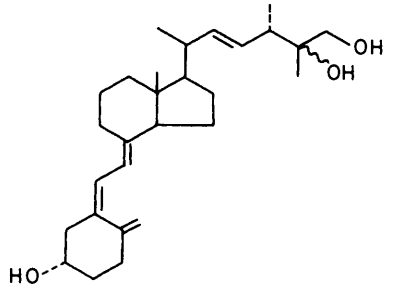
Synthesis of 25ξ,26-Dihydroxyvitamin D_2

By **Mary A. Gilhooly, David S. Morris,** and **Dudley H. Williams,*** University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW

Both acetylenic alcohols available from the attack of propynylmagnesium bromide on a steroidal C-22 aldehyde were utilised to generate a common γδ-unsaturated ester *via* a Claisen rearrangement. This ester was then converted into the title compound in a reaction sequence in which C-25 hydroxylation was achieved *via* oxygenation of the enolate anion of the C-26 ester function.

It has been shown that vitamin D_2 is converted into 25-hydroxyvitamin D_2 in the liver.[1] Further hydroxylation of this metabolite can occur to yield dihydroxylated metabolites.[1] 25,26-Dihydroxyvitamin D_2 [25,26-$(OH)_2$-D_2 (1), the product of 26-hydroxylation, has only

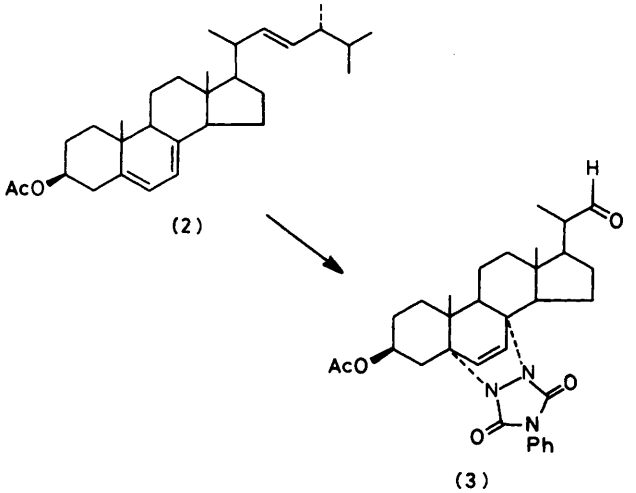


(1)

recently been isolated and was identified only tentatively.[2] Positive identification of this vitamin D_2 metabolite is required and this is best achieved by the chemical synthesis of 25,26-$(OH)_2$-D_2.

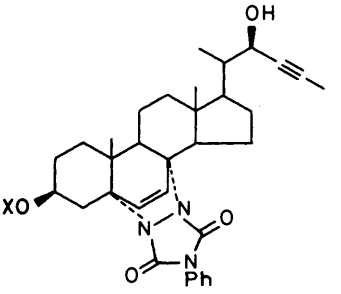
RESULTS AND DISCUSSION

Synthesis of the Acetylenic Alcohols (4) *and* (5).—Ergosterol acetate (2) was converted into the protected aldehyde (3) by well established procedures.[3,4] Initial attempts to synthesise the acetylenic alcohols (4) and (5) were conducted with propynyl-lithium as the nucleophile.

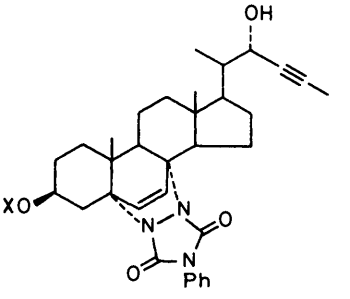


(2) (3)

At −78 °C, a solvent mixture of tetrahydrofuran (THF) and hexamethylphosphoramide (HMPA) was required to dissolve the propynyl-lithium and achieve good yields of compounds (4) and (5). At 0 °C with THF as solvent the major products were the dihydroxy-compounds (6) and (7). A more satisfactory method, avoiding the use of HMPA, employed propynylmagnesium bromide as the attacking nucleophile at −78 °C with THF as solvent. Using three equivalents of Grignard reagent and quenching the reaction at −78 °C, the yield of the more polar alcohol (4) was 46% and that of the less polar alcohol (5) was 37%. No evidence of epimerisation at C-20 appeared in the n.m.r. spectra of these compounds.



(4) X = Ac
(6) X = H



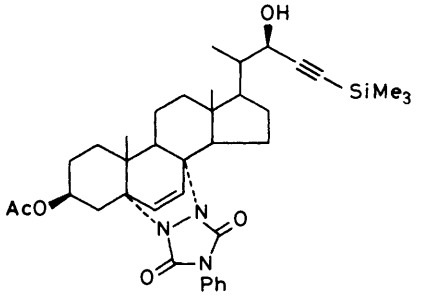
(5) X = Ac
(7) X = H

Precedent for the addition of lithium alkyls and Grignard reagents suggested that the major product would be the 22(*R*)-alcohol (4).[5] This was verified by correlation of the diastereoisomeric alcohols from these reactions with a C-22 alcohol of known structure.

X-Ray crystallography of the *p*-bromobenzoate derivative of compound (8) established the absolute configuration at C-22 as being *R*.[6] This compound was desilylated (in D_2O, although incorporation of deuterium at C-24 was incidental in the present work),[7] the 3β-acetate group was removed, and the product was protected as its 3β,22-bismethoxymethyl derivative. The

acetylenic anion was quenched with methyl iodide, and subsequent removal of the methoxymethyl groups afforded the diol (6) with known *R*-configuration at C-22.

The diastereoisomeric C-22 alcohols (4) and (5) were converted separately into the dihydroxy-compounds (6) and (7) by treatment with potassium carbonate in ethanol or methanol. Comparison of these products with the known diol established that the major, more polar, acetylenic alcohol (4) from the Grignard reaction did indeed have the *R* configuration at C-22.



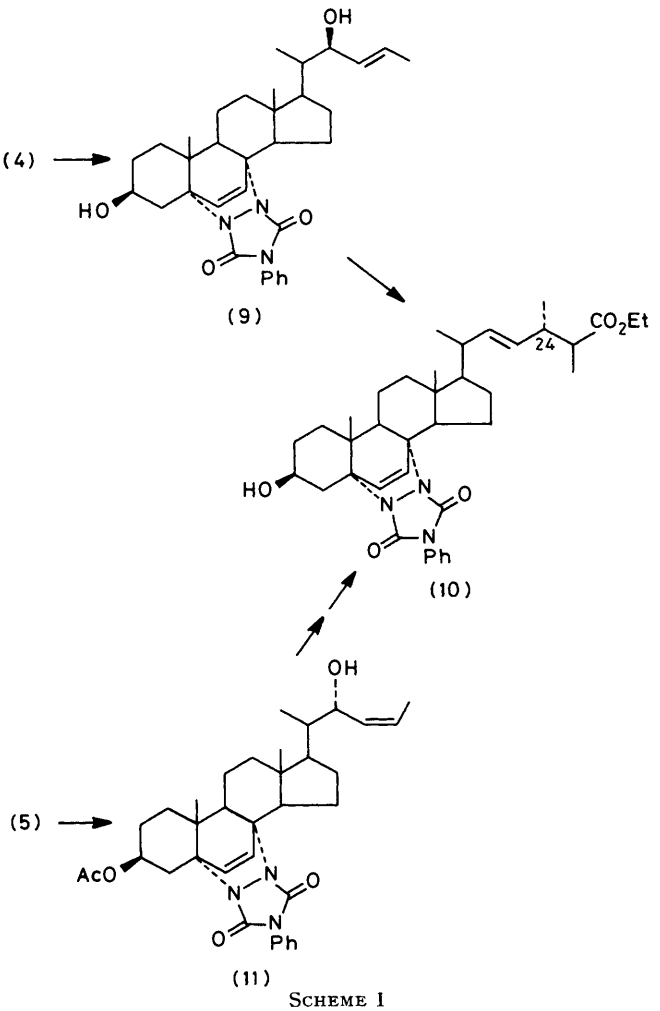
(8)

Synthesis of the γδ-Unsaturated Ester (10).—Knowledge of the C-22 stereochemistry of compounds (4) and (5) permitted the use of both these acetylenic alcohols in stereospecific syntheses of the γδ-unsaturated ester (10). The Claisen rearrangement is known to proceed through a chair-like transition state.[8] In the allyl vinyl ether intermediates derivable from the reaction of the allylic alcohols (9) and (11) with ethyl orthopropionate, the bulky steroid group prefers to be equatorial in the chair-like transition state. This ensures an (*E*)-22,23-olefin product and, given the olefin geometry of the starting material, the configuration at C-22 of the starting material determines that at C-24 in the product.[9] The (*E*)-allylic alcohol with *R* stereochemistry at C-22, (9), and the (*Z*)-allylic alcohol with *S* stereochemistry at C-22, (11), will both give a γδ-unsaturated ester with the *R* configuration at C-24 (Scheme 1).

The (*E*)-double bond of the alcohol (9) was produced upon lithium aluminium hydride treatment of the acetylenic alcohol (4). The reaction conditions cleaved the 3β-acetate and removed the ring B protecting group. Immediate reprotection of the diene was achieved by titration with the triazolinedione. The allylic alcohol (9) was obtained in 53% yield. Subjection of the alcohol (9) to the Claisen rearrangement (ethyl orthopropionate, propionic acid, reflux in benzene) led smoothly to the production of the γδ-unsaturated ester (10).

The (*Z*)-double bond of the alcohol (11) was obtained by hydrogenation of the acetylenic alcohol (5) over Lindlar catalyst. Claisen rearrangement of the alcohol (11), followed by removal of the 3β-acetate protecting group by means of potassium carbonate in absolute ethanol, afforded the ester (10).

Insertion of oxygen at C-25 was accomplished by quenching the enolate of the ester (10) with molecular



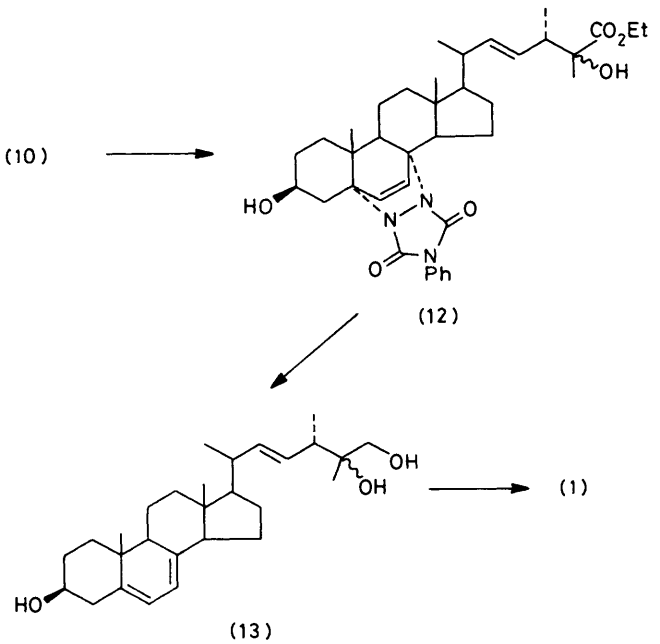
SCHEME 1

oxygen at −78 °C.[10] Reductive cleavage of the resulting peroxide with triethyl phosphite gave the dihydroxy-ester (12) in 69% yield from compound (10). Treatment of the dihydroxy-ester with lithium aluminium hydride in THF at 35 °C gave the provitamin (13) in 65% yield (Scheme 2).

The provitamin was dissolved in a mixture of methanol, THF, and diethyl ether and was subjected to sensitized irradiation (using fluorenone as a triplet sensitizer).[11] The previtamin was separated by preparative thick-layer chromatography (p.l.c.) and was then heated for 2½ h in 95% ethanol at 80 °C. P.l.c. then gave 25ξ,26-$(OH)_2$-D_2 (1) in 29% yield from the provitamin (13). The spectral properties were entirely consistent with those expected for the vitamin, although it should be noted that the product is expected to be a mixture of C-25 epimers. The overall yield of the vitamin from the aldehyde (3) was 3.5%.

EXPERIMENTAL

^{1}H N.m.r. spectra were recorded at 100 MHz on a Varian HA-100 spectrometer, at 90 MHz on a Varian EM-390



SCHEME 2

spectrometer, or at 80 MHz on a Varian CFT-20 spectrometer with internal tetramethylsilane as reference. Mass spectra were obtained on AEI-Kratos MS 902 or MS 30 spectrometers, the latter being used for high-resolution measurements. (−RDA) Refers to retro-Diels–Alder loss of 4-phenyl-1,2,4-triazoline-3,5-dione. I.r. spectra were recorded on a Perkin-Elmer 257 grating spectrometer using 0.5 mm solution cells with chloroform as solvent. U.v. spectra were recorded on a Unicam SP 1800 spectrometer for ethanol solutions.

M.p.s were recorded with a Kofler hot-stage microscope and are uncorrected.

Analytical thin-layer chromatography (t.l.c.) was carried out on Merck plates pre-coated with Keiselgel $60F_{254}$. Preparative thick-layer chromatography (p.l.c.) was carried out on plates 20 cm × 20 cm coated to a thickness of 1.3 mm with Keiselgel PF_{254}. Merck Keiselgel 60 (230—400 mesh ASTM) was used for column chromatography, and columns were eluted under a pressure of nitrogen at such a rate as to decrease the solvent level in the column at *ca.* 2 in/min.

Light petroleum = petroleum fraction of b.p. 60—80 °C.

22(R)-*Hydroxy*-5α,8α-(3,5-*dioxophenyl*-1,2,4-*triazolidino*)-26,27-*dinorcholest*-6-en-23-*yn*-3β-*yl Acetate* (4) *and its* 22(S)-*Epimer* (5).—Ethyl bromide (3.7 cm³, 50 mmol) was added dropwise to a vigorously stirred suspension of magnesium (1.08 g, 45 mg-atom) in dry THF (25 cm³) at 0 °C under argon. When all the magnesium had dissolved, the ethylmagnesium bromide solution was added to a saturated solution of propyne in dry THF (50 cm³) at 0 °C. A propyne flow through the solution was maintained during the addition, and for a further 10 min after completion of the addition. A portion of the propynylmagnesium bromide solution (38 cm³, 23 mmol) was added slowly to a stirred solution of the aldehyde (3) (4 g, 7.2 mmol) in THF (100 cm³) at −78 °C. The reaction was quenched after 2 h by addition of saturated aqueous ammonium chloride (80 cm³) and the product was then extracted with ethyl acetate (2 × 200 cm³). The combined organic extracts were washed with saturated brine (100 cm³), dried (Na_2SO_4), and the solvent was removed under reduced pressure to yield a foam. The product was purified by column chromatography (12.5% diethyl ether–chloroform as eluant).

The first compound eluted was the aldehyde (3) (0.39 g, 10% recovery). Continued elution afforded the less polar *acetylenic alcohol* (5) (1.6 g, 37%), which was crystallised from light petroleum–ethyl acetate to give material of m.p. 201—203 °C; δ ($CDCl_3$) 7.35 (5 H, m, Ph), 6.3 and 6.2 (2 H, ABq, *J* 8 Hz, 6- and 7-H), 5.6 and 5.35 (1 H, m, 3α-H), 4.4br (1 H, s, 22-H), 3.3 and 3.1 (1 H, dd, *J* 14 and 5 Hz, 9-H), 2.0 (3 H, s, OAc), 1.8 (3 H, d, *J* 2.5 Hz, C≡CMe), 1.0 (3 H, s, 19-H), and 0.85 (3 H, s, 18-H_3); 410 $(M - RDA)^+$, (10), 408 $(M - RDA - H_2)^+$, (4), 350 $(M - RDA - AcOH)^+$, (100), 348 $(M - RDA - H_2 - AcOH)^+$, (24), 335, 333, and 177 [Found: $(M - RDA - AcOH)^+$, 350.2611. $C_{25}H_{34}O$ requires *m/z* 350.2610]; $\nu_{max.}$ 3 600, 2 960, 1 750, 1 730, 1 695, and 1 410 cm^{-1}.

Continued elution afforded the more polar *acetylenic alcohol* (4) (1.95 g, 46%), which was crystallised from light petroleum–ethyl acetate, m.p. 194—197 °C; δ ($CDCl_3$) 7.35 (5 H, m, Ph), 6.3 and 6.2 (2 H, ABq, *J* 8 Hz, 6- and 7-H), 5.7—5.2 (1 H, m, 3α-H), 4.35br (1 H, s, 22-H), 3.35 and 3.15 (1 H, dd, *J* 14 and 5 Hz, 9-H), 2.0 (3 H, s, OAc), 1.85 (3 H, d, *J* 2.5 Hz, C≡CMe), 1.0 (3 H, s, 19-H_3), and 0.85 (3 H, s, 18-H_3); *m/z* 350 $(M - RDA - AcOH)^+$, (100), 348 (36), 335 $(M - RDA - AcOH - Me - H_2O)^+$, (5), and 177 [Found: $(M - RDA - AcOH)^+$, 350.2617. $C_{25}H_{34}O$ requires *m/z*, 350.2610]; $\nu_{max.}$ 3 600, 2 950, 1 750, 1 735, 1 695, and 1 410 cm^{-1}.

[24-^{2}H]-5α,8α-(3,5-*Dioxo*-4-*phenyl*-1,2,4-*triazolidino*)*chol*-6-*en*-23-*yne*-3β,22(R)-*diol*.—Anhydrous potassium carbonate (100 mg) was added to a stirred solution of the 3β-acetate (200 mg, 0.35 mmol), derived by desilylation–deuteriation [7] of compound (8), in dry methanol (10 cm³). After 29 h diethyl ether (10 cm³) was added, the solution was decanted, and was washed with distilled water (2 × 5 cm³) containing 2M hydrochloric acid (2 drops), then with saturated aqueous sodium hydrogen carbonate (2 × 5 cm³) and brine (2 × 5 cm³). The aqueous washings were extracted with diethyl ether (10 cm³) and the extract was washed as before. The combined extract and mother liquor were dried (Na_2SO_4) and evaporated to dryness to give the *title compound* (158 mg, 85%); δ ($CDCl_3$) 7.4 (5 H, m, Ph), 6.45 and 6.25 (2 H, ABq, *J* 8 Hz, 6-, and 7-H), 4.7—4.4 (total 2 H, m, 3α and 22-H), 3.4—3.1 (1 H, m, 9-H), 1.0 (3 H, s, 19-H_3), and 0.85 (3 H, s, 18-H_3); *m/z* 355 $(M - RDA)^+$, (48), 353 (36), and 337 $(M - RDA - H_2O)^+$, (17%).

[24-^{2}H]-3α,22(R)-*Bis*(*methoxymethoxy*)-5α,8α-(3,5-*dioxo*-4-*phenyl*-1,2,4-*triazolidino*)*chol*-6-*en*-23-*yne*.—*NN*-Di-isopropylethylamine (0.4 cm³, 2.3 mmol) was added to a solution of the deuteriated diol (250 mg, 0.47 mmol) in dry dichloromethane (5 cm³). After 5 min chloromethyl methyl ether (0.17 cm³, 2.3 mmol) was added and the mixture was stirred for 12 h. Dichloromethane (5 cm³) was added and the organic phase was washed in turn with ice-cold 0.5M hydrochloric acid (2 × 5 cm³), saturated aqueous sodium hydrogen carbonate (2 × 5 cm³), and brine (2 × 5 cm³). The organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure to yield the *title compound* (236 mg, 81%) as an orange foam; δ ($CDCl_3$) 7.35 (5 H, m, Ph), 6.35 and 6.15 (2 H, ABq, *J* 8 Hz, 6- and 7-H), 4.9—4.0 (total 6 H, m, 3α- and 22-H and 2 × OCH_2O), 3.25 (total 7 H, m, 9-H and 2 × OMe), 0.9 (3 H, s, 19-H), and 0.75 (3 H,

s, 18-H); *m/z* 443 (M − RDA)$^+$, (10), 411 (3), 411 (M − RDA − CH_3OH)$^+$, (11), 409 (6), and 381 (M − RDA − CH_3OCH_2OH)$^+$, (100%) [Found: (M − RDA − CH_3OCH_2OH)$^+$, 381.2778. $C_{26}H_{35}DO_2$ requires *m/z*, 381.2778]; $\nu_{max.}$ 2 950, 2 825, 2 600, (C–D str.), 1 750, 1 695, 1 410, and 1 150 cm^{-1}.

3β,22(R)-*Bis*(*methoxymethoxy*)-5α,8α-(3,5-*dioxo*-4-*phenyl*-1,2,4-*triazolidino*)-26,27-*dinorcholest*-6-*en*-23-*yne*.—n-Butyl-lithium (1.6M in hexane; 5 cm^3, 8 mmol) was added to a stirred solution of *NN*-di-isopropylamine (0.67 cm^3, 4.8 mmol) in anhydrous THF (2 cm^3) under nitrogen at 0 °C. After 10 min the solution was cooled to −78 °C and a portion (0.77 cm^3, 0.48 mmol) was added to a solution of the [24-^{2}H]acetylene (200 mg, 0.32 mmol) in dry THF (5 cm^3) under nitrogen at −78 °C and the mixture was stirred for 1 h. Methyl iodide (0.4 cm^3, 6.4 mmol) was added and, after 18 h, the mixture was poured into saturated aqueous ammonium chloride (10 cm^3). Extraction with ethyl acetate (3 × 10 cm^3), washing of the extracts with 0.5M hydrochloric acid (10 cm^3), then with saturated aqueous sodium hydrogen carbonate (10 cm^3), and brine (10 cm^3), drying (Na_2SO_4), and removal of the solvent under reduced pressure afforded the *title compound* (185 mg, 92%) as a yellow oil; δ ($CDCl_3$) 7.3 (5 H, s, Ph), 6.35 and 6.20 (2 H, ABq, *J* 8 Hz, 6- and 7-H), 4.9—4.3 (total 6 H, m, 3α and 22-H and 2 × OCH_2O), 3.45—3.00 (total 7 H, m, 9-H and 2 × OMe), 1.8 (3 H, d, *J* 2 Hz, C≡CMe), 0.9 (3 H, s, 19-H_3), and 0.8 (3 H, s, 18-H_3); *m/z* 456 (M − RDA)$^+$, (23), 454 (10), 424 (M − RDA − CH_3OH)$^+$, (10), 422 (5), and 394 (M − RDA − CH_3OCH_2OH)$^+$, (100%).

5α,8α-(3,5-*Dioxo*-4-*phenyl*-1,2,4-*triazolidino*)-26,27-*dinorcholest*-6-*en*-23-*yne*-3β,22(R)-*diol* (6).—(a) *From the* 3β,22(R)-*bismethoxymethoxy-compound*.—Toluene-*p*-sulphonic acid monohydrate (105 mg, 0.554 mmol) and the bismethoxymethoxy-compound (159 mg, 0.252 mmol) were dissolved in methanol (7 cm^3) and the resulting solution was heated under reflux at 70 °C. After 3½ h the solvent was removed, the residue was extracted with ethyl acetate (15 cm^3), and the extract was washed with saturated aqueous sodium hydrogen carbonate (2 × 5 cm^3) and with brine (5 cm^3). The aqueous washings were extracted with ethyl acetate (10 cm^3). The organic layers were combined, dried (Na_2SO_4), and the solvent was removed under reduced pressure to give the *title compound* (129 mg, 94%) which was crystallised from dichloromethane–ethyl acetate, m.p. 204—205 °C; δ ($CDCl_3$) 7.35 (5 H, m, Ph), 6.3 and 6.1 (2 H, ABq, *J* 7.5 Hz, 6- and 7-H), 4.5—4.2br (total 2 H, s, 3α-, and 22-H), 3.18 and 3.00 (1 H, dd, *J* 14 and 5 Hz, 9-H), 1.8 (3 H, d, *J* 2 Hz, C≡CMe), 0.95 (3 H, s, 19-H), and 0.85 (3 H, s, 18-H)$_3$; *m/z* 368 (M − RDA)$^+$, (100), 366 (53), 350 (M − RDA − H_2O)$^+$, (8), 348 (51), and 335 (M − RDA − H_2O − CH_3)$^+$, (75%) [Found: (M − RDA)$^+$, 368.2728. $C_{25}H_{36}O_2$ requires *m/z*, 368.2716]; $\nu_{max.}$ 3 600, 3 450, 2 950, 1 750, 1 695, and 1 410 cm^{-1}.

(b) *From the* 3β-*acetate*-22(R)-*alcohol* (4). A mixture of anhydrous potassium carbonate (90 mg) and the ester (4) (175 mg, 0.299 mmol) in absolute ethanol (5 cm^3) was stirred for 72 h. Diethyl ether (10 cm^3) was added and the solution was decanted, washed in turn with water (5 cm^3) containing 2M hydrochloric acid (2 drops), saturated aqueous sodium hydrogen carbonate (5 cm^3), and brine (5 cm^3), and was then dried (Na_2SO_4). Evaporation of the solvent under reduced pressure afforded the title compound (97 mg, 60%) which was crystallised from light petroleum–acetone, m.p. 204—206 °C. A mixed m.p. of the compounds obtained from parts (a) and (b) of this experiment was 204—206 °C. Spectral details of the compound derived in part (b) were identical with those in part (a) with the following exception: [Found: (M − RDA)$^+$ 368.2684. $C_{25}H_{36}O_2$ requires *m/z*, 368.2716].

5α,8α-(3,5-*Dioxo*-4-*phenyl*-1,2,4-*triazolidino*)-26,27-*dinorcholest*-6-*en*-23-*yne*-3β, 22(S)-*diol* (7).—This compound was prepared from the ester (5) (8 mg, 0.01 mmol) in an analogous manner to the preparation of the diol (6) from the ester (4), with methanol (2 cm^3) as solvent and a reaction time of 2½ h. The *product* showed δ ($CDCl_3$) 7.35 (5 H, m, Ph), 6.3 and 6.1 (2 H, ABq, *J* 7.5 Hz, 6-, and 7-H), 4.5—4.2br (total 2 H, s, 3α- and 22-H), 3.18 and 3.00 (1 H, dd, *J* 14 and 5 Hz, 9-H), 1.8 (3 H, d, *J* 2 Hz, C≡CMe), 0.95 (3 H, s, 19-H_3), and 0.85 (3 H, s, 18-H_3); *m/z* 368 (M − RDA)$^+$, (100), 366 (53), 350 (M − RDA − H_2O)$^+$, (8), 348 (51), and 335 (M − RDA − H_2O − Me)$^+$, (75%).

5α,8α-(3,5-*Dioxo*-4-*phenyl*-1,2,4-*triazolidino*)-26,27-*dinorcholest*-6,23(E)-*diene*-3β,22(R)-*diol* (9).—A solution of the more polar acetylenic acetate (4) (1 g, 1.7 mmol) in dry THF (20 cm^3) was added slowly to lithium aluminium hydride (300 mg, 8.8 mmol) in the same solvent (15 cm^3). The mixture was heated under reflux at 75 °C for 2 d and was then cooled, and ethyl acetate (70 cm^3) and a saturated aqueous solution of Rochelle salt (50 cm^3) were added. The resulting mixture was shaken and the aqueous layer was separated and extracted with ethyl acetate (2 × 50 cm^3). The combined organic extracts were washed with saturated brine (1 × 50 cm^3) and dried (Na_2SO_4). A solution of 4-phenyl-1,2,4-triazoline-3,5-dione in ethyl acetate was added dropwise to the dried extracts until a pink colour persisted. Removal of the solvent under reduced pressure, followed by purification of the residue [p.l.c.; 45% acetone–light petroleum as developer], afforded the *title compound* (9) (487 mg, 53%) which was crystallised from dichloromethane–light petroleum, m.p. 193—194 °C; δ ($CDCl_3$) 7.35 (5 H, m, Ph), 6.35 and 6.15 (2 H, ABq, *J* 8 Hz, 6- and 7-H), 5.65—5.4 (2 H, m, 23- and 24-H), 4.6—4.25 (1 H, m, 3α-H), 4.25—4.1br (1 H, s, 22-H), 3.15 and 3.00 (1 H, dd, *J* 14 and 5 Hz, 9-H), 1.7—1.55 (3 H, m, C=CMe), 0.95 (3 H, s, 19-H_3), and 0.8 (3 H, s, 18-H_3); *m/z* 370 (M − RDA)$^+$, (88), 368 (8), 352 (M − RDA − H_2O)$^+$, (34), 337 (M − RDA − H_2O − CH_3)$^+$, (100), 335 (5), 319 (M − RDA − H_2O − CH_3 − H_2O)$^+$, (44%), and 177 [Found: (M − RDA)$^+$, 370.2905. $C_{25}H_{38}O_2$ requires *m/z*, 370.2871]; $\nu_{max.}$ 3 600, 3 450, 3 000, 2 950, 1 745, 1 690, and 1 405 cm^{-1}.

Ethyl 24(S)-3β-*Hydroxy*-5α,8α-(3,5-*dioxo*-4-*phenyl*-1,2,4-*triazolidinoergosta*-6,22(E)-*dien*-26-*oate* (10).—Triethyl orthopropionate (3 cm^3, 15 mmol) and propionic acid (37 μl, 0.5 mmol) were added to a solution of the allylic alcohol (9) (410 mg, 0.75 mmol) in dry benzene (10 cm^3) under oxygen in a Dean–Stark apparatus containing 5A molecular sieves. The solution was heated under reflux for 24 h and the solvent was then removed under reduced pressure. The residue was dissolved in ethyl acetate (25 cm^3) and the solution was washed in turn with 2M hydrochloric acid (2 × 25 cm^3), saturated aqueous sodium hydrogen carbonate (2 × 25 cm^3), and brine (25 cm^3). The aqueous washings were re-extracted with ethyl acetate (25 cm^3). A solution of 4-phenyl-1,2,4-triazoline-3,5-dione in ethyl acetate was added dropwise to the combined extracts until a permanent, faint pink colour was obtained. The resulting solution was dried (Na_2SO_4), the solvent was removed under reduced pressure, and the product was purified [p.l.c.; 33% acetone–light petroleum as developer] to yield the *title compound* (10) (334 mg, 71%);

δ ($CDCl_3$) 7.35 (5 H, m, Ph), 6.4 and 6.2 (2 H, ABq, *J* Hz, 6- and 7-H), 5.4—5.12 (2 H, m, 22- and 23-H), 4.6—4.26 (1 H, m, 3α-H), 4.12 (2 H, q, *J* 8 Hz, OCH_2CH_3), 3.27 and 3.12 (1 H, dd, *J* 14 and 5 Hz, 9-H), 1.3 (3 H, t, *J* 8 Hz, OCH_2CH_3), 0.96 (3 H, s, 19-H_3), and 0.82 (3 H, s, 18-H); *m/z* 454 (M − RDA)$^+$, (52), 452 (4), 421 (M − RDA − H_2O − CH_3)$^+$, (48), 253 (100), and 251 (72%) [Found: (M − RDA)$^+$, 454.3418. $C_{30}H_{46}O_3$ requires *m/z*, 454.3447]; $\nu_{max.}$ 3 610, 2 950, 1 750, 1 730, 1 695, and 1 405 cm^{-1}.

22(S)-*Hydroxy*-5α,8α-(3,5-*dioxo*-4-*phenyl*-1,2,4-*triazolidino*)-26,27-*dinorcholesta*-6,23(Z)-*dien*-3β-*yl Acetate* (11).—A suspension of Lindlar catalyst (750 mg) in 95% ethanol (25 cm^3) was stirred vigorously under hydrogen until absorption of hydrogen had ceased. A solution of the acetylene (5) (750 mg, 1.3 mmol) in 95% ethanol (50 cm^3) was then added and vigorous stirring was continued for 7 d, after which the catalyst was filtered off and the solvent was removed under reduced pressure to yield a white foam (700 mg). Purification of the product [p.l.c.; 22% diethyl ether–chloroform as developer] afforded the *title compound* (11) (525 mg); δ ($CDCl_3$) 7.35 (5 H, m, Ph), 6.4 and 6.2 (2 H, ABq, *J* 8 Hz, 6- and 7-H), 5.68—5.2 total (3 H, m, 3α-, 23-, and 24-H), 4.2—4.0 (1 H, m, 22-H), 3.3—3.15 (1 H, dd, *J* 14 and 5 Hz, 9-H), 2.0 (3 H, s, OAc), 1.6br (3 H, s, C=CMe), 0.95 (3 H, s, 19-H_3) and 0.8 (3 H, s, 18-H_3); *m/z* 412 (M − RDA)$^+$, (6), 410, 394 (M − RDA − H_2O)$^+$, (5), 392, 352 (M − RDA − AcOH)$^+$, (29), 350 (M − RDA − H_2 − AcOH)$^+$, (100), 337 (M − RDA − AcOH − Me)$^+$, (23), 335 (16), 319 (M − RDA − AcOH − Me − H_2O)$^+$, (34%), and 177 [Found: (M − RDA − AcOH)$^+$, 352.2765. $C_{25}H_{36}O$ requires *m/z*, 352.2784]; $\nu_{max.}$ 3 610, 3 010, 2 960, 1 750, 1 735, 1 695, and 1 410 cm^{-1}.

Ethyl 3β-*Acetoxy*-5α,8α-(3,5-*dioxo*-4-*phenyl*-1,2,4-*triazolidino*)*ergosta*-6,22(E)-*dien*-26-*oate*.—The title compound was prepared from the allylic alcohol (11) (350 mg, 0.6 mmol)in the same manner as described for the preparation of the ester (10). The crude product was purified [p.l.c.; 25% acetone–light petroleum as developer] to yield the *title compound* (210 mg, 52%); δ ($CDCl_3$) 7.35 (5 H, m, Ph), 6.4 and 6.2 (2 H, ABq, *J* 8 Hz, 6- and 7-H), 5.65—5.0 (total, 3 H, m, 3α-, 22-, and 23-H), 4.11 (2 H, q, *J* 7 Hz, OCH_2CH_3), 3.4—3.1 (1 H, m, 9-H), 2.0 (3 H, s, OAc), 1.3 (3 H, t, *J* 8 Hz, OCH_2CH_3), 1.0 (3 H, s, 19-H_3), and 0.84 (3 H, s, 18-H_3); *m/z* 494 (M − RDA − H_2)$^+$, (3), 436 (M − RDA − AcOH)$^+$, (100), 434 (34), 421 (M − RDA − AcOH − Me)$^+$, (6), 419 (22), 251 (59%), and 177 [Found: (M − RDA − H_2)$^+$, 494.3398. $C_{32}H_{46}O_4$ requires *m/z*, 494.3396]; $\nu_{max.}$ 2 960, 1 750, 1 730, 1 695, and 1 405 cm^{-1}.

The diester was converted into compound (10) as follows. Anhydrous potassium carbonate (100 mg) was added to a stirred solution of the diester (191 mg, 0.285 mmol) in absolute ethanol (3.7 cm^3). After 52 h diethyl ether (10 cm^3) was added and the solution was decanted and washed in turn with distilled water (10 cm^3) containing 2M hydrochloric acid (2 drops), saturated aqueous sodium hydrogen carbonate (5 cm^3), and brine (5 cm^3). The aqueous washings were extracted with diethyl ether (10 cm^3). The combined extracts dried (Na_2SO_4). Removal of the solvent under reduced pressure gave compound (10) as a foam (145 mg, 79%).

Ethyl 3β,25-*Dihydroxy*-5α,8α-(3,5-*dioxo*-4-*phenyl*-1,2,4-*triazolidino*)*ergosta*-6,22(E)-*dien*-26-*oate* (12).—n-Buty-llithium (1.6M in hexane; 2 cm^3) was added to a stirred solution of *N*-isopropylcyclohexylamine (0.35 cm^3, 2.08 mmol) in dry THF (2.65 cm^3) under nitrogen at 0 °C. After 15 min the solution was cooled to − 78 °C and a portion (2 cm^3, 0.84 mmol) was added to a stirred solution of the ester (10) (210 mg, 0.33 mmol) in dry THF (5 cm^3) under a stream of nitrogen at −78 °C. After 1 h the dry nitrogen stream was replaced by a dry oxygen stream, the temperature being maintained at −78 °C. After 30 min triethyl phosphite (0.16 cm^3, 0.88 mmol) was added followed, after 15 min, by saturated aqueous ammonium chloride (20 cm^3). Extraction of the product with ethyl acetate (3 × 20 cm^3), and successive washing of the resulting extracts with 2M hydrochloric acid (20 cm^3), saturated aqueous sodium hydrogen carbonate (20 cm^3), and brine (20 cm^3), gave a solution which was dried (Na_2SO_4). The residue obtained upon evaporation under reduced pressure was purified [p.l.c.; 33% acetone–light petroleum as developer] to give the *α-hydroxy-ester* (12) (147 mg, 69%); δ ($CDCl_3$) 7.37 (5 H, m, Ph), 6.35 and 6.15 (2 H, ABq, *J* 8 Hz, 6- and 7-H), 5.4—5.1 (2 H, m, 22- and 23-H), 4.5—3.9 (total 3 H, 3α-H and OCH_2CH_3), 3.2 and 3.03 (1 H, dd, *J* 14 and 4 Hz, 9-H), 1.28 (3 H, s, 27-H_3), 0.93 (3 H, s, 19-H), and 0.83 (3 H, s, 18-H_3); *m/z* 470 (M − RDA)$^+$, (58), 468 (10), 452 (M − RDA − H_2O)$^+$, (6), 450 (6), 437 (M − RDA − H_2O − CH_3)$^+$, (55), 435 (3), 253 (65), 251 (100%), and 177 [Found: (M − RDA)$^+$, 470.3384. $C_{30}H_{46}O_4$ requires *m/z*, 470.3396].

Ergosta-5,7,22(E)-*triene*-3β,25,26-*triol* (13).—Lithium aluminium hydride (90 mg, 2.6 mmol) was added to a solution of the hydroxy-ester (12) (90 mg, 0.14 mmol) in dry THF (15 cm^3) under argon at 0 °C. The mixture was stirred at 35 °C for 24 h, then was cooled to room temperature and water was added dropwise to destroy excess of the hydride. A saturated aqueous solution of Rochelle salt (15 cm^3) was added and the mixture was extracted with ethyl acetate (3 × 25 cm^3). The combined extracts were washed with saturated brine (20 cm^3), dried (Na_2SO_4), and the solvent was removed under reduced pressure. Purification [p.l.c.; 40% acetone–light petroleum as developer] yielded the *provitamin* (13) (39 mg, 65%); δ ($CDCl_3$) 5.65—5.2 (total, 4 H, m, 6-, 7-, 22-, and 23-H), 3.8—3.3 (total 3 H, 3α-H and CH_2OH), 0.95 (3 H, s, 19-H_3), and 0.62 (3 H, s, 18-H_3); *m/z* 428 (M^+), (98), 410 (M − H_2O)$^+$, (20), 395 (M − H_2O − CH_3)$^+$, (100), 253 (78), and 251 (68%) [Found: M^+, 428.3301. $C_{28}H_{44}O_3$ requires M, 428.3290]; $\nu_{max.}$ 3 600 and 2 950 cm^{-1}; $\lambda_{max.}$ 271, 282, and 293 nm.

25,26-*Dihydroxyvitamin* D_2 (1).—A solution of the provitamin (13) (39 mg) in a mixture of methanol (15 cm^3), THF (60 cm^3), and diethyl ether (200 cm^3) was degassed with argon for 1 h and was then irradiated at 0 °C with a Hanovia medium-pressure mercury vapour lamp. After 30 min, fluorenone (30 mg) was added and the solution was irradiated for a further 15 min. The solvent was removed under reduced pressure at a temperature ≤10 °C. P.l.c. (30% acetone–light petroleum as developer) in the dark under argon afforded the previtamin ($\lambda_{max.}$ 261 nm).

The purified product was dissolved in 95% ethanol (5 cm^3) and the solution was heated to 80 °C under argon for 2½ h. Removal of solvent under reduced pressure and p.l.c. (20% diethyl ether–light petroleum) of the residue afforded the *vitamin* (1) (11.5 mg, 29%); δ ($CDCl_3$) 6.24 and 6.00 (2 H, ABq, *J* 11 Hz, 6- and 7-H), 5.4—5.2 (2 H, m, 22- and 23-H), 5.03 (1 H, m, 19-H), 4.82 (1 H, m, 19-H), 4.0—3.8 (1 H, m, 3α-H), 3.47 (2 H, m, CH_2OH), 1.25 (3 H, s, 27-H_3), and 0.55 (3 H, s, 18-H_3); *m/z* 428 (M^+), (100), 410 (M − H_2O)$^+$, (7), 395 (M − H_2O − Me)$^+$, (42), 271

(78), and 253 (88%) [Found: M^+, 428.3271. $C_{28}H_{44}O_3$ requires M, 428.3290]; $\lambda_{max.}$ 264 nm.

We thank the S.E.R.C. for financial support.

[2/062 *Received*, 13*th January*, 1982]

REFERENCES

[1] G. Jones, H. K. Schnoes, and H. F. DeLuca, *Biochemistry*, 1975, **14**, 1250.
[2] A. Hay and G. Jones, *Clin. Chem.*, 1979, **25**, 473.
[3] D. H. R. Barton, T. Shioiri, and D. A. Widdowson, *J. Chem. Soc. C*, 1971, 1968.
[4] S. C. Eyley, Ph.D. Thesis, University of Cambridge, 1975.
[5] D. M. Paitak and J. Wicha, *Chem. Rev.*, 1978, **78**, 199.
[6] F. H. Allen, W. B. T. Cruse, and O. Kennard, *Acta Crystallogr., Sect. B*, 1980, **36**, 2342.
[7] J. D. Meadows and D. H. Williams, *Tetrahedron Lett.*, 1980, **21**, 4373.
[8] G. Frater, A. Habich, H. J. Hansen, and H. Schmid, *Helv. Chim. Acta*, 1969, **52**, 335.
[9] B. Lythgoe, D. A. Roberts, and I. Waterhouse, *J. Chem. Soc., Perkin Trans. 1*, 1977, 2608.
[10] H. H. Wasserman and B. H. Lipschutz, *Tetrahedron Lett.*, 1975, 1731.
[11] S. C. Eyley and D. H. Williams, *J. Chem. Soc., Chem. Commun.*, 1975, 858.