-Me), 2.04 (s, 3 H, Ac), 3.64—4.08 (m, 1 H, $W_{\frac{1}{2}}$ 19.5 Hz, 3-H), 4.21 (dd, 1 H, *J* 12.6 and 4.2 Hz, 22-H), 4.92—5.12 (m, 1 H, $W_{\frac{1}{2}}$ 7.2 Hz, 1-H), and 5.55 (m, 1 H, 6-H) (Found: C, 71.75; H, 8.95. $C_{30}H_{44}O_6$ requires C, 71.97; H, 8.86%).

(20R,22R)-3β,20-*Dihydroxy*-1-*oxowitha*-5,24-*dienolide* (23).—To a solution of the diol (15) (30 mg) in dimethylformamide (1 ml) was added pyridinium dichromate (458 mg) at −5 °C. After being stirred for 8 h, water was added to the reaction mixture. Work-up gave a crude product, which was then treated with 75% aqueous acetic acid (2 ml) in THF (0.8 ml) at 40 °C for 1 h. Work-up (ethyl acetate for extraction) gave a solid, which was recrystallized from ethyl acetate to afford the *hydroxy ketone* (23) (15 mg, 61%), m.p. 189—190 °C; δ($CDCl_3$) 0.89 (s, 3 H, 13 Me), 1.28 (s, 3 H, 20-Me), 1.29 (s, 3 H, 10-Me), 1.88 and 1.96 (each s, each 3 H, 24- and 25-Me), 3.68—4.02 (m, 1 H, $W_{\frac{1}{2}}$ 19 Hz, 3-H), 4.22 (dd, 1 H, *J* 12 and 4.1 Hz), and 5.60 (m, 1 H, 6-H) (Found: C, 73.4; H, 8.95. $C_{28}H_{40}O_5$ requires C, 73.65; H, 8.83%).

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