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Synthesis of (22*R*)- and (22*S*)-22,25-Dihydroxyvitamin D_3 and Determination of Their Biological Activity[1]

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Bisnorcholenal 3-tetrahydropyranyl ether (**1**), obtained from bisnorcholenic acid in 3 steps, was coupled with lithium acetylide derived from 2-methyl-3-butyn-2-ol tetrahydropyranyl ether to give in 74% yield a 1:1 mixture of the cholest-5-ene-3β,22,25-trihydroxy-23-yne 3,25-bis(tetrahydropyranyl)ethers, **2a** and **2b**. After resolution of the C-22 epimers and determination of their configurations, both isomers were converted to 22,25-dihydroxycholesterol 3,22-diacetates, **5a** and **5b**, and to 25-hydroxy-22-methoxycholesterol 3-acetate, **8a** and **8b**. These compounds were converted to (22*R*)- and (22*S*)-22,25-dihydroxyvitamin D_3, **12a** and **12b**, and to 25-hydroxy-22-methoxyvitamin D_3, **14a** and **14b**, through the corresponding cholest-5,7-diene-3β,22,25-triol 3,22-diacetates (**9a** and **9b**) and cholest-5,7-diene-22-methoxy-3β,25-diol 3-acetates (**10a** and **10b**). According to preliminary biological testes, 22,25-$(OH)_2D_3$ and 22-CH_3O-25-OH-D_3 have neither vitamin D activity nor antivitamin D activity.

Keywords——(22*R*)- and (22*S*)-22,25-dihydroxyvitamin D_3; 22-methyl ethers of (22*R*)- and (22*S*)-22,25-dihydroxyvitamin D_3; vitamin D_3 activity; conformation of steroidal side chain; bisnorcholenal; coupling reaction with lithium acetylide

A number of structural analogs of 1,25-dihydroxyvitamin D_3, the hormonal form of vitamin D_3, have recently been synthesized in an effort to increase and also modify the activity of vitamin D_3. The structure-activity relationship of those analogs has been investigated.[2,3] The 1α-hydroxyl group is essential for biological activity, and the hydroxyl groups at the 3 and 25 positions and the proper side chain length contribute to the biological potency. Although the effect of the conformation of the A-ring has been discussed on the basis of a ^{1}H-nuclear magnetic resonance (NMR) analysis,[4] the conformational effect of the side chain on the activity has not been clarified. As we reported that (22*R*)- and (22*S*)-substituted sterol side chains have different conformations,[5] the introduction of a hydroxyl or methoxyl group at the C-22 position might be useful in determining the conformational effect on the biological activity.

Bisnorcholenal 3-tetrahydropyranyl (THP) ether (**1**), readily available from bisnorcholenic acid,[6] was coupled with lithium acetylide derived from 2-methyl-3-butyn-2-ol THP ether to give a 1:1 mixture of the acetylenic compounds **2a** and **2b** in 74% yield. After separation by recrystallization and column chromatography on silica gel, both isomers were separately converted into the 3,25-bis-THP ethers **3a** and **3b** by catalytic hydrogenation over platinum oxide. These were then converted to the triols **4a** and **4b** by acid hydrolysis. Acetylation of **4a** and **4b** gave the 3,22-diacetates **5a** and **5b** (96% yield from **2a** and **2b**).

The stereochemistry at C-22 was determined by comparison of the melting points of the 3,22,25-triols **4a** and **4b** and the diacetates **5a** and **5b** with the published data ("Experimental").[7] It was concluded that the less polar compounds (**4a** and **5a**) have the 22*R* configurations, while the more polar isomers (**4b** and **5b**) have the 22*S* configurations. These compounds showed the usually observed polarities of epimeric pairs of 22-alcohols although a reversal of the polarities is observed in the isomethyl ether analogs.[8]

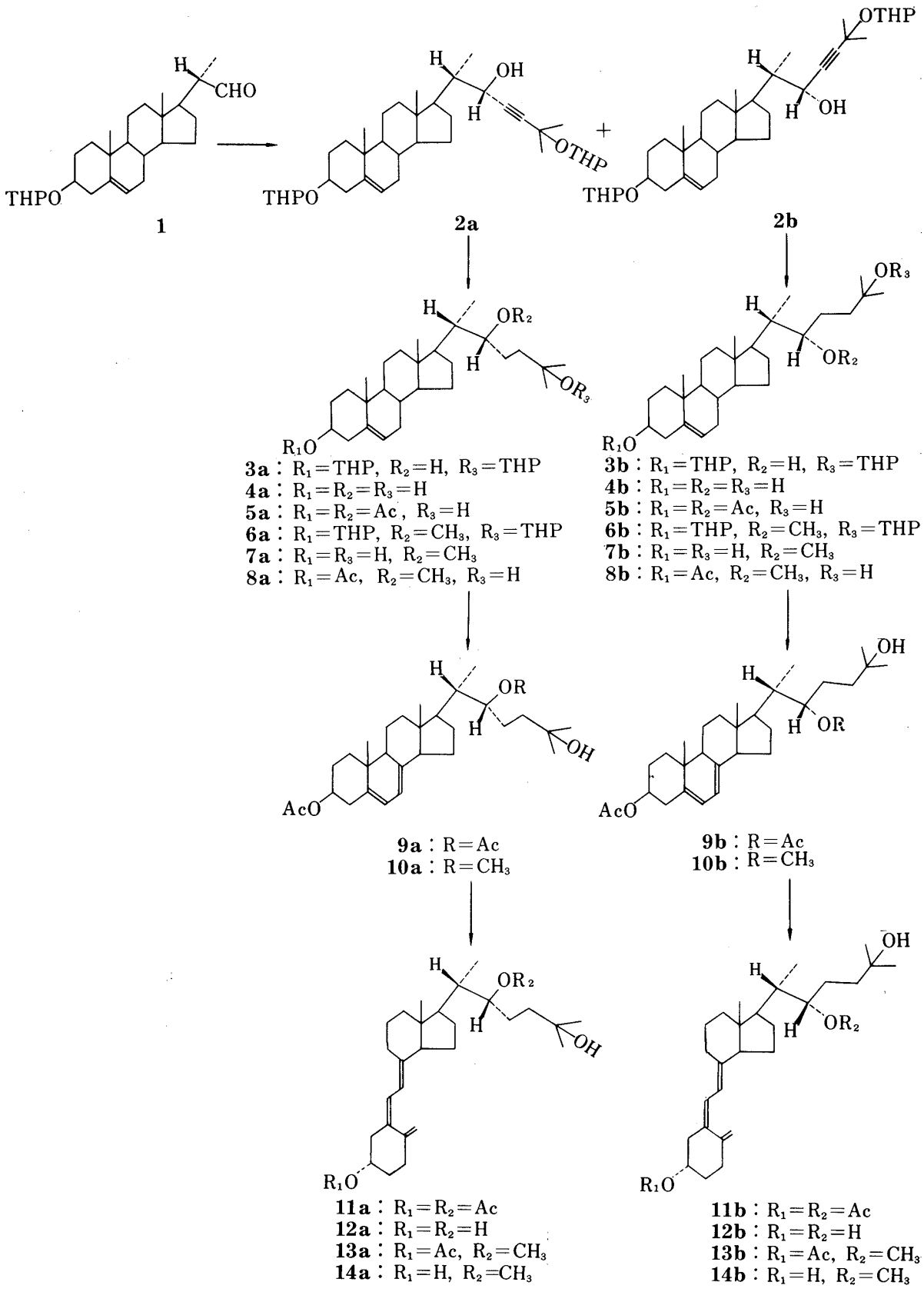

Chart 1

The configuration of C-22 in the (22*S*)-bis-THP ether **3b** could be inverted by Corey's method.[9] Thus, the corresponding mesylate was treated with potassium superoxide in the presence of dicyclohexyl-18-crown-6 in dimethylsulfoxide and dimethylformamide to give the (22*R*)-hydroxyl compound **3a** in 73% yield.

The 22-methyl ethers **8a** and **8b** were prepared by treatment of the 22-hydroxy compounds **3a** and **3b** with sodium hydride and methyl iodide in refluxing tetrahydrofuran followed by acid hydrolysis and then acetylation in 92% yield.

These 22,25-substituted cholesterols **5a**, **5b**, **8a** and **8b** were transformed to the corresponding calciferols **12a**, **12b**, **14a** and **14b** by the established method of vitamin D synthesis. Thus, the diacetate **5a** was brominated with N-bromosuccinimide in carbon tetrachloride followed by dehydrobromination with s-collidine in xylene to give a mixture of the 5,7- and 4,6-dienes. The desired 5,7-diene **9a** was isolated by treatment with *p*-toluenesulfonic acid in acetone followed by thin layer chromatographic separation in 16% yield. The 5,7-diene **9a** in benzene and ethanol was irradiated with a medium pressure mercury lamp and then refluxed to effect

Table I. Lack of Effect of 22,25-$(OH)_2D_3$ Isomers on Intestinal Calcium Transport and Serum Calcium Concentration of Vitamin D-deficient Rats

Compound given	Intestinal Ca transport ^{45}Ca serosal/^{45}Ca mucosal	Serum Ca mg/100 ml
Ethanol	1.7 ± 0.1*[a]	4.3 ± 0.2[a]
22*R*,25-$(OH)_2D_3$(**12a**)	1.6 ± 0.1[b]	3.8 ± 0.3[b]
22*S*,25-$(OH)_2D_3$(**12b**)	1.7 ± 0.4[b]	4.1 ± 0.3[b]
25-OH-D_3	2.7 ± 0.7[c]	6.4 ± 0.4[c]

* Standard deviation of the mean.
Significance of difference *b*) from *a*) N.S. *b*) from *a*) N.S.
c) from *a*) $p<0.005$. *c*) from *a*) $p<0.001$.

Rats fed a low-calcium, vitamin D-deficient diet for 3 weeks were given 325 p mol of one of the compounds dissolved in 0.1 ml of 95% ethanol intrajugularly 19 h prior to sacrifice. Rats in the control group received the vehicle alone. Each group contained 5—6 rats. They were killed by decapitation and the blood was centrifuged to yield serum. The serum calcium concentration was measured in the presence of 0.1% lanthanum chloride by means of a Perkin-Elmer atomic absorption spectrometer, model 403. The duodena were used for measurement of intestinal calcium transport activity.

Table II. Lack of Antivitamin D Activity of 22,25-$(OH)_2D_3$ Isomers in the Stimulation of Intestinal Calcium Transport and Increase of Serum Calcium Concentration

Compound given		Intestinal Ca transport ^{45}Ca serosal/^{45}Ca mucosal	Serum Ca mg/100 ml
First dose	Second dose		
Ethanol	Ethanol	2.4 ± 0.4*[a]	3.9 ± 0.2[a]
Ethanol	22*R*,25-$(OH)_2D_3$ (**12a**)	2.0 ± 0.5[b]	3.8 ± 0.4[b]
Ethanol	22*S*,25-$(OH)_2D_3$ (**12b**)	2.2 ± 0.4[b]	3.5 ± 0.4[b]
22*R*,25-$(OH)_2D_3$	25-OH-D_3	5.5 ± 0.6[c]	4.8 ± 0.3[c]
22*S*,25-$(OH)_2D_3$	25-OH-D_3	5.9 ± 1.7[c]	5.6 ± 0.3[c]
Ethanol	25-OH-D_3	7.5 ± 2.1[d]	5.0 ± 0.2[d]

* Standard deviation of the mean.
Significance of difference *b*) from *a*) N.S. *b*) from *a*) N.S.
c) and *d*) from *a*) $p<0.005$. *c*) and *d*) from *a*) $p<0.001$.
c) from *d*) N.S. *c*) from *d*) N.S.

Rats fed a low-calcium, vitamin D-deficient diet for 3 weeks were into 6 groups of 5—6 rats. They received the first dose of 2.6 n mol of 22*R*,25-$(OH)_2D_3$ or 2.6 n mol 22*S*,25-$(OH)_2D_3$ dissolved in 0.1 ml of ethanol or ethanol vehicle only intrajugularly. One h later, they received a second dose of 0.1 ml of ethanol containing 2.6 n mol of 22*R*,25-$(OH)_2D_3$ or 2.6 n mol of 22*S*,25-$(OH)_2D_3$ or 325 p mol of 25-OH-D_3 by the same route. Twenty four h after the second dose, the animals were killed and intestinal calcium transport activity and serum calcium concentration were measured as described in Table I.

thermal isomerization of the resulting precalciferol. Saponification followed by thin layer chromatography gave in 27% yield (22*R*)-22,25-dihydroxyvitamin D_3 **12a** which showed the characteristic vitamin D *cis*-triene absorption with λ_{max} at 263 nm and λ_{min} at 228 nm.

By essentially the same method as described for the (22*R*)- compound **9a**, the (22*S*)-hydroxy compound **9b** and (22*R*)- and (22*S*)- methyl ethers **10a** and **10b** were converted into the corresponding cholecalciferol derivatives **12b**, **14a** and **14b**, respectively.

Preliminary biological tests of 22,25-$(OH)_2D_3$ and the 22-methyl ether indicated that neither isomer has vitamin D activity or antivitamin D activity, as shown in Tables I, II and III. Although the real conformations of these compounds are not known, it is interesting to note that 22-hydroxyvitamin D_3[10] has no biological activity. However, 24-hydroxy-[11] and 26-hydroxyvitamin D_3[12] have similar activity to 25-hydroxyvitamin D_3, while the introduction of a second hydroxy group into the side chain [*e.g.* 24,25- and 25,26-$(OH)_2D_3$], diminished the activity.[2]

Table III. Lack of Effect of 22-CH_3O-25-OH-D_3 on Intestinal Calcium Transport and Serum Calcium Concentration of Vitamin D-deficient Rats

Compound given	Intestinals Ca transport ^{45}Ca serosal/^{45}Ca mucosal	Serum Ca mg/100 ml
EtOH (control)	2.1 ± 0.6*[a]	3.6 ± 0.1[d]
22R-CH_3O-25-OH-D_3 (**14a**)	2.3 ± 0.3[b]	3.5 ± 0.1
22S-CH_3O-25-OH-D_3 (**14b**)	2.0 ± 0.5	3.4 ± 0.2
25-OH-D_3	4.9 ± 0.8[c]	4.9 ± 0.3[e]

* Standard deviation of the mean.
Significance of difference *b*) from *a*) N.S.
c) from *a*) $p<0.001$.
e) from *a*) $p<0.001$.

Rats fed a low-calcium, vitamin D-deficient diet for 4 weeks were divided into 4 groups of 5—6 rats. They were given 650 p mol of a test compound in 0.1 ml of 95% ethanol intrajugularly 22 h prior to sacrifice (see Table I).

Experimental

Melting points were determined on a hot stage microscope and are uncorrected. UV spectra were recorded on a Shimadzu UV-200 apparatus with ethanol as the solvent. Proton NMR spectra were run on a Hitachi R-24A or a JEOL JNM-PS-100 spectrometer with $CDCl_3$ as the solvent and with tetramethylsilane as an internal reference. Mass spectra were determined in a Shimadzu LKB-9000S machine. Column chromatography was carried out with Merck Kieselgel 60. Thin-layer chromatography (TLC) was carried out on Merck precoated plates, Kieselgel 60, F_{254}. High pressure liquid chromatography (HPLC) was run on a Shimadzu LC-1 machine with a UV-detector. The following abbreviations are used: THF=tetrahydrofuran; THP=tetrahydropyranyl; s=singlet; d=doublet; m=multiplet; b=broad.

(22*R* and 22*S*)-3β,25-Bis(tetrahydropyranyloxy)-cholest-5-en-23-yn-22-ol (2a and 2b)——*n*-Butyllithium (10.5 ml of 1.6 mmol *n*-hexane solution) was added to a solution of 2-methyl-3-butyn-2-ol THP ether (2.68 g, 16.0 mmol) in THF (5 ml) through a syringe at 0°C under an argon atmosphere, and the mixture was stirred at 0°C for 10 min. The aldehyde 1 (3.57 g, 8.6 mmol) in THF (2 ml) was added to the acetylide solution and the mixture was stirred at −78°C for 30 min. Then sat. NH_4Cl solution and water were added and the mixture was extracted with ether. The organic layer was washed with sat. $NaHCO_3$ solution and brine, and dried over $MgSO_4$. Removal of the solvent by evaporation gave a syrup (4.5 g), which showed two spots (*Rf* 0.4 and 0.43) on TLC (benzene–AcOEt, 5:1) in a *ca.* 1:1 ratio. Recrystallization from methanol gave the more polar compound, the (22*R*)-alcohol (**2b**) (1.21 g), mp 159—161°C, NMR ($CDCl_3$) δ: 0.69 (3H, s, 18-Me), 1.00 (3H, s, 19-Me), 1.25 (6H, s, 26,27-Me_2), 4.69 (1H, m, 2′-H of 3-OTHP), 5.01 (1H, m, 2′-H of 25-OTHP), 5.30 (1H, m, 6-H). MS *m/e*: 480 (M–THPOH), 462 (M–THPOH–H_2O), 414 (C_{22}–C_{23} cleavage-1H), 378 (M–THPOH×2), 360 (M–THPOH×2-H_2O), 255, 253. *Anal.* Calcd for $C_{37}H_{58}O_5$: C, 76.24; H, 10.03 Found: C, 76.37; H, 10.03. The mother liquor was concentrated and chromatographed on silica gel. Elution with benzene–ethyl acetate (100:1) gave the (22*S*)-alcohol **2a** (2.05 g) and further elution with benzene–ethyl acetate (50:1) gave **2b** (0.44 g). The MS and NMR spectra showed the same patterns as those of **2a**.

(22*R* and 22*S*)-3β,25-Bis(tetrahydropyranyloxy)-cholest-5-en-22-ol (3a and 3b)——A mixture of platinum black (400 mg) and the 23-yne (**2a**) (700 mg) in methanol (20 ml) was stirred under a hydrogen atmosphere

for 40 min at room temperature. The catalyst was removed by filtration. The filtrate was evaporated to dryness, and the product was chromatographed on silica gel. The benzene–ethyl acetate (40: 1) fraction afforded the (22*R*)-alcohol **3a** (585 mg); mp 89—91°C (from methanol, needles). NMR ($CDCl_3$) δ: 0.68 (3H, s, 18-Me), 1.00 (3H, s, 19-Me), 1.21 (6H, s, 26,27-Me_2), 5.30 (1H, m, 6-H). MS *m/e*: 484 (M–THPOH), 382 (M–THPOH×2), 364 (M–THPOH×2–H_2O), 331 (C_{22}–C_{23} cleavage-THPOH), 313 (331–H_2O). *Anal.* Calcd for $C_{37}H_{62}O_5$: C, 75.72; H, 10.65. Found: C, 75.74; H, 10.56. By the same procedure, the 23-yne (**2b**) (850 mg) was converted to the (22*S*)-alcohol (**3b**) (605 mg); mp 141.5—142.5°C (from hexane–ether). NMR ($CDCl_3$) δ: 0.70 (3H, s, 18-Me), 1.01 (3H, s, 19-Me), 1.22 (6H, s, 26,27-Me_2), 5.30 (1H, m, 6-H). The mass spectrum showed the same pattern as that of **3a**.

(22*R* and 22*S*)-3β,22-Diacetoxycholest-5-en-25-ol (5a and 5b)——A mixture of **3a** (127 mg) and a few drops of dil. HCl in methanol–THF (5: 1, 30 ml) was stirred for 1 h at room temperature. The reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with sat. $NaHCO_3$ solution and brine, anp dried over $MgSO_4$. Removal of the solvent by evaporation gave a solid (90 mg), which was recrystallized from $CHCl_3$-methanol to give **4a**: mp 224—227°C (dec.) (described in ref.,[5] 253—255°C). MS *m/e*: 418 (M^+), 385 (M–H_2O–Me), 382 (M–H_2O×2), 367 (M–H_2O×2–Me), 349 (M–3H_2O–Me), 331 (C_{22}–C_{23} cleavage), 284 (C_{20}–C_{22} cleavage-H), 269 (284–Me).

The triol **4a** (90 mg) was acetylated with acetic anhydride (5 ml) in pyridine (5 ml) at room temperature. The reaction mixture was poured into ice-water and extracted with ether. Recrystallization of the product from hexane–ether gave **5a** (105 mg), mp 150—152.5°C. NMR ($CDCl_3$) δ: 0.68 (3H, s, 18-Me), 1.01 (3H, s, 19-Me), 1.24 (6H, s, 26,27-Me_2), 4.56 (1H, m, 3-H), 4.88 (1H, m, 22-H), 0.92 (3H, d, J=7.2 Hz, 21-Me). MS *m/e*: 502 (M^+), 442 (M–AcOH), 424 (M–AcOH–H_2O), 409 (M–AcOH–H_2O–Me), 382 (M–AcOH×2), 364 (M–AcOH×2–H_2O), 349 (M–AcOH×2–H_2O–Me), 313 (M-side chain-2H), 255, 253. *Anal.* Calcd for $C_{31}H_{50}O_5$: C, 74.06; H, 10.03. Found: C, 73.86; H, 9.99.

The diTHP ether **3b** (100 mg) was converted to the triol **4b** (74 mg) by the same procedure as described for **4a**; mp 185.5—187.5°C (MeOH). The mass spectrum showed the same pattern as that of **4a**. The triol **4a** (72 mg) was acetylated to give 86 mg of **5b**; mp 165—166.5°C (from MeOH). NMR ($CDCl_3$) δ: 0.69 (3H, s, 18-Me), 1.01 (3H, s, 19-Me), 1.23 (6H, s, 26,27-Me_2), 4.56 (1H, m, 3-H), 4.96 (1H, m, 22-H), 5.36 (1H, m, 6-H), 0.98 (3H, d, J=7.0 Hz, 21-Me). The mass spectrum showed the same pattern as that of **5a**. *Anal.* Calcd for $C_{31}H_{50}O_3$: C, 74.06; H, 10.03. Found: C, 74.12; H, 10.04.

Inversion of the Configuration at C-22——A mixture of the (22*S*)-alcohol **3b** (703 mg), methanesulfonyl chloride (0.4 ml) and pyridine (4 ml) was stirred at room temperature for 2 h. The reaction mixture was extracted with ether and the organic layer was washed with 2 N HCl, sat. $NaHCO_3$ solution and brine, dried over $MgSO_4$ and evaporated to dryness to give the mesylate (780 mg). NMR ($CDCl_3$) δ: 0.70 (3H, s, 18-Me), 1.00 (3H, s, 19-Me), 1.22 (6H, s, 26,27-Me_2), 2.99 (3H, s, OSO_2Me), 5.30 (1H, m, 6-H).

A solution of the mesylate (780 mg) in dimethylsulfoxide (25 ml) and dimethylformamide (10 ml) was treated with potassium superoxide (250 mg), and dicyclohexyl-18-crown-6 (1.20 g). The mixture was stirred at room temperature for 4 h, and then extracted with ethyl acetate. The organic layer was washed with dil. HCl, sat. $NaHCO_3$ solution and brine, and evaporated to dryness. The oily residue was chromatographed on silica gel with benzene–ethyl acetate (40: 1) to give the (22*R*)-alcohol **3a** (513 mg), which was converted to the 3,22-diacetate **5a**. The melting point, chromatographic behavior, NMR and mass spectra were identical with those of **5a** synthesized above.

(22*R* and 22*S*)-3β,25-Bis(tetrahydropyranyloxy)-22-methoxycholest-5-ene (6a and 6b)——Sodium hydrid (150 mg) and methyl iodide (2 ml) were added to a solution of the (22*R*)-alcohol **3a** (292 mg) in THF (4 ml) and the mixture was stirred under reflux for 1 h under an argon atmosphere. Then sat. NH_4Cl solution was added and the mixture was extracted with ether. The organic layer was washed with brine and dried over $MgSO_4$. Removal of the solvent by evaporation gave a yellow residue, which was chromatographed on Silica gel with benzene–ethyl acetate (100: 1) to give the (22*R*)-methoxide **6a** (275 mg), mp 96—105°C (methnol); NMR ($CDCl_3$) δ: 0.70 (3H, s, 18-Me), 1.00 (3H, s, 19-Me), 1.21 (6H, s, 26,27-Me_2), 3.09 (1H, m, 22-H), 3.26 (3H, s, 22-OMe), 5.30 (1H, m, 6-H), 4.67 (2H, m, 2′-H of THP). *Anal.* Calcd for $C_{38}H_{64}O_5$: C, 75.95; H, 10.74. Found: C, 75.96; H, 10.70.

The (22*S*)-alcohol **3b** (212 mg) was converted to the (22*S*)-methoxide **6b** (176 mg) in the same way as described above; mp 132—133°C (methanol); NMR ($CDCl_3$) δ: 0.68 (3H, s, 18-Me), 1.00 (3H, s, 19-Me), 1.2 (6H, s, 26,27-Me_2), 3.10 (1H, m, 22-H), 3.30 (3H, s, 22-OMe), 4.67 (2H, m, 2′-H of THP), 5.30 (1H, m, 6-H). *Anal.* Calcd for $C_{38}H_{64}O_5$: C, 75.95; H, 10.74. Found: C, 76.00; H, 10.73.

(22*R* and 22*S*)-3β-Acetoxycholest-5-ene-22-methoxy-25-ol (8a and 8b)——A solution of the di-THP ether **6a** (170 mg) and dil.HCl (2 drops) in MeOH–THF (5: 1, 30 ml) was stirred at room temperature for 1 h. The reaction mixture was extracted with ethyl acetate and the organic layer was washed with sat. $NaHCO_3$ solution and brine, then dried over $MgSO_4$. Removal of the solvent gave the diol **7a** (165 mg), mp 192—194°C (hexane–ether), NMR ($CDCl_3$+C_5D_5N) δ: 0.70 (3H, s, 18-Me), 1.00 (3H, s, 19-Me), 1.34 (6H, s, 26,27-Me_2), 3.15 (1H, m, 22-H), 3.26 (3H, s, 22-OMe), 3.65 (1H, m, 3-H), 5.30 (1H, m, 6-H). MS *m/e*: 432 (M^+), 417 (M–Me), 414 (M–H_2O), 400 (M–MeOH), 385 (M–Me–MeOH), 382 (M–MeOH–H_2O), 367 (M–Me–MeOH–H_2O), 345 (C_{22}–C_{23} cleavage), 327 (345–H_2O), 313 (345-MeOH), 295, 273, 271, 255 and 253. *Anal.* Calcd for $C_{28}H_{48}O_3$: C, 77.72; H, 11.18. Found: C, 77.72; H, 11.27.

The diol **7a** (77 mg) was acetylated with acetic anhydride (3 ml) and pyridine (3 ml) at room temperature. The product was chromatographed on Silica gel to give the acetate **8a** (82 mg), mp 134.5—136°C (hexane–ether). NMR ($CDCl_3$) δ: 0.70 (3H, s, 18-Me), 1.00 (3H, s, 19-Me), 1.19 (6H, s, 26,27-Me_2), 1.99 (3H, s, 3-OAc), 3.10 (1H, m, 22-H), 3.23 (3H, s, 22-OMe), 4.53 (1H, m, 3-H), 5.30 (1H, m, 6-H). MS *m/e*: 474 (M^+), 414 (M–AcOH), 396 (M–AcOH–H_2O), 387 (C_{22}–C_{23} cleavage), 383 (M–AcOH–H_2O–Me), 364 (M–AcOH–H_2O–MeOH), 327 (387-AcOH), 315, 313, 295, 253. *Anal.* Calcd for $C_{28}H_{48}O_3$: C, 77.72; H, 11.18. Found: C, 77.64; H, 10.96.

The diTHP ether **6a** (156 mg) was converted to the diol **7b** (99 mg) in the same way as described for **7a**, mp 187—190°C (methanol), NMR ($CDCl_3$) δ: 0.68 (3H, s, 18-Me), 1.00 (3H, s, 19-Me), 1.21 (6H, s, 26,27-Me_2), 3.10 (1H, m, 22-H), 3.30 (1H, m, 3-H), 3.31 (3H, s, 22-OMe), 5.30 (1H, m, 6-H). The mass spectrum showed the same pattern as that of **7a**. *Anal.* Calcd for $C_{28}H_{48}O_3$: C, 77.72; H, 11.18. Found: C, 77.65; H, 11.18.

The diol **7b** (92 mg) was converted to the acetate **8b** (99 mg) in the same way as described for **8a**, mp 152—154°C (methanol); NMR ($CDCl_3$) δ: 0.68 (3H, s, 18-Me), 1.01 (3H, s, 19-Me), 1.21 (6H, s, 26,27-Me_2), 2.00 (3H, s, 3-OAc), 3.10 (1H, m, 22-H), 3.31 (3H, s, 22-OMe), 4.60 (1H, m, 3-H), 5.30 (1H, m, 6-H). The mass spectrum showed the same pattern as that of **8a**. *Anal.* Calcd for $C_{30}H_{50}O_4$: C, 75.90; H, 10.62. Found: C, 75.92; H, 10.76.

(22*R* and 22*S*)-3β,22-Diacetoxycholest-5,7-dien-25-ol (9a and 9b)——N-Bromosuccinimide (10 ml) was added to a refluxing solution of the diacetate **5a** (20 mg) in carbon tetrachloride (2 ml), and the mixture was stirred for 55 min under an argon atmosphere until succinimide precipitated. The reaction mixture was then cooled in an ice-water bath and filtered to remove the resulting precipitates. The filtrate was evaporated to dryness *in vacuo* below 40°C. A solution of the residue in xylene (1 ml) was added to a refluxing solution of collidine (0.5 ml) and xylene (1.5 ml) and the mixture was stirred under an argon atmosphere for 10 min. The reaction mixture was extracted three times with ethyl acetate and the organic layer was washed with dil.HCl, sat. $NaHCO_3$ solution and brine, then dried over $MgSO_4$. The solvent was evaporated off. A solution of the residue in acetone (10 ml) containing a catalytic amount of *p*-toluenesulfonic acid was stirred at room temperature for 16 h under an argon atmosphere in the dark. The reaction mixture was extracted with ethyl acetate and the product was purified by preparative TLC on Silica gel developing twice with benzene–ethyl acetate (3: 1). The product band (Rf=0.64) was scraped off and eluted with ethyl acetate. Removal of the solvent by evaporation gave the (22*R*)-5,7-diene **9a** (5.25 mg), UV λ_{max}^{EtOH}: 272, 282 and 294 nm. MS *m/e*: 500 (M^+), 440 (M–AcOH), 422 (M–AcOH–H_2O), 407 (M–AcOH–H_2O–Me), 380 (M–2AcOH), 365 (M–AcOH–Me), 347 (M–2AcOH–H_2O–Me), 253 and 251.

The diacetate **5b** (20 mg) was converted to the (22*S*)-5,7-diene **9b** (5.42 mg) by the method described above. UV λ_{max}^{EtOH}: 272, 282 and 294 nm. The mass spectrum showed the same pattern as that of **9a**.

(22*R* and 22*S*)-22,25-dihydroxyvitamin D_3 (12a and 12b)——A solution of the 5,7-diene **9a** (3.25 mg) in benzene (90 ml) and ethanol (40 ml) was irradiated with a medium pressure mercury lamp through a Vycor filter at 0°C for 2.5 min while argon was bubbled through the solution. Then the solution was refluxed for 1 h under an argon atmosphere. Removal of the solvent by evaporation gave a yellow residue, which was purified by preparative TLC on silica gel developing four times with benzene–ethyl acetate (10: 1). The product band (Rf=0.39) was scraped off and eluted with ethyl acetate. Removal of the solvent by evaporation gave the (22*R*)-22,25-dihydroxyvitamin D_3 diacetate **11a** (770 μg); UV λ_{max}^{EtOH}: 264 nm; λ_{min}^{EtOH}: 227 nm. MS *m/e*: 500 (M^+), 482 (M–H_2O), 440 (M–AcOH), 425 (M–AcOH–Me), 422 (M–AcOH–H_2O), 380 (M–2AcOH), 365 (M–2AcOH–Me), 362 (M–2AcOH–H_2O), 118 (C_7–C_8 cleavage-AcOH).

A mixture of the diacetoxyvitamin D_3 **11a** (770 μg), 5% KOH–MeOH (2 ml) and THF (1 ml) was allowed to stand at room temperature for 16 h under an argon atmosphere in the dark. The reaction mixture was extracted with ethyl acetate and the extract was purified by HPLC with 2%MeOH–CH_2Cl_2 to give (22*R*)-22,25-dihydroxyvitamin D_3 (**12a**) (228 μg); UV λ_{max}^{EtOH}: 263 nm, λ_{min}^{EtOH}: 228 nm. MS *m/e* 416 (M^+), 398 (M–H_2O), 383 (M–Me–H_2O), 365 (M–Me–2H_2O), 357 (C_{24}–C_{25} cleavage), 311 (C_{22}–C_{23} cleavage-H_2O), 300 (C_{20}–C_{22} cleavage), 269, 267, 253, 251 and 118.

The (22*S*)-5,7-diene **9b** (3 mg) was converted to the (22*S*)-diacetoxyvitamin D_3 (**11b**) (574 μg) in the same way as described above. The UV and mass spectra showed the same patterns as those of **11a**. The (22*S*)-diacetate **11b** (574 μg) was converted to the (22*S*)-22,25-dihydroxyvitamin D_3 (**12b**) (259 μg) in the same way as described above. The UV and mass spectra showed the same patterns as those of **12a**.

(22R and 22S)-3β-Acetoxycholest-5,7-dien-25-ol (10a and 10b)——The 5-ene **8a** (15.6 mg) was treated with N-bromosuccinimide (8.2 mg) in CCl_4 (2 ml) followed by treatment with *p*-toluenesulfonic acid in acetone (10 ml) in the same way as described for **9a**. The product band (Rf=0.36) on preparative TLC on Silica gel developing twice with benzene–ethyl acetate (5: 1) was scraped off and eluted with ethyl acetate. Removal of the solvent by evaporation gave the (22*R*)-5,7-diene **10a** (4.09 mg), UV λ_{max}^{EtOH}: 271, 282 and 293 nm; MS *m/e*: 472 (M^+), 412 (M–AcOH), 394 (M–AcOH–H_2O), 380 (M–AcOH–MeOH), 365 (M–AcOH–MeOH–Me), 362 (M–AcOH–MeOH–H_2O), 325 (C_{22}–C_{23} cleavage-AcOH), 281 (C_{20}–C_{22} cleavage-AcOH), 253 and 251.

The 5-ene **8b** (20 mg) was converted to the (22*S*)-5,7-diene **10b** (3.48 mg) in the same way as described for **9a**. The UV and mass spectra exhibited the same patterns as those of **10a**.

(22R and 22S)-25-Hydroxy-22-methoxyvitamin D_3 (14a and 14b)——A solution of the 5,7-diene **10a** (4.09 mg) in benzene (90 ml) and ethanol (40 ml) was irradiated and refluxed in the same way as described

for **11a**. The product was purified by TLC on Silica gel with benzene–ethyl acetate (5: 1), developing four times. The product band ($Rf=0.5$) was scraped off and eluted with ethyl acetate. Removal of the solvent by evaporation gave the (22*R*)-vitamin D_3 acetate **13a** (948 μg); UV λ_{max}^{EtOH}: 265, λ_{min}^{EtOH}: 227 nm; MS *m/e*: 472 (M^+), 454 (M–H_2O), 412 (M–AcOH), 397 (M–AcOH–Me), 394 (M–AcOH–H_2O), 380 (M–AcOH–MeOH), 365, 362, 325 (C_{22}–C_{23} cleavage-AcOH), 281 (C_{20}–C_{22}–AcOH), 253, 251 and 118 (C_7–C_8 cleavage-AcOH). The acetate **13a** (948 μg) was treated with 5% KOH–MeOH (2 ml) and THF (1 ml) in the same way as described for **12a**. The mixture was extracted with ethyl acetate, and the extract was washed, dried and evaporated to dryness. Purification by HPLC with 0.5% MeOH–CH_2Cl_2 gave (22*R*)-25-hydroxy-22-methoxyvitamin D_3 (**14a**) (449 μg); UV λ_{min}^{EtOH}: 267, λ_{max}^{EtOH}: 228 nm, MS *m/e*: 430 (M^+), 412 (M–H_2O), 398 (M–MeOH), 397 (M–H_2O–Me), 383 (M–Me–MeOH), 380 (M–H_2O–MeOH), 379 (M–2H_2O–Me), 365 (M–H_2O–MeOH–Me), 362 (M–2H_2O–MeOH), 347 (M–2H_2O–MeOH–Me), 343 (C_{22}–C_{23} cleavage), 325 (343-H_2O), 293 (343-H_2O–MeOH), 271, 269, 253, 251, 136 (C_7–C_8 cleavage) and 118.

The 5,7-diene **10b** (3.48 mg) was converted to the (22*S*)-vitamin D_3 acetate **13b** (595 μg) in the same way as described for **11a**. The UV and mass spectra showed the same patters as those of **13a**.

The (22*S*)-vitamin D_3 acetate **13b** (595 μg) was converted to the (22*S*)-vitamin D_3 **14b** (174 μg) in the same way as described for **12a**. The UV and mass spectra showed the same patterns as those of **14a**.

Measurement of Biological Activity——Weanling male rats were obtained from Holtzman Co., Madison, Wis., and were fed a vitamin D-deficient low-calcium diet[13] and allowed water *ad libitum*. Intestinal calcium transport was measured by the everted gut sac technique as described by Martin and DeLuca[14] (see Tables I, II and III).

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References and Notes

1) This paper comprises "Steroid Studies Part 77." Part 76: S. Takatsuto, B. Ying, M. Morisaki, and N. Ikekawa, *Chem. Pharm. Bull.*, **29**, 903 (1981).
2) H.F. DeLuca and H.K. Schnoes, *Ann. Rev. Biochem.*, **45**, 631 (1976).
3) N. Ikekawa and T. Takeshita, *J. Org. Syn. Chem. Japan*, **37**, 755, 809 (1979).
4) A.W. Norman, D.A. Procsal, W.H. Okamura, and R.M. Wing, *J. Steroid Biochem.*, **6**, 461 (1975).
5) M. Nakane and N. Ikekawa, *J. Chem. Soc., Perkin I*, 1977, 1426.
6) M. Ishiguro, H. Saito, A. Sakamoto, and N. Ikekawa, *Chem. Pharm. Bull.*, **26**, 3715 (1978).
7) B.M. Trost and Y. Matsumura, *J. Org. Chem.*, **42**, 2036 (1977).
8) J.R. Wiersig, N.W. Sarcevic, and C. Djerassi, *J. Org. Chem.*, **44**, 3374 (1979) and references cited therein.
9) E.J. Corey, K.C. Nicolaou, M. Shibasaki, Y. Machida, and C.S. Shiner, *Tetrahedron Lett.*, **1975**, 3183.
10) D.R. Crump, *J. Chem. Soc.*, **1973**, 2731.
11) N. Ikekawa, M. Morisaki, N. Koizumi, M. Sawamura, Y. Tanaka, and H.F. DeLuca, *Biochem. Biophys. Res. Comm.*, **62**, 485 (1975); Y. Tanaka, H. Frank, H.F. DeLuca, N. Koizumi, and N. Ikekawa, *Biochemistory*, **14**, 3293 (1975); Y. Tanaka, H.F. DeLuca, A. Akaiwa, M. Morisaki, and N. Ikekawa, *Arch. Biochem. Biophys.*, **177**, 615 (1976).
12) J. Redel, C. Rebut-Bonnetan, and F. Delbarre, *J. Steroid Biochem.*, **9**, 1179 (1978); J. Redel, L. Miravet, Y. Calando, and M. Carre, *FEBS Letters*, **96**, 216 (1978).
13) T. Suda, H.F. DeLuca, and Y. Tanaka, *J. Nutr.*, **100**, 1049 (1970).
14) D.L. Martin and H.F. DeLuca, *Am. J. Physiol.*, **216**, 1351 (1969).