Syntheses of 1β-Hydroxyvitamin D_2 and D_3

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(Received July 30, 1988)

Synopsis. Reduction of 1-oxo-3,5-cyclovitamin D_2 and D_3 was carried out under various conditions. $LiAlH_4$ reduction in ether preferentially afforded 1β-hydroxy-3,5-cyclovitamin D_2 and D_3, which were converted to 1β-hydroxyvitamin D_2 and D_3.

It has been established that the hydroxyl group at C-1 of vitamins D_2 and D_3 plays an important role in eliciting their biological activity.[1] In order to investigate the relationship between the molecular configuration and the physiological activity, DeLuca[2] and Mazur[3] synthesized 1β-hydroxyvitamin D_3 (**2**), the C_1 epimer of 1α-hydroxyvitamin D_3 (**1**), by oxidation of **1** followed by the reduction of the formed 1-oxo-previtamin D_3, and evaluated its biological activity.

DeLuca et al. reported that 1α-hydroxyvitamin D_2 (**3**) is about ten-times less toxic than **1**, but appears to be almost equally potent regarding vitamin D activity.[4] Thus, it is noteworthy to compare the physiological activity of the C_1 epimer of **3** with that of **1**.

In the present paper, the reduction of 1-oxo-3,5-cyclovitamin D_3 (**5**) and D_2 (**6**)[5] under various conditions and the conversion of the formed 1β-hydroxy-cyclovitamin D_3 (**8**) and D_2 (**10**) into the corresponding 1β-hydroxyvitamin D_3 (**2**) and D_2 (**4**), are described.

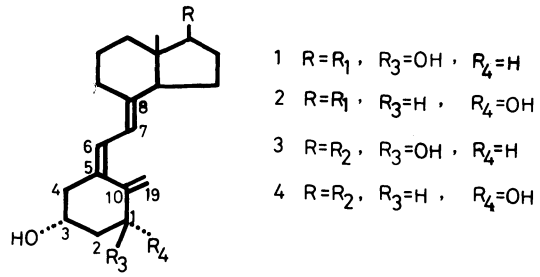
The 1-oxo-3,5-cyclovitamin D_3 (**5**) and D_2 (**6**) employed for the reduction were prepared from the corresponding 3,5-cyclovitamin D_3 and D_2 by modifying the procedure reported by DeLuca et al.[5] 1-Hydroxycyclovitamin D_3 and D_2 produced in the above mentioned reaction were converted to **5** and **6** by further oxidation with activated manganese oxide.

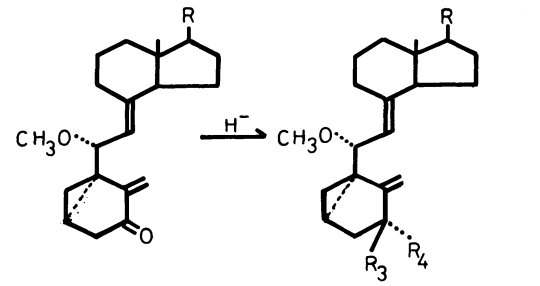
As indicated in Table 1, the stereochemistry of the reduction of **5** and **6** is highly dependent on the reagent and solvent used.[6,7] The reduction of **5** and **6** with lithium aluminum hydride ($LiAlH_4$) in ether gave 1β-hydroxy isomer (**8** and **10**) and 1α-hydroxy isomer (**7** and **9**) in a ratio of 5/1. On the other hand, the selectivity (**8**/**7** and **10**/**9**) was decreased when tetrahydrofuran (THF) was employed as solvent. The reduction with $Na[AlH_2(OCH_2CH_2OCH_3)_2]$ (sodium dihydridobis(2-methoxyethoxy)aluminate) gave similar results. These results may be well explained by assuming the coordination of the methoxyl group (at C-6) and carbonyl group (at C-1) of **5** and **6** to the Al atom of the reagent. Consequently, the approach of the hydride from the α-side of **5** and **6** appears to be hindered due to the chelation described above. Accordingly, the β-side attack of the hydride may be facilitated to yield a higher amount of 1β-hydroxy isomer (**8** and **10**). In contrast, THF may coordinate to the reducing agent, which probably makes the chelation between the Al atom and the oxygen atoms of A ring of vitamin D more difficult compared with the reduction in ether. As a result, this seems to lead to a lower ratio of 1β-hydroxy

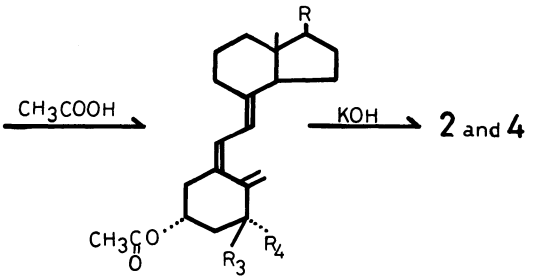
Table 1. Reduction of 1-Oxo-3,5-cyclovitamin D_3 (**5**) and D_2 (**6**) under Various Conditions

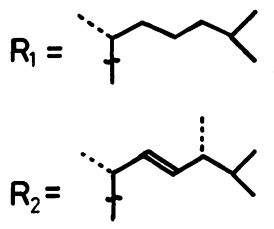
Entry	R	Reducing reagent	Temp	Solvent	Ratio (**8**/**7** or **10**/**9**)[a]
1	R_1	$LiAlH_4$	rt[b]	Ether	5.0
2	R_1	$LiAlH_4$	rt	THF	2.5
3	R_1	$LiAlH_4$	0 °C	Ether	4.5
4	R_1	$LiAlH_4$	0 °C	THF	2.5
5	R_1	$NaBH_4$	rt	Ether-EtOH	1.8
6	R_1	$NaBH_4$	rt	Ether-THF	2.1
7	R_1	DIBAL	rt	Ether	2.0
8	R_1	DIBAL	rt	THF	1.4
9	R_1	DIBAL	rt	Ether	1.6[c]
10	R_1	DIBAL	rt	THF	1.0[c]
11	R_1	DIBAL	−20 °C	Ether	1.5[c]
12	R_2	$LiAlH_4$	rt	Ether	5.1
13	R_2	$LiAlH_4$	rt	THF	2.7
14	R_2	$NaBH_4$	rt	Ether-EtOH	1.7
15	R_2	DIBAL	rt	Ether	1.5
16	R_2	DIBAL	rt	THF	1.1
17	R_2	DIBAL	rt	Ether	1.3[c]
18	R_2	DIBAL	rt	THF	1.1[c]
19	R_2	$Na[AlH_2(OCH_2CH_2OCH_3)_2]$	rt	Ether	5.0
20	R_2	$Na[AlH_2(OCH_2CH_2OCH_3)_2]$	rt	THF	1.4

a) Formation ratio was determined by means of high pressure liquid chromatography (HPLC) after 1-hydroxycyclovitamin D_3 and D_3 (**7**—**10**) were converted to corresponding 3β-acetoxy-1-hydroxyvitamin D_3 and D_2 (**11**—**14**). b) rt: room temperature. c) DIBAL solution was added to the solution of 1-oxo-3,5-cyclovitamin D_3 (**5**) and D_2 (**6**).









isomer (**8** and **10**) to 1α-hydroxy isomer (**7** and **9**). The effect of temperature was not relevant to the reduction selectivity.

Other reducing reagents, such as sodium borohydride ($NaBH_4$) and diisobutylaluminum hydride (DIBAL), showed no appreciable stereoselectivity in the reduction of **5** and **6** compared with $LiAlH_4$. It seems that DIBAL may be too bulky to form the chelation with the oxygen atoms of A ring moiety. Especially, the reduction with DIBAL in THF gave almost the same isomer ratio (1β-ol/1α-ol=1). However, this system (DIBAL-THF) can be an effective way to provide radioisotope labeled 1α-hydroxyvitamin D_2 and D_3 at position C-1, which seems to serve as convenient tools for a number of detailed biomedical experiments.[8,9]

Thus, the obtained 1β-hydroxy-3,5-cyclovitamin D_3 (**8**) and D_2 (**10**) were solvolyzed in acetic acid to yield 3β-acetoxy-1β-hydroxyvitamin D_3 (**12**) and D_2 (**14**), which were subsequently hydrolyzed upon treatment with ethanolic potassium hydroxide to produce 1β-hydroxyvitamin D_3 (**2**) and D_2 (**4**), respectively.

The inversion of the hydroxyl group at C-1 of **7** or **9** by a Mitsunobu reaction was not successful.[10] The procedure described here may be applied to the syntheses of other 1β-hydroxyvitamin D derivatives from the corresponding vitamin D derivatives.

Experimental

Melting points were uncorrected. UV spectra were taken on a Hitachi 320 spectrometer. ^1H NMR spectra were recorded in $CDCl_3$ on a JNF-FX200 spectrometer with TMS as an internal standard. Mass spectra were measured on a Hitachi M-80 mass spectrometer. HPLC were performed on a Hitachi 615-11 liquid chromatography equiped with a UV detector (set at 265 nm) and a Hibar column (prepacked column, LiChrosorb Si 60 (5 μm); 4 mm$\times$25 mm) with 20% hexane in dichloromethane as a mobile phase at a flow rate 1.0 ml min^{-1} (pressure: 30 kg cm^{-2}). Solvents were removed under reduced pressure.

1-Oxo-3,5-cyclovitamin D_3 (5) and D_2 (6). To a stirred suspension of SeO_2 (280 mg) in dichloromethane (100 ml), was added 4 ml of 3 M (1 M=1 mol dm^{-3}) 2,2,4-trimethylpentane solution of *t*-BuOOH. After the mixture was stirred for 1 h at room temperature, a dichloromethane solution (100 ml) of 3,5-cyclovitamin D_3[5] (1.0 g) was added dropwise. The mixture was allowed to react for 40 min at room temperature. The solution was washed with a 10% NaOH solution (40 ml). The dichloromethane solution was separated and concentrated to a heavy oil. The residue was extracted with ether, washed with brine, and dried over sodium sulfate. The ether solution was evaporated and the residue was chromatographed on silica gel. Elution with ethyl acetate/hexane (5/95, v/v) gave **5** as oil. 435 mg (42%). λ_{max} 248 nm (ε=4200, EtOH) (lit,[5] ε=3500).

Further elution with ethyl acetate/hexane (1/4, v/v) afforded 1α-hydroxycyclovitamin D_3 (**7**). 312 mg (25%). **7** (312 mg) was dissolved in dichloromethane (30 ml) and allowed to react with (activated) manganese oxide (900 mg) at room temperature for 1 h. The mixture solution was filtered on celite and concentrated. The residue was chromatographed on silica gel. Elution with ethyl acetate/hexane (5/95, v/v) gave **5**. 234 mg (75%).

Treatment of 3,5-cyclovitamin D_2[5] (1.0 g) in a similar way involving the oxidation process of 1α-hydroxycyclovitamin D_2 (**9**) provided 1-oxo-3,5-cyclovitamin D_2 (**6**). 620 mg (60%). λ_{max} 248 nm (ε=4000, EtOH).

1β-Hydroxyvitamin D_3 (2). To an ether solution (20 ml) containing $LiAlH_4$ (200 mg) was added dropwise an ether solution (10 ml) of 1-oxo-3,5-cyclovitamin D_3 (**5**) (400 mg) at room temperature. The mixture was stirred for 30 min. After decomposition of the excess $LiAlH_4$ with H_2O, the ether solution was washed with brine, dried (Na_2SO_4) and evaporated to dryness. The residue was purified by silica

gel chromatography (eluted with hexane/ethyl acetate, 4/1) to give a mixture of 1β- and 1α-hydroxycyclovitamin D_3[5] (**8**/**7**=5.0). 296 mg (74%).

The 1-hydroxycyclovitamin D_3 (296 mg) was dissolved in acetic acid (6 ml) and the mixture was stirred at 55 °C for 20 min. The mixture was extracted with ether, washed with a sat. $NaHCO_3$ solution and brine, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel. Elution with hexane/ethyl acetate (4/1) afforded 3β-acetoxy-1β-hydroxyvitamin D_3 (**12**). 205 mg (65%). λ_{max} 264 nm (ε=17500, EtOH) (lit,[3] ε=18000).

The actate (**12**) (205 mg) was dissolved in ethanolic potassium hydroxide (KOH 150 mg, EtOH 5 ml) and the mixture was stirred for 15 min at room temperature. The ethanol solution was extracted with ether, washed with brine, dried (Na_2SO_4) and concentrated to dryness. The residue was purified by silica gel chromatography (chloroform/ethyl acetate, 9/1) to yield 1β-hydroxyvitamin D_3 as oil. 149 mg (80%). λ_{max} 264 nm (ε=18500, EtOH) (lit,[3] ε=18000).

1β-Hydroxyvitamin D_2 (4). 1-Oxocyclovitamin D_2 (**6**) (400 mg) was reduced with $LiAlH_4$ (200 mg) in ether and treated in a similar manner described above to give 1-hydroxy-3,5-cyclovitamin D_2 (**10**/**9**=5.1). 280 mg (70%). *m/z* 426 (M^+).[5]

1-Hydroxycyclovitamin D_2 (280 mg) was treated with acetic acid (6 ml) at 60 °C for 15 min. After the usual work up, the residue was chromatographed (hexane/ethyl acetate, 4/1) to afford 3β-acetoxy-1β-hydroxyvitamin D_2 (**14**). 182 mg (61%): *m/z* 454 (M^+), 394 (M^+-CH_3COOH); ^1H NMR δ=6.33—6.00 (2H, ABq, *J*=12 Hz, H-6, H-7), 5.37 (1H, m, H-19E), 5.20 (2H, m, H-22, H-23), 5.00 (1H, m, H-19Z), 4.99 (1H, m, H-3), 4.17 (1H, bs, H-1), 2.05 (3H, s, $COCH_3$).

The acetate (**14**) (182 mg) was hydrolyzed in ethanolic potassium hydroxide (KOH 150 mg, EtOH 5 ml) at room temperature for 15 min. The solution was extracted with ether, washed with brine, dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel (chloroform/ethyl acetate, 4/1) to produce 1β-hydroxyvitamin D_2 (**4**). 124 mg (75%): Calcd for $C_{28}H_{44}O_2$: M, 412.3339. Found: *m/z* 412.3338; ^1H NMR δ=6.01—6.42 (2H, ABq, *J*=12Hz, H-6, H-7), 5.26 (1H, m, H-19E), 5.17 (2 H, m, H-22, H-23), 4.96 (1H, m, H-19Z), 4.30 (1H, bs, H-1), 4.07 (1H, m, H-3).

1α-Hydroxyvitamin D_2 (3) from 6. A 1 M hexane solution of DIBAL (10 ml) was added to a THF solution (20 ml) of 1-oxo-3,5-cyclovitamin D_2 (**6**) (400 mg) at room temperature and the mixture was stirred for 30 min. The solution was washed with brine and filtered on Celite. The solution was dried over Na_2SO_4 and evaporated. Purification on silica gel (hexane/ethyl acetate, 4/1) gave 1-hydroxycyclovitamin D_2 (**10**/**9**=1). 180 mg (45%).

The 1-hydroxycyclovitamin D_2 (180 mg) was treated with acetic acid (5 ml) at 60 °C for 20 min. The mixture was extracted with ether. The ether solution was washed with sat. $NaHCO_3$ and brine, and concentrated to dryness. The residue was purified by silica-gel chromatography. Elution with hexane/ethyl acetate (9/1) afforded 3β-acetoxy-1β-hydroxyvitamin D_2 (**14**) (60 mg, 31%). Further elution with the same developing solvent gave 3β-acetoxy-1α-hydroxyvitamin D_2 (**13**) (65 mg, 34%).

The acetate (**13**) (65 mg) was hydrolyzed with ethanolic potassium hydroxide (KOH 40 mg, EtOH 5 ml). The solution was extracted with ether, washed with brine and evaporated. Chromatography on silica gel (chloroform/ethyl acetate, 4/1) and crystallization (hexane-ethyl acetate) yielded **3**. 26 mg (44%). mp 140—141 °C (lit, [5] 138—140 °C).

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10) Treatment of 1α-hydroxycyclovitamin D_3 (**7**) (or 1α-hydroxycyclovitamin D_2, (**9**)) (1 mmol) with formic acid (or benzoic acid), triphenylphosphine and diethyl azodicarboxylate (2 mmol, each) in dry tetrahydrofuran (10 ml) at room temperature for 24 h afforded no desired product and only starting material (**7** or **9**) was recovered. (cf. W. H. Okamura and M. R. Pirio, *Tetrahedron Lett.*, **1975**, 4317).