Proc. Natl. Acad. Sci. USA
Vol. 75, No. 5, pp. 2080–2081, May 1978
Chemistry

Direct C-1 hydroxylation of vitamin D compounds: Convenient preparation of 1α-hydroxyvitamin D_3, 1α,25-dihydroxyvitamin D_3, and 1α-hydroxyvitamin D_2

(1α-hydroxyvitamin D synthesis/selenium dioxide oxidation/3,5-cyclovitamin D/cholecalciferol analogs)

HERBERT E. PAAREN, DAVID E. HAMER, HEINRICH K. SCHNOES, AND HECTOR F. DELUCA

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

Communicated by Henry Lardy, February 24, 1978

ABSTRACT **An efficient procedure for the direct C-1 hydroxylation of vitamin D compounds has been developed. The method involves conversion of vitamin D_3 tosylates to 3,5-cyclovitamin D derivatives, allylic oxidation with selenium dioxide, and acid-catalyzed solvolysis to the 1α-hydroxyvitamin D analogs. When applied to vitamin D_3, 25-hydroxyvitamin D_3, and vitamin D_2, this sequence gives the corresponding 1α-hydroxylated derivatives in 10–15% yield.**

The central role of 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2D_3$] in the regulation of calcium metabolism, the high biological potency of other 1α-hydroxyvitamin D analogs, and their promise as therapeutic agents for the treatment of various bone disorders have stimulated considerable interest in the synthesis of these compounds (1, 2). Most published routes involve the preparation of a suitably substituted steroidal 5,7-diene followed by the well-known photochemical and thermal isomerization to the desired 1α-hydroxyvitamin analog (2). A conceptually attractive alternative to these fairly complex and multistep processes is C-1 hydroxylation of vitamin D by allylic oxidation, an approach that, in principle, would offer the most direct access to 1α-hydroxy analogs from available vitamin D precursors (e.g., vitamins D_2, D_3, etc.). We have investigated this problem and document here an experimentally simple procedure of broad scope which, applied specifically to vitamin D_3, 25-hydroxyvitamin D_3 (25-OH-D_3), and vitamin D_2, affords the corresponding 1α-hydroxy analogs in 10–15% overall yield.

RESULTS AND DISCUSSION

Direct allylic oxidation of vitamin D is feasible but, because of difficulty in controlling the site, extent, and stereochemistry of hydroxylation, it is not (at least as yet) an efficient process. For example, oxidation [0.25 eq of SeO_2, 2 eq of *t*-butylhydroperoxide, CH_2Cl_2, 5.5 hr, 0° or 25° (3, 4)] of vitamin D_3 (**1a**) gave, in 4–5% yield, a mixture of monohydroxylated compounds from which 1α-hydroxyvitamin D_3 (1α-OH-D_3, **9a**), 1β-hydroxyvitamin D_3 (5, 6), 5,6-*trans*-1α-hydroxyvitamin D_3 (**13a**), and 5,6-*trans*-1β-hydroxyvitamin D_3 were isolated in 0.5–1% yield each. These products were characterized by UV, nuclear magnetic resonance (NMR), and mass spectral and chromatographic comparison with authentic materials. Under these reaction conditions, considerable starting material (35–55%) was recovered as well as a series of more polar products. Longer reaction times decreased the amount of recovered starting material and increased the proportion of polar products without substantially improving the yield of desired C-1 hydroxylated derivatives. Low temperatures (−78°, −10°) resulted in impractically slow reaction rates. Oxidation of 25-OH-D_3 (**1b**) gave the analogous series of products [1α,25-$(OH)_2D_3$, 1β,25-$(OH)_2D_3$ (6), and the two corresponding 5,6-*trans* isomers, identified by spectral and chromatographic comparison with authentic material] in similar yields. Our observations essentially confirm the recently published work of Pelc (7) (known to us, unfortunately, only after completion of our study) who obtained similar yields of 1-hydroxylated products upon SeO_2 oxidation of vitamin D_3 under somewhat different conditions.

These results led us to explore a more indirect approach—oxidation of 3,5-cyclovitamin D_3 (**2a**), available (*ca* 50%) by buffered methanolysis of vitamin D_3 3-tosylate and also reconvertible to the vitamin by acid-catalyzed solvolysis (8). As substrates for oxidation, derivatives of type **2** have some attractive features: one less allylic position, and the isolation of the C-1 target site within a strained [3.1.0] bicyclic system.

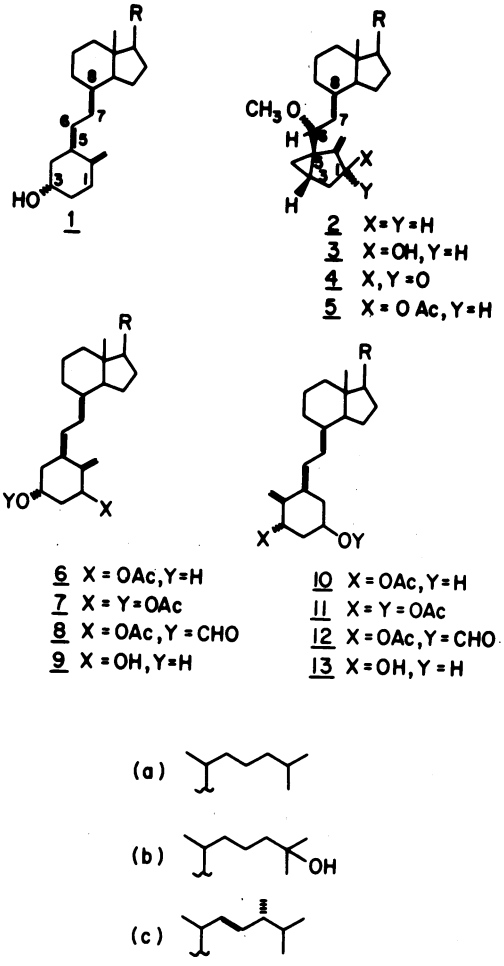
Oxidation of **2a*** (0.5 eq of SeO_2, 2.0 eq of *t*-BuOOH, CH_2Cl_2, 25°) was complete after 15 min and yielded **3a** as the major product (50%): *m/e* 414 (M^+, 30), 382 (70), 135 (65); ^1H NMR (270 MHz, $CDCl_3$), 3.26 (3H, s, 6-OCH_3), 4.18 (1H, d, J = 9.0 Hz, 6-H), 4.22 (1H, m, 1-H), 4.25 (1H, d, J = 9.0 Hz, 7-H), 5.17 (1H, d, J = 2.2 Hz, 19(Z)-H), 5.25 (1H, d, J = 2.2 Hz, 19(*E*)-H). A less polar minor product (20%) proved to be ketone **4a**: UV λ_{max} 248 nm (3500); *m/e* 412 (M^+, 35), 380 (40), 133 (100); ^1H NMR 3.30 (3H, s, 6-OCH_3), 4.06 (1H, d, J = 9.0 Hz, 6-H), 5.02 (1H, d, J = 9.0 Hz, 7-H), 5.62 (1H, 19(Z)-H), 6.04 (1H, 19(E)-H). Hydride reduction ($LiAlH_4$ or $NaBH_4$) of ketone **4a** gave **3a** in >80% yield. This product distribution was surprising because formation of the 1α-alcohol requires hydride attack on the more hindered face of the bicyclic system.

As a protective measure, **3a** was quantitatively acetylated to **5a** and then treated with 0.3 eq of *p*-toluenesulfonic acid in 75% aqueous dioxane for 15 min at 55°. Preparative thin-layer chromatography gave **6a** in 40% yield: UV, λ_{max} 265 nm; *m/e* 442 (M^+, 70), 382 (70), 134 (100); ^1H NMR, 0.52 (3H, s, 18-H_3), 2.03 (3H, s, 1-$OCOCH_3$), 4.19 (1H, m, 3-H), 5.04 (1H, d, J = 1.5 Hz, 19(Z)-H), 5.31 (1H, m (sharp), 19(E)-H), 5.49 (1H, m, 1-H), 5.93 (1H, d, J = 11.3 Hz, 7-H), 6.37 (1H, d, J = 11.3 Hz,

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: 1α-OH-D_3, 1α-hydroxyvitamin D_3; 1α,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; 25-OH-D_3, 25-hydroxyvitamin D_3; 1α-OH-D_2, 1α-hydroxyvitamin D_2; NMR, nuclear magnetic resonance.

* Stereochemical assignments (8) are based on the analogy between solvolysis of D_3 tosylate and the well-known cholesteryl tosylate → cholesteryl *i*-ether conversion. Sheves and Mazur (8) report the formation of a 6(*R*)/6(*S*) epimer mixture in the ratio of 4.5:1. Based on NMR analysis, our product contained the 6 *R*/*S* epimers in a ratio of *ca* 15:1; for the purposes of the synthesis described here, separation is not necessary.



6-H). The corresponding *trans* isomer **10a** (8%) and considerable amounts of less polar decomposition products also were obtained.

Secondary decomposition reactions during the cycloreversion process can be minimized by conducting the reaction in acetic or formic acid. For example, brief heating of **5a** in glacial acetic acid (55°, 15 min) gave a 70% yield of **7a** and **11a** in a 3:1 ratio. Separation of the *cis/trans* isomeric mixture, either as the diacetates or the corresponding diols, is difficult, however, and requires careful chromatography. In 98% formic acid/dioxane (1:1) at 55° (15 min), **5a** was solvolyzed in good yield to the acetoxy formates **8a** and **12a** (2:1 ratio). Selective mild hydrolysis of the crude mixture (5.0 eq of K_2CO_3, aq. MeOH, 25°, 5 min) followed by simple preparative thin layer chromatography gave **6a** and **10a** in yields of 45% and 22%, respectively (from **5a**).

Reductive ($LiAlH_4$) or hydrolytic (KOH, aq. MeOH, 40°, 1 hr) cleavage of acetate converted **6a** to 1α-OH-D_3 (**9a**) identical in all respects to an authentic sample.

The analogous sequence of tosylation, solvolysis, and oxidation applied to 25-OH-D_3 (**1b**) produced the key intermediate **3b** in *ca* 25% yield†: *m/e* 430 (M^+, 15), 398 (12), 135 (70), 59 (100); ^1H NMR 3.25 (3H, s, 6-OCH_3), 4.17 (1H, d, J = 9.2 Hz, 6-H), 4.20 (1H, m, 1-H), 4.95 (1H, d, J = 9.2 Hz, 7-H), 5.19 (1H, d, J = 1.9 Hz, 19(Z)-H), 5.22 (1H, d, J = 1.9 Hz, 19(*E*)-H).

† The C-1 ketone again was a minor reaction product.

Acetylation to **5b**(25-OAc) required more vigorous conditions (Ac_2O pyridine, 95°, 22 hr) because of the hindered 25-OH function. Solvolysis of the diacetate with *p*-toluenesulfonic acid gave a 35% yield of **6b**(25-OAc): UV λ_{max} 265 nm; *m/e* 500 (M^+, 25), 440 (55), 422 (15), 398 (10), 380 (45), 134 (100); ^1H NMR 0.52 (3H, s, 18-H_3), 1.97 (3H, s, 25-$OCOCH_3$), 2.03 (3H, s, 1-$OCOCH_3$), 4.18 (1H, m, 3-H), 5.03 (1H, d, J = 1.1 Hz, 19(Z)-H), 5.31 (1H, m (sharp), 19(*E*)-H), 5.49 (1H, m, 1-H), 5.93 (1H, d, J = 11.7 Hz, 7-H), 6.37 (1H, d, J = 11.7 Hz, 6-H). The corresponding 5,6-*trans* isomer (**10b**) was isolated as a minor product (10%). When treated with $LiAlH_4$, **6b**(25-OAc) was reduced to 1α,25-$(OH)_2D_3$ (**9b**), identified by UV, NMR, and mass spectral and chromatographic comparison with authentic material. Cycloreversion of **5b** in formic acid (which does not require protection of the 25-OH group) to the mixture of acetoxy formates **8b** and **12b** (*ca* 2:1 ratio) followed by hydrolysis to **6b** and **10b**, thin-layer chromatographic separation, and removal of the acetate increased the yield of **9b** to 42%.

Similarly, 1α-hydroxycyclovitamin D_2 (**3c**) [*m/e*, 426 (M^+, 55), 394 (75), 135 (95); ^1H NMR 3.26 (3H, s, 6-OCH_3), 4.18 (1H, d, J = 9.6 Hz, 6-H), 4.21 (1H, m, 1-H), 4.94 (1H, d, J = 9.6 Hz, 7-H), 5.17 (1H, m (sharp), 19(Z)-H), 5.19 (2H, m, 22-H and 23-H), 5.24 (1H, m (sharp), 19(*E*)-H] was obtained in 28% yield from vitamin D_2 (**1c**). Under the oxidation conditions, the allylic positions in the vitamin D_2 side chain were found to be inert.† Acetylation and acid-catalyzed solvolysis gave the vitamin analog **6c** (40%) and its 5,6-*trans* isomer **10c** (8%). Hydrolysis of **6c** led to the 1α-hydroxylated vitamin **9c**, the physical properties (NMR, UV, and mass spectrum) of which are in full accord with those of 1α-hydroxyvitamin D_2 prepared by a different method (9).

These examples suggest that the allylic oxidation process *via* cyclovitamin intermediates should be generally applicable to the preparation of a broad spectrum of 1α-hydroxyvitamin D analogs. Depending on the availability of the required vitamin starting material, the new route may in fact represent the experimentally most efficient and convenient approach to these compounds, especially since a more systematic study of reaction conditions can be expected to improve upon the yields that we report.

Our work was supported by National Institutes of Health Grant AM-14881, the Harry Steenbock Research Fund, the Wisconsin Alumni Research Foundation, and a National Institutes of Health postdoctoral fellowship (to H.E.P.).

1. DeLuca, H. F. & Schnoes, H. K. (1976) *Annu. Rev. Biochem.* **45**, 631–666.
2. Georghiou, P. E. (1977) *Chem. Rev.* **6**, 83–107.
3. Fieser, M. & Fieser, L. (1966) *Reagents for Organic Synthesis*, (Wiley, New York), Vol. 4, p. 362.
4. Umbreit, M. A. & Sharpless, K. B. (1977) *J. Am. Chem. Soc.* **99**, 5526–5528.
5. Sheves, M., Friedman, N. & Mazur, Y. (1977) *J. Org. Chem.* **42**, 3597–3599.
6. Paaren, H. E., Schnoes, H. K. & DeLuca, H. F. (1977) *J. Chem. Soc. Chem. Comm.*, 890–892.
7. Pelc, B. (1977) *Steroids* **30**, 193–201.
8. Sheves, M. & Mazur, Y. (1975) *J. Am. Chem. Soc.* **97**, 6249–6250.
9. Lam, H.-Y. P., Schnoes, H. K. & DeLuca, H. F. (1974) *Science* **186**, 1038–1040.