Ring Opening in the Dehydrocholesterol−Previtamin D System Studied by Ultrafast Spectroscopy

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The rate of the electrocyclic ring opening of 7-dehydrocholesterol to previtamin D is investigated by transient absorption spectroscopy with a time resolution better than 300 fs. The dehydrocholesterol, which is a derivative of 1,3-cyclohexadiene, is excited around 267 nm. The primary product, the s-cis,*Z*,s-cis conformer of the triene previtamin D, appears with a time constant of 5.2 ps. This result is consistent with literature data. We found that it is temperature independent. So there is no activation energy for this electrocyclic ring opening. A barrierless process, on the other hand, is expected to proceed much faster (in about 10^{-13} s), unless there is an entropy of activation. The results suggest that the molecule reaches within a few femtoseconds the lowest excited state ($2A_1$) of the product, from where it goes on through an entropic bottleneck. The primary product isomerizes thermally to the stable s-cis,*Z*,s-trans conformer of previtamin D within 125 ps in ethanol at room temperature. This time is much longer than the reported corresponding time in the cyclohexadiene/hexatriene system. We found that it depends on the solvent viscosity and on the temperature. The activation energy was determined to be 15.5 ± 1.0 kJ/mol.

1. Introduction

The main feature of the conversion of 7-dehydrocholesterol (cholesta-5,7-dien-3β-ol) to previtamin D (Figure 1) is a ring opening from a cyclohexadiene structure to an open-chain hexatriene. It is a prototype of the photochemical "electrocyclic" reactions. Electrocyclic reactions are cyclizations or reverse cyclizations of polyenes with a concerted shift of double and single bonds. The net result is the conversion of a π bond to a σ bond or vice versa. Like the other pericyclic reactions, this rearrangement is controlled by the principle of conservation of orbital symmetry (Woodward−Hoffmann rules).[1] In the particular case of a photochemical reaction, these rules result in a correlation of the excited state of the educt with the excited state of the product, whereas each ground state tends to correlate with a higher excited state. The latter correlation is interrupted by an avoided crossing (see Figure 1).

Since these rules are only correlation rules, they do not predict details of the potential surfaces and the actual dynamics: Is it a direct reaction on a purely repulsive surface (expected rate constant about 10^{13} s^{-1}), or is there a potential barrier? Where on the potential energy surfaces does the molecule cross over to the ground state? Do other states play a role? Questions of this kind led several groups to investigate such systems.

The time constant of the product formation of dehydrocholesterol was for the first time determined as 4.3 ps by end product analysis as a function of the excitation pulse duration:[2] When dehydrocholesterol was illuminated with short pulses (4 ps), the consecutive photoreaction of the primary product was partially suppressed and the yield of the corresponding secondary product, tachysterol, was significantly reduced compared to the case with long pulses (5 ns). Reid et al.[3−6] investigated the analogous system 1,3-cyclohexadiene and its electrocyclic ring opening by resonance Raman scattering. They presented evidence that these molecules undergo a rapid (approximately 10 fs) radiationless decay from the initially excited state $1B_2$ to the "dark" $2A_1$ potential energy surface. (The $2A_1$ state is connected with the ground state by a smaller transition moment

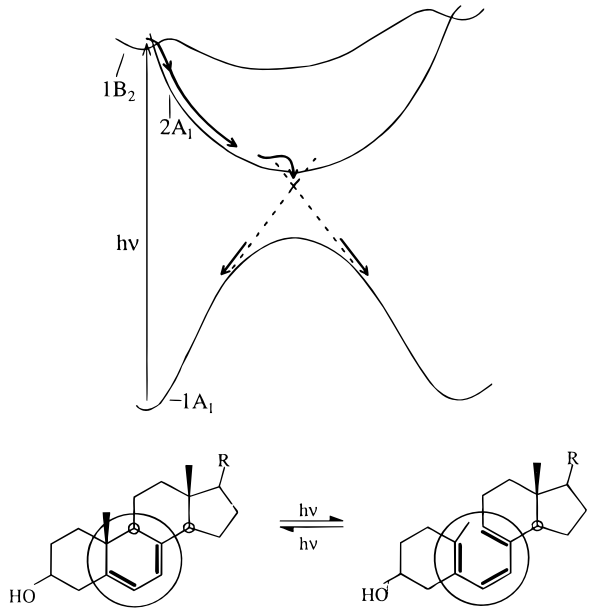

Figure 1. Schematic potential energy diagram for the photochemical ring opening of dehydrocholesterol. The horizontal axis has a large projection onto the single bond to be opened; it is the reaction coordinate, until the molecule arrives at the minimum. Then the molecule leaves the plane of the drawing in the direction of the conical intersection,[15] which is indicated by the broken lines (projection into the plane of paper). Also the maximum of $1A_1$ is actually in a different plane.[15] The subscripts 1 and 2 for the symmetry types should be dropped outside the Franck−Condon region, since the conrotatory reaction does not conserve the (approximate) symmetry planes of the reactant. The large circle in the formulas encloses the reaction center. R = 1,5-dimethylhexyl (C_8H_{17}).

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and is probably very close to $1B_2$ in cyclohexadiene;[7] for other polyenes see refs 8−12. Both states correspond to $\pi\pi^*$ excitation.) The ring opening propagates on this surface, until 6 ps after the initial photoexcitation the ground state hexatriene appears, which due to its high vibrational temperature then rapidly isomerizes to its stable conformer.[5,6]

These time constants of the ring openings are in reasonable agreement with each other, but too long for a direct reaction. The reason for the delay can be an activation energy or barrier, an activation entropy or statistical factor, or another bottleneck. The traditional view of a groundstate forbidden, photochemically allowed reaction is that there is an avoided crossing, which creates a minimum in the excited potential surface along the reaction coordinate—the so-called pericyclic minimum—and a corresponding maximum of the ground state potential.[13] This is shown in Figure 1, which is based (with some modifications) on an early quantum mechanical calculation.[14] The energy gap between minimum and maximum might present a bottleneck for the reaction. However, in a more recent quantum mechanical investigation, Celani et al.[15] found that with an additional distortion of the molecular frame, the surfaces can come much closer and in fact touch each other in a conical intersection (Figure 1). More work of this kind[16−18] has shown that such intersections are very frequent in photochemistry. A conical intersection is not a bottleneck. The molecule passes through it very rapidly.[18,19]

In the conical region the energy is a function of two critical coordinates. One of them corresponds to the distance of the two C atoms between which the ring opening occurs (C_1 and C_6, see Figure 6). The second coordinate characterizes a shift of C_1 in the direction of C_5.[15] The conical intersection is located a bit away from the $2A_1$ minimum along the second coordinate (Figure 1). Celani et al.[15] calculated a reaction path as follows: After the rapid relaxation from the $1B_2$ state to the $2A_1$ state, the excited cyclohexadiene propagates over a negligible barrier along this potential surface to the $2A_1$ minimum. From there to the conical intersection the molecule requires an activation energy of about 4 kJ/mol, which together with a small activation entropy can explain the observed time constant of several picoseconds.

The two quantum mechanical calculations differ strikingly in the prediction for the barrier. The older one[14] suggested a substantial barrier near the initial geometry, although its exact height depends on the way of interpolation between the few calculated points of the surfaces. The more recent calculation[15] predicted a small, but noticeable, barrier only about half-way along the reaction coordinate. Since such calculations are not quantitatively reliable for excited states of molecules with this size, a qualitative consideration also appears appropriate: The electronic excitation almost only affects the π system. (The $2A_1$ state is also a $\pi\pi^*$ state.) It will change primarily the lengths and torsional angles between the π-bonded atoms. The σ bond between C_1 and C_6 can initially not interact, because it is too low in energy and is nearly in the plane of the π-bonded atoms. If this bond is, however, stretched (and thus raised in energy) and twisted out of the plane by a thermal activation, the σ bond begins to interact with the π bonds and the potential energy begins to decrease. So this consideration suggests an early barrier.

From their resonance Raman scattering measurements, Trulson et al.[20] found evidence that the single bond is instantaneously stretched and twisted, i.e. accelerated in the direction of the reaction coordinate. This might suggest the absence of a barrier. The observation is, however, also compatible with an excited state minimum, shifted in this direction and followed by a barrier. An early barrier is required to explain that fluorescence of 7-dehydrocholesterol has been observed in a low-temperature matrix,[21] although at room temperature it is not observed; cyclohexadiene fluoresces at room temperature with a quantum yield of only 2×10^{-6}.[20] So the existing evidence in part suggests an early barrier (the fluorescence, the qualitative consideration, and the early quantum mechanical calculation), in part no early barrier (the resonance Raman experiments) and in part a late barrier (the recent quantum mechanical calculation). Therefore, it seemed important to us to measure the activation energy by studying the temperature dependence of the reaction rate.

In this work, we use the technique of femtosecond transient absorption spectroscopy to study the ring-opening reaction of dehydrocholesterol. This time-resolved measurement of transient excited and intermediate state absorption provides a direct observation of the time required for the electrocyclic reaction. We found that it is temperature independent, whereas the subsequent conformational rearrangement depends on the temperature and on the solvent viscosity in the expected way.

2. Experimental Section

The photochemical reaction of 7-dehydrocholesterol to previtamin D was studied by transient absorption measurements with femtosecond pulses. Dehydrocholesterol was excited by intense, frequency-tripled pulses (pump pulses) of a Ti-sapphire laser system, and the resulting transmission change of the sample was monitored by weak pulses (probe pulses) at the same wavelength as a function of the delay time. Only in a few measurements did we employ for the probe longer wavelengths (302−316 nm) than for the pump (267 nm), which were not absorbed by the dehydrocholesterol. They were synthesized by frequency-quadrupling the signal from a commercial (Topas) optical parametric generator (OPG; details to be published later). In one more experiment, we used the 267 nm for probing after pumping at 292 nm. This wavelength was synthesized by addition of the idler from the OPG to the Ti-sapphire frequency and subsequent frequency doubling.

All experiments were performed with 7-dehydrocholesterol (Sigma-Chemie) dissolved in UV grade ethanol (for some experiments, methanol) at a concentration of 160 mg/dm^3 (0.42 mmol/dm^3). The samples were kept under a nitrogen atmosphere to avoid oxidation. The solution was exposed to the laser radiation in a reaction cell of 500 μm length with UV grade CaF_2 windows. The transverse flow of the solution was sufficient to replace the irradiated volume between subsequent laser pulses. The reaction cell was thermally isolated by a surrounding vacuum chamber with CaF_2 windows. The temperature of the solution was varied from 217 to 323 K with an accuracy of ±2 K during a measuring period. For determination of the temperature a Ni−CrNi thermocouple was placed in the main stream directly after the cell.

Part of the experimental system is depicted schematically in Figure 2. Intense femtosecond pulses of tunable wavelength between 780 and 830 nm were generated in a laser system consisting of an argon ion laser pumped Ti-sapphire oscillator (Spectra Physics) and a Q-switched Nd:YLF laser pumped Ti-sapphire amplifier (Quantronix) with grating stretcher and compressor unit. The amplified pulses generated at a repetition rate of 1 kHz had in this spectral region an energy of up to 800 μJ and a pulse duration of 100 fs (from autocorrelation measurements). The transverse mode profile—checked by a CCD camera—was close to Gaussian.

For the initiation of the photochemical reaction and the study of the transient absorption of dehydrocholesterol and its

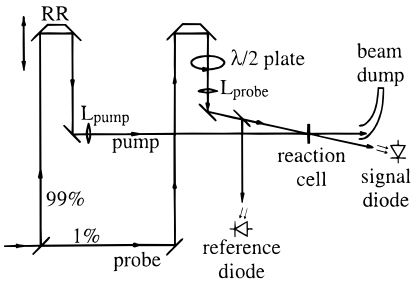

Figure 2. Schematic of the experimental system. RR: variable delay line with retroreflector.

products, ultrashort UV pulses were employed. These pulses were generated from the laser pulses described above by frequency doubling and tripling, using a commercial unit (CSK). This system—consisting of a 1.5 mm lithium borate (LBO) crystal for frequency doubling and a 1 mm barium β-borate (BBO) crystal for the generation of the third harmonic—provided UV pulses from 260 to 277 nm by tuning the oscillator and the compressor−stretcher unit. The pulse duration was 300 fs, and more than 10% of the energy of the fundamental wavelength was converted to the UV.

A beam splitter was inserted after the tripler unit. The reflected beam (99%) served for pumping, the transmitted beam (1%), for probing. After passing separate delay units, the pulses were focused by two lenses: L_{pump} (focal length 100 cm) and L_{probe} (50 cm). The beams overlapped in the sample with an intersection angle of 50 mrad. The foci had an almost perfect Gaussian transversal shape with 100 μm diameter for the pump and 50 μm for the probe pulse. The average energy of the pump pulses was about 3 μJ in front of the cell.

The delay unit of the pump beam was controlled by a stepper motor with a personal computer, and a retroreflector (RR) was used for high pointing stability. The delay zero and the pulse duration were determined by recording the correlation function between pump and probe pulses.

The direction of the polarization of the probe beam was rotated by a half-wave plate, selected for a small wedge angle.

The energy of the probe pulses was monitored via beam splitters and photodiodes before and after the reaction cell. The diode signals were recorded via integrators and digital lock-in amplifiers. A time constant of 300 ms was chosen to restrict the signal fluctuation to 0.1%. All data were stored by the computer.

3. Results

We report two series of measurements of the electrocyclic ring opening: (1) the transient absorption kinetics of dehydrocholesterol after excitation at room temperature and (2) its temperature dependence.

(1) Transient Absorption at Room Temperature. The absorption spectrum of dehydrocholesterol (D) dissolved in ethanol[22,23] is presented in Figure 3 (solid line). The strong absorption band between 250 and 310 nm, which shows some vibrational structure, is due to the $\pi\pi^*$ transition ($1A_1 \rightarrow 1B_2$) of D. We used pump pulses at three different wavelengths (262, 267, 272 nm) to excite dehydrocholesterol to this state.

A typical trace of the time evolution of the transient absorption $\Delta A = -\ln(T/T_0)$ after excitation, probed by a weak delayed pulse—at the same wavelength as the pump pulse—is shown in Figure 4 for a wavelength of 262 nm (filled circles; T, T_0, transmission of the sample with and without excitation). The polarization of the probe beam relative to the pump beam encloses the ("magic") angle of 54.7° to eliminate the influence of orientational relaxation on the observed transients.[24] We

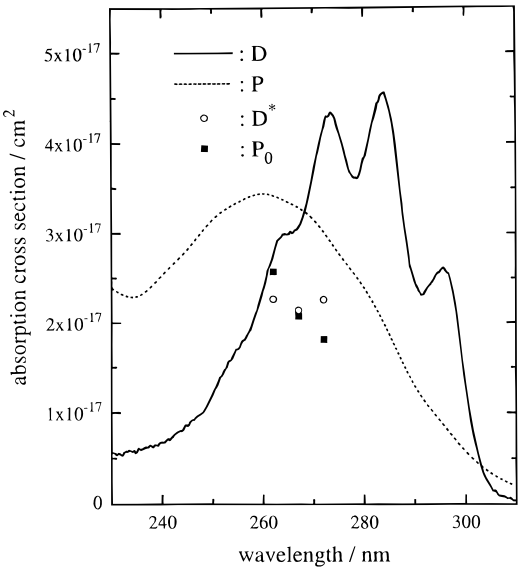

Figure 3. Ultraviolet absorption spectra[22,23] of 7-dehydrocholesterol (D, solid curve) and previtamin D (P, dashed curve) in ethanol at room temperature. Preliminary values for absorption cross sections of excited D (D*) and for the primary conformer of P (P_0) are also indicated.

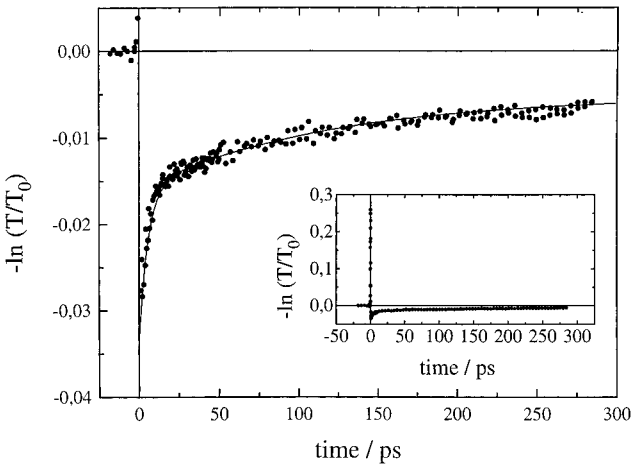

Figure 4. Time-resolved transient absorption of dehydrocholesterol (0.4 mmol/dm^3) in ethanol at 262 nm after excitation at the same wavelength. The change of absorption $\Delta A = -\ln(T/T_0)$ is plotted versus the delay time between the femtosecond excitation and the probe pulse (points; T_0 and T are the transmissions of the sample before and after excitation, respectively). A fast and a slow recovery component are readily seen. The solid line is the best fit of a doubly exponential form (eq 1). The total absorption change of the sample including the two-photon absorption in the solvent is depicted in the inset.

estimate that the pump pulse promotes approximately 40% of the molecules in the irradiated volume to the electronically excited state. Within the correlation width of pump and probe beam, there is a rapid transient absorption peak (see inset of Figure 4), which allows us to determine the width of our UV pulses. This peak, also observed in pure ethanol at all three wavelengths, can be attributed to a two-photon absorption primarily in the solvent and (to a small extent and with opposite sign) to a degenerate four-wave mixing process in the CaF_2 windows.[25] The two-photon absorption in ethanol also results in a small residual increase of the absorption, which lasts longer than the effects we are interested in. We subtracted it from the measured absorptions.

In Figure 4 the excitation of dehydrocholesterol results in a maximum absorption decrease of 3%. The following recovery of ΔA shows a double-exponential behavior. ΔA increases at first with a time constant $t_1 = 5.2 \pm 0.5$ ps and then more slowly

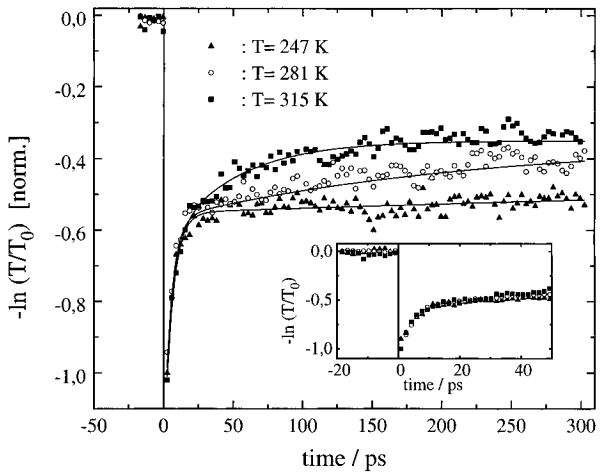

Figure 5. Picosecond absorption change ΔA (normalized to 1 at time 0) of dehydrocholesterol in ethanol (0.4 mmol/dm^3) at 272 nm after excitation at the same wavelength for three different temperatures $T =$ 247 (triangles), 281 (circles), and 315 K (squares). The solid lines are doubly exponential fits. The inset shows that the fast recovery time is temperature independent. The second (slow) decay time is clearly temperature dependent.

with $t_2 = 125 \pm 20$ ps. The absorption does not completely restore even at long delay times. Similar time evolutions of the transient absorption were observed at 267 and 272 nm, indicating that the time constants are independent of the specific wavelength within the experimental accuracy. This was also supported by tuning the probe wavelength to 304, 306, or 316 nm, after pumping at 267 nm. In this case, we observed a transient increase of absorption, since dehydrocholesterol does not noticeably absorb beyond 300 nm. So the longer wavelength component exhibits the same temporal behavior, and for example, the 5.2 ps exponential is not the result of a superposition of several decays with similar, but different, lifetimes. The same time constants were also observed when the excitation was at 292 nm (near the 0−0 transition of the dehydrocholesterol band) and the probe at 267 nm. This indicates that the rate constants do not depend on the initial vibrational excess energy.

Orientational relaxation cannot be seen in the applied, "magic" orientation of pump and probe polarizations and can be ignored under these conditions. Using the method of ref 24, we deduced from the difference of the transient absorptions with parallel and perpendicular polarization (pump versus probe) an orientational relaxation time of 240 ± 60 ps for dehydrocholesterol dissolved in ethanol. This value is in the same range as for molecules with a comparable size, e.g. rhodamine 6G.[26,27]

(2) The Effect of Temperature on the Transient Dynamics. The transient absorption at different temperatures between 217 and 323 K was measured at the pump and probe wavelength 272 nm. Figure 5 presents the results for three different temperatures. The normalized absorption change is plotted versus the delay time between pump and probe pulses. All measurements show a sudden change of absorption at $t = 0$ ps. The following increase is found to be double exponential. The first time constant is 5.2 ± 0.5 ps at all temperatures. This is clearly shown in the magnified inset of Figure 5.

Quite different is the temperature dependence of the second time constant t_2 of the double exponential decay. In Figure 5, the first rapid decrease of the transient absorption change is seen to be followed by a slower one, whose time constant varies between 60 ps at 315 K and 1300 ps at 247 K.

4. Discussion

First we will consider here which species can contribute to the observed transient absorption (i.e. a kinetic model) and then present some further evidence for our assignment. After that, we will discuss the temperature dependence of the observed rates.

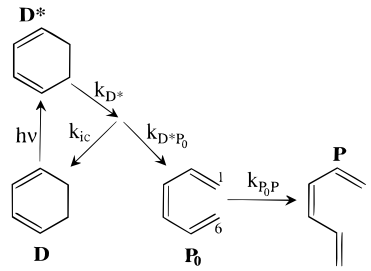

Figure 6. Suggested reaction steps of the photochemical ring opening of dehydrocholesterol after UV excitation. The figure shows the reaction center only.

(1) Kinetic Model. The experimental time-dependent absorption change $\Delta A(t)$ can be well fitted by

$$\Delta A(t) = A_0 + A_1 \exp(-t/t_1) + A_2 \exp(-t/t_2) \qquad (1)$$

This indicates that there are at least two consecutive reactions. It is suggestive to assume the first step to be the photoinduced ring opening of dehydrocholesterol (D) to the s-cis,*Z*,s-cis conformer of previtamin D (P_0), which then in a second step rearranges thermally to the s-trans,*Z*,s-cis conformer (P). Figure 6 summarizes in more detail the relevant reaction steps. Dehydrocholesterol is excited by the pump pulse from the ground state D to the electronically excited state D*, whose nature will be discussed in section 5. This excited molecule can undergo either the ring opening (rate constant $k_{D^*P_0}$) or an internal conversion (rate constant k_{ic}) to the ground state. The sum of the two rate constants is k_{D^*}, the rate constant of the overall depopulation of D* (see Figure 6). The primary product P_0 then converts to the final product P by a cis−trans isomerization around a single bond with a rate constant k_{P_0P}. After photoexcitation all the time-dependent population densities of the four involved species D, D*, P_0, and P can contribute to the absorption. The solution of the rate equations results in a time dependence of the form of eq 1 with

$$t_1^{-1} = k_{D^*},\ t_2^{-1} = k_{P_0P} \qquad (2)$$

In the latter equation, an additive term k_{PP_0} for the back reaction has been neglected. At all investigated wavelengths and wavelength combinations we found $t_1 = 5.2 \pm 0.5$ ps for the ring opening (depopulation of D*, appearance of P) and $t_2 = 125 \pm 20$ ps for the single-bond cis−trans isomerization at room temperature.

In the quantitative evaluation of the amplitudes A_i in eq 1 we have assumed that $k_{D^*P_0}/k_{ic} = 0.34/0.66 = 0.52$. (0.34 is the reported quantum yield for the reaction).[28,29] We checked whether this value is unchanged also for femtosecond excitation: We determined the number of product molecules P by measuring the UV spectra before and after irradiating under the same conditions as in the pump−probe experiment; comparing this value with the number of absorbed photons calculated from the cross section, we found for this ratio 0.39−0.47, in reasonable agreement with the literature value. In the spectral region between 240 and 350 nm, we have not observed any other products, e.g. those resulting from photoexcitation of P_0 or P (such as tachysterol). However, more D molecules were consumed in this experiment than P molecules produced. This has already previously been observed at high intensity,[30] and the corresponding product has been identified as toxisterol B, resulting from the addition of a solvent molecule to the double

bonds; it absorbs only at shorter wavelengths.[30] This reaction can be attributed to two-photon absorption[30] (although this has also been doubted[31]), giving rise to radical cations, which can add a solvent molecule.[32]

The radical cations themselves might, however, have a UV absorption with a time dependence of its own. The reactions of such species, such as ring opening[33−35] or addition of the solvent, would be ground state reactions (unless a third photon is absorbed). They will be associated with an activation energy and therefore be slow (nanoseconds or longer). So they will not have any influence on the observed time constants. In fact, when we varied the pump intensity by a factor of 2, we did not observe any change of rates. However, the radical cations lead to an uncertainty in the evaluation of the absorption cross section of D* and P_0, which are contained in the amplitudes A_1 and A_2 in eq 1. Thus, our values (e.g. at 267 nm $\sigma(D^*) = 2.1 \times 10^{-17}$ cm^2 and $\sigma(P_0) = 1.7 \times 10^{-17}$ cm^2) are only preliminary.

(2) Evidence for the Assignment. Although this model appears plausible, further evidence for the assignment would be desirable, since other processes with similar time constants are conceivable. For example, the vibrational cooling will occur in a few picoseconds.[36] Note that around 267 nm we do not excite the 0−0 transition and that the electronic energy is converted to nuclear motion during the reaction. On the other hand, with excitation at 292 nm (near the 0−0 transition) t_1 was again measured as 5 ± 1 ps. The strongest support that the first time constant is due to the ring opening comes from two independent methods, which found similar values for this process.

(i) By investigating the yield ratio of the product P to the secondary product, tachysterol (T), (produced by excitation of P_0 or P) as a function of the pulse duration, we found previously a time constant of 4.3 ± 0.5 ps.[2] This number depends on the values for the quantum yields of the photoreactions $P_0 \rightarrow T$ and $P \rightarrow T$. Only the latter one is known.[28] If the former is assumed to be slightly smaller, the agreement would be even better.

(ii) Time-resolved resonance Raman studies of the electrocyclic ring openings in 1,3-cyclohexadiene and its 2-methyl-5-isopropyl derivative gave time constants of 6 and 11 ps, respectively.[4,5]

For the second step, the orientational relaxation has already been excluded as an alternative, since it cannot be observed at the magic angle. The assignment as a thermal cis−trans isomerization is also confirmed by the dependence on the temperature and the viscosity of the solvent (see below).

(3) Temperature Dependence of t_1. According to transition state theory, a unimolecular rate constant k can be written as a function of the enthalpy ΔH_a and entropy ΔS_a of activation:

$$k = A \exp(-\Delta H_a/k_B T) \quad (3)$$

$$A = (k_B T/h) \exp(\Delta S_a/k_B) \quad (4)$$

where k_B and h are the Boltzmann and Planck constant, respectively. At $T = 295$ K, the first factor in eq 4 equals 6×10^{12} s^{-1}. There is no obvious reason why the activation entropy of the ring opening should be large. (An example where it would be large and negative would be the back reaction: In the transition state, several torsional degrees of freedom must be frozen, which in the open-chain molecule correspond to coordinates of isomerization to other conformers.) From the temperature independence of k (Figure 5) and from our limit of accuracy, we could conclude that $\Delta H_a < 1$ kJ/mol, a very small value. So k should be equal to $k_B T/h$. However, according to our results, it is 30 times smaller. What can be the reason?

A first consideration is that there might be a barrier, but that the molecules might not be in thermal equilibrium with the solvent, so that its temperature would have a negligible influence only. The vibrational cooling can take several picoseconds.[36] The initial excitation at 267 nm is more than 4100 cm^{-1} ≈ 50 kJ/mol above the vibrational band origin (probably near 300 nm), so that a small initial barrier could be overcome. Therefore, we employed in one experiment a pump wavelength of 292 nm, which leaves a vibrational excess energy of only about 900 cm^{-1} in D*. But the time constants did not change. Another source of vibrational excess energy might be the fact that, during the movement on the potential surface $2A_1$, electronic energy is converted to nuclear motion (vibration). So a late barrier could be overcome, because the molecule might be internally hot (as in cyclohexadiene[5,6]), independent of the temperature of the surroundings. This possibility seems to infer that we cannot exclude a late barrier. A barrier of 4 kJ/mol has been predicted just before the conical intersection.[15] A certain excess energy might, however, be unimportant for another reason: The redistribution of vibrational energy within the molecule can be much faster than the cooling. According to ref 37, it requires between 200 and 500 fs. Even with an excess energy of 100 kJ/mol (the calculated energy release before the conical intersection[15]), for each vibrational degree of freedom there would be less than 1 kJ/mol (or a correspondingly small temperature rise), not sufficient to climb a barrier. So a late barrier of much larger magnitude is not probable.

Furthermore, the vibrational excess energy cannot explain the slowness of the rate. According to the RRKM theory, much above a barrier the reaction rate would be equal to the frequency factor A, which is identical to the preexponential factor A in eq 3.

So we prefer to assume an activation entropy ($\Delta S_a \approx -3.4 k_B$), in spite of the preliminary consideration above. Celani et al. calculated $\Delta S_a \approx -2 k_B$ for the passage through an activated state immediately before the conical intersection.[15] A negative entropy of activation means that some vibrations, which initially are thermally excited, are not excited anymore in the activated state, because their frequency has increased. An equivalent view is that the exit channel is very narrow. The exit valley is generated by the electronic stabilization due to the Woodward−Hoffmann principle. So this stabilization seems to be very sensitive to small distortions of the geometry.

(4) t_2 and Its Temperature Dependence. In Figure 4 after the rapid ring-opening reaction a slower process is observed with a time constant of 125 ± 20 ps. We attributed it to the thermal s-cis → s-trans isomerization $P_0 \rightarrow P$. This time dependence allows for the first time a ready distinction between the two molecular conformers P_0 and P. This is in contrast to cyclohexadiene, where the cooling rate appeared to be not fast enough to prevent an immediate (7 ps) isomerization of the initially produced conformer.[5,6] It has been suggested[5] that the cooling rate during a reaction is fast if the moving parts of the molecule experience a strong friction in the solvent (see the following paragraph), as in cis−trans isomerization of stilbenes and certainly also in our example, and is slower if the friction is weaker, as in the cyclohexadiene ring opening. As another reason for the rapid conformational isomerization in the unsubstituted system, Reid et al.[6] suggested steric hindrance in the starting molecule, which would reduce the energy of activation. However, the slower reaction in our more highly substituted system (as well as the activation energy; see below) rules out this interpretation.

To verify that this reaction is an isomerization around a single bond as sketched in Figure 6, we measured the dependence of

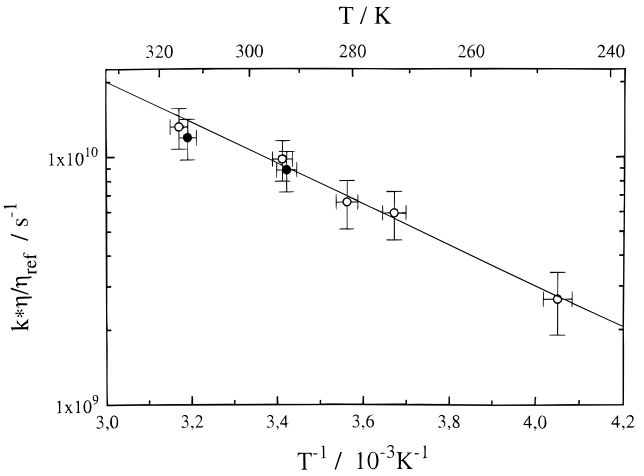

Figure 7. Plot of $\ln(k\eta_T/\eta_{ref})$ versus T^{-1} for the conformational isomerization $P_0 \rightarrow P$ in ethanol (open circles) and methanol (closed circles). $\eta_{ref} = 1.1$ mPa s (viscosity of ethanol at 295 K). The solid line is the best fit of the form of eq 5.

t_2 on the solvent viscosity and on the temperature. The latter gives information about the activation energy, which can be compared to similar isomerizations, whereas the dependence on the viscosity η is typical for reactions in which large parts of the molecule move within the solvent with friction.[38,39] From studies in alcoholic solvents, the rate constant k of isomerization reactions was found to be proportional to η^{-a} with $0 \leq a \leq 1$.[38,40] $a = 1$ corresponds to the strong coupling case between the solute and the solvent molecules, which is typical if the moving parts of the solute are bulky. Including the temperature dependence, the rate constant of the isomerization reaction can then be described by an Arrhenius-type expression:[38]

$$k = (\eta_{295K}/\eta_T)A \exp(-\Delta H_a/k_a T) \qquad (5)$$

where η_T is the temperature dependent solvent viscosity, A is the Arrhenius preexponential factor, and ΔH_a is the thermal intramolecular activation enthalpy for the isomerization reaction.

Transient absorption measurements of dehydrocholesterol were carried out at different temperatures ranging between 247 and 315 K. Two different alcohols, ethanol and methanol, served as solvent. Figure 5 shows three examples of the measurements in ethanol. The isomerization rate of P_0 varied by a factor of 20 in this range. According to eq 5, we plotted in Figure 7 $\ln(k\eta_T/\eta_{ref})$ versus T^{-1}, where η_{ref} is η_{295K} for ethanol. η_T was taken from ref 41. At room temperature, methanol has half the viscosity of ethanol. Obviously at all temperatures, the rates in the two solvents lie on the same line. So $k \propto \eta^{-1}$. This is just the strong coupling case, which is expected for such an isomerization.

From the slope in Figure 7 we can derive an internal activation energy of $\Delta H_a = 15.5 \pm 1$ kJ/mol. This value is in good agreement with barriers for isomerizations about single bonds in polyenes, which are on the order of 16 kJ/mol.[42,43] A molecular mechanics calculation of the previtamin D conformers found about 33 kJ/mol;[44] this deviation is still acceptable for such a calculation. The preexponential factor A equals $(6 \pm 2) \times 10^{12}$ s^{-1} in ethanol. This is just equal to $k_B T/h$. That is, according to eq 4, $\Delta S_a = 0$, not unexpected for such a reaction.

In summary, the temperature and viscosity dependence of t_2 strongly supports our assignment that this process is the isomerization $P_0 \rightarrow P$ (Figure 6).

5. Summary and Concluding Remarks

We found that the electrocyclic ring-opening reaction of dehydrocholesterol (D) to previtamin D (P) can clearly be divided into two consecutive steps, the ring opening itself and the rearrangement from the primary conformer (P_0) to the stable one (P). P_0, whose geometry is still ringlike, has repeatedly been postulated as the precursor for the photochemical ring closure of previtamin D and explains the wavelength dependence of this reaction (see, for example, refs 45 and 46; for another interpretation see ref 47. The conformational isomerization proceeds in the electronic ground state over a barrier of 15.5 kJ/mol, with a time constant of 125 ps in ethanol at room temperature. This is in contrast to the analogous reaction of 1,3-cyclohexadiene, where the energy released in the photochemical ring opening carries the molecule almost without delay (time constant 7 ps, i.e. similar to the ring opening; cooling time 9 ps) over the barrier to the final conformer of hexatriene.[5,6] Obviously, in the D/P system, the cooling is much faster than 9 ps. For interpreting this difference, one should notice that the moving molecular parts in the s-cis → s-trans isomerization $P_0 \rightarrow P$ are much larger than in the unsubstituted hexatriene, so that the coupling to the solvent will be stronger.

During the first step, the ring opening and the crossover from the excited potential surface to the ground state surface occur in 5.2 ps. This time constant is temperature independent. It was also unchanged when the excitation was near the band origin, so that there was nearly no excess vibrational energy. So one can infer that there is no barrier higher than 1 kJ/mol for the ring opening. It is worth noting that the photochemical electrocyclic ring closure of s-cis dienes to cyclobutenes has been observed in a matrix at 15 K.[48] So this reaction also seems to have no activation energy. The barrierless ring opening is in contrast to the expectation on the basis of a qualitative consideration and to the early quantum mechanical calculation,[14] whereas the deviation from ref 15 is only minor. A more serious contrast, however, exists with the fluorescence, which can be observed at 77 K in a matrix,[2] but not at room temperature. So there seems to be a barrier which makes the radiationless depopulation of the initially excited state at 77 K slow enough that the fluorescence (time constant in the nanosecond range) can compete with it, at least in the matrix. We believe that an additional barrier is generated by the matrix, which can prevent the movement or large amplitude vibrations of bulky groups. The fluorescence requires such an effect near the Franck−Condon region, where all atoms have experienced only very little displacement. The associated activation volumes can be estimated to several cm^3/mol, which would yield a reasonable matrix-induced barrier of several kJ/mol, if the compressibility of the matrix is around 1 GPa^{-1}.

Other barrierless processes in photochemistry are some $Z \rightarrow E$ (or cis → trans) isomerizations. For example, octatetraene can isomerize at 4.2 K in a hexane matrix.[49] In larger molecules such as stilbenes (see, for example, ref 50) or cyanines (see for example, ref 51), the process is slowed down by the solvent viscosity.

The fluorescence spectrum of 7-dehydrocholesterol overlaps with the absorption spectrum, whereas in lumisterol (which has only a slightly different geometry) there is a small gap between the two.[21] As argued in ref 10, this means that in the latter the $1B_2$ state relaxes to the slightly lower lying $2A_1$ state before emission, whereas in the dehydrocholesterol the $2A_1$ state seems to lie higher than the $1B_2$ state (in the Franck−Condon region). Nevertheless, the relaxation $1B_2 \rightarrow 2A_1$ will not be a bottleneck for the reaction, which is assumed to proceed initially on the 2A surface. The crossover to the ground state surface 1A in the region of the conical intersection will also be extremely fast.[15] If there is no electronic bottleneck, one expects for energies above a barrier (or if there is no barrier) a rate of $k_B T/h$

$= 6 \times 10^{12}\ s^{-1}$, unless there is an activation entropy ΔS_a. Since the observed ring opening is 30 times slower, there must be a ΔS_a of about $-3.4k_B$. A negative ΔS_a means that the electrocyclic reaction with its aromatic transition state can easily be perturbed by some thermally excited vibrations.

Surprisingly, this value of ΔS_a is about the same as for the (thermal) ring closure of hexatriene to cyclohexadiene and derivatives,[52−54] although due to free internal rotation in the open-chain compound, one expects for the ring closure much more change of order than for the ring opening. ΔS_a is slightly more negative than calculated by Celani and co-workers[15] for a place on the surface, before the molecule turns to the conical intersection.

An experimental hint that the (entropy) bottleneck for the reaction is not close to the starting point of the reaction along the $2A_1$ potential surface, but close to a much lower minimum, comes from the very low fluorescence yield at room temperature (2×10^{-6} for cyclohexadiene[20]): If the molecule would remain in the Franck−Condon region for 5 ps, either in the $1B_2$ state (radiative lifetime 6 ns[20]) or in the $2A_1$ state (radiative lifetime about 15−55 times longer, depending on the geometry[14]), it should fluoresce with a quantum yield of 10^{-3} to 2×10^{-5}. If it, however, proceeds rapidly (e.g. in 10 fs) to a much lower energy state on the potential surface, as suggested by the calculations,[15] then the low quantum yield of UV fluorescence is understandable. So probably, the molecule moves in a very short time on a purely repulsive surface to the pericyclic minimum, from where it proceeds within 5.2 ps through an entropic bottleneck (probably again without barrier) down to a conical intersection and from there to the product P_0 or the educt D.

In any case, the D* observed by us by excited state absorption corresponds to a state with a lifetime of 5.2 ps. This state would be ionized by a UV photon if its energy is still close to the $1B_2$ or $2A_1$ state; but if it is much lower in energy (e.g. near the pericyclic minimum, as suggested above), the UV photon would not be sufficient for ionization. Since the spectral dependence of σ(D*) is probably different for the two cases, extending the measurements of σ(D*) can probably distinguish between them and thus help to locate the reaction bottleneck.

Whereas the ring opening is slow compared to the expectation for a barrierless unimolecular process, it is worth noting that it is much faster than any bimolecular reaction, caused by collisions between two solute molecules. Nevertheless, photodimers have been observed for dehydrocholesterol as well as for cyclohexadiene, in particular with excitation at long wavelengths and at high concentrations (40 mmol/dm^3).[45,55] In the products, the ring has not been opened. The obvious explanation is that the photodimers are formed by excitation of van der Waals dimers, which will be in equilibrium with the monomers at higher concentrations.

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References and Notes

(1) Woodward, R. B.; Hoffmann, R. *The Conservation of Orbital Symmetry*, 3rd ed.; Verlag Chemie: Weinheim, 1981.
(2) Gottfried, N.; Kaiser, W.; Braun, M.; Fuss, W.; Kompa, K. L. *Chem. Phys. Lett*. **1984**, *110*, 335.
(3) Trulson, M. O.; Dollinger, G. D.; Mathies, R. A. *J. Am. Chem. Soc*. **1987**, *109*, 587.
(4) Reid, P. J.; Doig, S. J.; Mathies, R. A. *Chem. Phys. Lett*. **1989**, *156*, 163.
(5) Reid, P. J.; Doig, S. J.; Wickham, S. D.; Mathies, R. A. *J. Am. Chem. Soc*. **1993**, *115*, 4754.
(6) Reid, P. J.; Lawless, M. K.; Wickham, S. D.; Mathies, R. A. *J. Phys. Chem*. **1994**, *98*, 5597.
(7) MacDiarmid, R.; Sabljić, A.; Doering, J. P. *J. Chem. Phys*. **1985**, *83*, 2147.
(8) Kohler, B. E. *J. Chem. Phys*. **1990**, *93*, 5838.
(9) MacDiarmid, R. *Int. J. Quantum Chem*. **1986**, *29*, 875.
(10) Hudson, B. S.; Kohler, B. E.; Schulten, K. In *Excited States*; Lim, K., Ed.; Academic Press: New York, 1982; Vol. 6, p 1.
(11) Buma, W. J.; Kohler, B. E.; Song, K. *J. Chem. Phys.* **1990**, *92*, 4622.
(12) Buma, W. J.; Kohler, B. E.; Song, K. *J. Chem. Phys*. **1991**, *94*, 4691.
(13) Klessinger, M.; Michl, J. *Excited States and Photochemistry of Organic Molecules*; Verlag Chemie: Weinheim, 1995.
(14) Share, P. E.; Kompa, K. L.; Peyerimhoff, S. D.; van Hemert, M. C. *Chem. Phys*. **1988**, *120*, 411.
(15) Celani, P.; Ottani, S.; Olivucci, M.; Bernardi, F.; Robb, M. A. *J. Am. Chem. Soc*. **1994**, *116*, 10141.
(16) Bernardi, F.; De, S.; Olivucci, M.; Robb, M. A. *J. Am. Chem. Soc*. **1990**, *112*, 1737.
(17) Bernardi, F.; Olivucci, M.; Ragazos, I. N.; Robb, M. A. *J. Am. Chem. Soc*. **1992**, *114*, 8211.
(18) Klessinger, M. *Angew. Chem*. **1995**, *107*, 597.
(19) Manthe, U.; Köppel, H. *J. Chem. Phys*. **1990**, *93*, 1659.
(20) Trulson, M. O.; Dollinger, G. D.; Mathies, R. A. *J. Chem. Phys.* **1989**, *90*, 4274.
(21) Havinga, E.; de Kock, R. J.; Rappoldt, M. P. *Tetrahedron* **1960**, *11*, 276.
(22) Sternberg, J. C.; Stillo, H. S.; Schwendeman R. H. *Anal. Chem*. **1960**, *32*, 84.
(23) Gliesing, S.; Reichenbächer, M.; Ilge, H.-D.; Fassler, D. *J. Prakt. Chem*. **1987**, *329*, 311.
(24) Lessing, H. E.; Jena, A. v. *Chem. Phys. Lett*. **1976**, *42*, 213.
(25) Laubereau, A. In *Ultrashort Laser Pulses*; Kaiser, W., Ed.; Springer: Berlin, 1988.
(26) Fleming, G. R.; Morris, J. M.; Robinson, G. W. *Chem. Phys*. **1976**, *17*, 91.
(27) Myers, A. B.; Hochstrasser; R. M. *IEEE J. Quantum Electr*. **1986**, *QE-22*, 1482.
(28) Gliesing, S.; Reichenbächer, M.; Ilge, H.-D.; Fassler, D. *Z. Chem*. **1989**, *29*, 21.
(29) Jacobs, H. J. C.; Gielen, J. W. J.; Havinga, E. *Tetrahedron Lett*. **1981**, *22*, 4013.
(30) Bogoslowskii, H. A.; Berik, I. K.; Gundorov, S. I.; Terenetskaya, I. P. *High Energy Chem*. **1989**, *23*, 218.
(31) Terenetskaya, I. P.; Gundorov, S. I.; Kravchenko, V. I.; Berik, I. K. *Sov. J. Quantum Electron*. **1988**, *18*, 1323.
(32) Orlov, A. I.; Mikhailova, N. P.; V'yunov, K. A. *Khim. Prirod. Soedin*. **1989**, 225.
(33) Shida, T.; Egawa, Y.; Kubodera, H.; Kato, T. *J. Chem. Phys*. **1980**, *73*, 5963.
(34) Shida, T.; Kato, T.; Nosaka, Y. *J. Phys. Chem*. **1977**, *81*, 1095.
(35) Bondybey, V. E.; English, J. H.; Miller, T. A. *J. Mol. Spectrosc*. **1980**, *80*, 200.
(36) Seilmeier, A.; Kaiser, W. In *Ultrashort Laser Pulses*; Kaiser, W., Ed.; Springer: Berlin, 1988.
(37) Weiner, A. M.; Ippen, E. P. *Chem. Phys. Lett*. **1985**, *116*, 656.
(38) Keery, K. M.; Fleming, G. R. *Chem. Phys. Lett*. **1982**, *93*, 322.
(39) Hicks, J. M.; Vandersall, M. T.; Sitzmann, E. V.; Eisenthal, K. B. *Chem. Phys. Lett*. **1987**, *135*, 413.
(40) Nikowa, L.; Schwarzer, D.; Troe, J.; Schroeder, J. *J. Chem. Phys*. **1992**, *97*, 4827.
(41) Lide, D. R., Ed. *Handbook of Chemistry and Physics*, 74th ed.; CRC Press: Boca Raton, 1993.
(42) Ackermann, J. R.; Kohler, B. E. *J. Chem. Phys*. **1984**, *80*, 45.
(43) Carreira, L. A. *J. Chem. Phys.* **1975**, *62*, 3851.
(44) Dauben, W. G.; Funhoff, D. J. H. *J. Org. Chem*. **1988**, *53*, 5070.
(45) Jacobs, H. J. C.; Havinga, E. *Adv. Photochem*. **1979**, *11*, 305.
(46) Pfoertner, K. *Helv. Chim. Acta* **1972**, *55*, 937.
(47) Dauben, W. G.; Disanayaka, B.; Funhoff, D. J. H.; Kohler, B. E.; Schilke, D. E.; Zhou, B. *J. Am. Chem. Soc*. **1991**, *113*, 8367.
(48) Squillacote, M.; Semple, T. C. *J. Am. Chem. Soc*. **1990**, *112*, 5546.
(49) Granville, M. F.; Holtom, G. R.; Kohler, B. E. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 31.
(50) Waldeck, D. H. *Chem. Rev.* **1991**, *91*, 415.
(51) Åberg, U.; Åkesson, E.; Alvarez, J.-L.; Fedchenia, I.; Sundström, V. *Chem. Phys*. **1994**, *183*, 269.
(52) Marwell, E. N.; Caple, G.; Schatz, B.; Pipin, W. *Tetrahedron* **1973**, *29*, 3781.
(53) Gaasbeek, C. J.; Hogeveen, H.; Volger, H. C. *Recl. Trav. Chim. Pays-Bas* **1972**, *91*, 821.
(54) Lewis, K. E.; Steiner, H. *J. Chem. Soc*. **1964**, 3080.
(55) Havinga, E. *Experientia* **1973**, *29*, 1181.

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