Reaction of Diethyl Azodicarboxylate with 3β-Acetoxycholesta-5,7-diene (2). To a solution of **2** (1 g) dissolved in sodium-dried benzene (10 mL) was added diethyl azodicarboxylate (1 g), and the solution was refluxed under nitrogen for 4 h. Removal of the solvent and of the excess ester under reduced pressure gave a crude solid which on crystallization from hexane yielded 3β-acetoxy-7α-(1,2-dicarbethoxyhydrazo)cholesta-5,8-diene (**3**): 0.770 g; mp 138–139 °C (from hexane; lit.[7] mp 138–139.5 °C); $[\alpha]^{20}_D$–76°; IR 3470, 1755, 1715, 1708 cm^{-1}; ^1H NMR δ 6.12 (1 H, m, NH), 5.45 (1 H, m, 6-H), 5.18 (1 H, m, 7β-H), 4.05–4.85 (5 H, overlapping, 3α-H and 2 $COOCH_2CH_3$), 2.00 (3 H, s, CH_3COO), 1.21 (3 H, s, 19-CH_3) 0.65 (3 H, s, 18-CH_3); mass spectrum, *m/e* 424 (2%, M – $C_6H_{12}N_2O_4$), 365 (100), 349 (15).

Anal. Calcd for $C_{35}H_{56}N_2O_6$: C, 70.0; H, 9.4; N, 4.7. Found: 69.9; H, 9.3; N, 4.7).

Evaporation of the mother liquor under reduced pressure gave a residue which was purified by preparative TLC (20% EtOAc/toluene) to afford **3** (95 mg) and 3β-acetoxy-7α-(1,2-dicarbethoxyhydrazo)cholesta-5,8(14)-diene (**4**): 250 mg; mp 74–75 °C (amorphous); IR 3470, 1755, 1705 cm^{-1}; ^1H NMR δ 6.30 (1 H, m, NH), 5.30 (1 H, m, 6 H), 5.06 (1 H, m, 7β-H), 4.05–4.85 (5 H, overlapping, 3α-H and 2 $COOCH_2CH_3$), 2.00 (3 H, s, CH_3COO), 0.86 (6 H, s, 18- and 19-CH_3); mass spectrum, *m/e* 424 (3%, M – $C_6H_{12}O_4N_2$), 365 (100).

Anal. Calcd for $C_{35}H_{56}N_2O_6$: C, 70.0; H, 9.4; N, 4.7. Found: C, 69.8; H, 9.5; N, 4.7.

Synthesis of Cholesta-5,8-dien-3β-ol (1a). 3β-Acetoxy-7α-(1,2-dicarbethoxyhydrazo)-5,8-diene (**3**, 0.500 g) dissolved in ethylamine (20 mL) was treated with lithium (0.200 g), and the mixture was stirred at –20 °C for 30 min longer than required for the initial appearance of a blue color. The usual workup afforded cholesta-5,8-dien-3β-ol (**1a**): 208 mg; mp 106–107 °C (from methanol); $[\alpha]^{20}_D$ –4.5; IR 3400 cm^{-1}; ^1H NMR δ 5.48 (1 H, m, 6-H), 3.55 (1 H, m, 3α-H), 2.54 (2 H, m, 7-H_2), 2.36 (1 H, m, 4α-H), 2.28 (1 H, m, 4β-H), 1.18 (3 H, s, 19-CH_3), 0.66 (3 H, s, 18-CH_3); mass spectrum, *m/e* (relative intensity) 384 (60, M^+), 351 (100, M – (H_2O + Me)), 325 (20), 271 (20, M – C_8H_{17}), 253 (20, M – (C_8H_{17} + H_2O)), 217 (20), 211 (23).

Anal. Calcd for $C_{27}H_{44}O$: C, 84.3; H, 11.5. Found: C, 84.4; H, 11.4.

Acetylation of **1a** with acetic anhydride–pyridine afforded 3β-acetoxycholesta-5,8-diene (**1b**): mp 100–101 °C lit.[1] 98–100 °C; from methanol $[\alpha]^{20}_D$ –17°; IR 1740, 1250 cm^{-1}; ^1H NMR δ 5.48 (1 H, m, 6-H), 4.62 (1 H, m, 3α-H), 2.54 (2 H, m, 7-H_2), 2.42 (1 H, m, 4α-H), 2.35 (1 H, m, 4β-H), 2.02 (3 H, s, CH_3COO), 1.20 (3 H, s, 19-CH_3), 0.66 (3 H, s, CH_3); mass spectrum, *m/e* (relative intensity) 426 (5, M^+), 366 (87, M – AcOH), 351 (100), 253 (20), 211 (30).

Anal. Calcd for $C_{29}H_{46}O_2$: C, 81.6; H, 10.9. Found: C, 81.5; H, 10.8.

Reduction of Cholesta-5,8-dien-3β-ol (1a). A solution of the dienol **1a** (100 mg) in ethanol (10 mL) containing Raney nickel (200 mg) was shaken in hydrogen. The usual workup afforded 5α-cholest-8-en-3β-ol: 80 mg (from methanol); mp 127–128 °C; $[\alpha]^{23}_D$ + 48°; identical by mixture melting point with an authentic sample.[13]

Acknowledgment. We acknowledge support from the Ministero della Pubblica Istruzione.

Registry No. 1a, 70741-38-7; **1b**, 17137-76-7; **2**, 1059-86-5; **3**, 3914-89-4; **4**, 77965-72-1; 5α-cholest-8-en-3β-ol, 566-97-2.

(13) Barton, D. H. R.; Cox, J. D. *J. Chem. Soc.* **1949**, 214.

Structure and Synthesis of 25-Hydroxycholecalciferol-26,23-lactone, a Metabolite of Vitamin D

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The aldehyde **6**, prepared from ergosterol, underwent addition with vinylmagnesium bromide. Construction of the carbon side chain of the title compound was completed with a Claisen rearrangement. After conversion to the hydroxy acid **11**, halolactonization and subsequent dehalogenation gave the desired five-membered lactones. Separation of all four possible diastereoisomers was achieved by high-pressure liquid chromatography, and these were carried through to the provitamin stage. By chemical correlation and solution of two X-ray structures, the absolute stereochemistry of all four products was established. Irradiation in the presence of fluorenone as a triplet sensitizer and thermal isomerization gave the four target molecules. The natural product was identified by NMR comparison with the isolated metabolite and consideration of the biochemical pathway which leads to it.

In 1979, the isolation and identification of 23,25-dihydroxycholecalciferol-26,23-lactone (**1**), a new metabolite of vitamin D_3, was reported.[1] The stereochemistry at C-23 and C-25 was undetermined. The lactone was obtained from the plasma of chicks and became a major circulating metabolite of vitamin D_3 under conditions of hypervitaminosis. A synthesis of all four possible diastereoisomeric lactones has recently been reported, and it has been shown that one of these compounds is identical with the natural product, thus confirming the gross structure.[2] However, since the stereochemistries at C-23 and C-25 of the four lactones were not established, the stereochemistry of the natural product at these sites remained unestablished. We now report independent syntheses of the four possible lactones and experiments which establish the stereochemistries at C-23 and C-25 in each case. These experiments establish that the natural product has the 23*R*,25*S* stereochemistry. Biological testing of all four metabolites is also reported.

(1) Wichmann, J. K.; DeLuca, H. F.; Schnoes, H. K.; Horst, R. L.; Shepard, R. M.; Jorgensen, N. A. *Biochemistry* **1979**, *18*, 4775.
(2) Wichmann, J. K.; Paaren, H. E.; Fivizzani, M. A.; Schnoes, H. K.; DeLuca, H. F. *Tetrahedron Lett.* **1980**, 4667.

Results and Discussion

Our synthetic strategy involved the synthesis of **2** (Scheme I), in which the 3β-OH and ring B diene functionalities would be suitably protected, with both *R* and *S* stereochemistries present at C-25. This last feature should be accessible by oxygenation of an anion (sp^2 hybridization) adjacent to carbonyl. We then planned to

0022-3263/81/1946-3422$01.25/0

Scheme I

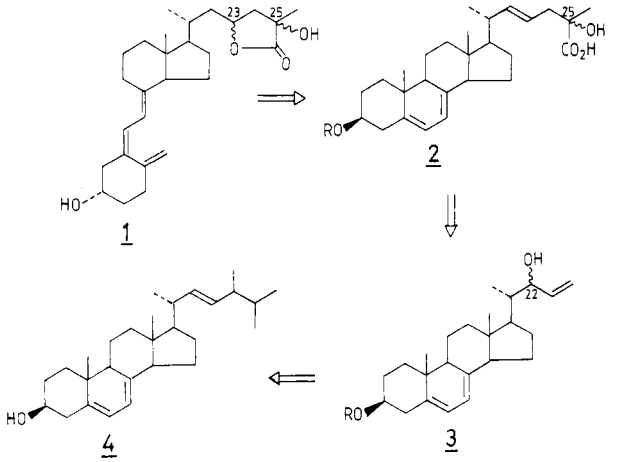
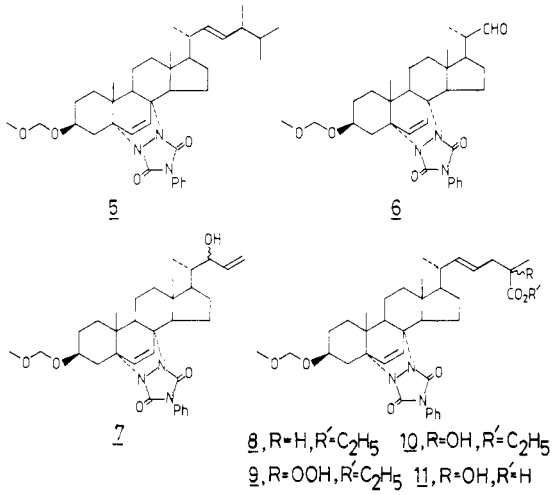


Chart I



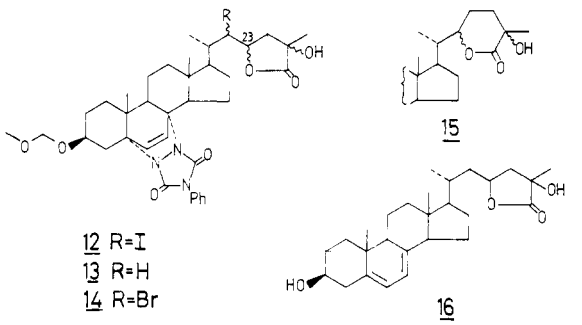
lactonize **2** via bromo- or iodolactonization and finally to produce **1** by reduction, followed by the sequence of photochemical and thermal isomerization.[3] The desired precursor of **2** should be available by Claisen rearrangement of an allylic alcohol **3**, in which either or both C-22 stereoisomeric alcohols could be utilized. It was envisaged that **3** would be obtained from a C-22 aldehyde, which would in turn be available by ozonolysis of a suitable derivative of ergosterol (**4**).[4]

The ring B diene of ergosterol was protected as the Diels–Alder adduct with 4-phenyl-1,2,4-triazoline-3,5-dione,[4] and the 3β-hydroxyl group was converted to the methoxymethyl derivative by reaction with chloromethyl methyl ether to give **5** (Chart I). Selective cleavage of the side-chain double bond of **5** was achieved by ozonolysis at −70 °C to give **6**, in a manner analogous to that previously reported for the corresponding 3β-acetate.[4] The nuclear magnetic resonance spectrum of **6** exhibited a doublet at δ 9.57 for the aldehyde proton. This indicated that no epimerization had occurred at C-20. The allylic alcohol **7** was obtained in 89% yield by adding 1.25 equiv of vinylmagnesium bromide to **6** at −78 °C and quenching the reaction at low temperature with ammonium chloride. The mixture of C-22 diastereoisomers which was obtained was used directly in the ensuing Claisen reaction. The Claisen rearrangement was carried out in benzene under reflux

(3) Eyley, S. C.; Williams, D. H. *J. Chem. Soc., Chem. Commun.* **1975**, 858.

(4) Barton, D. H. R.; Shiori, T.; Widdowson, D. A. *J. Chem. Soc. C* **1971**, 1968.

Chart II



12 R=I
13 R=H
14 R=Br

Table I. Yields (in Percent) of Lactones 13 from 11 via Bromo- and Iodolactonization

						total	
lactone 13	I	II	III	IV	6-ring	γ	$\gamma + \delta$
via iodo lactones	12	5	21	23		61	61
via bromo lactones	21	5	24	8	6	58	64

with a 10-fold excess of triethyl orthopropionate and a catalytic quantity of propionic acid. The resulting γ,δ-unsaturated ester **8**, obtained in 88% yield, was converted to the enolate anion at −78 °C, and molecular oxygen was passed through the solution for 1 h to yield the peroxide **9**. Immediate reduction of the peroxide with triethyl phosphite at −78 °C gave the α-hydroxy ester **10** in 93% yield from **8**.

A separation of the diastereoisomeric forms of **10** at this stage was desirable, but prolonged attempts, using HPLC, failed. The existence of a diastereoisomeric mixture (both *R* and *S* stereochemistries at C-25) was, however, established by a ^{1}H NMR study of **10** in the presence of the chiral shift reagent tris[3-[(heptafluoropropyl)hydroxymethylene]-*d*-camphorato]europium(III). Under these conditions, the 25-methyl resonance split into two signals of approximately equal intensity.

Alkaline hydrolysis of the mixture of diastereoisomers of **10** gave the mixture of acids **11**. Without further purification, this mixture of acids was treated with iodine and potassium iodide under basic conditions to give the iodo lactones **12** (Chart II). At this stage, three iodo lactone components were separated by preparative HPLC. These were labeled iodo lactones I, II, and III, in order of elution. Reduction of each of these iodo lactones with an excess of tri-*n*-butyltin hydride at room temperature gave the dehalogenated lactones **13**. Iodo lactone I gave lactone I; iodo lactone II gave two lactones, II and III, separable by preparative thin-layer chromatography; iodo lactone III gave lactone IV. Thus, all four possible diastereoisomers of **13** had been isolated. The mass spectra and infrared spectra of lactones I–IV (**13**) were essentially identical, and the only major difference in their ^{1}H NMR spectra was the chemical shift of the C-23 proton; this proton resonated as a multiplet at δ 4.6 in lactones I and II, as compared with a multiplet at δ 4.4 for lactones III and IV.

Bromolactonization of **11** was also carried out to give the mixture of bromo lactones **14**, and again separation of three components was possible by preparative HPLC; these were labeled bromolactones I, II, and III, in order of elution. Reduction of these compounds with an excess of tri-*n*-butyltin hydride again gave the lactones **13**. Bromo lactone I gave two lactones on reduction, one of which was lactone I (carbonyl stretching frequency at 1775 cm^{-1}) and the other a six-membered-ring lactone **15** (carbonyl stretching frequency at 1730 cm^{-1}). Bromo lactone II also gave two

Scheme II

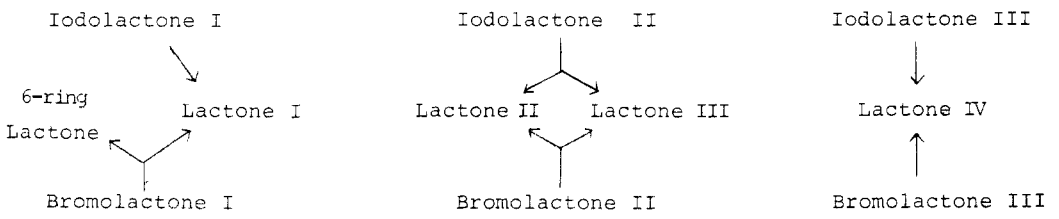


Table II. Yields (in Percent) (%) of Products en Route from 13 to 1

process	starting material 13 I	II	III	IV
removal of methoxymethyl group	100	100	100	100
removal of triazolinedione group	61	54	74	57
photochemical and thermal isomerizations	23	48	55	51

products, lactones II and III. Bromo lactone III gave lactone IV. Thus, all four possible diastereoisomeric γ-lactones **13** were separated. The relationships between the various lactones are summarized in Scheme II, and the yields are given in Table I.

The remaining four synthetic steps were executed separately on the four separated diastereoisomers. Removal of the 3β-methoxymethyl group with *p*-toluenesulfonic acid in methanol, followed by deprotection of the ring B diene by heating the product under reflux in collidine,[5] gave **16**. This 5,7-diene was irradiated with UV light in a mixed solvent (ether/THF/MeOH), after which fluorenone was added as a triplet sensitizer[3] and irradiation continued. The resulting provitamins were isolated by preparative thin-layer chromatography and subjected to a [1,7] thermal sigmatropic shift of hydrogen to give the target molecules **1**. The yields obtained in each case for these last four steps are summarized in Table II.

It was then necessary to determine the stereochemistry at C-23 and C-25 of each of the vitamin lactones (**1I**, **1III**, **1IIII**, and **1IV**) respectively obtained from the lactones I, II, III, and IV (**13**). In order to attempt to determine which pairs of the four lactones **13** had a common stereochemistry at C-23, we subjected each of the lactones **13** to dehydration with thionyl chloride in pyridine at 60 °C. From each diastereoisomer, two products, **17** and **18**, were obtained.

17 **18**

The butenolides **18** from lactones I and III had the same R_f value. This R_f value was different from the R_f value of the butenolides **18** from lactones II and IV. However, the R_f value of **18** from the latter pair was the same. We therefore conclude that lactones I and III have the same stereochemistry at C-23 and that this stereochemistry is opposite to that of lactones II and IV, which have the same stereochemistry as each other at C-23. The complete elucidation of stereochemistry at C-23 and C-25 in all four lactones was therefore reduced to determining an X-ray structure of either lactone I or lactone III and one X-ray structure of either lactone II or lactone IV. All four lactones **13** were isolated in crystalline form, but crystals of lactones I and II proved more amenable to X-ray analysis.[6]

(5) Anastasia, M.; Derossi, M. *J. Chem. Soc., Chem. Commun.* **1979**, 164.

Table III. Absolute Configurations at C-23 and C-25 in Lactones I-IV (13)

lactone	configuration C-23	C-25
I	*R*	*R*
II	*S*	*S*
III	*R*	*S*
IV	*S*	*R*

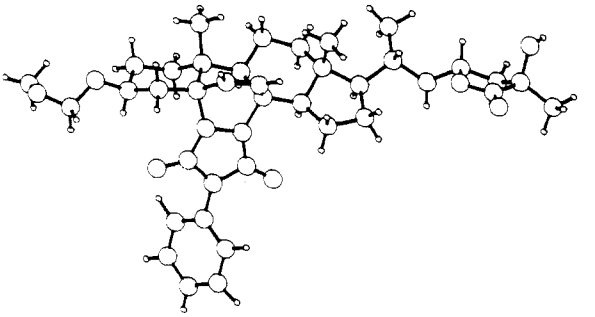


Figure 1. X-ray structure of the lactone **13I**.

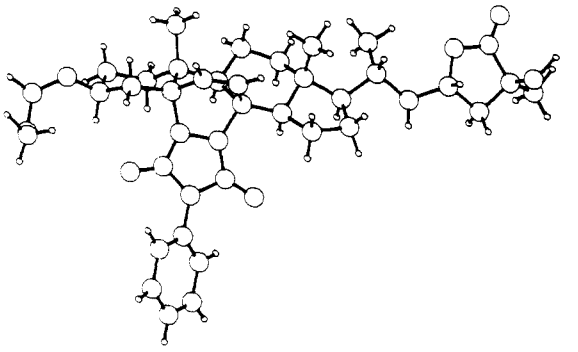


Figure 2. X-ray structure of the lactone **13II**.

The derived stereochemistries are given in Table III.

It remains to determine which of these lactones affords the vitamin hydroxy lactone **1** corresponding to the natural metabolite. Wichmann et al.[1] report that the ^{1}H NMR spectrum of the naturally occurring metabolite contains the C-23 proton resonance at δ 4.46.[1] This value is, as required, in very close agreement with the resonance position (4.44 ppm) of this proton in a synthetic sample which cochromatographs with the natural metabolite. In our synthetic products, **1I**, **1III**, **1IIII**, and **1IV**, the C-23 proton resonances occur at 4.76, 4.72, 4.45, and 4.44 ppm, respectively. These data establish that the natural product has the 23*R*,25*S* or 23*S*,25*R* stereochemistry. Additionally, it has recently been shown that 25,26-dihydroxycholecalciferol is a biosynthetic precursor of the naturally occurring lactone **1**,[7] to which it is converted (among other processes) by oxidation of the C-26 hydroxyl group. Since 25,26-dihydroxycholecalciferol has the *S* stereochemistry

(6) Egert, E.; Cruse, W. B. T.; Kennard, O., paper in preparation.
(7) Hollis, B. W.; Ross, B. A.; Lambert, P. S. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 520.

at C-25,[8,10] the natural metabolite is (23*R*,25*S*)-25-hydroxycholecalciferol-26,23-lactone.

It is noteworthy that in the pairs of compounds **13I/13II** and **1I/III**, the C-23 proton resonance is at relatively low field (4.6–4.76 ppm), relative to its positions in the pairs **13III/13IV** and **1III/1IV** (4.4–4.45 ppm). Thus, the resonance is found at low field in the *R,R* and *S,S* compounds. The X-ray structures of **13I** and **13II** (Figures 1 and 2) establish that in these cases (*R,R* and *S,S*, respectively), the C-23 proton and C-25 hydroxyl are in a pseudo-"1,3-diaxial" relationship. It seems likely that the low-field shift of the C-23 proton is caused by its proximity to the C-25 oxygen and, further, that in these compounds (Figures 1 and 2) the hydroxyl groups occupy the pseudoaxial positions to avoid the dipole–dipole repulsive interaction which would occur between a pseudoequatorial hydroxy group and an adjacent carbonyl group.

Biological testing of the metabolites has no far shown that none of them has more than 1% of the activity of vitamin D in bringing about intestinal calcium absorption. Further tests are in progress.

Experimental Section

Routine nuclear magnetic resonance (NMR) spectra were recorded at 90 MHz on a Varian EM 390 spectrometer; 60-MHz spectra were obtained on a Varian EM 360 spectrometer, 80-MHz spectra on a Varian CFT-20 spectrometer, and 400-MHz spectra on a Bruker WH 400 spectrometer. All resonances are quoted on the δ scale in parts per million downfield from internal Me_4Si (δ 0). Coupling constants (J) are in hertz, and splitting patterns are indicated by the symbols d = doublet, q = quartet, t = triplet, and m = multiplet.

Infrared spectra were recorded on a Perkin-Elmer 257 grating spectrophotometer using 0.5-mm solution cells with chloroform as the solvent; frequencies (ν) are quoted in reciprocal centimeters. Ultraviolet spectra were recorded in 95% ethanol solutions and wavelengths are quoted in nanometers.

Mass spectra were obtained on AEI-Kratos MS 30 and MS 902 spectrometers. Results are presented in the following form: m/z (fragment, percent of base peak). "RDA" refers to retro-Diels–Alder loss of 4-phenyl-1,2,4-triazoline-3,5-dione.

Column chromatography was performed by using the "flash chromatography" technique developed by Still.[9] The grade of silica used was 40–63 μm (E. Merck No. 9585). Preparative thin-layer chromatography (TLC) was carried out on silica GF_{254}. High pressure liquid chromatography (HPLC) was carried out on a Lichrosorb silica column (10-μm silica, length 45 cm).

Petroleum ether refers to the petroleum ether fraction boiling in the range 60–80 °C. Diethyl ether is abbreviated to ether.

3β-(Methoxymethoxy)-5α,8α-(4-phenyl-1,2-urazolo)bisnorchola-6-en-22-al (6). A solution of 4-phenyl-1,2,4-triazoline-3,5-dione (9.0 g, 51.4 mmol) in acetone (70 cm^3) was added dropwise to a stirred solution of ergosterol (20 g, 50.5 mmol) in 50% acetone/benzene (750 cm^3) until a faint pink coloration persisted. Evaporation of solvent under reduced pressure gave a pale yellow foam (29.0 g), crystallized from aqueous acetone (25.5 g, 45.5 mmol, 88%).

N,N-Diisopropylethylamine (8.0 cm^3, 46 mmol) and chloromethyl methyl ether (35 cm^3, 46 mmol) were added to a stirred solution of the ergosterol adduct (20 g, 35 mmol) in dry dichloromethane (150 cm^3). After 3 h the reaction was quenched with ice-cold 0.5 M hydrochloric acid (200 cm^3). The aqueous phase was extracted with dichloromethane (100 cm^3), and the organic phases were washed with ice-cold 0.5 M hydrochloric acid (150 cm^3). Drying over sodium sulfate and solvent removal under reduced pressure gave a pale yellow foam (21.5 g) which was crystallized from methanol (18.8 g, 32 mmol, 91%).

A saturated solution of ozone in 1% pyridine/dichloromethane (425 cm^3) at −78 °C was added to a stirred solution of the methoxymethyl ergosterol adduct (2.5 g, 4.07 mmol) in the same solvent (100 cm^3) at the same temperature. The blue color was immediately discharged. Dimethyl sulfide (5 cm^3) was added and the solution allowed to warm to room temperature. The reaction mixtures from four similar oxidations were combined, washed with ice-cold 2% hydrochloric acid (400 cm^3) and water (400 cm^3), and dried over sodium sulfate. The products were purified by column chromatography. First eluted was the starting material (3.0 g, 30%) followed by the aldehyde **6** (3.5 g), crystallized from ether as white needles: 3.2 g (5.85 mmol, 36%); mp 173–175 °C; NMR ($CDCl_3$, 90 MHz) δ 9.57 (1 H, d, J = 4 Hz, 22-H), 7.41 (5 H, m, Ar), 6.41, 6.21 (2 H, AB q, J = 8 Hz, 6,7-H), 4.84, 4.72 (2 H, AB q, J = 7 Hz, OCH_2O), 4.6–4.1 (1 H, m, 3α-H), 3.4 (s, 3 H, OMe), 3.4–3.2 (m, 4 H, 9-H), 1.17 (d, J = 7 Hz, 21-H), 1.01 (s, 3 H, 19-H), 0.87 (s, 3 H, 18-H); mass spectrum, m/z 372 (M^+ − RDA, 11), 370 (M^+ − RDA − H_2), 310 (M^+ − RDA − $MeOCH_2OH$, 100), 295 (M^+ − RDA − $MeOCH_2OH$ − Me, 44), 177; $C_{24}H_{36}O_3$ requires m/z 372.2665, found (M^+ − RDA) m/z 372.2666.

3β-(Methoxymethoxy)-5α,8α-(4-phenyl-1,2-urazolo)chola-6,23-dien-22-ol (7). Vinyl bromide (3.8 cm^3, 5.78 g, 0.055 mol) was added to dry tetrahydrofuran (50 cm^3) at 0 °C. The vinyl bromide solution was added in portions to a mixture of magnesium turnings (1.2 g, 0.005 mol) in tetrahydrofuran (50 cm^3) together with a catalytic quantity of iodine at 0 °C. When all the magnesium had dissolved the Grignard solution concentration was 0.5 mmol cm^{-3}, and this solution was cooled to 0 °C. The Grignard solution (26 cm^3, 13 mmol) was added to a solution of the aldehyde **6** (5.25 g, 9.6 mmol) in dry tetrahydrofuran (50 cm^3) at −78 °C. After the mixture was stirred for 2 h, the reaction was quenched by adding ammonium chloride solution (20 cm^3) at −78 °C. The reaction mixture was allowed to warm to room temperature, extracted into dichloromethane (2 × 250 cm^3), washed with brine solution (100 cm^3), and dried over anhydrous sodium sulfate. Removal of solvent under reduced pressure gave a white foam (6.6 g). Purification of the crude product by column chromatography (chloroform/ether (3:1) as eluant) gave the allyl alcohol **7**: 4.9 g (89%); NMR ($CDCl_3$, 90 MHz) δ 7.36 (5 H, m, Ar), 6.38, 6.18 (2 H, AB q, J = 8 Hz, 6, 7-H), 5.85 (1 H, ddd, J = 18, 10, 4.5 Hz, 23-H), 5.3–5 (2 H, m, 24-H), 4.77, 4.64 (2 H, AB q, J = 6.5 Hz, OCH_2O), 4.25 (1 H, m, 3α-H), 3.32 (3 H, s, OMe), 0.94 (3 H, s, 19-H), 0.74 (3 H, s, 18-H); mass spectrum, m/z 400 (M^+ − RDA, 16), 398 (M^+ − RDA − H_2, 12), 366 (M^+ − RDA − H_2 − MeOH, 12), 353 (M^+ − RDA − MeOH − Me, 4), 338 (M^+ − RDA − $MeOCH_2OH$, 100), 336 (M^+ − RDA − H_2 − $MeOCH_2OH$, 17), 323 (M^+ − RDA − $MeOCH_2OH$ − Me, 23), 321 (M^+ − RDA − H_2 − $MeOCH_2OH$ − Me, 6), 177; $C_{26}H_{40}O_3$ requires m/z 400.2977, found (M^+ − RDA) m/z 400.2995; IR ν_{max} 3600, 3500, 2950, 1745, 1690 cm^{-1}.

26-(Carboethoxy)-3β-(methoxymethoxy)-5α,8α-(4-phenyl-1,2-urazolo)cholesta-6,22-diene (8). Triethyl orthiopropionate (13.9 cm^3, 70 mmol) and propionic acid (90 μL, 1.2 mmol) were added to a solution of the allyl alcohol **7** (4 g, 6.96 mmol) in dry benzene (70 cm^3) under nitrogen in a Dean–Stark apparatus containing 5-Å molecular sieves. The solution was heated under reflux for 18 h. Ethyl acetate (250 cm^3) was then added, and the mixture washed with 1.5 M hydrochloric acid (2 × 150 cm^3), saturated sodium hydrogen carbonate solution (100 cm^3), and saturated brine (100 cm^3). The aqueous washings were extracted with ethyl acetate (100 cm^3) in each case. The organic layers were dried over sodium sulfate, and the solvent was removed under reduced pressure. The resulting foam was dissolved in dichloromethane (100 cm^3) and stirred, and a solution of 4-phenyl-1,2,4-triazoline-3,5-dione in acetone was added dropwise until a faint pink coloration persisted. The solvent was removed under reduced pressure and the crude product purified by column chromatography (40% ethyl acetate/60% petroleum ether as

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(9) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(10) A referee has informed us that other workers, in work not currently published, have not been able to establish that (25*S*)-25,26-dihydroxyvitamin D_3 is the precursor of 25-hydroxycholecalciferol-26,23-lactone. Our conclusion that the lactone possesses the 23*R*,25*S* stereochemistry relies on the only published evidence available to us.[7] If this evidence is invalidated by subsequent publications, then the possibility that the lactone has the 23*S*,25*R* stereochemistry will still exist.

eluant) to afford the ester 8 as a white foam: 4.04 g (88%); NMR ($CDCl_3$, 90 MHz) δ 7.35 (5 H, m, Ar), 6.35, 6.15 (2 H, AB q, J = 8 Hz, 6, 7-H), 5.22 (2 H, m, 22, 23-H), 4.77, 4.64 (2 H, AB q, J = 6.5 Hz, OCH_2O), 4.25 (1 H, m, 3α-H), 4.06 (2 H, q, J = 7 Hz, $CO_2CH_2CH_3$), 3.32 (3 H, s, OMe), 3.25 (1 H, m, 9-H), 1.2 (3 H, t, J = 7 Hz, $CO_2CH_2CH_3$), 0.96 (3 H, s, 19-H), 0.78 (3 H, s, 18-H); mass spectrum, m/z 484 (M^+ - RDA, 9), 482 (M^+ - RDA - H_2, 8), 450 (M^+ - RDA - H_2 - MeOH, 5), 437 (M^+ - RDA - Me, 6), 422 (M^+ - RDA - $MeOCH_2OH$, 100), 407 (M^+ - RDA - $MeOCH_2OH$ - Me, 22.5), 177% IR ν_{max} 2960, 1750, 1730, 1695 cm^{-1}. Anal. Calcd for $C_{39}H_{53}O_6N_3$: C, 71.0; H, 8.1; N, 6.3. Found: C, 70.9; H, 8.1; N, 6.4.

26-(Carboethoxy)-3β-(methoxymethoxy)-5α,8a-(4-phenyl-1,2-urazolo)cholesta-6,22-dien-25-ol (10). n-Butyllithium (1.6 M in hexane, 12 cm^3) was added to a stirred solution of N-isopropylcyclohexylamine (3.4 cm^3, 20 mmol) in dry tetrahydrofuran (40 cm^3) under argon at 0 °C. After 30 min, a portion of this solution (24 cm^3, 8 mmol) was cooled to -78 °C and added to a stirred solution of the ester 8 (3.5 g, 5.3 mmol) in dry tetrahydrofuran (40 cm^3) under argon at -78 °C. After 40 min, a stream of dry oxygen was bubbled through the orange solution of the anion, maintaining the temperature at -78 °C. After a further 1 h, triethyl phosphite (1.95 cm^3, 9.3 mmol) was added, followed, after 20 min, by addition of a saturated ammonium chloride solution (20 cm^3). The solution was allowed to warm to room temperature, extracted with ethyl acetate (2 × 300 cm^3), washed with brine (100 cm^3), and dried over sodium sulfate. Purification of the product by column chromatography (45% ethyl acetate/55% petroleum ether as eluant) gave the hydroxy ester **10** as a white foam: 3.35 g (93%); NMR ($CDCl_3$) δ 7.36 (5 H, m, Ar), 6.35, 6.15 (2 H, AB q, J = 8 Hz, 6, 7-H), 5.3 (2 H, m, 22, 23-H), 4.76, 4.64 (2 H, AB q, J = 6 Hz, OCH_2O), 4.2 (1 H, m, 3α-H), 4.15 (2 H, q, J = Hz, $CO_2CH_2CH_3$), 3.32 (3 H, s, OMe), 3.17 (1 H, m, 9-H), 1.3 (s, 27-H); mass spectrum, m/z 500 (M^+ - RDA, 11), 498 (M^+ - RDA - H_2, 4), 466 (M^+ - RDA - H_2 - MeOH, 2), 453 (M^+ - RDA - MeOH - Me, 4), 438 (M^+ - RDA - $MeOCH_2OH$, 44), 423 (M^+ - RDA - $MeOCH_2OH$ - Me, 28), 177; IR ν_{max} 3530, 2960, 1745, 1730, 1695 cm^{-1}; UV λ_{max} 258, 207 nm. Anal. Calcd for $C_{39}H_{53}O_7N_3$: C, 69.3; H, 7.9; N, 6.2. Found: C, 69.0; H, 7.9; N, 5.9.

26-Carboxy-3β-(methoxymethoxy)-5α,8α-(4-phenyl-1,2-urazolo)cholesta-6,22-dien-25-ol (11). A 10% aqueous solution of potassium hydroxide (50 cm^3) was added to a solution of the hydroxy ester **10** (3.36 g, 4.98 mmol) in tetrahydrofuran (60 cm^3) and the mixture stirred vigorously at room temperature for 36 h, by which time no starting material remained. The mixture was poured into ice-cold 0.5 M hydrochloric acid (300 cm^3) and extracted with ethyl acetate (3 × 300 cm^3). The combined ethyl acetate layers were washed with saturated brine (250 cm^3) and dried (sodium sulfate). Removal of solvent gave the acid **11** (3.35 g, 100%) as a white foam: NMR ($CDCl_3$, 80 MHz) δ 7.32 (5 H, m, Ar), 6.34, 6.14 (2 H, AB q, J = 8 Hz, 6, 7-H), 5.3 (2 H, m, 22, 23-H), 4.75, 4.63 (2 H, AB q, J = 6 Hz, OCH_2O), 4.25 (1 H, m, 3α-H), 3.34 (3 H, s, OMe), 3.2 (1 H, m, 9-H), 1.34 (3 H, s, 27-H), 0.93 (3 H, s, 19-H), 0.76 (3 H, s, 18-H); mass spectrum, m/z 472 (M^+ - RDA, 13), 470 (M^+ - RDA - H_2, 12), 454 (M^+ - RDA - H_2O, 7), 452 (M^+ - RDA - H_2 - H_2O, 3), 438 (M^+ - RDA - H_2 - MeOH, 2), 410 (M^+ - RDA - $MeOCH_2OH$, 100), 408 (M^+ - RDA - H_2 - $MeOCH_2OH$, 48), 395 (M^+ - RDA - $MeOCH_2OH$ - Me, 35), 177; $C_{29}H_{44}O_5$ requires m/z 472.3189; found (M^+ - RDA) m/z 472.3195; IR ν_{max} 3500, 3250, 2800, 1745, 1690 cm^{-1}.

22-Iodo-3β-(methoxymethoxy)-5α,8α-(4-phenyl-1,2-urazolo)cholesta-6-en-25-ol-26,23-lactone (12). A 0.5 M aqueous solution of sodium bicarbonate (26 cm^3) was added to a solution of the acid **11** (1.69 g, 2.61 mmol) in tetrahydrofuran (28 cm^3). A solution (13 cm^3) of iodine (4.8 g, 18.9 mmol) and potassium iodide (10 g, 60 mmol) in water was added to the acid anion, and the mixture stirred in the dark at room temperature for 2 days. The mixture was extracted with dichloromethane (2 × 250 cm^3) and washed with saturated sodium thiosulfate solution (100 cm^3) and water (100 cm^3), in each case reextracting the aqueous phases with more dichloromethane (100 cm^3). The combined dichloromethane layers were dried over sodium sulfate, the solvent was removed under reduced pressure, and purification of the product was achieved by column chromatography (60% ethyl acetate/40% petroleum ether as eluant). The three iodo lactones of 12, I (271 mg, 14%), II (601 mg, 30%), and III (547 mg, 27%), were separated by preparative HPLC.

Iodo lactone I: NMR ($CDCl_3$, 80 MHz) δ 7.38 (5 H, m, Ar), 6.38, 6.21 (2 H, AB q, J = 8 Hz, 6, 7-H), 4.79, 4.67 (2 H, AB q, J = 6 Hz, OCH_2O), 4.7 (1 H, m, 23-H), 4.25 (1 H, m, 3α-H), 4-3.7 (1 H, m, 22-H), 3.36 (3 H, s, OMe), 3.2 (1 H, m, 9-H), 1.5 (3 H, s, 27-H), 0.95 (3 H, s, 19-H), 0.87 (3 H, s, 18-H); mass spectrum, m/z 598 (M^+ - RDA, 8), 596 (M^+ - RDA - H_2, 11), 581 (M^+ - RDA - H_2 - Me, 2), 580 (M^+ - RDA - H_2O, 1), 564 (M^+ - RDA - H_2 - MeOH, 5), 551 (M^+ - RDA - MeOH - Me, 9), 536 (M^+ - RDA - $MeOCH_2OH$, 100), 534 (M^+ - RDA - H_2 - $MeOCH_2OH$, 47), 521 (M^+ - RDA - Me - $MeOCH_2OH$, 24), 177; IR ν_{max} 3560, 3370, 2960, 1785, 1750, 1695 cm^{-1}.

Iodo lactone II: NMR ($CDCl_3$, 80 MHz) δ 7.38 (5 H), 6.38, 6.21 (2 H), 4.97, 4.67 (2 H), 4.6-3.9 (3 H, m, 22-H, 23-H, 3α-H), 3.36 (3 H), 1.47 (3 H), 0.95 (3 H), 0.87 (3 H); mass spectrum, m/z 598 (10), 598 (13), 581 (2), 580 (1), 564 (7), 551 (12), 536 (100), 5.34 (52), 521 (16), 177; IR ν_{max} 3560, 3370, 2960, 1785, 1750, 1695 cm^{-1}.

Iodo lactone III: NMR ($CDCl_3$, 80 MHz) δ 7.37 (5 H), 6.37, 6.19 (2 H), 4.79, 4.67 (2 H), 4.4, 3.9 (3 H), 3.35 (3 H), 1.44 (3 H), 0.94 (3 H), 0.79 (3 H); mass spectrum, m/z 598 (11), 596 (10), 581 (3), 580 (2), 564 (6), 551 (8), 536 (100), 534 (33), 521 (12), 177; IR ν_{max} 3560, 3370, 2960, 1785, 1750, 1695 cm^{-1}.

22-Bromo-3β-(methoxymethoxy)-5α,8α-(4-phenyl-1,2-urazolo)cholesta-6-en-25-ol-26,23-lactone (14). A 0.5 M aqueous solution of sodium bicarbonate (27 cm^3) was added to a stirred solution of the acid 11 (1.6 mg, 247 mmol) in tetrahydrofuran (27 cm^3). This mixture was cooled in an ice-bath and, after 15 min, bromine (ca. 0.2 cm^3) was added dropwise until the orange color persisted. After 2 h at 0 °C, the mixture was extracted with dichloromethane (2 × 200 cm^3) and washed with saturated sodium thiosulfate (100 cm^3) and water (100 cm^3), in each case reextracting the aqueous phases with dichloromethane (100 cm^3). The combined organic layers were dried over sodium sulfate, the solvent was removed under reduced pressure, and purification of the product was achieved by column chromatography (67% chloroform/33% ether). The three bromo lactaones of **14**, I (505 mg, 28%), II (667 mg, 37%), and III (155 mg, 9%), were separated by preparative HPLC.

Bromo lactone I: NMR ($CDCl_3$, 80 MHz) δ 7.38 (5 H, m, Ar), 6.39, 6.21 (2 H, AB q, J = 8 Hz, 6, 7-H), 4.79, 4.67 (2 H, AB q, J = 6 Hz, OCH_2O), 4.7-4.2 (3 H, m, 22-H, 23-H, 3α-H), 3.36 (3 H, s, OMe), 3.25 (1 H, m, 9-H), 1.49 (3 H, s, 27-H), 1.03 (3 H, d, J = 6 Hz, 21-H), 0.95 (3 H, s, 19-H), 0.84 (3 H, s, 18-H); mass spectrum, m/z 552 (3), 550 (5), 548 (2) (M^+ - RDA/M^+ - RDA - H_2), 535 (1.5), 533 (1) (M^+ - RDA - H_2 - Me), 518 (2), 516 (1.5, M^+ - RDA - H_2 - MeOH), 505 (5), 503 (4, M^+ - RDA - MeOH - Me), 490 (92), 488 (100, M^+ - RDA - $MeOCH_2OH$), 475 (25), 473 (27, M^+ - RDA - $MeOCH_2OH$ - Me), 177; IR ν_{max} 3570, 3400, 2960, 1785, 1750, 1695 cm^{-1}.

Bromo lactone II: NMR ($CDCl_3$, 80 MHz) δ 7.37 (5 H), 6.38, 6.21 (2 H), 4.79, 4.67 (2 H), 4.7-4.3 (3 H), 3.35 (3 H), 3.25 (1 H), 1.47 (3 H), 1.00 (3 H), 0.95 (3 H), 0.84 (3 H); mass spectrum, m/z 552 (1.5), 550 (8), 548 (6), 535 (1), 533 (1), 518 (4), 516 (3), 505 (10), 503 (9), 490 (72), 488 (100), 475 (18), 473 (22); IR ν_{max} 3570, 3400, 2960, 1785, 1750, 1695 cm^{-1}.

Bromo lactone III: NMR ($CDCl_3$, 80 MHz) δ 7.37 (5 H), 6.38, 6.2 (2 H), 4.79, 4.67 (2 H), 4.7-4.5 (3 H), 3.35 (3 H), 3.25 (1 H), 1.45 (3 H), 1.16 (3 H), 0.95 (3 H), 0.8 (3 H); mass spectrum, m/z 552 (6), 550 (9), 548 (5), 535 (2), 533 (2.5), 518 (6), 516 (5), 505 (8), 503 (8), 490 (100), 488 (65), 486 (42), 475 (47), 473 (35), 471 (10); IR ν_{max} 3570, 3400, 2960, 1785, 1750, 1695 cm^{-1}.

3β-(Methoxymethoxy)-5α,8α-(4-phenyl-1,2-urazolo)-cholesta-6-en-25-ol-26,23-lactone (13) from Iodo Lactone 12. Freshly distilled tri-n-butyltin hydride was added dropwise to iodo lactone **12I** (250 mg, 0.32 mmol) in dry tetrahydrofuran (4 cm^3) at room temperature under an argon atmosphere. After 12 h, TLC showed no starting material remained. Ethyl acetate (50 cm^3) and water (25 cm^3) were added, and the mixture was shaken. The water layer was separated and reextracted with ethyl acetate (2 × 25 cm^3). The ethyl acetate layers were combined, washed with saturated brine solution (30 cm^3), and dried (sodium sulfate), and the solvent was removed under reduced pressure. Preparative TLC with 65% ethyl acetate/35% petroleum ether gave lactone I (**13I**; 176 mg, 85%). The same procedure was used to reduce

iodo lactones II and III. Iodo lactone II (590 mg, 0.76 mmol) gave lactone II (85 mg, 18%) and lactone III (348 mg, 71%). Iodo lactone III (540 mg, 0.70 mmol) gave lactone IV (378 mg, 83%).

Lactone I (13I) was crystallized from ether as needles: mp 191.5–194 °C; NMR ($CDCl_3$) δ 7.37 (5 H, m, Ar), 6.38, 6.21 (2 H, AB q, J = 8 Hz, 6, 7-H), 4.97, 4.67 (2 H, AB q, J = 6.5 Hz, OCH_2O), 4.7–4.5 (1 H, m, 23-H), 4.3 (1 H, m, 3α-H), 3.35 (3 H, s, OMe), 3.2 (1 H, m, 9-H), 1.48 (3 H, s, 27-H), 0.95 (3 H, s, 19-H), 0.8 (3 H, s, 18-H); mass spectrum, m/z 472 (M^+ – RDA, 15), 470 (M^+ – RDA – H_2, 16), 455 (M^+ – RDA – H_2 – Me, 1.5), 454 (M^+ – RDA – H_2O, 1), 438 (M^+ – RDA – H_2 – MeOH, 7), 425 (M^+ – RDA – MeOH– Me, 12), 410 (M^+ – RDA – $MeOCH_2OH$, 100), 408 (M^+ – RDA – H_2 – $MeOCH_2OH$, 47), 395 (M^+ – RDA – $MeOCH_2OH$ – Me, 37), 177; IR ν_{max} 3550, 3450, 2960, 1770, 1750, 1695 cm^{-1}. Anal. Calcd for $C_{37}H_{49}O_7N_3$: C, 68.6; H, 7.6; N, 6.5. Found: C, 68.4; H, 7.3; N, 6.5.

Lactone II (13II) was crystallized from ether as rhombohedra: mp 181.5–185 °C; NMR ($CDCl_3$) δ 7.37 (5 H), 6.37, 6.21 (2 H), 4.79, 4.67 (2 H), 4.7–4.5 (1 H), 4.3 (1 H), 3.36 (3 H), 3.2 (1 H), 1.48 (3 H), 0.95 (3 H), 0.8 (3 H); mass spectrum, m/z 472 (31), 470 (29), 454 (3), 438 (21), 425 (29), 410 (100), 408 (31), 395 (34), 369 (33), 177; $C_{29}H_{44}O_5$ requires m/z 472.3189, found (M^+ – RDA) m/z 472.3193; IR ν_{max} 3550, 3450, 2960, 1770, 1750, 1695 cm^{-1}.

Lactone III (13III) was crystallized as needles from methanol: mp 203–204 °C; NMR ($CDCl_3$, 80 MHz) δ 7.36 (5 H), 6.37, 6.20 (2 H), 4.78, 4.66 (2 H), 4.5–4.2 (2 H, m, 23-H, 3α-H), 3.34 (3 H), 3.2 (1 H), 1.45 (3 H), 0.94 (3 H), 0.85 (3 H); mass spectrum, m/z 472 (28), 470 (28), 455 (3), 438 (23), 425 (27), 410 (100), 408 (34), 395 (39), 369 (24), 177; IR ν_{max} 3550, 3450, 2960, 1775, 1750, 1695 cm^{-1}. Anal. Calcd for $C_{37}H_{49}O_7N_3$: C, 68.6; H, 7.6; N, 6.3. Found: C, 68.3; H, 7.6; N, 6.3.

Lactone IV (13IV) was crystallized as needles from ether: mp 182–184 °C; NMR ($CDCl_3$, 80 MHz) δ 7.36 (5 H), 6.36, 6.20 (2 H), 4.78, 4.66 (2 H), 4.4–4.2 (2 H, m), 3.34 (3 H), 3.2 (1 H), 1.44 (3 H), 0.94 (3 H), 0.79 (3 H); mass spectrum, m/z 472 (13), 470 (13), 455 (1.5), 454 (2.5), 438 (7), 410 (100), 408 (64), 395 (44), 369 (17), 177; IR ν_{max} 3550, 3450, 2960, 1775, 1750, 1695 cm^{-1}. Anal. Calcd for $C_{37}H_{49}O_7N_3$: C, 68.6; H, 7.6; N, 6.5. Found: C, 68.6; H, 7.9; N, 6.4.

Synthesis of 13 from Bromo Lactone 14. Tri-*n*-butyltin hydride (0.3 cm^3, 2.4 mmol) was added dropwise to a solution of bromo lactone **14I** (495 mg, 0.68 mmol) in dry tetrahydrofuran (5 cm^3) at room temperature under an argon atmosphere. After 12 h at 40 °C, ethyl acetate (60 cm^3) and water (30 cm^3) were added, and the mixture was shaken. The aqueous layer was separated and reextracted with ethyl acetate (2 × 40 cm^3). The ethyl acetate layers were combined, washed with saturated brine solution (30 cm^3), and dried (sodium sulfate), and the solvent was removed under reduced pressure. Preparative TLC with 65% ethyl acetate/35% petroleum ether gave lactone I (**13I**; 321 mg, 74%) and the six-membered lactone **15** (94 mg, 21%). The same procedure was used to reduce bromo lactones II and III. Bromo lactone II (656 mg, 0.9 mmol) gave lactone II (67.3 mg, 11.6%) and lactone III (376 mg, 64%). Bromo lactone III (155 mg, 0.21 mmol) gave lactone IV (121 mg, 89%). The spectral details of lactones I–IV were entirely consistent with those recorded for the same lactones obtained by reduction of the iodo lactones **12**.

3β-(Methoxymethoxy)-5α,8α-(4-phenyl-1,2-urazolo)-cholesta-6-en-25-ol-26,22-lactone (15). This compound isolated as above was crystallized as needles from ether: mp 197–201 °C; NMR ($CDCl_3$, 80 MHz) δ 7.35 (5 H, m, Ar), 6.36, 6.2 (2 H, AB q, J = 8 Hz, 6, 7-H), 4.76, 4.65 (2 H, AB q, J = 6.5 Hz, OCH_2O), 4.3 (2 H, 22-H, 3α-H), 3.35 (3 H, s, OMe), 3.2 (1 H, m, 9-H), 1.47 (3 H, s, 27-H), 0.95 (3 H, s, 19-H), 0.8 (3 H, s, 18-H); mass spectrum, m/z 472 (M^+ – RDA, 10), 470 (M^+ – RDA – H_2, 9), 455 (M^+ – RDA–H_2 – Me, 1), 438 (M^+ – RDA – H_2 – MeOH, 5), 425 (M^+ – RDA – MeOH – Me, 5), 410 (M^+ – RDA – $MeOCH_2$ OH, 100), 408 (M^+ – RDA – H_2 – $MeOCH_2OH$, 34), 395 (M^+ – RDA – $MeOCH_2OH$ – Me, 31), 369 (13), 177; IR ν_{max} 3570, 3400, 2960, 1750, 1730, 1695 cm^{-1}.

Cholesta-5,7-diene-3β,25-diol-26,23-lactone (16). *p*-Toluenesulfonic acid (325 mg, 1.71 mmol) was added to a stirred solution of **13I** (400 mg, 0.62 mmol) in methanol (14 cm^3)/tetrahydrofuran (8 cm^3). The temperature was maintained at 30 °C for 2 days. The mixture was extracted with ether acetate (2 × 120 cm^3), washed with sodium hydrogen carbonate solution (60 cm^3) and brine solution (60 cm^3), and dried (sodium sulfate), the aqueous phases being reextracted with ethyl acetate (60 cm^3). Removal of solvent under reduced pressure gave **3β-hydroxy-5α,8α-(4-phenyl-1,2-urazolo)cholesta-6-en-25-ol-26,23-lactone** (375 mg, 100%; subsequently abbreviated to dihydroxy lactone I). The same procedure was used to hydrolyze the methoxymethyl protecting group in lactones II, III, and IV, and in each case 100% yields were obtained of the dihydroxy lactones II, III, and IV.

Dihydroxy Lactone I. This compound crystallized as needles from acetone: mp 182.5–184 °C; NMR ($CDCl_3$, 80 MHz) δ 7.35 (5 H, m, Ar), 6.36, 6.16 (2 H, AB q, J = 8 Hz, 6, 7-H), 4.65 (1 H, m, 23-H), 4.35 (1 H, m, 3α-H), 3.1 (1 H, m, 9-H), 1.46 (3 H, s, 27-H), 1.0 (3 H, d, J = 6 Hz, 21-H), 0.94 (3 H, s, 19-H), 0.8 (3 H, s, 18-H); mass spectrum, m/z 428 (M^+ – RDA, 83), 426 (M^+ – RDA – H_2, 28), 408 (M^+ – RDA – H_2 – H_2O, 65), 395 (M^+ – RDA – H_2O – Me, 100), 393 (M^+ – RDA – H_2 – H_2O – Me, 20), 369 (53), 177. $C_{27}H_{40}O_4$ requires m/z 428.2926, found (M^+ – RDA) m/z 428.2928; IR ν_{max} 3570, 3450, 2950, 1770, 1750, 1690 cm^{-1}; UV λ_{max} 254, 206 nm.

Dihydroxy Lactone II. The product was crystallized as needles from ether: mp 176–184; NMR ($CDCl_3$, 80 MHz) δ 7.35 (5 H), 6.36, 6.16 (2 H), 4.65 (1 H), 4.35 (1 H), 3.1 (1 H), 1.46 (3 H), 0.95 (3 H), 0.81 (3 H); mass spectrum, m/z 428 (89), 426 (43), 408 (70), 395 (100), 393 (51), 336 (51) 177; IR ν_{max} 3570, 3450, 2950, 1770, 1750, 1690 cm^{-1}; UV λ_{max} 254, 206 nm.

Dihydroxy Lactone III. The product was crystallized as needles from methanol: mp 181–182.5 °C; NMR ($CDCl_3$, 80 MHz) δ 7.38 (5 H), 6.36, 6.16 (2 H), 4.4 (2 H, m, 3α-H, 23-H), 3.15 (1 H), 1.45 (3 H), 0.94 (3 H), 0.8 (3 H); mass spectrum, m/z 428 (92), 426 (60), 408 (93), 395 (100), 393 (25), 336 (52), 177; IR ν_{max} 3560, 3450, 2950, 1775, 1750, 1690 cm^{-1}; UV λ_{max} 254, 205 nm.

Dihydroxy Lactone IV. The product was crystallized as needles from acetone: mp 208–209.5 °C; NMR ($CDCl_3$, 80 MHz) δ 7.37 (5 H), 6.36, 6.16 (2 H), 4.4 (2 H, m), 3.15 (1 H), 1.43 (3 H), 0.93 (3 H), 0.79 (3 H); mass spectrum, m/z 428 (71), 426 (45), 408 (68), 395 (100), 393 (35), 336 (82), 177; IR ν_{max} 3560, 3450, 2950, 1775, 1750, 1690 cm^{-1}; UV λ_{max} 254, 207 nm.

Dihydroxy lactone I (180 mg) was dissolved in freshly distilled collidine (30 cm^3) and heated under reflux in an argon atmosphere for 15 min. After the mixture cooled, the product was extracted into ethyl acetate (250 cm^3), and this solution washed with ice-cold 1 M hydrochloric acid (4 × 100 cm^3) and saturated sodium bicarbonate (100 cm^3) and dried (sodium sulfate). Removal of the solvent and crystallization of the product from acetone gave **16I** (109 mg, 61%).

The same procedure was used to remove the ring B diene protecting group in the remaining three diastereoisomers.

(23*R*,25*R*)-Cholesta-5,7-diene-3β,25-diol-26,23-lactone (16I): NMR ($CDCl_3$, 80 MHz) δ 5.7–5.25 (2 H, m, 6, 7-H), 4.7 (1 H, m, 23-H), 3.6 (1 H, m, 3α-H), 1.49 (3 H, s, 27-H), 1.04 (3 H, d, J = 6 Hz, 21-H), 0.93 (3 H, s, 19-H), 0.63 (3 H, s, 18H); mass spectrum, m/z 428 (M^+, 76), 410 (M^+ – H_2O, 86), 395 (M^+ – H_2O – Me, 100), 369 (55), 253 (M^+ –sidechain – H_2O, 33). $C_{27}H_{40}O_4$ requires m/z 428.2927, found (M^+) m/z 428.2932; IR ν_{max} 3570, 3400, 2940, 2870, 1770 cm^{-1}; UV λ_{max} 294, 282, 271, 262 nm.

(23*S*,25*S*)-Cholesta-5,7-diene-3β,25-diol-26,23-lactone (16II): NMR ($CDCl_3$, 80 MHz) δ 5.6–5.25 (2 H), 4.65 (1 H), 3.6 (1 H), 1.48 (3 H), 0.93 (3 H), 0.63 (3 H); mass spectrum, m/z 428 (46), 410 (7), 408 (9), 395 (100), 369 (86), 253 (44); $C_{27}H_{40}O_4$ requires m/z 428.2927, found (M^+) m/z 428.2921; IR ν_{max} 3570, 3400, 2870, 1770 cm^{-1}; UV λ_{max} 293, 282, 271, 262 nm.

(23*R*,25*S*)-Cholesta-5,7-diene-3β,25-diol-26,23-lactone (16III): NMR ($CDCl_3$, 80 MHz) δ 5.7–5.25 (2 H), 4.4 (1 H), 3.6 (1), 1.47 (3 H), 0.93 (3 H), 0.64 (3 H); mass spectrum, m/z 428 (83), 410 (11), 408 (15), 395 (100), 369 (52), 253 (16); $C_{27}H_{40}O_4$ requires m/z 428.2927, found (M^+) m/z 428.2927; IR ν_{max} 3570, 3400, 2940, 2870, 1775 cm^{-1}; UV λ_{max} 293, 282, 271, 262 nm.

(23*S*,25*R*)-Cholesta-5,7-diene-3β,25-diol-26,23-lactone (16IV): NMR ($CDCl_3$, 80 MHz) δ 5.65–5.25 (2 H), 4.4 (1 H), 3.6 (1 H), 1.47 (3 H), 0.93 (3 H), 0.62 (3 H); mass spectrum, m/z 428 (89), 410 (12), 408 (14), 395 (100), 369 (57), 253 (18); $C_{27}H_{40}O_4$ requires m/z 428.2927, found (M^+) m/z 428.2931; IR ν_{max} 3570, 3450, 2945, 2870, 1775 cm^{-1}; UV λ_{max} 293, 282, 271, 262 nm.

(23*R*,25*R*)-25-Hydroxycholecalciferol-26,23-lactone (1I). A solution of **16I** (50 mg) in methanol (10 cm^3)/tetrahydrofuran (60 cm^3)/ether (260 cm^3) was degassed with argon for 1 h. The

solution was irradiated at 0 °C with an internal water-cooled Hanovia medium-pressure mercury vapor lamp for 40 min under an argon atmosphere while the temperature was maintained below 5 °C. Fluorenone (45 mg) was added, and the solution was irradiated for a further 20 min. Solvent was then removed under reduced pressure, keeping the temperature below 10 °C. The product (λ_{max} 264 nm) was isolated be preparative TLC with 25% acetone/petroleum ether as the eluant in the dark under an argon atmosphere. The product was dissolved in 95% ethanol (6 cm^3) and heated at 80 °C for 2.5 h. Removal of ethanol and preparative TLC with 85% ether/15% petroleum ether as the eluant gave the target molecule **1I** (11.5 mg, 23%). This was repeated for the other three diastereoisomers. **1I**: NMR ($CDCl_3$, 400 MHz) δ 6.23, 6.02 (2 H, AB q, J = 10 Hz, 6, 7-H), 5.04, 4.81 (2 H, m, 19-H), 4.77 (1 H, m, 23-H), 3.96 (1 H, m, 3α-H), 1.52 (3 H, s, 27-H), 1.02 (3 H, d, J = 6 Hz, 21-H), 0.57 (3 H, s, 18-H); mass spectrum, m/z 428 (M^+, 98), 413 (M^+ - Me, 6), 411 (6), 410 (M^+ - H_2O, 22), 395 (M^+ - H_2O - Me, 100), 369 (28), 253 (54); UV λ_{max} 265 nm.

(23*S*,25*S*)-25-Hydroxycholecalciferol-26,23-lactone (1II): NMR ($CDCl_3$, 400 MHz) δ 6.22, 6.02 (2 H), 5.04 (1 H), 4.8 (1 H), 4.72 (1 H), 3.95 (1 H), 1.52 (3 H), 1.03 (3 H), 0.57 (3 H); mass spectrum m/z 428 (100), 413 (8), 411 (7), 395 (71), 369 (15), 253 (10); UV λ_{max} 265 nm.

(23*R*,25*S*)-25-Hydroxycholecalciferol-26,23-lactone (1III): NMR ($CDCl_3$, 400 MHz) δ 6.22, 6.03 (2 H), 5.04 (1 H), 4.8 (1 H), 4.45 (1 H), 3.94 (1 H), 1.51 (3 H), 1.01 (3 H), 0.56 (3 H); mass spectrum, m/z 428 (98), 413 (7), 411 (6), 410 (21), 395 (100), 369 (34), 253 (54); IR ν_{max} 3570, 2945, 1778, 1205 cm^{-1}; UV λ_{max} 265 nm.

(23*S*,25*R*)-25-Hydroxycholecalciferol-26,23-lactone (1IV): NMR ($CDCl_3$, 400 MHz) δ 6.22, 6.03 (2 H), 5.05 (1 H), 4.82 (1 H), 4.43 (1 H), 3.95 (1 H), 1.51 (3 H), 1.03 (3 H), 0.56 (3 H); mass spectrum, m/z 428 (97), 413 (10), 411 (9), 410 (25), 395 (100), 369 (28), 253 (22); UV λ_{max} 265 nm.

3β-(Methoxymethoxy)-5α,8α-(4-phenyl-1,2-urazolo)-cholesta-6,25-diene-26,23-lactone (18). Thionyl chloride (0.1 cm^3) was added to a solution of the lactone **13I** (20 mg, 0.031 mmol) in pyridine (2 cm^3). Under an argon atmosphere, the mixture was heated to 60 °C, and after 8 h the reaction was carefully quenched with ice. The products were extracted into ethyl acetate (2 × 10 cm^3) and washed with water (5 cm^3), saturated copper sulfate solution (4 × 8 cm^3), and brine solution (8 cm^3). The organic phase was dried with sodium sulfate, the solvent was removed under reduced pressure, and two products were isolated by preparative TLC with 55% ethyl acetate/45% petroleum ether as the eluant. The higher R_f product was the chloride.

25-Chloro-3β-(methoxymethoxy)-5α,8α-(4-phenyl-1,2-urazolo)cholesta-6-ene-26,23-lactone (**17**, 3.4 mg) and the lower R_f product the butenolide **18** (10.4 mg).

In a similar manner, lactones **13** (II, III, and IV) were dehydrated to give mixtures of chloride and butenolide. The chlorides **17** from all four lactones showed very similar spectroscopic properties. Details are recorded for the chloride from lactone I: NMR ($CDCl_3$, 80 MHz) δ 7.37 (5 H, m, Ar), 6.39, 6.20 (2 H, AB q, J = 8 Hz, 6, 7-H), 4.8, 4.66 (2 H), AB q, J = 6.5 Hz, OCH_2O), 4.7–4.2 (2 H, m, 3α,23-H), 3.36 (3 H, s, OMe), 1.78 (3 H, s, 27-H), 0.95 (3 H, s, 19-H), 0.81 (3 H, s, 18-H); mass spectrum m/z 492 (2), 490 (7.5), 489 (1.5), 488 (4.5) (M^+ - RDA)/(M^+ - RDA - H_2), 458 (1.5), 456 (2.5) (M^+ - RDA - MeOH)/(M^+ - RDA - H_2 - MeOH), 445 (3.5), 443 (5.5) (M^+ - RDA - MeOH - Me)/(M^+ - RDA - H_2 - MeOH - Me), 430 (33), 429 (36), 428 (100), 427 (29), 426 (30) (M^+ - RDA - $MeOCH_2OH$)/(M^+ - RDA - $MeOCH_2OH$ - Me), 415 (10), 414 (9), 413 (29), 412 (4.5), 411 (10) (M^+ - RDA - $MeOCH_2OH$ - Me)/(M^+ - RDA - H_2 - $MeOCH_2OH$ - Me), 177. $C_{29}H_{43}O_4Cl$ requires 490.2850, found (M^+ - RDA) m/z 490.2838; IR ν_{max} 3500, 2950, 1780, 1750, 1695 cm^{-1}.

(23*R*)-3β-(Methoxymethoxy)-5α,8α-(4-phenyl-1,2-urazolo)cholesta-6,25-diene-26,23-lactone. The butenolide was isolated from dehydration of lactones I and III: NMR ($CDCl_3$, 80 MHz) δ 7.36 (5 H, m, Ar), 6.9 (1 H, m, 24-H), 6.38, 6.21 (2 H, AB q, J = 8 Hz, 6, 7-H), 4.8 (1 H, m, 23-H), 4.79, 4.66 (2 H, AB q, J = 6.5 Hz, OCH_2O), 4.25 (1 H, m, 3α-H), 3.35 (3 H, s, OMe), 1.88 (3 H, br s, 27-H), 1.03 (3 H, d, J = 6 Hz, 21-H), 0.95 (3 H, s, 19-H), 0.8 (3 H, s, 18-H); mass spectra m/z 454 (M^+ - RDA, 11), 452 (M^+ - RDA - H_2, 12), 420 (M^+ - RDA - H_2 - MeOH, 5), 407 (M^+ - RDA - MeOH - Me, 8), 392 (M^+ - RDA - $MeOCH_2OH$, 100), 390 (M^+ - RDA - H_2 - $MeOCH_2OH$, 56), 377 (M^+ - RDA - $MeOCH_2OH$ - Me, 41), 375 (M^+ - RDA - H_2 - $MeOCH_2OH$ - Me, 22), 177; IR ν_{max} 3500, 2950, 1750, 1690 cm^{-1}; R_f 0.46 (55% ethyl acetate/45% petroleum ether).

(23*S*)-3β-(Methoxymethoxy)-5α,8α-(4-phenyl-1,2-urazolo)cholesta-6,25-diene-26,23-lactone. The butenolide was isolated from dehydration of lactones II and IV: NMR ($CDCl_3$, 80 MHz) δ 7.37 (5 H), 6.9 (1 H), 6.38, 6.2 (2 H), 4.7 (1 H), 4.79, 4.66 (2 H), 4.25 (1 H), 3.35 (3 H), 1.89 (3 H), 1.03 (3 H), 0.95 (3 H), 0.8 (3 H); mass spectrum, m/z 454 (13), 452 (14), 420 (7), 407 (8), 392 (100), 390 (59), 377 (42), 375 (27), 177; IR ν_{max} 3500, 2950, 1950, 1690 cm^{-1}; R_f 0.41 (55% ethyl acetate/45% petroleum ether).

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Registry No. **1 I**, 77714-49-9; **1 II**, 77714-46-6; **1 III**, 77714-48-8; **1 IV**, 77714-47-7; **5**, 78108-99-3; **6**, 77248-17-0; **7**, 78109-00-9; **8**, 78109-01-0; **10**, 78109-02-1; **11**, 78109-03-2; **12**, 78109-04-3; **13 I**, 78109-05-4; **13 II**, 78183-81-0; **13 III**, 78183-82-1; **13 IV**, 78183-83-2; **14**, 78109-06-5; **15**, 78109-07-6; **16 I**, 77698-45-4; **16 II**, 77698-42-1; **16 III**, 77698-44-3; **16 IV**, 77698-43-2; **17**, 78109-08-7; **18**, 78109-09-8; 3β-hydroxy-5α,8α-(4-phenyl-1,2-urazolo)cholesta-6-en-25-ol-26,23-lactone I, 78109-10-1; 3β-hydroxy-5α,8α-(4-phenyl-1,2-urazolo)-cholesta-6-en-25-ol-26,23-lactone II, 78183-84-3; 3β-hydroxy-5α,8α-(4-phenyl-1,2-urazolo)cholesta-6-en-25-ol-26,23-lactone III, 78183-85-4; 3β-hydroxy-5α,8α-(4-phenyl-1,2-urazolo)cholesta-6-en-25-ol-26,23-lactone IV, 78183-86-5; (23*R*)-3β-(methoxymethoxy)-5α,8α-(4-phenyl-1,2-urazolo)cholesta-6,25-diene-26,23-lactone, 78109-11-2; (23*S*)-3β-(methoxymethoxy)-5α,8α-(4-phenyl-1,2-urazolo)cholesta-6,25-diene-26,23-lactone, 78183-87-6; vinyl bromide, 593-60-2; 4-phenyl-1,2,4-triazoline-3,5-dione, 4233-33-4; ergosterol, 57-87-4.