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KINETICS OF THERMAL [1,7A]-SIGMATROPIC SHIFT OF HEXAFLUORO VITAMIN D_3 AND VITAMIN D_3 DERIVATIVES. EVALUATION OF CONFORMATIONS OF THE A RING AFFECTED BY 1-OH AND 3-OH GROUPS.

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Abstract: The quantitative evaluation of the [1,7a]-sigmatropic rearrangement of vitamin D_3 and its analogs affected by the conformations of the A ring using the ^{1}H-NMR method was described. Although the side chain of the D ring had no effect on the hydrogen migration, the rearrangement was influenced by the hydroxy groups of the A ring.

It is well known that a key step in the primary metabolic pathway leading to physiologically active 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$)[1)] is the transformation of previtamin D_3 (preD_3) to vitamin D_3 (D_3) (Figure 1). The kinetics and thermodynamics of this isomerization for the specific case of preD_3 has been studied in detail by Hanewald et al.[2a)] and others.[2b,c)] The equilibrium ratio of previtamin to vitamin is temperature dependent and the reaction follows reversible and first-order kinetics. Extensive work has been completed to evaluate the conformational features of preD_3 and D_3,[3)] however, little attention has been paid to the relationship between the conformation of the A ring affected by the 1- and 3-OH groups and the kinetic study under the same reaction conditions. ST-630 (26,26,26,27,27,27-hexafluoro-1,25-dihydroxyvitamin D_3) is one of a few analogs that have greater biological activity than 1,25-$(OH)_2D_3$ *in vivo* in the vitamin D-deficient rat[4)] and chick,[5)] and its action is longer-lasting than that of 1,25$(OH)_2D_3$. ST-232 (26,26,26,27,27,27-hexafluoro-1,23(S),25-trihydroxyvitamin D_3) is the major metabolite of ST-630.[6)] The reason for the enhanced biological activities[4)] was explained in part by a decreased metabolic inactivation by way of 26- and 27-hydroxylations due to the

substituted fluoro groups at the 26- and 27-carbons.

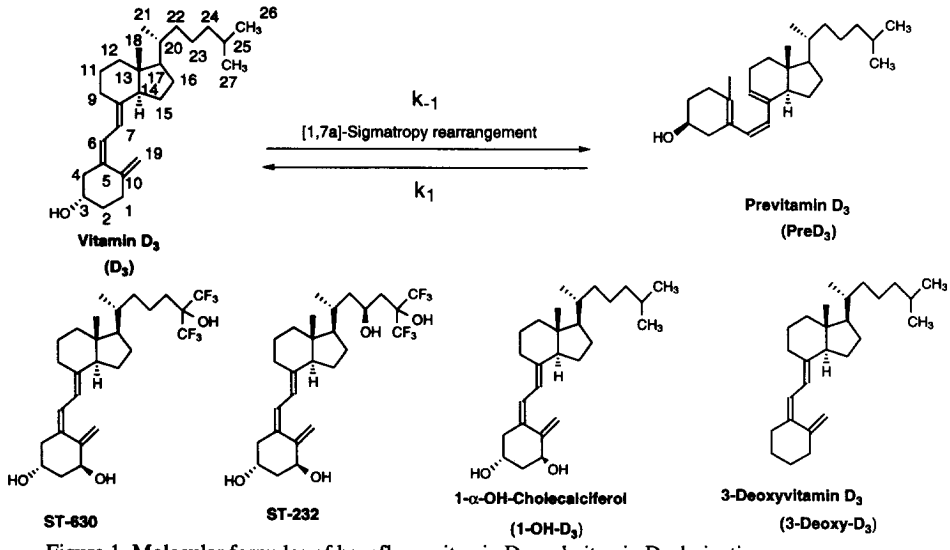

Figure 1. Molecular formulas of hexafluorovitamin D_3 and vitamin D_3 derivatives with the numbering system used in this paper

It is the purpose of this article to describe the quantitative evaluation of the rearrangement of hexafluorovitamin D_3 and vitamin D_3 derivatives. This would provide the information about the effects of the hydroxyl substituents of the A ring, which relates the conformation of the A ring, during the [1,7a]-sigmatropic hydrogen shift.

The ^{1}H-NMR experiments were done using a JEOL A-500 (500 MHz) nmr spectrometer operating in the pulsed fourier transform mode with a DEC station 3200 computer and 32 K data points. Crystalline D_3 and 1α-hydroxycholecalciferol (1-OH-D_3) were purchased from Solvay Duphar (Amsterdam, the Netherlands) and used without further purification. ST-630, ST-232 and 3-deoxyvitamin D_3 (3-deoxy-D_3) were synthesized[7] and then stored in the dark below -20 °C. Spectroscopic grade ethanol-d_6, which was obtained from E. Merck (Darmstadt, Germany), was used as the solvent with TMS serving as the internal reference. For the kinetic studies, solutions of ST-630, ST-232, D_3, 1-OH-D_3 and 3-deoxy-D_3 were dissolved in ethanol-d_6 such that the final concentration was approximately 5 mg/ml. These solutions were then cooled to -78 ℃.

For a kinetic run, a sample was placed in the precalibrated ^{1}H-NMR probe, which was preset to a specific temperature. After thermal equilibration of the sample, the ^{1}H-NMR spectra were recorded at regular time intervals. For the isomerization of ST-630, ST-232, D_3, 1-OH-D_3 and 3-deoxy-D_3 to the thermodynamically less stable previtamin form, the rate of the reaction was monitored by following the disappearance of the H-6 signal (ST-630: δ 6.27, ST-232: δ 6.26, D_3: δ 6.25, 1-OH-D_3: δ 6.27, 3-deoxy-D_3: δ 6.16). As a cross check, the H-7 signal (ST-630: δ 6.08, D_3: δ 6.03, ST-232: δ 6.08, 1-OH-D3: δ 6.09, 3-deoxy-D_3: δ 6.04) or the H-19 Z signal (ST-630: δ 5.29, ST-232: δ 5.27, D_3: δ 5.05, 1-OH-D_3: δ 5.27, 3-deoxy-D_3: δ 4.99) was also periodically monitored to measure the reliability of the integration data. Measurements were made at three different temperatures (45 °C, 60 °C, 75 °C).

The reversible first-order rate constants of the reaction between the previtamin form and vitamin form are defined[2a] by $\ln m/(m-x)=(k_1 + k_{-1})t$, where $m=(k_1a-k_{-1}b)/(k_1+k_{-1})$, and a and b are the concentrations of the previtamin form at t=0, respectively. x is the change in concentration. To calculate m, m=(a-Kb)/(1+K) is needed, where K is the equilibrium constant (k_1/k_{-1}). K, m, and x are obtained from the NMR results. In a plot of ln m/(m-x) versus time (s), the slope of the line is the sum of k_1 and k_{-1}. Thus k_1 and k_{-1} are obtained. The activation parameters were calculated from an Arrhenius plot of the natural logarithm of the rate constants for the previtamin form to vitamin form conversion (k_1) versus the reciprocal of the absolute temperature. A kinetic study of their [1,7a]-sigmatropic hydrogen shifts was carried out according to the ^{1}H-NMR analytical method developed by Okamura et al.[8] Integration of the H-6 or H-7 siganal of the previtamin versus the H-6 or H-7 signal of the vitamin in the ^{1}H-NMR spectrum could be used to quantify the relative amounts of previtamin and vitamin. Assuming a reversible, first-order kinetic rate law and following the reaction to 7-15 half-lives, with separate determination of the equilibrium constants for the preD_3-D_3 interconversion over the same temperature range, the results summarized in Tables I and II were obtained.

The kinetic data and activation parmeters for the transformation of the D_3 at 80 °C were essentially identical to the value previously reported by Hanewald et al[2a]. The rate constant for the [1,7a]-hydrogen migration of 1-OH-D_3 at 80 °C was calculated to be 5.65×10^{-4} s^{-1}, which is comparable to the 5.63×10^{-4} s^{-1} value calculated for the isomerization of 1, 25-$(OH)_2D_3$.[9] The rate constants and activation parameters for the isomerization of ST-630 most closely resembled those of ST-232 and 1-OH-D_3 but different from those of D_3,

especially Keq and the enthalpy of activation. Moreover, Keq and the enthalpy of activation of D_3 were different from 3-deoxy-D_3.

Table I. Kinetic Data for the Transformation of Vitamin D to Previtamin D[a]

Substrate	k_1[b] x 10^4	k_{-1}[b] x 10^4	Keq[c]
3-Deoxy-vitamin D_3	4.83 (0.11)	1.58 (0.17)	3.06 (0.31)
Vitamin D_3	5.04 (0.19)	1.36 (0.23)	3.71 (0.25)
1-α-OH-Cholecalciferol	5.65 (0.28)	1.09 (0.13)	5.18 (0.18)
ST-232	5.46 (0.33)	1.07 (0.29)	5.10 (0.35)
ST-630	5.46 (0.25)	1.07 (0.22)	5.10 (0.26)

[a] At 80 °C. Standard deviations are given in parentheses.
[b] k_1 and k_{-1} is given in s^{-1}. [c]Keq is defined as k_1/k_{-1} where the forward process is for the isomerization of previtamin D to vitamin D.

Table II. Activation Parameters for the Transformation of Vitamin D to Previtamin D[a]

Substrate	Ea[b]	log A[c]	$\Delta G^{\dagger}$ [b]	$\Delta H^{\dagger}$ [b]	$\Delta S^{\dagger}$ [d]
3-Deoxy-vitamin D_3	23.5 (0.2)	10.8 (0.1)	26.9 (0.5)	21.8 (0.2)	-14.4 (0.1)
Vitamin D_3	23.6 (0.1)	10.7 (0.2)	27.0 (0.6)	22.9 (0.1)	-11.8 (0.4)
1-α-OH-cholecalciferol	24.5 (0.4)	11.3 (0.09)	27.2 (0.8)	23.9 (0.4)	-9.3 (0.4)
ST-232	24.5 (0.1)	11.2 (0.1)	27.2 (0.7)	23.8 (0.1)	-9.5 (0.3)
ST-630	24.5 (0.5)	11.2 (0.3)	27.2 (0.5)	23.8 (0.3)	-9.5 (0.2)

[a] At 80 °C. Standard deviations are given in parentheses. [b] Units=kcal/mol. [c] A is given in s^{-1}.
[d] Units=cal/mol K.

These data indicated that the 1- and 3-OH groups of the A-ring affected the [1,7a]-sigmatropic hydrogen migration and the nature of the side chain of the D-ring had essentially no effect on the isomerization.

These results agreed with the mechanism of the [1,7a]-sigmatropic migration because the A-, seco-B-, C- and D-ring are associated with the transition states of the isomerization and the side chain of the D-ring is far apart from the structure. The fact that the Keq (or the free energy of activation) and the enthalpy of activation of ST-630, D_3, 3-deoxy-D_3 were decreased slightly in this order meant that the vitamin form of 3-deoxy-D_3 was more easily converted to its pre-form than ST-630 and D_3. No significant changes in the entropy of activation for this transformation were observed among ST-630, ST-232, D_3, 1-OH-D_3, and 3-deoxy-D_3.

A conformational equilibrium of the A ring between two chair forms must be occurring. Wing[3a,b] reported that the introduction of a hydroxyl group at 1α in D_3 slightly shifts the conformational equilibrium to favor an equatorial 1α-OH-D_3. We thought that the A ring of 3-deoxy-D_3 consists of an equilibrium mixture of almost an equal population of the two chair conformers, because it has no hydroxy groups at the A Ring. The [1,7a]-sigmatropic rearrangement would be influenced by the conformation of the A ring. We concluded that the shifts in the conformational equilibrium between the two chair forms of the A ring resulted in the difference of the equilibrium constants and the enthalpy of activation among the active type of D_3, D_3, and 3-deoxy-D_3. Although the presence of systematic experimental error can not be ruled out, the equilibrium constant and the enthalpy of activation of D_3 is bigger than 3-deoxy-D_3 and smaller than the active type of D_3 under the same reaction conditions. It was suggested that these data imply that the origin of the observed difference in the rate constant lies in a relative population of the active type of D_3, D_3, and 3-deoxy-D_3.

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