(21) M. Christl and J. D. Roberts, *J. Amer. Chem. Soc.*, **94**, 4565 (1972).
(22) Alternative explanations suggested by Christl and Roberts[21] are a small amount of protonation of the carbonyl carbon of the amino acid residue bound to the C-terminal unit in acidic solution and stabilization of the cis configuration by attraction between the charged terminal groups in the zwitterion form. The lack of any change in the chemical shift of the carbonyl carbon of *N,N*-dimethylacetamide over the pH range 5.2 to 1.0[23] suggests that the deshielding is not a result of carbonyl oxygen protonation. In addition, carboxylate group pK_a's calculated from the carbonyl carbon shifts agree with those calculated from chemical-shift data for other carbons. Results described above for peptides in which both cis and trans isomers can be seen indicate that the second explanation does not account for the observations. Also, such an interaction is not possible for acetylsarcosine.
(23) C. A. Evans and D. L. Rabenstein, unpublished results.
(24) Although the resonances for the cis isomers of the proline dipeptides were difficult to observe in the most acidic solution because of the low population, it could be seen in less acidic solution and was not shifting as the carboxylate group was being titrated.
(25) D. L. Rabenstein and S. Libich, *Inorg. Chem.*, **11**, 2960 (1972).

Elucidation of the Solution Conformation of the A Ring in Vitamin D Using Proton Coupling Constants and a Shift Reagent

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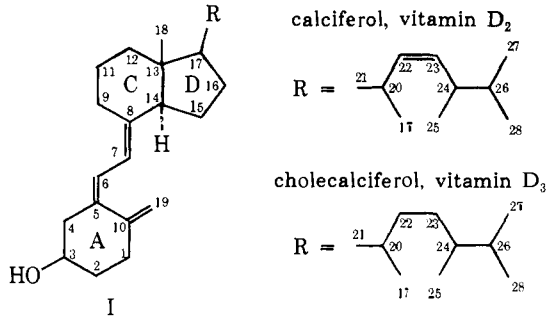
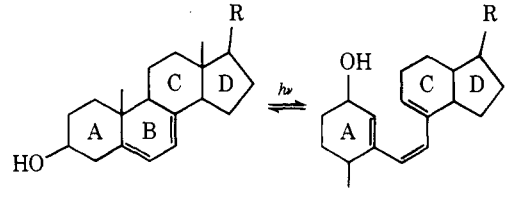
Abstract: The proton spin-spin splittings of vitamin D_2, calciferol, have been analyzed in terms of the solution conformation of the A ring. Using the known coupling constants for cyclohexanol, the observed multiplets are shown to be consistent with a dynamic equilibrium between approximately equal amounts of the α and β chair forms for the A ring. Resonances on the A ring not observable in the pure compound were resolved with the aid of the shift reagent $Eu(dpm)_3$. Analysis of the $Eu(dpm)_3$-induced dipolar shifts confirms the presence of the 1:1 mixture of conformers. The insensitivity of the proton multiplet structure to the presence of $Eu(dpm)_3$ suggests that the equilibrium is not perturbed significantly upon coordination. Conformational analysis as a function of temperature, using the induced dipolar shifts, indicates that the β chair is thermodynamically slightly more stable. The merits and problems of quantitative *vs.* qualitative use of shift reagents in solution conformational analysis of large flexible molecules are discussed.

The class of D vitamins comprises a family of fat-soluble compounds[2-5] derived photochemically from steroids, members of which have long been recognized for their antirachitic activity. Although the exact role is yet to be elucidated, the essence of vitamin D activity[2,6] is to elevate the plasma calcium and phosphate content to supersaturated levels. The most important members of this family of vitamins[4] are ergocalciferol or calciferol, also called vitamin D_2, and cholecalciferol, also known as vitamin D_3, both of which have the skeleton I. They differ only in the nature of the side chain at the 17 position. The vitamins, as indicated in I, must first be metabolized[6] to the 1,25-dihydroxy derivatives, which are thought to be the circulating active form.

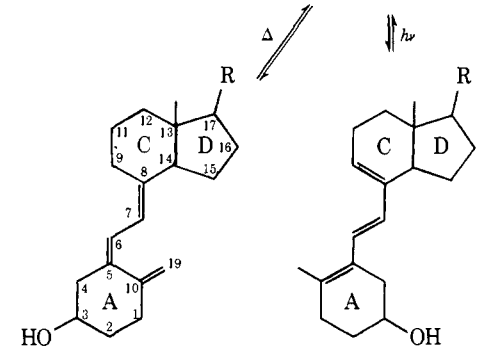


I

The well-documented role of photochemical activity[2-8] in the interconversion among various forms of the D vitamins and their precursors, as found, for example, in the conversion of sterol to calciferol[6] in the skin upon uv irradiation, has led to close scrutiny of the photochemistry of this class of compounds. The currently accepted photochemical and thermal pathways for interconverting among several important intermediates are illustrated[4,5] in II for vitamin D_2.



IIa, provitamin D, ergosterol — IIb, previtamin D, precalciferol



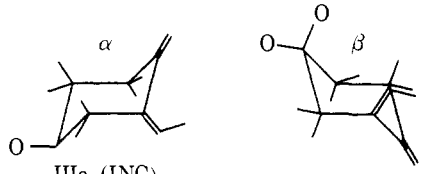
IIc, vitamin D, calciferol — IId, tachysterol

The thermal equilibrium involving previtamin D and vitamin D is thought[4,5] to be in favor of vitamin D in spite of the instability of exocyclic double bonds in cyclohexane rings due to the influence of the strained configuration[5] of the five-membered D ring.

Because of their importance, considerable attention has been devoted toward elucidating the structure of vitamin D_2 derivatives or analogs. In tachysterol, the trans,trans,cis form, as opposed to the all-trans forms, for the A–C ring bridge was established[8] by solution NOE measurements.

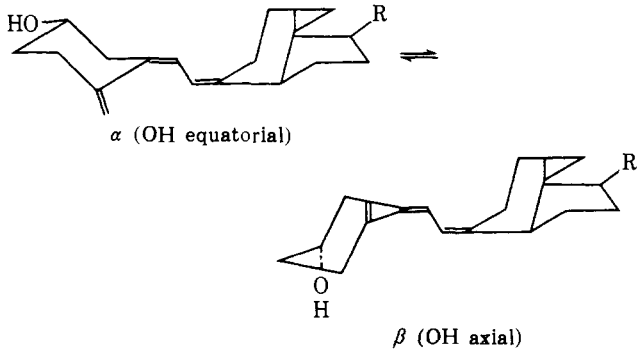
X-Ray crystallographic data have been reported for two derivatives of vitamin D_2, the calciferol 4-iodo-5-nitrobenzoate,[9] INC, and 3,20-bis(ethylenedioxy)calciferol,[10] ECF. An important structural result which has arisen from these studies[9,10] is the verification of the expected 6,7-trans form, as depicted in IIc, the trans fusion of the C and D ring, the twisting of the diene system such that the planes defined by 6,7,8,9,14 and 4,5,6,7,10 make an angle of ~12° (the 5,10,19 and 5,6,10 planes make an angle of 56–61°), and the surprisingly nearly regular, slightly flattened chair configuration of the A and C six-membered rings.

However, the two structural studies, although concurring on the regular chair configuration for the A ring, differ as to the form of the chair. The INC structure yielded[9] the α form[5] of the A ring, *i.e.*, IIIa, while the ECF structure gave[10] the β form[5] of the chair, *i.e.*, IIIb. It was first suggested[10] that the difference between the conformations of the A ring in these two structures must have resulted from conversion in one of the two compounds.



IIIa (INC) IIIb (ECF)

Originally, little attention was paid to the exact nature of the A ring in solution, although its conformation could have a marked effect on the topology of some of the irradiation products of vitamin D. Experimental work by Bakker, *et al.*,[11] has shown recently that the minor products also isolated upon irradiation of vitamin D_3, the so-called "over-irradiation" products,[4,5] have structures which can be explained only by postulating an equilibrium between α and β forms of the A ring, as illustrated in IV. Aside from these references,[4,5,11] there is available neither qualitative nor quantitative data as to the position of the equilibrium depicted in IV.



IV

As an aid toward understanding more fully the detailed solution structure of the D vitamins, we have undertaken a proton nmr study of calciferol in order to determine the conformation of the A ring, and if possible, to provide insight into other structural features of this molecule. Conformational information can be obtained from proton multiplet structure due to the angular dependence of vicinal coupling constants as described by the Karplus relation.[12,13] Unfortunately, as indicated in earlier reports,[4,14] the poor resolution of most of the methylene ring resonances precludes such an analysis for the pure compound at 100 MHz. In order to provide the necessary spectral resolution required to assign multiplets, we have employed the paramagnetic shift reagent[15,16] $Eu(dpm)_3$ (dpm = dipivaloylmethane).

In addition to providing spectral resolution, the relative lanthanide-induced paramagnetic shift[15,16] is used as a guide for determining the conformation of the A ring in solution. The paramagnetic shift, which arises from the dipolar interaction,[17] is proportional to the geometric factor $(3\cos^2\theta - 1)r^{-3}$, where θ is the angle between the Eu–proton vector and the Eu–O vector, and r is the length of the former vector.[17,18] Hence the relative shift for any pair of nonequivalent protons is equal to the relative values for the geometric factors. Since both r and θ are highly sensitive to the conformation of the A ring, very useful information on the solution conformation can be obtained. Though the use of shift reagents for detailed conformational analysis has received wide attention in the past few years,[15,16,18-20] we approached the quantitative use of the induced shifts with caution in view of the fact that axial symmetry may not be applicable,[20] and that the presence of contact shift contributions have been established[19] in enough cases so as to raise serious questions about a quantitative structure derived in any case where the structure is truly unknown. However, the availability of independent estimates of the solution conformation from the coupling constants[12,13] and the shift ratios[15,16] should provide a reasonable check on the consistency of our interpretation.

Experimental Section

Materials. Crystalline vitamin D_2 (calciferol) was purchased from Nutritional Biochemical Corp. and used without further purification; it was stored in the dark under nitrogen at 0°. $Eu(dpm)_3$, obtained from Norell Chemical Co., was stored over $CaCl_2$ prior to use. Spectroscopic grade CCl_4 (Mallinckrodt) and $CDCl_3$ (Merck Sharpe and Dohme) were used as solvents with TMS serving as internal reference.

Sample Preparation. Samples containing $Eu(dpm)_3$ and calciferol were prepared by adding 0.50 ml of a 0.097 or 0.142 *M* CCl_4 solution of calciferol to weighed amounts of $Eu(dpm)_3$, varying the $Eu(dpm)_3$ from 0 to the maximum allowed by solubility. A plot of the observed nmr shift *vs.* $Eu(dpm)_3$ concentration exhibited a straight line which did not pass through the origin. This failure to pass through the origin can be traced to preferential coordination of impurities by $Eu(dpm)_3$ in the calciferol or in the $Eu(dpm)_3$ or both.

Variable-temperature nmr data were obtained on a 0.109 *M* CCl_4 solution of calciferol containing enough $Eu(dpm)_3$ to give a well-resolved spectrum, with the 3-H shift at −11.6 ppm from TMS at 29°.

Nmr Measurements. The spectra in CCl_4 solution were obtained on a Jeol JNH-MH-100 spectrometer operating at 100 MHz. In the variable-temperature studies, the probe temperature was controlled by a Jeol JES-VT-3 unit which was calibrated with a thermocouple within a nmr tube. Decoupling experiments were performed using the standard JNH-MH-100 decoupling procedures.

Although it was possible to obtain most coupling constants from the CW single traces of the CCl_4 solution in calciferol, it was desirable to obtain better signal:noise ratios using computer-averaging techniques. Since it was noted that multiplet structures in CCl_4 and $CDCl_3$ solution were the same (although chemical shifts for some of the peaks differed), a proton FT spectrum of a 0.1 *M* calciferol solution in $CDCl_3$ was obtained with considerably improved S:N ratio and resolution which permitted more accurate determination of coupling constants. Those peaks for which coupling constants could be obtained accurately in CCl_4 in a single CW trace (peaks a, b, f, g, h, i), were unchanged on going to $CDCl_3$.

All shifts are given in parts per million relative to TMS; coupling constants are given in hertz. Relative shifts for protons q and r are defined as $[\nu_q(\text{obsd}) - \nu_q(\text{diam})]/[\nu_r(\text{obsd}) - \nu_r(\text{diam})]$, where $\nu(\text{obsd})$ is the shift observed in the presence of $Eu(dpm)_3$, and $\nu(\text{diam})$ is the shift in the absence of $Eu(dpm)_3$. It was noted that none of the shifts were altered more than 1–2 Hz upon addition of $La(dpm)_3$, indicating that the use of the free calciferol as reference is valid.

Calculations. The relative geometric factors were computed using a rigid model of calciferol constructed using the scale 5.0 cm

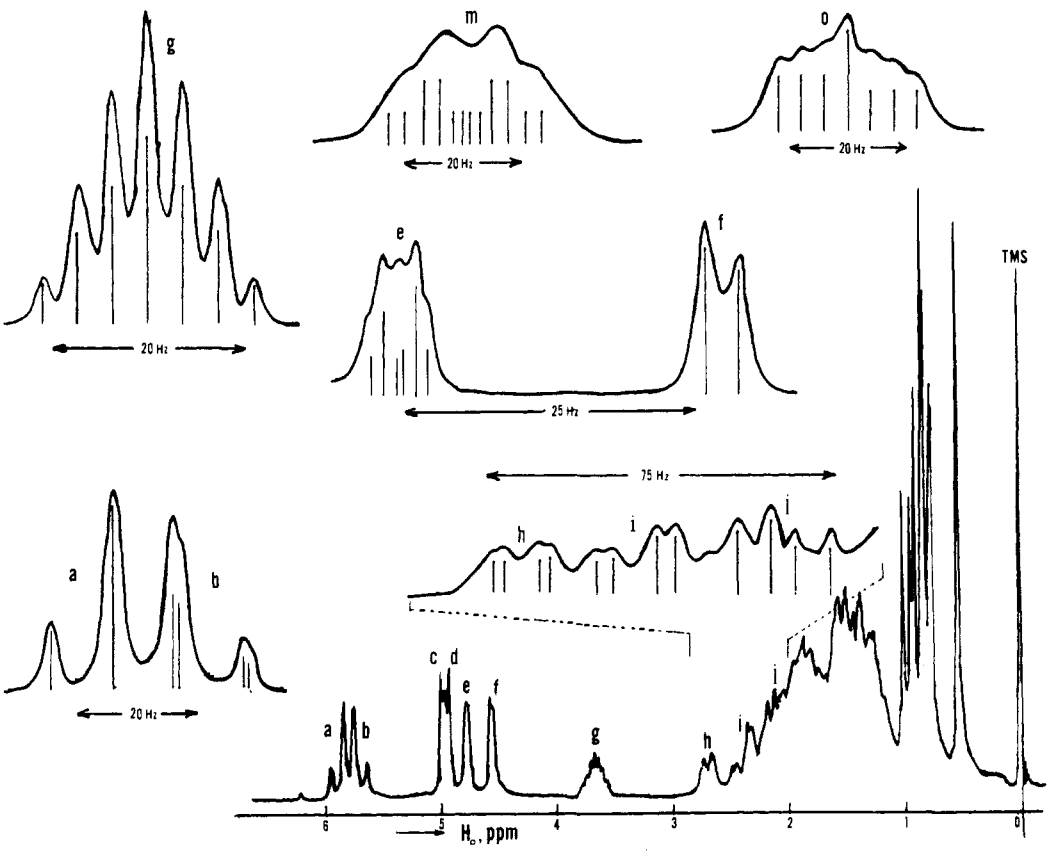


Figure 1. Proton nmr trace of a 0.2 *M* CCl_4 solution of calciferol at 29°. The peaks a,b,e–j are given in expanded form above. The broadened multiplet structure of peaks l and o, resolved in the presence of $Eu(dpm)_3$, are also included in the upper-right portions. The stick diagrams are included to indicate the extent of agreement between the observed multiplets and those predicted for the 1:1 α:β chair mixture using the cyclohexanol coupling constants for the A ring found in Table II. The labeling for resonances is as listed in Table I.

= 1.0 Å. Both the α and β chair configuration were used, assuming a regular chair geometry, but adopting all other dimensions for the INC structure.[9] Geometric factors were calculated as a function of the rotational angle about the CO bond.

Results and Discussion

Assignment and Analysis of Coupling Constants. The 100-MHz proton nmr trace of a 0.2 *M* solution of calciferol in CCl_4, illustrated in Figure 1, reveals well-resolved resonance primarily for the three pairs of vinylic protons, peaks a–f, which have been assigned previously,[4,14] plus the 3-H resonance, peak g. Three peaks, h, i, and j, at the low-field side of the methylene proton envelope can also be discerned. The expanded proton of Figure 1 permits the assignment of peaks i and j to the 4-protons on the basis that irradiating g (3-H) reduces both i and j to members of an AB doublet with the typical[21] $J_{gem} \sim 13$ Hz. The g peak (3-H) analyzes as a triplet of triplets with $J_1 \simeq 7.4$ and $J_2 \simeq 3.7$ Hz, where the couplings to the axial and equatorial 2-H and 4-H are degenerate. Assignments and peak positions are listed in Table I.

It is observed here, however, that in spite of the regular chair configuration found in both X-ray studies,[9,10] the larger J(3-H,4-H) ~ 7.4 Hz, is considerably less than the value for J_{axax} and larger than the values for either J_{axeq} or J_{eqeq} obtained from cyclohexanol.[13,22] The unusually low value of 7.4 Hz, however, can be explained by assuming the presence of both the α and β chair conformation in solution so that the larger J(3-H,4-H) coupling would be some average of J_{axax} and J_{eqeq}, while the smaller J(3-H,4-H) coupling is J_{axeq}. Using $J_{axax} = 11.1$, $J_{eqeq} = 2.7$, and $J_{axeq} \sim 3.7$ Hz,[22] the observed multiplets for peaks g, i, and j *suggest ~50:50 mixture*[23] *of the* α *and* β *chairs.*

The presence of both chair forms in solution is also indicated by peak e (19-H*), which, in addition to the 2.4-Hz cis splitting due to f (19-H), exhibits identical coupling to *two* protons with $J \simeq 1.0$ Hz; this splitting arises from the two 1-protons. However, the angular dependence of such allylic coupling constants[24] with a single chair configuration predicts unequal coupling to these two protons with $J = 0$ for the equatorial 1-H and $J \sim 2$–3 Hz for the axial 1-H. The identical couplings to both 1-protons indicates that

Table I. Observed Multiplet Structure and Assignments for Resolved Resonances of Calciferol

Peak	Diamagnetic shift, δ	Multiplet[a]	Assignment
a	6.14	D (11.3)	6-H
b	5.94	D of D (11.3, ~0.8)	7-H[b]
c, d	5.17	Complex multiplet	22-H, 23-H
e	4.97	D of T (2.4, ~1.0)	19-H*[c]
f	4.73	D (2.4)	19-H
g	3.82	~T of T (~7.4, ~3.7)	3-H
h	2.80	D of D (10.5, 2.5)	14-H
i	2.51	D of D (13.0, 3.7)	4-H'
j	2.24	D of D (13.0, 7.4)	4-H
k	~1.5	S	3-OH
l	~2.5	Not observable	2-H'
m	~1.8	D of T of D (~12, ~7, ~4)[d]	2-H
n	~2.0	Not observable	1-H'
o	~2.1	D of D of D (~12, ~8, ~4)[d]	1-H
p	0.57	S	18-CH_3

[a] S = singlet, D = doublet, T = triplet; J is given in parentheses. [b] Secondary splitting of ~0.8 Hz to the axial 9-H. [c] 19-H* corresponds to proton cis to 5,6 double bond. [d] Observable only in the presence of $Eu(dpm)_3$.

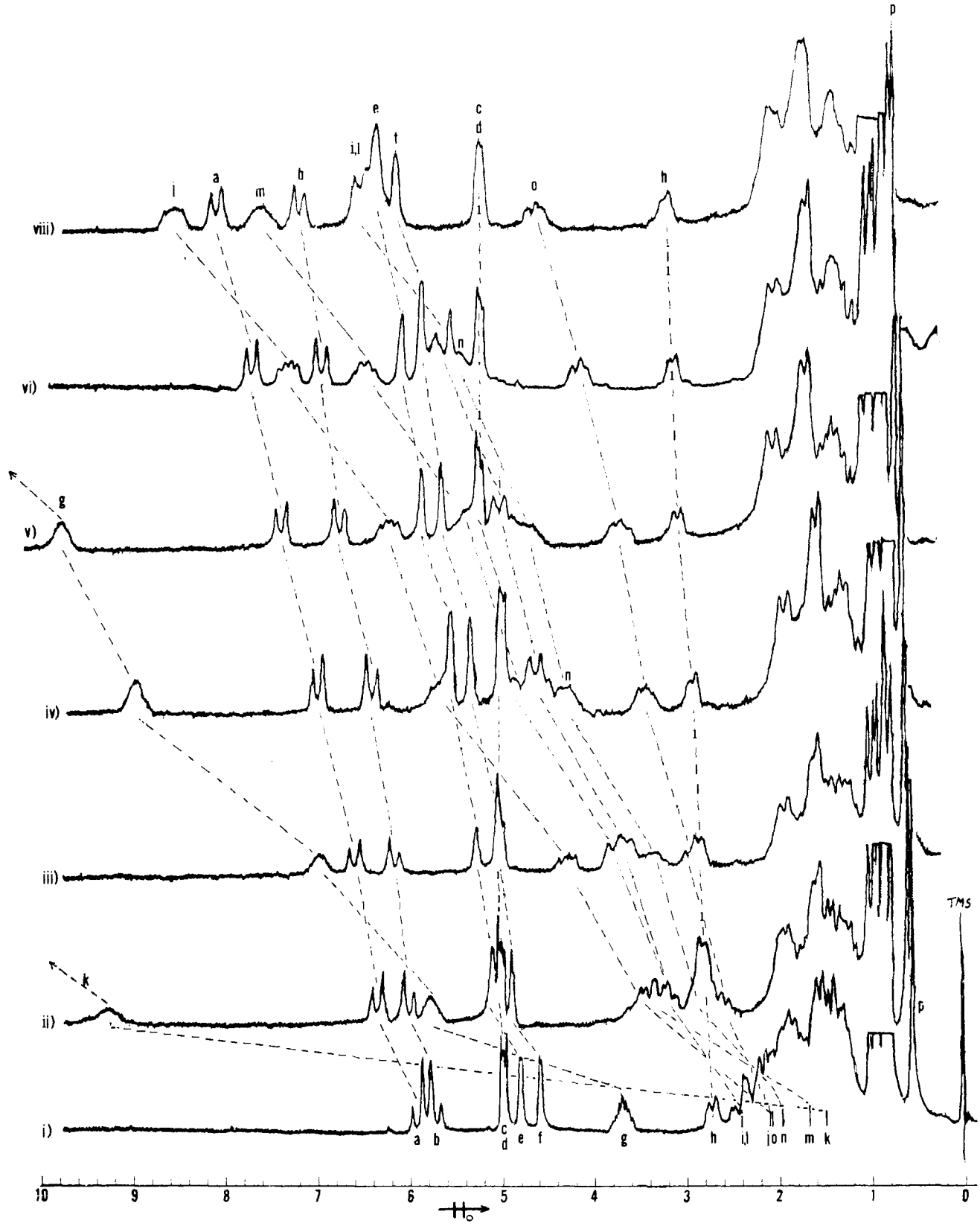


Figure 2. Proton nmr trace of calciferol in CCl_4 at 29° in the presence of increasing amounts of $Eu(dpm)_3$; the assignment of the peaks is given in Table I. Actual calibration of the shifts in these spectra is only approximate.

there is rapid averaging over equal amounts of the two conformations for which the allylic couplings[24,25] interchange. Since J(transoid) > J(cisoid) for exocyclic methylene groups,[24] peak e is assigned to the 19-H which is cis to the 5,6 double bond and is indicated as 19-H*.

The coupling constant data for peaks e, g, i, and j therefore strongly support the presence of both α and β chair forms in approximately equal amounts. Other multiplets which can be resolved only upon addition of $Eu(dpm)_3$ similarly require the presence of both forms. In Table II we list the multiplets predicted for each A-ring methylene proton using the averaged α and β chair coupling constants derived

Table II. Predicted Multiplet Structure for A-Ring Protons Based on Cyclohexanol Coupling Constants and a 1 : 1 Mixture of α and β Chairs

Position	Predicted multiplet	Coupled protons	Peak in Figure 2
3-H	T of T (7.0, 3.6)[a,b]	(2,4-H, 2,4-H′)	g
4-H	D of D (12, 7.0)[a]	(4-H′, 3-H)	j
4-H′	D of D (12, 3.6)[b]	(4-H, 3-H)	i
2-H	D of T of D (12, 7.0, 3.6)[c]	(2-H′, 1,3-H, 1-H′)	l
2-H′	D of D of T (12, 7.0, 3.6)	(2-H, 1-H′,1,3-H)	m
1-H′	D of D of D (12, 7.0, 3.6)	(1-H, 2-H′, 2-H)	n
1-H	D of D of D (12, 7.0, 3.6)	(1-H′, 2-H, 2-H′)	o
19-H*	D of T (2.4, 1.0)	(19-H, 1-H,H′)	e

[a] Spectrum in Figure 1 indicates coupling is 7.4, not 7.0 Hz. [b] Spectrum in Figure 1 indicates coupling is 3.7 not 3.6 Hz. [c] Spectrum in Figure 1 indicates coupling is 8 and 4, not 7.0 and 3.6 Hz.

from cyclohexanol.[22] Detailed analysis of the shifts indicated by $Eu(dpm)_3$ will provide additional confirmation of the presence of both chair forms. In order to discuss coupling between protons which are dynamically averaged over the α and β conformations, we will refer to protons which are *axial* in the α form as H (*i.e.*, 4-H for peak j), and protons which are equatorial[25] in α as H′ (*i.e.*, 4-H′ for peak i). Hence in the β chair, axial and equatorial protons are designated H′ and H, respectively.

A number of resonances, which could not be resolved in Figure 1 (peaks k–o) or were resolved but could not be assigned (peaks h and p), were easily assigned with the aid of $Eu(dpm)_3$. The selected spectra in Figure 2, which illustrate the effect of the addition of increasing amounts of $Eu(dpm)_3$, permit resolution of all A-ring protons, although peaks i and l always remain approximately degenerate. The observed peaks a–p are listed in Table I, along with their observed multiplet structure and either the observed or extrapolated diamagnetic positions. The nature of the observed multiplets may be compared to those predicted[22] by a 50:50 α:β chair mixture, as listed in Table II.

Peak m yields a multiplet which is consistent with a proton on C_2 and axial in the α chair (*i.e.*, 2-H) as illustrated in the insert in Figure 1. Peaks n and o are unaffected by irradiating g (3-H) and hence ≥four bonds removed from 3-H. Decoupling m (2-H) deletes an ~8-Hz coupling from o, leaving a doublet of doublets (~12 Hz, ~4 Hz), indicating that o is also axial in the α chair (*i.e.*, o = 1-H). The consistency between the observed o multiplet and that predicted for 1-H in Table II is illustrated in the insert in Figure 1. Irradiation of n deletes the ~12-Hz coupling from o (1-H), indicating that n and o are geminal (*i.e.*, n = 1-H′). Peak l, which is always under i (4-H), as indicated by relative areas, must belong to 2-H′, the only remaining A-ring proton. The consistency of these assignments is further confirmed by the very similar slopes of the shift *vs.* [Eu(dpm)] for the pairs of resonances 4-H,2-H, and 4-H′,2-H′ (*vide infra*) which occupy spatially comparable positions relative to the OH group (and hence the Eu ion) in both the α and β chairs.

The resonance h is slightly affected by $Eu(dpm)_3$ but is not coupled to any A-ring proton and must therefore belong to ring C. The small effect due to $Eu(dpm)_3$ suggests it is either 9-H or 14-H. The latter assignment is favored since the primary splitting of 10.8 Hz is less than that generally found for geminal protons[21] (≥12 Hz), indicating that the proton is attached to a tertiary carbon. The low-field diamagnetic position of h is also consistent[26] with this assignment. The two splittings observed for 14-H therefore arise from the two protons on C-15. The one methyl peak p, which exhibits a sensitivity toward $Eu(dpm)_3$, must originate from 18-CH_3, the methyl group closest to the A ring.

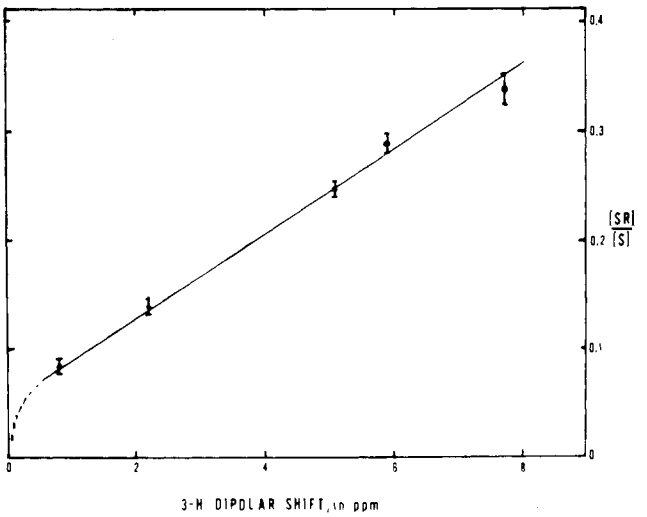


Figure 3. Peak of the 3-H (peak g) shift as a function of the [$Eu(dpm)_3$]:[calciferol] mole ratio. The failure to extrapolate to the diamagnetic position at [$Eu(dpm)_3$] = 0 is due to the presence of small impurities in the sample which coordinate preferentially to $Eu(dpm)_3$.

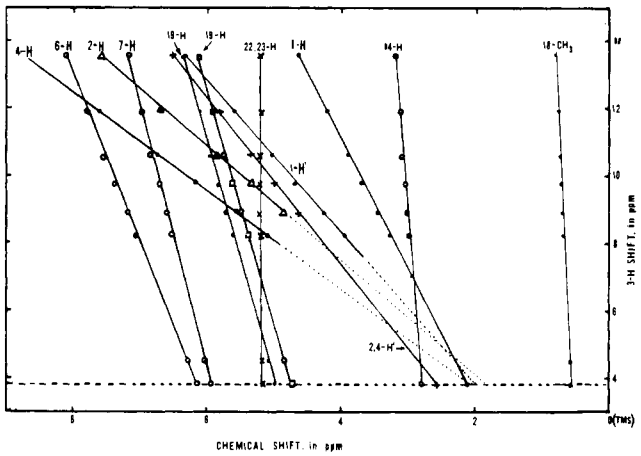


Figure 4. Plot of the observed shift relative to TMS of all resolved resonance *vs.* the shift of the 3-H proton as a function of increasing amounts of $Eu(dpm)_3$. Extrapolation of the shifts to a shift of 3.82 ppm below TMS for 3 H (indicated by the horizontal dashed line) permits the determination of the diamagnetic position of resonance not resolvable in the absence of $Eu(dpm)_3$.

Analysis of $Eu(dpm)_3$-Induced Shifts. Figure 2 illustrates the effect on the calciferol spectrum upon the addition of increasing amounts of $Eu(dpm)_3$. A plot of the 3-H shift *vs.* the $Eu(dpm)_3$/calciferol = [SR]/[S] mole ratio is found in Figure 3. The data yield a straight line, indicating that only a single species is formed, although the line does not extrapolate to the diamagnetic position for 3-H. This curvature in the line at very low [SR]/[S] ratios arises because of very small amounts of impurities in the calciferol, probably water, which preferentially interact with $Eu(dpm)_3$. Hence a plot such as found in Figure 3 cannot be used to obtain the diamagnetic position of these resonances which can be resolved only in the presence of $Eu(dpm)_3$. The correct extrapolated diamagnetic position for all protons can be obtained, however, for a plot of the shift of any position *vs.* that of the 3-H peak as a function of increasing $Eu(dpm)_3$. Such a plot is given in Figure 4. All lines in this graph are straight, which indicates that all shift ratios are independent of the fraction of calciferol coordinated to $Eu(dpm)_3$.

The relative shifts due to $Eu(dpm)_3$ for the assigned positions, with the degenerate pair 2-H's 4-H′ taken as 10.0, are

Table III. Calculated Relative Geometric Factors for α-Chair Conformation for A Ring in Calciferol-Eu(dpm)$_3$ Complex[a]

Position	Obsd rel. shifts at 29°	Relative geometric factors[b] $\phi = 0$	±45°	±90°	±135°	±180°
3-H	23.4	16.1	17.9	21.7	17.2	15.2
4-H	16.0	9.5	11.3	10.4	8.3	8.9
2-H	15.1	9.5	11.3	10.4	8.3	8.9
2-H', 4-H'	10.0	10.0	10.0	10.0	10.0	10.0
1-H'	11.0	3.1	4.6	3.5	1.6	1.8
1-H	6.2	3.2	5.7	5.7	4.7	5.0
19-H, H* [d]	3.3	2.0	2.1	1.4	*c*	*c*
6-H	4.7	1.9	4.0	1.2		
7-H	3.0	1.7	1.8	2.4		
14-H	0.9	0.4	0.7	0.5		
18-H		0.3	0.3	0.6		

[a] Regular chair geometry adopted for A ring, otherwise the structural data for INC employed; Eu–O distance = 2.6 Å, Eu–O–C angle = 130°. [b] Geometric factor for 2-H', 4-H' normalized to 10.0 in each case. [c] ϕ defined as in text; the most probable value is $\phi = 0$; $\phi \geq \pm 90$ not physically possible, so no values were calculated. [d] Observed shift ratios, as well as calculated geometric factors, are the same for 19-H and 19-H*.

Table IV. Calculated Relative Geometric Factors for β-Chair Conformation for A Ring in Calciferol-Eu(dpm)$_3$ Complex[a]

Position	Obsd rel. shifts at 29°	Relative geometric factors[b] $\phi = 0$	±45°	±90°	±135°	±180°
3-H	23.4	26.3	27.8	21.7	23.8	23.3
4-H	16.0	18.5	13.9	13.3	16.9	18.8
2-H	15.1	18.5	13.9	13.3	16.9	18.8
2-H',4-H'	10.0	10.0	10.0	10.0	10.0	10.0
1-H'	11.0	18.6	17.9	17.6	15.7	25.7
1-H	6.2	8.5	7.6	−3.2	−1.7	−8.5
19-H, H* [d]	3.3	4.2	4.3	1.1	*c*	*c*
6-H	4.7	6.6	4.3	−5.8		
7-H	3.0	4.7	4.0	−1.0		
14-H	0.9	1.6	0.5	−1.0		
18-H		0.6	1.7	−0.7		

[a–d] See corresponding footnotes in Table III.

given in the first column of Tables III and IV. 2-H',4-H' were chosen on the reference instead of the more obvious 3-H peak to eliminate any problems which may arise because of significant contact shift contribution. Such contribution would be most important for OH and decrease with the number of intervening bonds.[27]

Although both X-ray structures[9,10] on calciferol derivatives agree on a regular, slightly flattened chair conformation, differing only in an α chair for INC and a β chair for ECF, the individual bond distances and angles differed sufficiently[9,10] so as not to provide a totally unambiguous set of parameters from which to calculate the geometric factors. Sample calculation using the X-ray geometry[9,10] for one of the derivatives as well as the regular chair geometry for the A ring yielded results which were very similar, particularly since the Eu–O–C bond angle and Eu–O bond distance must be estimated.[28,29] In view of our interest in only a semiquantitative picture of the solution structure, we selected to perform detailed calculations of geometric factors only for a geometry based on the bond distances, bond angles, and dihedral angles of a regular chain (*vide infra*). Test calculations suggest that geometric factors for the A ring using these data differed insignificantly from those based on the structure[10] of ECF.

The geometric factors were computed as a function of the rotational angle ϕ, which describes the rotation of the chair about the C–O bond; ϕ is the angle between the H_3–C_3–O and C_3–O–Eu planes, with $\phi = 0$ corresponding to the case where H_3, C_3, O, and Eu are in the same plane and H_3 and Eu are cis to each other. The Eu–O distance was selected as a reasonable 2.6 Å,[28] and the Eu–O–C bond angle[29] as 130°. Substantial variations in the angle left the qualitative shift patterns unaltered. Since the slopes of the shifts *vs.* Eu(dpm)$_3$ are nearly identical for the 2-H and 4-H, as well as for the 2-H' and 4-H' pairs, it appears that conformations with the rotational angle $\pm\phi$ are approximately equally probable. A significant preference for a positive or negative ϕ would result in large differences in dipolar shifts for the 2-H' and 4-H' pair on the 2-H and 4-H pair. Molecular models[30] confirm this effective symmetry for ϕ. The computed relative geometric factors as a function of $\pm\phi$ for assigned protons, with the geometric factor for 2-H',4-H' pair normalized to 10.0, are found in Tables III and IV for the α and β chair forms of calciferol, respectively.

Comparison of the experimental relative shifts, also included in Tables III and IV, with the calculated geometric factors for either the α or the β chair reveals large discrepancies for any choice of ϕ, as well as for any combination of ϕ's for a single chair configuration. Furthermore, inspection of space-filling models[30] indicates that the preferred orientation of the OH for minimal steric interaction between the A ring of calciferol and the dpm ligands in Eu(dpm) occurs at $\phi = 0$ for both the α and β chairs. We will therefore make the reasonable assumption that the preferred orientation is with $\phi = 0$ for both α and β chairs and instead consider the presence of both chair forms.

The discrepancies between the experimental data and the calculated geometric factors for either chair form can be removed by considering a dynamic equilibrium between the α and β forms in solution. A search was made for that mixture of the α and β forms which most closely reproduced the observed shift pattern.[31] In column 4 of Table V, we give the calculated relative geometric factors for a 45:55 mixture of the α and β chairs, respectively, which provides the best fit[32] to the observed data at 29°; agreement is most satisfactory. It is noteworthy that the best fit for the mixture based on the relative dipolar shifts is essentially the same as that determined above from the averaged coupling constants.

The possibility that the position of the equilibrium in III is affected significantly by coordination to Eu(dpm)$_3$ was investigated. A sensitive test of this is to monitor that multiplet structure of 4-H as a function of the Eu(dpm)$_3$/calciferol = [SR]/[S], mole ratio. If there is a significant difference in the position of the equilibrium for the free and coordinated, then J(4-H,3-H), the averaged J_{axax} and J_{eqeq}, will change by approximately 0.8 Hz for every 10% change in α:β mixture.[33]

There are three regions in [SR]/[S] ratio where J(4-H,3-H) can be clearly observed. At [SR]/[S] = 0, J(4-H,3-H) = 7.4 Hz; at [SR]/[S] ~ 0.22 (*i.e.*, the 3-H shift is −8 ppm from TMS), J(4-H,3-H) = 7.3 Hz; and at [SR]/[S] ~ 0.3 (the 3-H shift is ~10 ppm below TMS), J(4-H,3-H) = 7.1 Hz. The geminal J(4-H,4-H') is invariant. At [SR]/[S] = 0.3, the α:β ratio has changed $\lesssim$3%, in spite of the fact that this ratio reflects a 30% coordination of the calciferol for a 1:1 SR:S complex or 60% coordination if the dominant species is the 1:2 adduct. The insensitivity of the shift ratios to the [SR]/[S] ratio also argues strongly against a change in the α:β ratio with Eu(dpm)$_3$. Hence the equilibrium in IV is not significantly affected upon coordination to Eu(dpm)$_3$.

Effect of Temperature on Shift Ratios. Since the fit of the 29° shift ratio data to the calculated geometric factors of a mixture of α and β forms indicated a slight preference for the β chair, it may be reasonable to expect that this ratio of conformers will be sensitive to temperature. Such tempera-

Table V. Fit of Observed Shift Ratios to Relative Geometric Factors Computed for α,β Chair Mixtures[a]

	Rel geom fact.[b]		Shift ratios[b]					
			T = 29°		T = −26°		T = 66°	
	α chair	β chair	Obsd	Calcd for $\alpha:\beta$ = 45:55	Obsd	Calcd for $\alpha:\beta$ = 30:70	Obsd	Calcd for $\alpha:\beta$ = 50:50
3-H[c]	17.0	26.4	23.4	22.2	22.6	23.6	23.0	21.7
4-H	9.5	18.5	16.0	14.5	17.9	15.8	14.5	14.0
2-H	9.5	18.5	15.1	14.5	15.6	15.8	14.1	14.0
2-H′, 4-H′	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
1-H′	3.1	18.6	11.0	11.6	13.5	14.6		10.8
1-H	3.2	8.5	6.2	6.1	6.7	6.9	5.9	5.9
19-H	2.0	4.2	3.3	3.2	3.8	3.6	3.0	3.1
6-H	1.9	6.6	4.7	4.5	5.5	5.2	4.3	4.3
7-H	1.7	4.7	3.0	3.4	3.6	3.8	2.7	3.2
14-H	0.4	1.6	0.9	1.0	1.2	1.2	0.9	1.0
18-CH_3	0.3	0.6	0.5	0.5	0.5	0.4	0.5	0.4

[a] Optimum fit determined for ring A protons only. [b] Normalized in each case for 2-H′, 4-H′ shift of 10.0. [c] Umprimed protons are axial in α chair, primed protons are axial in β chair.

ture effects could yield thermodynamic data on the conversion between the two chairs as well as provide a more stringent test of our interpretation of the solution conformation.

The effect of temperature on the observed shift ratios is illustrated in Figure 5. From this figure it is clear that the shift ratios do vary, with the sensitivity to temperature depending on the location of the proton. It is also apparent from this figure that the shift ratios change with lower temperatures so as to resemble more closely the pattern predicted for the β chair.

In Table V, we present the best fit[32,34] of the conformer mixture to the observed shift ratios at the extreme temperatures, −26 and 66°, in addition to 29°. Good fits are obtained only by increasing the fraction of β on lowering the temperature and increasing the fraction of α on raising the temperature. The three fits in Table V yield values for equilibrium constants for the equilibrium depicted in IV of 2.3, 1.2, and 1.0 at −26, 29, and 66°, respectively, which affords estimates of $\Delta H \sim -1.5$ kcal, and $\Delta S \sim -5$ eu. Very little reliance can be placed on these numbers, however, since the fits between the data and the calculated geometric factors were based on a perfectly regular chair geometry. The qualitative conclusions, however, that the two chairs are present in comparable amounts, with the β form thermodynamically slightly more stable, appear unambiguous.

Although attempts were made to obtain confirming evidence of the temperature effect on the equilibrium by monitoring coupling constants, unambiguous data could not be obtained because of either the severe line broadening which sets in at low temperatures owing to viscosity effects or the overlapping of the resonance which occurs upon raising the temperature.

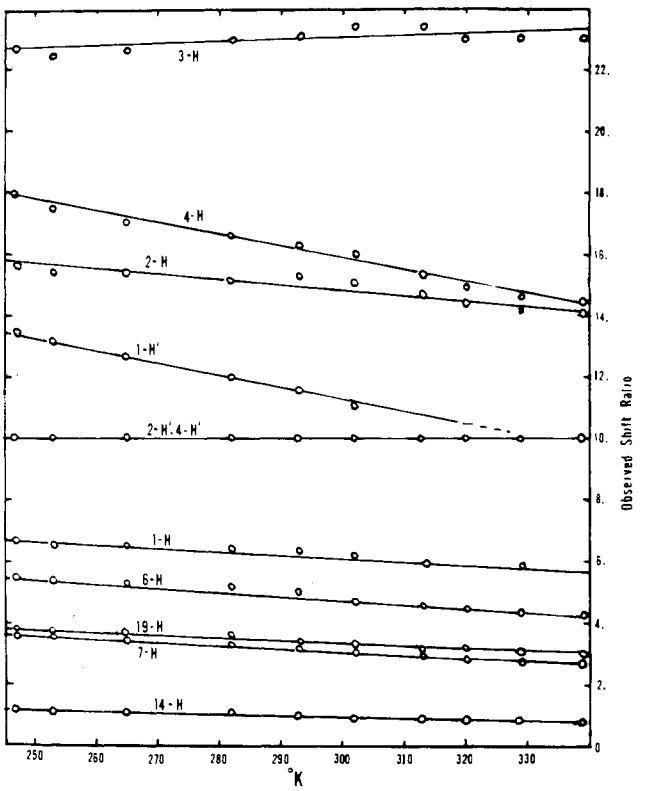


Figure 5. Plot of the change in the ratio of observed shifts *vs.* temperature, normalizing all shifts to 10.00 for the degenerate 2-H′, 4-H′ peaks.

Conclusions

Analysis of both the A-ring proton spin–spin splittings,[12,13] as well as the lanthanide-induced dipolar shift ratios,[15,16] leads us to the conclusion that vitamin D_2 is present in CCl_4 solution in a mixture of conformers differing in the nature of the chair configuration of the A ring. The similarity of the nmr spectra in CCl_4 and $CHCl_3$ indicates that a similar mixture of conformers is present. Moreover, the observation of nearly the same 3-H multiplet pattern for cholecalciferol, vitamin D_3, suggests that the equilibrium may be a general characteristic of the D vitamins.

It is estimated that the mixture contains approximately equal amounts of the vitamin with the A ring in the α and β chair configurations, with the β chair slightly favored thermodynamically. It is not known at this time whether the presently observed change in the position of this $\alpha \rightleftarrows \beta$ chair equilibrium with temperature plays any role in the previously reported[4,5] sensitivity of the nature of the photochemical reactions on temperature. The present results do suggest that similar studies on a variety of the vitamin D intermediates and precursors[4] may shed new light on the relationship between solution conformation and chemical or photochemical reactivity of these molecules. Several of these studies are currently in progress in this laboratory.

Comments

Based on the apparent success of our interpretation of the A-ring conformation vitamin D_2, it may be tempting to try to derive either additional structural information on the C and D rings or the side chain at the 17 position, or to obtain more quantitative estimates of the thermodynamics of the chair conversion. After careful consideration, we conclude that such additional and/or more refined interpretation, although obviously possible since the system is highly unde-

termined, is likely to lead to highly ambiguous and possibly misleading conclusions.

These preliminary pessimistic conclusions are based on two lines of evidence. (1) Uncertainties in bond distances and angles multiply, even within a fixed "conformation," and rapidly lead to large errors in geometric factors for protons near the extremity of the molecule. This is particularly true for the A–C ring bridge, where small changes in the dihedral angle between the 5,6,10 and 5,10,19 planes can cause substantial changes in geometric factors for the vinyl-bridge and C-ring protons. This angle differs by 5° in the two structurally characterized calciferol derivatives[9,10] and would normally not be considered a parameter in a computer search for an optimum fit.

(2) Rotamers with ϕ other than 0 are not insignificant. Without a knowledge of the shape of this rotational barrier, a quantitative calculation of averaged geometric factors is not possible. The importance of this effect is clearly demonstrated in the present case. The nonequivalence of the 2-H and 4-H shifts suggests a small preference of the $Eu(dpm)_3$ for one side of the A ring. This nonequivalence is minimized at high temperature when the 4-H:2-H shift ratio is 1.03. However, this ratio is temperature dependent, increasing to 1.15 at −26°, which clearly indicates a higher asymmetry in the preference for a given $+\phi$ *vs.* $-\phi$. The observed trend in this shift ratio is consistent with a preference for the $Eu(dpm)_3$ to be on the opposite side of the A ring from the vinyl bridge in the β chair, as might be anticipated from molecular models.[30]

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(25) Note that axial protons in the α chair become the equatorial protons in the β chair, and *vice versa*.
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(31) The 2,4-H shifts were used as reference in order to minimize errors from possible contact shifts which may not be negligible for the protons two or three bonds removed from the metal (*i.e.*, OH or 3-H). Another advantage in using the 2,4-H peaks as reference is that they are observable over the whole range of SR:S ratios. The comparison of the observed shift ratios with the calculated shift ratios for either the α or β chairs are unaffected by the choice of reference. If 3-H is used as reference, the calculated α:β chair mixture does not change significantly for those given in Table V.
(32) The "best fit" was determined by a least-squares program which minimized the square of the difference between the observed shift ratios and the calculated shift ratios for an α,β chair mixture as a function of the ratio of the two conformations.
(33) The data in ref 22 for cyclohexanol yield values for the averaged J(4-H,3-H) as a function of the α:β ratio are as follows: 70:30, J = 8.6; 60:40, J = 7.8; 50:50, J = 7.0; 40:60, J = 6.1; 30:70, J = 5.3 Hz. Thus the change in J(4-H,3-H) from 7.4 to 7.1 reflects a change in the ratio from ~55:45 to 52:48.
(34) Since the errors in calculated geometric factors increase with distance from the metal for our model of calciferol owing to ambiguities in angles and distances which do not depend on conformation, the fits we performed to the averaged geometric factors of a mixture of α and β chairs were done using only the A-ring proton data. The fact that very reasonable fits are simultaneously obtained for the bridge and two B-rin_ protons confirms the validity of our interpretation.