(26 mg, 0.044 mmol) in 1 mL of absolute methanol. The mixture was allowed to stand at room temperature for 3 h, rendered neutral with Dowex 50W-X8 cation-exchange resin (H^+). The resin was filtered and the solvent was evaporated to dryness in vacuo. The residue was purified by preparative TLC to afford the free *C*-nucleoside **19** (10 mg, 81.5%) as a white solid, mp 180–182 °C; $[\alpha]^{24.3}_D$ +52.9 (*c* 0.59, methanol); MS, *m/e* 279 (M^+); ^1H NMR (Me_2SO-d_6) δ 3.47–4.08 (m, 5, H-2′, H-3′, H-4′, H-5′), 5.68 (d, 1, H-1′, $J_{1',2'}$ = 6 Hz), 7.67–7.97 (m, 2, H-4, H-5), 8.10 (dd, 1, H-6, $J_{5,6}$ = 6 Hz, $J_{4,6}$ = 2 Hz); ^{13}C NMR (Me_2SO-d_6) δ 61.89 (C-5′), 71.37, 78.39, 78.51, 84.94 (C-1′, C-2′, C-3′, C-4′), 122.15, 128.99, 132.62, 133.09, 134.43 (Ar–C), 169.13, 169.71 (C=O); IR ($CHCl_3$) 3580, 3320, 3210 cm^{-1}.

Anal. Calcd for $C_{13}H_{13}NO_6$: C, 55.91, H, 4.70; N, 5.02. Found: C, 56.07; H, 4.55; N, 4.99.

3-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)phthalimide (20). Ethyl orthoformate (0.1 mL, 0.6 mmol) was added to a well-stirred suspension of **19** (9 mg, 0.032 mmol) in acetone (1 mL) containing *p*-toluenesulfonic acid monohydrate (4.6 mg) and the mixture was allowed to stand at room temperature for 12 h. Then sodium bicarbonate was added, and the mixture was stirred for 15 min. The solid was collected by filtration and thoroughly washed with acetone. The filtrates were combined and evaporated in vacuo to a syrup which was purified by preparative TLC: MS, *m/e* 319 (M^+); ^1H NMR ($CDCl_3$) δ 1.35 (s, 3, isopropylidene CH_3), 1.65 (s, 3, isopropylidene CH_3), 2.94 (br, 1, OH), 3.89 (m, 2, H-5′), 4.23 (q, 1, H-4′, $J_{3',4'}$ = 8 Hz, $J_{4',5'}$ = 4 Hz), 4.68 (t, 1, H-2′, $J_{1',2'}$ = $J_{2',3'}$ = 5 Hz), 4.91 (dd, 1, H-3′, $J_{2',3'}$ = 5 Hz, $J_{3',4'}$ = 8 Hz), 5.69 (d, 1, H-1′, $J_{1',2'}$ = 5 Hz), 7.63–8.04 (m, 3, Ar H), 10.71 (br, 1, NH); IR ($CHCl_3$) 3645, 3410, 2980, 1760, 1720, 1620 cm^{-1}.

Registry No. **1**, 86528-49-6; **2** (isomer 1), 89196-63-4; **2** (isomer 2), 89299-52-5; **3**, 89196-64-5; **4**, 89196-65-6; **6**, 86528-50-9; **7** (isomer 1), 89254-78-4; **7** (isomer 2), 89254-79-5; **8**, 89196-66-7; **9**, 89196-67-8; **11**, 89196-68-9; **12**, 89196-69-0; **13**, 89196-70-3; **14**, 89196-71-4; **15**, 89196-72-5; **16**, 89196-73-6; **17**, 89196-74-7; **18**, 89196-75-8; **19**, 89196-76-9; **20**, 89196-77-0; dimethyl acetylenedicarboxylate, 762-42-5; maleimide, 541-59-3.

Studies of Vitamin D Oxidation. 4. Regio- and Stereoselective Epoxidation of Vitamin D

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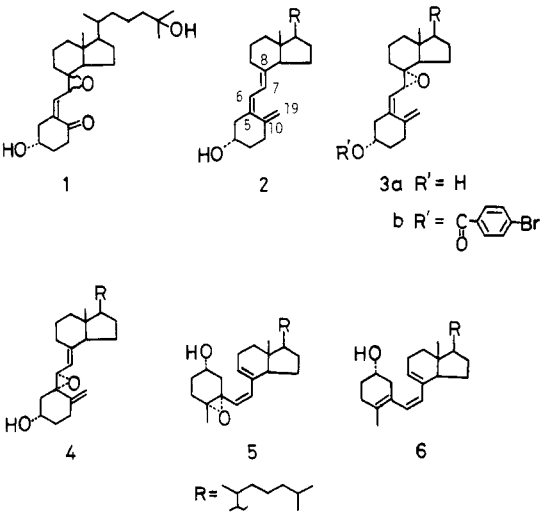
Regio- and stereoselective epoxidations of vitamin D_3 at the 7,8- and 5,6-double bonds were performed. Epoxidation with *m*-chloroperbenzoic acid gave exclusively (7*R*)-7,8-epoxyvitamin D_3 (81%) while epoxidation with *tert*-butyl hydroperoxide catalyzed by $VO(acac)_2$ afforded (5*S*)-5,6-epoxyvitamin D_3 in excellent yield (90%). The structures of the epoxides were confirmed by spectral analysis and by single-crystal X-ray analysis.

By extensive studies of the metabolism of vitamin D, more than twenty metabolites of vitamin D_3 (**2**) have been isolated and identified.[1] The structural alterations of vitamin D by metabolism can be classified into two groups, hydroxylation at the 1α-position under conditions of vitamin D deficiency and oxidation at the side chain mostly under conditions of vitamin D supplementation. Oxidation of the conjugated triene part of vitamin D has not been observed in the metabolites isolated so far, except for 7,8-epoxy-25-hydroxy-19-nor-10-oxovitamin D_3 (**1**),[2] although one may expect such oxidation in vivo as in other unsaturated fat-soluble biological compounds such as fatty acids[3] and vitamin A.[4] We have been studying the oxidation of the conjugated triene part of vitamin D in conjunction with biological oxidation and have reported its oxidation with singlet oxygen.[5]



1 2 3a R′ = H b R′ = 4-bromobenzoyl 4 5 6

Selective epoxidation of vitamin D derivatives is known. It has been reported that epoxidation of 3,5-dinitro-

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benzoate of vitamin D_2 with peracids proceeded regio- and stereoselectively to yield one of the corresponding 7,8-epoxides,[6,7] whereas epoxidation of vitamin D_2 with a combination of hydrogen peroxide and benzonitrile gave exclusively the corresponding (5*S*)-5,6-epoxide.[7] Aiming at the synthesis of a new metabolite of vitamin D_3 possessing an epoxy function, we have reinvestigated regio- and stereochemical consequences of the epoxidation of vitamin D using two epoxidation reagents, *m*-chloroperbenzoic acid and *tert*-butyl hydroperoxide, in the presence of transition metal complexes. We report here the results including unambiguous determination of the stereochemistry of the resulting epoxides with the aid of X-ray crystallography.

Epoxidation of vitamin D_3 (**2**) with *m*-chloroperbenzoic acid (CH_2Cl_2, −70 to 0 °C) occurred with exclusively high regio- and stereoselectivity to yield the single epoxide (7*R*)-7,8-epoxyvitamin D_3 (**3a**) in 81% isolated yield. The structure of **3a** was fully established by the spectral data as well as by the X-ray analysis. The UV spectrum (227.5 nm, ϵ 7330) shows a conjugated diexo diene chromophore[8] indicating that the epoxidation occurred at the 7,8-double bond. The mass, ^{1}H NMR and ^{13}C NMR spectra support the assigned structure. Stereochemical assignment and confirmation of the structure were achieved by single-crystal X-ray analysis of the *p*-bromobenzoate **3b**. The stereoscopic view of the molecule of **3b** drawn by the PLUTO program[9] is shown in Figure 1. The result indicates that the oxygen atom of the epoxide ring is oriented in the α-direction. Thus it was established that the epoxidation of the conjugated triene function of vitamin D with peracids occurs exclusively at the terminal 7,8-double bond from the sterically less hindered α-side of the molecule. Similarly regio- and stereoselective oxidation of the 7,8-double bond has been observed in the reaction of vitamin D with potassium permanganate.[10] This regioselectivity can be attributed to the thermodynamic stability of the resultant conjugated diene derivatives. The 7,8-double bond rather than the 10,19-double bond was attacked selectively probably because the former bond is more electron rich than the latter.

We next turned our attention to epoxidation with *tert*-butyl hydroperoxide (TBHP) catalyzed by vanadium and molybdenum complexes[11] expecting regioselective epoxidation of the 5,6-double bond by the directive effect of the 3β-hydroxyl group situated homoallylic to the double bond. As expected, treatment of vitamin D_3 (**2**) with anhydrous TBHP in benzene in the presence of $VO(acac)_2$ (0.04 equiv) at room temperature gave (5*S*)-5,6-epoxyvitamin D_3 (**4**) as the sole product in 90% isolated yield. The structure of **4** was confirmed by the mass, ^{1}H NMR, ^{13}C NMR, and UV spectra, of which the ^{1}H NMR and UV spectra were closely related to those reported for 5,6-epoxyvitamin D_2.[7] The stereochemistry of the epoxide ring was elucidated on the basis of the well-established stereochemical course of the epoxidation,[11] the oxygen being oriented to the side of the homoallylic hydroxyl group. Epoxidation of vitamin D_3 with TBHP (benzene) using molybdenum hexacarbonyl (0.04 equiv) as the catalyst did not proceed at room temperature. Refluxing of the reaction gave a mixture of three epoxides, **3a** (24%), **4** (47%), and (5*R*)-5,10-epoxyprevitamin D_3 (**5**) (24%). The structure of **5** was determined by spectral analysis. The UV spectrum (243 nm, ϵ 5400) is in good agreement with that reported for 5,10-epoxyprevitamin D_2.[6] The stereochemistry of the epoxide ring is also based on the stereochemical course of the epoxidation, the oxygen being oriented to the same side of the 3β-hydroxyl group. The low selectivity of the epoxidation catalyzed by molybdenum complex is clearly due to the forced reaction conditions. The formation of 5,10-epoxyprevitamin D_3 (**5**) indicates an equilibrium between vitamin D_3 (**2**) and previtamin D_3 (**6**) under the reaction conditions.

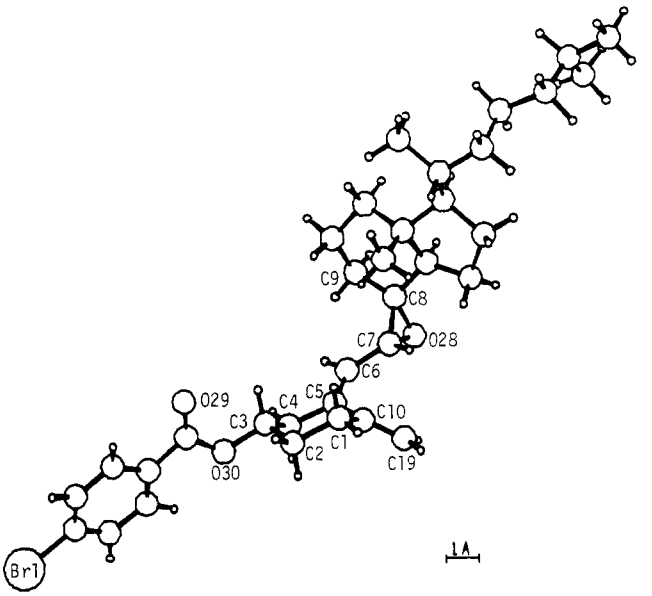

Figure 1. Stereoscopic view of the molecule of **3b** drawn by PLUTO program.

Experimental Section

Melting point was determined on a Yanaco micro melting point apparatus and is uncorrected. Infrared (IR) spectra were obtained on a Hitachi 215 spectrophotometer. Proton magnetic resonance (^{1}H NMR) spectra were recorded with a Varian XL-100 spectrometer with tetramethylsilane as an internal standard. Carbon magnetic resonance (^{13}C NMR) spectra were recorded with a Varian XL-100 spectrometer at 25.16 MHz. The solvent for ^{13}C NMR spectra was $CDCl_3$ with tetramethylsilane as an internal reference, the deuterium of the solvent providing the internal lock signal. Mass spectra were recorded with a JEOL JMS-D300 GC-MS instrument with interfaced computer. Ultraviolet (UV) spectra were recorded with a Hitachi 200-10 double beam spectrophotometer in a 95% ethanol solution unless otherwise noted.

(7*R*)-7,8-Epoxy-9,10-seco-5,10(19)-cholestadien-3β-ol (3a). To a solution of vitamin D_3 (**2**) (1.0 g, 2.6 mmol) in dry dichloromethane (30 mL) was added 80% *m*-chloroperbenzoic acid (562 mg, 2.6 mmol) at −70 °C. The solution was stirred for 2.5 h while the temperature of the solution was allowed to raise to 0 °C. The reaction mixture was diluted with dichloromethane, washed with 5% $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel (20 g) with ethyl acetate–hexane (3:7) as the eluent to give epoxide **3a** (844 mg, 81%) as a colorless oil: high-resolution MS $C_{27}H_{44}O_2$ requires, *m/z* 400.3341; found, *m/z* 400.3358; MS, *m/z* 400 (M^+), 382, 367, 364, 287, 269, 251, 247; ^{1}H NMR ($CDCl_3$) δ 0.67 (3 H, s, H-18), 0.85 (6 H, d, J = 6 Hz, H-26 and H-27), 3.88 (1 H, d, J = 9 Hz, H-7), 3.93 (1 H, m, H-3), 4.93 (1 H, br s, H-19), 5.00 (1 H, br s, H-19), 5.20 (1 H, d, J = 9 Hz, H-6); ^{13}C NMR ($CDCl_3$)

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δ 145.5 (s, C-5 or C-10), 145.0 (s, C-10 or C-5), 121.4 (d, C-6), 112.2 (t, C-19), 69.1 (d, C-3), 65.6 (s, C-8), 56.7 (d, C-17), 56.3 (d, C-14), 54.1 (d, C-7), 46.0 (t, C-4), 46.0 (s, C-13), 39.5 (t, C-12 and C-24), 36.1 (C-22), 35.7 (C-20), 35.1 (C-2), 32.0 (C-1), 30.8 (C-9), 28.0 (C-25), 27.4 (C-16), 23.9 (C-23), 22.8 (C-15), 22.6 (C-27), 22.3 (C-26), 20.0 (C-11), 18.8 (q, C-21), 12.6 (q, C-18); IR ($CHCl_3$) 3610, 3440, 2950, 2865 cm^{-1}.

(7*R*)-7,8-Epoxy-9,10-seco-5,10(19)-cholestadien-3β-yl *p*-Bromobenzoate (3b). *p*-Bromobenzoyl chloride (609 mg, 2.78 mmol) was added to a solution of epoxide **3a** (740 mg, 1.85 mmol) in dry pyridine (3 mL) at −20 °C. The mixture was kept standing at −20 °C for 5 min and then at 0 °C for 1 h. The reaction mixture was diluted with ethyl acetate, washed with brine, dried over Na_2SO_4, and evaporated. The crude product was chromatographed on silica gel (20 g) with ethyl acetate–hexane (1:9) as the eluent to give *p*-bromobenzoate **3b** (668 mg, 62%): mp 121–122 °C (from dichloromethane–methanol); high-resolution MS $C_{34}H_{47}O_3Br$ requires, m/z 582.2706; found, m/z 582.2695; MS, m/z 584 and 582 (M^+), 566, 564, 382, 364, 349, 269, 251, 247; ^1H NMR ($CDCl_3$) δ 0.72 (3 H, s, H-18), 0.87 (6 H, d, J = 6 Hz, H-26 and H-27), 3.93 (1 H, d, J = 9.5 Hz, H-7), 5.02 (1 H, br s, H-19), 5.10 (1 H, br s, H-19), 5.26 (1 H, d, J = 9.5 Hz, H-6), 7.57 (2 H, d, J = 8.5 Hz, H-aromatic), 7.88 (2 H, d, J = 8.5 Hz, H-aromatic); UV (hexane–95% EtOH, 1:1) 243 nm (ε 43 200); IR (KBr) 2955, 2930, 2860, 1720 cm^{-1}.

(5*S*)-5,6-Epoxy-9,10-seco-7,10(19)-cholestadien-3β-ol (4). To a solution of vitamin D_3 (**2**) (100 mg, 0.26 mmol) and $VO(acac)_2$ (3 mg, 1.1×10^{-2} mmol) in dry benzene (2 mL) was slowly added 4.98 M anhydrous TBHP (104 μL, 0.52 mmol) benzene solution at 5 °C. During the addition of the peroxide, the color of the reaction mixture turned from green to dark red. The solution was allowed to warm to room temperature and then stirred for 3 h at that temperature. After addition of aqueous Na_2SO_3, the mixture was extracted with benzene, the extracts were washed with brine, dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel (3 g) with ethyl acetate–hexane (3:7) as the eluent to give epoxide **4** (94 mg, 90%) as a colorless oil: high-resolution MS $C_{27}H_{44}O_2$ requires, m/z 400.3341; found, m/z 400.3346; MS, m/z 400 (M^+), 385, 382, 357, 315, 287; ^1H NMR ($CDCl_3$) δ 0.47 (3 H, s, H-18), 0.85 (6 H, d, J = 6 Hz, H-26 and H-27), 3.62 (1 H, d, J = 9 Hz, H-6), 3.90 (1 H, m, H-3), 4.65 (1 H, d, J = 9 Hz, H-7), 4.91 (2 H, br s, H-19); ^{13}C NMR ($CDCl_3$) δ 148.0 (s, C-10), 142.9 (s, C-8), 114.5 (d, C-7), 108.9 (t, C-19), 69.1 (d, C-3), 64.3 (s, C-5), 61.4 (d, C-6), 56.5 (C-17), 56.0 (C-14), 45.3 (s, C-13), 44.8 (t, C-4), 40.2 (C-12), 39.4 (C-24), 36.0 (C-20 and C-22), 35.5 (C-2), 30.7 (C-1), 29.3 (C-9), 27.9 (C-25), 27.5 (C-16), 23.8 (C-15 and C-23), 22.8 (C-27), 22.5 (C-26), 21.8 (C-11), 18.8 (q, C-21), 11.6 (q, C-18); UV λ_{max} (95% EtOH) <220 nm; IR ($CHCl_3$) 3610, 3420, 2950, 2870 cm^{-1}.

Molybdenum Hexacarbonyl Catalyzed Epoxidation of Vitamin D_3 (2). To a solution of vitamin D_3 (**2**) (100 mg, 0.26 mmol) and $Mo(CO)_6$ (3 mg, 1.1×10^{-2} mmol) in dry benzene (2 mL) was added 4.98 M anhydrous *tert*-butyl hydroperoxide (104 μL, 0.52 mmol) benzene solution at room temperature. The mixture was refluxed for 1.5 h and then poured into aqueous Na_2SO_3. The mixture was extracted with benzene, and the extracts were washed with brine, dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel (7 g) with ethyl acetate–hexane (2:8) as the eluent to give **5** (25 mg, 24%), **4** (47 mg, 45%), and **3a** (25 mg, 24%) in this order. **5**: high-resolution MS $C_{27}H_{44}O_2$ requires, m/z 400.3341; found, m/z 400.3348; MS, m/z 400 (M^+), 385, 382, 364, 357, 343, 287, 269; ^1H NMR ($CDCl_3$) δ 0.71 (3 H, s, H-18), 0.88 (6 H, d, J = 7 Hz, H-26 and H-27), 0.96 (3 H, d, J = 7 Hz, H-21), 1.31 (3 H, s, H-19), 3.71 (1 H, m, H-3), 5.46 (1 H, d, J = 12 Hz, H-6 or H-7), 5.58 (1 H, m, H-9), 5.86 (1 H, d, J = 12 Hz, H-7 or H-6); IR ($CHCl_3$) 3450, 2950, 2870 cm^{-1}.

X-ray Structure Determination of 3b. The prismatic crystals several mm long were grown in a dichloromethane–methanol solution with space group $P2_1$. The crystal was cut into several pieces with approximate dimensions of 0.08 × 0.12 × 0.5 mm and used for the intensity measurement. The cell parameters and diffraction intensities were measured on a Philips PW 1100 diffractometer using Cu Kα radiaion monochromated by a graphite plate. The crystal data were a = 17.312 (10) Å, b = 6.407 (4) Å, c = 15.814 (9) Å, β = 116.19 (6)° for Z = 2, and a calculated density of 1.231 g/cm^3. Intensities decreased during the measurement due to deterioration of the crystal structure upon X-ray irradiation. Therefore the specimen was changed whenever the intensities of three standard reflections decreased by 10–20%. A total of 6515 reflections were measured in the 2Θ range of 6° through 160° using 6 crystals and they were reduced by 3484 independent reflections (including 691 Friedel reflections). The agreement of $|F|$ between the equivalent reflections was R = 0.047 while that between the Friedel reflections was 0.052.

The structure was solved by the heavy atom method and refined by the block-diagonal-matrix least-squares calculations to an R value of 0.062 including all the 47 hydrogen atoms with isotropic temperature factors which were located on the difference electron-density maps. The absolute configuration was determined by the anomalous dispersion method. The structure factors were calculated with dispersion corrections f' = 0.767, f'' = 1.283 for the atomic scattering factor of bromine (with Cu Kα radiation) and the ratios of $|F(hkl)|/|F(\bar{h}\bar{k}\bar{l})|$ were compared between observed and calculated values. Of the total of 216 Friedel pairs for which both the observed and calculated ratios differ more than 1% from unity, 203 pairs consistently indicated the absolute configuration shown in Figure 1.

Acknowledgment. We are indebted to H. Nakai and T. Furusawa for their assistance in thhe experimental work.

Registry No. **2**, 67-97-0; **3a**, 89231-90-3; **3b**, 89231-91-4; **4**, 89231-92-5; **5**, 89231-93-6; BrC_6H_4-*p*-COCl, 586-75-4.

Supplementary Material Available: Final positional parameters, thermal parameters, bond distances, and bond angles in **3b** (6 pages). Ordering information is given on any current masthead page.