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LINEAR DICHROISM STUDY OF RETINOIDS

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Abstract—Linear dichroism spectra of several retinoids and related polyenes incorporated in stretched polyethylene films were determined. It is suggested that the retinoids are oriented with the plane of the ring parallel to the stretching direction of the film, the long polyene chain being displaced from that direction.

Polarized absorption spectroscopy of organic molecules incorporated in stretched polymer films has become, in the last two decades, a powerful technique for the investigation of polarizations of electronic transitions and of the orientation of solute molecules in these matrices. In our first reports on linear dichroism of molecules incorporated in stretched polyethylene films, we showed that the polarization data of cholestane derivatives possessing the α,β-unsaturated ketone chromophore in different positions of the steroidal skeleton can be analysed by using a model developed by Fraser and Beer.[1a,b] This model defines either a system in which the molecules are uniaxially distributed both around the stretching direction of the polymer and around one of the molecular axes, or a distribution in which a fraction of the incorporated molecules is perfectly oriented in the direction of stretching and the remaining fraction is randomly distributed.[2,3] The close fit between the experimental polarization data and the values calculated using this model allowed us to determine the direction of the electronic transition moments of various chromophores contained in the steroidal skeleton.[1]

We have used the same model, not only for steroids possessing rodlike symmetry, but also for planar elongated molecules which do not possess this symmetry. In this case we assume a partition of molecules between two different phases of the polyethylene, one in which the molecules are randomly distributed, and the other in which they are perfectly oriented with their planes and longitudinal axes parallel to the stretching direction. We have shown that this model is consistent not only with the linear dichroism results, but also with fluorescence polarization data obtained from the same oriented samples.[4]

We have previously suggested that the randomly distributed molecules are contained in the amorphous regions of the stretched film, while the oriented molecules are in the crystalline or paracrystalline domains of the film. It appears that our assumption that solute molecules enter the crystalline region was unbased, since present experimental evidence show that the crystalline region is inaccessible for solute molecules.[5] However, this does not affect the validity of our model, provided that the fraction of oriented molecules is located in other sites of the stretched polyethylene. An experimental support for a partition of the solute molecules between oriented and unoriented regions of the polymer has recently been found, using ESR measurements of spin labelled elongated molecules incorporated in stretched polyethylene films.[6] Thus, we feel justified in using the two-phase distribution model for rodlike molecules, as well as for planar molecules possessing either disc-like or elongated shape.[7]

Although this simple working model which utilizes only one parameter of orientation does not necessarily describe in ultimate detail the actual distribution of molecules incorporated in stretched polyethylene films, it interprets correctly our accumulated linear dichroism and fluorescence polarization data. It takes into consideration the heterogeneous morphology of the stretched polyethylene, and therefore, gives us an insight into the geometrical distribution of molecules incorporated in these oriented films. The main advantage of our method is that it allows dealing with non-symmetrical organic molecules, and thus, to draw information on structures, configurations and conformations of organic molecules. The use of this model also enables the study of molecular association and related phenomena.

Our two-phase model differentiates between molecules which align in different ways in the stretched polymer. Until now, we have described the orientation of two groups of molecules:[4]

(a) *Non-planar elongated molecules of cylindrical symmetry.* These molecules (exemplified by steroids) have one of the three orthogonal axes much longer than the other two (rodlike shape), and are aligned in the oriented fraction with their long axes parallel to the direction of stretching.

(b) *Planar molecules.* These molecules orient themselves with their planes parallel to the direction of stretching. Planar molecules of elongated shape, such as anthracene, have one in-plane orthogonal axis considerably longer than the other, and will be oriented with the long axis parallel to the stretching direction. Planar disc-like molecules such as coronene will be oriented only with their plane parallel to film stretching.

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In addition, there is another group of molecules capable of giving high orientation in the stretched film which cannot be included in either group described above. These molecules possess a planar aromatic ring system to which an extended rigid aliphatic chain is attached and which does not lie in the plane containing the ring. These are exemplified by *p*-alkoxybenzoic acids which are oriented in the film with their planes parallel to the stretching direction, their extended chains being displaced from that direction.[8]

Retinoids also contain a ring system, and an extended chain, but unlike *p*-alkoxybenzoic acids, the chromophore of the retinoids is located in the long chain and not in the ring.

In this paper we report a linear dichroism study of retinoids incorporated in stretched polyethylene films. The results and conclusions might assist in a better understanding of the effect that the unique conformations of these important biological molecules have on their ability to orient in membranes and other organized natural systems.

EXPERIMENTAL

The polyethylene film used was Suprathen 200, supplied by Kalle and Co. AG. The technique for incorporating the absorbing compounds into the polyethylene films has previously been described.[9] Due to the lability of some of the measured compounds, care was taken in preparing the samples in the dark and using O_2-free solns.

The linear dichroic spectra were measured in Cary 118 and UVKON 810 spectrophotometers, using the previously described PNP method.[9] In this method, two successive scans of the absorption spectra are recorded, one with light polarized parallel to the stretching direction of the film ($OD_\|$) and the other with non-polarized light (OD). The dichroic ratio (d_o), defined as the ratio of the absorption intensities for light polarized parallel ($OD_\|$) and perpendicular ($OD_\perp$) to the stretching direction of the film, is therefore calculated using the following expression:

$$d_o = \frac{OD_\|}{OD_\perp} = \frac{OD_\|}{OD - \log(1 + 10^{-\Delta} - 10^{OD - OD_\| - \Delta})}$$

where $\Delta(\lambda)$ is a correction due to the dichroism of the stretched polyethylene matrix.

RESULTS AND DISCUSSION

The structures of the compounds studied, as well as all the experimental dichroic ratios, are presented in Table 1. Due to the fact that many of the compounds show broad and structureless absorption bands, the dichroic ratio values reported were calculated at the wavelength of maximum absorption (λ_{max}).

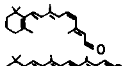
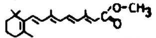
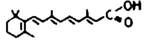
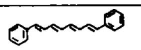
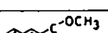
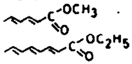
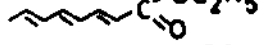
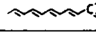
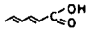
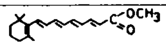
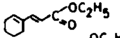
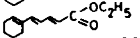
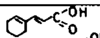
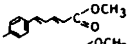
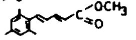
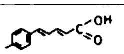
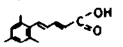
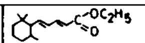
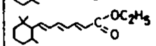
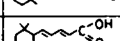
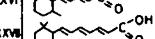
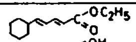
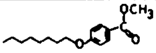
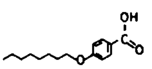
11-*cis*-Retinal (I) in stretched polyethylene showed a dichroic ratio of d_o = 1.8. All-*trans* retinal (II) (Fig. 1), retinol (vitamin A, III), retinyl acetate (IV) and methyl retinoate (V) show similar d_o values: 1.8, 1.7, 1.6 and 1.8, respectively. Although the low value of d_o of 11-*cis*-retinal could be attributed to a low degree of its orientation in the film, due to its non-linear shape, the values obtained for the all-*trans* retinoids were found to be surprisingly low, since another polyene of comparable length (1,8-diphenyl-1,3,5,7-octatetraene, VII) shows d_o = 14.5 (Fig. 2).[10]

Relatively low values of dichroic ratios were previously observed in carotenoids. Thus, the linear dichroic spectrum of *β*-carotene, reported by Eckert and Kuhn in 1960, shows a dichroic ratio of *ca* 4.0.[11] It is interesting to note that retinoic acid (VI) also shows the same value of dichroic ratio, which is, however, much larger than that of its methylester (d_o = 4 vs d_o = 1.6). This indicates that retinoic acid exists in the film as dimeric H-bonded species which probably has the same shape as *β*-carotene.

The low dichroic ratio values of the all-*trans* retinoids, may be attributed to different factors which can affect either the orientation of these molecules or the polarization of the electronic transition. In order to establish the influence of the geometrical factors on the linear dichroism of retinoids, we measured the linear dichroic spectra of a number of structurally related polyenes (Table 1).

The linear dichroic spectra of the esters of linear polyenic acids of different lengths (VIII–X), showed progressively increasing values of d_o. The longest compound measured, methyl ester of deca-tetraenoic acid (X), which possesses four conjugated double bonds in a linear chain similar to that of retinal, showed a dichroic ratio of d_o = 10. This large value indicates that

Table 1. Dichroic ratio values of polyenic compounds incorporated in stretched polyethylene films

Compound		λ_{max}, nm	d_o
I		375	1.8
II		380	18
III		332	17
IV		330	16
V		363	18
VI		376	4.0
VII		381	14.5
VIII		258	2.6
IX		297	61
X		333	10
XI		266	4.8
XII		313	12
XIII		360	3.1
XIV		262	1.8
XV		302	2.8
XVI		367	6.3
XVII		270	2.8
XVIII		317	7.4
XIX		378	8.4
XX		317	6 1
XXI		314	2 2
XXII		330	7.2
XXIII		328	3.4
XXIV		262	1 3
XXV		302	17
XXVI		271	1.4
XXVII		313	1 8
XXVIII		261	1.8
XXIX		269	4 7
XXX		311	5.4
XXXI		255	4 2
XXXII		264	21

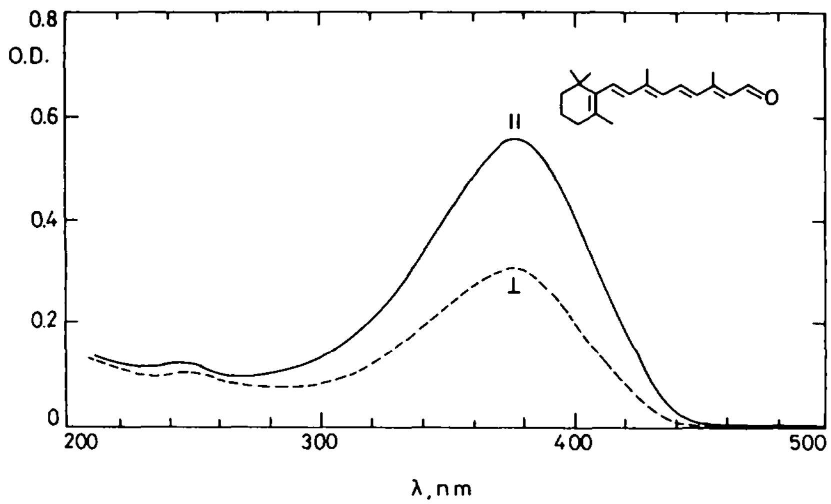


Fig. 1. Linear dichroic spectrum of all-*trans* retinal (II): (——) the curve of optical densities obtained by light polarized in the direction of stretching; (---) the curve obtained by light polarized perpendicular to the direction of stretching.

this polyene is highly oriented with its transition moment in the direction of stretching. The d_0 values of the respective polyenic acids (XI and XII) also increased with the increase of the chain length. Their higher d_0 values, as compared with those of the respective methylesters, points to the presence of dimeric associated species. The comparatively high dichroic ratio observed in aliphatic polyenic acids and their esters indicates that the presence of the cyclohexene and Me groups in retinoids might be responsible for their low dichroic ratio.

We have observed that methyl des-methyl retinoate (XIII) in which the two Me groups at the polyene chain of methyl retinoate (V) were removed shows only slightly higher d_0 than its parent compound (3.1 vs 1.6). On the other hand, the analogs of retinoate (V) lacking, in addition, the remaining three Me groups, show a significant increase in dichroism. Thus, the ester of a polyenic acid having four conjugated double bonds (XVI) has a d_0 value of 6.3. Therefore, the direction of the transition moment of the polyene chromophore in this compound is, to a high degree, in the direction of stretching, indicating its considerable orientation in the film. The series of analogous compounds, XIV–XVI, and XVII–XIX, also show the characteristic increase of dichroic ratios with the increasing polyene chain length.

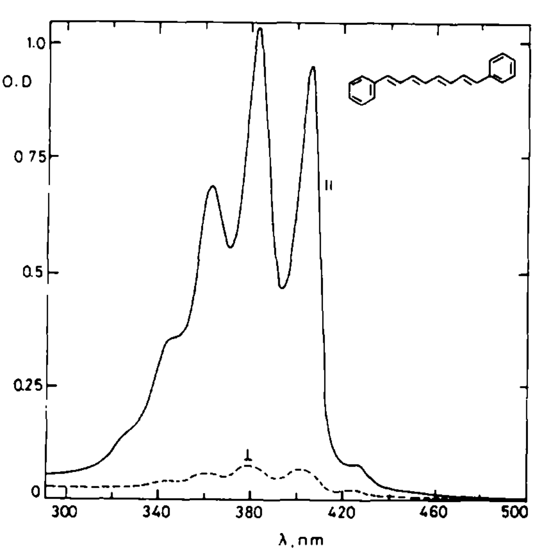


Fig. 2. Linear dichroic spectrum of 1,8-diphenyl-1,3,5,7-octatetraene (VII): (——) the curve of optical densities obtained by light polarized in the direction of stretching; (---) the curve obtained by light polarized perpendicular to the direction of stretching.

Thus, it appears that in the retinoids, the non-coplanarity of the polyene chain and the cyclohexene ring system are responsible for the low dichroic ratios of these compounds. It was established by X-ray studies that in the retinal molecule there is a twist of about 60° around the C_6—C_7 single bond, due to the steric hindrance between the *ortho*-Me substituents and the chain.[12] Therefore, removal of these Me groups leads to a more planar molecule which orients itself with its chain along the direction of stretching. It may be noted that, unlike in the retinoids, the absorption spectra of compounds XVI and XIX show a considerable vibrational fine structure characteristic of a planar polyene chromophore.

The influence of coplanarity between the ring and the linear chain on the orientation of a molecule in stretched polyethylene film is evident from the comparison of dichroic ratios of *p*-tolyl-pentadienoic acid and its ester (XX and XXII) with their analogs having additional Me groups in *ortho* positions (XXI and XXII). These Me groups prevent the coplanarity of the molecule and thus decrease considerably the d_0 values.

We have also measured the linear dichroism of a series of compounds possessing the polyene chain bound to saturated cyclohexane ring systems. The low d_0 values of compounds XXIV–XXVII can be attributed to their non-planar shape. Their analogs lacking both the endocyclic double bond and the ring

Me substituents (XXVIII–XXX) exhibit an increase in the dichroism, although their d_0 values are smaller than those of the corresponding compounds possessing the endocylic double bond.

On the basis of the experimental results reported here, we assume that a considerable fraction of the retinoid molecules is highly oriented with the plane of the ring parallel to the stretching direction. In this orientation, the π–π^* transition moment of the polyene chromophore makes a large angle with the direction of stretching, resulting in a low value of the dichroic ratio.

Support for a high orientation of retinoid molecules derives from the time-resolved linear dichroism measurements in the picosecond time domain.[13] All *trans*-retinal incorporated in stretched polyethylene was excited with a 352 nm pulse of 6 ps duration. Within less than 10 ps, a transient S_n–S_m absorption was observed with a dichroic ratio value of 4. This large dichroic ratio value, compared to that of the ground state retinal molecules, $d_0 = 1.8$, indicates a change in the direction of the transition moment of the excited molecules towards the stretching direction of the film. This can be explained, assuming a change in conformation of the oriented retinal molecules on excitation, by the molecules becoming more planar than in the ground state. These results corroborate our assumption that retinal molecules are located in the oriented phase of the polyethylene. The alternative suggestion, that the molecules are distributed only in the amorphous region of the polyethylene, would involve their diffusion in the polyethylene from one site to another, which is not likely to occur in as short a time as 10 ps. Additional evidence in favour of a high degree of order in retinoids could be obtained from the linear dichroic spectra of *p*-octoxybenzoic acid (XXXII). This compound appears in the crystal as a centrosymmetric H-bonded dimer.[14] Its alkyl chain has extended conformation, out of the plane of the benzene ring. Since the transition moment responsible for the strong charge-transfer π–π^* transition is localized in the plane of the ring,[1c] the very high values measured for d_0 indicate that this compound is highly oriented in the film with the plane of the ring parallel to the direction of stretching and, therefore, with the long alkoxy chain considerably tilted from that direction. A schematic representation of the suggested orientation of *p*-octoxybenzoic acid, as well as that of retinoic acid and of a desmethylated derivative in stretched polyethylene, is shown in Fig. 3.

The retinoids studied in this paper are structurally related to many natural pigments which play key roles in important photo-biological processes.

Since all these processes occur in oriented molecular environments, we believe that the conclusions of this work might be of assistance when analysing the molecular mechanisms of these crucial biological processes.

CONCLUSIONS

Comparison of the linear dichroism spectra of natural retinoids incorporated in stretched polyethylene films with those of many other polyene model compounds, shows that the low dichroic ratio values of the retinoids can be attributed to their characteristic distribution in the oriented matrix. It is suggested that this distribution involves a fraction of retinoid molecules oriented with the plane of the ring parallel to the direction of stretching, the long polyene chain remaining deviated from that direction. This orientation should be taken into consideration in the explanation of the biological processes in which such molecules are involved.

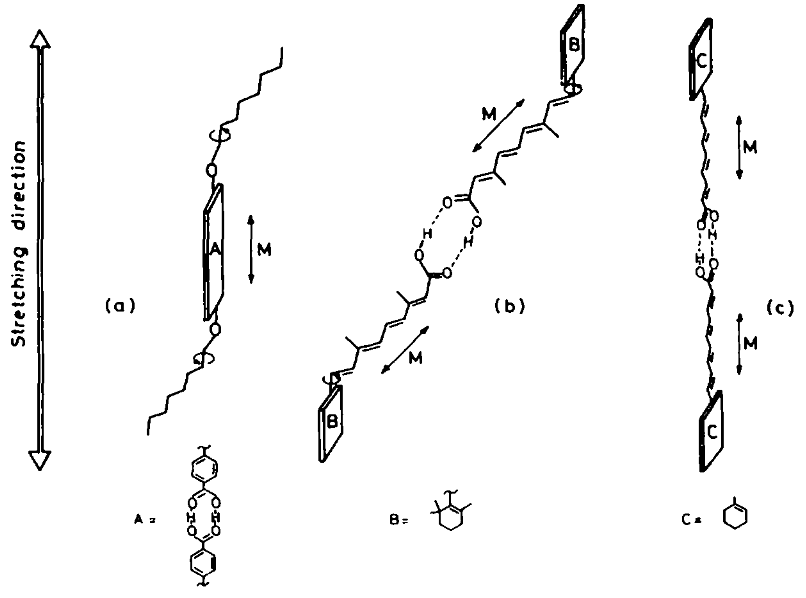


Fig. 3. Schematic representation of suggested orientations in stretched polythene: (a) *p*-octoxy-benzoic acid (XXXII); (b) retinoic acid (VI); (c) desmethylated derivative of retinoic acid (XIX). M: transition moment vector of the π–π^* transition.

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