extrema in $\Delta C_p^{\ddagger}$ for these reactions might be,[12] our positive heat capacities of activation at $n_{H_2O} = 0.98$ do accord with the occurrence of strong hydrophobic interaction between the substrate and 2-BE.[13] It is well known that hydrophobic hydration of relatively nonpolar solutes like **1** and **2** in water is accompanied by large and positive heat capacities.[2,3,14,15] Hence, hydrophobic interaction, implying destructive overlap of the hydrophobic hydration envelopes of the solute and the cosolvent,[16] will give rise to negative heat capacities. This effect will be much stronger for the nonpolar initial state than for the polar transition state. In TA solutions of extreme heterogeneity, like 2-BE–H_2O, the overall result will be a positive maximum in $\Delta C_p^{\ddagger}$ at the solvent composition of maximum hydrophobic hydration ($n_{H_2O} = 0.98$). In addition, the maximum in $\Delta C_p^{\ddagger}$ will be most pronounced for the most hydrophobic substrate (**2**) as is borne out by experiment. Interestingly, strong $\Delta H^{\ddagger}$–$\Delta S^{\ddagger}$ compensation is also found for the hydroxide-ion catalyzed reaction of **2** in 2-BE–H_2O (stopped-flow technique; $n_{H_2O} = 1.00$, $\Delta H^{\ddagger} = 5.4$ kcal mol^{-1}, $\Delta S^{\ddagger} = -25$ eu; $n_{H_2O} = 0.98$, $\Delta H^{\ddagger} = 0.3$ kcal mol^{-1}, $\Delta S^{\ddagger} = -46$ eu; $n_{H_2O} = 0.95$, $\Delta H^{\ddagger} = 3.4$ kcal mol^{-1}, $\Delta S^{\ddagger} = -38$ eu). However, within the limits of experimental error a linear Eyring plot is obtained at $n_{H_2O} = 0.98$. This difference with the water-catalyzed process presumably reflects the smaller differences in hydrophobicity between initial state and transition state for the hydroxide-ion catalyzed reaction.

In conclusion, the present results either demonstrate an unprecedented solvent effect on $\Delta C_p^{\ddagger}$ for the neutral hydrolysis of **1** and **2** in a TA solution or may well demand an improved interpretation of the changes in $\Delta C_p^{\ddagger}$ found previously for solvolytic reactions of neutral substrates in water-rich TA mixtures. We are pursuing further kinetic studies to distinguish between these possibilities.

Supplementary Material Available: Pseudo-first-order rate constants for the neutral hydrolysis of **1** and **2** in *t*-BuOH–H_2O and 2-*n*-butoxyethanol–H_2O as a function of solvent composition and at a series of temperatures (Table II) and Gibbs free energies, enthalpies, and entropies of activation for the neutral hydrolysis of **1** and **2** in *t*-BuOH–H_2O in the range $n_{H_2O} = 0.925$–1.000 (Table III) (4 pages). Ordering information is given on any current masthead page.

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(9) At higher temperatures, rates are higher than expected on the basis of an Eyring plot of kinetic data obtained at lower temperatures, despite the increase of $\Delta H^{\ddagger}$ with temperature. This is inherent in transition-state theory, which can be shown as follows. If θ is a reference temperature and $T > \theta$, then $\Delta G_T^{\ddagger} = \Delta H_T^{\ddagger} - T\Delta S_T^{\ddagger} = \Delta G_\theta^{\ddagger} + \Delta C_p^{\ddagger}(T - \theta) - T\Delta C_p^{\ddagger} \ln (T/\theta)$. Thus, $d(\Delta G_T^{\ddagger})/dT - d(\Delta G_\theta^{\ddagger})/dT = -\Delta C_p^{\ddagger} \ln (T/\theta)$. Owing to the dominant entropy term, the deviation of the Gibbs free energy of activation from that expected on basis of the Eyring equation differs in sign from the temperature dependence of $\Delta H^{\ddagger}$.

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Received February 8, 1980

Formation of Vitamin D_3 in Synthetic Lipid Multibilayers. A Model for Epidermal Photosynthesis

Sir:

Formation of vitamin D_3 (cholecalciferol, **3**) in fluid solution photolysis of 7-dehydrocholesterol (7-DHC, **1**) has been studied in considerable detail.[1] The locus of in vivo photobiogenesis of **3** is the epidermis.[2a,c,3] Several important differences exist between the two systems. The most obvious is that, once the primary photoprocess takes place in epithelial cells, circulation may remove the product to sublayer sites where further absorption of light is precluded. Thus a true photoequilibrium may not occur. As would be expected, the biological reaction is more economic in terms of selectivity and specificity. Holick et al. have shown that previtamin D_3 (precholecalciferol, **2** is essentially the only photoproduct formed from **1** in rat skin epithelial cells.[3] Apparently photochemical ring opening of the provitamin **1** to previtamin D_3 (**2**) and subsequent thermal conversion at body temperature into the vitamin occurs specifically in the skin, while in solution competing photoreactions of close quantum yield lead to photoequilibria involving tachysterol$_3$ (**4**), lumisterol$_3$ (**5**), and more complicated photoisomers[4] as well as solvent addition products[5] (Scheme I).

At least in part the differences between the two systems might share a conformational origin ultimately based upon the ordered milieu of the epidermis vs. the relatively disordered environment of fluid solution. Another important point is that the atmosphere absorbs essentially all radiation below λ 300–310 nm.[6] The absorption maximum of **1** occurs at shorter wavelength, namely, 280 nm. A series of weak absorption bands of the diene which occur around 330 nm (ϵ 50) may account for epidermal photosynthesis, and the mechanism of the ring opening is possibly different from that occurring at shorter wavelengths. Accordingly, as a step toward probing both the medium effect as well as the wavelength variation, the photolysis of **1** at ~280 and 310 nm in the anisotropic environment of synthetic ordered lipid multibilayers as a model for the epidermal photosynthesis was studied.

The first objective was to prepare a series of saturated synthetic lipid multibilayers incorporating 7-dehydrocholesterol (**1**) as a structural component and determine that these

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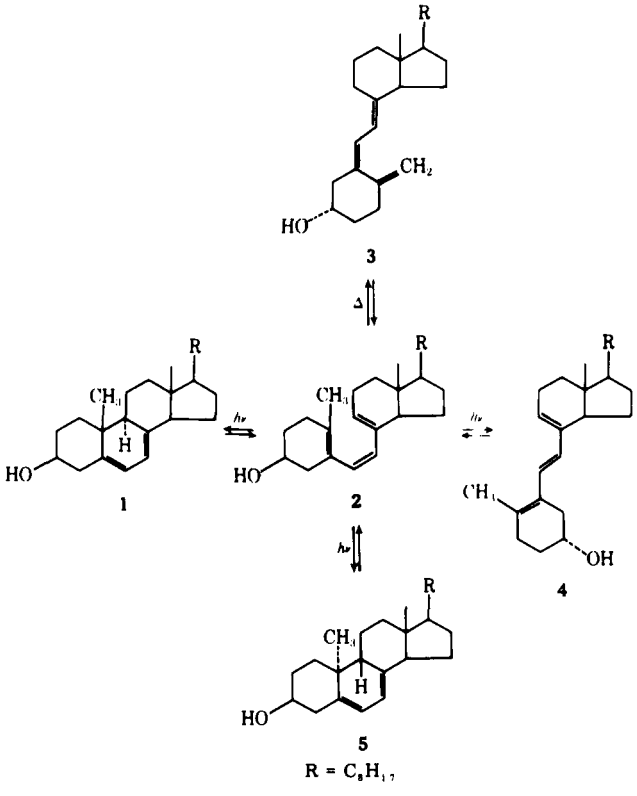
Table I. Photoproducts from Irradiation of 7-Dehydrocholesterol (**1**) (7-DHC) in Ordered Lipid Multibilayers

entry	system	chain length	**2**, %[d]		**3**, %		**5**, %		**4**, %		**1**, %	
			A[b]	B[c]	A	B	A	B	A	B	A	B
1	dilauroyl-L-α-phosphatidylcholine[d]	C_{12}	8.2	6.45	0.6	0.43	2.1	7.9	0.95	2.4	88.1	82.8
2	distearoyl-L-α-phosphatidylcholine	C_{18}	7.0	6.15	0.87	0.97	2.0	6.7	0.9	2.8	89.2	83.3
3	dimyristoyl-L-α-phosphatidylcholine	C_{14}	9.2	6.3	1.9	1.1	2.7	9.1	1.0	1.6	85.2	81.9
4	dipalmitoyl-L-α-phosphatidylcholine	C_{16}	7.2	6.2	1.3	1.3	2.2	8.5	0.6	1.9	88.5	81.9
5	7-DHC thin film[e]		2.7	0	0.87	0	0	0	0	0	96.4	100
6	hexane solution		20.7	21.	4.9	3.3	2.1	8.5	44.9	19.9	27.2	46.4

[a] Analyses were performed by LC using a μ-Porasil column, CH_2Cl_2, 2 mL/min. Internal standards of each photoproduct were used for standardization. Initial separation was carried out by TLC to remove the lipid part. Typically 30–40% of the 1 μmol of sterol is accounted for after photolysis, TLC and LC. [b] Irradiation using a medium-pressure mercury lamp for 15 min through quartz under nitrogen at room temperature. [c] Irradiation as described in note *c* but with a Pyrex filter for 45 min. [d] Each membrane contained 1 μmol (384 μg) of 7-DHC in 20 mol % sterol:80 mol % lipid ratio. The average thickness of the membranes determined by optical microscopy was 1.5×10^{-2} mm. [e] The thin film is formed by evaporation of a chloroform solution of **1** on a glass slide.

Scheme I. The Photochemistry and Thermal Reaction of 7-Dehydrocholesterol (**1**)



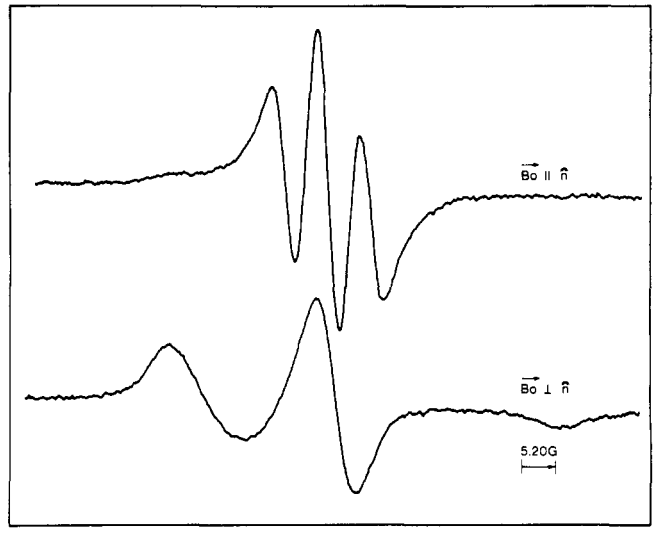


Figure 1. EPR X-band spectrum of 3-nitroxycholestane in 20 mol % 7-dehydrocholesterol (**1**) and 80 mol % dilauroyl-L-α-phosphatidylcholine at 21 °C.

membranes were *ordered*. Furthermore, it was necessary to delineate the concentration range over which the sterol multibilayer membranes possessed ordered structures. EPR measurements using 3-spiro-2′-*N*-oxyl-4,4-dimethyloxazolidinecholestane as a spin probe reveal ordering by the difference in the spectrum at perpendicular and parallel orientations of the external magnetic field with respect to the bilayer normal. In the anisotropic environment the long axis of the cholestane spin probe is normal to the surface of the film. Smith and Butler have shown that addition of various steroids preserve the ordering of hydrated lipid multibilayers probably via a specific interaction of the rather flat steroid molecule with the linear hydrocarbon chains of the fatty acid.[7,8] Hydrated lipid multibilayers containing up to 20 mol % **1** in dilauroyl-L-α-phosphatidylcholine, distearoyl-L-α-phosphatidylcholine, dimyristoyl-L-α-phosphatidylcholine, and dipalmitoyl-L-α-phosphatidylcholine were prepared according to the procedure of Shimoyama et al.[9,10] EPR measurements of each containing the cholestane spin probe were recorded with the external magnetic field $\vec{B}_0$ parallel (∥) and perpendicular (⊥) to the normal ($\hat{n}$) of the hydrated bilayer. Figure 1 depicts typical behavior which demonstrates clearly the anisotropy of the system at these concentrations.

Turning to the photoproduction of vitamin D_3 (**3**), it is well established that in fluid solution photochemically induced ring opening of the ring-B diene present in 7-dehydrocholesterol (**1**) yields the *cZc* triene previtamin D_3 (**2**).[1d] Thermal 1,7-antarafacial hydrogen shift yields **3**.[11] Results of photolysis of **1** at low conversions in the four membrane systems described above (entries 1–4) at ~280 and 310 nm are presented in Table I.

Reference to Table I reveals that the membrane reactions are different from those observed in a thin film in which a chloroform solution of **1** is simply evaporated on a glass slide (entries 1–4 relative to 5). Low conversions and the absence of **4** and **5** are significant in the latter. More importantly a striking difference exists between the membrane system and isotropic fluid solution (entries 1–4 relative to 6). In the membrane, tachysterol (**4**) is a minor product. Just the reverse obtains for the formation of **4** in hexane. The "quasi" photostationary concentrations of **2**:**4**:**1** in solution are normally 1:2:1.[12b,c]

Furthermore, there is a wavelength dependency for the

formation of tachysterol$_3$ (**4**). In the membrane, irradiation at a longer wavelength results in an increase in **4**, while the reverse holds true in solution. It is known from solution photolyses that lumisterol$_3$ (**5**) is formed photochemically from precholecalciferol by cyclization in the reverse stereochemical sense (9β hydrogen, 10α methyl),[12a] while *cEc* tachysterol$_3$[1c] is formed by a photochemical $Z \rightarrow E$ isomerization of the C_{6-7} double bond of *cZc* (**2**).

First addressing the environmental aspect of these differences: we envisage a model for the membrane–7-dehydrocholesterol (**1**) system in which the steroid is located along the straight hydrocarbon chains with the C_3 hydroxyl group aimed toward the aqueous interface. Much data on microviscosity,[13] cation, anion, and neutral molecule permeability, and ion channel formation agree with this picture.[14] Both vitamin D$_3$ (**3**) and tachysterol$_3$ (**4**) possess rather elongated molecular shapes which are drastically different from those of the planar and compact steroidal shape of **1** and **2**. In fact, some evidence exists that vitamin D$_3$–lipid multibilayers form *disordered* systems.[8] The membrane effect in the present study is explicable on the basis of initial ring opening of the diene **1** $\rightarrow$ **2** yielding previtamin D$_3$ (**2**) locked in the relatively restricted hydrophobic environment of the lipid multibilayer. The A ring of **2** initially lies above the plane of the C–D rings. Conversion into the alternative helical conformation (A ring below the C–D rings) which is the one leading to lumisterol$_3$ (**5**) may be achieved by torsion about the C_{5-6} bond preserving the cisoid structure of **2** (either conformation may yield **3**). This cisoid conformation in fluid solution is known to be a low energy form which rapidly equilibrates even at -100 °C between the two helical trienes.[15] In hexane solution, which we consider the isotropic counterpart of the membrane, unrestricted rotation of **2** is allowed and subsequent isomerization to **4** and **3** is favored. After photolysis the membrane remains ordered as determined by EPR, but this is not surprising because of the relatively large amount of 7-dehydrocholesterol remaining in the membrane.

A clue to the variations in products vs wavelength in the membrane relative to hexane solution is provided by comparison of the ultraviolet spectrum of **1** in the two media. The hexane spectrum is sharply defined and relatively narrow; in contrast, the membranous spectra are comparatively broader with greater absorbancy at longer wavelength. The epidermal system may absorb more strongly at wavelengths at ~300 nm relative to isotropic hydrocarbon solution.[16]

A final point deals with the nature of the electronic transition involved in the observed photochemistry. The absorption of **1** between 250 and 310 nm [λ_{max} 281 nm (ϵ 12 000)] corresponds to a $\pi \rightarrow \pi^*$ transition. Photochemistry involving $n \rightarrow \pi^*$ transitions, for example with ketones in the lipid micelles[17a,b] or vesicles,[17c] leads to photoattachment to the lipid due to biradicaloid intermediates. To probe the possible photoattachment of **1** to the membrane, [3α-^{3}H]-7-dehydrocholesterol was synthesized (specific activity, 1.4 Ci/mmol).[18] Labeled samples of **1** were introduced into the membranes listed in Table I and these were photolyzed in the standard way. The lipid part was separated by thin layer chromatography and rechromatographed on silica gel to a constant DPM. Essentially all of the tritium remained in the steroid portion.[18] This result agrees with no covalent bonding to the lipid bilayer and corresponds to concerted electrocyclizations of the dienes and trienes with essentially no biradicaloid component. There was no indication of bis steroid formation[19] or photofragmentation.

We conclude that photoformation of vitamin D$_3$ (**3**) in hydrated lipid multibilayers is a relevant model for in vivo photosynthesis and the conformational restraints imposed by the lipid geometry inhibits the biogenetically unimportant channel leading to tachysterol$_3$ (**4**).

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Received November 26, 1979

Longitudinal Restrictions of the Binding Site of Opsin As Measured with Retinal Isomers and Analogues

Sir:

Of the 12 presently known geometric isomers of retinal, 9 (11-cis, 9-cis,[1,2] 9,13-dicis,[2] 7-cis, 7,9-dicis, 7,13-dicis, 7,9,13-tricis,[3] 7,11-dicis,[4] and 9,11-dicis[5]) are known to form rhodopsin or its isomers when incubated with cattle opsin, one (11,13-dicis[1]) gives ambiguous results, and two (the all-trans and 13-cis isomers) are known with certainty not to form stable

0002-7863/80/1502-4259$01.00/0