Registry No. **1a**, 1930-56-9; **1b**, 49739-65-3; **1c**, 298-81-7; **1d**, 2009-24-7; **2b**, 1930-59-2; **2c**, 3779-03-1; **2d**, 68123-30-8; **3**, 86885-06-5; **4**, 15294-25-4; **5**, 73640-81-0; **6**, 3902-71-4; **7**, 21902-10-3; **8**, 1930-58-1; **9**, 86885-07-6; cyclohexene, 110-83-8.

Electrophilic Addition of OsO_4 to 25-Hydroxycholecaliferol and Its 3,5-Cyclo Derivative[1]

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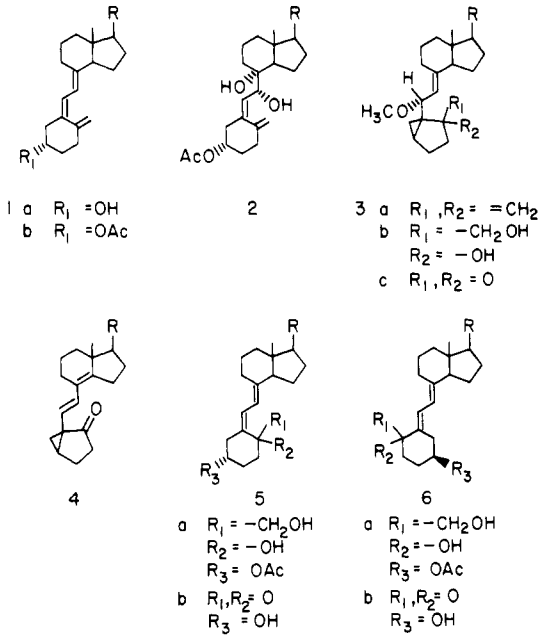
Received March 10, 1983

The organic chemistry of vitamin D has been largely confined to the synthesis and characterization of many of its biologically revelant metabolites, with the main efforts being directed at 1α-hydroxylation and side-chain modification schemes.[3] The chemistry of the conjugated triene, which typifies the vitamin D skeleton, and methods for its selective modification have not been explored systematically, although the s-cis character of the 5,6- and 10(19)-double bonds has been exploited for the formation of adducts with $Fe_2(CO)_9$[4] and in reactions with dienophiles (SO_2, triazoline, and singlet oxygen).[5-8] Hydroboration of the vitamin with the bulky borohydride 9-BBN exhibits high regioselectivity for the 10(19)-double bond of the triene and gives the 19-hydroxy-10(19)-dihydrovitamin analogues in excellent yields.[9]

In connection with our biochemical work, we were interested in preparing chemically or photochemically reactive probes (affinity labels) for the various macromolecular binding protein (e.g., the D-transport protein, or the cytosolic receptor protein) of the vitamin D endocrine system. We report here on the reactions of 25-hydroxycholecalciferol and its cyclovitamin derivative with osmium tetraoxide in pyridine that illustrate aspects of the electrophilic chemistry of the vitamin D triene and led to two 10-oxo derivatives with potential utility as covalent markers in biochemical systems.

When 25-hydroxycholecalciferol 3-acetate (**1b**) in pyridine was treated with a 1.2-fold excess of a freshly prepared OsO_4/pyridine reagent (100 mg/mL) at room temperature, a rapid reaction ensued and TLC analysis revealed the total absence of starting material within 10 min. A 75% yield of compound **2** was obtained, characterized as the 7,8-vicinal diol by NMR and mass spectral analysis. The stereochemistry of the 7,8-diol can be assigned by assuming α-face attack of the osmium reagent due to the presence of the axial β-face C-18 methyl group. The downfield NMR shift (from 0.54 to 0.80 ppm) for the C-18 methyl group in the 7,8-diol product also argues for an α-orientation of the 8-hydroxyl group.



The unique regiospecificity of this reaction on the normal vitamin skeleton changes dramatically when the 3,5-cyclovitamin analogue **3a** is utilized as a substrate. When **3a** was treated as above, the reaction was equally rapid and afforded a predominant product in 70% yield. NMR and mass spectroscopy established the cyclovitamin adduct as the 10,19-diol **3b**.

The pronounced change in the olefinic reactivity toward osmium tetraoxide can be rationalized on the basis of the known preference for osmic acid addition to strained, but sterically accessible, double bonds.[10-12] The normal vitamin triene possesses a great deal of conformational and rotational flexibility with only the 7,8-double bond rigidly fixed and exocyclic to the C ring. The β-face of the C-D ring system is sterically shielded by the axial orientation of the C-18 methyl group. In the cyclovitamin derivative the steric environment of the 7,8-double bond is drastically altered. The presence of the 6(*R*)-methoxy functionality makes it inaccessible to reagent approach, while 10(19)-olefin is conformationally fixed and strained by the [3.1.0] A-ring system and thus becomes the preferred target for the reagent.

The 10,19-dihydroxycyclovitamin **3b** was envisioned as the precursor for the 10-oxo-19-nor vitamin analogue **5b**. This $\alpha,\beta,\gamma,\delta$-unsaturated ketone (a structural relative of the 10-oxovitamin D_2 analogue previously synthesized as an intermediate in the partial synthesis of vitamin D_2[13]), has all the attributes of an endo-photoaffinity label,[14] i.e., a photoreactive chromophore absorbing at long wavelengths (310 nm) with a high extinction coefficient (15 000) and located on a part of the molecule that is within the binding domain of the macromolecule.

The conversion of the cyclovitamin-10,19-diol to the 10-oxo analogue **3c** was accomplished smoothly by treatment of **3b** with $NaIO_4$ in MeOH. However, reaction of **3c** with glacial HOAc gave diene **4**, resulting from the

(1) Supported in part by Program Project Grant No. 14881 from the National Institutes of Health and by the Harry Steenbock Fund of the Wisconsin Alumni Research Foundation.
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acid-catalyzed loss of methanol, as the major product, and none of the expected cycloreversion products **5b** and/or **6b**. This type of allylic elimination reaction has been noted in other cycloreversion reactions studied in our laboratories (S. H. Lee, unpublished results); however, the 6,8(14)-diene cyclovitamin was never more than a minor product in those cases.

This difficulty was circumvented by performing the cycloreversion reaction directly on the 10,19-diol cyclovitamin **3b** to yield the (5*Z*)- and (5*E*)-diols **5a** and **6a** in 58% and 18% yield, respectively. Reaction of either **5a** or **6a** with $NaIO_4$ in methanol followed by basic hydrolysis gave the corresponding 10-oxo-19-norcholecalciferols **5b** and **6b**.

Experimental Section

Mass spectra were run on an AEI/MS9 instrument at 70 eV. UV spectra were taken in absolute ethanol on a Beckman Model 24 spectrophotometer. Proton NMR spectra were recorded with a Bruker WH-270 pulse Fourier transform instrument in $CDCl_3$ solutions, with $CHCl_3$ as an internal standards.

7,8,25-Trihydroxy-7,8-dihydrocholecalciferol 3-Acetate (2). To a solution of 250 mg of 25-hydroxycholecalciferol 3-acetate (**1b**) in 2.0 mL of dry pyridine was added 1.67 mL of a 10% solution of OsO_4 in pyridine. After 15 min all the starting material had been consumed and 10 mL of 10% $NaHSO_3$ was added. This solution was stirred for 30 min at room temperature and then diluted with 50 mL of 10% $NaHSO_3$ and extracted with ether (3 × 25 mL). The ether extracts were washed with water (2 × 25 mL), 1 N HCl (2 × 25 mL), saturated $NaHCO_3$ (2 × 25 mL), and water (1 × 50 mL), dried over Na_2SO_4, and concentrated to an oil in vacuo. Purification of preparative TLC (silica gel, 5% $MeOH/CHCl_3$) or preparative HPLC (6.2 × 250 mm Zorbax-Sil column, 15% 2-propanol/hexanes) gave 185 mg of **2** as an oil that eluted at 15 mL and possessed the following spectral characteristics: mass spectrum, *m/z* (relative intensity) 476 (M^+, 3), 458 (5), 416 (10), 298 (25), 245 (20), 136 (100), 59 (75); NMR δ 0.80 (3 H, s, 18-H_3), 0.92 (3 H, d, *J* = 6.0 Hz, 21-H_3), 1.23 (6 H, s, 26-H_3 and 27-H_3), 2.04 (3 H, s, 3-$OCOCH_3$), 4.81 (1 H, m, 3-H), 4.91 (1 H, d, *J* = 9.5 Hz, 7-H), 4.95 (1 H, s, 19(*Z*)-H), 5.03 (1 H, s, 19(*E*)-H), 5.58 (1 H, d, *J* = 9.5 Hz, 6-H).

(6*R*)-25-Hydroxy-3,5-cyclocholecalciferol 6-Methyl Ether (3a). A solution of 300 mg of 25-hydroxycholecalciferol (**1a**) and 350 mg of *p*-toluenesulfonyl chloride in 2.0 mL of dry pyridine was allowed to react for 48 h at 5 °C with stirring. The solution was then quenched with saturated $NaHCO_3$ and the aqueous phase extracted with ether (3 × 30 mL). The ether extracts were washed with 1 N HCl (2 × 20 mL), saturated $NaHCO_3$ (2 × 30 mL), and H_2O (1 × 50 mL), dried over $MgSO_4$, and concentrated in vacuo. The resulting crude 3β-tosylate derivative was taken up in 15.0 mL of anhydrous methanol containing 800 mg of $NaHCO_3$ and heated to 55 °C for 6.0 h. At the end of this period the reaction mixture was cooled, concentrated to ~5 mL, diluted with ether, and washed with water (3 × 30 mL). After drying over $MgSO_4$, the ether solution was concentrated to an oil that was shown to be 80% 25-hydroxy-3,5-cyclovitamin D_3 (**3a**) by TLC analysis and suitable for subsequent reactions.

(6*R*)-10,19-Dihydro-10,19,25-trihydroxy-3,5-cyclocholecalciferol 6-Methyl Ether (3b). To 462 mg of **3a** in 3.0 mL of dry pyridine was added 3.1 mL of a 10% OsO_4 solution in pyridine and the reaction was continued for 10 min, after which it was quenched with 15 mL of 10% $NaHSO_3$. After 30 min the reaction mixture was further diluted with 50 mL of $NaHSO_3$ and extracted with ether (3 × 30 mL). Workup and purification as for **2** above gave, in 70% yield, product **3b** as a colorless oil: mass spectrum, *m/z* (relative intensity) 448 (M^+, 3), 430 (5), 416 (45), 398 (15), 367 (40), 269 (40), 245 (35), 59 (100); NMR δ 0.32 (1 H, m, 3-H), 0.52 (2 H, m, 4-H_2), 0.56 (3 H, s, 18-H_3), 0.90 (3 H, d, *J* = 6.0 Hz, 21-H_3), 1.23 (6 H, s, 26-H_3 and 27-H_3), 3.25 (3 H, s, 6-OCH_3), 3.63 (2 H, m, 19-H_2), 4.60 (1 H, d, *J* = 9.2 Hz, 6-H), 4.78 (1 H, d, *J* = 9.2 Hz, 7-H).

(6*R*)-25-Hydroxy-10-oxo-3,5-cyclo-19-norcholecalciferol 6-Methyl Ether (3c). A solution of 20 mg of **3b** in 1.5 mL of methanol was treated with 500 μL of saturated $NaIO_4$ in H_2O and warmed to 55 °C for 3.5 h. At the end of this time the reaction mixture was diluted with ether (30 mL), washed with water (3 × 15 mL), dried over $MgSO_4$, and concentrated to an oil in vacuo. The crude material was subjected to HPLC purification (6.2 × 250 mm Zorbax-Sil, 5% 2-propanol/hexanes) to give **3c**, eluting at 27 mL, in 52% yield: mass spectrum, *m/z* (relative intensity) 416 (M^+, 3), 398 (5), 384 (55), 255 (40), 139 (100), 59 (35); NMR δ 0.52 (3 H, s, 18-H_3), 0.96 (3 H, d, *J* = 6.0 Hz, 21-H_3), 1.23 (6 H, s, 26-H_3 and 27-H_3), 3.22 (3 H, s, 6-OCH_3), 4.58 (1 H, d, *J* = 9.6 Hz, 6-H), 4.72 (1 H, d, *J* = 9.6 Hz, 7-H).

(*E*)-10-Oxo-3,5-cyclo-19-nor-9,10-seco-6,8(14)-cholestadien-25-ol (4). A solution of 10 mg of **3c** in 0.5 mL of glacial acetic acid was heated to 55 °C for 45 min. The reaction mixture was cooled, quenched with ice/$NaHCO_3$, and extracted with ether (3 × 20 mL). The organic phase was washed with water (2 × 25 mL), dried over $MgSO_4$, and concentrated in vacuo. HPLC purification (6.2 mm × 250 mm Zorbax-Sil, 4% 2-propanol/hexanes) gave product **4**, eluting at 24 mL, in 65% yield: UV λ_{max} 254 nm; mass spectrum, *m/z* (relative intensity) 384 (M^+, 10), 366 (10), 255 (18), 59 (100); NMR δ 0.89 (3 H, s, 18-H_3), 0.96 (3 H, d, *J* = 6.0 Hz, 21-H_3), 1.23 (6 H, s, 26-H_3 and 27-H_3), 5.92 (1 H, d, *J* = 16 Hz, 7-H), 6.28 (1 H, d, *J* = 16 Hz, 6-H).

(5*Z*)- and (5*E*)-10,19,25-Trihydroxy-10,19-dihydrocholecalciferol 3-Acetate (5a and 6a). A solution of 300 mg of 10,19-diol **3b** in 3.0 mL of glacial acetic acid was heated to 55 °C for 154 min and then quenched by adding dropwise to ice/saturated $NaHCO_3$. The ether extraction (3 × 25 mL) was washed with H_2O (2 × 30 mL), dried over $MgSO_4$, and concentrated in vacuo. The oily crude product, subjected to HPLC purification (6.2 × 250 mm, Zorbax-Sil, 8% 2-propanol/hexanes), gave **5a**, eluting at 49 mL, in 48% yield [UV λ_{max} 252 nm; mass spectrum, *m/z* (relative intensity) 476 (M^+, 5), 458 (20), 416 (35), 398 (25), 245 (30), 185 (60), 134 (100), 59 (60); NMR δ 0.55 (3 H, s, 18-H_3), 0.96 (3 H, d, *J* = 6.0 Hz, 21-H_3), 1.23 (6 H, s, 26-H_3 and 27-H_3), 2.05 (3 H, s, 3-$OCOCH_3$), 3.72 (2 H, m, 19-H_2), 4.74 (1 H, m, 3-H), 5.82 (1 H, d, *J* = 11.2 Hz, 7-H), 6.63 (1 H, d, *J* = 11.2 Hz, 6-H)] and the 5*E* isomer, eluting at 27 mL, in 18% yield [UV λ_{max} 250 nm; mass spectrum, *m/z* (relative intensity) 476 (M^+, 2) 458 (6), 416 (30), 398 (30), 245 (25), 185 (40), 134 (100), 59 (80); NMR δ 0.46 (3 H, s, 18-H_3), 0.98 (3 H, d, *J* = 6.2 Hz, 21-H_3), 1.22 (6 H, s, 26-H_3 and 27-H_3), 2.03 (3 H, s, 3-$OCOCH_3$), 3.67 (2 H, q AB, *J* = 11.0 Hz, 19-H_2), 4.7 (1 H, m, 3-H), 6.02 (1 H, d, *J* = 15 Hz, 7-H), 6.30 (1 H, d, *J* = 15 Hz, 6-H)].

(5*Z*)- and (5*E*)-10-Oxo-25-hydroxy-19-norcholecalciferol (5b and 6b). A solution of 50 mg of (**5a**) in 1.5 mL of methanol was treated with 0.5 mL of a saturated solution of $NaIO_4$ in H_2O. The reaction mixture was heated to 50 °C for 2.5 h, diluted with H_2O, and extracted with ether (3 × 30 mL). The ether extracts were washed with H_2O (2 × 20 mL), dried over $MgSO_4$, and concentrated in vacuo. The crude oily material was taken up in 3.0 mL of ethanol and treated with 1.0 mL of 5% methanolic NaOH for 30 min at room temperature. The reaction mixture was neutralized with 1 N HCl, concentrated in vacuo, and diluted with ether (50 mL). The organic phase was washed with H_2O (2 × 20 mL), dried over $MgSO_4$, and taken to an oil in vacuo, which was purified via HPLC (6.2 × 250 mm Zorbax-Sil, 14% 2-propanol/hexanes) to give **5b**, eluting at 37 mL, in 72% yield: UV λ_{max} 310 nm (ε 15 000); mass spectrum, *m/z* (relative intensity) 402 (M^+, 35), 384 (30), 369 (10), 359 (45), 341 (15), 273 (35), 177 (50), 135 (70), 133 (100), 59 (60); NMR δ 0.55 (3 H, s, 18-H_3), 0.96 (3 H, d, *J* = 6.0 Hz, 21-H_3), 1.22 (6 H, s, 26-H_3 and 27-H_3), 4.2 (1 H, M, 3-H), 5.87 (1 H, d, *J* = 12.6 Hz, 6-H), 7.61 (1 H, d, *J* = 12.6 Hz, 7-H).

Similar treatment of the (5*E*)-triol 3-acetate **6a** afforded the 5(*E*)-10-oxo analogue **6b**, which on HPLC eluted at 34 mL in 14% 2-propanol/hexane and exhibited the following physical characteristics: UV λ_{max} 307 (ε 24 000); mass spectrum, *m/e* (relative intensity) 402 (M^+, 30), 384 (30), 369 (20), 359 (40), 273 (40), 177 (60), 135 (40), 133 (100), 59 (40); NMR δ 0.56 (3 H, s, 18-H_3) 0.95 (3 H, d, *J* = 6.0 Hz, 21-H_3), 1.23 (6 H, s, 26-H_3 and 27-H_3), 4.2 (1 H, m, 3-H), 6.65 (2 H, q AB, *J* = 11.8 Hz, 6-H and 7-H).

Registry No. **1a**, 19356-17-3; **1b**, 52993-60-9; **2**, 86823-83-8; **3a**, 86852-06-4; **3b**, 86834-44-8; **3c**, 86823-84-9; **4**, 86823-85-0; **5a**, 86823-86-1; **5b**, 86852-07-5; **6a**, 86823-87-2; **6b**, 85925-90-2; osmium tetraoxide, 20816-12-0.