BIOORGANIC CHEMISTRY 6, 203–218 (1977)

Synthesis and Bioassay of 3-Deoxy-1α-Hydroxyvitamin D_3, an Active Analog of 1α,25-Dihydroxyvitamin D_3

B. L. ONISKO, H.-Y. LAM,[1] L. E. REEVE, H. K. SCHNOES, AND H. F. DELUCA

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin–Madison, Madison, Wisconsin 53706

Received December 10, 1976

Two synthetic routes to 3-deoxy-1α-hydroxyvitamin D_3, an analog of 1α,25-dihydroxyvitamin D_3, are described. One involved the six-step conversion of 1α,2α-epoxy-6,6-ethylenedioxy-5α-cholestan-3-one to 1α-acetoxycholest-5-ene, whereas, in the second, the same intermediate was prepared from 1α-hydroxycholesterol. Conversion of the Δ^5-sterol to the required 5,7-diene was accomplished most efficiently via 7-keto and 7-tosylhydrazone intermediates. Bioassay of 3-deoxy-1α-hydroxyvitamin D_3 in the rat establishes that the analog can fulfill all common vitamin D functions including stimulation of intestinal calcium transport, mobilization of calcium and phosphate from bone, stimulation of growth, and calcification of bone. Direct comparison indicates the compound to have 1/20 to 1/50 of the activity of 1α-hydroxyvitamin D_3, but it acts with a time course indistinguishable from the latter.

It is well established that the biological activity of vitamin D_3 is due to metabolic conversion to 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2D_3$, **1**],[2] a hormone responsible for inducing intestinal calcium transport, bone calcium mobilization, and intestinal phosphate transport (*1*). Vitamin D_3 is hydroxylated at C-25 (primarily in the liver) to produce 25-hydroxyvitamin D_3 (25-OH-D_3, **2**), which is subsequently converted to 1α,25-$(OH)_2D_3$ by a kidney mitochondrial enzyme as a response to hypocalcemia or hypophosphatemia. This two-step transformation naturally raises the question of the relative functional significance of the various hydroxy groups in 1α,25-$(OH)_2D_3$.

Comparative biological activity data on vitamin D_3 metabolites and several structural analogs have established that the 1α- and 25-hydroxyl groups fulfill important haptophoric functions. Thus, 25-OH-D_3 administered in physiologic amounts is inactive *in vivo* when 1α-hydroxylation is prevented by nephrectomy, and the compound is capable of inducing calcium transport in isolated, vascularly perfused rat intestine (*2*, *3*), of stimulating release of calcium from fetal rat bone in organ culture (*4*), and of effective competition with 1α,25-$(OH)_2D_3$ for intestinal receptor sites (*5–7*) only at concentrations two to three orders of magnitude higher than those required for 1α,25-$(OH)_2D_3$. Similarly, the 25-deoxy analog, 1α-hydroxyvitamin D_3 (1α-OH-D_3, **3**) is about 1000 times less active than 1α,25-$(OH)_2D_3$ in the *in vitro* bone resorption

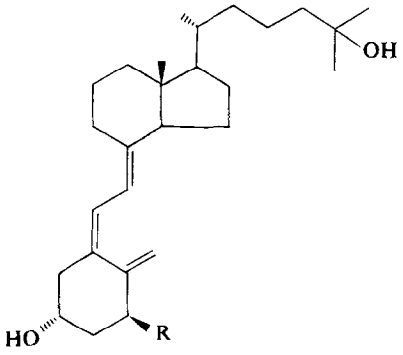
[1] Present address: Manitoba Institute of Cell Biology, 700 Bannatyne Avenue, Winnipeg, Canada R3E OV9.

[2] Abbreviations used: 1α,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; 25-OH-D_3, 25-hydroxyvitamin D_3; 1α-OH-D_3, 1α-hydroxyvitamin D_3; 3-deoxy-1α-OH-D_3, 3-deoxy-1α-hydroxyvitamin D_3.

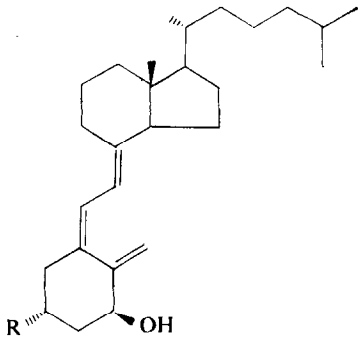
ISSN 0045-2068

system (*8*) and the intestinal receptor assay (*5–7*), and the pronounced *in vivo* potency of the substance [approximately that of 1α,25-$(OH)_2D_3$ (*9*, *10*)] is now known to be a consequence of its rapid metabolism in rats and chicks to 1α,25-$(OH)_2D_3$ (*11*, *12*).

To assess the functional significance of the 3β-hydroxyl group, we prepared 3-deoxy-1α-hydroxyvitamin D_3 (3-deoxy-1α-OH-D_3, **4**). The present paper describes synthetic details of our work and provides an extension of the preliminary biological data communicated earlier (*13*). In the interim, preliminary reports of a preparation of **4**, and its bioassay in the chick gut calcium transport system (*14*), and details of the synthesis of the corresponding provitamin (*15*) have appeared.



1: R = OH, 1α,25-$(OH)_2D_3$
2: R = H, 25-OH-D_3



3: R = OH, 1α-OH-D_3
4: R = H, 3-deoxy-1α-OH-D_3

EXPERIMENTAL

Materials and Methods

Mass spectra were obtained on an AEI Model MS-902 mass spectrometer at 70 eV using a direct probe for introduction of samples (source temperature, 100–150°C above ambient); high resolution mass spectra were measured on the same instrument coupled to an AEI Model DS-50 data system and using perfluorokerosene as an internal mass standard. Ultraviolet absorption spectra were recorded on a Beckman Model 25 instrument; nuclear magnetic resonance spectra were taken on either a Bruker 90 MHz, a Varian T-60, or a Bruker 270 MHz spectrometer using $CDCl_3$ as solvent and tetramethylsilane as internal standard; infrared absorption spectra were obtained with a Perkin–Elmer Model 567 instrument; optical rotations were measured on a Perkin–Elmer Model 141 polarimeter with a Brinkmann Instruments Model K-2/R temperature controller. Gas–liquid chromatography was carried out on an F and M Model 402 chromatograph equipped with a glass column (1.1 m × 3 mm) packed with 3% SE-30 on 100–120 mesh Gas Chrom Q, operated isothermally at 240°C at a He flow rate of 80 ml/min. For thin layer chromatography, air-dried silica gel G plates (0.25 mm thick) were used. Silica gel (Davison Chemical, grade 923, 100–200 mesh) was used for column chromatography. High-pressure liquid chromatography was performed on a Dupont Model 830 liquid chromatograph with a Waters Model U6K injector; two Zorbax-SIL

(Dupont, 25 cm × 2.1 mm) columns were used in series. Commercial Skellysolve B was distilled and the fraction boiling at 67–69°C was used. Pyridine and benzene were distilled before use and dried over molecular sieves. Other solvents were reagent grade and used as such. Melting points were measured on a Thomas Hoover apparatus and are uncorrected. Elemental analyses were performed by Micro-Tech Laboratories, Skokie, Illinois.

Synthesis of 3-Deoxy-1α-Hydroxyvitamin D_3 (**4**)

6,6-Ethylenedioxy-5α-cholest-2-en-1α-ol (**6**). A solution of 2.0 g of epoxide **5**[3] [mp 98.5–100°C; nmr ($CDCl_3$) δ 3.9 (*m*, 4*H*), 3.46 (*d*, 1*H*, $J = 4.2$ Hz), 3.2 (*d*, 1*H*, $J = 4.2$ Hz); mass spectrum *m/e* (relative intensity) 458 (M^+, 30), 291 (186), 99 (100); Anal. Calcd for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.71; H, 10.27] in 15 ml of hydrazine hydrate was refluxed for 15 min, diluted with 50 ml of H_2O, and extracted three times with ether. The ether layer was washed with 20 ml of H_2O, dried over Na_2SO_4, and evaporated. The residue was applied to a silicic acid column (180 g) in Skellysolve B. Elution with mixtures of ethyl acetate/Skellysolve B (200 ml of 2% ethyl acetate in Skellysolve B, followed by 200 ml of 5%, 300 ml of 10%, 200 ml of 15%, 600 ml of 20%, and finally 200 ml of 30% ethyl acetate in Skellysolve B) gave (in the 20% ethyl acetate eluant) 720 mg (35%) of the oily 1α-hydroxy compound **6** which was homogeneous on tlc. After several weeks at –4°C, this oily compound solidified: mp 84–90°C; nmr, δ 5.85 (*m*, 2*H*, C-2,3), 3.93 (*m*, 4*H*, ketal), 3.66 (*m*, 1*H*, C-1); mass spectrum *m/e* (relative intensity) 444 (M^+, 66), 375 (95), 291 (100).

6,6-Ethylenedioxy-5α-cholestan-1α-ol (**7**). A solution of 720 mg of allylic alcohol **6** in 60 ml of cyclohexane was hydrogenated under 1 atm of H_2 at room temperature for 4 hr, using 700 mg of 5% Pd/C as catalyst. The solution was then filtered, the filtrate was evaporated to dryness, and the residue was recrystallized from Skellysolve B to give 660 mg (92% yield) of **7**: mp 96–98°C; nmr, δ 3.93 (*m*, 4*H*, ketal), 3.65 (*m*, 1*H*, C-1); ir ($CHCl_3$) 3480 (OH), 1205–1400 cm^{-1} (four bands, ketal); mass spectrum, *m/e* (relative intensity) 446 (M^+, 39), 291 (100). Anal. Calcd for $C_{29}H_{50}O_3$: C, 77.94; H, 11.28. Found: C, 78.07; H, 11.54.

1α-Hydroxy-5α-cholestan-6-one (**8**). Ketal **7** (660 mg) was dissolved in 8 ml of MeOH and 10 ml of 95% EtOH containing 40 mg of *p*-toluene sulfonic acid monohydrate was added. This solution was stirred at room temperature for 16 hr. A 5% $NaHCO_3$ solution was then added and after extraction with ether, drying (Na_2SO_4), and evaporation of the solvent, the residue was recrystallized from methanol/ether to give colorless crystals of **8**: mp 181–183°C (quantitative yield); nmr, δ 3.75 (*m*, 1*H*, C-1); ir ($CHCl_3$) 3480 (OH), 1700 cm^{-1} (ketone); mass spectrum, *m/e* (relative intensity) 402 (M^+, 83), 384 (86), 369 (20), 367 (23), 331 (49), 289 (43), 271 (23), 247 (23), 229 (60),

1α-Acetoxy-5α-cholestan-6-one (**9**). Alcohol **8** was acetylated in 12 ml of Ac_2O and 2 ml of pyridine at 50°C for 36 hr. After addition of 25 ml of H_2O, the product was extracted into ether. Drying (Na_2SO_4) and evaporation of the ether gave, after recrystallization of the residue from hot methanol, 400 mg of white crystals (**9**): mp 104–105°C; nmr ($CDCl_3$) δ 4.98 (*m*, 1*H*, C-1), 2.13 (*s*, 3*H*, acetate); ir ($CHCl_3$) 1710 (ketone), 1730 cm^{-1} (acetate); mass spectrum, *m/e* (relative intensity) 444 (M^+, 49),

[3] An intermediate available from our earlier synthesis of 1α-OH-D_3 (see Refs. *9* and *16*).

384 (84), 366 (48), 229 (26). Anal. Calcd for $C_{29}H_{48}O_3$: C, 78.33; H, 10.88. Found: C, 77.56; H, 11.04.

1α-Acetoxy-5α-cholestan-6β-ol (**10**). To a solution of 300 mg of compound **9** in 20 ml of isopropanol, 64 mg of $NaBH_4$, dissolved in 5 ml of isopropanol, was added. After stirring at room temperature for 16 hr, 20 ml of H_2O containing 1 drop of 3% H_2SO_4 was added. Extraction with ether, drying (Na_2SO_4), and evaporation of the combined ether layers gave a residue which was chromatographed on silicic acid (15 g). Elution with 20% ethyl acetate in Skellysolve B yielded 260 mg (86%) of pure 6β-alcohol **10**. Crystallization from MeOH gave white needles: mp 127–128°C; nmr ($CDCl_3$) δ 4.75 (*m*, 1*H*, C-1); 3.86 (*m*, 1*H*, C-6), 2.06 (*s*, 3*H*, acetate); ir ($CHCl_3$) 3480 (alcohol), 1725 cm^{-1} (acetate); mass spectrum *m/e* (relative intensity) 446 (M^+, 1.2), 428 (13), 386 (39), 368 (100), 255 (25), 231 (23), 228 (52), 213 (45).

1α-Acetoxycholest-5-ene (**11**). To an ice-cold pyridine solution (0.5 ml) of 255 mg of hydroxy compound **10**, 0.8 ml of $POCl_3$ was added dropwise. The solution was then kept at room temperature for 5.5 hr. After addition of ice water, the solution was extracted with ether and the combined ether fractions were dried over Na_2SO_4 and evaporated. The residue was applied to a 10-g silicic acid column in Skellysolve B. Elution with 60 ml of Skellysolve B and then 10% ethyl acetate in Skellysolve B gave, in fraction 10–12 (8-ml fractions), the 1α-acetoxy compound **11** (yield, 230 mg, 94%). Crystallization from ethanol produced white crystals of mp 65–66°C; $[\alpha]_D^{20} - 17°$ (*c* 2.2, $CHCl_3$); nmr, δ 5.45 (*m*, 1*H*, C-6), 4.98 (*m*, 1*H*, C-1), 2.05 (*s*, 3*H*, acetate); ir ($CHCl_3$) 1725 cm^{-1} (acetate); mass spectrum *m/e* (relative intensity) 428 (M^+), 368 (100), 255 (39), 247 (34), 219 (24), 213 (28). Anal. Calcd for $C_{29}H_{48}O_2$: C, 81.25; H, 11.29. Found: C, 81.42; H, 11.38.

1α-Acetoxy-5,7-cholestadiene (**12**). Derivative **11** (150 mg), dissolved in 2 ml of Skellysolve B and 2 ml of benzene, was treated with 75 mg of *N,N'*-dibromo-5,5-dimethylhydantoin. The solution was kept at 70°C for 10 min and then cooled (ice bath, 5 min), filtered, and evaporated and the residue was taken up in xylene (2 ml) and added dropwise to a preheated solution of 0.25 ml of trimethyl phosphite in 1 ml of xylene at 135°C. After 1.75 hr at 135°C, the solvent was evaporated under reduced pressure and the products were separated on a column of Alumina (10 g). Elution with 5% ether in Skellysolve B furnished 9.3 mg (6.2% yield) of the 7-dehydrocholesterol derivative **12**: uv (ethanol) λ_{max} 295, 283, 273 nm; mass spectrum *m/e* (relative intensity) 426 (M^+, 12), 366 (100), 351 (23), 253 (24), 226 (26), 211 (61), 199 (44).

1α-Acetoxycholesteryl acetate (**14**). A solution of 2.42 g of 1α-hydroxycholesterol (**13**) (see Footnote 3) [nmr ($CDCl_3$) δ 5.60 (*m*, 1*H*, C-6), 4.04 (*t* of *t*, $J_1 = 11$, $J_2 = 5.5$ Hz, 1*H*, C-3) 3.84 (*d* of *d*, $J_1 = J_2 = 3$ Hz, 1*H*, C-1) 1.03 (*s*, 3*H*, C-19), 0.94 (*d*, $J = 6$ Hz, 3*H*, C-21), 0.87 (*d*, $J = 6$ Hz, 6*H*, C-26, 27), 0.69 (*s*, 3*H*, C-18); mass spectrum *m/e* (relative intensity) 402 (M^+, 54), 387 (20), 384 (100), 366 (49), 351 (15), 289 (13), 271 (26), 253 (19); homogeneous on tlc ($R_f = 0.05$, 20% ethyl acetate in Skellysolve B)] in 14 ml each of pyridine and acetic anhydride was heated at 70°C for 5 hr. Solvents were removed under vacuum at 75°C, and the oil was taken up in diethyl ether, washed twice with 2% aqueous HCl, once with saturated NaCl, and dried (Na_2SO_4). Evaporation of solvent produced an oil which solidified after several days at 0°C to provide 2.90 g (99%) of 1α-acetoxycholesteryl acetate (**14**): mp 91.5–93°C; ir (CCl_4) 1738 and 1240 cm^{-1} (acetate); nmr ($CDCl_3$) δ 5.53 (*m*, 1*H*, C-6), 5.05 (*d* of *d*, $J_1 = J_2 = 2.6$ Hz,

1*H*, C-1), 4.90 (*m*, 1*H*, C-3), 2.05 (*s*, 3*H*, acetate), 2.02 (*s*, 3*H*, acetate), 1.08 (*s*, 3*H*, C-19), 0.90 (*d*, $J = 5.6$ Hz, 3*H*, C-21), 0.86 (*d*, $J = 6.1$ Hz, 6*H*, C-26, 27), 0.67 (*s*, 3*H*, C-18); mass spectrum, *m/e* (relative intensity) 486 (M^+, 0.1), 366 (92), 351 (17), 253 (9), 118 (100); homogeneous on glc and tlc ($R_f = 0.70$, 20% ethyl acetate in Skellysolve B); Anal. Calcd for $C_{31}H_{50}O_4$: C, 76.50; H, 10.36. Found: C, 76.65; H, 10.59.

1α-Acetoxycholesterol (**15**). To 1α-acetoxycholesteryl acetate (**14**, 2.25 g) dissolved in 15 ml of diethyl ether, 15 ml of 0.1 *M* KOH/MeOH was added and the mixture was stirred at 0°C for 3 hr. After neutralization with acetic acid and addition of H_2O, the aqueous phase was extracted three times with diethyl ether (30 ml each). The combined ether phases were washed with saturated salt solution, dried (Na_2SO_4), and applied to a 100-g column of silica gel. Elution with 800 ml of 25% ethyl acetate in Skellysolve B followed by crystallization from methanol afforded 1.76 g (90%) of 1α-acetoxycholesterol (**15**): mp 88–89°C; ir (CCl_4) 3620, 1040, and 1020 cm^{-1} (3-β-alcohol), 1736 and 1241 cm^{-1} (acetate); nmr ($CDCl_3$) δ 5.51 (*m*, 1*H*, C-6), 5.04 (*d* of *d*, $J_1 = J_2 = 3.0$ Hz, 1*H*, C-1), 3.82 (*m*, 1*H*, $W_{1/2} = 22$ Hz, C-3), 2.03 (*s*, 3*H*, acetate), 1.07 (*s*, 3*H*, C-19), 0.89 (*d*, $J = 6.6$ Hz, 3*H*, C-21), 0.86 (*d*, $J = 6.1$ Hz, 6*H*, C-26,27), 0.66 (*s*, 3*H*, C-18); mass spectrum, *m/e* (relative intensity) 444 (M^+, 3), 384 (100), 366 (16), 271 (11), 253 (9); homogeneous on glc and tlc ($R_f = 0.21$, 20% ethyl acetate in Skellysolve B); Anal. Calcd for $C_{29}H_{48}O_3$: C, 78.31; H, 10.89. Found: C, 78.41; H, 10.95.

1α-Acetoxycholesteryl tosylate (**16**). To a solution of 1.68 g of 1α-acetoxycholesterol (**15**) in 17 ml of pyridine was added 2.02 g of tosyl chloride. The mixture was stirred for 16 hr at room temperature in the dark. After addition of cold 4% aqueous $KHCO_3$ (40 ml) and extraction with ethyl ether (3 × 30 ml), the combined ether extracts were washed (3×) with 2% HCl, once with saturated NaCl, dried over Na_2SO_4, and evaporated. Crystallization of the residue from MeOH gave 2.27 g (100%) of the acetoxy tosylate (**16**): mp 121–124°C; uv (ethyl ether) λ_{max} 261 nm (ε 590); ir (CCl_4) 1740 and 1241 cm^{-1} (acetate), 1373 and 1182 cm^{-1} (sulfonate); nmr ($CDCl_3$) δ 7.82 and 7.27 (*AB*, $J = 8$ Hz, 4*H*, tosylate), 5.51 (*m*, 1*H*, C-6), 4.95 (*d* of *d*, $J_1 = J_2 = 3$ Hz, 1*H*, C-1), 4.59 (*m*, 1*H*, C-3), 2.45 (*s*, 3*H*, CH_3 of tosylate), 1.97 (*s*, 3*H*, acetate), 1.02 (*s*, 3*H*, C-19), 0.85 (*d*, $J = 6.4$ Hz, 6*H*, C-26, 27), 0.64 (*s*, 3*H*, C-18); mass spectrum, *m/e* (relative intensity) 426 (M^+, 6), 384 (3), 366 (100); homogeneous on tlc ($R_f = 0.66$, 20% ethyl acetate in Skellysolve B). Anal. Calcd for $C_{36}H_{54}O_5S$: C, 72.18; H, 9.09. Found: C, 72.34; H, 9.23.

5-Cholesten-1α-ol (**17**). Into a flask equipped with heater, condensor, and drying tube was added 0.700 g of acetoxy tosylate **16** dissolved in 10 ml of dry benzene, followed by 7.0 ml of a 70% benzene solution of sodium bis(2-methoxyethoxy)-aluminum hydride. After refluxing under N_2 for 18 hr, the flask was transferred to an ice bath and 60 ml of H_2O followed by 3 ml of 10% NaOH and finally 100 ml of H_2O were added. The white precipitate was removed by filtration and washed with ethyl ether. The aqueous phase of the filtrate was extracted three times with ethyl ether (30 ml each) and the combined ether extracts were washed once with 50 ml of 10% NaOH and then with saturated salt solution. The dried solution (Na_2SO_4) was evaporated, and the residue was applied to silica gel (120 g) and eluted with 300 ml of 10% ethyl acetate in Skellysolve B. Removal of solvent afforded 0.250 g (55%) of **17** as an amorpous solid. [Continued elution with ethyl acetate provided 0.24 g of 1α-hydroxycholesterol (**13**) which can be recycled.] Crystallization from MeOH gave white needles of 5-cholesten-

1α-ol (**17**): mp 95–97°C; $[\alpha]_D^{20} = -44°$ (*c* 4.3, $CHCl_3$)[4]; ir (CCl_4) 3370 cm^{-1} (alcohol); nmr ($CDCl_3$) δ 5.54 (*m*, 1*H*, C-6), 3.74 (*m*, 1*H*, C-1), 1.0 (*s*, 3*H*, C-19), 0.92 (*d*, *J* = 5 Hz, 3*H*, C-21), 0.87 (*d*, *J* = 6.1 Hz, 6*H*, C-26, 27), 0.68 (*s*, 3*H*, C-18); mass spectrum, *m/e* (relative intensity) 386 (M^+, 100), 371 (26), 368 (50); homogeneous on glc and tlc ($R_f = 0.80$, 20% ethyl acetate in Skellysolve B).

1α-Acetoxycholest-5-ene (**11**). To 5.0 ml of dry pyridine and 5.0 ml of acetic anhydride was added 0.300 g of 5-cholesten-1α-ol (**17**). The mixture was heated to 60°C for 16 hr. Solvents were evaporated at 65°C, and the oil was dissolved in ethyl ether and washed twice with 5% HCl, followed by saturated NaCl solution. After drying (Na_2SO_4) and evaporation, the residue was chromatographed on 30-g silica gel. Elution with 100 ml of 5% ethyl acetate in Skellysolve B yielded 0.334 g (100%) of 1α-acetoxycholest-5-ene (**11**). Crystallization from ethanol gave white plates of **11** identical in physical properties with material prepared by dehydration of 6β-alcohol **10**, described above.

1α-Acetoxy-5-cholesten-7-one (**18**). To an efficiently stirred mixture of 0.740 g of dry pyridine and 12 ml of dichloromethane was added 0.460 g of chromium trioxide. After stirring for 15 min at room temperature, 0.200 g of 1α-acetoxy-cholest-5-ene (**11**) in 4 ml of dichloromethane was introduced. After stirring for 17 hr at room temperature, more preformed complex was added (0.310 g of CrO_3 into 0.490 g of pyridine/8 ml of dichloromethane, stirred for 15 min at room temperature). After 7 hr, 90 ml of diethyl ether was added, and the solution was washed with saturated $NaHCO_3$ (6 × 20 ml), 5% HCl (3 × 30 ml), and 5% $NaHCO_3$, and then with saturated NaCl solution. The dried solution (Na_2SO_4) was evaporated and the residue was chromatographed on 110-g silica gel. Elution with 200 ml of 15% ethyl acetate in Skellysolve B provided 0.050 g (25%) of starting material and 0.072 g (35%) of the title compound **18**. Crystallization from MeOH gave material of mp 138–140°C, $[\alpha]_D^{20} = -66°$ (*c* 2.89, $CHCl_3$); ir (CCl_4) 1737 and 1240 cm^{-1} (acetate), 1673 cm^{-1} (enone); nmr ($CDCl_3$) δ 5.75 (*s*, 1*H*, C-6), 5.01 (*d* of *d*, $J_1 = J_2 = 3$ Hz, 1*H*, C-1), 2.03 (*s*, 3*H*, acetate), 1.23 (*s*, 3*H*, C-19), 0.92 (*d*, *J* = 4 Hz, 3*H*, C-21), 0.86 (*d*, *J* = 6.4 Hz, 6*H*, C-26, 27), 0.67 (*s*, 3*H*, C-18); mass spectrum, *m/e* (relative intensity) 442 (M^+, 22), 400 (45), 382 (75), 367 (67); homogeneous on tlc ($R_f = 0.50$, 20% ethyl acetate in Skellysolve B). Anal. Calcd for $C_{29}H_{46}O_3$: C, 78.68; H, 10.47. Found: C, 78.78; H, 10.71.

1α-Acetoxycholest-5-ene-7-tosylhydrazone (**19**). To 2.5 ml of methanol was added 43 mg of **18** and tosylhydrazine (86 mg). After 10 hr of reflux, methanol was evaporated, the residue was dissolved in 6 ml of 30% ethyl acetate in Skellysolve B and cooled to 0°C, and excess tosylhydrazine was removed by filtration. The filtrate was applied to a column of silica gel (50 g) and eluted with 300 ml of 30% ethyl acetate in Skellysolve B. This gave 56 mg (95%) of **19** as an amorphous solid: uv (ether) λ_{max} 238 nm (ε 14,500), 271 nm (ε 8,900); $[\alpha]_D^{20} = -140°$ (*c* 1.87, $CHCl_3$); ir (CCl_4) 3220 (N-H), 1739 and 1241 (acetate), 1170 cm^{-1} (sulfonamide); nmr ($CDCl_3$) δ 7.85 and 7.29 (*AB*, *J* = 8.3 Hz, 4*H*, tosylhydrazone), 6.02 (*s*, 1*H*, C-6), 4.93 (*m*, 1*H*, C-1), 2.43 (*s*, 3*H*, CH_3 of tosyl), 1.95 (*s*, 3*H*, acetate), 1.12 (*s*, 3*H*, C-19), 0.89 (*d*, *J* = 6.3 Hz, 6*H*, C-26, 27), 0.63 (*s*, 3*H*, C-18); mass spectrum, *m/e* (relative intensity) 610 (M^+, 13), 426 (16), 366 (100), 253 (25); homogeneous on tlc ($R_f = 0.25$, 20% ethyl acetate in Skellysolve B).

1α-Acetoxy-5,7-cholestadiene (**12**). Lithium hydride (50 mg) was added to a solution of 50 mg of **19** in 2.8 ml of toluene. After refluxing under N_2 for 8 hr and cooling to

[4] Predicted values are: 5-cholesten-1α-ol, –45.1°C; 5-cholesten-1β-ol, –58.5°C (*17*).

room temperature, excess lithium hydride was removed by filtration. The solids were washed with ethyl ether and the filtrate was washed with 5 ml each of 5% H_2SO_4, 5% $NaHCO_3$, saturated NaCl, and H_2O. The dried solution (Na_2SO_4) was evaporated, and the residue was applied to a column of silicic acid (15 g). Elution with 400 ml of 8% ethyl acetate in Skellysolve B provided 15.5 mg (44%) of 1α-acetoxy-5,7-cholestadiene (**12**) as an oil: $[\alpha]_D^{20} = -32°$ (*c* 1.54, $CHCl_3$); uv (EtOH) λ_{max} 270, 281, 292 nm; ir (CCl_4) 1739 and 1246 cm^{-1} (acetate), 1600 and 1654 cm^{-1} (diene); nmr ($CDCl_3$) δ 5.62 (*d*, $J = 5.9$ Hz, 1*H*, C-6), 5.40 (*m*, 1*H*, C-7), 4.88 (*m*, 1*H*, C-1), 2.06 (*s*, 3*H*, acetate), 0.98 (*s*, 3*H*, C-19), 0.93 (*d*, $J = 6.1$ Hz, 3*H*, C-21), 0.86 (*d*, $J = 6.1$ Hz, 6*H*, C-26, 27), 0.61 (*s*, 3*H*, C-18); mass spectrum *m/e* (relative intensity) 426 (M^+, 11), 366 (100), 351 (19), 253 (18), 211 (42); homogeneous on glc and tlc ($R_f = 0.79$, 10% ethyl acetate in Skellysolve B).

1α-Hydroxy-5,7-cholestadiene (**20**). To a solution of **12** (3.5 mg) in 1.0 ml of ethyl ether was added 1.0 ml of 1.0 *M* KOH in MeOH. After stirring at room temperature for 16 hr, the reaction was quenched with acetic acid. Addition of H_2O (15 ml) and extraction with ethyl ether gave, after evaporation, a residue which was chromatographed on 3 g of silicic acid. Elution with 70 ml of 10% ethyl acetate in Skellysolve B afforded 2.5 mg of **20** (79%) as an oil: uv (ethanol) λ_{max} 260, 270, 281, 293 nm; ir (CCl_4) 3610 and 1060 cm^{-1} (alcohol), 1610 and 1650 cm^{-1} (diene); nmr ($CDCl_3$) δ 5.66 (*d* of *d*, $J_1 = 5.6$ Hz, $J_2 = 1.0$ Hz, 1*H*, C-6), 5.35 (*d* of *d* of *d*, $J_1 = 5.6$ Hz, $J_2 = J_3 = 2.6$ Hz, 1*H*, C-7) 3.62 (*m*, 1*H*, C-1), 0.94 (*d*, $J = 5.9$ Hz, 3*H*, C-21), 0.93 (*s*, 3*H*, C-19), 0.87 (*d*, $J = 6.3$ Hz, 6*H*, C-26, 27), 0.62 (*s*, 3*H*, C-18); mass spectrum *m/e* (relative intensity) 384 (M^+, 100), 366 (42), 351 (20), 271 (10), 253 (16), 211 (30); homogeneous on glc and tlc ($R_f = 0.50$, 10% ethyl acetate in Skellysolve B).

3-Deoxy-1α-hydroxyvitamin D_3 (**4**) *from alcohol* **20**. Diene **20** (1.35 mg in 100 ml of ethyl ether) was irradiated under N_2 for 6 min using an ice bath, vigorous stirring, Vycor filter, water-cooled quartz irradiation apparatus and a mercury arc lamp (Hanau TQ 150 Zz). The solvent was evaporated, and the residue was purified by high-pressure liquid chromatography (1% 2-propanol in Skellysolve B, flow rate 1.4 ml/min). The previtamin derivative (0.66 mg, λ_{max} 260 nm) eluted between 17 and 20 min. Solvents were evaporated, the oil was taken up in 1.0 ml of ethanol and heated to 60°C under N_2 for 2.0 hr. After removing the solvent, the residue was chromatographed on high-pressure liquid chromatography (0.75% 2-propanol in Skellysolve B, flow rate 1.35 ml/min). Three additional passes on high-pressure liquid chromatography afforded 0.21 mg (15%) of the desired 3-deoxy-1α-OH-D_3 (**4**) as an oil: uv (EtOH) λ_{max} 262 nm, λ_{min} 230 nm (Fig. 2); nmr (Fig. 3) δ 6.29 and 6.02 (*AB*, $J = 11$ Hz, 2*H*, C-6 and C-7), 5.29 (*m*, 1*H*, C-19), 4.94 (*m*, 1*H*, C-19), 4.14 (*d* of *d* of *d*, $J_1 = 12$ Hz, $J_2 = 6$ Hz, $J_3 = 3$ Hz, 1*H*, C-1), 0.93 (*d*, $J = 5$ Hz, 3*H*, C-21), 0.86 (*d*, $J = 6$ Hz, 6*H*, C-26, 27), 0.55 (*s*, 3*H*, C-18); high-resolution mass spectrum (Fig. 2) *m/e* (relative intensity, composition, *m/e* calcd) 384.3372 (10, $C_{27}H_{44}O$, 384.3392), 366.3299 (2, $C_{27}H_{42}$, 366.3287), 271.2053 (3, $C_{19}H_{47}O$, 271.2062), 259.2419 (3, $C_{19}H_{31}$, 259.2426), 136.0889 (100, $C_9H_{12}O$, 136.0888); homogeneous on high-pressure liquid chromatography (0.75% 2-propanol in Skellysolve B, flow rate 1.35 ml/min, $t_R = 22.0$ min).

3-Deoxy-1α-hydroxyvitamin D_3 (**4**) *from acetate* **12**. An ether solution (200 ml) of 2.3 mg of the 5,7-diene **12** was irradiated for 1.5 min at 0°C. The products were separated on a column of $AgNO_3$-impregnated silicic acid prepared as a slurry in Skellysolve B.

Elution with 5% ether in Skellysolve B gave two main fractions. The nonpolar fraction (tubes 8–11, 3.2-ml fractions), exhibiting uv absorption at λ_{max} 260 nm and λ_{min} 235 nm, contained the desired previtamin D derivative. Warming for 3 hr in ethanol (2 ml) under

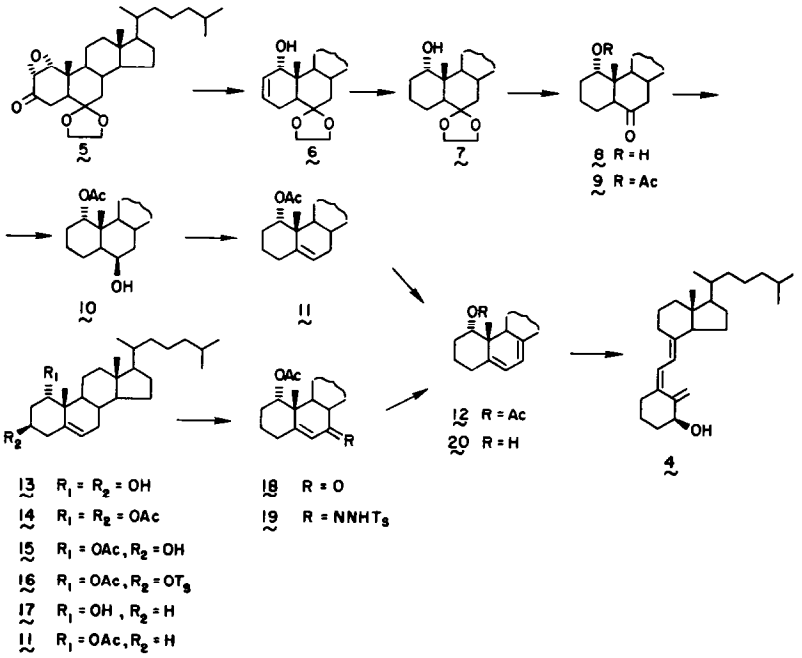


FIG. 1. Synthetic routes to 3-deoxy-1α-OH-D_3 (4) from 6,6-ethylenedioxy-1α,2α-epoxy-5α-cholestan-3-one (5) and from 1α-hydroxycholesterol (13).

FIG. 2. Mass spectrum and ultraviolet spectrum (inset) of 3-deoxy-1α-OH-D_3 (4). The ultraviolet spectrum shown covers the range from 218 to 342 nm; $\lambda_{min} = 230$ nm, $\lambda_{max} = 262$ nm.

N_2 at 80°C produced an enhancement of the optical density indicating isomerization of the previtamin to the vitamin skeleton. The mixture was then saponified by the addition of two drops of 0.9 *N* KOH in methanol and warming to 60°C for 10 min. Addition of water, extraction with $CHCl_3$, drying of the $CHCl_3$ phases, and evaporation gave, after chromatography of the residue on Sephadex LH-20 (20 g) and elution with $CHCl_3$/Skellysolve B (1:1), the desired product, 3-deoxy-1α-hydroxyvitamin D_3.

(**4**), identical in physical properties (uv, ms, glc, and hplc) to material prepared as described above.

Trimethylsilyl ether of 3-deoxy-1α-OH-D_3. To 2.5 μg of 3-deoxy-1α-OH-D_3 (**4**) dissolved in 5 μl of diethyl ether was added 15 μl of bis(trimethylsilyl) trifluoro acetamide containing 1% trimethyl chlorosilane (Regisil, RC-2). The mixture was kept at room

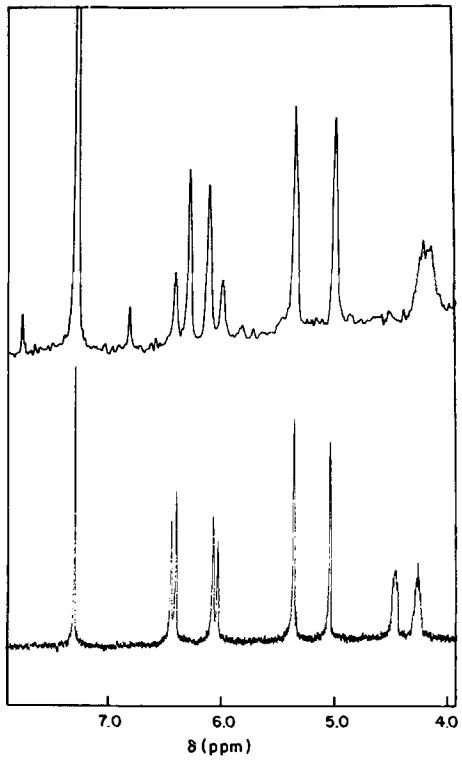


FIG. 3. Partial nmr spectra of 3-deoxy-1α-OH-D_3 (top, 90 MHz) and 1α-OH-D_3 (bottom, 270 MHz). The chloroform signal appears at δ 7.26; resonances at δ 6.87 and 7.39 in the upper spectrum are spinning side bands. See Experimental for exact resonance positions and assignments.

temperature for 0.5 hr. The mass spectrum of the product showed peaks at *m/e* (relative intensity) 456 (M^+, 17), 441 (5), 366 (5), 253 (6), 208 (100); glc of this material produced two peaks (pyro and isopyro derivatives, retention times of 4.8 and 5.3 min) and established the purity to be at least 98%.

Biological Assays

Animals. Male albino weanling rats were obtained from the Holtzman Company (Madison, Wisconsin). They were housed in overhanging wire cages and fed *ad libitum* one of the vitamin D-deficient diets described by Suda *et al.* (*18*). The diets were (I) low calcium (0.02%), normal phosphorus (0.3%) or (II) high calcium (1.2%), low phosphorus (0.1%). The animals were maintained on these diets for 2 weeks prior to their use in bioassay experiments. At this time the rats were divided into groups of five to six animals.

Determination of intestinal calcium transport, bone calcium mobilization, and growth stimulation (Table 1). Animals maintained on diet (I) received the test compound dissolved in 0.05 ml of 1,2-propanediol. Controls received the vehicle only. Doses were

TABLE 1

BIOLOGICAL ACTIVITY OF 1α-OH-D_3 AND 3-DEOXY-1α-OH-D_3 IN VITAMIN D-DEFICIENT RATS ON A LOW CALCIUM DIET[a]

Analog	Dose (ng/day)	Intestinal Ca transport[b]	Bone Ca mobilization[c]	Growth stimulation[d]
1,2-Propanediol	—	2.4 ± 0.2	3.4 ± 0.1	14 ± 2
3-Deoxy-1α-OH-D_3	5	2.3 ± 0.1	3.3 ± 0.1	11 ± 3
1α-OH-D_3	5	4.5 ± 0.6[e]	3.6 ± 0.1	19 ± 3
3-Deoxy-1α-OH-D_3	50	3.4 ± 0.3[e]	3.4 ± 0.1	16 ± 3
1α-OH-D_3	50	5.3 ± 0.4[e]	4.2 ± 0.2[e]	27 ± 4[e]
3-Deoxy-1α-OH-D_3	500	5.6 ± 0.5[e]	3.9 ± 0.1[e]	20 ± 1[e]

[a] Values are expressed as the mean ± SE; five to six rats per group.
[b] Serosal ^{45}Ca/mucosal ^{45}Ca.
[c] Serum Ca (mg/100 ml).
[d] Weight gain, grams per 7 days.
[e] Significant difference from control, $P < 0.025$.

administered intraperitoneally each day for 7 days. The animals were sacrificed 24 hr after the last dose and their blood and duodena were obtained. Bone calcium mobilization was determined by measuring serum calcium levels: 0.1-ml aliquots of serum were diluted with 1.9 ml of 0.1% aqueous $LaCl_3$ and calcium was measured by atomic absorption spectroscopy (Perkin–Elmer Model 412 atomic absorption spectrometer). Results in Table 1 are expressed as milligrams of Ca per 100 ml of serum (mg%). Intestinal calcium transport was determined using the everted gut sac technique of Martin and DeLuca (*19*). For ^{45}Ca counting (Packard Tri-Carb Model 3375 liquid scintillation counter), aliquots from the serosal and mucosal media were spotted on filter paper discs, dried, and placed in 20 ml counting vials containing 10 ml of scintillation counting solution [2 g of 2,5-diphenyl-oxazole and 0.1 g of 1,4-bis-2-(4-methyl-5-phenyl-oxazolyl) benzene per liter of toluene]. Calcium transport activity in Table 1 is expressed as the ratio of ^{45}Ca counts in the serosal to those in the mucosal fluid. All animals were weighed before and after treatment.

Determination of bone phosphate mobilization and calcification (Table 2). Animals maintained on diet (II) were dosed as described above. The animals were sacrificed 24 hr after the last dose and their blood, radii, and ulnae were obtained. Bone phosphorus mobilization was determined by measuring serum inorganic phosphorus levels (*20*). Radii and ulnae were examined for degree of endochondral calcification by the line test method (*21*).

Bone calcium mobilization versus time (Fig. 4). Animals maintained on diet (I) received the test compound dissolved in 0.05 ml of 95% ethanol. Controls received the vehicle only. Doses were administered intrajugularly, and the animals were sacrificed

TABLE 2

BIOLOGICAL ACTIVITY OF 1α-OH-D_3 AND 3-DEOXY-1α-OH-D_3 IN VITAMIN D-DEFICIENT RATS ON A LOW PHOSPHATE DIET[a]

Analog	Dose (ng/day)	Serum phosphate (mg/100 ml)	Calcification score
1,2-Propanediol	—	3.8 ± 0.2	0.3 ± 0.2
3-Deoxy-1α-OH-D_3	50	3.7 ± 0.3	0.8 ± 0.5
3-Deoxy-1α-OH-D_3	150	4.3 ± 0.2	1.7 ± 0.5[b,c]
3-Deoxy-1α-OH-D_3	500	5.4 ± 0.3[b]	>5.0, all animals
1α-OH-D_3	8	5.3 ± 0.2[b]	4.4 ± 0.5[b,d]

[a] Values are expressed as the mean ± SE; five to six rats per group.
[b] Significant difference from control, $P < 0.025$.
[c] Equivalent to 2.3 IU/μg.
[d] Equivalent to 110 IU/μg.

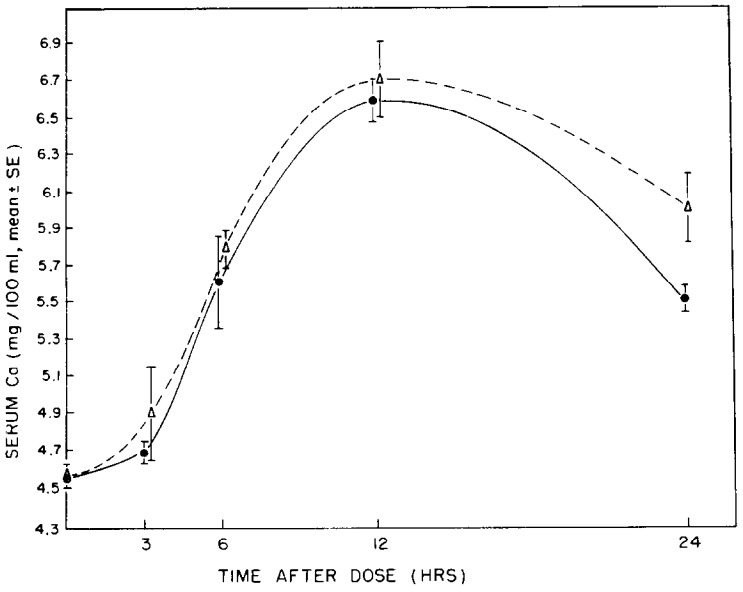


FIG. 4. Time course of serum calcium elevation in vitamin D-deficient rats given a single intrajugular dose of 125 ng of 1α-OH-D_3 (3, —●—) or 1.9 μg of 3-deoxy-1α-OH-D_3 (4, ---△---).

at the indicated times after injection. Serum calcium levels were determined as described above.

RESULTS

Synthesis

Both routes to 3-deoxy-1α-hydroxyvitamin D_3 (3-deoxy-1α-OH-D_3) (**4**) outlined in Fig. 1 make use of intermediates (compounds **5** and **13**) available from an earlier preparation of 1α-OH-D_3 (*9*, *16*). Both sequences lead, via unambiguous conversions,

to 1α-acetoxycholesta-5,7-diene (**12**)—the required steroidal intermediate for subsequent photochemical generation of the vitamin D skeleton.

Epoxyketone **5**, upon treatment with hydrazine hydrate (*22*), provided allylic alcohol **6** and subsequent catalytic reduction gave crystalline 1α-hydroxy-derivative **7**. Removal of the ketal and acetylation afforded **9**, which, after reduction ($NaBH_4$) to the 6β-alcohol **10** and dehydration with $POCl_3$ (*23*), yielded the desired Δ^5-cholestene intermediate **11** in 23% overall yield from **5**. Preparation of the required 5,7-diene intermediate (**12**) then involved the usual allylic bromination and dehydrobromination sequence (*24*), which, applied to **11** gave, after careful chromatography, pure **12**, but in rather poor yield (6%).

The same compound was obtained from 1α-hydroxycholesterol (**13**) by a preparatively cleaner and more efficient sequence. Acetylation of **13** to diacetate **14**, followed by selective hydrolysis (KOH/MeOH) to 1α-acetoxycholesterol (**15**) and tosylation, gave **16**. Reduction of **16** and reacetylation of the resulting 5-cholesten-1α-ol (**17**) then afforded **11** in ca. 50% overall yield from **13**. Allylic oxidation of **11** (CrO_3/pyridine; *25*, *26*) to the 7-ketone (**18**), conversion to the corresponding tosylhydrazone (**19**), and decomposition with LiH in boiling toluene (*27*, *28*) led to diene **12** (15% from **11**) uncontaminated by other double-bond isomers. Hydrolysis of **12** then led to the corresponding 5,7-cholestadiene-1α-ol (**20**).

Dienes **12** or **20** were converted to analog **4** by the usual photochemical route. Brief irradiation of an ether solution of **12** yielded the previtamin D derivative which, after purification, was isomerized by gentle heating to the vitamin skeleton. Subsequent hydrolysis (KOH, MeOH) provided 3-deoxy-1α-OH-D_3 (**4**). Alternatively, irradiation of 1α-hydroxycholesta-5,7-diene (**20**) followed by thermal isomerization of isolated previtamin led to analog **4** directly. The alternate preparative routes described here (i.e., **5** → **11** → **12** → **4** and **13** → **11** → **18** → **19** → **12** → **20** → **4**) yielded identical products as judged by uv and mass spectrometry and high-pressure liquid chromatography retention times. Our bioassay data are based on material synthesized by the second approach.

Synthetic 3-deoxy-1α-OH-D_3 (**4**) gave the expected physical data. The gas–liquid chromatogram of the trimethylsilyl ether derivative of 3-deoxy-1α-OH-D_3 showed the twin peaks due to thermal rearrangement to the corresponding pyro and isopyro derivatives (*29*, *30*). This observation and the uv absorption spectra of **4** (Fig. 2) are evidence for the 5,6-*cis*-triene structure. The high-resolution mass spectrum of **4** (Fig. 2) established the correct molecular formula (calcd for $C_{27}H_{44}O$: 384.339; found: 384.337). The base peak (*m/e* 136, $C_9H_{12}O$) represents the characteristic cleavage between carbons 7 and 8 (*31*). The downfield nmr spectrum of **4** is shown in Fig. 3. The 1β-proton (δ 4.14) can be interpreted as an eight-line pattern ($J_{1\beta,2\alpha} = 12$ Hz, $J_{1\beta,2\beta} = 6$ Hz, $J_{\text{allylic}} = 3$ Hz). For comparison, part of the nmr spectrum of 1α-OH-D_3 (**3**) is also included in Fig. 3. The 1β-proton (δ 4.43) appears as a doublet of doublet of doublet of doublets ($J_{1\beta,2\alpha} = 7.7$ Hz, $J_{1\beta,2\beta} = 4.5$ Hz, $J_{1\beta,19Z} = 1.0$ Hz, $J_{1\beta,19E} = 1.3$ Hz). These couplings have been verified by homonuclear decoupling experiments performed at 270 MHz. Since 1α-OH-D_3 like vitamin D_3 itself, is known to consist of a nearly equal mixture of ring A chair conformers (*32–34*), the 7.7-Hz coupling represents an average of J_{ax-ax} and J_{eq-eq} [11.1 and 2.7 Hz, (*35*)] the 4.5-Hz coupling is an average of J_{ax-eq} and J_{eq-ax} [5.5 and 2.6 Hz (*36*)], and both allylic coupling constants

(1.0 and 1.3 Hz) are averages of J_{ax-19} and J_{eq-19} [2–3 and 0 Hz (*37*)]. If 3-deoxy-1α-OH-D_3 existed entirely as one chair conformer with the 1α-hydroxyl function equatorially oriented, the expected coupling constants would be: $J_{1\beta,2\alpha} = 11.1$ Hz, $J_{1\beta,2\beta} = 5.5$ Hz, and $J_{allylic} = 2$–3 Hz. The above interpretation of the 1-β-proton signal in the spectrum of 3-deoxy-1α-OH-D_3, therefore, implies an equatorial 1α-hydroxyl with little conformational averaging. A conformationally biased A-ring has also been deduced from nmr data on 1α-hydroxy-3-*epi*vitamin D_3. but in that case the axial 1α-hydroxy conformer predominates (*38*, *39*).

Biological Activity

Analog **4** was tested in the rat by measuring its effect on intestinal calcium transport, mobilization of calcium from bone, serum phosphate elevation, bone calcification, and weight gain. Vitamin D-deficient animals, maintained on either low calcium or low phosphate diets, received graded doses of either compound **4** or 1α-OH-D_3 (**3**, serving as comparative positive control) or of the vehicle (1,2-propanediol) by daily intraperitoneal injections for 7 days. One day after the last dose, the various vitamin D responsive physiological parameters were measured. Results are summarized in Tables 1 and 2, whereas Table 3 presents a comparison of the relative potencies of 3-deoxy-1α-OH-D_3 (**4**) and 1α-OH-D_3 (**3**). 3-Deoxy-1α-OH-D_3 exhibited biological activity in all

TABLE 3

SUMMARY AND INTERCOMPARISON OF BIOLOGICAL ACTIVITIES OF 1α-OH-D_3 AND 3-DEOXY-1α-OH-D_3

Biological assay	Dose (ng/day) of 1α-OH-D_3 which gives half-maximal response	Dose (ng/day) of 3-deoxy-1α-OH-D_3 which gives half-maximal response	Activity ratio 1α-OH-D_3 to 3-deoxy-1α-OH-D_3
Intestinal calcium transport	3[a] (2, lit.)[b]	60[a]	20–30
Bone calcium mobilization	6[a] (6, lit.)[b]	200[a]	30–40
Serum phosphate elevation	—	200[a]	40–50[c]
Endochondral calcification	4.5 (3.3, lit.)[d]	220	50–60

[a] Values obtained from interpolation of data in Tables 1 and 2.
[b] Values obtained from data in Ref. *10*.
[c] Ratio of doses required to produce equivalent response.
[d] Value from Ref. *11*.

assays. As shown in Table 1, a dose of 50 ng/day of **4** administered to vitamin D-deficient animals on a low calcium diet produced a significant stimulation of intestinal calcium transport and 500 ng/day gave maximal responses. Although, in this assay, 3-deoxy-1α-OH-D_3 is clearly less effective than 1α-OH-D_3, it is interesting to note that its time course of response is nearly identical to that produced by 1α-OH-D_3

(Fig. 4). The 3-deoxy analog also stimulated the release of bone calcium as measured by the rise in serum calcium (Table 1) and was effective in promoting the growth of these calcium-deficient animals (Table 1), although, in both assays, significant effects were observed only at the high-dose levels.

In rats maintained on a low phosphate diet, the ability of analog **4** to produce a significant rise in serum phosphorus (Table 2) is in accord with the analogous serum calcium data of Table 1 and confirmed the effectiveness of the compound in mobilization of bone mineral. Table 2 also includes results of a "line test" assay for the calcification of bone of rachitic rats on a low phosphate diet. The experimentally measured calcification scores which translate into antirachitic potencies of 110 IU/μg for 1α-OH-D_3 and 2.3 IU/μg for 3-deoxy-1α-OH-D_3 characterize the analog as a weakly active antirachitic agent.

Examination of the comparative data of Tables 1 and 2 clearly shows 3-deoxy-1α-OH-D_3 to be much less active than 1α-OH-D_3 in all assays. Activity ratios between the two compounds, summarized in Table 3, indicate the 3-deoxy analog to possess about 1/20 to 1/60 of the biological potency exhibited by 1α-OH-D_3 (**3**), depending on the specific response assayed.

DISCUSSION

The syntheses described here represent two unambiguous routes to 3-deoxy-1α-OH-D_3 (**4**). Both routes lead to identical products, for which stereochemical assignments are assured by the known stereochemistry of the starting materials and the nmr spectra and optical rotations of various intermediates (see Experimental).

Worthy of comment also is the preparation of 5,7-diene **12** from the corresponding Δ^5-steroid **11** via intermediates **18** and **19**. In the 3-deoxy-steroid series, this approach proved superior to the more standard Hunzicker–Müllner (*24*) allylic bromination/dehydrobromination sequence. The latter furnished mixtures of dienes, from which the desired $\Delta^{5,7}$-intermediate could be obtained only in low yields (5–7%) and after careful chromatography. In contrast, allylic oxidation of **11** to 7-ketone **18** proceeded with high regio-specificity (no Δ^5-4-one was detected, *26*) and reasonable yield (35%); subsequent tosylhydrazone formation to **19** (95%) and decomposition of **19** with LiH gave **12** (44%), containing no other double-bond isomers.

Our bioassay data establish the *in vivo* potency of 3-deoxy-1α-OH-D_3 and thereby prove that the 3-β-hydroxyl of the natural hormone [1α,25-$(OH)_2D_3$] is not an essential functional group. The biological activity of 5,6-*trans*-vitamin D_3 (*40*) and dihydrotachysterol$_3$ (*41*) supports this conclusion.

The activity of 3-deoxy-1α-OH-D_3 is probably due to its metabolic conversion to 3-deoxy-1α,25-$(OH)_2D_3$. This is suggested by three lines of evidence: (1) 3-Deoxy-1α-OH-D_3 responds with a time course identical to 1α-OH-D_3 (see Fig. 4), and the latter is known to be rapidly converted to 1α,25-$(OH)_2D_3$ in the rat with maximal concentrations of 1α,25-$(OH)_2D_3$ appearing in the target tissues several hours before the maximal biological response is observed (*11, 12*). (2) Considering bone calcium mobilization, 3-deoxy-1α-OH-D_3 appears much more active when tested *in vivo* than in the *in vitro* assay measuring release of calcium from cultured fetal rat bones (*8*). Since

1α,25-$(OH)_2D_3$ is twice as active as 1α-OH-D_3 in the mobilization of bone calcium *in vivo* (*10*), and 1α-OH-D_3 is 30 times more active than 3-deoxy-1α-OH-D_3 (this report), 1α,25-$(OH)_2D_3$ can be estimated to be 60 times more effective than 3-deoxy-1α-OH-D_3 when tested *in vivo*. In the *in vitro* bone assay, 3-deoxy-1α-OH-D_3 has been shown to be inactive even at concentrations 3×10^3 times higher than required for 1α,25-$(OH)_2D_3$.[5] Such a relationship strongly supports metabolism of 3-deoxy-1α-OH-D_3 *in vivo*. (3) Other compounds possessing a 1α-hydroxyl function (or its geometric equivalent) and a cholesterol side chain are known to be 25-hydroxylated *in vivo* [e.g,, dihydrotachysterol (*42*, *43*), 5,6-*trans*-vitamin D_3 (*43*), and 1α-OH-D_3 (*11*, *12*, *44*, *45*)].

The lower potency of 3-deoxy-1α-OH-D_3 when compared to 1α-OH-D_3 *in vivo* could be a consequence of both inefficient 25-hydroxylation of 3-deoxy-1α-OH-D_3 and the lack of the normal 3β-OH-receptor binding interaction. Recent receptor binding experiments, using the chick intestinal cytosol binding protein for 1α,25-$(OH)_2D_3$, in which a 30-fold difference in binding affinity between 1α-OH-D_3 and analog **4** has been measured suggest that the latter explanation is correct (*46*).

ACKNOWLEDGMENTS

This work was supported by Grant No. AM-14881, Contract No. 72-2226, and Training Grant No. GM-00236 BCH from the National Institutes of Health, as well as contributions from the Wisconsin Alumni Research Foundation. The departmental 270 MHz nmr facility is supported by NSF Grant No. BMS74-23826. We thank Mr. Sean Hehir for the nmr data and Mrs. Barbara Gallagher for the preparation of certain synthetic intermediates.

REFERENCES

1. H. F. DeLuca, *Fed. Proc.* **33**, 2211 (1974); H. F. DeLuca and H. K. Schnoes, *Annu. Rev. Biochem.* **45**, 631 (1976).
2. E. B. Olson and H. F. DeLuca, *Science* **165**, 405 (1969).
3. J. L. Omdahl and H. F. DeLuca, *Physiol. Rev.* **53**, 327 (1973).
4. L. G. Raisz, C. L. Trummel, M. F. Holick, and H. F. DeLuca, *Science* **175**, 768 (1972).
5. P. F. Brumbaugh and M. R. Haussler, *Life Sci.* **13**, 1737 (1973).
6. D. A. Procsal, W. H. Okamura, and A. W. Norman, *J. Biol. Chem.* **250**, 8382 (1975).
7. J. A. Eisman, A. J. Hamstra, B. E. Kream, and H. F. DeLuca, *Arch. Biochem. Biophys.* **176**, 235 (1976).
8. P. H. Stern, C. L. Trummel, H. K. Schnoes, and H. F. DeLuca, *Endocrinology* **97**, 1552 (1975).
9. M. F. Holick, E. J. Semmler, H. K. Schnoes, and H. F. DeLuca, *Science* **180**, 190 (1973).
10. M. F. Holick, P. Kasten-Schraufrogel, T. Tavela, and H. F. DeLuca, *Arch. Biochem. Biophys.* **166**, 63 (1975).
11. M. F. Holick, B. M. Gallagher, S. A. Holick, T. Tavela, H. K. Schnoes, and H. F. DeLuca, *J. Biol. Chem.* **251**, 1020 (1976).
12. M. F. Holick, S. A. Holick, T. E. Tavela, H. K. Schnoes, and H. F. DeLuca, *J. Biol. Chem*, **251**, 1025 (1976).

[5] Even after further increasing the concentration of 3-deoxy-1α-OH-D_3 by two orders of magnitude (to 10^{-4} *M*) in this assay, little, if any, bone resorption activity is detectable. We thank Professor Paula Stern (Department of Pharmacology, Northwestern University Medical School) for these data.

13. H. Y. Lam, B. L. Onisko, H. K. Schnoes, and H. F. DeLuca, *Biochem. Biophys. Res. Commun.* **59**, 845 (1974).
14. W. H. Okamura, M. N. Mitra, R. M. Wing, and A. W. Norman, *Biochem. Biophys. Res. Commun.* **60**, 179 (1974); *Science* **188**, 1013 (1975).
15. M. N. Mitra, A. W. Norman, and W. H. Okamura, *J. Org. Chem.* **39**, 2931 (1974).
16. E. J. Semmler, Ph.D. thesis, University of Wisconsin, Madison (1974).
17. L. F. Fieser and M. Fieser, "Steroids," pp. 177–180. Reinhold, New York, 1959.
18. T. Suda, H. F. DeLuca, and Y. Tanaka, *J. Nutr.* **100**, 1049 (1970).
19. D. L. Martin and H. F. DeLuca, *Amer. J. Physiol.* **216**, 1351 (1969).
20. P. S. Chen, T. Y. Toribara, and H. Warner, *Anal. Chem.* **28**, 1756 (1956).
21. U. S. Pharmacopoeia, 15th revision, p. 889. Mack, Easton, Penn., 1955.
22. C. Djerassi, D. H. Williams, and B. Berkoz, *J. Org. Chem.* **27**, 2205 (1962).
23. D. H. R. Barton and W. J. Rosenfelder, *J. Chem. Soc.* 1047 (1951).
24. F. Hunzicker and F. X. Müllner, *Helv. Chim. Acta* **41**, 70 (1958).
25. R. Ratcliffe and R. Rodehorst, *J. Org. Chem.* **35**, 4000 (1970).
26. W. G. Dauben, M. Lorber, and D. S. Fullerton, *J. Org. Chem.* **34**, 3587 (1969).
27. L. Caglioti, P. Grasselli, and G. Maina, *Chim. Ind. (Milan)* **45**, 559 (1963).
28. W. G. Dauben and D. S. Fullerton, *J. Org. Chem.* **36**, 3277 (1971).
29. E. Havinga, *Experientia* **29**, 1181 (1973).
30. T. Kobayashi and M. Yasumura, *J. Vitaminol. (Kyoto)* **18**, 78 (1972).
31. G. R. Waller (Ed.), "Biochemical Applications of Mass Spectrometry," pp. 505–510. Wiley, New York, 1972.
32. R. M. Wing, W. H. Okamura, A. Rego, M. R. Pirio, and A. W. Norman, *J. Amer. Chem. Soc.* **97**, 4980 (1975).
33. R. M. Wing, W. H. Okamura, M. R. Pirio, S. M. Sine, and A. W. Norman, *Science* **186**, 939 (1974).
34. G. N. LaMar and D. L. Budd, *J. Amer. Chem. Soc.* **96**, 7317 (1974).
35. F. A. L. Anet, *J. Amer. Chem. Soc.* **84**, 1053 (1962).
36. D. H. Williams and N. S. Bhacca, *J. Amer. Chem. Soc.* **86**, 2742 (1964).
37. L. M. Jackman and S. Sternhell, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," pp. 316–325. Pergamon Press, Oxford, 1969.
38. M. Sheves, E. Berman, D. Freeman, and Y. Mazur, *Chem. Commun.* 643 (1975).
39. W. H. Okamura and M. R. Pirio, *Tetrahedron Lett.* 4317 (1975).
40. M. F. Holick, M. Garabedian, and H. F. DeLuca, *Biochemistry* **11**, 2715 (1972).
41. T. Suda, R. B. Hallick, H. F. DeLuca, and H. K. Schnoes, *Biochemistry* **9**, 1651 (1970).
42. R. B. Hallick and H. F. DeLuca, *J. Biol. Chem.* **247**, 91 (1972).
43. D. E. M. Lawson and P. A. Bell, *Biochem. J.* **142**, 37 (1974).
44. M. F. Holick, S. A. Holick, T. E. Tavela, B. Gallagher, H. K. Schnoes, and H. F. DeLuca, *Science* **190**, 576 (1975).
45. K. Fukushima, Y. Suzuki, Y. Tohira, I. Matsunaga, K. Ochi, N. Nagano, Y. Mishii, and T. Suda, *Biochem. Biophys. Res. Commun.* **66**, 632 (1975).
46. J. A. Eisman and H. F. DeLuca, *Steroids*, in press (1975).