

The Absorption Spectra of Monolayers of Fat-soluble Vitamins and the Orientations of Their Chromophores

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(Received October 11, 1979)

The UV absorption spectra at normal incidence and the polarized spectra at 45° incidence of monolayers of vitamin K₁, α -tocopherylquinone, vitamin E, ergosterol, and vitamin D₂ were measured. The absorption peaks of the monolayers of vitamin K₁, α -tocopherylquinone, and vitamin D₂ shifted to wavelengths longer than those in a hexane solution, while vitamin E and ergosterol showed little shift. The orientations of their chromophores in the monolayers were investigated by means of the dichroic ratios obtained from their polarized spectra. The molecular axes of vitamin K₁, α -tocopherylquinone, and vitamin E were found to be oriented in the range from 0° to about 30° against the normals of their monolayer planes. The molecular axis of ergosterol was found to tilt about 24° against the normal of the monolayer plane. For vitamin D₂, no reliable conclusion could be obtained as to its orientation.

Fat-soluble vitamins and their related compounds used in this study, vitamin K₁, α -tocopherylquinone (α -TQ), vitamin E, ergosterol, and vitamin D₂, have a quinone or a linear polyene moiety as their chromophores and form stable monolayers at air-water interfaces. These compounds exist in biological membranes and are involved in some biochemical processes. Vitamin K₁, α -TQ, and vitamin E are contained in chloroplasts and are thought to play a part in the electron-transfer system. Vitamin D₂ and ergosterol, which are steroid hormones, are known to have strong affinities for biological membranes. Their chromophores are thought to be important in their biological functions.

The states and the interactions of chromophores of these molecules and other fat-soluble vitamins in monolayers are interesting from both biological and physical points of view, but they have not been well studied except for the vitamin A group,¹⁾ although the surface pressure-area curves, surface potentials, and other properties of their monolayers have been investigated by several workers.²⁻⁷⁾ The main difficulty in such a study is that the intensities of the absorption spectra of chromophores in monolayers are generally very small. Several methods of measuring the absorption spectra of monolayers were devised by Jacob *et al.*,⁸⁾ Trurnit and Colmano,⁹⁾ and Tweet.¹⁰⁾ These methods, however, are all more or less troublesome. More recently, Kuhn *et al.*¹¹⁾ designed a high-sensitivity spectrophotometer for thin layers and investigated the absorption spectra of monolayers of ionic cyanine dyes substituted with long hydrocarbon chains. We constructed in our laboratory a spectrophotometer similar to theirs because the absorption spectra of monolayers can be measured more easily with it than with any other methods. We have then measured the polarized absorption spectra of monolayers of vitamin K₁, α -TQ, vitamin E, vitamin D₂, and ergosterol in order to get information about the interactions and the orientations of their chromophores in monolayers.

Experimental

Materials. Guaranteed-reagent-grade vitamin K₁,

vitamin D₂, ergosterol, and β -cholesterol were purchased from Nakarai Chemicals, Ltd. The vitamin E (Wako first grade) and phytol were obtained from Wako Pure Chemical Industries, Ltd., and the α -TQ, from the Nutritional Biochemicals Corporation. The absorption spectra of the fat-soluble vitamins in organic solutions were identical with the reported values in peak positions and extinction coefficients as follows: Vitamin K₁ (in hexane): within 1 nm and 4% compared with the values reported for petroleum ether¹²⁾ and isooctane(2,2,4-trimethylpentane)¹³⁾ solutions; α -TQ (in ethanol): within 1 nm and 5% compared with the values reported for an ethanol solution;¹⁴⁾ vitamin E (in hexane): within 1 nm and 3% compared with the values reported for a cyclohexane solution;¹⁵⁾ vitamin D₂ (in hexane): within 0.5 nm and 2% compared with the values reported for a hexane solution;¹⁶⁾ ergosterol (in ethanol): within 0.5 nm and 3% compared with the values reported for an ethanol solution.¹⁷⁾ All the above chemicals were stored in the dark at about -20°C in a reduced-pressure desiccator in which air had been replaced with nitrogen. Guaranteed-reagent-grade Na₂HPO₄, KH₂PO₄, and spectroscopic-grade hexane were purchased from Nakarai Chemicals, Ltd. All the chemicals were used without further purification. Twice-distilled water (pH=5.6—5.9) was used in all the experiments. Optically flat quartz slides (50×24×0.8 mm³) were obtained from the Takahashi Giken Co.

Methods. The UV-absorption spectra of the above compounds in a hexane solution were recorded on an MPS-5000 spectrophotometer (Shimadzu Co.). The absorption spectra of monolayers were measured with a high-sensitivity spectrophotometer using the single-beam and sample in-sample out technique developed by Kuhn *et al.*¹¹⁾ The apparatus constructed in our laboratory was the same as theirs in fundamental design. It consists of an automatic scanning monochromator, a self-exciting oscillator system, a light-detecting system (a photomultiplier and a preamplifier), a high-tension control system which feeds a highly negative voltage to the photomultiplier, a lock-in amplifier, and an X-Y recorder. The self-exciting oscillator system consists of a mechanical oscillator made of a long, thin iron plate (95×16×1 mm³) which is fixed vertically on a rigid frame made of iron and attached at its top with a holder to mount a quartz slide vertically against the iron plate, two electro-magnets to drive the oscillation of the oscillator, a coil to detect the frequency and the amplitude of the oscillation, and a negative-feedback electric circuit fed with the signal from the coil to provide the magnets with the a.c. current with the resonance frequency of the oscillator and a constant amplitude. The oscillator, with a quartz slide

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mounted in it, was oscillated at a resonance frequency of about 40 Hz and with a constant amplitude (measured at the center of the quartz slide) of about 10 mm. In measurements of the absorption spectra of monolayers, one half of the quartz slide was covered with a monolayer. Then the sample area of the slide (covered with the monolayer) and the reference area (not covered with it) passed the light beam alternately. The transmitted and modulated light was directed to the photomultiplier. The a.c. signal from the pre-amplifier was amplified by the lock-in amplifier, and the absorbance was recorded. The reference signal for the lock-in amplifier was taken from the self-exciting oscillator system. The following minor modifications were made in our apparatus: The light from the exit slit of the monochromator was focused on the quartz slide not with lenses, but with two mirrors, in order to eliminate the chromatic aberration. The integrated signal from the pre-amplifier was used to regulate the high tension fed into the photomultiplier, because the absorptions of the monolayers are very small.

The a.c. signal from the pre-amplifier was assumed to be a rectangular wave. This assumption is plausible because the boundary between the sample area and the reference area most quickly passes the light beam. The peak-to-peak value of the rectangular wave is proportional to the absorbance because the absorptions of monolayers are very small. The conversion factor from absorption(%) to absorbance is $\log_{10}(e/100)$. The high tension fed into the photomultiplier was adjusted to make the output from the pre-amplifier 50 mV for the case of a 5% absorption. In the lock-in amplifier, the first term of the Fourier series of the rectangular wave is synchronously rectified in the full wave and the rms value of the rectified wave is obtained. The conversion factor from the rms value of the rectified wave to the peak-to-peak value of the rectangular wave is $\pi/\sqrt{2}$. Therefore, the absorbance is obtained by multiplying the output of the lock-in amplifier (volt) by $(\pi/\sqrt{2})\log_{10}e (=0.9647)$. The absorbance measured with our apparatus agreed with that measured with usual spectrophotometers within a 4% error in the range of optical density (OD) of $(3-4) \times 10^{-3}$.

The sensitivity of our apparatus was limited by noises which could not be synchronized by the lock-in amplifier. The noise level was 1×10^{-4} OD/layer. The systematic noises caused by the magnetic field and the mechanical vibration from the oscillator system could, however, be suppressed to a level which did not exceed the above random noise level by making a magnetic shielding of the photomultiplier and a mechanical insulation of the oscillator to prevent dissipation of the vibration energy to other parts of the apparatus. Therefore, measurements were possible up to the range of the 2.5×10^{-3} OD/layer on a full scale with a noise level below 4%.

In the measurements of the polarized absorption spectra at 45° incidence, a polarizer (a Glan-Thomson prism made of calcite) was set just before the slide. The polarizer was set in such a manner as to make the plane of polarization parallel to the plane of incidence (p-spectrum) or perpendicular to it (s-spectrum).

The Blodgett method¹⁸⁾ was used for preparing spectroscopic samples of monolayers. A monolayer was spread on twice-distilled water or a 10^{-3} mol/dm³ phosphate buffer (pH=7.8-8.0) at $20 \pm 1^\circ \text{C}$ by dropping a fresh solution in hexane (5-10 mg/10 cm³). A clean quartz slide held vertically was moved slowly into the water until the lower half of it sank below the air-water interface. At this time the monolayer was not transferred because the slide was hydrophilic. Then surface pressure was applied to the

monolayer by using oleic acid (30 mN/m) or a mixture of ethyl myristate and liquid paraffin (6.3- or 16.3-mN/m)¹⁹⁾ as the piston oil, and the slide was raised slowly from the water. One monolayer was thereby transferred on each side of the lower half of the slide. The absorption spectra were recorded on the high-sensitivity spectrophotometer described above as soon as the slide became dry. All the operations handling monolayers were performed under dim red lights to avoid photochemical decomposition.

The quartz slide was cleaned by immersing it in a hot chromic acid mixture for about one hour. The slide was then washed with a great quantity of city water and immersed in a NaOH solution (pH=*ca.* 12) for about one hour. It was then taken out and washed first with much city water and then with distilled water. Finally, it was rinsed sufficiently with twice-distilled water and dried in an electric dryer. Its cleanliness was checked by light scattering in the dark.

Results and Discussion

In the spectrophotometer with the single-beam and sample in-sample out technique, a transparent plate, half of which is covered with a monolayer, is oscillated at a constant frequency so that the sample area (covered with the monolayer) and the reference area (not covered with it) pass the light beam alternately. The transmitted and modulated light is directed into a photomultiplier. The a.c. signal from it is amplified by a lock-in amplifier, and the absorbance of the monolayer is recorded. Therefore, the measured absorption includes the apparent absorption due to the difference in reflectivity between the monolayer and the plate when their reflectivities do not coincide. The difference between the two reflectivities can be expected to become larger at shorter wavelengths in the UV region because the dispersions of their refractive indices become larger there. Because of the high sensitivity of the apparatus, a very small difference in the reflectivities and even an inhomogeneousness in the quartz slide which can not be detected by usual optical techniques may produce detectable apparent absorptions.

The apparent absorption of a phytol monolayer was subtracted from the apparent absorptions of the monolayers of vitamin K₁, α -TQ, and vitamin E, which have a phytol chain or a phytol-like chain, and the apparent absorption of a β -cholesterol monolayer was subtracted from the apparent absorptions of the monolayers of vitamin D₂ and ergosterol, which have skeletons similar to it, as a correction to cancel out the apparent absorption due to the differences in reflectivity between the monolayers and the quartz slide and to the inhomogeneousness in the slide. Most peculiarities observed in the apparent absorptions of monolayers were eliminated by this correction. For instance, the difference between the apparent p- and s-spectra in the order of the 4×10^{-4} OD/layer, which almost all the monolayers showed in the region of wavelengths longer than 400 nm, were eliminated by this correction. The negative absorption in the order of the 1×10^{-3} OD/layer, which the spectra of the α -TQ and ergosterol monolayers showed in the wavelength region of 220-240 nm, also became positive in ergosterol after this

correction. However, the α -TQ monolayer still showed a small negative absorption at wavelengths shorter than 235 nm (Fig. 2) even after this correction. This seems to be due to the smaller refractive index of the α -TQ monolayer than that of the phytol monolayer in this wavelength region because the long chain of α -TQ does not have the double bond which phytol has. The error due to the difference in the refractive index, which is attributed to the difference in the structure of the long-chain moiety or the skeleton from phytol or β -cholestanol, may become significant in the wavelength region shorter than 250 nm, but its effect does not seem serious in the longer-wavelength region, where the absorption peaks of the chromophores exist.

The polarized absorption spectra at normal and 45° incidences were measured in order to investigate the anisotropy of the monolayers. In the case of the normal incidence, the spectra of monolayers were examined with the plane of polarization set at the angles 0°, $\pm 45^\circ$, and 90° against the direction of the withdrawal of the slide from the monolayer on the air-water interface. All the monolayers examined showed no significant difference in the polarized spectra at normal incidence, indicating that no anisotropy in the layer plane existed. In the case of the 45° incidence, however, remarkable differences between the p- and s-spectra were observed, indicating that the molecules in all the monolayers were oriented uniaxially. The apparent dichroic ratio, $R_{app} = A_s/A_p$, was obtained from A_s and A_p , the absorbances of the s- and p-spectra, which were corrected for the apparent absorption as has been described above. We calculated the true dichroic ratio corrected for the interference effect as follows.

The refractive indices of the long-chain moieties of the monolayers, which are almost identical with those of phytol or β -cholestanol, can be assumed to be equal to that of quartz at wavelengths longer than 250 nm, because the apparent absorptions of the phytol and β -cholestanol monolayers due to the differences in their reflectivities from that of the quartz slide were below 4×10^{-4} OD/layer and about one order smaller than the absorption of the chromophores, which amounted to $(1.5-5.0) \times 10^{-3}$ OD/layer. Therefore, we can use the equations of correction for the interference at the substrate-air interface proposed by Kuhn *et al.*¹¹⁾ In the case of a monolayer with a thickness much smaller than the wavelength of the measuring light, the true absorbances are given by:

$$\bar{A}_s = \frac{A_s}{1 + r_s} \quad (1)$$

$$\bar{A}_p = \frac{A_p}{1 + r_p}, \quad (2)$$

where r_s and r_p are the reflection coefficients for the s- and p-polarized lights respectively at a 45° incidence. We will assume hereafter that the refractive indices of the monolayers are isotropic and coincide with that of quartz. According to Fresnel's equations, r_s and r_p are given by:

$$r_s = \frac{n \cdot \cos \beta - \cos \alpha}{n \cdot \cos \beta + \cos \alpha} \quad (3)$$

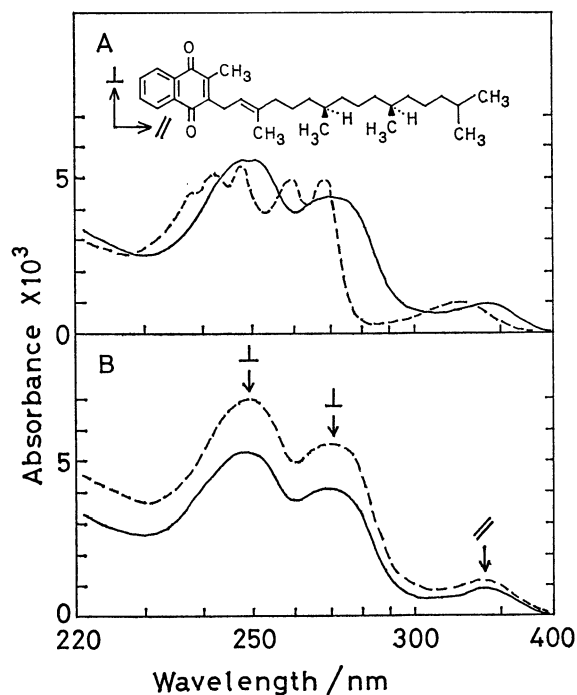


Fig. 1. Absorption spectra of vitamin K_1 monolayer spread on 10^{-3} mol/dm³ phosphate buffer pH 7.8–8.0 and transferred to quartz slide under the surface pressure of 6.3 mN/m.

(A) —: Absorption of the monolayer at normal incidence (n-spectrum), ----: absorption of the hexane solution in an arbitrarily chosen scale. (B) Polarized spectra of the monolayer at 45° incidence. —: The plane of polarization is parallel to the plane of incidence (p-spectrum), ----: the plane of polarization is perpendicular to it (s-spectrum). The symbols \perp and \parallel indicate the directions of the transition moments.

$$r_p = \frac{n \cdot \cos \alpha - \cos \beta}{n \cdot \cos \alpha + \cos \beta}, \quad (4)$$

where n is the refractive index of quartz; $\alpha (=45^\circ)$, the angle of incidence, and β , the angle of refraction. The refractive index of air is assumed to be unity. Finally, the true dichroic ratio is obtained by:

$$R = \frac{\bar{A}_s}{\bar{A}_p} = \frac{R_{app}(1 + r_p)}{1 + r_s}. \quad (5)$$

The values of n at the absorption peaks of the monolayers are obtained from the data of Jerrard and Turpin²⁰⁾ and Malitson²¹⁾ for the dispersion of the refractive index of quartz. For instance, n is 1.507 at 250 nm and 1.478 at 340 nm.

We show in Figs. 1 to 5 the observed absorption spectra of the monolayers which are corrected for the apparent absorption. We summarize in Table 1 the positions of the absorption peaks in the hexane solution and in the monolayers and the shifts of the peaks between monolayer and solution.

The spectra of vitamin K_1 and α -TQ monolayers were red-shifted compared with those of their solutions, and the vibronic structures seen in solution disappeared (Figs. 1 and 2). The spectra of the vitamin E mono-

TABLE 1. SPECTRAL DATA AND ORIENTATION ANGLES OF THE MONOLAYERS

Compound	$\lambda_{\text{max}}/\text{nm}$		Shift cm^{-1}	$R_{\text{app}}^{\text{a})}$	$R^{\text{a})}$	γ	θ
	Solution	Monolayer					
Vitamin K ₁	239	250	820	1.34 ± 0.02	1.12 ± 0.02	$0-38^\circ$	$64-90^\circ$
	242						
	248						
	260	270	840	1.35 ± 0.02	1.13 ± 0.02		
	269						
	326						
	340	1260	1.35 ± 0.03	1.13 ± 0.02			
α -TQ	250	270	840	1.39 ± 0.04	1.16 ± 0.03	$0-34^\circ$	$67-90^\circ$
	260						
	268						
Vitamin E	291	292	—	1.42 ± 0.01	1.19 ± 0.01	$0-30^\circ$	$69-90^\circ$
	298						
Ergosterol	262	262	—	0.33 ± 0.02	0.28 ± 0.02	$21.7 \pm 0.7^\circ$ $22.5 \pm 0.8^\circ$ $25.1 \pm 0.6^\circ$ $26.6 \pm 1.1^\circ$	0°
	271	270	—	0.35 ± 0.02	0.30 ± 0.02		
	282	282	—	0.42 ± 0.02	0.35 ± 0.02		
	294	294	—	0.46 ± 0.04	0.39 ± 0.03		
Vitamin D ₂	265	267	280	1.28 ± 0.02	1.07 ± 0.01	—	56°

a) The average of the results for two samples prepared independently.

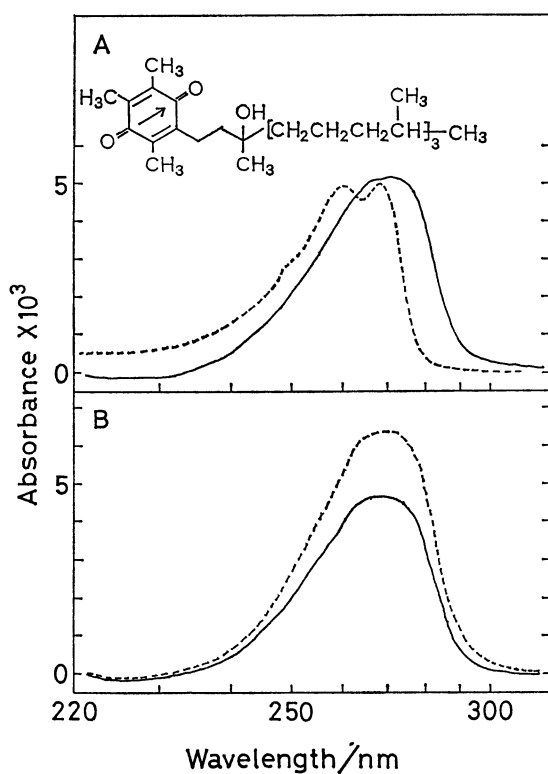


Fig. 2. Absorption spectra of α -tocopherylquinone monolayer spread on 10^{-3} mol/dm³ phosphate buffer pH 7.8—8.0 and transferred to quartz slide under the surface pressure of 16.3 mN/m. The arrow indicates the direction of the transition moment. (A) —: The n-spectrum of the monolayer, ----: the spectrum in hexane solution in an arbitrarily chosen scale. (B) Polarized spectra of the monolayer at 45° incidence. —: The p-spectrum, ----: the s-spectrum.

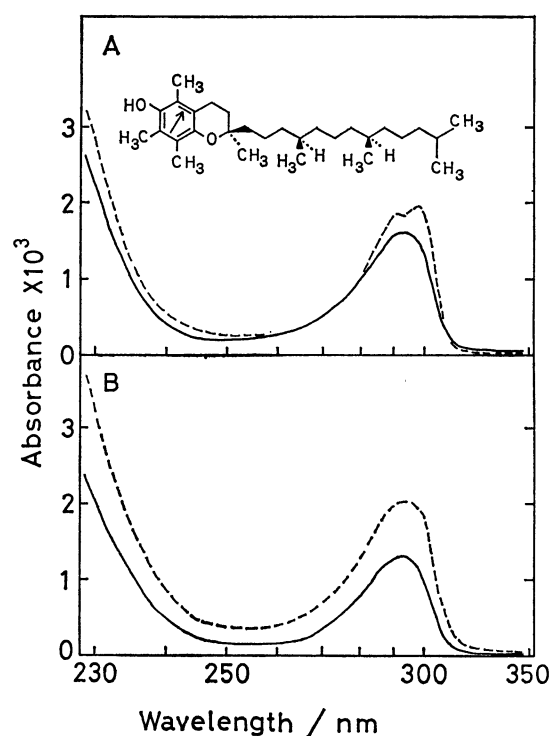


Fig. 3. Absorption spectra of vitamin E monolayer spread on 10^{-3} mol/dm³ phosphate buffer pH 7.8—8.0 and transferred to quartz slide under the surface pressure of 16.3 mN/m. The arrow indicates the direction of the transition moment. (A) —: The n-spectrum of the monolayer, ----: the spectrum in hexane solution in an arbitrarily chosen scale. (B) Polarized spectra of the monolayer at 45° incidence. —: The p-spectrum, ----: the s-spectrum.

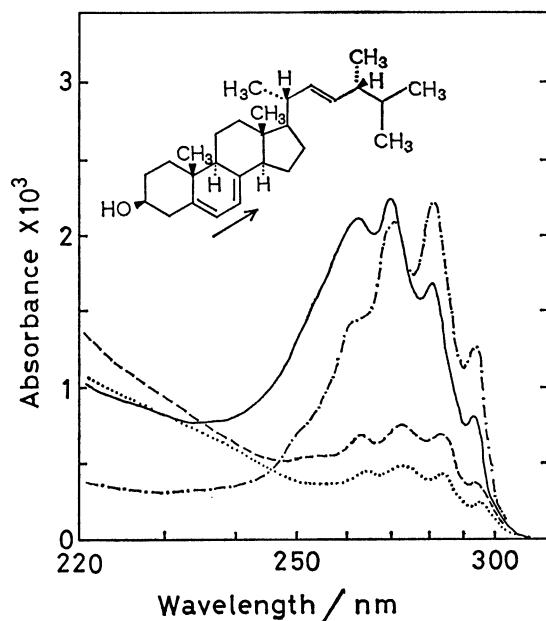


Fig. 4. Absorption spectra of ergosterol monolayer spread on twice distilled water and transferred to quartz slide under the surface pressure of 30 mN/m.: Absorption at normal incidence (n-spectrum), —: polarized p-spectrum at 45° incidence, ----: polarized s-spectrum at 45° incidence, — · —: the spectrum in hexane solution in an arbitrarily chosen scale. The arrow indicates the direction of the transition moment.

layers (Fig. 3) showed little shift, but the vibronic structure disappeared. The spectra of the ergosterol monolayers (Fig. 4) also showed little shift, but the relative intensities of the vibronic peaks changed greatly compared with those in solution. The spectra of the vitamin D₂ monolayers were slightly red-shifted (Fig. 5).

Concerning the polarized spectra at a 45° incidence, the strengths of the s-spectra were larger than those of the p-spectra in the vitamin K₁, α -TQ, vitamin E, and vitamin D₂ monolayers. On the other hand, the strength of the p-spectrum is about three times as large as that of the s-spectrum in the ergosterol monolayers. The strength of the non-polarized spectrum at a normal incidence (n-spectrum) is smaller than that of the s-spectrum. This indicates that the transition moment of ergosterol tilts only a little against the normal of the layer plane.

The apparent dichroic ratios, R_{app} 's, and the true ones, R 's, at the absorption peaks of the monolayers are listed in Table 1. The listed values are the averages of the results for two samples prepared independently.

We calculated the orientation angles of the molecules by assuming a model (Fig. 6A) the same as that of Akutsu *et al.*,²²⁾ because the transition moments in the monolayers were found to be oriented uniaxially. In Fig. 6A, the x-y plane is the layer plane and the y-z plane is the plane of light incidence. Now we assume that the molecules are rigid and have principal axes fixed to them (the 2nd rotation axes). As to the orientation of the whole molecule, it is assumed

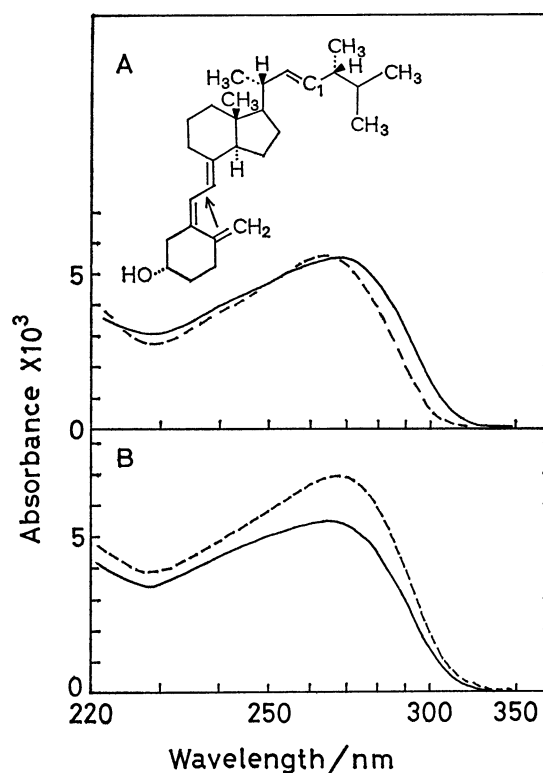


Fig. 5. Absorption spectra of vitamin D₂ monolayer spread on twice distilled water and transferred to quartz slide under the surface pressure of 30 mN/m. (A) —: The n-spectrum of the monolayer, ----: the spectrum in hexane solution in an arbitrarily chosen scale. (B) Polarized spectra at 45° incidence. —: The p-spectrum, ----: the s-spectrum. The arrow indicates the direction of the transition moment.

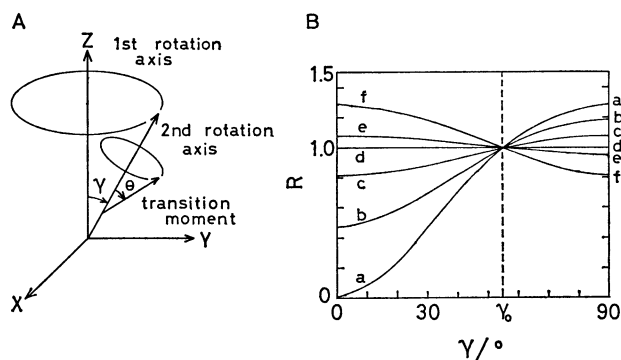


Fig. 6. (A) The model of uniaxial orientation of transition moments in a monolayer. The x-y plane is the monolayer plane and the y-z plane is the plane of light incidence. (B) The true dichroic ratio R as functions of the orientation angle γ at different θ 's, where n is 1.50 and α is 45°.

Curve a: $\theta=0^\circ$, b: 30° , c: 45° , d: $\arcsin\sqrt{2/3}$ ($\approx 54.7^\circ$), e: 60° , f: 90° . The angle γ_0 is $\arcsin\sqrt{2/3}$.

that the 2nd rotation axis tilts against the normal of the layer plane (the 1st rotation axis) by a fixed angle, γ , and is distributed uniformly around it. The transition moment in the chromophore is assumed to tilt against the 2nd rotation axis by a fixed angle,

θ , and to be distributed uniformly around it. Then the dichroic ratio is given by:

$$R = \frac{B}{(1 - 3 \sin^2 \beta)B + 4 \sin^2 \beta}, \quad (6)$$

where:

$$B = 2 \sin^2 \theta + 2 \sin^2 \gamma - 3 \sin^2 \theta \sin^2 \gamma. \quad (7)$$

We show in Fig. 6B how the dichroic ratio, R , depends on γ at different θ 's when α is 45° and n is 1.50. As can be seen in Fig. 6B, the relation between R and γ is sensitively dependent on θ . We may note that there is a special value of θ , 54.7° , independently of n , where R equals unity independently of γ . Therefore, if θ is near 54.7° , it is impossible to make a reliable determination of γ from the observation of R . When θ has a definite value not close to 54.7° , we can determine γ from the observed value of R . However, if there is any uncertainty in the value of θ , a unique determination of γ is not possible and we can determine it only in a certain range. There is an ambiguity in the definition of the θ angle in vitamin K_1 , α -TQ, and vitamin E because their chromophores may make an internal rotation against the long chain moiety and θ may depend on the conformation of the molecules. On the other hand, in ergosterol and vitamin D_2 , the chromophores are fixed to rigid molecular skeletons, and so we can determine θ by quantum mechanical calculations for the directions of the transition moments.

The directions of the transition moments in the chromophore of vitamin K_1 were assumed to be parallel to the O-O direction for the bands at 250- and 270-nm and to be perpendicular to it for the band at 340 nm, as is shown in Fig. 1, according to Kuboyama's calculation of 1,4-naphthoquinone by the simple MO method.²³⁾ The dichroic ratios obtained by us were nearly equal for these two groups of transition (Table 1), where the directions of the transition moments are perpendicular to each other. The γ angle must have the same value for all the transitions. Therefore, the θ 's for these transition moments must be nearly equal in order for their R 's to be nearly equal. This shows that the 2nd rotation axis must lie on the plane which is perpendicular to the naphthoquinone plane and which bisects the orthogonal angle formed with the two groups of the transition moments. Then, the θ angle may have a value within the range from 45° to 90° . The δ angle between the 2nd rotation axis and the naphthoquinone plane is then given by:

$$\cos \delta = \sqrt{2} \cdot \cos \theta. \quad (8)$$

By curves a and a' in Fig. 7 we show the relations between the θ and γ angles which give the observed values of 1.12 and 1.13 of the R 's for the transitions at 250- and 270-nm respectively. Because of the slight difference in n for these transitions, the two curves are not identical, but the difference between them is small. The curve corresponding to the transition at 340 nm is almost identical with the a' curve, and so it is not shown in Fig. 7. The dependence of δ on θ is also shown in Fig. 7. We can see in Fig. 7 that the θ and γ angles consistent with the observed value of R range from 64° to 90° and from 0° to 38° respectively. The tilt angle of the naphthoquinone plane

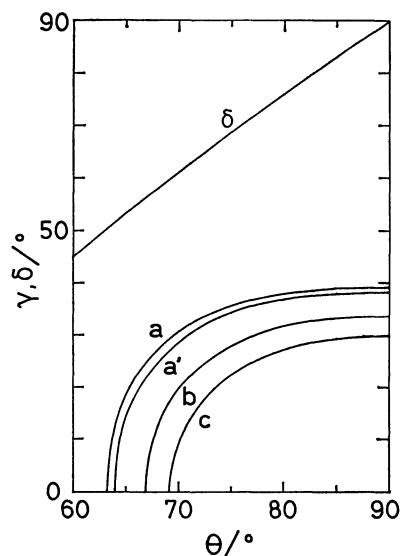


Fig. 7. The trajectories of the angles γ and θ to give the observed values of the dichroic ratio R .

Curve a: $R=1.12$ and $n=1.507$ for the 250 nm band of vitamin K_1 , a': $R=1.13$ and $n=1.498$ for the 270 nm band of vitamin K_1 , b: $R=1.16$ and $n=1.498$ for the 270 nm band of α -TQ, c: $R=1.19$ and $n=1.491$ for the 292 nm band of vitamin E. The θ dependence of the tilt angle δ of naphthoquinone plane against the 2nd rotation axis in vitamin K_1 monolayer is also shown in the upper side.

against the normal of the layer plane (the 1st rotation axis) is distributed in the range from $\delta - \gamma$ to $\delta + \gamma$. If γ has a value not near 0° , then the tilt angle of the naphthoquinone moiety is distributed over a wide range, for instance, from 32° to 92° for $\gamma=30^\circ$ and from 52° to 128° for $\gamma=38^\circ$. As a result, the hydrophilic naphthoquinone moieties in a vitamin K_1 monolayer have to make contacts with the water surface with contact angles distributed over a wide range. Considering the structure of naphthoquinone, it seems to be unnatural that naphthoquinone moieties can make contacts with the water surface with widely distributed contact angles without any meaningful effect on the interaction energy of the monolayer with water. Therefore, it seems likely that γ has a value near the lower boundary, 0° , of its admissible range. When γ is near 0° , θ and δ nearly equal 64° and 51° respectively, and the naphthoquinone plane contacts with the water surface at an almost constant angle of about 51° . As can be seen from Fig. 7, θ is limited in a narrow range from 64° to 71° , even if γ varies from 0° to 30° . Hence, it seems reasonable to guess that γ is smaller than 30° and that θ is close to 64° .

The spectral shifts in vitamin K_1 monolayers can be explained qualitatively by the molecular exciton theory by assuming $\gamma \approx 0^\circ$. McRae and Kasha²⁴⁾ showed that a linear-stacked array of chromophores has a spectrum with a blue shift compared with its monomer spectrum when $\eta < \arcsin \sqrt{1/3} = 35.3^\circ$ (η is the complementary angle between the transition moment and the direction of the linear arrangement) and one with a red shift when $\eta > \arcsin \sqrt{1/3}$. The red shift in the spectrum of the vitamin K_1 monolayers can be

explained by this theory, because the η angle is near 64° if the naphthoquinone moieties make stacked linear arrays with their orientation angle, γ , near 0° . Thus, the naphthoquinone moieties in a vitamin K_1 monolayer appear to be stacked at least in a short range.

In α -TQ, the direction of the transition moment was assumed to be parallel to the O—O direction, as is shown in Fig. 2, according to Kuboyama's calculation of benzoquinone.²³⁾ The θ and γ values, which give the observed R value of 1.16, are in the following two ranges: $\theta=0-34^\circ$ and $\gamma=67-90^\circ$, and $\theta=67-90^\circ$ and $\gamma=0-34^\circ$. Because of the symmetry of Eq. 6 for θ and γ , there are always two sets of solutions in which θ and γ are exchanged when only an R for a transition is available for the determination of γ . Since the 2nd rotation axis should agree roughly with the axis of the long-chain moiety, the former set of solutions is inadequate because it implies that the long chains of some molecules are almost parallel to the water surface. Therefore, we adopt the latter set. The shape of the admissible θ - γ curve for this case is shown by Curve b in Fig. 7. The orientation angle of the plane of the hydrophilic group of α -TQ can not be determined because at least two observable transitions with different directions of the transition moments are necessary for its determination. However, if γ is not near 0° , then the O—O direction of the α -TQ chromophore should make contact angles with the water surface over a wide range from $\theta-\gamma$ to $\theta+\gamma$, for instance, from 56° to 124° for $\gamma=34^\circ$. This also seems to be unnatural, and so we may guess that the value of γ is perhaps on the side near 0° in its admissible range. The red shift in the spectrum of the α -TQ monolayers can be explained in the same manner as in vitamin K_1 if γ is near 0° and if the chromophores of α -TQ are stacked at least in a short range. The η angle then becomes about 67° , which yields a red shift in the spectrum.

The direction of the transition moment in the chromophore of vitamin E was assumed to be perpendicular to the O—OH direction for the band at 292 nm, as is shown in Fig. 3, according to Nishimoto and Fujishiro's calculation of hydroquinone by the ASMO method.²⁵⁾ The θ and γ angles which give the observed R value of 1.19 are in the following two ranges: $\theta=0-30^\circ$ and $\gamma=69-90^\circ$, and $\theta=69-90^\circ$ and $\gamma=0-30^\circ$. The former set seems to be inadequate for the same reason as in the case of α -TQ. Similarly, we may guess that γ has a value near 0° in its admissible range, shown by Curve c in Fig. 7. The spectrum of the vitamin E monolayers showed little shift in contrast with those of vitamin K_1 and α -TQ. The spectrum must show a red shift if γ is near 0° and if the chromophores are stacked in a short range, for η becomes about 69° when $\gamma \approx 0^\circ$. This suggests that the chromophores in a monolayer of vitamin E are not stacked even in a short range.

In ergosterol, the chromophore for the band from 262 nm to 294 nm is an *s-cis*-butadiene structure. The direction of its transition moment, calculated by the HMO method, roughly agrees with the direction of the molecular axis, as is shown in Fig. 4. We assume

for this molecule that $\theta=0^\circ$. From the observed values of R for the vibronic peaks at 262-, 270-, 282-, and 294-nm (Table 1), their γ 's are determined to be 22° , 23° , 25° , and 27° respectively. The obtained values of γ agree nicely within an error of 5° . These values are also in good agreement with the tilt angle, 25° , suggested by Langmuir *et al.*²⁶⁾ from a comparison of the thickness of the built-up film of ergosterol with the X-ray data of its crystal. This provides evidence in support of our method for the determination of the orientation angles. In ergosterol with $\theta=0^\circ$, the η angle is equal to γ . The fact that the spectrum of the ergosterol monolayers showed little shift may be explained by the closeness of the orientation angle, $\gamma \approx 24^\circ$, to the critical angle, $\eta=35.3^\circ$, where the molecular exciton theory predicts no-shift. The observed change in the relative absorption intensities at the three vibronic peaks suggests that the coupling between electrons and nuclear vibrations in the excited state suffers a change upon the formation of a monolayer.

The chromophore of vitamin D_2 for the band at 267 nm has a hexatriene structure. We calculated the direction of the transition moment by the HMO method. The chromophore moiety was regarded as plane, and the angles between the adjacent carbon atoms were assumed to be 120° . The result is shown by the arrow in Fig. 5. If the straight line joining the oxygen atom with the carbon atom, C_1 , in Fig. 5 is regarded as the 2nd rotation axis, θ is about 56° . This θ value is very close to the angle, 54.7° , at which R equals unity independently of γ , and it is difficult to determine γ from R unless R is measured with a very high accuracy. If we put $\theta=56^\circ$, we can not find the γ angle, which gives the observed value, 1.07 of R . This seems to be due to the insufficient accuracy of the measurement of R . Therefore, we can not get any information about the orientation of the vitamin D_2 molecule from the dichroic ratio. Vitamin D_2 in a monolayer, however, might have an orientation angle, γ , roughly as large as that of ergosterol, because its monolayer area is only slightly larger than that of ergosterol, according to the data of Langmuir *et al.*²⁶⁾

We summarize in Table 1 the orientation angles, γ and θ , or their admissible ranges, as determined from the dichroic ratio. The orientation angle, γ , of the principal axis of the ergosterol molecule was determined precisely as 24° (the average of the four values) within an error of 5° . This value is in good agreement with the value, 25° , suggested by Langmuir *et al.* For vitamin K_1 , α -TQ, and vitamin E, only the admissible ranges for the orientation angles, γ and θ , were determined. The determined ranges for γ are similar for these three molecules. This suggests that they have a similar arrangement of the molecular axis in their monolayers, perhaps because of the similarity of their molecular structures. However, the shifts in their monolayer spectra suggest that the chromophores of vitamin K_1 and α -TQ are stacked at least in a short range, while those of vitamin E are not even in a short range. In vitamin D_2 , no con-

clusion about the orientation of the molecules in its monolayer could be obtained because the value of θ is close to the angle, 54.7° , where the dichroic ratio, R , is independent of γ .

We would like to thank Professor Hans Kuhn for kindly sending us information about the electric circuit of the high-sensitivity spectrophotometer made in his laboratory.

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