was consumed. The residue, dissolved in DMF (4 mL), was added to pyridine (62 mL) heated at 60 °C. The pyridine solution, after being stirred for 48 h at 60 °C, was evaporated under reduced pressure. The residue was extracted with $CHCl_3$, washed with water, dried (Na_2SO_4), and evaporated. The product was obtained by gel chromatography (Sephadex LH-20; MeOH), 83 mg (47%): mp 164-166 °C; $[\alpha]^{25}_D$ -19.2° (c 0.83, MeOH); ^1H NMR ($CDCl_3$) δ 1.75 (m, 12 H), 2.07 (s, 9 H), 3.63 (m, 9 H), 4.08 (part of AB q, 3 H), 4.35 (br s, 3 H), 4.82 (s, 6 H), 7.35 (s, 15 H), 7.63 (d, 3 H, NH), 7.87 (t, 3 H, NH).

Cyclotri(N^5-acetyl-N^5-hydroxy-L-ornithylglycyl) (2). Compound **21** (0.18 g, 0.19 mmol) was hydrogenated with 10% Pd-C (0.18 g) in MeOH/H_2O (20 mL/10 mL), as described for **3**, to afford the product, 0.12 g (84%): $[\alpha]^{25}_D$ -14.9° (c 1.0, MeOH); IR (KBr) 1650 cm^{-1}; ^1H NMR (DMSO-d_6 at 50 °C) δ 1.50 (m, 6 H), 1.70 (m, 6 H), 1.96 (s, 9 H), 3.45 (t, 6 H), 3.78 (AB q, 6 H), 4.22 (q, 3 H), 8.15 (br s, 6 H), 9.54 (br s, 3 H). Anal. Calcd for $C_{27}H_{45}N_9O_{12}{}^3/_2H_2O$: C, 45.37; H, 6.77; N, 17.64. Found: C, 45.58; H, 6.78; N, 17.31.

Cyclo(triglycyltri(N^5-acetyl-N^5-(benzyloxy)-L-ornithyl)) (22). Compound **19** (0.30 g, 0.27 mmol) was hydrolyzed with 1 N NaOH (1.0 mL, 1.0 mmol) in MeOH (4 mL) to give a crude product, 0.227 g (77%). The product was esterified with HOSu (0.50 g, 0.43 mmol) with DEC·HCl (81 mg, 0.42 mmol) in DMF (3 mL)/CH_2Cl_2 (5 mL) to give 0.185 g (74%). The OSu ester (0.156 mmol) in CH_2Cl_2 (2 mL) was treated with TFA (2.68 g, 23 mmol) and then dissolved in DMF (4 mL). Addition of the DMF solution into pyridine (52 mL) heated at 60 °C gave the cyclic product, 74 mg (49%): mp 176-179 °C; R_f 0.37; IR (KBr) 1660 cm^{-1}.

Compound **22** (74 mg, 0.076 mmol) was hydrogenated with 10% Pd-C (74 mg) in MeOH/H_2O (15 mL/5 mL) and purified with Sephadex G-10 to give compound **1**, 45 mg (83%): IR (KBr) 1660 cm^{-1}; ^1H NMR (DMSO-d_6 at 50 °C) gave signals virtually at the same positions as those of **1**. Anal. Calcd for $C_{27}H_{45}N_9O_{12}{}^3/_2H_2O$: C, 45.37; H, 6.77; N, 17.63. Found: C, 45.33; H, 6.59; N, 17.16.

Iron(III) Binding Ratio. A sample (14-15 mg) of each hexapeptide was dissolved in water (10.0 mL); 1.0 mL of the sample solution and 1.0 mL of 0.2 N aqueous KNO_3 solution were mixed. An appropriate amount of a standardized aqueous ferric nitrate solution (3.07×10^{-3} M) was added to this. The pH of the mixture was adjusted to 4.0 or 5.0 with 0.01 or 0.1 N KOH and diluted to 10.0 mL before spectral determination.

Spectral Determination of the 1:1 Mixture. An accurately weighed sample (1.2 mg) of an analogue was mixed with an equimolar amount of ferric nitrate solution (3.07×10^{-3} M) and diluted to 10.0 mL. The pH of a 3.0-mL solution was adjusted to an appropriate value with 0.1 or 0.01 N KOH or 0.01 N HNO_3, and after 1 h, the visible spectrum was measured.

Iron(III) Exchange Reactions. Iron complex solutions of hexapeptides were prepared by dissolving an accurately weighed sample (6-8 mg) in an equivalent amount of ferric nitrate solution (3.07×10^{-3} M) and an equal volume of 0.2 M aqueous KNO_3 solution. The solution was made pH 5.0 with aqueous KOH before dilution to 5.0 mL with 0.1 M KNO_3 solution.

EDTA in buffer solution was made by dissolving EDTA·$2Na^+$·$2H_2O$ in acetate buffer solution (ionic strength 0.1, pH 5.42) to give a concentration of 9.27×10^{-3} M.

Iron(III) exchange reaction was followed by observing the decrease of an absorbance at 425 nm by repeat scanning with a Hitachi 320 S spectrophotometer. Each run was carried out in a cuvette with 3.0 mL of solution containing an iron complex and EDTA with a ratio of 1/20. A constant temperature (25 °C) was maintained. The first-order rate constant (k_{obsd}) was obtained from the plot of ln $[(OD_0 - OD_\infty)/(OD_t - OD_\infty)]$ vs time.

Proton Dissociation and Iron Stability Constants for Compound 2. Compound **2** (25.5 mg) was dissolved in degassed and deionized water (25 mL), and 20 mL of the solution was titrated with 0.01 N NaOH (carbonate free) under an atmosphere of argon at 25 °C and ionic strength 0.10 ($NaClO_4$). The proton dissociation constants (pK_1, pK_2, and pK_3) were obtained by computer calculation with aid of Gran's plot.

The iron complex stability constant was obtained by using the iron stability constant of EDTA[18] and the pK's of the hydroxamic acid groups, after determination of an apparent equilibrium point in a mixture of the iron complex of **2** and EDTA at pH 5.3, ionic strength 0.1, and 25 °C.

Aluminum Complex Formation. A solution of compound **2**-Al(III) complex was prepared by dissolving **2** (10 mg) in DMSO-d_6 (0.5 mL), followed by the addition of aluminum hydroxide (10 equiv). The mixture was sonicated with slight heating and filtered to remove excess of the hydroxide. The precipitate was washed with DMSO-d_6 (0.3 mL), and the DMSO-d_6 solution was combined for NMR spectral measurement.

Acknowledgment. We thank the Asahi Glass Foundation for Industrial Technology for partial financial support of this work.

Hydrotitanation-Protonation of Vitamin D_2 and Its Analogues: An Efficient Method for the Preparation of 10,19-Dihydrovitamins D_2 Including Dihydrotachysterol$_2$†

J. G. Cota, M. C. Meilán, A. Mouriño,* and L. Castedo*

Departamento de Química Orgánica, Facultad de Química y Sección de Alcaloides del CSIC, Santiago de Compostela, Spain

Received February 9, 1988

In this study we describe an easy and efficient method for the preparation of the known 10,19-dihydrovitamins D_2 **2b** (DHV_2-II), **2c** (DHV_2-IV), **3c** (dihydrotachysterol$_2$, DHT_2), and the new dihydrovitamins D_2 **2f** and **2g**. This method is based on the regioselective hydrometalation reaction of vitamin D_2 and its derivatives with the system Cp_2TiCl_2-$LiAlH_4$ or Cp_2TiCl_2-Red-Al (Aldrich). Under optimal conditions, the reaction with the former of these hydrometalating systems takes place with a high degree of stereoselectivity and allows labeling at C-19.

Introduction

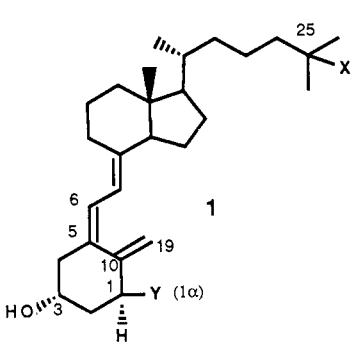
Dihydrovitamins D are a class of compounds derived by reduction of the natural vitamin D_3 (**1a**), the unnatural vitamin D_2 (**2a**), and their 5E isomers (5,6-trans derivatives) (Chart I). Among them, dihydrotachysterol$_2$ (DHT_2, **3c**), first isolated by von Werder,[1] is considered an interesting analogue of 1α,25-dihydroxyvitamin D_3 (**1b**), the hormonal form of vitamin D_3, because the former's 3β-OH

† This work was taken in part from the Ph.D. Thesis of J.G.C. and was presented as a communication in the Sixth Workshop on Vitamin D, Merano, Italy, 1985.

(1) Von Werder, F. *Hoppe-Seyler's Z. Physiol. Chem.* **1939**, *260*, 119.

0022-3263/88/1953-6094$01.50/0

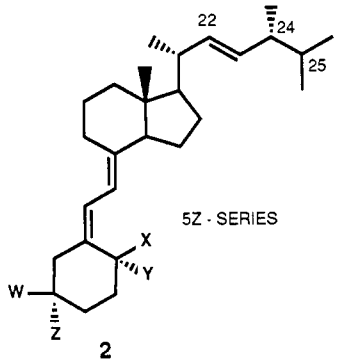
Chart I



1

1a, X=Y=H

1b, X=Y=OH



2

2a, X,Y=CH_2, Z=OH, W=H

2b, X=Me, Y=W=H, Z=OH (DHV_2-II)

2c, X=W=H, Y=Me, Z=OH (DHV_2-IV)

2d, X,Y=CH_2, W=H, Z=OTBDMS

2e, X,Y=CH_2, Z=H, W=OH

2f, X=Me, Y=Z=H, W=OH

2g, X=Z=H, Y=Me, W=OH

2h, X,Y=CH_2, W=OCOpNO$_2$Ph, Z=H

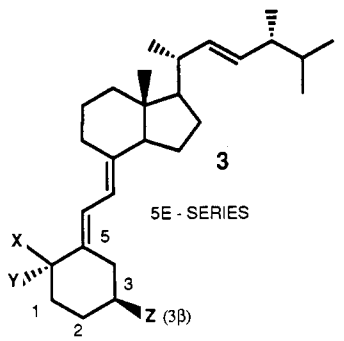
2i, X,Y=CH_2, W=OCOPh, Z=H

2j, X=CH_2OH, Y=Z=H, W=OCOPh

2k, X=CH_2OTs, Y=Z=H, W=OCOPh

2l, X=Z=H, Y=CH_2OH, W=OCOPh

2m, X=Z=H, Y=CH_2OTs, W=OCOPh



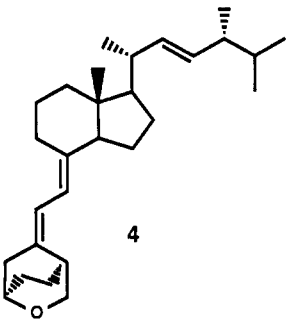
3

3a, X,Y=CH_2, Z=OH

3b, X=Me, Y=H, Z=OH (DHV_2-III)

3c, X=H, Y=Me, Z=OH (DHT_2)

3d, X,Y=CH_2, Z=OTBDMS



4

group has a similar topological orientation to that of the latter's 1α-OH.[2] In spite of the availability of 1α,25-dihydroxyvitamin D_3, DHT_2 is still widely used in the treatment of hypoparathyroidism and to prevent bone disorders attending renal failure.[3] Interestingly, it has also been found that this vitamin D analogue is able to act synergistically with indolebutyric acid (IBA) to cause a pronounced increase in root formation in cuttings.[4]

In the past, the considerable effort put into the search for efficient ways of preparing DHT_2 and related dihydrovitamins D has largely been unsuccessful, due mainly to the lability of the triene system of starting materials such as vitamin D and 5,6-*trans*-vitamin D and to the production of complicated reaction mixtures.[1,5] In fact, it was not until 1978 that the definitive stereochemistry of DHT_2 (**3c**) and other 10,19-dihydrovitamins D_2 (**3b**, **2b**, **2c**) was fully established.[6] Even recent research in this area[7] has obtained only low yields of DHT from 5*E* isomers of vitamin D (D_2 and/or D_3 series): 6% for hydroboration–protonation,[8] 34% for rhodium-catalyzed hydrogenation,[6,9] and 36% for hydrozirconation–protonation.[10] The therapeutic value of DHT_2,[3] the interest of biochemists in elucidating its mode of action,[11] the discovery of 19-hydroxylated dihydrovitamins D_3 with antivitamin D_3 properties,[12] and the possibility of preparing 19-substituted DHT derivatives as haptens for the induction of antibodies for 1α,25-dihydroxyvitamin D_3 determination nevertheless prompted us to pursue this topic further.

Results and Discussion

It has previously been reported that $LiAlH_4$ or Red-Al (Aldrich) ($NaAlH_2(OCH_2CH_2OCH_3)_2$) in the presence of a catalytic amount of bis(cyclopentadienyl)titanium dichloride (Cp_2TiCl_2) are excellent systems for the reduction

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Table I[a]

entry	"S"	"Al-H"	molar ratio "S":"Ti":"Al-H"	solvent	rctn time, h	% yield 2b	2c	3b	3c	2f	2g	convrsn, %
1	**2a**	$LiAlH_4$	1:0.5:2	THF	55	50 (22)	50 (7)					70
2	**2a**	Red-Al	1:1:4	THF	4.5	40	60					100
3	**2a**	Red-Al	1:2:4	THF	1.5	40 (40)	60 (40)					100
4	**2a**	$LiAlH_4$	1:2:2.3	THF	2.5	90 (84)	10 (76)					100
5	**2a**	$LiAlH_4$	1:2:2.5	THF	2.5	85	15					100
6	**2a**	Red-Al	1:2:4	PhH	2	50	50					75
7	**2a**	$LiAlH_4$	1:2:2.3	PhH	2.5	50	50					75
8	**2a**	$LiAlH_4$	1:2:2.3	THF	0.5	90 (89)	10					90
9	**2a**	$LiAlH_4$	1:2:2.3	THF	3	90 (88)	10					100
10	**2a**	$LiAlH_4$	1:2:2.3	THF	50	75 (45)	25 (40)					100
11	**2a**	$LiAlH_4$	1:2:2.3	THF	2.5	90 (57)	10 (50)					100
12	**2d**	Red-Al	1:2:3.2–4	THF	2	45	55					100
13	**2d**	$LiAlH_4$	1:2:1.8–2.3	THF	2	45	55					100
14	**3a**	Red-Al	1:2:4	THF	1.5			38	57			100
15	**3a**	$LiAlH_4$	1:2:2.3	THF	2.5			6.5	86			100
16	**3d**	$LiAlH_4$	1:2:2	THF	2			52	43			100
17	**2e**	$LiAlH_4$	1:2:2.3	THF	2.5					8	90	100
18	**2e**	Red-Al	1:2:4	THF	1.5					59	39	100

[a] "S", substrate; "Ti", Cp_2TiCl_2; "Al-H", aluminum hydride source. Yields (±5) are means of at least three experiments. For amounts of reaction products greater than 15 mg, yields were determined by weight. For amounts less than 15 mg, yields were determined by quantitative UV. Numbers in parentheses refer to the deuteriated product isolated after quenching with D_2O. For entry 11, an H_2 atmosphere was used.

of terminal nonconjugated olefins.[13,14] However, we found that reaction of these hydrometalating systems with vitamin D_2 (**2a**) under the reported conditions[13] was very slow and gave the 10,19-dihydrovitamins **2b** and **2c** in low yield due to decomposition of the violet hydrometalating agents after prolonged reaction times. When $LiAlH_4$ was used as the hydride source in THF as solvent, it was necessary to increase the amount of Cp_2TiCl_2 to obtain reasonable yields of the above dihydrovitamins D_2 after 50 h (entry 1, Table I). When either $LiAlH_4$ or Red-Al was used as the hydride source in THF as solvent, the reaction rate was considerably accelerated by further increasing the amount of Cp_2TiCl_2 relative to vitamin D_2 (for representative experiments, see entries 2–5). In these conditions, a near-quantitative combined yield of 10,19-dihydrovitamins **2b** and **2c** was obtained after protonation and flash chromatography separation. Interestingly, the stereochemical bias of the reaction was reversed on changing from Red-Al to $LiAlH_4$. It is worth noting that when $LiAlH_4$ was used and under the optimal conditions shown in entry 4, the reduction of the 10,19-double bond of vitamin D_2 took place with a remarkable degree of stereoselectivity, affording the dihydrovitamin D_2 **2b** as the main product.[15] This behavior has no precedent in previous methods used for the regioselective reduction of the triene system of vitamin D.[5,6,8–10] The reaction in benzene led to incomplete conversion of the starting materials after similar reaction times, and no appreciable degree of stereoselectivity was observed either with $LiAlH_4$ or with Red-Al as the hydride source (entries 6 and 7).

The stereochemical ratio obtained under the optimal conditions (entry 4) for the 10,19-dihydrovitamins **2b** and **2c**, which are respectively trans and cis with respect to ring A, encouraged us to study the reaction with other vitamin D_2 derivatives such as (5*E*)-vitamin D_2 (**3a**) and epivitamin D_2 (**2e**). Treatment of **3a**, prepared by iodine-catalyzed isomerization of vitamin D_2 by a slightly modified form of Okamura's method,[6,16] with $LiAlH_4$, followed by the addition of Cp_2TiCl_2 in THF at room temperature, afforded, after 2.5 h and after protonation and chromatography, 86% and 6.5% yields of the (5*E*)-10,19-dihydrovitamins D_2 **3c** (DHT_2) and **3b** (DHV_2-III) respectively (entry 15).[17] Application of these reaction conditions to epivitamin D_2 (**2e**) (prepared by Mitsunobu inversion of vitamin D_2)[18] gave the new 10,19-dihydroepivitamins D_2 **2g** and **2f** in 90% and 8% yield respectively (entry 17).[19]

At this point it was clear that when $LiAlH_4$ is used as the metal hydride in THF under noncatalytic conditions, there is a significant bias toward reduction of the 10,19-double bonds of vitamin D_2, epivitamin D_2, and (5*E*)-vitamin D_2 from the face nearer to the hydroxyl group. In an attempt to determine the role that the hydroxyl group might play in this reaction, we subjected *tert*-butyldimethylsilyl ethers of vitamin D_2 and (5*E*)-vitamin D_2, **2d** and **3d**, individually to similar reaction conditions (entries 12, 13, 16). In these cases, the ratio between the corresponding dihydrovitamins obtained after deprotection (48% HF, acetonitrile) indicated that no stereoselection took place. These results are evidence of the hydroxyl-assisted reduction of the 10,19-double bond of vitamin D_2 and the above analogues.[20]

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(14) (a) It has been also reported that treatment of conjugated dienes with Cp_2TiCl_2–$HAl(NR_2)_2$ under catalytic conditions in THF produces isomerization and a moderate yield of monoalkene: Ashby, E. C.; Noding, S. A. *J. Org. Chem.* **1979**, *44*, 4364. (b) Deoxygenation of allyl and benzyl alcohols with the system Cp_2TiCl_2–$LiAlH_4$ has been reported: Sato, F.; Tomuro, Y.; Ishikawa, H.; Oikawa, T.; Sato, M. *Chem. Lett.* **1980**, 103.

(15) Larger amounts of $LiAlH_4$ decrease the reaction rate and the stereoselectivity.

(16) We have recently found that the isopropyl ether used in Okamura's method[6] for the purification of (5*E*)-vitamin D_2 must be distilled from Na. The use of flash chromatography (eluent: hexanes–10% EtOAc/hexanes) gives better results.

(17) When Red-Al was used instead of $LiAlH_4$, the reaction took place without appreciable stereoselection (entry 14 of Table I); the dihydrovitamin **3b** (DHV_2-III) was easily separated from dihydrotachysterol$_2$ by flash chromatography.

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(19) The stereochemistry of the new dihydrovitamins **2g** and **2f** was confirmed by following the procedure of Okamura:[6] the epivitamin D_2 benzoate **2i** was subjected to hydroboration–oxidation with 9-BBN/H_2O_2 to afford a mixture of the 19-hydroxydihydroepivitamin D_2 benzoates **2j** and **2l**. Tosylation of these individual compounds followed by superhydride reduction of the corresponding tosylates **2k** and **2m** gave respectively the cyclic ether **4** and **2g**.

In order to determine the viability of this procedure for the preparation of labeled dihydrovitmains D_2, and to gain insight into the mechanism of the reaction, we proceeded to carry out deuteriation experiments. After reduction of **2a** under the optimal conditions shown in entry 4, quenching of the reaction mixture with deuterium oxide led to reaction products **2b** and **2c** with respectively 84% and 76% of deuterium incorporation as measured by mass spectral analysis.[21] The ^1H NMR spectra of these compounds showed a decrease in the intensity of the doublets at 1.10 and 1.07 respectively corresponding to the deuteriated 19-methyl groups. These results are consistent with the formation of hydrometalated intermediates with the metal (Ti or Al) bound to the terminal 19-carbon atom.[14a,22] In order to determine whether the formation of the hydrometalated species corresponding to **2b** and **2c** from **2a** was an equilibrium process, we proceeded to quench with D_2O aliquots of the reaction mixture taken at several different times (entries 8–10). Early in the reaction (entry 8), the ratio between the dihydrovitamins D_2 **2b** and **2c** was 90:10 and the former exhibited 89% deuterium incorporation. The same result was obtained after the reaction was complete (entry 9), but after 50 h, the trans:cis ratio had dropped to 75:25 and deuterium incorporation decreased to approximately 45% (entry 10). The low trans:cis ratio observed after 50 h indicates that a very slow equilibrium process took place. The loss of deuterium incorporation after prolonged reaction times and the small amount of protonated dihydrovitamins D_2 observed during the early stages of the reaction may be explained in terms of the breaking of the C–metal (C–Ti or C–Al) bonds by hydrogen or other hydride species present in the reaction medium. A hydrogen atmosphere in the early stages of the reaction gave rise to a significant decrease in deuterium incorporation (entry 11).

Conclusion

Under appropriate and noncatalytic conditions, the systems Cp_2TiCl_2–$LiAlH_4$ and Cp_2TiCl_2–Red-Al provide an excellent means for the preparation of 10,19-dihydrovitamin D_2 derivatives. Particularly noteworthy is the stereoselectivity observed when Cp_2TiCl_2–$LiAlH_4$ is used as the hydrometalating system. Under optimal conditions this system appears to be ideal for the preparation of the clinically important dihydrotachysterol$_2$ and its C-19 labeled analogue. Further research is needed to determine whether stereoselective hydrometalation by coordination with hydroxyl groups is performed by this hydrometalating system equally effectively when it is applied to other conjugated alkenes or simple alkenols.

Experimental Section

The general procedures that were employed have been described previously.[23] In the present work, high-pressure liquid chromatography (HPLC) was carried out by using a Waters 6000 A solvent delivery system, a Waters 490 multiwavelength UV detector, and a Spectra-Physics SP4290 integrator. The column used was a Whatman M9 10/50 Partisil (eluent 16% EtOAc/hexane) or a ZorbaxSIL 4.6/25 (eluent 1% 2-propanol/hexane). Bis(cyclopentadienyl)titanium dichloride was purchased from Alfa Products and used without further purification. Solutions of 9-BBN, $NaAlH_2(OCH_2CH_2OCH_3)_2$, and lithium triethylborohydride were purchased from Aldrich. Clear solutions of $LiAlH_4$ in THF were prepared as per Brown and co-workers.[24]

(5*E*)-Vitamin D_2 (5,6-*trans*-Vitamin D_2, 3a). Five milliliters of a solution of iodine in Et_2O, prepared by dissolving 20 mg of iodine in 100 mL of Et_2O, was added to a solution of vitamin D_2 (1 g) in normal Et_2O (500 mL). The resulting solution was allowed to stand for 2.5 h, washed successively with a saturated solution of Na_2SO_3 (2 × 50 mL), H_2O (100 mL), and brine (50 mL), dried (Na_2SO_4), filtered, and concentrated under vacuum. The resulting residue was transferred to a chromatography column packed with flash-type silica gel previously washed with hexanes. Elution with hexanes–10% EtOAc/hexanes afforded, after concentration and high-vacuum drying, 0.58 g of **3a** (58%) identical with an authentic sample (^1H NMR, TLC). Crystallization from isopropyl ether afforded material with mp 87–90 °C (lit.[25] mp 99–101 °C) and UV (EtOH) λ_{max} 273 nm (ϵ 20 400) [lit.[25] λ_{max} 272–273 nm (ϵ 24 400)].

General Procedure for the Preparation of 10(*S*),19-Dihydrovitamin D_2 (2b), 10(*R*),19-Dihydrovitamin D_2 (2c), 10(*S*),19-Dihydro-(5*E*)-vitamin D_2 (DHT_2, 3c), and 10(*R*),19-Dihydro-(5*E*)-vitamin D_2 (3b). Reaction of Vitamin D_2 with the System Cp_2TiCl_2–Red-Al. A solution of $NaAlH_2(OCH_2CH_2OCH_3)_2$ in toluene (280 μL, 1.01 mmol, 3.63 M) was slowly added to a solution of vitamin D_2 (100 mg, 0.25 mmol) in dry THF (1 mL) under argon. After the mixture was stirred for 3 min at room temperature, solid Cp_2TiCl_2 (128 mg, 0.50 mmol) was added at once; gas was rapidly evolved, and the mixture turned violet. The walls of the reaction flask were washed with THF (1 mL). The reaction mixture was stirred for 1.5 h and then quenched by the slow addition of drops of water at 0 °C. The resulting mixture was transferred to a separation funnel containing water (50 mL) and extracted with Et_2O (2 × 30 mL). The combined organic layers were washed (brine), dried ($MgSO_4$), filtered, and concentrated in vacuo to afford a residue, which was flash chromatographed (1.5 × 20 cm, 10% Et_2O/hexanes) to give, after concentration and high-vacuum drying, 40 mg of **2b** (DHV_2-II) and 60 mg of **2c** (DHV_2-IV). Crystallization of **2b** from hexanes afforded material with mp 105–105.5 °C (lit.[6] mp 104–105 °C) and UV (EtOH) λ_{max} 252 nm (ϵ 32 500). Crystallization of **2c** from acetone/drops of H_2O afforded material with mp 80–81.5 °C (lit.[6] mp 83.5–86 °C) and UV (EtOH) λ_{max} 252 nm (ϵ 33 300).

Reaction of (5*E*)-Vitamin D_2 (5,6-*trans*-Vitamin D_2, 3a) with the System Cp_2TiCl_2–$LiAlH_4$. A clear solution of $LiAlH_4$ in THF (264 μL, 0.58 mmol, 2.19 M) was slowly added via syringe to a solution of **3a** (100 mg, 0.25 mmol) in dry THF (1 mL) under argon. After the mixture was stirred for 3 min, solid Cp_2TiCl_2 (128 mg, 0.50 mmol) was added at once. The walls of the reaction flask were washed with THF (1 mL). The resulting violet solution was stirred for 2.5 h. Workup and flash chromatography as above afforded 86 mg of **3c** (DHT_2, determined by weight, 86%) and 6.5 mg of **3b** (DHV_2-III, 6.5%, determined by HPLC). Crystallization of **3c** from hexanes afforded material with mp 131–132 °C (lit.[6] mp 123–125 °C) and UV (EtOH) λ_{max} 252 nm (ϵ 37 000). Crystallization of **3b** obtained by the Cp_2TiCl_2–Red-Al procedure from acetone afforded material with mp 108–110 °C (lit.[6] mp 108–110 °C). The four dihydrovitamins **2b**, **2c**, **3b**, and **3c** were identical (^1H NMR, TLC) to authentic samples obtained by Okamura's procedure.[6]

Protection of Vitamin D_2 (2a) and (5*E*)-Vitamin D_2 (3a) to the *tert*-Butyldimethylsilyl Ethers 2d and 3d. Vitamin D_2 (1 g, 2.52 mmol) was added to a solution of $ClSiMe_2$-*t*-Bu (0.65 g, 4.3 mmol) and imidazole (0.5 g, 7.5 mmol) in dry DMF (10 mL).

(20) (a) A similar effect has been postulated in the titanium-assited carbometalation of alkenols: Richey, H. G., Jr.; Moses, L. M.; Hangeland, J. J. *Organometallics* **1983**, *2*, 1545. (b) Because the silyl ether group adopts an equatorial orientation (Andrews, D. R.; Barton, H. R.; Cheng, K. P.; Finet, J. P.; Hesse, R. H.; Johnson, G.; Pechet, M. M. *J. Org. Chem.* **1986**, *51*, 1637), we cannot discard the possibility of this group to direct also the hydrometalation in other systems.

(21) These results were obtained by MS analysis after addition of D_2O to the reaction mixture and workup. Similar results were obtained by reversed quenching.

(22) This observation is consistent with previous findings of Ashby and co-workers concerning the catalytic hydrometalation of simple terminal alkenes.[13c] Further research is needed to see whether the hydrometalating species involved in these reactions are the same.

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The resulting mixture was isolated from light and stirred for 2.5 h. The mixture then resulting from addition of water (150 mL) was extracted with Et_2O (3 × 30 mL). The organic extracts were washed with HCl (1 M, 2 × 50 mL) and saturated aqueous $NaHCO_3$ solution (100 mL). Drying (Na_2SO_4), filtration, and removal of solvent in vacuo afforded 1.35 g of vitamin D_2 *tert*-butyldimethylsilyl ether (**2d**) (95%, oil). The same procedure was used for the preparation of (5*E*)-vitamin D_2 *tert*-butyldimethylsilyl ether (**3d**).

General Procedure for the Reaction of Vitamin D_2 or (5*E*)-Vitamin D_2 *tert*-Butyldimethylsilyl Ethers (2d or 3d) with Cp_2TiCl_2–$LiAlH_4$ and Cp_2TiCl_2–Red-Al. The individual reactions were carried out as above by using 170–200 mg of substrate and the molar substrate: Cp_2TiCl_2:"aluminum hydride" ratios listed in Table I. After the indicated reaction time, HPLC (Partisil column/hexane) showed 100% conversion of starting material to the corresponding protected dihydrovitamins D_2. Deblocking of the silyl ether was accomplished by stirring of the crude substrate with a solution of HF (48%, 1 mL) in acetonitrile (19 mL) for 3 h. Workup ($NaHCO_3$, Et_2O) and flash chromatography afforded the desired dihydrovitamins **2b** (45%) and **2c** (55%) or **3b** (52%) and **3c** (43%).

Epivitamin D_2 *p*-Nitrobenzoate (2h). Solutions of *p*-nitrobenzoic acid (186 mg, 1.11 mmol) in dry THF (2 mL) and diethyl azodicarboxylate (190 μL, 1.11 mmol) in dry benzene (2 mL) were successively added to a solution of vitamin D_2 (400 mg, 1.01 mmol) and PPh_3 (291 mg, 1.11 mmol) in dry benzene (5 mL) at room temperature under argon. The resulting reaction mixture was stirred for 3 h and then concentrated in vacuo. Extraction of the residue with hexanes (2 × 50 mL), washing (saturated solution of $NaHCO_3$), drying (Na_2SO_4), filtration, concentration in vacuo, and flash chromatography of the residue (1.5 × 20 cm, 0–3% EtOAc/hexanes) afforded **2h** (132 mg, 24%, foam). Crystallization from methanol/drops of EtOAc afforded material with mp 111–112 °C: ^{1}H NMR δ 8.28 and 8.19 (4 H, m, Ar H), 6.27 and 6.06 (2 H, AB q, J = 11.4 Hz, H-6, H-7), 5.21–5.17 (3 H, m, H-22, H-23, H-3β), 5.11 and 4.90 (2 H, br s, 2H-19); MS, m/e 546 (M + 1, 18), 545 (M^+, 48), 379 (30), 378 (100), 253 (57). Anal. Calcd for $C_{35}H_{47}O_4N$: C, 77.00; H, 8.69; N, 2.56. Found: C, 77.17; H, 8.58; N, 2.62.

Epivitamin D_2 Benzoate (2i). Epivitamin D_2 benzoate (**2i**) was prepared in 24% yield as above. Crystallization from methanol/drops of Et_2O afforded material with mp 92–94 °C: ^{1}H NMR δ 8.02 and 7.46 (5 H, m, Ar H), 6.26 and 6.07 (2 H, AB q, J = 11.3 Hz, H-6, H-7), 5.27–5.10 (3 H, m, H-22, H-23, H-3β), 5.09 and 4.88 (2 H, br s, 2H-19); MS, m/e 500 (M^+, 1.2), 3.78 (M^+ – PhCOOH, 16), 253 (10), 263 (13), 211 (25), 157 (14), 143 (20), 135 (21), 133 (12), 131 (12), 129 (13), 119 (83), 118 (98), 117 (23), 69 (100). Anal. Calcd for $C_{35}H_{48}O_2$: C, 83.93; H, 9.66. Found: C, 83.65; H, 9.83.

Epivitamin D_2 (2e). A solution of $LiAlH_4$ (500 μL, 0.75 mmol, 1.5 M) was added to a solution of epivitamin D_2 *p*-nitrobenzoate (**2h**, 430 mg, 0.79 mmol) in dry Et_2O (10 mL) under argon. The reaction mixture was stirred for 1.5 h at room temperature and quenched at 0 °C by the slow addition of ice pellets. The mixture was extracted with Et_2O (2 × 30 mL), and the combined organic layers were washed with brine, dried (Na_2SO_4), and filtered. Removal of solvent under reduced pressure afforded a residue, which was flash chromatographed (2 × 20 cm, 3–6% EtOAc/hexanes) to give 275 mg of **2e**[18] (89%). Crystallization from methanol afforded material with mp 46–58 °C: ^{1}H NMR δ 6.23 and 6.04 (2 H, AB q, J = 11.3 Hz, H-6 and H-7), 5.19 (2 H, m, H-22, H-23), 5.05 and 4.83 (2 H, br s, 2H-19), 3.88 (1 H, m, H-3β); UV (EtOH) λ_{max} 265 nm (ε 18 000), λ_{min} 228 nm; MS, m/e 397 (M + 1, 31), 396 (M^+, 100), 378 (13), 363 (32), 271 (37), 253 (48); HRMS calcd for $C_{28}H_{44}O$ 396.3394, found 396.3354. This compound was distinguished from vitamin D_2 by HPLC (Zorbax-SIL/1% 2-propanol–hexane; flow rate, 2 mL/min; retention time, 12 min). Vitamin D_2 runs slightly faster than epivitamin D_2.

10(*S*),19-Dihydroepivitamin D_2 (2f) and 10(*R*),19-Dihydroepivitamin D_2 (2g). Epivitamin D_2 (100 mg, 0.25 mmol) was treated with the system Cp_2TiCl_2–$LiAlH_4$ or Cp_2TiCl_2–Red-Al as above to give **2f** and **2g**. Workup and flash chromatography (1.5 × 20 cm, 10% Et_2O/hexanes) afforded the pure dihydroepivitamins **2g** and **2f** (see yields and reaction times in Table I). **2g** [R_f 0.59, 40% Et_2O/hexanes; mp 86–87 °C (hexanes)]: ^{1}H NMR δ 6.09 and 5.84 (2 H, AB q, J = 11.3 Hz, H-6, H-7), 5.19 (2 H, m, H-22, H-23), 4.05 (1 H, m, H-3β), 1.09 (3 H, d, J = 7.2 Hz, CH_3-19); MS, m/e 399 (M + 1, 6), 398 (M^+, 22), 273 (17), 255 (22), 205 (21), 173 (10), 161 (18), 121 (100); HRMS calcd for $C_{28}H_{46}O$ 398.3549, found 398.3539; UV (EtOH) λ_{max} 242, 250 (ε 33 100), 260 nm. Anal. Calcd for $C_{28}H_{46}O$: C, 84.33; H, 11.65. Found: C, 84.60; H, 11.65. **2f** [R_f 0.38, mp 111–113 °C (acetone)]: ^{1}H NMR δ 6.08 and 5.82 (2 H, AB q, J = 11.4 Hz, H-6, H-7), 5.19 (2 H, m, H-22, H-23), 3.56 (1 H, m, H-3β), 1.09 (3 H, d, J = 7.2 Hz, CH_3-19); MS, m/e 399 (M + 1, 14), 398 (M^+, 50), 273 (39), 255 (34), 173 (13), 161 (24), 147 (50), 121 (100); HRMS calcd for $C_{28}H_{46}O$ 398.3549, found 398.3559; UV (EtOH) λ_{max} 242, 250 (ε 31 600), 260 nm. Anal. Calcd for $C_{28}H_{46}O$: C, 84.33; H, 11.65. Found: C, 83.97; H, 12.02.

19-Hydroxy-10(*S*),19-dihydroepivitamin D_2 Benzoate (2j) and 19-Hydroxy-10(*R*),19-dihydroepivitamin D_2 Benzoate (2l). A solution of 9-BBN (1.36 mL, 0.68 mmol, 0.5 M) in THF was added via syringe to a solution of epivitamin D_2 benzoate (**2i**, 243 mg, 0.486 mmol) in THF (8 mL). The reaction mixture was stirred under argon at room temperature for 3 h. The resulting mixture was cooled to 0 °C and quenched by the addition of methanol (1 mL). The ice/H_2O bath was removed, and aqueous NaOH (0.5 mL, 3 M) and H_2O_2 (0.5 mL, 30%) were successively added. The mixture was stirred for 0.5 h and then poured onto water. Extraction with Et_2O (2 × 40 mL), drying (Na_2SO_4), filtration, and removal of solvent under reduced pressure afforded a residue, which was flash chromatographed (2 × 20 cm, 4% EtOAc/hexanes) to give, after concentration and high-vacuum drying, 124 mg of **2j** [49%, R_f 0.56 (40% Et_2O/hexanes), mp 154.5–155.5 °C (acetone)] and 91 mg of **2l** [36%, R_f 0.27 (40% Et_2O/hexanes)]. **2j**: ^{1}H NMR δ 8.06–7.41 (5 H, m, Ar H), 6.43 and 5.90 (2 H, AB q, J = 10.8 Hz, H-6, H-7), 5.20 (2 H, m, H-22, H-23), 4.93 (1 H, m, H-3β), 3.71 (2 H, m, 2H-19); MS, m/e 396 (M^+ – benzoic acid, 17), 271 (8), 147 (12), 145 (8), 137 (14), 135 (16), 133 (11), 119 (18), 108 (10), 107 (100). Anal. Calcd for $C_{35}H_{50}O_3$: C, 81.01; H, 9.73. Found: C, 81.31; H, 9.69. **2l**: ^{1}H NMR δ 8.03–7.38 (5 H, m, Ar H), 6.31 and 5.91 (2 H, AB q, J = 11 Hz, H-6, H-7), 5.33 (1 H, m, H-3β), 5.20 (2 H, m, H-22, H-23), 3.73 (2 H, m, 2H-19); MS, m/e 518 (M^+, 0.2), 396 (M^+ – benzoic acid, 17), 378 (11), 353 (11), 241 (15), 149 (18), 173 (22), 147 (14), 137 (16), 133 (14), 119 (26), 107 (100); HRMS calcd for $C_{35}H_{50}O_3$ 518.3762, found 518.3731.

Benzoyloxy Tosylates 2k and 2m. A mixture of hydroxy benzoate **2j** (79 mg, 0.152 mmol) and *p*-toluenesulfonyl chloride (100 mg, 0.52 mmol) in dry pyridine (2.5 mL) was allowed to stand in the refrigerator for 48 h. Ice was added, and the resulting precipitate was separated by filtration. The solid was washed with cold water and then dissolved in Et_2O. Drying (Na_2SO_4), filtration, removal of solvent in vacuo, and high-vacuum drying afforded 92 mg of **2k** [90%, mp 111–112 °C (hexanes), R_f 0.80 (40% Et_2O/hexanes)]: ^{1}H NMR δ 8.10–7.33 (9 H, m, Ar H), 6.28 and 5.74 (2 H, AB q, J = 11.5 Hz, H-6, H-7), 5.21 (2 H, m, H-22, H-23), 4.87 (1 H, m, H-3β), 4.19 (1 H, t, J = 9.8 Hz, H-19), 4.00 (1 H, q, J = 9.8 Hz, J' = 6.6 Hz, H-19), 2.43 (3 H, s, $ArCH_3$); MS, m/e 672 (M^+, 0.4), 500 (25), 396 (30), 379 (21), 378 (70), 368 (18), 253 (88), 119 (100). Anal. Calcd for $C_{42}H_{56}O_5S$: C, 74.95; H, 8.40. Found: C, 74.74; H, 8.75. Application of the same procedure to the hydroxy benzoate **2l** (100 mg, 0.193 mmol) afforded **2m** [100%, mp 104–105 °C (hexanes), R_f 0.65 (40% Et_2O/hexanes)]: ^{1}H NMR δ 8.00–7.31 (9 H, m, Ar H), 6.16 and 5.75 (2 H, AB q, J = 11.5 Hz, H-6, H-7), 5.25–5.19 (3 H, m, H-22, H-23, H-3β), 4.15 (1 H, t, J = 9.8 Hz, H-19), 3.98 (1 H, q, J = 9.8 Hz, J' = 6.3 Hz, H-19), 2.43 (3 H, s, $ArCH_3$); MS, m/e 550 (M^+ – benzoic acid, 0.5), 500 (M^+ – *p*-TsOH, 2), 378 (M^+ – $PhCO_2H$ – *p*-TsOH, 10), 253 (19), 145 (16), 133 (22), 119 (98), 105 (98), 91 (65), 77 (52), 69 (100). Anal. Calcd for $C_{42}H_{56}O_5S$: C, 74.95; H, 8.40. Found: C, 74.55; H, 8.70.

Reduction of Benzoyloxy Tosylate 2m to 10(*R*),19-Dihydroepivitamin D_2 (2g). A solution of lithium triethylborohydride in THF (1.5 mL, 1.5 mmol, 1 M) was added via syringe to a solution of **2m** (106 mg, 0.158 mmol) in dry THF (8 mL) at 0 °C under argon. The mixture was successively stirred at room temperature overnight and at reflux for 2 h. Water (0.5 mL), aqueous NaOH (1.5 mL, 3 M), and H_2O_2 (30%, 3 mL) were added, and the resulting mixture was refluxed for 0.5 h. The mixture was cooled to room temperature and extracted with Et_2O (2 ×

30 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated in vacuo to afford a solid, which was flash chromatographed (1.5 × 10 cm, 5% Et_2O/hexanes) to give, after concentration and high-vacuum drying, 55 mg of **2g** (88%), which proved identical (^1H NMR, TLC) with that obtained by reduction of epivitamin D_2 with Cp_2TiCl_2–"aluminum hydride".

Reduction of Benzoyloxy Tosylate 2k to the Ether 4. A solution of lithium triethylborohydride in THF (850 μL, 0.85 mmol, 1 M) was added via syringe to a solution of **2k** (80 mg, 0.12 mmol) at 0 °C under argon. The resulting mixture was stirred at room temperature for 2 h and then at reflux for 4 h. Quenching and workup as above gave, after flash chromatography (1.5 × 20 cm, 7% Et_2O/hexanes), 47 mg of **4** (100%): ^1H NMR δ 6.17 and 5.76 (2 H, AB q, J = 11.3 Hz, H-6, H-7), 5.19 (2 H, m, H-22, H-23), 3.97–3.90 (3 H, m, 2H-19, H-3β); UV (Et_2O) λ_{max} 246, 254, 264 nm; MS, m/e 396 (M^+, 26), 271 (24), 149 (20), 147 (16), 145 (13), 137 (32), 133 (40), 107 (100); HRMS calcd for $C_{28}H_{44}O$ 396.3392, found 396.3360.

Acknowledgment. We gratefully acknowledge the financial support of the Dirección General de Investigación Cientifica y Tēcnica (DGICYT) Project PB87-0478 and the generous gift of vitamin D_2 by Hoffmann la Roche (Basel). We thank R. B. Rookhuizen for the development of the modified procedure for the preparation of 5,6-*trans*-vitamin D_2.

Registry No. **2a**, 50-14-6; **2b**, 65377-86-8; **2c**, 807-27-2; **2d**, 104846-62-0; **2e**, 116559-84-3; **2f**, 116559-85-4; **2g**, 116559-86-5; **2h**, 116559-87-6; **2i**, 116467-93-7; **2j**, 116559-88-7; **2k**, 116561-02-5; **2l**, 116559-89-8; **2m**, 116561-03-6; **3a**, 51744-66-2; **3b**, 65377-91-5; **3c**, 67-96-9; **3d**, 115540-26-6; **4**, 116559-90-1.

Studies of Extended Quinone Methides. Design of Reductive Alkylating Agents Based on the Quinazoline Ring System

Robert H. Lemus and Edward B. Skibo*[1]

Department of Chemistry, Arizona State University, Tempe, Arizona 85287-1604

Received May 31, 1988

This report discusses the quinone methide reactivity, electrochemistry, and xanthine oxidase alkylation properties of a quinazoline-based reductive alkylating agent. The design of this alkylating agent involved functionalizing the quinazoline ring as a quinone with a leaving group placed so as to afford a quinone methide species upon reduction. pH–rate profiles, nucleophile-trapping studies, and product studies indicate the presence of a steady-state quinone methide species. The quinone methide species reacts by either trapping nucleophiles or ketonizing to a quinone. It is concluded that the fate of this and similar quinone methides can be predicted from the redox potential of the quinone resulting from quinone methide ketonization. If a low potential quinone is the ketonization product, ketonization is thermodynamically favored over nucleophile trapping. The opposite is true if a high redox potential quinone (such as the quinazoline-based system) results from ketonization. Finally, the reductive alkylation of the xanthine oxidase active site is demonstrated with the title systems.

The low reduction potentials exhibited by some tumor cells[2] has generated an interest in reductive alkylating agents as selective antitumor agents.[3,4] Reductive alkylating agents are quinones functionalized with a leaving group so as to permit quinone methide formation upon reduction. The quinone methide species can trap nucleophiles important to cellular function as well as ketonize to a quinone derivative. Indeed, many naturally occurring quinone antitumor agents such as mitomycin[5] and the anthracyclines[6] act as reductive alkylating agents.

Efforts in this laboratory have been direct toward the design of reductive alkylating agents based on heterocyclic ring systems. Thus the benzimidazole[7] and imidazo[4,5-*g*]quinazoline[8,9] ring systems have been functionalized as such. The goals of these efforts have been to gain insights into the structure–reactivity relationship for quinone methide fate (nucleophile trapping vs ketonization) and to design enzyme-directed reductive alkylating agents. Most, if not all, naturally occurring reductive alkylating agents are directed toward DNA rather than enzymes. However, the imidazo[4,5-*g*]quinazoline agents alkylate the active site of xanthine oxidase and thus represent the first enzyme-directed reductive alkylation system.[9]

The present report discusses the quinone methide reactivity, electrochemistry, and xanthine oxidase alkylation

(1) National Institutes of Health research career development award recipient 1988–1993 (CA01349).

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