(3:1); ^{1}H NMR ($CDCl_3$) δ 0.9 (3 H, t, J = 6 Hz, CH_3), 1.1-2.5 (29 H, m), 2.6-2.9 (2 H, m), 3.1 (1 H, br s), 3.38 (2 H, t, J = 6 Hz, CH_2O), 4.0 (1 H, br, C*H*OH), 5.3-5.6 (2 H, m, *trans*-C*H*=C*H*C(OH)), 5.9-6.2 (2 H, m, *cis*-CH=CH); IR (neat) 3410 (OH), 1730 (C=O), 1140 (C-O) cm^{-1}.

Compound **23**: 100% yield; R_f 0.31, hexanes:ethyl acetate (3:1); ^{1}H NMR ($CDCl_3$) δ 0.87 (3 H, t, J = 5 Hz, CH_3), 1.0-2.4 (32 H, m), 3.32 (2 H, t, J = 6 Hz, CH_2O), 3.67 (1 H, br s, CHO), 4.0 (1 H, br, C*H*OH), 6.5-6.6 (2 H, m, *trans*-CH=CH); IR (neat) 3410 (OH), 1730 (C=O) cm^{-1}.

Synthesis of 18 and 24. The synthesis of **24** is representative. Compound **23** (158 mg, 0.389 mmol) and 0.22 g (3.9 mmol) of KOH were refluxed for 2.5 h in 10 mL of 4:1 methanol-H_2O. After cooling, the reaction mixture was diluted with ether, acidified with dilute HCl, washed with brine, and dried over magnesium sulfate. Flash chromatography afforded 105 mg (77%) of **24**: R_f 0.26, hexanes:ethyl acetate:acetic acid (30:15:1); ^{1}H NMR ($CDCl_3$) δ 0.87 (3 H, t, J = 5 Hz, CH_3), 1.1-2.5 (23 H, m), 3.33 (2 H, t, J = 6 Hz, CH_2O), 3.67 (1 H, br s, CHO), 4.1 (1 H, br, C*H*OH), 5.5 (2 H, m, CH=CH), 8.3 (2 H, br s, OH, CO_2H); IR (neat) 3400 br (OH), 1720 (C=O), 1160 (C-O) cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 177.96, 133.51, 131.23, 131.10, 82.07, 73.08, 68.67, 46.17, 37.07, 33.95, 31.67, 30.89, 29.52, 25.69, 25.03, 24.45, 22.50, 20.68, 17.04, 13.92, 13.79, 12.62; MS, m/z 332.23676; calcd for $C_{21}H_{32}O$ ($M^+ - H_2O$); 332.23515.

Compound **18**: 82% yield; R_f 0.29, hexanes:ethyl acetate:acetic acid (30:15:1); ^{1}H NMR ($CDCl_3$) δ 0.85 (3 H, t, J = 5 Hz, CH_3), 1.0-2.0 (15 H, m), 2.1-2.4 (4 H, m), 2.5-2.9 (2 H, m), 3.0 (1 H, br s, CHO), 3.38 (2 H, t, J = 6 Hz, CH_2O), 3.8-4.1 [1 H, br, C*H*OH], 5.2-5.5 (2 H, m, *trans*-CH=CH), 5.9-6.2 (2 H, m, *cis*-CH=CH); IR (neat) 3420 br (OH), 1715 (C=O), 1090 (C-O) cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 178.96, 137.86, 134.94, 134.03, 132.86, 86.23, 73.09, 69.00, 50.59, 47.67, 47.47, 46.95, 46.43, 46.24, 37.07, 33.95, 31.60, 29.46, 25.69, 25.04, 24.45, 22.57, 13.98; MS, m/z 332.23574; calcd for $C_{21}H_{32}O_3$ ($M^+ - H_2O$), 332.23515.

Acknowledgment. We gratefully acknowledge the National Institutes of Health (AM21795) for financial support, Proctor and Gamble for fellowship support for D. R. Leach, and Johnson Matthey Inc. and Engelhard Industries for generous loans of palladium salts.

Synthesis of 25-Hydroxyvitamin D_2 and Its 24-Epimer

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Received November 22, 1983

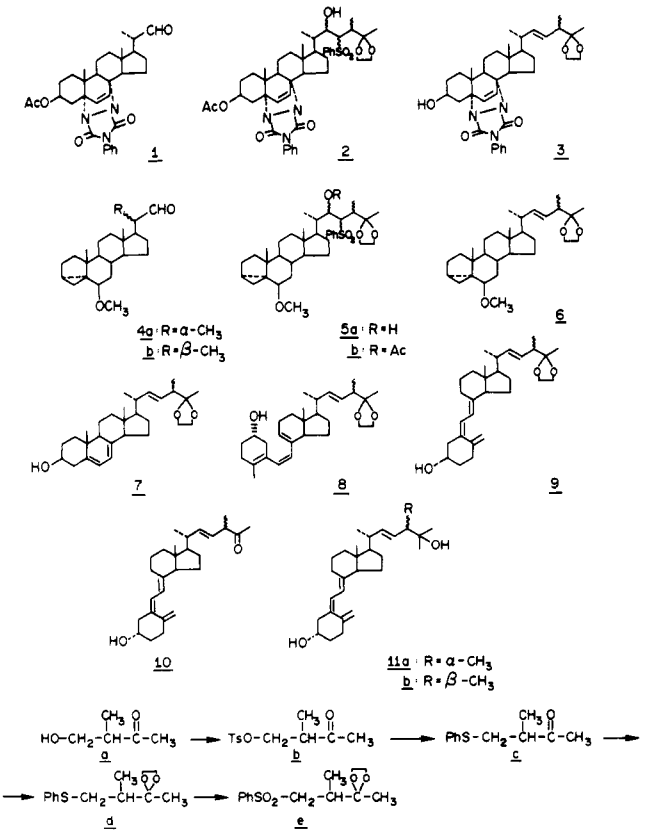
The synthesis of 25-hydroxyvitamin D_2 (25-hydroxyergocalciferol, **11a**) and its 24-epimer **11b** is described. The synthetic product **11a** proved to be identical in all respects with the natural metabolite of vitamin D_2.

Studies on the metabolism of vitamin D_2 (ergocalciferol) have shown that it undergoes both 25-hydroxylation and 1α-hydroxylation during its conversion to the active calcium mobilizing hormone.[1] This activation pathway is the same as that described for vitamin D_3.[2,3] The major circulating metabolite of vitamin D_2, 25-hydroxyergocalciferol (25-hydroxyvitamin D_2, 25-OH-D_2, **11a**), was first isolated in 1969 from the blood of hogs fed massive doses of vitamin D_2, and its structure was established by spectral correlations based on its mass spectrum, ^{1}H NMR characteristics, and ultraviolet absorption.[4] A synthesis of 25-OH-D_2, **11a**, has been achieved some year ago by chemists of the Upjohn Company,[5] using 6β-methoxy-3,5α-cyclocholesta-22,24-diene described by Salmond[6] and Hutchins[7] as starting material for side chain elaboration by selective epoxidation and C-24 alkylation, but other than a brief mention of it[6], that work has not been published to date.

In initiating our own work on the synthesis of 25-OH-D_2 we were guided by two principal objectives, namely, to obtain material for a thorough assessment of its biological activity and properties, and to devise a route that would be conveniently adaptable to the generation of highly radiolabeled material required for metabolite assays and further metabolism studies.

The latter objective, in particular, dictated a different synthetic approach from that used by the Upjohn chemists[5,6] in which complete side chain elaboration precedes generation of the 5,7-diene. In the case of the vitamin D_2 series, the usual allylic bromination/dehydrobromination sequence for introduction of the 7,8-double bond[8] suffers from fairly low yields presumably due to side reactions evolving the 22,23-double bond. More satisfactory for our

Chart I



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0022-3263/84/1949-2148$01.50/0

purposes was a scheme in which completion of side chain synthesis was deferred so as to provide the opportunity for radiolabel introduction at a very late step in the synthesis. This concept is realized by the synthetic sequence illustrated by structures 1 to 11 (Chart I). in which the C-25 ketone **10** serves as the common intermediate for the generation of unlabeled or labeled (^{14}C, ^{13}C, ^{3}H, ^{2}H) 25-OH-D_2 by a final Grignard reaction. The details of our synthesis of 25-OH-D_2 (**11a**) and its 24-epimer (**11b**) are the subject of this report.

The starting materials for this synthesis were the known PTAD diene protected 22-aldehyde **1**[9] and the side chain fragment, sulfone e. Compound e was obtained in 56% overall yield from 4-hydroxy-3-methyl-2-butanone (a). The tosylate b derived from a was converted to the thioether c (PhSH, *t*-BuOK, DMF, room temperature), and after protection of the carbonyl group, treatment of ketal d with *m*-chloroperbenzoic acid afforded the desired sulfone e in quantitative yield.

Sulfone e was combined[10,11] with aldehyde **1** to give the α-hydroxy sulfones **2** as a mixture of the diastereoisomers at C-22, C-23, and C-24. Although literature examples[12] involving reactions of similar C-22 steroidal aldehydes with a variety of carbanions argued against epimerization at C-20, the retention of configuration at that asymmetric center was unequivocally established at a later stage (see below). The commonly used[10,11] methods to generate carbanions, such as reactions with LDA or *n*-BuLi, failed to give a good yield of the condensation product **2**, but ethylmagnesium bromide proved effective. When this reagent was used, the condensation of sulfone e with aldehyde **1** was accomplished in about 50% yield. However, it is possible to recover unreacted substrates and, after one recycling, to enhance the yield of **2** to 70%. The same reaction of the *i*-steroidal 22-aldehyde **4a**[13] works better, does not require recycling, and uses a smaller excess of sulfone e.

The α-hydroxy sulfones (**2** or **5a**) were subjected to sodium amalgam reduction[14] in buffered methanol at 4 °C. The phenylsulfonyl and hydroxyl groups were removed together under these conditions to afford the olefins (**3** or **6**, respectively). Such reductive elimination reactions of α-hydroxy sulfones or their derivatives are known to produce predominantly or exclusively trans olefins.[15] We have found that derivatization of intermediary α-hydroxy sulfone (for example acetylation of **5a** to **5b**) does not improve the yield of the reduction (which is, however, quite satisfactory—about 70%).

In order to prove that stereochemical integrity at C-20 had been maintained during side chain construction, the newly formed 22,23-double bond in compound **6** was cleaved by treatment with an excess of ozone in 1% pyridine-methylene chloride, followed by a reductive workup under conditions which resulted in no epimerization. The product of this reaction was compared with the pure (20*S*)-aldehyde **4a** and the mixture of (20*R*)- and (20*S*)-epimers (**4a** and **4b**) obtained by filtration of **4a** through an alumina column.[9] Thin-layer chromatography did not show any traces of (20*R*)-aldehyde **4b** among the ozonolysis products. The ^1H NMR spectra of the ozonolysis product and the aldehyde **4** were identical, and there were no resonances attributable to the (20*R*)-isomer, proving that the desired stereochemistry at C-20 was present in compound **6** (and, hence, in **3**).

The protecting group of the 5,7-diene was removed by lithium aluminum hydride reduction of adduct **3** in boiling THF for 10 h. The resulting mixture of 24-epimers of provitamin **7** appeared homogeneous on TLC or by HPLC (this is also true for the 24-epimers of **3** and **6**). Compound **7** was irradiated with a mercury arc lamp fitted with a Vycor filter to form previtamin **8** (44%). After separation from the other photoisomers by high-pressure liquid chromatography (compound **8** also gives a single, sharp peak on HPLC), **8** was thermally isomerized to the vitamin D_2 analogue **9** (82%). In order to deprotect the carbonyl group at C-25, compound **9** was treated with *p*-toluenesulfonic acid in aqueous ethanol. The reaction did not proceed at room temperature, but heating under reflux for 90 min removed the protecting group without double bond isomerization to afford the desired ketone **10** in almost quantitative yield. Both compounds, ketal **9** and ketone **10**, are homogeneous by TLC and HPLC. Finally, reaction of ketone **10** with methylmagnesium iodide gave, in very good yield, a mixture of 25-OH-D_2 (**11a**) and 24-epi-25-OH-D_2 (**11b**), resolvable into two components by HPLC, of which the more polar one comigrated with an authentic sample of 25-OH-D_2. When 1% of 2-propanol in hexane was used as an eluent (Zorbax-SIL column, 6.2 mm × 25 cm, flow rate 2 mL/min), the separation of epimers was complete (24-epi-25-OH-D_2 (**11b**) 175 mL and 25-OH-D_2 (**11a**)/186 mL) and the ratio of **11b**:**11a** was determined as 48:52. Preparative separation on HPLC (with a 2% 2-propanol in hexane) afforded material of high purity, after a 2-fold chromatography.

The infrared, ^1H NMR, and mass spectra of both epimers are nearly identical and in full accord with the assigned structures, though they do not provide additional evidence concerning the configuration at C-24. Both compounds showed a band 971 cm^{-1} in the infrared spectrum for the 22-trans-double bond and the typical and very characteristic pattern of fragmentation in the mass spectrum dominated by the ring A fragments at m/z 136 and 118. The mass spectrum of 25-OH-D_2 obtained by synthesis was essentially identical with that of the product isolated by Suda et al.[4] from hog blood. Likewise, the ^1H NMR spectra of the synthetic compound agree with the data reported by Suda et al. for the natural product, although detailed comparison was not possible since the data of Suda et al. are limited to the side chain and angular methyl groups.

(1) Jones, G.; Schnoes, H. K.; DeLuca, H. F. *Biochemistry* **1975**, *14*, 1250.
(2) DeLuca, H. F.; Paaren, H. E.; Schnoes, H. K. In "Topics in Current Chemistry"; Springer Verlag: New York, 1979; Vol. 83, p 1.
(3) DeLuca, H. F. *Biochem. Soc. Trans.* **1981**, *10*, 147.
(4) Suda, T.; DeLuca, H. F. ; Schnoes, H. K.; Blunt, J. W. *Biochemistry* **1969**, *8*, 3515.
(5) Campbell, J. A.; Squires, D. M., unpublished results (see also ref 6).
(6) Salmond, W. G.; Sobala, M. C. *Tetrahedron Lett.* **1977**, 1695.
(7) Hutchins, R. F. N.; Thompson, M. .; Svoboda, J. A. *Steroids* **1970**, *15*, 113.
(8) (a) Hunziker, F.; Müllner, F. Y. *Helv. Chim. Acta* **1958**, *41*, 70. (b) Esvelt, R. P.f Fivizzani, M. A.; Paaren, H. E.; Schnoes, H. K.; DeLuca, H. F. *J. Org. Chem.* **1981**, *46*, 456. (c) Lam, H.-Y.; Schnoes, H. K.; DeLuca, H. F. *Steroids* **1977**, *30*, 671.
(9) Barton, D. H. R.; Shioiri, T.; Widdowson, D. A. *J. Chem. Soc. C* **1971**, 1968.
(10) (a) Yamada, S.; Nakayama, K.; Takayama, H. *Tetrahedron Lett.* **1981**, *22*, 2591. (b) Yamada, S.; Nakayama, K.; Takayma, H. *Chem. Pharm. Bull.* **1981**, *29*, 2393.
(11) (a) Kocienski, P. J.; Lythogoe, B.; Ruson, S. *J. Chem. Soc. Perkin Trans. 1* **1978**, 829. (b) Kocienski, P. J.; Lythgoe, B.; Roberts, D. A. *Ibid.* **1978**, 834.
(12) (a) Eyley, S. C.; Williams, D. H. *J. Chem. Soc. Perkin Trans 1* **1976**, 727, 731. (b) Wiersig, J. R.; Waespe-Sarcevic, N.; Djerassi, C. *J. Org. Chem.* **1979**, *44*, 3374.
(13) Heyl, F. W.; Centolella, A. P. U.S. Patent 2 623 052, 1952; *Chem. Abstr.* **1953**, *47*, 10016c.
(14) Julia, M.; Paris, J. M. *Tetrahedron Lett.* **1973**, 4833.
(15) Kocienski, P. J.; Lythogoe, B.; Waterhouse, I. *J. Chem. Soc. Perkin Trans. 1* **1980**, 1045.

The above sequence represents an experimentally quite convenient synthesis of the natural metabolite; the reaction of keto intermediate **10** with commercially available labeled Grignard reagents provides, in addition, a most direct and efficient route to side chain labeled material.[16]

Experimental Section

NMR spectra were taken with a Bruker WH-270 FT spectrometer by using $CDCl_3$ solutions with Me_4Si as internal standard. Infrared spectra were recorded on a Nicolet MX-1 as $CHCl_3$ solutions or as KBr pellets. Mass spectra were obtained at 110–120 °C above ambient temperature at 70 eV with an AE1 MS-9 spectrometer coupled to a DS-50 data system. Ultraviolet (UV) absorption spectra were recorded in absolute ethanol with a Hitachi Model 100-60 recording spectrophotometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter. High-pressure LC was performed on a Waters Associates Model ALC/GPC 204 using a Zorbax-SIL (Dupont) 6.2 mm × 25 cm column, monitoring at 310 nm for preparative samples or 260 mm for analytical samples.

Column chromatography was performed on silica gel 60, 70–230 mesh ASTM (Merck). Almost all reactions were carried out under an argon atmosphere, and unless specified otherwise, organic solutions were dried over anhydrous Na_2SO_4.

Preparation of Known Compounds. The C-22 aldehyde 1 was obtained by degradation of ergosterol acetate (in which the ring B diene system has been protected by Diels–Alder addition of 4-phenyl-1,2,4-triazoline-3,5-dione) according to Barton's procedure.[9] The aldehyde **4a** was synthesized from stigmasterol by a known method.[13] The epimeric aldehyde **4b** was obtained by partial isomerization of **4a** on alumina.[9]

Synthesis of the Side Chain Fragment. To a stirred solution of 4-hydroxy-3-methylbutan-2-one (a) (12.75 g, 0.125 mol) in pyridine (100 mL) was added *p*-TsCl (33.25 g, 0.175 mol) in portions, and after standing for 14 h at room temperature, the reaction mixture was poured into water and extracted with CH_2Cl_2. The extract was washed several times with aqueous $CuSO_4$ solution and water and then dried over anhydrous sodium sulfate. Removal of solvent under reduced pressure gave the crude tosylate b, which was used directly for the next reaction.

Thiophenol (14 g) dissolved in DMF (100 mL) was treated with *t*-BuOK (14 g). To this reagent, tosylate b was added and after 12 h at room temperature, the reaction mixture was poured into water and extracted with CH_2Cl_2. The extract was washed with aqueous Na_2CO_3 solution and water and then dried. Evaporation of solvent gave an oily residue which was purified by column chromatography. Pure phenyl sulfide c was eluted with benzene (yield 15 g).

To compound c (15 g) in benzene (100 mL) were added ethylene glycol (6 g) and *p*-TsOH (20 mg), and the reaction mixture was heated under a Dean–Stark trap for 3 h. After cooling, it was extracted with Na_2CO_3 solution and water and then dried, and the solvent was evaporated. The product, ketal d, was chromatographically homogeneous and was used in the subsequent transformation without further purification.

Crude ketal d in dichloromethane (250 mL) solution was treated with *m*-CPBA (80–85%, 27 g; added in portions). The temperature of the reaction mixture was maintained below 30 °C. After addition of all reagent the reaction was allowed to stand at room temperature with occasional shaking. When the reaction reached completion (about 1.5 h), the aromatic acids were removed by extraction with aqueous NH_3, and the organic layer was washed with water and dried. Evaporation of the solvent gave the oily sulfone e in essentially quantitative yield (19 g). The product was substantially pure (homogeneous by TLC) and was used without any further purification: ^1H NMR δ 1.18 (d, J = 7 Hz, 3 H), 1.19 (s, 3 H), 3.84 (m, 4 H), 7.3–7.6 and 7.6–7.9 (m, 5 H); IR (KBr) ν_{max} 1305, 1147, 1082 cm^{-1}; mass spectrum, m/z (relative intensity) 255 (M^+ – Me, 21), 184 (66), 87 (92), 43 (100). This compound (mp 88–89 °C) has been prepared previously by a different procedure.[17]

Coupling of Sulfone e to Aldehyde 1: Hydroxy Sulfone 2 and Olefin 3. Grignard reagent was prepared from Mg (535 mg, 22.22 mmol) and ethyl bromide in ether (10 mL), and the vigorously stirred solution was treated with sulfone e (6 g, 22.22 mmol) in benzene (6 mL). The precipitate formed was ground with a spatula, stirring was continued, and after 15 min the aldehyde 1 (2.0 g) was added in benzene (10 mL). The reaction mixture was stirred at room temperature for 24 h, then poured into aqueous $(NH_4)_2SO_4$ solution, and extracted with benzene. The organic layer, after washing with water, drying, and evaporation, gave an oily residue which was chromatographed on silica gel. In the benzene–ether fraction (8:2), excess sulfone was recovered (4.5 g); elution with benzene–ether (3:1) afforded unreacted aldehyde 1 (1.0 g); the reaction products 2 were eluted with ethyl acetate.

The crude mixture of steroidal α-hydroxy sulfones 2 was dissolved in methanol (200 mL) saturated with Na_2HPO_4. Sodium amalgam (5.65%, 15 g) was added and the reaction mixture was stirred at 4 °C for 15 h. To increase yield, unreacted aldehyde, as recovered above, was recycled through the sulfone addition, and the resulting α-hydroxy sulfones were, as in the previous case, treated with sodium amalgam in buffered methanol.

The reaction mixtures (after both Na/Hg reductions) were combined and, after removal of mercury by filtration and of methanol by evaporation under reduced pressure, water was added and the organic material was extracted with benzene. After drying and evaporation of solvent, the oil residue was chromatographed on a silica gel column. Elution with benzene–ether (1:4) gave compound **3** (1.0 g, 44%) as a colorless foam: ^1H NMR δ 0.80 (s, 18-H), 0.97 (s, 19-H), 1.22 (s, 26-H), 3.93 (m, 4 H, ketal H), 4.44 (m, 1 H, 3α-H), 5.25–5.45 (m, 2 H, 22-H and 23-H), 6.23 and 6.39 (doublets, J = 8 Hz, 2 × 1 H, 7-H and 6-H), 7.25–7.45 (m, 5 H, C_6H_5); IR ($CHCl_3$) ν_{max} 3603 (OH), 1749, 1692 (C=O), 1406, 1038 cm^{-1}; mass spectrum, m/z 440 (M^+ – triazoline, 24), 87 (100).

Coupling of Sulfone e to Aldehyde 4a: Hydroxy Sulfone 5a and Olefin 6. Grignard reagent was prepared from Mg (75 mg, 3.1 mmol) and ethyl bromide in ether (10 mL). To the stirred solution of ethylmagnesium bromide, sulfone e (891 mg, 3.3 mmol) in benzene (5 mL) was added. After the resulting suspension was stirred at room temperature for 15 min, a solution of aldehyde **4a** (290 mg) in benzene (5 mL) was added. The reaction was continued for 2.5 h, then quenched with saturated $(NH_4)_2SO_4$ solution (5 mL), and dilutedd with ether. The separated organic layer was washed with water, dried, and evaporated. The oily residue containing **5a** was treated with acetic anhydride (2 mL) and pyridine (2 mL). The reaction mixture was allowed to stand for 24 h, poured into water, and extracted with benzene. The benzene extract was washed with an aqueous solution of $CuSO_4$ and water, dried, and evaporated. The crude product (acetate **5b**) was dissolved in methanol saturated with Na_2HPO_4 an sodium amalgam (5.65%, 8 g) was added. The reaction mixture was stirred at 4 °C for 16 h. After the reaction, mercury was removed by filtration, methanol was evaporated, and water and benzene were added to dissolve the residue. The benzene layer was dried and evaporated. The oily residue was chromatographed over silica gel. Elution with a benzene–ether mixture (93:7) afforded compound **6** (206 mg, 54%): ^1H NMR δ 0.74 (s, 18-H), 1.04 (s, 19-H), 1.25 (s, 26-H), 2.78 (m, 1 H, 6α-H), 3.34 (s, 3 H, OCH_3), 3.97 (m, 4 H, ketal H), 5.25–5.45 (m, 2 H, 22-H and 23-H); IR (KBr) ν_{max} 3470 (OH), 1095 cm^{-1}; mass spectrum, m/z (relative intensity) 456 (M^+, 1), 441 (M^+ – Me, 45), 87 (100).

Ozonolysis of Compound 6. Compound **6** (100 mg) was treated with an excess of ozone at −70 °C in 1% pyridine–methylene chloride (50 mL). Dimethyl sulfide (1 mL) was added at −70 °C and the mixture warmed to room temperature, then washed with 1% hydrochloric acid and water, and dried. Evaporation gave the crude product which was purified by column chromatography. Elution with benzene–ether (85:15) afforded an aldehyde **4a**. Comparison of ^1H NMR spectra and TLC R_f values of the compound obtained with those an authentic sample of the aldehyde **4a** and those of the mixture of **4a** and **4b** proved unequivocally the retention of the configuration on C-20 during synthesis of **6**.

(16) Napoli, J. L.; Fivizzani, M. A.; Hamstra, A. J.; Schnoes, H. K.; DeLuca, H. F. *Anal. Biochem.* **1979**, *96*, 481. Suda, T.; DeLuca, H. F.; Hallick, R. B. *Ibid.* **1971**, *43*, 139.

(17) Inomata, K.; Nakayama, Y.; Tsutsumi, M.; Katake, H. *Heterocycles* **1979**, *12*, 1467.

Removal of the PTAD Protecting Group: 5,7-Diene 7. A mixture of the compound **3** (1 g) and lithium aluminum hydride (1.8 g) in THF (120 mL) was heated under reflux for 10 h. After cooling, excess reagent was destroyed with a few drops of water, the mixture was dried over anhydrous $MgSO_4$ and filtered, and solvent was evaporated to give a colorless crystalline material. Crude diene **7** was repeatedly crystallized from ethanol; first and second crops were combined (415 mg). The mother liquor was chromatographed on a silica gel column to give with benzene–ether (7:3) 120 mg of **7**: total yield, 535 mg (79%); mp 132–134 °C (ethanol); ^1H NMR δ 0.63 (s, 18-H), 0.95 (s, 19-H), 1.23 (s, 26-H), 3.63 (m, 1 H, 3α-H), 3.95 (m, 4 H, ketal H), 5.20–5.50 (m, 3 H, 22-H, 23-H, and 7-H), 5.57 (m, 1 H, 6-H); IR (KBr) ν_{max} 3430 (OH), 1063, 1038 cm^{-1}; mass spectrum, m/z (relative intensity) 440 (M^+, 50), 407 ($M^+ - H_2O$ – Me, 11), 87 (100); UV (EtOH) λ_{max} 282 nm (ϵ 11 000).

Irradiation of Compound 7: Previtamin Analogue 8. A solution of diene **7** (50 mg) in 150 mL of benzene–ether (1:4) was cooled on ice and deoxygenated with argon for 20 min. The reaction mixture was irradiated for 18 min with a mercury arc lamp (Hanovia SA-1) fitted with a Vycor filter. The solvent was evaporated and the residue was chromatographed on HPLC (6.2 mm × 25 cm Zorbaz-SIL, 4 mL/min, 1400 psi) and eluted with 2% 2-propanol in hexane to yield 22 mg (44%) of previtamin **8**: ^1H NMR δ 0.73 (s, 18-H), 1.24 (s, 26-H), 1.64 (s, 19-H), 3.96 (m, 5 H, ketal H and 3α-H), 5.35 (m, 2 H, 22-H and 23-H), 5.50 (m, 1 H, 9-H), 5.69 and 5.94 (doublets, J = 11.5 Hz, 2 × 1 H, 6-H and 7-H); UV (EtOH) λ_{max} 263 nm (ϵ 8900).

Isomerization of 8 to the Vitamin Analogue 9. Previtamin **8** (22 mg) was dissolved in ethanol (40 mL) and heated under reflux for 150 min. The product was purified by HPLC to yield 18 mg (82%) of the pure vitamin **9**: ^1H NMR δ 0.75 (s, 18-H), 1.24 (s, 26-H), 3.94 (m, 5 H, ketal H and 3α-H), 4.81 and 5.04 (2 narrow m, 2 × 1 H, 19(*Z*)-H and 19(*E*)-H), 5.33 (m, 2 H, 22-H and 23-H), 6.03 (d, J = 11 Hz, 1 H, 7-H), 6.22 (d, J = 11 Hz, 1 H, 6-H); mass spectrum, m/z (relative intensity) 440 (M^+, 17), 87 (100); UV (EtOH) λ_{max} 265 nm (ϵ 17 000); m/z calcd for $C_{29}H_{44}O_3$, 440.3290; found, 440,3278.

Hydrolysis of the Ketal: Keto Vitamin D_2 Analogue 10. To the solution of **9** (18 mg) in ethanol (35 mL), *p*-toluene sulfonic acid (7.5 mg) in water (1 mL) was added and the reaction mixture was heated under reflux for 90 min (the reaction course was monitored by HPLC). The solvent was evaporated, and the residue was dissolved in benzene and extracted with water. The benzene solution was dried (anhydrous $MgSO_4$) and evaporated to yield product **10** (16 mg, 99%): ^1H NMR δ 0.57 (s, 18-H), 1.04 (d, J = 7 Hz, 21-H), 1.13 (d, J = 7 Hz, 28-H), 2.12 (s, 3 H, 26-H), 3.10 (m, 1 H, 24-H), 3.96 (m, 1 H, 3α-H), 4.82 and 5.05 (2 narrow m, 2 × 1 H, 19(*Z*)-h and 19(*E*)-H), 5.2–5.5 (m, 2 H, 22-H and 23-H), 6.03 (d, J = 11.5 Hz, 1 H, 7-H), 6.22 (d, J = 11.5 Hz, 1 H, 6-H); IR ($CHCl_3$) ν_{max} 3596 (0H), 1709 cm^{-1} (C=O); mass spectrum, m/z (relative intensity) 396 (M^+, 41), 363 ($M^+ - H_2O$ – Me, 13), 271 (M^+ – side chain, 16), 253 (M^+ – side chain – H_2O, 23), 136 (100), 118 (95); UV (EtOH) λ_{max} 265 nm (ϵ 17 900).

Reaction of Ketone 10 with Methylmagnesium Iodide: 25-OH-D_2, 11a, and Its Epimer 11b. Grignard reagent was prepared from magnesium (240 mg) and methyl iodide in anhydrous ether (20 mL). To one-tenth of this solution (2 mL, 0.5 M solutions of CH_3MgJ) was added ketone **10** (16 mg, 0.04 mmol) in ether (2 mL). The reaction mixture was stirred at room temperature for 2 h, then quenched with aqueous solution of NH_4Cl, diluted with benzene, andd washed with water. The organic layer was separated, dried, and evaporated. The crude product was first purified by silica gel column chromatography (elution with 20% ether in benzene) and the mixture of **11a** and **11b** (16 mg, 96%) thereby obtained was then repeatedly chromatographed on an HPLC column by using 2% 2-propanol in hexane as an eluent. The peaks of 24-epi-25-OH-D_2 (**11b**) and 25-OH-D_2 (**11a**) partially overlapped, but a second chromatographic pass afforded relatively pure material (purity about 95%). Chromatography and rechromatography of each stereosiomer yield 4 mg of **11b** (collected at 68 mL), 4 mg of **11a** (collected at 74 mL) and 7 mg of the mixture of both epimers.

25-OH-D_2 (11a): $[\alpha]^{25}_D$ +56.8° (*c* 0.2, EtOH); ^1H NMR δ 0.57 (s, 18-H), 1.00 (d, J = 7 Hz, 28-H), 1.04 (d, J = 7 Hz, 21-H), 1.15 and 1.17 (2 singlets, 26-H and 27-H), 3.95 (m, 1 H, 3α-H), 4.82 and 5.05 (2 narrow m, 2 × 1 H, 19(*Z*)- and 19(*E*)-H), 5.23–5.43 (m, 2 H, 22-H and 23-H), 6.05 and 6.22 (2 doublets, J = 11 Hz, 2 × 1 H, 7-H and 6-H); IR ν_{max} 3401 (OH), 1645, 1631 (C=C), 971 cm^{-1} (trans C=C); mass spectrum, m/z (relative intensity) 412 (M^+, 63), 394 ($M^+ - H_2O$, 10), 379 ($M^+ - H_2O$ – Me, 23), 271 (M^+ – side chain, 37), 253 (M^+ – side chain –H_2O, 43), 136 (100), 118 (86), 59 (99); UV (EtOH) λ_{max} 265 nm (ϵ 17 950); m/z calcd for $C_{28}H_{44}O_2$, 412.3341; found, 412.3344.

24-Epi-25-OH-D_2 (11b): $[\alpha]^{25}_D$ +50.7° (*c* 0.2, EtOH); ^1H NMR δ 0.57 (s, 18-H), 0.99 (d, J = 7 Hz, 28-H), 1.03 (d, J = 7 Hz, 21-H), 1.14 and 1.16 (2 singlets, 26-H and 27-H), 3.94 (m, 1 H, 3α-H), 4.82 and 5.03 (2 narrow m, 2 × 1 H, 19(*Z*)-H and 19(*E*)-H), 5.20–5.40 (m, 2 H, 22-H and 23-H), 6.04 and 6.22 (2 doublets, J = 11 Hz, 2 × 1 H, 7-H and 6-H); IR (KBr) ν_{max} 3401 (OH), 1643, 1630 (C=C), 971 cm^{-1} (trans C=C); mass spectrum, m/z (relative intensitiy) 412 (M^+, 62) 394 ($M^+ - H_2O$, 12), 379 ($M^+ - H_2O$ – Me, 31), 271 (M^+ – side chain, 44), 253 (M^+ – side chain – H_2O, 55), 136 (100), 118 (67), 59 (38); UV (EtOH) λ_{max} 265 nm (ϵ 17 300); m/z calcd for $C_{28}H_{44}O_2$, 412.3341; found, 412.3333.

Note: Starting from pure provitamin **7** further synthesis (irradiation, isomerization, deketalization, and Grignard reaction) may be accomplished without chromatographic purification of any intermediate. Careful column chromatography before the final separation on HPLC removes all byproducts. The overall yield of these four steps (**7** → **11**) is 34%.

Acknowledgment. This work was supported by Grant No. AM-32701 and a Program Project Grant No. AM-14881 from the National Institutes of Health and by the Wisconsin Alumni Research Foundation.