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*Review Article*

# Supramolecular Exciton Chirality of Carotenoid Aggregates

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*ABSTRACT* The conventional organic chemistry concept of chirality relates to single molecules. This article deals with cases in which exciton chirality is generated by the interaction of associated carotenoids. The handed property responsible for exciton signals in these systems is due to the alignment of neighboring molecules held together by secondary chemical forces. Their mutual positions are characterized by the overlay angle. Experimental manifestation is obtained by spectroscopic studies on carotenoid aggregates. Compared to molecular spectra, both UV/visible and circular dichroism spectroscopic observations reveal modified absorption bands and induced Cotton effects of opposite sign (exciton couplets), respectively. A new term, "supramolecular exciton chirality," is suggested for these phenomena, allowing the detection of weak chemical interactions not readily accessible for experimental studies, although highly important in the mechanism of biological processes. *Chirality 15:680–698, 2003.* 

*KEY WORDS:* circular dichroism; exciton signals; induced chirality; overlay angles; structured aggregates

The brilliant proposal that structural formulae should be considered in three dimensions[1] not only established stereochemistry, but somehow also created a belief that the concept of chirality relates to single molecules. The emphasis put on configuration led to the unanimous agreement that covalent bonds determine the structure and optical properties of molecules. It took well into the 20th century to appreciate secondary bonds as components of the chemical structure. Remote constraints between atoms that are not directly linked to each other can occur within a single molecule that may give rise to conformational chirality.[2] Secondary interactions between proteins and ligands induce chirality in configurationally achiral molecules.[3,4] Furthermore, secondary chemical forces may create a handed property by aligning neighboring chiral molecules. The last case involves carotenoid aggregates,[5] the chiroptical spectra of which contain Cotton effects several hundred times as strong as those of the individual molecule. The source of this sensitivity is the delocalization of excitation energy (exciton) over chromophores belonging to neighboring molecules. In the absence of chromophore, there is no exciton and the alignment of molecules cannot induce a signal observation by optical spectroscopy. If the alignment is not chiral, it will not give induced signals in the circular dichroism (CD) spectrum. Hence, for Cotton effects generated by the interaction of individual molecules, the introduction of a new term, "supramolecular exciton chirality," is suggested here.

The first indication of the change of absorption spectra due to self-assembly of molecules came from the work of Jelley,[6] who observed a bathochromic (red) shift upon aggregation. According to the molecular exciton model, a hypsochromic (blue) shift of the absorption spectral band is indicative of a tight association called the H-type, or card-pack aggregate, while a redshifted peak is related to a loose-type (J- or head-to-tail) association.[7,8] In CD spectroscopy the spectrum of two interacting chromophores is composed of two components (i and j) Cotton effects of opposite sign; these are separated by $V_{ij} = (1/2)(\sigma_i - \sigma_j)$, the Davydov split,[9] where $V_{ij}$ is the interaction energy while $\sigma_i$ and $\sigma_j$ are wavenumbers of the maxima of component Cotton effects. The sign of split Cotton effects defined by the sign of the long wavelength peak has been successfully applied for determination of absolute configurations,[10] a technique known as the exciton chirality method.[11–13] Accordingly, the order of positive and negative peaks in the band-pair of the CD exciton couplet tells the P or M sign of the torsion angle of covalently linked pairs of chromophores. Theoretical approaches yielded the definition of exciton chirality and derived the intensity of the split Cotton effect ($A = \Delta\varepsilon_1 - \Delta\varepsilon_2$; where $\Delta\varepsilon_1$ and $\Delta\varepsilon_2$ refer to first and second Cotton effects,

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respectively) as a nonsymmetrical sinusoid function of $\tau$, the torsion angle. Accordingly,[14] the A value is positive between 0° and 180° and has a maximum around $\tau = 70°$. Besides, A is inversely proportional to the square of the interchromophoric distance[14] and proportional to the square of the extinction coefficient.[15] Thus, exciton chirality depends on both energetic and geometric factors.

Spectral detection of associated aromatic dyes by CD, i.e., chirality induction created by aggregation, was reported in the 1980s[9] and the exciton-coupled CD spectra was interpreted as a consequence of interactions of chromophores in close vicinity.[17] Carotenoid dyes also associate in aqueous organic solutions,[5,18] demonstrating that the excitation energy corresponds to neighboring molecules in the aggregate. Thus, interaction between chromophores also occurs when they belong to different molecules. A recent study showed electronic interactions of two individual molecules to be manifest even when separated by 12 nm.[19]

## SUPRAMOLECULAR CHIRALITY

In order to achieve an appreciation, let us start with established ideas. A simple element of molecular chirality is the torsion angle $\tau$ described by the minimum number of parameters (four times three steric coordinates for atoms of the same kind, i.e., 13 parameters altogether, while a tetrahedral carbon atom with four different substituents needs 16), with the provision that its value satisfies $0° < \tau < 180°$ (Fig. 1a).

Let us keep the four atoms at their chiral positions and eliminate the bond for which the torsion angle is defined in Figure 1a; we arrive then to the simplest case of supramolecular chirality—the *overlay* of two bonds—provided that the same condition holds: $0° < \omega < 180°$ (Fig. 1b). In order to characterize the geometry of overlay, we should define $\omega$ as the *overlay angle*. For this purpose, assume that the overlaying molecules are of rigid rod shape consisting of more than unique bonds. Let these rods represent the direction of electric dipole transition moment. The rods should be projected along their distance from each other and seen from the direction perpendicular to both of them lying in parallel planes. Note that the projection of the two bonds in Figure 1b does not satisfy this requirement, but Figure 1c,d do so, allowing the overlay angles to be observed. Finally, consider the special case when the distance of the rods connects their centers (central overlay, Fig. 1e). Then the overlay angle is indefinite as to whether acute or obtuse and the arrangement becomes nonchiral for $\omega = 90°$. The sign of the overlay angle $\omega$ is defined in the usual way,[20] being positive if the front rod will cover the rear one by a clockwise turn around an axis coinciding with the distance of the rods. As P and M are descriptors for the torsion angles, $\pi$ and $\mu$ are suggested for right- and left-handed overlays, respectively. While the sign of overlay angles for Figure 1c and d is predictive of the exciton signal, in Figure 1e angles of both $\pi$ and $\mu$ signs are present with an algebraic sum of 180°. The exciton signals generated by this arrangement are detectable in the absorption spectrum, but significant internal compensation of Cotton effects will occur. In other words, the experimental signed order of the CD couplet will experimentally prove the nonsymmetric shape of the theoretically derived CD intensity curve,[14] indicating that the acute angle produces the more intense Cotton effect.

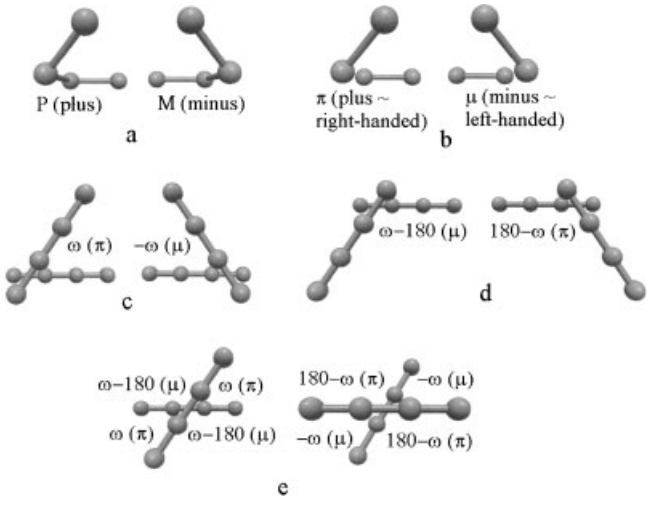


**Fig. 1.** Molecular and supramolecular chiralities. **a:** A pair of torsion angles in mirror-image relation. **b:** Mirror-image related overlays of two bonds. **c,d:** Mirror-image related overlays shown for hypothetical rigid rod molecules projected along their distance allowing the overlay angle to be defined. **e:** Mirror-image related central overlays; this chiral arrangement with overlay angles of opposite sign becomes nonchiral if $\omega = 90°$.

The chirality elements given in Figure 1 are unstable on their own; single bonds are flexible, and thus torsion angles are generally transient unless stabilized by intermolecular forces[4] (due to, e.g., a chiral protein surface, as seen for $\gamma$-aminobutyric acid bound to its receptor sites[21]). An overlay angle is even less restricted since the individual rods are able to move in three dimensions, and a dimer needs force holding the aligned molecules together.

### *"Hydrophobia"*

A common case bringing about a close approach of molecules is aggregation of a substance soluble in organic solvents only when their molecules stick together in aqueous solution. The contact of neighboring molecules makes it difficult to change mutual position, as shown for amino acid derivatives in order to elucidate structural requirements of $\beta$-sheet formation.[22] The *hydrophobic* label is generally used to describe such a phenomenon. However, Hildebrand[23] pointed out that the term is a misnomer: "there is no hydrophobia between water and alkanes; there only is not enough hydrophilia to pry apart the hydrogen bonds of water so that the alkanes can go into solution without assistance from attached polar groups." Current theoretical developments, such as a quasichemical theory of liquids[24] and the concept of hydrophobic hydration based on statistical thermodynamics,[25] as well as recent experimental findings strongly support Hildebrand's view; in a concentrated methanol–water solution believed to be homogeneous, neutron diffraction indicated incomplete mixing, as most of the water molecules are involved in hydrogen-bonded clusters existing among

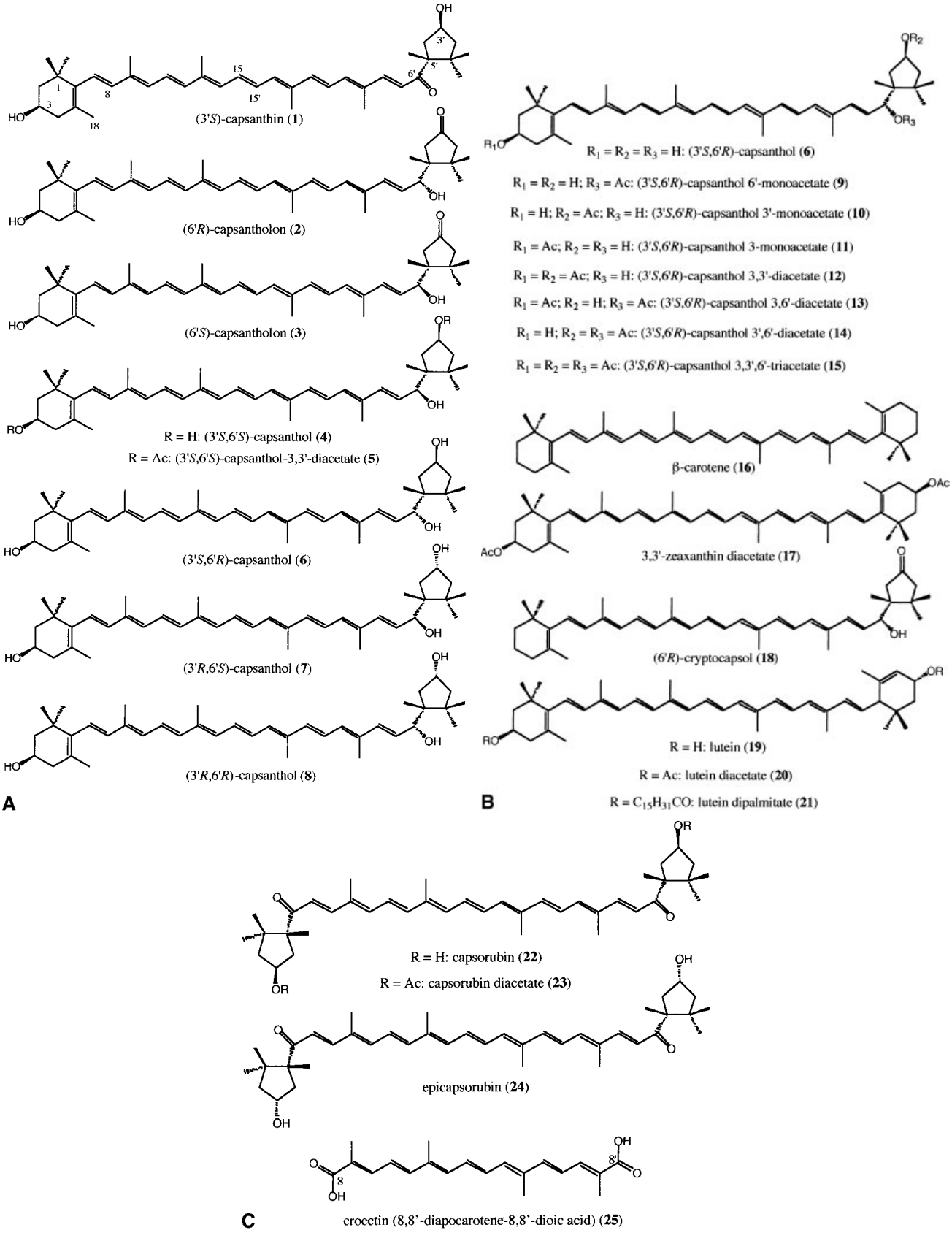


**Fig. 2.** Formulae of carotenoids and related compounds investigated.

closely packed methanol molecules.[26] Thus, aggregate formation does not necessarily require affinity between assembling molecules which may gather into clusters because they cannot mix with water-rich domains of the medium. Hence, the force creating overlay angles in the aggregate may come from the strength of aqueous association. There may be additional interaction between aggregated neighbors, however.

In order to provide an overall nonzero overlay angle for a macroscopic assembly, chiral discrimination should be operative in the alignment of neighboring molecules. This will defy statistical compensation by opposite angles, and provide excess for overlay angles of either $\pi$ or $\mu$ sign. Otherwise, as in a haystack, overlay angles of opposite sign would statistically lead to external compensation. It is analogous to a racemate in molecular terms. Measurable Cotton effects due to association require the participation of enantiomerically pure molecules giving preference for chiral overlays and capable of inducing chirality through noncovalent interaction. This phenomenon has been observed in diverse systems; optically active solutes create an excess of solvent molecules of the same helicity through intermolecular chirality transfer,[27] an achiral pentamethin dimer becomes chiral in the presence of a chiral host,[28] helical superstructures of J-aggregates are formed under the restricting influence of substituents.[29,30] The last case represents intramolecular chirality transfer[31] when substituents induce a shift in conformational equilibria. In a similar way, chiral influences of remote nucleotide units can be transmitted through noncovalent interactions to the duplex formation of peptide nucleic acids.[32] The provisions outlined for the geometry of chiral elements, i.e., $0° < \omega < 180°$ (and $\omega \neq 90°$ in the case of the central overlays of Fig. 1e) are necessary for the exciton couplet to appear in the CD spectra.[33,34]

Based on the progress achieved in the isolation[35] and semisynthetic modifications[36–38] of carotenoids from *Capsicum annuum* (red paprika), we investigated the properties of supramolecular exciton chirality generated by these pigments. This review summarizes our findings.

## PREPARATION AND CHARACTERIZATION OF CAROTENOIDS

In general, the carotenoids used were isolated from natural sources or prepared by semisynthesis from natural compounds. The isolation of natural carotenoids was carried out under nitrogen atmosphere in the darkness in order to avoid *trans* ⇒ *cis* isomerization. Solutions of iso-

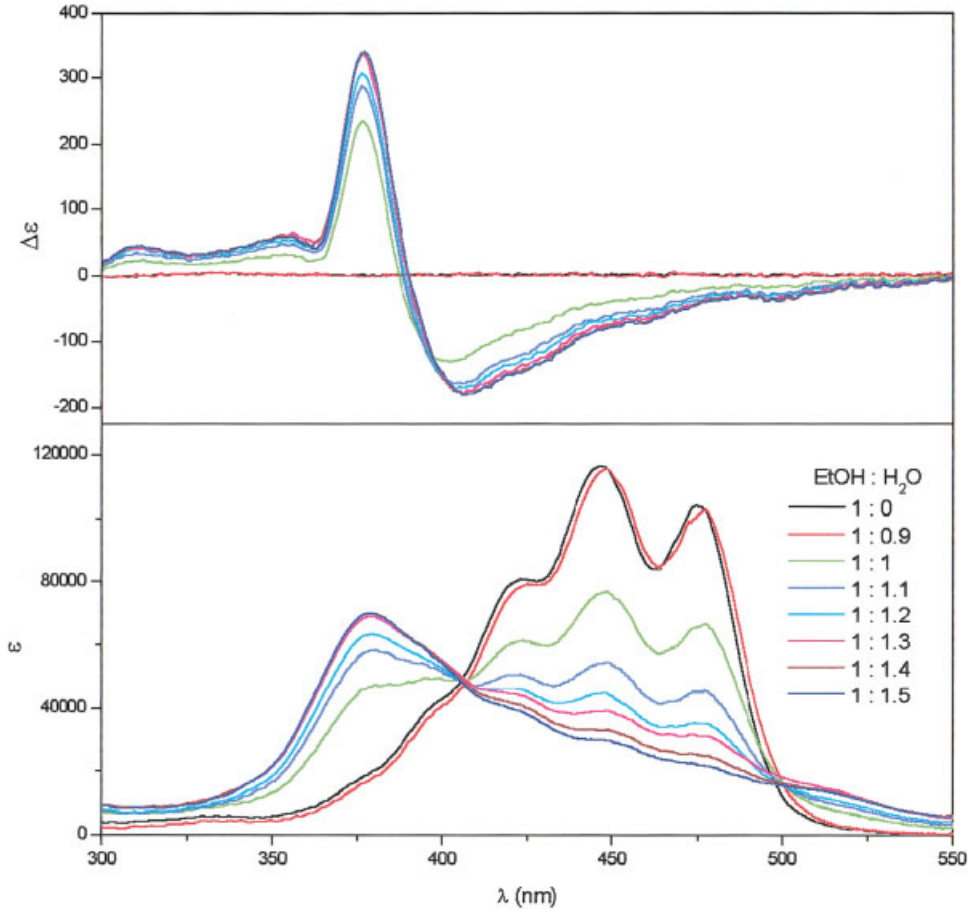


**Fig. 3.** The development of carotenoid self-assembly from (6′*R*)-capsantholon (**2**) by gradual dilution of the sample at 25°C. Inset: v/v ratio of ethanol to water (from Ref. 39).

Fig. 3 live 4/C

lated material were stored at −20°C under nitrogen away from light. Separation and purification of pigments were achieved by column chromatography on calcium carbonate. After rechromatography, the individual pigments were crystallized from benzene-hexane or benzene-methanol. The purity of all (isolated and semisynthetic) compounds (95% or higher) was checked by high-performance liquid chromatography (HPLC) and identity characterized by nuclear magnetic resonance (NMR), CD, UV-VIS, and mass spectra. Crystalline carotenoids were stored in sealed tubes under nitrogen.

The carotenoids with κ-end groups, i.e., capsanthin ((3*R*,3′*S*,5′*R*)-3,3′-dihydroxy-β,κ-caroten-6′-one, (**1**)), capsorubin ((3*S*,5*R*,3′*S*,5′*R*)-3,3′-dihydroxy-κ,κ-carotene-6,6′-dione, (**22**) and cryptocapsin ((3′*S*,5′*R*)-3′-hydroxy-β,κ-caroten-6′-one) were isolated from red spice paprika (*Capsicum annuum*). The reduction of the keto-carotenoids by $NaBH_4$ resulted in the appropriate diastereomer alcohols. In this way (3′*S*,6′*S*)- (**4**) and (3′*S*,6′*R*)-capsanthol (**6**), (6′S)- and (6′*R*)-cryptocapsol (**18**) were prepared[37] from capsanthin and cryptocapsin, respectively. The capsanthol epimers (**4, 6**) were subjected to Oppenauer oxidation ($Al(OPr^i)_3$ in acetone) yielding (6′*R*)- (**2**) and (6′*S*)-capsantholon (**3**). Subsequent reduction of these epimers by sodium borohydride resulted in the formation of (3′*R*,6′*S*)- (**7**) and (3′*R*,6′*R*)-capsanthol (**8**).[36] Diastereomer mixtures were separated by column chromatography on calcium carbonate or by thin-layer chromatography (TLC) on silica plate.

Preparation of mono-, di-, and triacetates of capsanthols required different strategies. The capsanthol-3,3′-diacetates (**5, 12**) were prepared by the acetylation of capsanthin (**1**) followed by $NaBH_4$ reduction. (6′*R*)-Capsanthol-3,3′,6′-triacetate (**15**) was obtained by the acetylation of (3′*S*,6′*R*)-capsanthol (**6**). The other di- and monoacetates were prepared from 3,3′,6′-triacetate and 3,3′-diacetate of (6′*R*)-capsanthol. Thus, dezacetylation of (6′*R*)-capsanthol-3,3′,6′-triacetate (**15**) by sodium borohydride gave (6′*R*)-capsanthol 3,6′-diacetate (**13**), 3′,6′-diacetate (**14**), and 6′-monoacetate (**9**), while dezacetylation of (6′*R*)-capsanthol-3,3′-diacetate (**12**) yielded (6′*R*)-capsanthol 3′-acetate (**10**) and 3-acetate (**11**).[38] All acetate derivatives were separated and purified by column chromatography on calcium carbonate column.

Lutein ([all-*E*,3*R*,3′*R*,6′*R*]-β,ε-carotene-3,3′-diol) (**19**) was isolated from green paprika (*Capsicum annuum*) according to the method described elsewhere.[35] Lutein diacetate (**20**) was obtained by acetylation of lutein, whereas lutein dipalmitate (Helenien) (**21**) was isolated from *Helianthus annuus*. Zeaxanthin 3,3′-diacetate (**17**) was prepared by acetyl chloride from zeaxanthin ([all-*E*,3*R*,3′*R*]-β,β-carotene-3,3-diol) isolated from *Lycium halimifolium*.

The formulae of carotene derivatives investigated are collected in Figure 2.

## CAROTENOID AGGREGATES

Our interest focused on the question: How does the structure of a carotenoid molecule influence the properties of self-assembly? In this pursuit the epimers of capsantholon (**2, 3**) and capsanthol (**4, 6, 7, 8**), the semisynthetic derivatives of natural capsanthin (**1**), played a central role. The creation of supramolecules was studied[39] by aqueous titration of the ethanolic solution of (6′*R*)-capsantholon (**2**) while taking UV/VIS and CD spectra (Fig. 3).

The spectroscopic detection displays the change brought about by aqueous dilution. In ethanol, the molecular absorption spectrum shows intensive peaks[40] (at 475.5; 447; 423 nm); the spacing of the absorption band is consistent with the superposition of 0-0, 0-1, 0-2 vibrational levels on the electronic excitation.[5] While **2** has molecular chirality, its CD spectrum above 300 nm is weak and appears as a baseline at the scale of Figure 3. It indicates that intramolecular chirality transfer[31] to the polyene chromophore is insubstantial, in agreement with other 3-hydroxycarotenoids.[41] Association starts at an ethanol/water ratio of 1:1 and gradually increases with higher dilution (Fig. 3, inset), as indicated by the appearance of a new absorption peak at 377 nm in the near-UV region, together with the gradual attenuation of intensity and vibrational structure superimposed on the molecular absorption bands, as well as by the appearance of an exciton couplet in the CD spectra. The crossover point in the CD does not coincide with the new absorption band as a consequence of different aggregates present. The dramatic change due to dilution indicates that the energy of dominant absorption in the aggregate is about 66 kJ/mol higher than the 0-0 level excitation energy of monomer molecules. This difference may slightly vary with the actual organic solvent, as the absorption frequency depends on the refractive index.[42] The intensity of Cotton effects arising from assembled carotenoid molecules (i.e., from supramolecular chirality) increases up to the 1:1.5 ethanol/water dilution ratio and is several hundred times higher than that of **2** in ethanol solution. The aggregate is of μ sign, mainly a tightly organized H-type (card pack) with significant contribution from J-type, or head-to-tail, assemblies, as indicated by the long redshifted tail of absorption band and the remnants of the vibrational fine structure in the CD. There was no sign of sample anisotropy in spectroscopic detection.

It should be noted that the shape of exciton CD bands depends on the method of dilution; by abruptly diluting a sample applying ethanol/water = 1:3 ratio, the card-pack character becomes more dominant and the exciton amplitude increases several times further within an hour,[40] during which most of the head-to-tail aggregates disappear (Fig. 4).[39] (Therefore, this ratio was chosen to be applied most generally.) The CD exciton peaks are found[40] at 372 and 388 nm and the crossing point nicely matches the absorption maximum, confirming the increased share of card-pack aggregate. The intensity of the split Cotton effects, $A \cong -2500$, is very high.[39] All these indicate that the card-pack aggregate can be built up on the account of the head-to-tail assembly.

In order to rationalize the intensity of the exciton couplet in Figure 4, we refer to experiments with porphyrin derivatives of molecules having remote stereogenic centers.[43] These chromophores providing powerful absorption ($\varepsilon_{419} = 350{,}000$) allowed measurements of

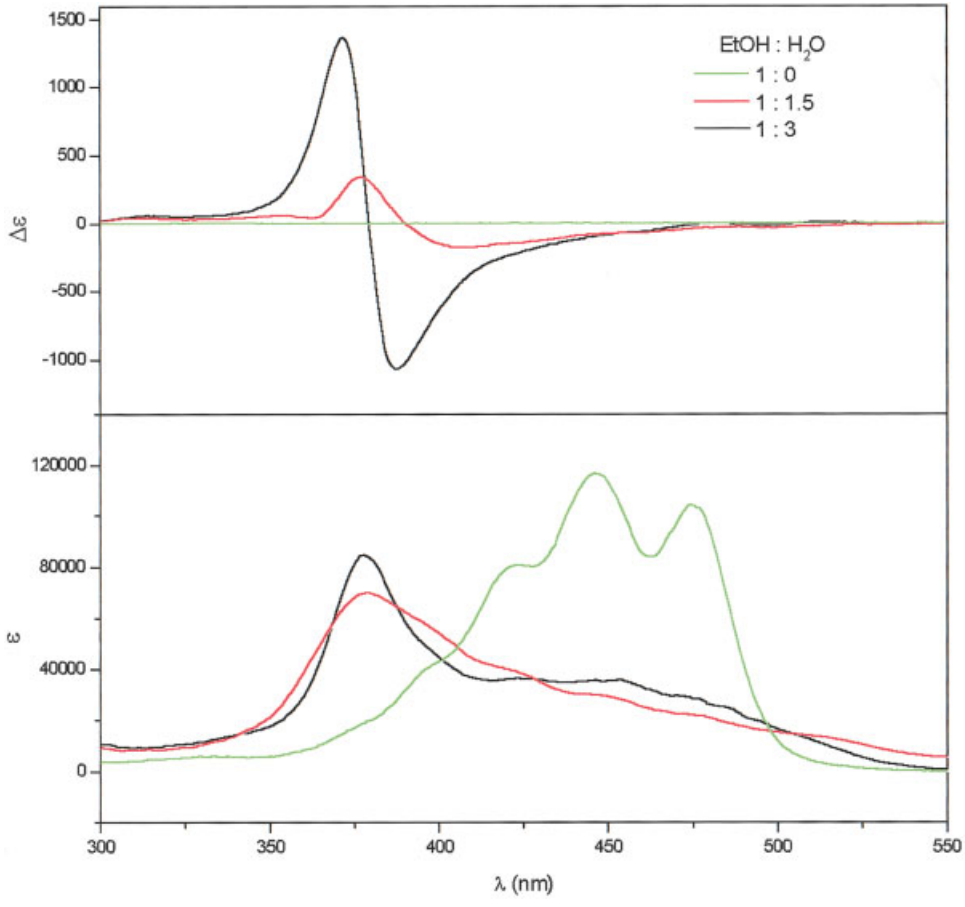


**Fig. 4.** Comparison of UV/VIS and CD spectra of **2** induced by gradual and abrupt aqueous dilution (red and black, respectively); for dilution ratios, see inset (from Ref. 39).

exciton CD spectra for cases[44] when the interacting chromophores were separated by a distance of 50 Å. The $\varepsilon_{max}$ value for carotenoids is around 100,000, so according to theory,[15] about 10 times smaller A values may be expected for identical distance and orientation. Aggregation of rod-like carotenoid molecules could be envisaged according to Figure 1e. While the end groups of most molecules, including (6′*R*)-capsantholon (**2**), are different, they hardly interact with the excitation of the polyene chain. So from the point of view of exciton coupling, carotenoid aggregates can be represented by the central overlay type (Fig. 1e), with significant internal compensation of overlay angles of opposite sign. Another consequence of carotenoid overlays is the absence of Cotton effects at ω = 90°, thus the intensity function derived for glycol bis-benzoates[14] predicting maximum at a dihedral angle of 70° cannot be applied here. On the other hand, aggregated carotenoid molecules pressed together by the strong association of water molecules closely approach each other, so the interchromophoric distance is substantially shorter than in covalent porphyrin derivatives.[44]

Although the 6′-OH group of capsantholon is ineffective for giving intense CD bands of a single molecule, its configuration dramatically influences the structure of the aggregate,[40] as shown by the spectra of (6′*S*)-capsantholon (**3**) in Figure 5. The spacing remains on the redshifted absorption band and appears on the likewise structured exciton couplet, corroborating the formation of head-to-tail aggregate in which individual molecules retain their vibrational freedom. Both **2** and **3** form self-assemblies of left-handed character (involving an excess of μ overlay angles), as shown in Figures 4 and 5 by the negative sign of the low-energy CD band.

The difference in supramolecular organization due to different configurations at the 6′ atom is not easy to reconcile. An attractive proposal put forward by Martin and colleagues[45,46] emphasized the role of hydrogen bonds. They have found the aggregate of capsorubin (**22**) to be of H-type with a small J-type contribution, while the aggregate of epicapsorubin (**24**) to be mixed with an increased share of head-to-tail structures.[45] This difference was connected with the observation of intramolecular hydrogen bonding in epicapsorubin which contains the carbonyl and OH groups in *cis*-configuration.[46] Supposing that intramolecular hydrogen bonding competes with intermolecular ones, the stability of epicapsorubin aggregates was suggested to be weaker than that of capsorubin.[45] However, the capsantholon epimers, **2** and **3,** are not in that relation: the carbonyl group is on the κ-ring and the aggregate spectra are basically different (no trace of a

Fig. 4 live 4/C

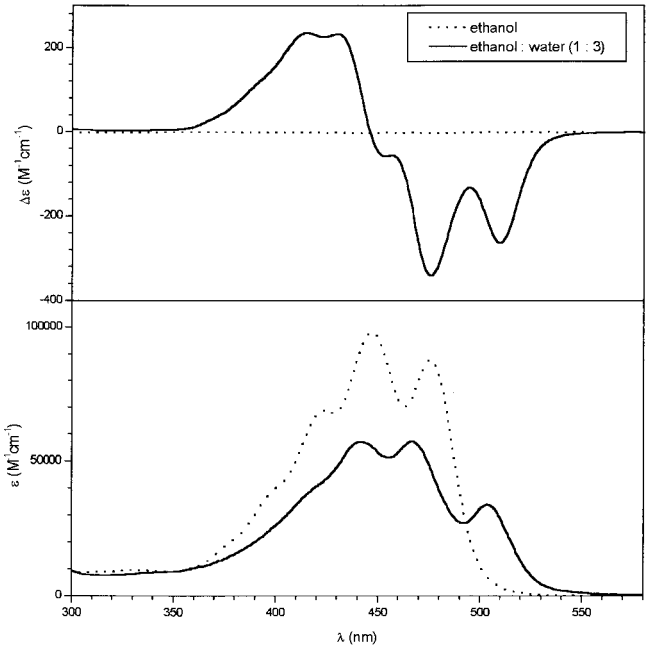


**Fig. 5.** UV/VIS and CD spectra of (6′*S*)-capsantholon (**3**) and its self-assembly (from Ref. 40 with permission from Elsevier Science Journals).

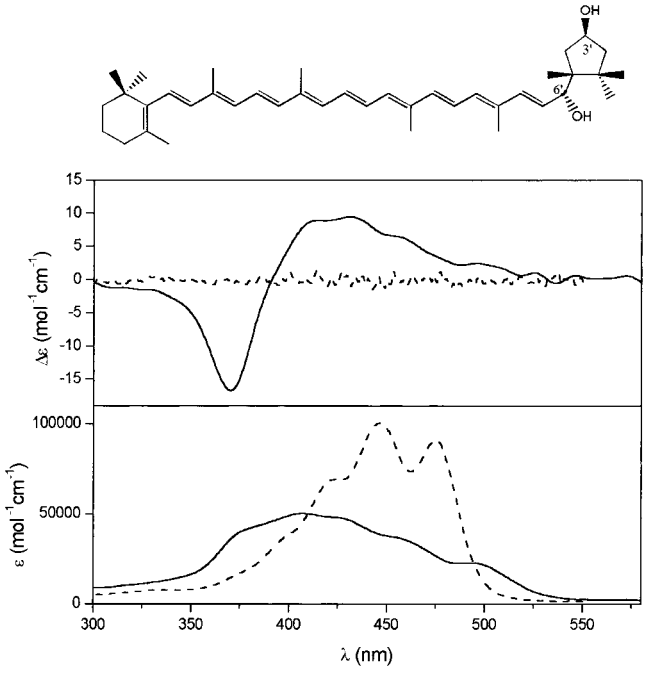


**Fig. 7.** UV/VIS and CD spectra of (6′*R*)-cryptocapsol in ethanol (dashed line) and upon aqueous dilution (solid line) (from Ref. 47).

card-pack assembly is seen in Fig. 5). Moreover, the infrared spectra of both **2** and **3** exclude intramolecular hydrogen bond formation (Holly S, unpubl.). Hence, this hypothesis cannot explain the reason for the different aggregate structures of capsantholon epimers.

In order to test the role of configuration at the 6′-carbon atom, the reduced derivatives, (3′*S*,6′*S*)- and (3′*S*,6′*R*)-capsanthols (**4, 6,** respectively), were further investigated. Similar to capsantholons, the 6′*S*-form (**4**) aggregated in head-to-tail and the 6′*R*-form (**6**) in card-pack fashion.[47] Peculiarly, the (3′*S*,6′*R*)-capsanthol CD spectra displayed slow supramolecular inversion[48] (from π to μ), as seen in Figure 6.

The kinetics of inversion is complex and cannot be explained by a simple **A** ⇋ **B** transformation,[48] in spite

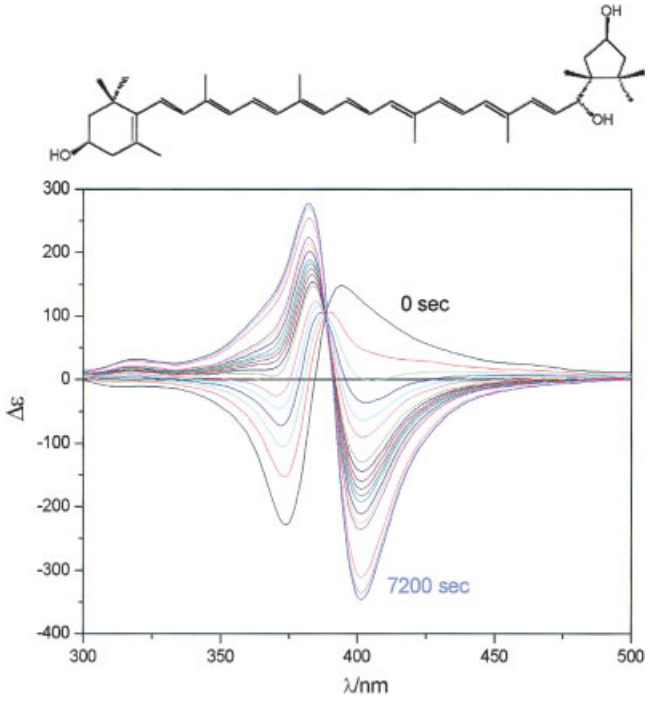


**Fig. 6.** Time-dependent inversion of the CD spectrum of (3′*S*,6′*R*)-capsanthol card-pack aggregate (from Ref. 48).

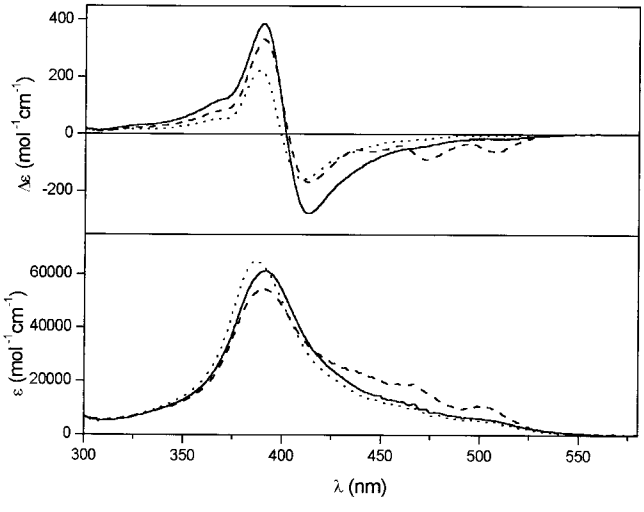


**Fig. 8.** UV/VIS an CD spectra of mixtures of aggregates by simultaneous aqueous dilution of solutions in ethanol; (6′*R*)-capsanthol:(6′*S*)-capsanthol in ratios of 2:1 (dotted line), 1:1 (solid line), 1:2 (dashed line) (from Ref. 47).

Fig. 6 live 4/C

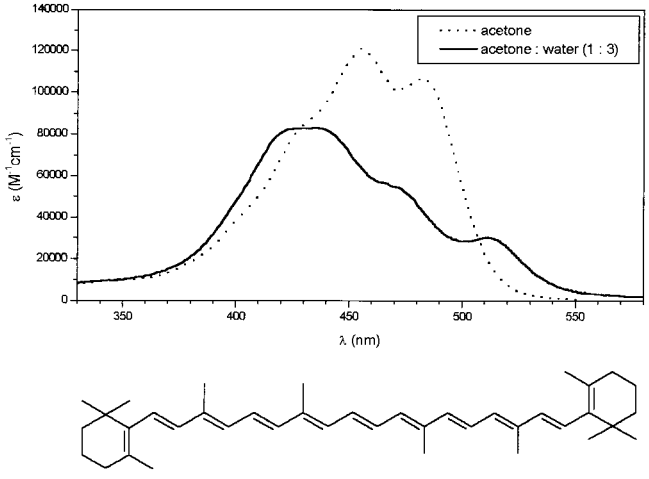


**Fig. 9.** UV/VIS spectra of molecular (dotted line) and aggregated (solid line) β-carotene (**16**) (from Ref. 40 by permission from Elsevier Science Journals).

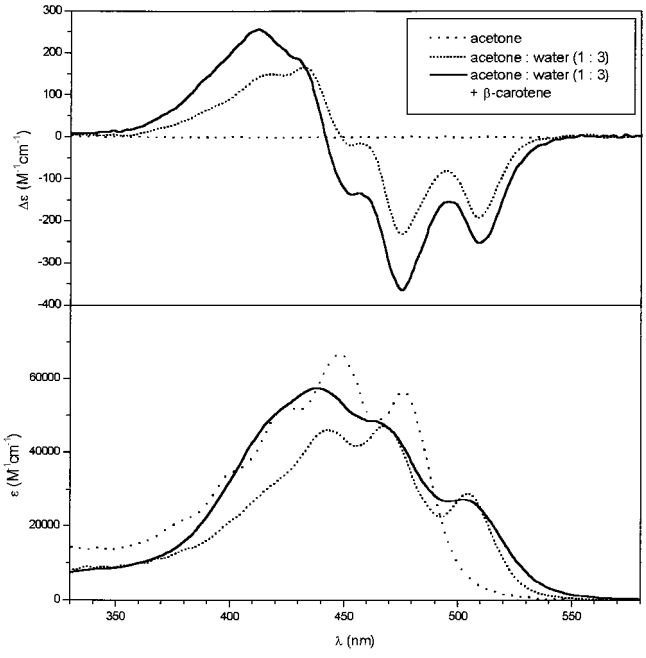


**Fig. 10.** Amplified Cotton effects of the aggregate of (6′*S*)-capsantholon (**3**) by β-carotene (**16**) (from Ref. 40 by permission from Elsevier Science Journals).

of the well-defined isobestic point that might suggest an equilibrium between two species, with the excess of the first being transformed in time to an excess of the second. Since the A value in Figure 6 (at 2 h) is less than half of that of (6′*R*)-capsantholon (Fig. 4), it is reasonable to assume external and internal compensations of Cotton effects; on the one hand, by simultaneous formation of both π and μ types of aggregates, as well as their alignment resembling Figure 1e on the other. As the slow transformation suggests, there might be some delay in the formation of intermolecular hydrogen bonds between the neighboring molecules that slightly changes the overlay angle, giving preference to the μ-type Cotton effects.

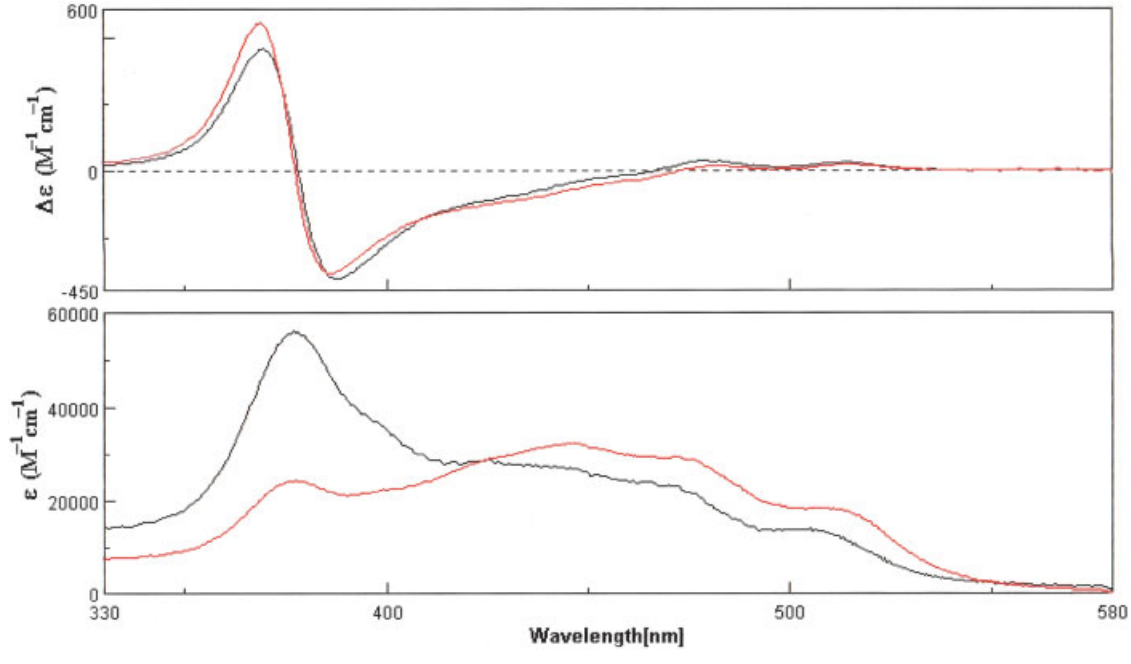


**Fig. 11.** (6′*R*)-Capsantholon aggregate developed by aqueous dilution of solutions in acetone in the absence (black) and in the presence (red) of equimolar amounts of β-carotene; measurements were taken after 160 min.

Fig. 11 live 4/C

As shown below, the closest approach of molecules requires a nonzero overlay angle. The complexity of transformation may be the consequence of a series of Van der Waals interactions as the molecules are adjusted in their mutual vicinity.

Another carotenoid, natural 6′*R*-cryptocapsol (**18**) differs from (3*S*,6′*R*)-capsanthol (**6**) in lacking the 3-OH group only. The consequence is the missing chiral preference in the population of torsion angles around the C(6)–C(7) bond that results in a low intensity for the exciton couplet.[47] As shown in Figure 7, both blue- and red-shifts occur in the UV/VIS spectrum upon aqueous dilution and the CD spectrum corroborates the presence of both types of aggregates.

Of the two types of aggregates, the card-pack structure has higher stability, as demonstrated by the simultaneous development of both types from a mixture of capsanthol 6′-epimers (**4, 6**). The card-pack aggregate incorporates the head-to-tail assembly to an approximately equimolar extent,[47] as seen in Figure 8. It seems to indicate that head-to-tail-forming (6′*S*)-capsanthol is inserted in such a way as to find only card-pack-structured (6′*R*)-capsanthol neighbors.

In light of the induction of the card-pack chirality shown in Figure 8, it is tempting to check whether achiral

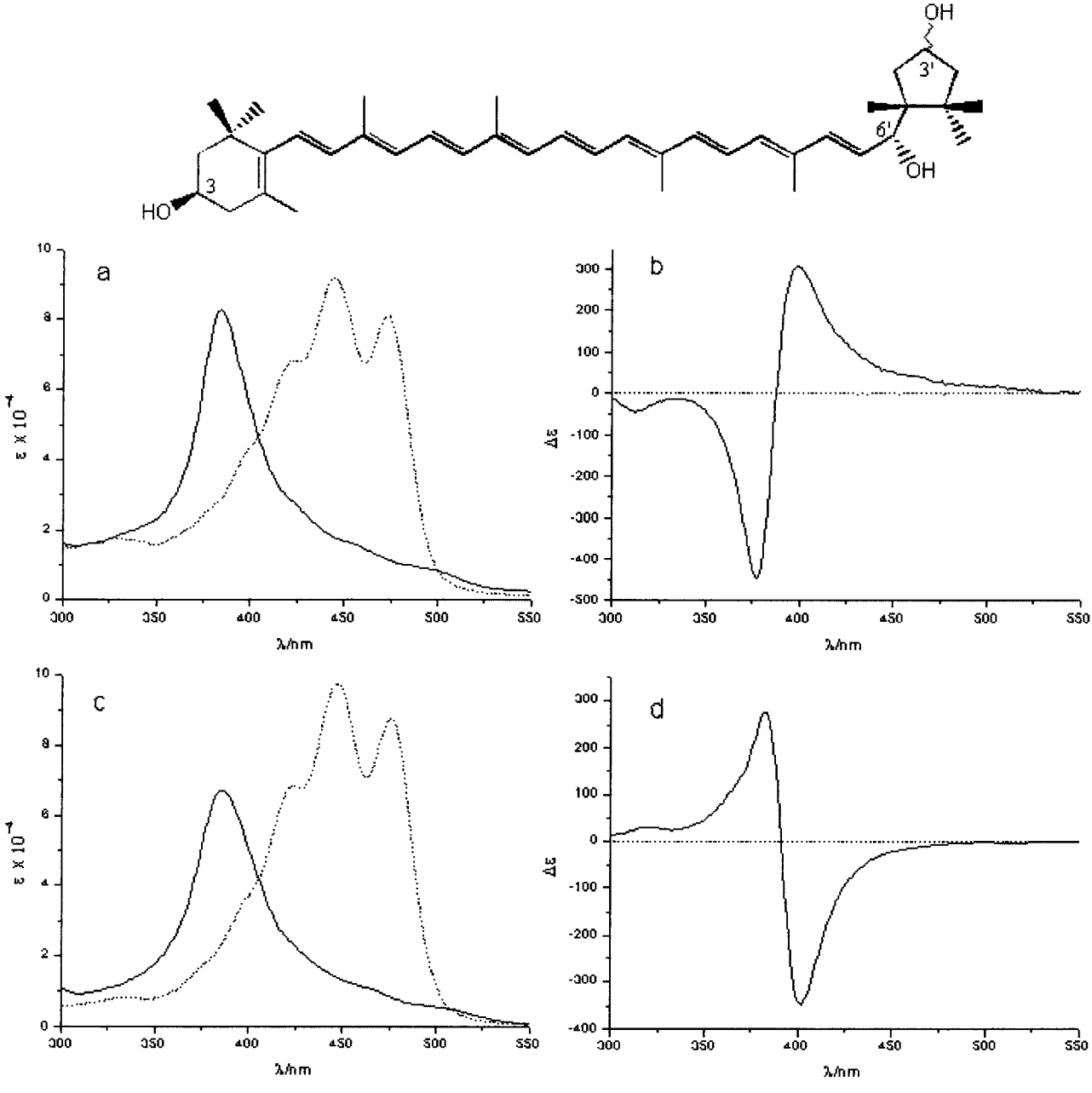


**Fig. 12.** UV/VIS and CD spectra of (6′*R*)-capsanthol-3′-epimers (**6, 8**). Dotted lines: molecular spectra (in ethanol), solid lines: spectra of aggregates **a,b:** 3′*R*-(**8**). **c,d:** 3′*S*-(**6**). Spectra recorded immediately (**a–c**) (independent of time), aggregate spectrum recorded after 2 h (**d**); cf. Fig. 6. (From Ref. 47.)

molecules could incorporate into chiral supramolecular structures. Natural β-carotene (**16**) is achiral and forms a mixture of J- and H-type aggregates in aqueous acetone,[40] as seen in the UV/VIS spectra (Fig. 9). There is no CD band, obviously as a consequence of the haystack character of the aggregate providing compensation by the statistically distributed overlay angles.

By simultaneous development of aggregates in the presence of β-carotene, the Cotton effects of the (6′*S*)-capsantholon (**3**) head-to-tail assembly become more intense at both the short and long wavelength sides of the spectrum.[40] This indicates that the J-type aggregate of (6′*S*)-capsantholon is stable enough to provide a template for β-carotene incorporation. At the same time the mixed character of β-carotene self-aggregates is retained (Fig. 10).

On the other hand, a similar experiment with (6′*R*)-capsantholon demonstrates that the card-pack aggregate can hardly incorporate β-carotene even after 160 min (Fig. 11). This emphasizes the compact character of card-pack organizations into which the hydrocarbon β-carotene is not readily able to enter. Obviously, β-carotene lacking hydroxyl groups cannot interfere with the secondary bonds holding neighboring (6′*R*)-capsantholon molecules together in the card-pack assembly, while such a resistance is not an attribute of the (6′*S*)-capsantholon head-to-tail organization.

The influence of configuration was tested further by investigations of (6′*R*)-capsanthol (**6, 8**) and (6′*S*)-capsanthol (**4, 7**) 3′-epimers. Again the 6′-center proved to be decisive in determining the type of aggregate, i.e., (3′*R*,6′*R*)-capsanthol (**8**) aggregated in card-pack fashion, while (3′*R*,6′*S*)-capsanthol (**7**) formed a head-to-tail assembly.[47] The 3′-epimers of (6′*S*)-capsanthol (**4, 7**) gave identical CD spectra resembling Figure 5, unlike the card-pack-forming 3′-epimers of (6′*R*)-capsanthol (**6, 8**), as shown in Figure 12.

Although (3′*S*,6′*R*)-capsanthol aggregate (Fig. 12d) builds up in a time-dependent fashion (as shown in Fig. 6), the almost exact mirror image-related spectra of card-pack aggregates (Fig. 12b,d) offer the possibility of checking their ability of compensate each other. To this end, aqueous dilution was made on the mixture of the two epimers in ethanol, allowing the aggregates to develop simultaneously. The result is shown in Figure 13.

The time-dependent extinction of Cotton effects due to supramolecular organization reflects the instantaneously formed right-handed aggregate (characteristic of (3′*R*,6′*R*)-capsanthol, **8**) and the simultaneous existence of time-dependent inversion from π to μ aggregate (resembling (3′*S*,6′*R*)-capsanthol, **6**).[48] It seems to be a strong indication of the coexistence of aggregates in mirror-image relation; hence, these card-pack structures have remarkable stability. This can be contrasted with the head-to-tail organization by the same experimental setup; (3′*S*,6′*S*)-capsanthol (**4**) instantaneously forms a left-handed aggregate, while its 3,3′-diacetate (**5**) produces a right-handed head-to-tail assembly that increases exciton intensity during the first hour. Their mixture gives an aggregate showing immediate compensation of supramolecular Cotton effects,[48] indicating that the weaker organization does not provide stability for the characteristic assemblies of component molecules (Fig. 14).

The above mixed aggregates may be characterized by crystallographic analogs: Figure 13 refers to the slow formation of a conglomerate type of mixture of aggregates, while Figure 14 resembles the rapid development of a racemic crystal.

## ROLE OF FREE HYDROXYL GROUPS

As the preparation of selectively acetylated carotenoids has been accomplished[38] (3*S*,6′*R*)-capsanthol (**6**), the card-pack-forming carotene-triol became a suitable model

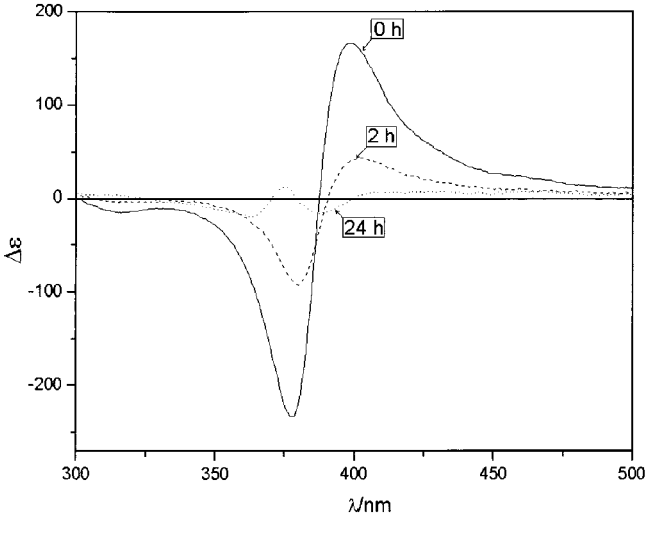


**Fig. 13.** Time-dependent CD of aggregates developed simultaneously from the equimolar mixture of **6** and **8** (from Ref. 48).

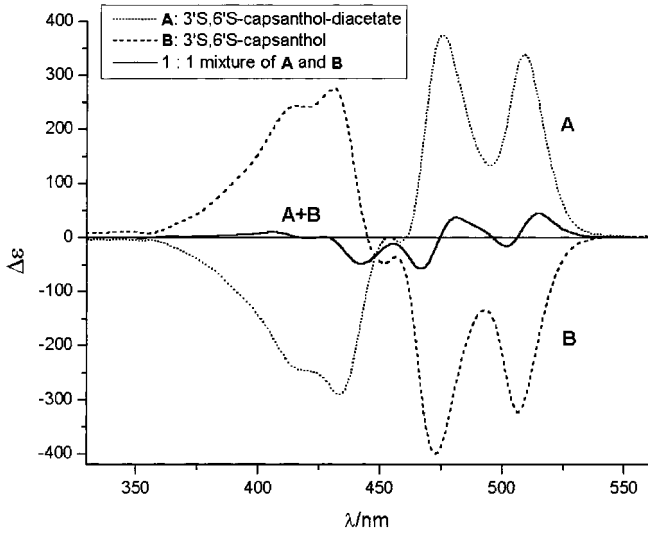


**Fig. 14.** Immediate extinction of Cotton effects due to supramolecular chirality; **A:** time-dependent; **B:** independent of time; **A+B:** equimolar mixture (independent of time) (from Ref. 48).

for studying the dependence of supramolecular organization on the structure of carotenoid monomers.[39] The various types of aggregates developed from monoacetates are shown in Figure 15.

Both 3′- and 6′-monoacetates organize into card-pack structures, but the 3-monoacetate forms a head-to-tail assembly. Hence, the loss of free OH at the β-ring prohibits card-pack formation. Figure 16 displays the spectra of aggregates from diacetates (**12–14**) and the triacetate (**15**).

As seen, only head-to-tail aggregates are formed from diacetates and the triacetate. In these compounds, at least one end of the molecule is devoid of free OH group(s). Thus, it can be concluded that whenever the free OH is converted to acetate at either end of the molecule, the supramolecule created by aqueous dilution is of a head-to-tail character. It complements the observation made on 6′*R*-cryptocapsol (**18**, cf. Fig. 7), strengthening the role of intermolecular hydrogen bonds[45] in structuring the card-pack aggregate.

In order to have a better insight into the formation of head-to-tail organization, 3,3′-diester structures have been compared. The zeaxanthin-3,3′-diacetate spectra[47] (**17**) are similar to those of capsanthol-3,3′-diacetates (Figs. 13, 15). Interestingly, the length of the esterifying acid played a role, as found for lutein-3.3′-diesters (**20, 21**). Although both are of head-to-tail organization, the aggregate of lutein-3,3′-dipalmitate showed Cotton effects of higher intensity, indicating increased chiral recognition and a somewhat more dense structure than the –3,3′-diacetate,[39] as the slightly blueshifted spectra of the former demonstrate (Fig. 17).

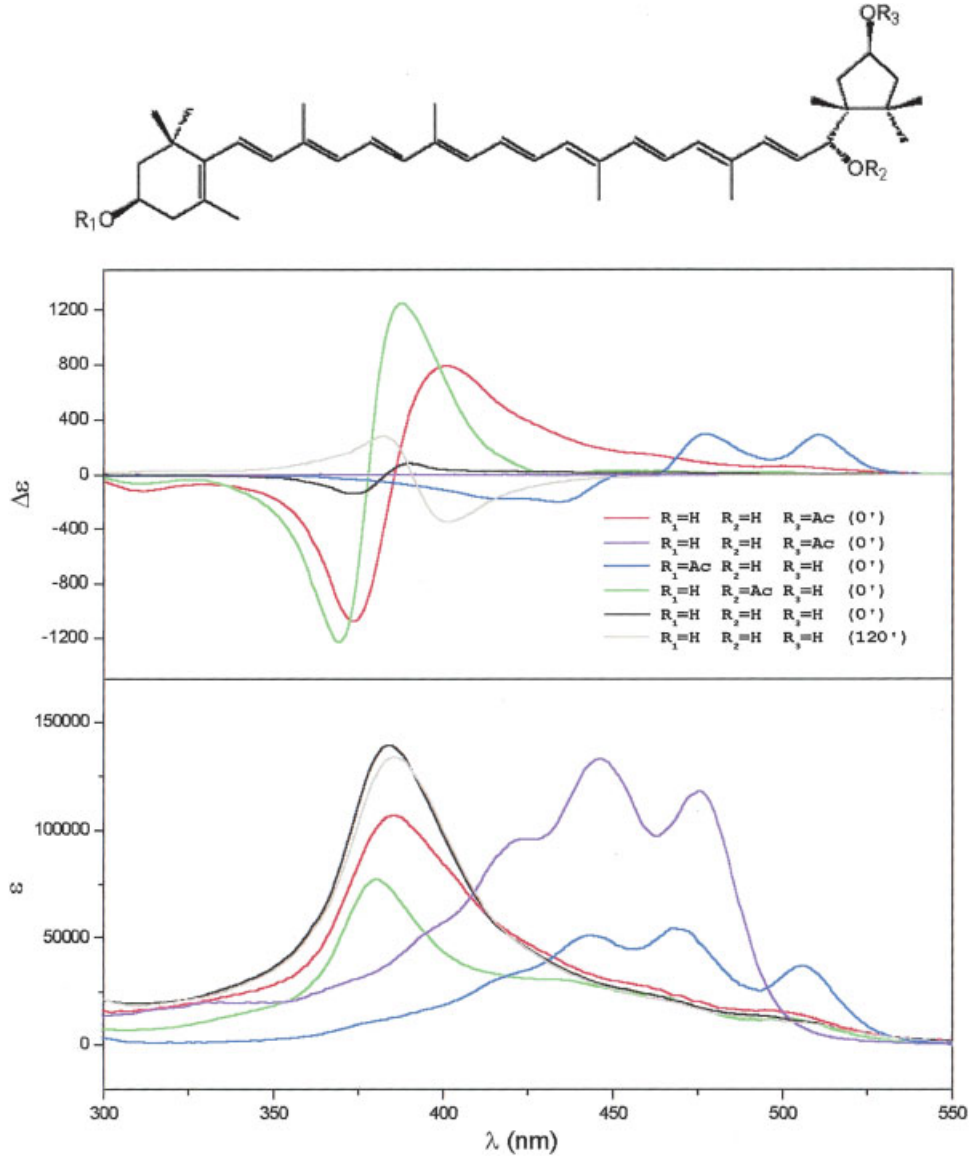


**Fig. 15.** UV/VIS and CD spectra of 3′*S*,6′*R*-capsanthol monoacetate aggregates: red, **9**; green, **10**; blue, **11**; molecular spectra: purple (from Ref. 39).

Fig. 15 live 4/C

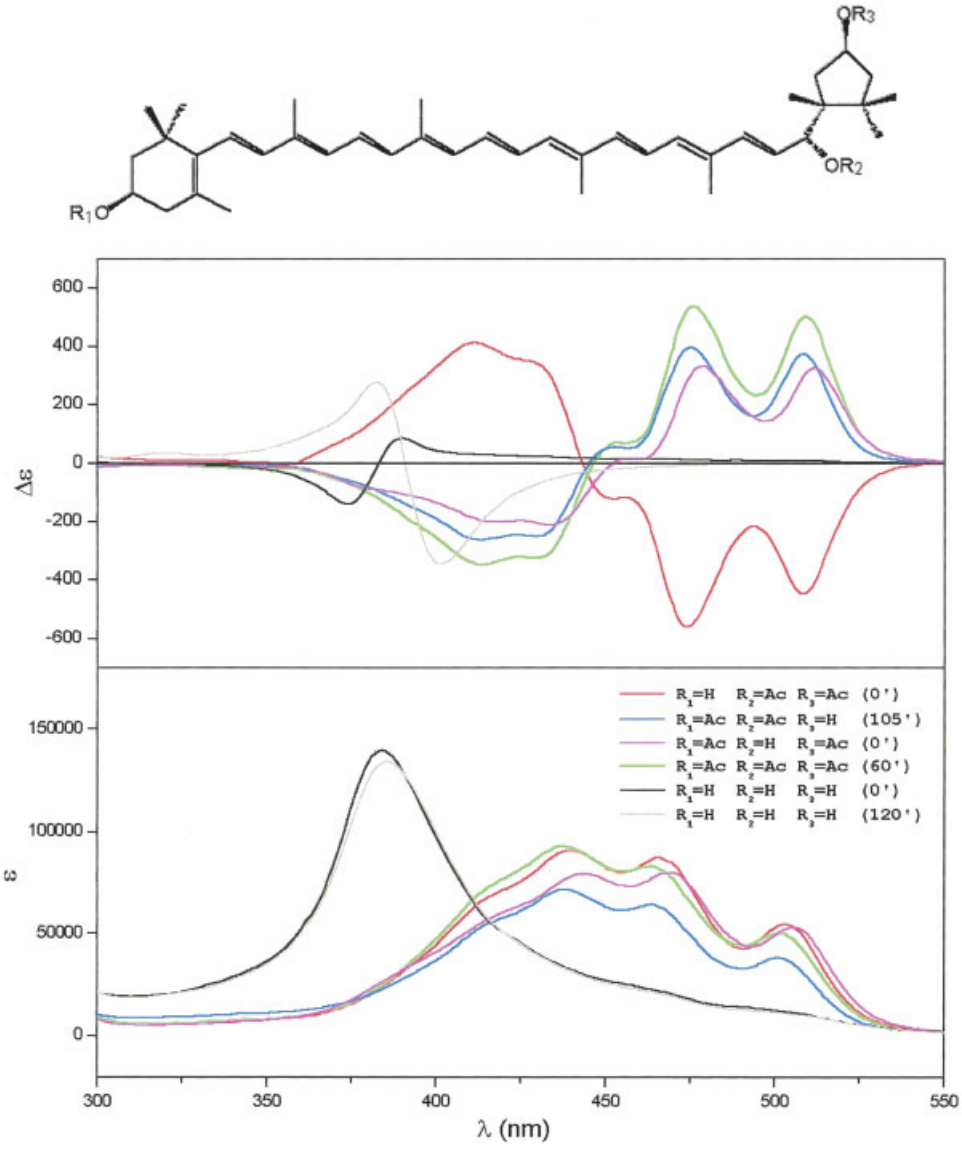


**Fig. 16.** UV/VIS and CD spectra of aggregates from 3*S*,6′*R*-capsanthol diacetates (blue, **12;** purple, **13;** red, **14**) and triacetate (green, **15**) (from Ref. 39).

Obviously, the long aliphatic chain reinforced chiral recognition in the self-assembly. This is also an example where the Cotton effect intensity of the head-to-tail aggregate is commensurate with that of the card pack.

Finally, there is a case where Cotton effects of the aggregate formed by a compound with free hydroxyl groups is weak. Figure 18 shows the spectra of aggregates of capsorubin (**22**) and of its 3,3′-diacetate (**23**). Whereas the card-pack character of the former is still more pronounced (slightly blueshifted bands), the Cotton effect intensity of the diacetate is several times higher. Besides, the diacetate supramolecule has a definite card-pack contribution. It certainly testifies that card-pack formation is not restricted to those carotenoids having free hydroxyl groups at both ends of the monomer molecules. In other words, close contacts are not confined to hydrogen bonding.[39]

## MORPHOLOGY OF CAROTENOID AGGREGATES

Morphology was studied in a pair of carotenoids, lutein (**19**) and lutein diacetate (**20**), that produce card-pack and head-to-tail self-assemblies, respectively. Again, the compound containing hydroxyl groups at both ends of the molecule formed the card-pack aggregate. Both types of aggregates produced in the liquid phase could be transformed into thin films that essentially retained their chiral organization on a quartz surface.[49] Morphology of the aggregates was revealed by atomic force microscopy. An image of the card pack is shown in Figure 19, and that of the head-to tail organization is shown in Figure 20.

Fig. 16 live 4/C

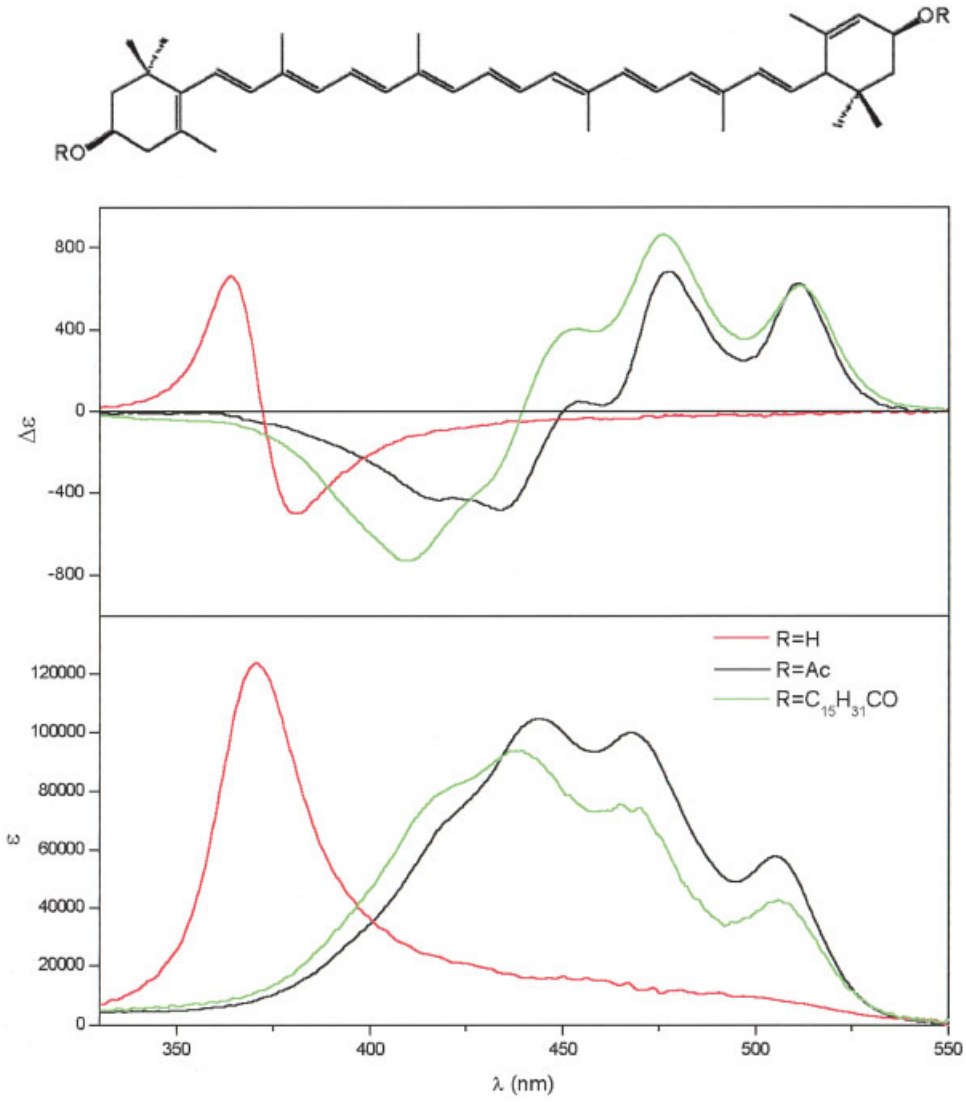


**Fig. 17.** Spectra of aggregates of lutein (**19,** red) and lutein diesters (**20,** 3,3′-diacetate, black; **21,** 3,3′-dipalmitate, green) (from Ref. 39).

The card-pack aggregate is concentrated into longitudinal rock-like structures that do not fill the screen (Fig. 19). In contrast, the head-to-tail assembly fills the screen by displaying a fiber-like texture. The images are in agreement with the closely packed organization of the card pack and the loosely attached character of the head-to-tail aggregates.

## PROPOSED MODELS FOR CAROTENOID AGGREGATES

Since carotenoid aggregates are mainly identified by their spectral properties, any effort at visualization should account for the structural reasons providing chiral discrimination of overlay angles. The shape of a carotenoid molecule can be characterized by its Conolly surface, defined by the closest approach of solvent molecules. This has been calculated for lutein (**19**) and is shown in Figure 21.

Due to the bulky end groups, carotenoid molecules cannot closely approach each other by parallel organization. Calculations revealed the interrelation between the distance in dimers and their overlay angle at energy minima, as given in Table 1.

In card-pack aggregates the closest approach allowed by the bulky end groups is combined with the lowest interface of dimers with the aqueous solvent. The following structures have been considered for 3*S*,6′*R*-capsanthol dimers (Fig. 22). As seen, the minimal surface is associated with an overlay angle of $\sim -20°$.

The high intensity of exciton spectra indicates the excess of one of the enantiomeric formations, but they are not characteristic of the details of organization; hence, a general model for the aggregates cannot be put forward. Nevertheless, on the basis of the collected experimental evidence an attempt was made to propose[39] the following model for the aggregate of 3′*S*,6′*R*-capsanthol (**6**): a continuous chain of strong hydrogen bonds connect the κ-ends

Fig. 17 live 4/C

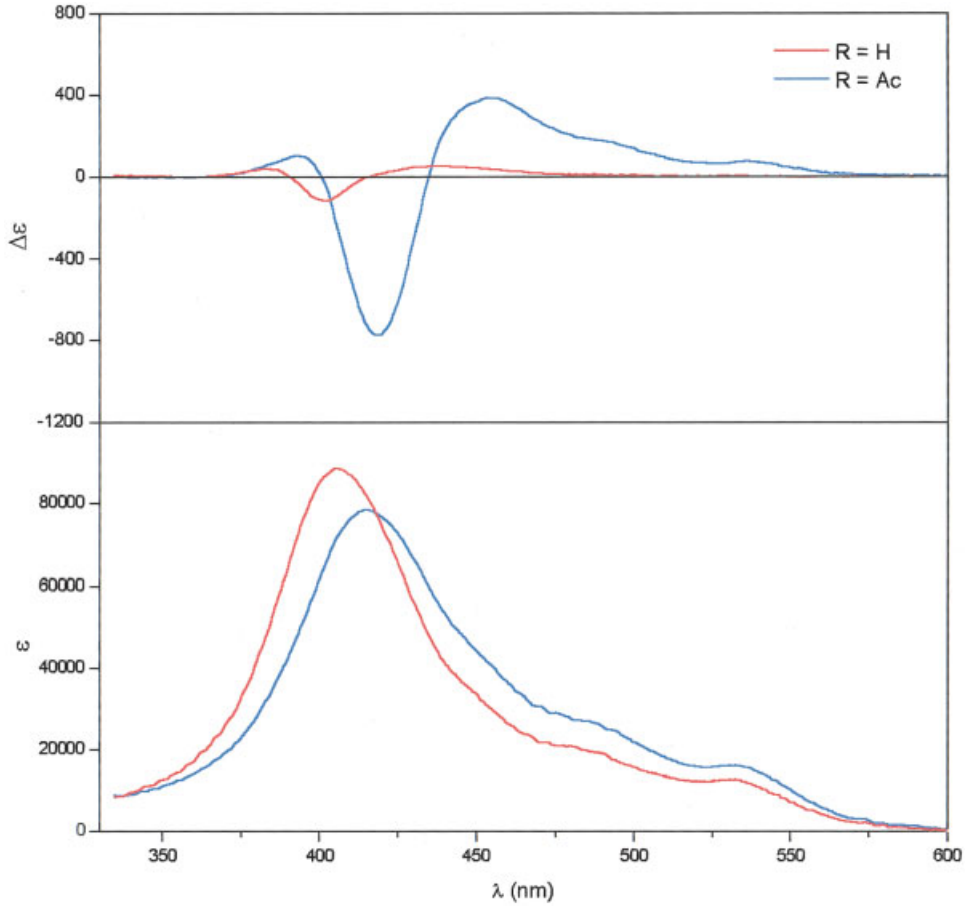


**Fig. 18.** UV/VIS and CD spectra of aggregates of capsorubin (**22,** red) and of capsorubin-3,3′-diacetates (**23,** blue) (from Ref. 39).

of the molecules (adjusted by an overlay angle of −20°) according to the following sequence:

$$(3' - \mathrm{OH})_{\text{1st molecule}} \rightarrow (6' - \mathrm{OH})_{\text{2nd molecule}} \text{---}$$

$$(3' - \mathrm{OH})_{\text{2nd molecule}} \rightarrow (6' - \mathrm{OH})_{\text{3rd molecule}} \text{---}$$

while the β-ends are aligned by weaker interactions (Fig. 23). The model expresses that molecules in the card-pack aggregate are both directly connected and oriented by their neighbors, providing the closely packed structure corresponding to both the lack of vibrational structure in the CD spectra and the compact morphology revealed by atomic force microscopy.

Although the resolution of the atomic force microscopic image (Fig. 19) is not at the molecular scale, it may be suggested that the helically organized molecules shown in Figure 23 associate further to form longitudinal bundles satisfying the observed morphology.

A model for the head-to-tail aggregate should be sought as a looser type of organization in which supramolecular chirality is created indirectly. The self-assembly providing movement and vibration to individual molecules is envisaged to form disk-like layers within which the parallel orientation of molecules provides neither close contact nor chiral discrimination. The ordering of molecules in the layers may be essentially two-dimensional, like a fish-bone organization.[50] The layers are pressed together by the strength of water structure so that they associate to form a long fibrous morphology, in accordance with the atomic force microscopic image (Fig. 20). On the basis of the intensity difference in CD exciton bands of lutein-3,3′-diacetate (**20**) and -dipalmitate (**21**), it is proposed that chirality is induced by the twist of neighboring layers directed by the orientation of 3 and 3′ centers to which acid sidechains are attached[39] (Fig. 24).

The envisaged morphology of the head-to-tail aggregate is similar to that of liquid crystals.[49]

## BIOLOGICAL IMPORTANCE OF CAROTENOID SELF-ASSEMBLIES

On bright days, there is a serious imbalance between absorbed energy and the photosynthetic capacity of plant leaves. Carotenoids in chloroplasts play the important photoprotective role of nonradiative dissipation of excess energy. Spectroscopic evidence proved that this process is associated with pigment–protein and pigment–pigment association.[51] The main carotenoid involved in this role is zeaxanthin (cf. **17**),[52] the aggregation of which produces

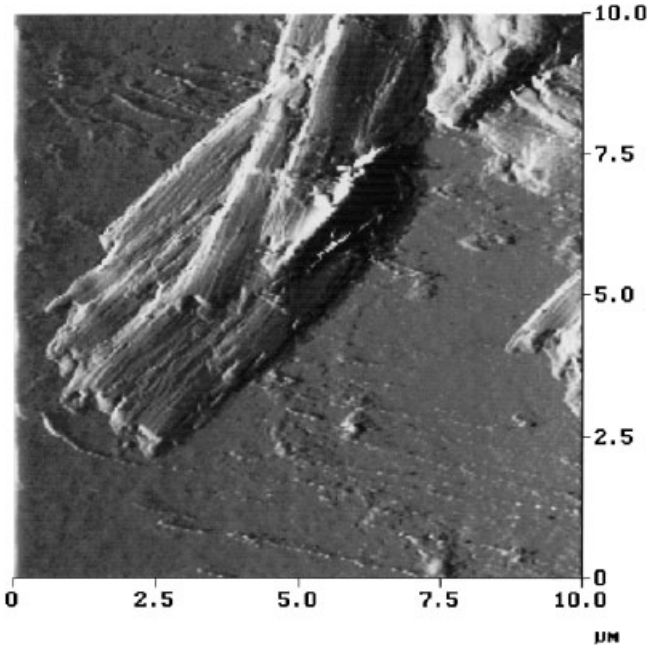


**Fig. 19.** Atomic force microscopic image of the film of a card-pack aggregate of lutein (**19**) on quartz slide (from Ref. 49 by permission of the American Chemical Society).

some head-to-tail structures besides the dominant card-pack aggregates,[53] ensuring light absorption in a wide spectral range.

Carotenoids are also available in chromoplasts.[54] The isolation of natural dyes, however, should affect the conditions of how these molecules are embedded in their

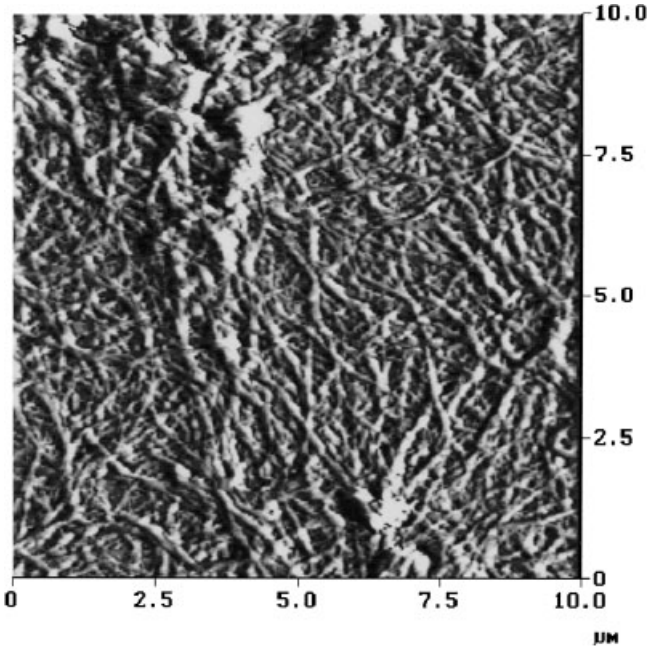


**Fig. 20.** Atomic force microscopic image of the film of a head-to-tail aggregate of lutein diacetate (**20**) on quartz slide (from Ref. 34 by permission of the American Chemical Society).

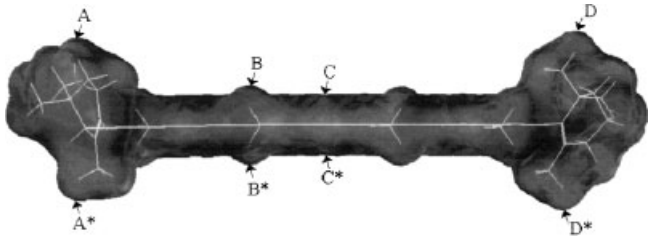


**Fig. 21.** Connolly surface of lutein (**19**); marked distances are, as follows: A↔A*, 8.2 Å; B↔B*, 4 Å; C↔C*, 3 Å; D↔D*, 9 Å; (from Ref. 34 by permission of the American Chemical Society).

environs. Hence, supramolecular organization in nature is best studied in original tissue. Since carotenoids are ubiquitous in the yellow petals of flowers, it was of interest to perform spectroscopic studies on living flowers. Indeed, CD spectra could be recorded on the petals of various species containing tubulous chromoplasts.[55] The Cotton effects were sometimes quite strong. It came as a surprise that these spectra were very similar to those of head-to-tail aggregates prepared in vitro in sample tubes. Figure 25 demonstrates the similarity.[47]

The optical activity provided by carotenoid self-assemblies may play an important role in plant life. The polarized pattern of light reflected by the petals is sensed by the compound eyes of insects performing fertilization. In this context, it may not be accidental that the structured spectral signal of the head-to-tail aggregate serves to perform the task, since it offers better recognition of the plant species than the sharp, unstructured spectrum of a card-pack aggregate would do.

## STUDY OF PROTEIN-BINDING SITES

The several hundred- to thousand-fold amplification of CD spectral intensity of molecular chirality brought about by intermolecular organization is highly tempting to develop sensitive probes for biological samples of low concentration. A possible application is an investigation of the topology of multiple protein binding sites. Indeed, a special case of chiral intermolecular interaction of carotenoid molecules is the binding of crocetin (**25**), a polyene dicarboxylic acid, to human serum albumin (HSA).[56] Crocetin occupies the fatty acid binding sites, of which six are crystallographically determined.[57] Although crocetin is achiral, the CD spectrum of crocetin bound to albumin is typical of the exciton type, indicating the interaction of at least two molecules, as shown in Figure 26. Here, intermolecular organization governed by the protein structure generates chirality.

In order to find out the position of the crocetin molecules responsible for exciton interaction, the X-ray

**TABLE 1. Optimal overlay angles (ω) of dimers at given distances (δ) between lutein molecules[49]**

| δ (Å) | 3.50 | 4.50 | 5.00 | 5.50 | 6.00 | 6.50 |
|---|---|---|---|---|---|---|
| ω (Degrees) | −36 | −35 | −23 | −18 | −17 | −15 |

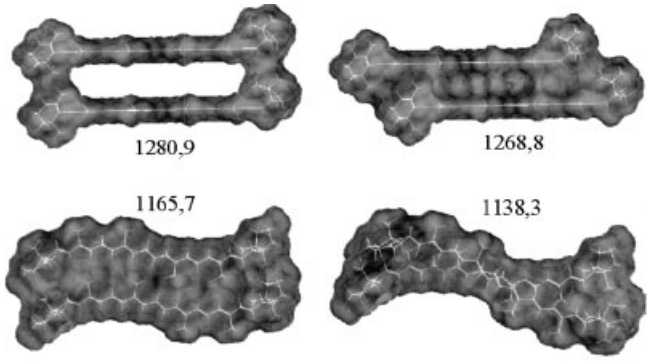


**Fig. 22.** Surface of 3′*S*,6′*R*-capsanthol dimers given in Å$^2$ for different geometries.

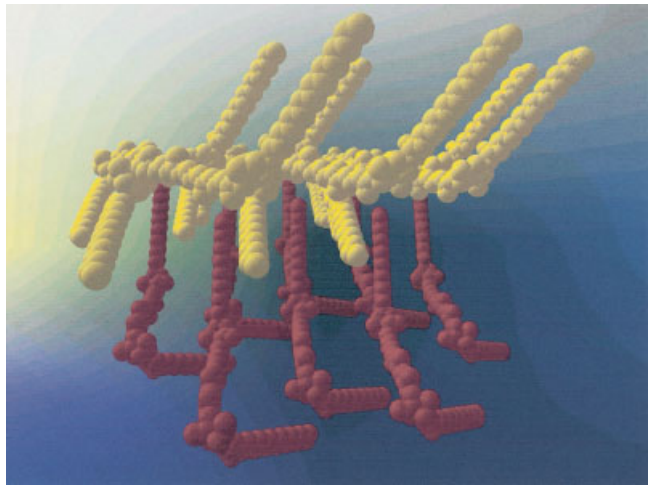

**Fig. 24.** Molecular model proposed for the head-to-tail aggregate on the basis **21;** neighboring layers labeled by different colors are twisted by ω ~+30° due to the 3 and 3′ centers. The palmitate sidechains intercalate into the matrix of the upper layer (from Ref. 39).

structure of HSA saturated by palmitic acid[57] was used to localize bound crocetin molecules (Fig. 27).

An analysis of the HSA structure (Fig. 27) and the μ-signed (left-handed) exciton couplet (Fig. 26) led us to identify sites 3 and 4, the occupation of which being responsible for the induced CD spectrum. The mutual positions of these crocetin molecules together with interacting amino acid sidechains are shown in Figure 28.

Although homology among albumins of different mammalian species is high,[58] their binding site topology is different. The above method is sensitive enough to detect minute changes. Crocetin molecules bound to porcine albumin produce a right-handed exciton couplet, while other species do not give exciton signals at all, reflecting slight differences in the location of fatty acid-binding sites.[59]

## CONCLUSION

Exciton-coupled Cotton effects of very high intensity are generated by the interaction of independent molecules that are located in their mutual vicinity in such a way that the polarization directions of their electric dipole transition moments form an overlay angle with dominant sign. This sign indicates that the positions of neighboring molecules escape complete compensation—external and internal alike, which occurs only partially or not at all. This is the consequence of either molecular chirality of the associated molecules, the Cotton effects of which become substantially amplified by the supramolecular organization, or the topology of protein-binding sites that can induce CD spectra by proper positioning of achiral molecules. We suggest here the term "supramolecular exciton

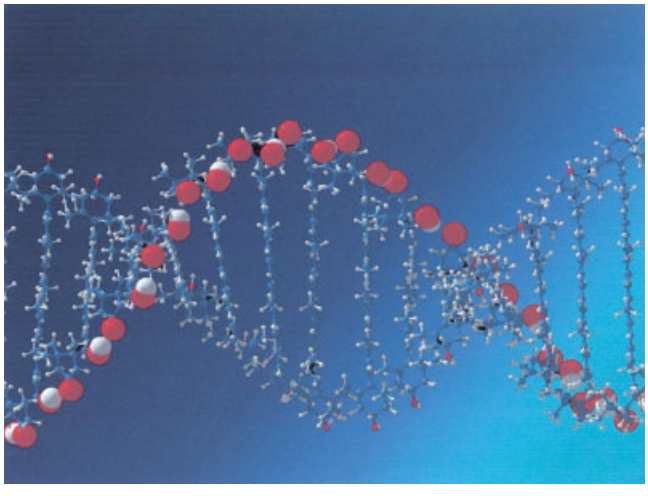

**Fig. 23.** Molecular model of the card-pack aggregate of **6;** enlarged oxygen (red) and hydrogen (white) atoms form a continuous chain of hydrogen bonds (from Ref. 39).

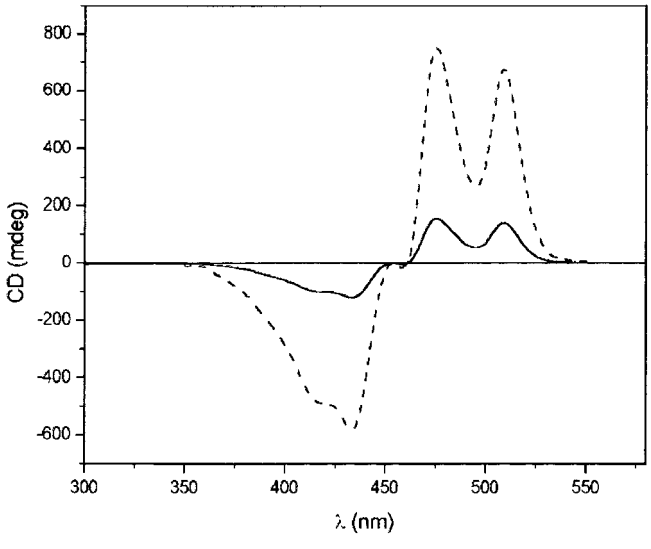


**Fig. 25.** CD spectra of *Caragana arborescense* living petal (solid line) and 3′*S*,6′*S*-capsanthol-3,3′-diacetate (**5,** dashed line).

Figs. 23 & 24 live 4/C

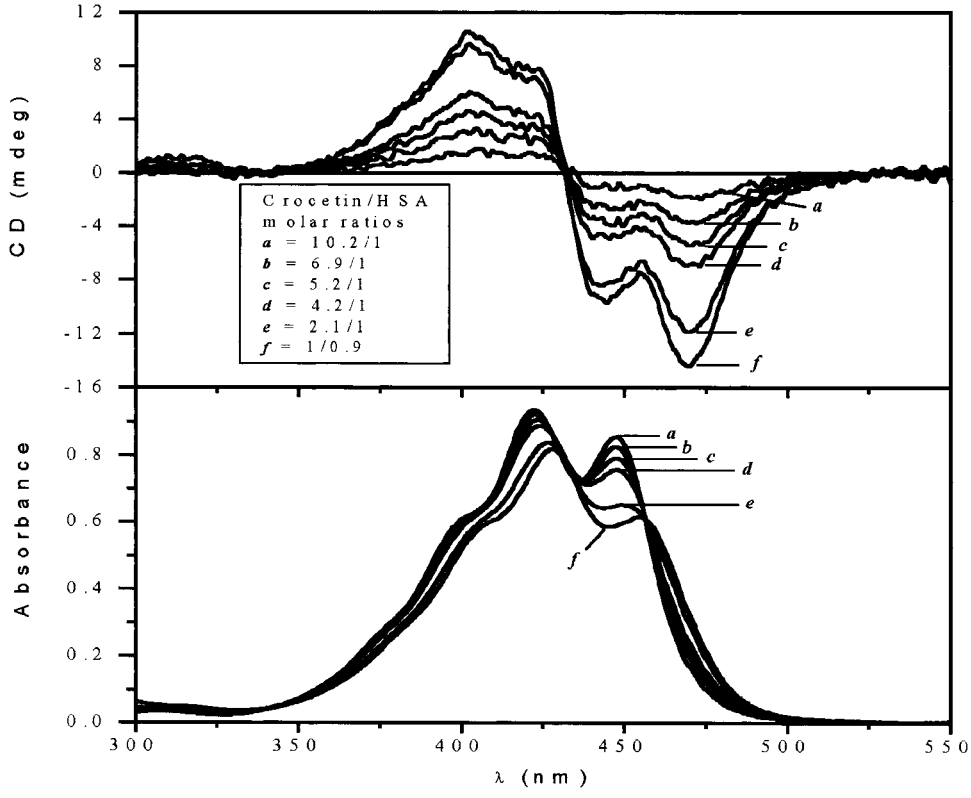


**Fig. 26.** UV/VIS and CD spectra of the crocetin-HSA complex (from ref. 56 by permission from Elsevier Science Journals).

chirality" for the above phenomena. This term refers to both the origin (chiral organization of molecular elements of supramolecules) and the method of observation (exciton coupling). The single molecule is not a closed box for excitation of a chromophore and exciton resonance does not *qualitatively* distinguish covalently attached chromophores from those of neighboring molecules. *Quantitative* differences to established rules of exciton chirality emerge, however, for centrally overlaying chromophores representing overlay angles of opposite sign. Further studies of supramolecular exciton chirality may shed light on intermo-

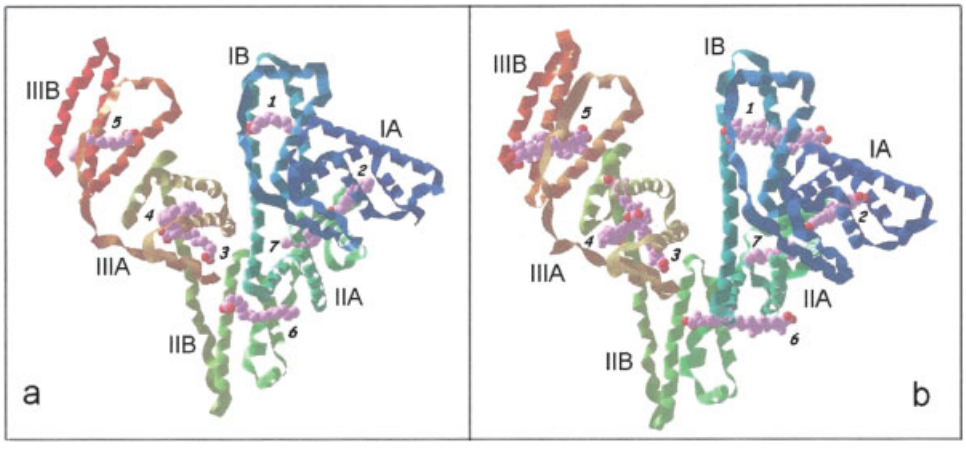


**Fig. 27.** X-ray crystallographic structure of HSA complexed with palmitic acid (**a**). Ligand molecules in space-filling mode are colored (carbon, pink; oxygen, red). **b:** Structure of crocetin–albumin complex by fitting six crocetin molecules to palmitic acid binding sites 1–6 (from ref. 56 by permission from Elsevier Science Journals).

Fig. 27 live 4/C

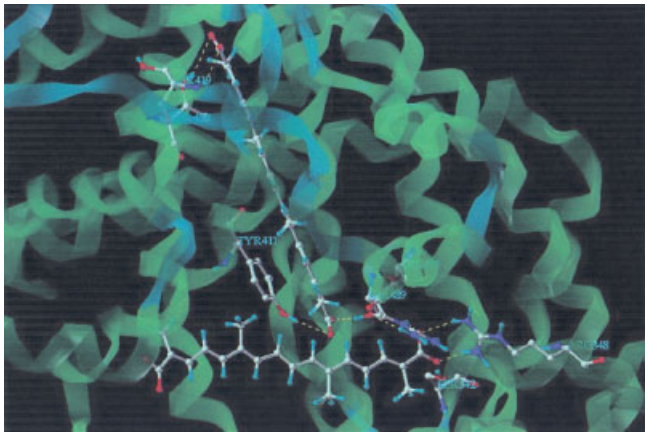

**Fig. 28.** Crocetin molecules docked to fatty acid-binding sites 3 and 4 of HSA. Amino acid sidechains stabilizing the position of bound crocetin molecules via hydrogen bonds are indicated (from ref. 56 by permission from Elsevier Science Journals).

lecular interactions and contribute to characterization of the secondary chemical bonds that are still the Cinderella of present-day concepts.

## ACKNOWLEDGMENTS

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## LITERATURE CITED

1. van't Hoff JH. Voorstel tot uitbreiding der tegenwoordig in de scheikunde gebruikte struktuurtformules in de ruimte; benevens een daarmee samenhangende opmerking omtrent het verband tusschen optisch actief vermogen en chemische constitutie van organische verbindingen. (A suggestion on the extension into space of the structural formulas at present used in chemistry; a note on the relation between the optical activity and the chemical constitution of organic compounds.) Utrecht: J. Greven; 1874.
2. Boiadijev SE, Lightner DA. Optical activity and stereochemistry of linear oligopyrroles and bile pigments. Tetrahedron Asymmetry 1999; 10:607–655.
3. Blauer G, Harmatz D, Snir J. Optical properties of bilirubin-serum albumin complexes in aqueous solution. I. Dependence on pH. Biochim Biophys Acta 1972;278:68–88.
4. Simonyi M. The concept of chiral conformers and its significance in molecular pharmacology. In: Testa B, Meyer UA, editors. Advances in drug research, vol. 30. London: Academic Press; 1997. p 73–110.
5. Salares VR, Young NM, Carey PR, Bernstein HJ. Excited-state (exciton) interactions in polyene aggregates. Resonance Raman and absorption spectroscopic evidence. J Raman Spectr 1977;6:282–288.
6. Jelley EE. Spectral absorption and fluorescence of dyes in the molecular state. Nature 1936;1009–1010.
7. Kasha M. Energy-transfer mechanisms and the molecular exciton model for molecular aggregates. Radiat Res 1963;20:55–71.
8. Kasha M, Rawls HR, El-Bayoumi MA. The exciton model in molecular spectroscopy. Pure Appl Chem 1965;11:371–392.
9. Davydov AS. Theory of molecular excitons. Translated by Kasha M, Oppenheimer M Jr. New York: McGraw Hill; 1962.
10. Harada N, Nakanishi K. A method for determining the chiralities of optically active glycols. J Am Chem Soc 1969;91:3989–3991.
11. Harada N, Nakanishi K. The exciton chirality method. Acc Chem Res 1972;5:257–263.
12. Harada N, Nakanishi K. Circular dichroism spectroscopy: exciton coupling in organic stereochemistry. Mill Valley, CA: University Science Books; 1983.
13. Berova N, Nakanishi K. Exciton chirality method: principles and application. In: Berova N, Nakanishi K, Woody RW, editors. Circular dichroism. Principles and applications. New York: John Wiley & Sons; 2000. p 337–382.
14. Harada N, Chen SL, Nakanishi K. Quantitative definition of exciton chirality and the distant effect in the exciton chirality method. J Am Chem Soc 1975;97:5345–5352.
15. Heyn MP. Dependence of exciton circular dichroism amplitudes on oscillator strength. J Phys Chem 1975;79:2424–2426.
16. Hoshino T, Matsumoto U, Goto T. Evidences of the self-association of anthocyanins. I. Circular dichroism of cyanine anhydrobase. Tetrahedron Lett 1980;21:1751–1754.
17. Hoshino T, Matsumoto U, Harada N, Goto T. Chiral exciton coupled stacking of anthocyanins: interpretation of the origin of anomalous CD induced by anthocyanin association. Tetrahedron Lett 1981;22: 3621–3624.
18. Buchwald M, Jencks WP. Optical properties of astaxanthin solutions and aggregates. Biochemistry 1968;7:834–843.
19. Hettich C, Schmitt C, Zitzmann J, Kühn S, Gerhardt I, Sandoghdar V. Nanometer resolution and coherent optical dipole coupling of two individual molecules. Science 2002;298:385–389.
20. Klyne W, Prelog V. Description of steric relationships across single bonds. Experientia 1960;16:521–523.
21. Simonyi M. Recognition of chiral conformations of the achiral neurotransmitter, gamma-aminobutyric acid. Enantiomer 1996;1:403–414.
22. Tsang KY, Diaz H, Graciani N, Kelly JW. Hydrophobic cluster formation is necessary for dibenzofuran-based amino acids to function as β-sheet nucleators. J Am Chem Soc 1994;116:3988–4005.
23. Hildebrand JH. Is there a "hydrophobic effect"? Proc Natl Acad Sci USA 1979;76:194.
24. Pratt LR, LaViolette RA, Gomez MA, Gentile ME. Quasi-chemical theory for the statistical thermodynamics of the hard-sphere fluid. J Phys Chem B 2001;105:11662–11668.
25. Pratt LR. Molecular theory of hydrophobic effects: "She is too mean to have her name repeated." Annu Rev Phys Chem 2002;53:409–436.
26. Dixit S, Crain J, Poon WCK, Finney JL, Soper AK. Molecular segregation observed in a concentrated alcohol-water solution. Nature 2002;416:829–832.
27. Gottarelli G, Osipov MA, Spada GP. A study of solvent effect on the optical rotation of chiral biaryls. J Phys Chem 1991;95:3879–3884.
28. Buss V, Reichardt C. Chiral dimers of a chiral pentamethine cyanine dye and of an achiral pentamethine cyanine dye in γ-cyclodextrin as chiral host. J Chem Soc Chem Commun 1992;1636–1638.
29. von Berlepsch H, Bottcher C, Ouart A, Burger C, Dahne S, Kirstein S. Supramolecular structures of J-aggregates of carbocyanine dyes in solution. J Phys Chem B 2000;104:5255–5262.
30. Spitz C, Dahne S, Ouart A, Abraham HW. Proof of chiralitys of J-aggregates spontaneously and enantioselectively generated from achiral dyes. J Phys Chem B 2000;104:8664–8669.
31. Kuball HG, Bruning H, Muller T, Turk O, Schonhofer A. Helical twisting power of chiral mono- and bis-aminoanthraquinones. J Mater Chem 1995;5:2167–2174.
32. Kozlov IA, Orgel LE, Nielsen PE. Remote enantioselection transmitted by an achiral peptide nucleic acid backbone. Angew Chem Int Ed 2000;39:4292–4295.
33. Rodger A, Nordén B. Circular dichroism & linear dichroism. Oxford: Oxford University Press; 1997. p 66–75.
34. Lightner DA, Gurst JE. Organic conformational analysis and stereochemistry from circular dichroism spectroscopy. New York: John Wiley & Sons; 2000. p 450.
35. Deli J, Molnár P. Paprika carotenoids: analysis, isolation, structure elucidation. Curr Org Chem 2002;6:1197–1219.
36. Deli J, Ősz E, Molnár P, Zsila F, Simonyi M, Tóth G. Reduction of capsorubin and cryptocapsin. Helv Chim Acta 2001;84:3810–3817.
37. Deli J, Ősz E, Visy J, Zsila F, Simonyi M, Tóth G. Stereoselective

Fig. 28 live 4/C

reduction of "capsanthol-3-ones" by complex hydrides. Helv Chim Acta 2001;84:263–270.

38. Molnár P, Ősz E, Zsila F, Simonyi M, Deli J. Preparation of partially acetylated carotenoids. Helv Chim Acta 2002;85:2349–2357.
39. Bikádi Z, Zsila F, Deli J, Mády G, Simonyi M. The supramolecular structure of self-assembly formed by capsanthin derivatives. Enantiomer 2002;7:67–76.
40. Zsila F, Bikádi Z, Deli J, Simonyi M. Configuration of a single center determines chirality of supramolecular carotenoid self-assembly. Tetrahedron Lett 2001;42:2561–2563.
41. Bucherer R, Noack K. Circular dichroism. In: Britton G, Liaanen-Jensen S, Pfander H, editors. Carotenoids, vol. 1B. Spectroscopy. Basel: Birkhäuser; 1995.
42. Milon A, Wolff G, Ourisson G, Nakatani Y. Organization of carotenoid-phospholipid bilayer systems. Helv Chim Acta 1986;69: 12–24.
43. Matile S, Berova N, Nakanishi K, Novkova S, Philipova I, Blagoev B. Porphyrins: powerful chromophores for structural studies by exciton coupled circular dichroism. J Am Chem Soc 1995;117:7021–7022.
44. Matile S, Berova N, Nakanishi K, Fleischhauer J, Woody RW. Structural studies by exciton coupled circular dichroism over a large distance: porphyrin derivatives of steroids, dimeric steroids and brevetoxin B. J Am Chem Soc 1996;118:5198–5206.
45. Auweter H, Benade J, Betterman H, Beutner S, Köpsel C, Lüddecke E, Martin HD, Mayer B. Association and self-assembly of carotenoids: structure and stability of cartenoid aggregates. In: Mosquera MIM, Galan MJ, Mendez DH, editors. Pigments in food technology. Sevilla: Dep Legal; 1999. p 197–201.
46. Martin HD, Werner T. Modified carotenoids: spectroscopy and conformation of capsorubin and related end group isomers. J Mol Structure 1992;266:91–96.
47. Zsila F, Bikádi Z, Deli J, Simonyi M. Chiral detection of carotenoid assemblies. Chirality 2001;13:446–453.
48. Zsila F, Deli J, Bikádi Z, Simonyi M. Supramolecular assemblies of carotenoids. Chirality 2001;13:739–744.
49. Zsila F, Bikádi Z, Keresztes Z, Deli J, Simonyi M. Investigation of the self-organization of lutein and lutein diacetate by electronic absorption, circular dichroism spectroscopy and atomic force microscopy. J Phys Chem B 2001;105:9413–9421.
50. Heckl WM, Smith DPE, Binnig G, Klagges H, Hänsch TW, Maddocks J. Two-dimensional ordering of the DNA base guanine observed by scanning tunneling microscopy. Proc Natl Acad Sci USA 1991;88:8003–8005.
51. Aspinall-O'Dea M, Wentworth M, Pascal A, Robert B, Ruban A, Horton P. In vitro reconstitution of the activated zeaxanthin state associated with energy dissipation in plants. Proc Natl Acad Sci USA 2002;99:16331–16355.
52. Ruban AV, Pascal AA, Robert B, Horton P. Activation of zeaxanthin is a key event in the regulation of light harvesting. J Biol Chem 2002; 277:7785–7789.
53. Ruban AV, Horton P, Young AJ. Aggregation of higher plant xanthophylls: differences in absorption spectra and in the dependency on solvent polarity. J Photochem Photobiol B 1993;21:229–234.
54. Camara B, Hugueney P, Bouvier F, Kuntz M, Moneger R. Biochemistry and molecular biology of chromoplast development. Int Rev Cytol 1995;163:175–247.
55. Zsila F, Deli J, Simonyi M. Color and chirality: carotenoid self-assemblies in flower petals. Planta 2001;213:937–942.
56. Zsila F, Bikádi Z, Simonyi M. Induced chirality of crocetin binding to human serum albumin: origin and nature. Tetrahedron Asymmetry 2001;12:3125–3137.
57. Bhattacharya AA, Grune T, Curry S. Crystallographic analysis reveals common modes of binding of medium and long-chain fatty acids to human serum albumin. J Mol Biol 2000;303:721–732.
58. Peters T. All about albumin: biochemistry, genetics and medical applications. San Diego: Academic Press; 1996. p 55–83.
59. Zsila F, Bikádi Z, Simonyi M. Further insight into the molecular basis of carotenoid-albumin interactions: circular dichroism and electronic absorption study on different crocetin-albumin complexes. Tetrahedron Asymmetry 2002;13:273–283.