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Δ^{22}-Unsaturated Analogs of Vitamin D_3 and Their C(1)-Hydroxylated Derivatives

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A stereospecific synthesis of (22*E*)- and (22*Z*)-dehydrocholecalciferols is described starting from C-22 steroidal aldehydes. Direct C(1) hydroxylation of these compounds was achieved via oxidation of the 3,5-cyclovitamin D intermediates with SeO_2/t-butylhydroperoxide, affording 1α-hydroxyvitamin D_3 with the Δ^{22}-unsaturated side chain. In addition, their 5,6-*trans* isomers and 1β epimers have been prepared. The (22*E*) isomer is as active as vitamin D_2 or D_3 in its ability to mobilize bone calcium and heal rickets in rats, whereas the 22*Z* compound is less effective. Bioassay of the corresponding 1α-hydroxylated analogs showed Δ^{22}-*trans*-1α-hydroxyvitamin D_3 to be equal to 1α-hydroxyvitamin D_3 in mobilizing bone calcium and healing rickets, whereas the corresponding *cis* isomer was less active. The C(1)-hydroxylated vitamins were tested for their ability to bind the chick intestinal receptor protein for 1,25-dihydroxyvitamin D_3. Both Δ^{22} isomers were found to be somewhat more potent than 1α-hydroxyvitamin D_3 in the displacement of tritiated 1,25-dihydroxyvitamin D_3.

Recent developments in vitamin D chemistry have been directed toward synthesis of naturally occurring metabolites and analogs (*1*). Advances in an understanding of structure–activity relationships for vitamin D compounds depend on the availability of related analogs that are structurally or stereochemically modified (*2, 3*). The purpose of such studies is to elucidate structural features of the vitamin D molecule necessary for biological activity and to investigate to what extent different activities (e.g., intestinal calcium transport, bone calcium mobilization, differentiation of leukemia cells) are affected by specific structural modifications. We turned our attention to the hydrocarbon side chain to determine the importance to biological activity of the C-22,23 double bond and its configuration as it occurs in vitamin D_2 compounds.

It is well known that birds discriminate against vitamin D_2 compounds, whereas mammals do not (*4–6*). The structural difference between vitamin D_2 and vitamin D_3 compounds is that the former have a *trans* double bond between carbons 22 and 23 and an additional methyl group at C-24. The D_2 derivative having the saturated ergosterol side chain (i.e., 24*S*-methyl cholecalciferol, or vitamin D_4) has been reported to express diminished biological activity in mammals (*7*),

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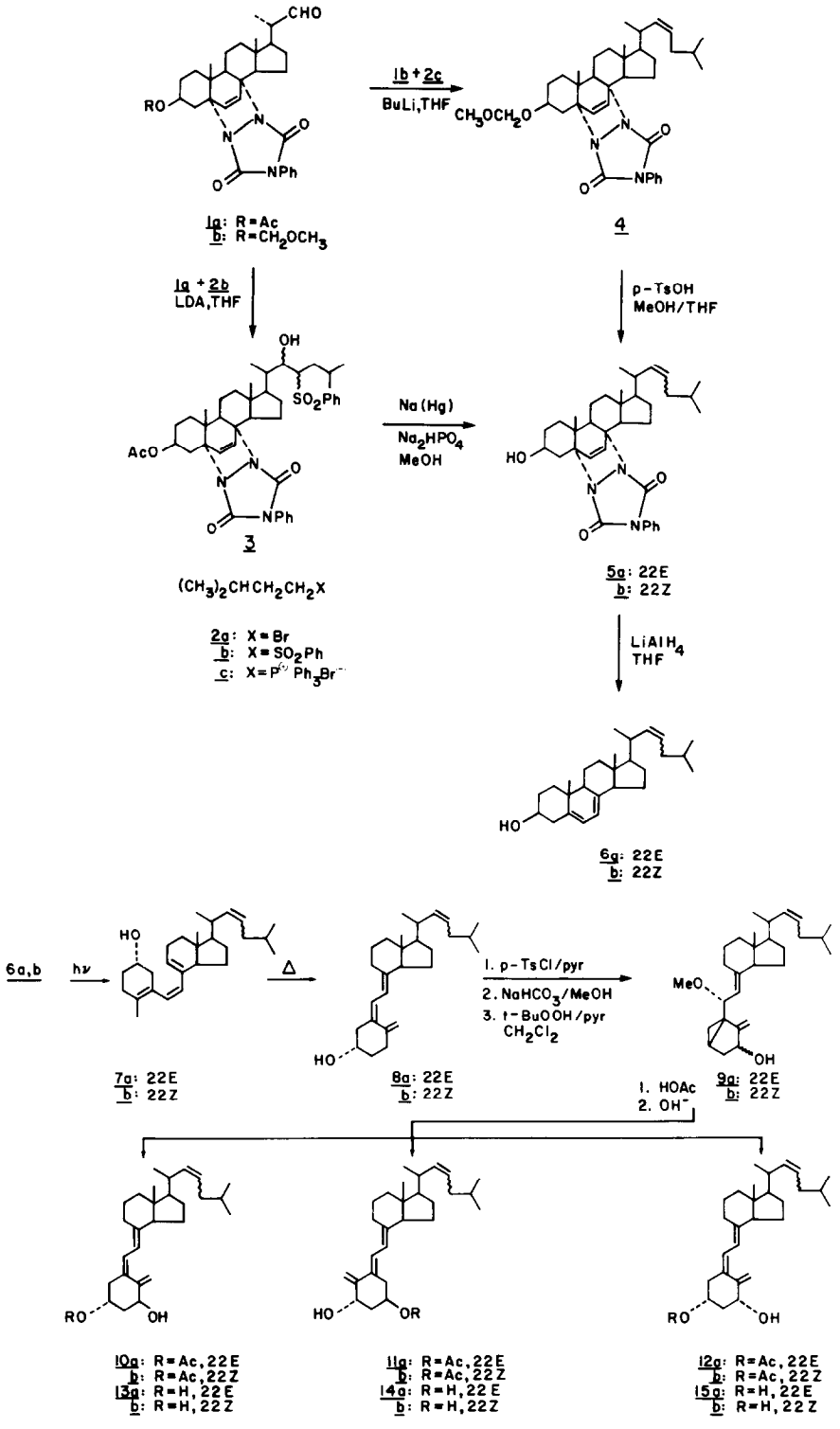
CHO
RO
N
O
NPh
1a: R=Ac
b: R=CH2OCH3
1b + 2c
BuLi,THF
CH3OCH2O
4
1a + 2b
LDA,THF
p-TsOH
MeOH/THF
OH
SO2Ph
AcO
3
Na(Hg)
Na2HPO4
MeOH
HO
5a: 22E
b: 22Z
(CH3)2CHCH2CH2X
2a: X=Br
b: X=SO2Ph
c: X=P+Ph3Br-
LiAlH4
THF
6a: 22E
b: 22Z
6a,b
hν
Δ
7a: 22E
b: 22Z
8a: 22E
b: 22Z
1. p-TsCl/pyr
2. NaHCO3/MeOH
3. t-BuOOH/pyr
CH2Cl2
MeO
9a: 22E
b: 22Z
1. HOAc
2. OH-
RO
OH
HO
OR
10a: R=Ac, 22E
b: R=Ac, 22Z
13a: R=H, 22E
b: R=H, 22Z
11a: R=Ac, 22E
b: R=Ac, 22Z
14a: R=H, 22E
b: R=H, 22Z
12a: R=Ac, 22E
b: R=Ac, 22Z
15a: R=H, 22E
b: R=H, 22Z

whereas 22-dehydro-25-hydroxycholecalciferol was found to be somewhat more potent than 25-OH-D_3 (*8*). To study the effect of unsaturation at C-22,23 more thoroughly, we have prepared Δ^{22}-*cis* and -*trans* isomers of vitamin D_3, their 1α-hydroxy derivative, and their 5,6-*trans* isomers. This paper reports the synthesis of these analogs and their biological activity in mammals.

RESULTS

A synthesis of the *cis*- and *trans*-22-dehydrocholecalciferols **8** has been reported (*9*); that preparation led to a mixture of the C-22,23 double bond geometric isomers, which were separated at an early stage of synthesis (provitamins **6**). Separation of the dienes **6a** and **6b** was achieved with some difficulty by column chromatography of their 3β-acetoxy derivatives using silver nitrate-impregnated silica gel. Since our preliminary results indicated that, in general, resolution of the double bond geometric isomers is not easily accomplished even with TLC or HPLC, we developed methods in which only one of the two possible olefins could be produced.

Construction of the side chain with a *trans*-C-22 double bond was accomplished by coupling the known PTAD-protected diene–aldehyde **1a** (*10*) with the sulfone **2b** and subsequent reduction of the resulting diastereoisomeric α-hydroxysulfone **3** with sodium amalgam in buffered methanol. It has been shown previously that such reductive removal of phenylsulfonyl and hydroxyl groups produces predominantly or exclusively *trans* olefin (*11–13*). In agreement with these findings we found that adduct **5a** was obtained in high yield and purity. Less than 5% of the 22*Z* isomer **5b** was detected by NMR; moreover epimerization at C-20 was not observed. Reduction of **5a** with lithium aluminum hydride removed the triazolinedione group and afforded crystalline diene **6a**.

For the synthesis of the corresponding *cis*-Δ^{22} compounds, a Wittig condensation was used. Despite the published data indicating a lack of stereospecificity in analogous reactions (*8, 9, 14*), we established that the desired 22-*cis* olefin was accessible by the selection of suitable reaction conditions. Since the presence of the 3β-acetoxy group in aldehyde **1a** resulted in diminished yields in the reaction with ylide generated from **2c**, the corresponding methoxymethyl derivative **1b** (*15*) was used. Wittig reaction of aldehyde **1b** resulted almost exclusively in the formation of the 22*Z* adduct **4** (95% purity). Removal of the 3β-methoxymethyl group with *p*-toluenesulfonic acid in methanol, followed by deprotection of the ring B-diene by lithium aluminum hydride reduction, gave crystalline diene **6b**. It is worth noting that attempted isomerization of the 22,23 double bond in adduct **4** by treatment with iodine in a manner analogous to that applied to a 5,22-choladiene derivative failed to give *trans* olefin.

The remaining synthetic steps were executed separately on the two double bond isomers (**6a** and **6b**). The 5,7-dienes **6** were irradiated with a UV lamp and the resulting previtamins **7** were isolated by preparative HPLC. Compounds **7** were further subjected to thermal equilibration by refluxing in ethanol to give the vita-

min D analogs **8** after HPLC purification. The isolation of previtamins **7** from the irradiation mixture could not be omitted in view of the similar retention volumes of the corresponding tachysterol analogs and vitamins **8**.

The synthetic route to the 1α-hydroxylated vitamins **13** utilized the method of Paaren *et al.* (*16*). Tosylation of vitamins **8** followed by bicarbonate-buffered methanolysis and subsequent allylic oxidation with selenium dioxide and t-butylhydroperoxide were accomplished without chromatographic purification of the intermediates and provided 1-hydroxycyclovitamins **9**. The yield of this last oxidation step was not affected by the presence of the isolated double bond at C-22,23. Based on the results of the subsequent acetic acid-catalyzed retrosolvolysis, the intermediate products **9**, though apparently homogeneous by TLC and NMR analysis, consisted of the 1α-hydroxy isomer and minor amounts (ca. 10%) of the 1β-hydroxy epimer. Cycloreversion of **9** and HPLC of the reaction mixtures afforded, in addition to the 3β-acetates **10** and **11**, the 1β-hydroxy epimer **12**. Hydrolysis of the isolated 3β-acetoxyvitamins with methanolic KOH gave the corresponding 1α-hydroxy-22-dehydrocholecalciferols **13**, their 5,6-*trans* isomers **14**, and the 1β-hydroxyvitamins **15**. The ultraviolet ^{1}H-NMR and mass spectra of all compounds are in agreement with the assigned structures. 1β-Hydroxyvitamins **15** also have the appropriate spectral properties, namely, an ultraviolet absorption maximum slightly shifted to the shorter wavelengths, characteristic relative intensities of the peaks *m/z* 134 and 152 in the mass spectra, and chemical shifts of the C-1 and C-3 protons in the ^{1}H-NMR spectra typical of 1β,3β-dihydroxyvitamins (*17, 18*). The 22*E* and 22*Z* isomers are easily distinguishable by their methyl resonances in ^{1}H-NMR spectra. 22*Z* Stereochemistry shifts the C-18 and C-26/C-27 methyl signals to lower field ($\Delta\delta$ ca. 0.03 ppm), and the C-21 methyl signal to higher field ($\Delta\delta$ ca. 0.05 ppm). On the other hand, the shift of C-22/C-23 olefinic signals in the ^{1}H-NMR spectra (*19*) and differences in the relative intensities at *m/z* 109 and 111 in the mass spectra (*9*) are not reliable criteria for distinguishing the geometric isomers.

Biological assays. Biological activity of both Δ^{22}-unsaturated vitamin D_3 analogs **8a** and **8b** was determined *in vivo* in rachitic rats and compared with that of vitamin D_2 or D_3. The assay included the determination of their antirachitic activity (line test) and the relative ability to increase serum calcium and/or inorganic phosphorus concentration, a measure of bone calcium mobilization activity. As shown in Table 1, the *trans* isomer **8a** at the 0.524-nmol dosage level was active in increasing serum calcium concentration of rats on a low-calcium diet, whereas the *cis*-isomer **8b** showed no activity at that level. Serum calcium concentration was elevated in response to both isomers at the 5.24-nmol level. Each of the analogs **8a** and **8b** was also tested for its ability to elevate serum inorganic phosphorus concentration of rachitic rats fed the low-phosphorus diet (Table 2). The 22*E* compound **8a** at the level of 0.26 nmol increased serum inorganic phosphorus concentration and caused endochondral calcification comparable to that produced by vitamin D_2 or vitamin D_3, whereas the *cis* isomer **8b** had no effect. Both vitamins at the level of 2.6 nmol elevated serum inorganic phosphorus concentration and caused calcification of rachitic cartilage.

In assaying the biological potency of the corresponding C(1)-hydroxylated ana-

TABLE 1

INCREASE IN SERUM CALCIUM CONCENTRATION IN RESPONSE TO THE ISOMERS OF Δ^{22}-VITAMIN D_3

Compound given	Dosage (nmol)	Serum calcium (mg/100 ml)
Control	—	3.9 ± 0.2^a
trans-Δ^{22}-Vitamin D_3 (**8a**)	0.524	4.8 ± 0.5^b
	5.24	5.0 ± 0.3^c
cis-Δ^{22}-Vitamin D_3 (**8b**)	0.524	4.3 ± 0.2^d
	5.24	4.8 ± 0.2^c
Vitamin D_2	0.524	4.8 ± 0.1^c
	5.24	5.2 ± 0.2^c

Note. Rats fed a low-calcium, vitamin D-deficient diet for 3 weeks were given a single dose of either compound dissolved in 0.05 ml of 95% ethanol intrajugularly 24 h prior to sacrifice. Rats in the control group received ethanol only. They were killed by decapitation and blood was centrifuged to yield serum. Each group had six rats. Serum calcium values are means ± SD.

$^{a-c}$ Significant difference: a from b, $P < 0.005$; a from c, $P < 0.001$. No difference: a from d, N.S.

TABLE 2

INCREASE OF SERUM INORGANIC PHOSPHORUS IN RESPONSE TO, AND THE ANTIRACHITIC ACTIVIY OF, A SINGLE DOSE OF Δ^{22}-VITAMIN D_3 COMPOUNDS

Compound given	Dosage (nmol)	Serum inorganic phosphorus (mg/100 ml)	Calcification score (units)
Control	—	2.7 ± 0.2^a	0 ± 0
trans-Δ^{22}-Vitamin D_3 (**8a**)	0.26	4.5 ± 0.3^b	4.0 ± 1.1
	2.6	5.3 ± 0.6^b	>5
cis-Δ^{22}-Vitamin D_3 (**8b**)	0.26	2.7 ± 0.2^c	0 ± 0
	2.6	4.6 ± 0.8^b	4.4 ± 0.2
Vitamin D_2	0.26	4.1 ± 0.3^b	3.8 ± 0.6
Vitamin D_3	0.26	4.4 ± 0.2^b	4.4 ± 0.4

Note. Rats fed a low-phosphorus, vitamin D-deficient diet for 3 weeks were given a single dose of either compound dissolved in 0.05 ml of 95% ethanol intrajugularly 7 days prior to sacrifice. Rats in the control group received ethanol only. They were killed by decapitation, blood was collected, and their radii and ulnae were removed to determine antirachitic activity as described in the text. Blood was immediately centrifuged to yield serum. Serum inorganic phosphorus (means ± SD) was determined by the colorimetric method as described in the text. Each group had six rats.

$^{a-c}$ Significant difference: a from b, $P < 0.001$. No difference: a from c.

logs, it was demonstrated (Tables 3 and 4) that the *cis* isomer (**13b**) was much less active in causing mineralization of rachitic rats or in stimulating mobilization of calcium from bone than the *trans* isomer (**13a**) which was as active as the standard reference compound (1α-OH-D_3). For comparison, 1α-OH-D_3 is 1000 times less competent than 1α,25-$(OH)_2D_3$ in binding to the chick intestinal receptor (*1*). As shown in Fig. 1, the binding affinities of the analogs for the 1α,25-$(OH)_2D_3$-receptor protein from chick intestine were determined in comparison with 1α-hydroxyvitamin D_3. It was found that **13b** is approximately three times more effective than 1α-OH-D_3 in displacement of 1α,25-$(OH)_2$-[26,27-^{3}H]D_3 from the receptor protein, while Δ^{22}-*trans*-vitamin **13a** is about as active as 1α-OH-D_3. It is possible that **13a** is either more poorly transported or 25-hydroxylated than **13b** *in vivo*. Alternatively, the presence of a 25-hydroxyl may result in a reverse in binding preferences. The corresponding 5,6-*trans* derivatives show the same trend. Because the displacement curves are not parallel, an exact comparison between **14a** and **14b** is not possible. However, **14b** and **14a** bind more poorly than 1α-OH-D_3. Both 1β-hydroxyvitamins **15a** and **15b** were a thousand times less active than 1α-OH-D_3 in displacement of 1,25-$(OH)_2$-[^{3}H]D_3 from the receptor protein. It is interesting that 1α-hydroxylated analogs having the *cis* configuration at the C-22,23 double bond showed higher affinity than the corresponding *trans* compounds in binding to the chick receptor protein, despite the fact that *trans*-Δ^{22} unsaturation represents the natural configuration as it exists in the vitamin D_2 side chain, and that the 1α-hydroxy-*trans*-Δ^{22} isomer is more active than the Δ^{22}-*cis*-1α-OH-D_3 in the *in vivo* assays. These results suggest that 25-hydroxylation of these compounds in the liver may differ substantially, with the *trans* configuration being preferred. An-

TABLE 3

ACTIVITY OF Δ^{22}-1α-OH-D_3 ANALOGS IN MOBILIZING CALCIUM FROM BONE OF RATS ON A LOW-CALCIUM DIET

Compound	Amount Given (pmol/day/7 days)	Number of animals	Serum calcium (mg%)
Control	0	5	3.7 ± 0.27
1α-OH-D_3	6.25	5	3.9 ± 0.08
	31.25	5	4.2 ± 0.27[a]
	62.5	5	5.0 ± 0.38[a]
Δ^{22}-*trans*-1α-OH-D_3 (**13a**)	6.25	4	3.8 ± 0.14
	31.25	5	4.1 ± 0.20
	62.5	5	4.9 ± 0.55[a]
Δ^{22}-*cis*-1α-OH-D_3 (**13b**)	—	—	—
	31.25	5	3.6 ± 0.28
	62.5	4	3.6 ± 0.08

Note. Animals were fed the low-calcium (0.02% calcium, 0.3% phosphorus) diet for 17 days and then dosed daily via subcutaneous minipump for 7 days. Animals in the control group received propylene glycol only. There were six animals per group. Values are means ± SD.

[a] Differs from control at $P < 0.001$.

TABLE 4

ABILITY OF Δ^{22}-1α-OH-D_3 COMPOUNDS TO SUPPORT MINERALIZATION OF BONE IN RACHITIC RATS

Diet 24 compound	Amount given (pmol/day/7 days)	Serum inorganic phosphorus (mg/100 ml)	Calcification score
Control	—	2.2 ± 0.25	0 − 0.1
1α-OH-D_3	6.25	2.1 ± 0.30	1.0 ± 0.5[a]
	31.25	2.8 ± 0.13[a]	4.3 ± 0.5[a]
	62.5	4.3 ± 1.0[a]	5.0 ± 0[a]
Δ^{22}-*trans*-1α-OH-D_3 (**13a**)	6.25	2.4 ± 0.22	0.1
	31.25	2.8 ± 0.20[a]	3.5 ± 0.5[a]
	62.5	3.2 ± 0.10[a]	5.5 ± 0.15[a]
Δ^{22}-*cis*-1α-OH-D_3 (**13b**)	—	—	—
	31.25	2.4 ± 0.34	0
	62.5	2.8 ± 0.24[a]	2.4 ± 0.7[a]
	125.0	3.3 ± 0.30[a]	5.0 ± 0.8[a]

Note. Experimental animals were fed the high-calcium (1.2%), low-phosphorus (0.1%) rachitogenic diet for 3 weeks. There were six animals per group. Doses of vitamin D were given daily for 7 days in 0.1 ml 95% propylene glycol, 5% ethanol. The vitamin D-deficient control animals received the vehicle alone. The data are expressed as means ± SEM.

[a] Differs from the propylene glycol control at $P < 0.001$.

other possibility is that the vitamin D binding protein may provide the *in vivo* discrimination between these compounds.

EXPERIMENTAL

General. NMR spectra were taken in $CDCl_3$ with a Bruker WH-270 FT spectrometer with TMS as internal standard. Ultraviolet spectra were recorded in

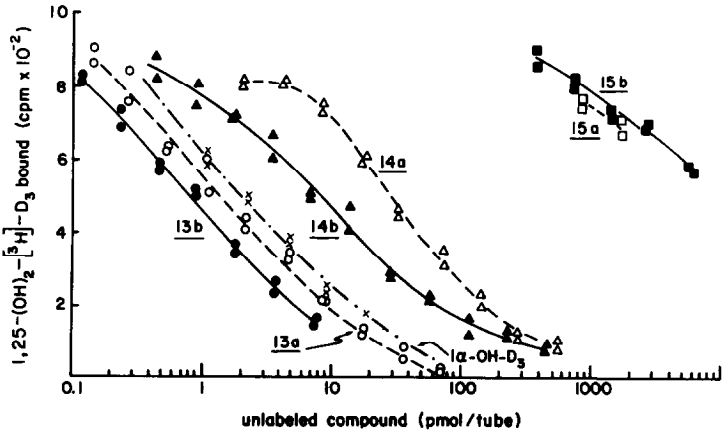

FIG. 1. Displacement of 1,25-$(OH)_2$-[^{3}H]D_3 from chick intestinal cytosol receptor protein for 1,25-$(OH)_2D_3$ by C(1)-hydroxylated analogs. The assay method is described in the text.

ethanol with a Hitachi Model 100-60 spectrophotometer. Infrared spectra were taken with Nicolet MX-1 spectrophotometer in KBr pellets. Mass spectra (MS) were obtained at 110–120°C above ambient temperature at 70 eV with an AEI MS-9 mass spectrometer. Results are presented in the following form: *m/z* (fragment, percentage of base peak). "RDA" refers to retro-Diels–Alder loss of 4-phenyl-1,2,4-triazoline-3,5-dione. Optical rotations were measured in $CHCl_3$ on a Perkin–Elmer Model 141 polarimeter. Melting points were taken on a Thomas–Hoover capillary melting-point apparatus and are uncorrected. High-performance liquid chromatography (HPLC) was performed on a Waters Associates Model ALC/GPC 204 using a Zorbax-Sil (DuPont) 6.2 mm × 25 cm column at a flow rate 4 ml/min and 1500 psi. Column chromatography was performed on silica gel 60, 70–230 mesh ASTM (Merck). Preparative thin-layer chromatography (TLC) was carried out on silica 60 PF-254 (20 × 20 cm plates, 1 mm silica gel). Irradiations were carried out using a Hanovia 608A36 mercury arc lamp fitted with a Vycor filter. Almost all reactions were performed under an argon atmosphere. All commercial reagents were purchased from Aldrich Chemical Company.

3-Methyl-1-butylphenylsulfone (**2b**). $PhSO_2Na$ (1.97 g, 12 mmol) was added to a stirred solution of 3-methyl-1-bromobutane **2a** (1.51 g, 1.2 ml, 10 mmol) in DMF (20 ml) at 75°C. The mixture was heated at 75°C for 5 h, then cooled, poured into water, and extracted with benzene. The organic layer was washed with 5% HCl, 5% $NaHCO_3$ and water, dried over Na_2SO_4, and evaporated. The oily sulfone **2b** (1.69 g, 80%) was substantially pure and was used without any purification. NMR, δ 0.88 (6H, d, J = 6.5 Hz, 2 × CH_3), 1.61 (3H, m, —CH_2—CH<), 3.08 (2H, m, SO_2–CH_2–), 7.50–7.95 (5H, m, Ar–*H*); IR, ν_{max} 1300 (br), 1147, 1088, 745, 692, 564, 539 cm^{-1}; MS, *m/z* 212 (M^+, 3), 143 (92), 77 (57), 71 (73), 70 (73), 55 (42), 43 (100), 41 (47).

(22E)-5α,8α-(4-Phenyl-1,2-urazolo)cholesta-6,22-dien-3β-ol (**5a**). *n*-Butyllithium (1.7 *M* solution in hexane, 4.12 ml, 7 mmol) was added to a stirred solution of diisopropylamine (707 mg, 1 ml, 7 mmol) in dry THF (14 ml). The mixture was stirred for 15 min at room temperature and sulfone **2b** (1.50 g, 7.07 mmol) in dry THF (11 ml) was added dropwise in 10 min. The solution was stirred at room temperature for an additional 15 min, then cooled to 0°C, and aldehyde **1a** (*10*) (545 mg, 1 mmol) in dry THF (7 ml) was added. The stirring was continued for 2 h at 0°C and the solution was slowly warmed to room temperature (30 min). The mixture was poured into a saturated solution of Na_2HPO_4 in methanol (200 ml), sodium amalgam (5.65%, 10 g) was added, and the reaction mixture was stirred at 4°C for 17 h. Precipitated mercury was filtered off and, after concentration of the reaction mixture to ~5 ml, it was diluted with water and extracted with methylene chloride. The organic extract was washed with water, dried (Na_2SO_4), and concentrated *in vacuo*, and the oily residue was chromatographed on a silica gel column. Excess of sulfone **2b** was eluted with benzene–ether (7 : 3) mixture. Elution with benzene–ether (6 : 4) afforded pure adduct **5a** (375 mg, 67%) as a foam: NMR, δ 0.81 (3H, s, 18-H_3), 0.86 (6H, d, J = 6.7 Hz, 26-H_3 and 27-H_3), 0.97 (3H, s, 19-H_3), 1.03 (3H, d, J = 6.8 Hz, 21-H_3), 3.16 (1H, dd, J_1 = 4.4 Hz, J_2 = 14 Hz, 9-H), 4.44 (1H, m, 3-H), 5.25 (2H, br m, 22-H and 23-H), 6.22 and 6.39 (2H, ABq, J = 8.5 Hz, 6-H and 7-H), 7.40 (5H, br m, Ar-*H*); IR, ν_{max} 3444, 1754, 1701,

1599, 1402, 969, 757 cm^{-1}; MS, *m/z* 557 (M^+, <1%), 382 (M^+-RDA, 70), 349 (M^+-RDA-H_2O-Me, 51), 253 (M^+-RDA-H_2O-side chain, 28), 251 (45), 119 (PhNCO, 83), 55 (100).

(22E)-Cholesta-5,7-dien-3β-ol (**6a**). The adduct **5a** (330 mg, 0.6 mmol) and lithium aluminum hydride (700 mg) in dry THF (40 ml) were heated under reflux for 18 h. The excess of reagent was decomposed with a few drops of water. Anhydrous Na_2SO_4 was added and the organic phase was decanted and evaporated to give crystalline residue which was purified on a column of silica gel. Elution with a benzene–ether (94 : 6) mixture gave pure diene **6a** (180 mg, 80%) which was crystallized from methanol: mp 119.5–122.5°C (lit. (*20*) 118–123°C); $[\alpha]_D^{24}$ −118° (*c* = 1.2, $CHCl_3$), lit. (20) $[\alpha]_D$ −111° (*c* = 1, $CHCl_3$); NMR, δ 0.63 (3H, s, 18-H_3), 0.87 (6H, d, *J* = 6.7 Hz, 26-H_3 and 27-H_3), 0.95 (3H, s, 19-H_3), 1.03 (3H, d, *J* = 6.8 Hz, 21-H_3), 3.64 (1H, m, 3-H), 5.25 (2H, br m, 22-H and 23-H), 5.38 and 5.57 (2H, ABq, *J* ~ 6 Hz, 7-H and 6-H); UVλ_{max} 281 nm; IR, ν_{max} 3436, 1461, 1382, 1366, 1062, 1036, 968 cm^{-1}; MS, *m/z* 382 (M^+, 100), 349 (M^+-H_2O-Me, 71), 323 (34), 271 (M^+-side chain, 16), 253 (M^+-side chain-H_2O, 32).

(22Z)-3β-(Methoxymethoxy)-5α,8α-(4-phenyl-1,2-urazolo)cholesta-6,22-diene (**4**). The phosphonium bromide **2c** (*21*) (1.67 g, 4.04 mmol) in dry THF (73 ml) was treated with *n*-butyllithium (1.7 M solution in hexane, 2.42 ml, 4.11 mmol) at 3–5°C with stirring. After it was stirred for 1 h at room temperature, the orange-red solution was cooled to 3°C and aldehyde **1b** (*15*) (1.84 g, 3.36 mmol) in dry THF (24 ml) was added. The colorless reaction mixture was stirred overnight at room temperature and then poured into water and extracted with benzene. The organic extract was washed with 5% HCl, saturated sodium bicarbonate, and water; dried (Na_2SO_4); and concentrated *in vacuo* to an oil, which was purified on a column of silica gel. Elution with benzene–ether (94 : 6) afforded adduct **4** (1.38 g, 68%) as a foam: NMR, δ 0.83 (3H, s, 18-H_3), 0.89 and 0.91 (6H, 2 × d, *J* = 6.8 Hz, 26-H_3 and 27-H_3), 0.97 (3H, d, *J* = 6.8 Hz, 21-H_3), 0.98 (3H, s, 19-H_3), 3.30 (1H, dd, J_1 = 4.4 Hz, J_2 = 14 Hz, 9-H), 3.38 (3H, s, –OCH_3), 4.33 (1H, m, 3-H), 4.70 and 4.81 (2H, ABq, *J* = 6.8 Hz, OCH_2O), 5.21 (2H, br m, 22-H and 23-H), 6.23 and 6.39 (2H, ABq, *J* = 8.5 Hz, 6-H and 7-H), 7.41 (5H, br m, Ar-*H*); IR, ν_{max} 1756, 1703, 1601, 1397, 1046 cm^{-1}; MS, *m/z* 601 (M^+, 1%), 426 (M^+-RDA, 4), 364 (M^+-RDA-MeOCH_2OH, 61), 349 (M^+-RDA-MeOCH_2OH-Me, 16), <253 (M^+-RDA-MeOCH_2OH-side chain, 18), 251 (18), 119 (PhNCO, 100).

(22Z)-5α,8α-(4-Phenyl-1,2-urazolo)-cholesta-6,22-dien-3β-ol (**5b**). A solution of adduct **4** (601 mg, 1 mmol) and *p*-toluenesulfonic acid (523 mg, 2.75 mmol) in a methanol (20 ml)–THF (12 ml) mixture was stirred for 2 days at room temperature. The reaction mixture was poured into saturated sodium bicarbonate solution and extracted several times with benzene. Extracts were washed with water, dried (Na_2SO_4), and evaporated under reduced pressure. Purification of the crude product by column chromatography (benzene–ether 70 : 30 as eluant) gave the hydroxy adduct **5b** (550 mg, 99%) as a foam: NMR, δ 0.83 (3H, s, 18-H_3), 0.89 and 0.91 (6H, 2xd, *J* = 6.8 Hz, 26-H_3 and 27-H_3), 0.95 (3H, s, 19-H_3), 0.98 (3H, d, *J* = 6.8 Hz, 21-H_3), 3.16 (1H, dd, J_1 = 4.4 Hz, J_2 = 14 Hz, 9-H), 4.44 (1H, m, 3-H), 5.22 (2H, br m, 22-H and 23-H), 6.22 and 6.39 (2H, ABq, *J* = 8.5 Hz, 6-H and 7-H), 7.40 (5H, br m, Ar-*H*); IR, ν_{max} 3447, 1754, 1700, 1600, 1396

cm^{-1}; MS, *m/z* 557 (M^+, <1%), 382 (35), 349 (33), 253 (20), 251 (33), 119 (100), 55 (82).

(22Z)-Cholesta-5,7-dien-3β-ol (**6b**). The adduct **5b** (530 mg, 0.95 mmol) was converted to the diene **6b** by the procedure described above for **5a** to **6a** using lithium aluminum hydride (1 g) in THF (60 ml). The product was purified by chromatography on silica (benzene–ether 94 : 6 as eluant) to afford pure diene **6b** (290 mg, 76%) after crystallization from ethanol: mp 148–151°C (lit. (9) 147–150°C); $[\alpha]_D^{24}$ −132° (*c* = 0.9, $CHCl_3$); NMR, δ 0.66 (3H, s, 18-H_3), 0.90 and 0.91 (6H, 2xd, *J* = 6.8 Hz, 26-H_3 and 27-H_3), 0.96 (3H, s, 19-H_3), 0.98 (3H, d, *J* = 6.9 Hz, 21-H_3), 3.64 (1H, m, 3-H), 5.20 (2H, br m, 22-H and 23-H), 5.39 and 5.57 (2H, ABq, *J* ~ 6 Hz, 7-H and 6-H); UV, λ_{max} 281 nm; IR, ν_{max} 3346, 1463, 1375, 1364, 1067, 1040, 831 cm^{-1}; MS, *m/z* 382 (M^+, 100), 349 (65), 323 (32), 271 (15), 253 (30).

Attempted isomerization of a C_{22}–C_{23} double bond in **5b**. Adduct **5b** (10 mg) was refluxed in benzene (20 ml) containing iodine (75 mg) for 6 h. The cooled solution was washed with sodium thiosulfate and water, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was partially purified by chromatography over silica (benzene–ether 6 : 4 as eluant) and eluted material (3.2 mg) was refluxed with excess lithium aluminum hydride in THF for 16 h. After the usual workup, the crude diene was subjected to HPLC using 2% 2-propanol in hexane as eluant. Compound collected at 57 ml (2 mg) was identical in all respects with the 22Z-diene **6b**. No trace of the *trans* isomer **6a** was detected.

(5Z,7E,22E)-9,10-Secocholesta-5,7,10(19),22-tetraen-3β-ol (*trans-Δ^{22}-vitamin D_3*) (**8a**). A solution of **6a** (100 mg, 0.26 mmol) in an ether (120 ml)–benzene (30 ml) mixture was degassed with argon for 40 min. The solution was irradiated at 0°C for 13 min in a quartz immersion well equipped with a UV lamp and filter. The solvent was removed under reduced pressure and the residue separated by HPLC using 1% 2-propanol in hexane as eluant. Pure previtamin **7a** (40.4 mg, 40%) was collected at 24 ml: NMR, δ 0.72 (3H, s, 18-H_3), 0.87 (6H, d, *J* = 6.7 Hz, 26-H_3 and 27-H_3), 1.04 (3H, d, *J* = 6.8 Hz, 21-H_3), 1.65 (3H, s, 19-H_3), 3.91 (1H, m, 3-H), 5.28 (2H, br m, 22-H and 23-H), 5.50 (1H, m, 11-H), 5.69 and 5.96 (2H, ABq, *J* = 12.5 Hz, 7-H and 6-H); UV λ_{max} 260.5 nm, λ_{min} 234 nm. The previtamin **7a** (40 mg, 0.1 mmol) in ethanol (100 ml) was heated under reflux for 3 h. After removal of solvent, the resulting mixture of **8a** and **7a** was separated by HPLC (elution with 1% 2-propanol in hexane). Yield of the vitamin **8a** (collected at 34 ml) was 30.8 mg (77%); mp (hexane) 99–101°C (lit. (9) 101–104°C); NMR, δ 0.56 (3H, s, 18-H_3), 0.88 (6H, d, *J* = 6.7 Hz, 26-H_3 and 27-H_3), 1.02 (3H, d, *J* = 6.6 Hz, 21-H_3), 3.96 (1H, m, 3-H), 4.82 and 5.05 (2H, 2 × narr m, 19-H_2), 5.27 (2H, br m, 22-H and 23-H), 6.03 and 6.24 (2H, ABq, *J* = 11.4 Hz, 7-H and 6-H); UV, λ_{max} 265 nm, λ_{min} 228 nm; IR, ν_{max} 3420, 1458, 1441, 1378, 1366, 1050, 970, 943, 891, 862 cm^{-1}; MS, *m/z* 382 (M^+, 22), 349 (M^+-H_2O-Me, 4), 271 (M^+-side chain, 8), 253 (M^+-side chain-H_2O, 13), 136 (100), 118 (80).

(5Z,7E,22Z)-9,10-Secocholesta-5,7,10(19),22-tetraen-3β-ol (*cis-Δ^{22}-vitamin D_3*) (**8b**). Irradiation of diene **6b** (150 mg, 0.39 mmol), under the conditions described in the preceding experiment, followed by HPLC of the resulting mixture afforded previtamin **7b** (56.9 mg, 38%) as a colorless oil: NMR, δ 0.75 (3H, s, 18-H_3), 0.90 and 0.91 (6H, 2xd, *J* = 6.7 Hz, 26-H_3 and 27-H_3), 0.99 (3H, d, *J* = 6.8 Hz, 21-H_3),

1.64 (3H, s, 19-H_3), 3.90 (1H, m, 3-H), 5.20 (2H, br m, 22-H and 23-H), 5.69 and 5.95 (2H, ABq, J = 12 Hz, 7-H and 6-H); UV, λ_{max} 261 nm, λ_{min} 234 nm. Thermal isomerization of **7b** (56 mg, 0.15 mmol) in refluxing ethanol gave an oily vitamin **8b** (43 mg, 77%) after separation by HPLC. NMR, δ 0.60 (3H, s, 18-H_3), 0.89 and 0.90 (6H, 2xd, J = 6.7 Hz, 26-H_3 and 27-H_3), 0.97 (3H, d, J = 6.6 Hz, 21-H_3), 3.96 (1H, s, 3-H), 4.82 and 5.05 (2H, each a narr m, 19-H_2), 5.20 (2H, br m, 22-H and 23-H), 6.04 and 6.24 (2H, ABq, J = 11.4 Hz, 7-H and 6-H); UV, λ_{max} 265.5 nm, λ_{min} 228 nm; IR, ν_{max} 3427, 1458, 1379, 1048, 966, 943, 892 cm^{-1}; MS, *m/z* 382 (M^+, 21) 349 (5), 271 (8), 253 (14), 136 (100), 118 (82).

(5Z,7E,22E)-3β-Acetoxy-9,10-secocholesta-5,7,10(19),22-tetraen-1α-ol (*trans-*Δ^{22}*-1α-hydroxyvitamin* D_3 *3-acetate*) (**10a**). Freshly recrystallized *p*-toluenesulfonyl chloride (50 mg, 0.26 mmol) was added to a solution of vitamin **8a** (30 mg, 0.08 mmol) in dry pyridine (300 μl). After 30 h at 4°C, the reaction mixture was poured into ice/saturated $NaHCO_3$ with stirring. The mixture was stirred for 15 min and extracted with benzene. The organic extract was washed with saturated $NaHCO_3$, saturated copper sulfate, and water; dried (Na_2SO_4); and concentrated *in vacuo* to an oil. The crude tosylate was treated with $NaHCO_3$ (150 mg) in anhydrous methanol (10 ml) and the mixture was stirred for 8.5 h at 55°C. After cooling and concentration to ~2 ml, the mixture was diluted with benzene (80 ml), washed with water, dried (Na_2SO_4), and evaporated under reduced pressure. The resulting oily 3,5-cyclovitamin was sufficiently pure to be used for the following oxidation step.

To a vigorously stirred suspension of SeO_2 (4 mg, 0.036 mmol) in dry CH_2Cl_2 (5 ml), t-butylhydroperoxide (13.2 μl, 0.094 mmol) was added. After 30 min, dry pyridine (40 μl) was added, and the mixture was stirred for an additional 25 min at room temperature, diluted with CH_2Cl_2 (3 ml), and cooled to 0°C. The crude 3,5-cyclovitamin product in CH_2Cl_2 (4.5 ml) was then added. The reaction proceeded at 0°C for 15 min and then was allowed to warm slowly (30 min) to room temperature. The mixture was transferred to a separatory funnel and shaken with 30 ml of 10% NaOH. Ether (150 ml) was added and the organic phase was washed with 10% NaOH and water and dried over Na_2SO_4. Concentration to dryness *in vacuo* gave a yellow oily residue which was purified on a silica gel TLC plate developed in 7 : 3 hexane : ethyl acetate (R_f 0.35) to give hydroxycyclovitamin **9a** (14.4 mg, 45%): NMR, δ 0.55 (3H, s, 18-H_3), 0.64 (1H, m, 3-H), 0.88 (6H, d, J = 6.9 Hz, 26-H_3 and 27-H_3), 1.03 (3H, d, J = 6.9 Hz, 21-H_3), 3.26 (3H, s, –OCH_3), 4.2 (2H, m, 1-H and 6-H), 4.95 (1H, d, J = 9.3 Hz, 7-H), 5.1–5.4 (4H, br m, 19-H_2, 22-H and 23-H); MS, *m/z* 412 (M^+, 27), 380 (M^+-MeOH, 46), 339 (22), 269 (M^+-side chain-MeOH, 29), 245 (18), 135 (100). A solution of cyclovitamin **9a** (12 mg) in glacial acetic acid (0.5 ml) was heated at 55°C for 15 min. The mixture was carefully poured into ice/saturated $NaHCO_3$ and extracted with benzene and ether. The combined extracts were washed with water, dried (Na_2SO_4), and evaporated. Products were subjected to HPLC (1.5% 2-propanol in hexane as eluant) yielding, in elution order, **12a** (0.87 mg, 7%, rv 38 ml), **10a** (4.90 mg, 38%, rv 42 ml), and **11a** (3.10 mg, 24%, rv 50 ml).

10a: NMR, δ 0.56 (3H, s, 18-H_3), 0.88 (6H, d, J = 7.0 Hz, 26-H_3 and 27-H_3), 1.02 (3H, d, J = 6.8 Hz, 21-H_3), 2.04 (3H, s, –OCOCH_3), 4.41 (1H, m, 1-H), 5.02 (1H,

narr m, 19-H), 5.1–5.4 (4H, br m, 3-, 19-, 22-, and 23-H), 6.03 and 6.35 (2H, ABq, J = 11.4 Hz, 7-H and 6-H); UV, λ_{max} 264 mm, λ_{min} 227.5 nm; MS, *m/z* 440 (M^+, 15), 380 (M^+-HOAc, 84), 362 (M^+-HOAc-H_2O, 9), 269 (M^+-side chain -HOAc, 40), 251 (15), 135 (100), 134 (94).

11a: NMR, δ 0.57 (3H, s, 18-H_3), 0.89 (6H, d, J = 7.0 Hz, 26-H_3 and 27-H_3), 1.03 (3H, d, J = 6.8 Hz, 21-H_3), 2.04 (3H, s, –OCOCH_3), 4.49 (1H, m, 1-H), 5.00 and 5.14 (2H, 2 × narr m, 19-H_2), 5.25 (3H, br m, 3-, 22-, and 23-H), 5.81 and 6.58 (2H, ABq, J = 12.0 Hz, 7-H and 6-H); UV, λ_{max} 269.5 nm, λ_{min} 228 nm; MS, *m/z* 440 (M^+, 6), 380 (47), 269 (15), 135 (100), 134 (62).

12a: NMR, δ 0.55 (3H, s, 18-H_3), 0.87 (6H, d, J = 6.9 Hz, 26-H_3 and 27-H_3), 1.01 (3H, d, J = 6.9 Hz, 21-H_3), 2.06 (3H, s, –OCOCH_3), 4.17 (1H, m, 1-H), 4.99 (2H, m, 3-H and 19-H), 5.1–5.4 (3H, br m, 19-, 22-, and 23-H), 6.00 and 6.38 (2H, ABq, J = 11.3 Hz, 7-H and 6-H); UV, λ_{max} 262.5 nm, λ_{min} 227 nm; MS, *m/z* 440 (M^+, 27), 380 (78), 362 (12), 269 (28), 251 (20), 135 (100), 134 (78).

(5Z,7E,22Z)-3β-Acetoxy-9,10-secocholesta-5,7,10(19),22-tetraen-1α-ol (cis-Δ^{22}*-1α-hydroxyvitamin D_3 3-acetate)* (**10b**). Tosylation of vitamin **8b** (50 mg, 0.13 mmol) and methanolysis of the 3-tosylate were achieved by the method described above for the 22*E* isomer **8a**. Oxidation of the crude 3,5-cyclovitamin product with SeO_2 (5.1 mg, 0.046 mmol) and t-butylhydroperoxide (16.5 μl, 0.118 mmol) in CH_2Cl_2 (12.5 ml) containing pyridine (50 μl) afforded after preparative TLC the oily hydroxycyclovitamin **9b** (20 mg, 37%): NMR, δ 0.59 (3H, s, 18-H_3), 0.63 (1H, m, 3-H), 0.89 and 0.90 (6H, 2xd, J = 6.9 Hz, 26-H_3 and 27-H_3), 0.96 (3H, d, J = 6.9 Hz, 21-H_3), 3.25 (3H, s, –OCH_3), 4.17 (2H, m, 1-H and 6-H), 4.96 (1H, d, J = 9.3 Hz, 7-H), 5.1–5.4 (4H, br m, 19-H_2, 22-H and 23-H); MS, *m/z* 412 (M^+, 26), 380 (48), 339 (22), 269 (28), 245 (20), 135 (100). The analogously performed cycloreversion of cyclovitamin **9b** (18 mg) in glacial acetic acid (0.8 ml) yielded, after HPLC separation (1.5% 2-propanol–hexane as eluant), pure 3β-acetoxyvitamins **12b** (1.44 mg, 7%, rv 36 ml), **10b** (6.60 mg, 34%, rv 42 ml), and **11b** (4.20 mg, 22%, rv 50 ml).

10b: NMR δ 0.60 (3H, s, 18-H_3), 0.90 and 0.92 (6H, 2xd, J = 7.0 Hz, 26-H_3 and 27-H_3), 0.97 (3H, d, J = 6.8 Hz, 21-H_3), 2.04 (3H, s, –OCOCH_3), 4.41 (1H, m, 1-H), 5.02 (1H, narr m, 19-H), 5.1–5.4 (4H, br m, 3-, 19-, 22- and 23-H), 6.03 and 6.35 (2H, ABq, J = 11.4 Hz, 7-H and 6-H); UV, λ_{max} 264.5 nm, λ_{min} 227.5 nm; MS, *m/z* 440 (M^+, 10), 380 (72), 362 (7), 269 (31), 251 (12), 135 (100), 134 (99).

11b: NMR δ 0.60 (3H, s, 18-H_3), 0.90 and 0.91 (6H, 2xd, J = 7.0 Hz, 26-H_3 and 27-H_3), 0.97 (3H, d, J = 6.9 Hz, 21-H_3), 2.05 (3H, s, –OCOCH_3), 4.49 (1H, m, 1-H), 5.00 and 5.14 (2H, 2 × narr m, 19-H_2), 5.20 (3H, br m, 3-, 22-, and 23-H), 5.82 and 6.59 (2H, ABq, J = 12.0 Hz, 7-H and 6-H); UV, λ_{max} 270 nm, λ_{min} 228 nm; MS, *m/z* 440 (M^+, 4), 380 (30), 269 (10), 135 (100), 134 (52).

12b: NMR, δ 0.58 (3H, s, 18-H_3), 0.89 and 0.90 (6H, 2xd, J = 6.9 Hz, 26-H_3 and 27-H_3), 0.96 (3H, d, J = 6.9 Hz, 21-H_3), 2.06 (3H, s, –OCOCH_3), 4.16 (1H, m, 1-H), 4.98 (2H, m, 3-H and 19-H), 5.1–5.4 (3H, br m, 19-, 22-, and 23-H); UV λ_{max} 263 nm, λ_{min} 227 nm; MS, *m/z* 440 (M^+, 32), 380 (78), 362 (21), 269 (28), 251 (19), 135 (100), 134 (82).

Hydrolysis of the 3β-acetoxy group in **10, 11,** *and* **12**. The same procedure was used to hydrolyze the acetoxy groups in vitamins **10**, **11**, and **12**. The solution of

3β-acetoxyvitamin (0.7–6 mg) in ethanol (0.1 ml) was treated with 10% KOH in methanol (0.8 ml) and the mixture was heated for 1 h at 50°C. After the usual workup and final HPLC purification (8% 2-propanol in hexane as eluant), the corresponding 1-hydroxyvitamins were obtained: **13a** (84%, rv 40 ml), **13b** (83%, rv 39 ml), **14a** (72%, rv 38 ml), **14b** (77%, rv 38 ml), **15a** (70%, rv 33 ml), and **15b** (70%, rv 32 ml).

13a: NMR, δ 0.56 (3H, s, 18-H_3), 0.87 (6H, d, J = 7.0 Hz, 26-H_3 and 27-H_3), 1.02 (3H, d, J = 6.8 Hz, 21-H_3), 4.22 (1H, m, 3-H), 4.42 (1H, m, 1-H), 5.00 (1H, narr m, 19-H), 5.1–5.4 (3H, br m, 19-, 22-, and 23-H), 6.01 and 6.38 (2H, ABq, J = 11.4 Hz, 7-H and 6-H); UV, λ_{max} 264.5 nm, λ_{min} 227.5 nm; MS, m/z 398 (M^+, 21), 380 (M^+-H_2O, 9), 287 (M^+-side chain, 6), 269 (M^+-side chain-H_2O, 8), 251 (5), 152 (38), 134 (100).

13b: NMR, δ 0.59 (3H, s, 18-H_3), 0.89 and 0.90 (6H, 2xd, J = 7.0 Hz, 26-H_3 and 27-H_3), 0.96 (3H, d, J = 6.8 Hz, 21-H_3), 4.23 (1H, m, 3-H), 4.43 (1H, m, 1-H), 5.00 (1H, narr m, 19-H), 5.1–5.4 (3H, br m, 19-, 22-, and 23-H), 6.02 and 6.39 (2H, ABq, J = 11.4 Hz, 7-H and 6-H); UV, λ_{max} 264.5 nm, λ_{min} 227.5; MS, m/z 398 (M^+, 21), 380 (8), 287 (6), 269 (7), 251 (5), 152 (36), 134 (100).

14a: NMR, δ 0.58 (3H, s, 18-H_3), 0.87 (6H, d, J = 7.0 Hz, 26-H_3 and 27-H_3), 1.03 (3H, d, J = 6.8 Hz, 21-H_3), 4.24 (1H, m, 3-H), 4.49 (1H, m, 1-H), 4.97 and 5.13 (2H, 2 × narr m, 19-H_2), 5.25 (2H, br m, 22-H and 23-H), 5.88 and 6.58 (2H, ABq, J = 11.5 Hz, 7-H and 6-H); UV, λ_{max} 273 nm, λ_{min} 229.5 nm; MS, m/z 398 (M^+, 21), 380 (5), 287 (6), 269 (5), 251 (4), 152 (33), 134 (100).

14b: NMR, δ 0.61 (3H, s, 18-H_3), 0.89 and 0.91 (6H, 2xd, J = 7.0 Hz, 26-H_3 and 27-H_3), 0.97 (3H, d, J = 6.9 Hz, 21-H_3), 4.25 (1H, m, 3-H), 4.51 (1H, m, 1-H), 4.98 and 5.13 (2H, 2 × narr m, 19-H_2), 5.21 (2H, br m, 22-H and 23-H), 5.89 and 6.59 (2H, ABq, J = 11.5 Hz, 7-H and 6-H); UV, λ_{max} 273 nm, λ_{min} 229.5 nm; MS, m/z 398 (M^+, 17), 380 (4), 287 (5), 269 (5), 251 (4), 152 (29), 134 (100).

15a: NMR, δ 0.56 (3H, s, 18-H_3), 0.87 (6H, d, J = 7.1 Hz, 26-H_3 and 27-H_3), 1.02 (3H, d, J = 6.8 Hz, 21-H_3), 4.10 (1H, m, 3-H), 4.35 (1H, m, 1-H), 5.01 (1H, d, J = 2 Hz, 19-H), 5.1–5.4 (3H, br m, 19-, 22-, and 23-H), 6.05 and 6.45 (2H, ABq, J = 11.3 Hz, 7-H and 6-H); UV, λ_{max} 263 nm, λ_{min} 226.5 nm; MS, m/z 398 (M^+, 19), 380 (14), 269 (11), 251 (10), 152 (100), 134 (58).

15b: NMR, δ 0.60 (3H, s, 18-H_3), 0.89 and 0.91 (6H, 2xd, J = 7.0 Hz, 26-H_3 and 27-H_3), 0.97 (3H, d, J = 6.9 Hz, 21-H_3), 4.10 (1H, m, 3-H), 4.36 (1H, m, 1-H), 5.01 (1H, d, J = 2 Hz, 19-H), 5.1–5.4 (3H, br m, 19-, 22-, and 23-H), 6.06 and 6.45 (2H, ABq, J = 11.3 Hz, 7-H and 6-H); UV, λ_{max} 262.5 nm, λ_{min} 226.5 nm; MS, m/z 398 (M^+, 20), 380 (19), 269 (11), 251 (10), 152 (100), 134 (60).

BIOASSAYS

Rats. Weanling male rats were purchased from the Holtzman Company (Madison, WI) and fed either a low-calcium/adequate-phosphorus (0.02% calcium, 0.3% phosphorus) vitamin D-deficient diet or a high-calcium/low-phosphorus (1.2% calcium, 0.1% phosphorus) vitamin D-deficient diet for 3 weeks. Each rat received

the dose test compound by osmotic minipump set to deliver the indicated daily dose of compound.

Determination of serum calcium and inorganic phosphorus concentration. Serum calcium was determined in the presence of 0.1% lanthanum chloride by means of a Perkin–Elmer atomic absorption spectrometer Model 403. Serum inorganic phosphorus was determined by the colorimetric assay of Chen *et al.* (*22*).

Measurement of antirachitic activity. Antirachitic activity was measured by the line test method as described in the U.S. Pharmacopeia (*23*). Radii and ulnae of rats were removed, split lengthwise, and stained in 1.5% silver nitrate solution to allow scoring of the new calcification in the epiphyseal plate.

COMPETITIVE RECEPTOR BINDING ASSAY

Displacement of 1,25-$(OH)_2$-[^3H]D_3 from chick intestinal cytosol receptor protein for 1,25-$(OH)_2D_3$ by analogs **13**, **14**, *and* **15**. Various concentrations of C(1)-hydroxylated compounds were dissolved in 50 μl of 95% ethanol and incubated for 16 h in an ice bath with the receptor preparation in the presence of 1α,25-$(OH)_2$-[26,27-^{3}H]D_3 as described by Shepard *et al.* (*24*). Vitamin D_2 and vitamin D_3 were purchased from Aldrich Chemical Company (Milwaukee, WI). 1α-OH-D_3 was a gift from Leo Pharmaceuticals (Ballerup, Denmark). 1α,25-$(OH)_2$-[26,27-^{3}H]D_3 was synthesized by a previously described method (*25*).

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