INFRARED SPECTRA OF CRYSTALLINE POLYSACCHARIDES

VI. EFFECT OF ORIENTATION ON THE TILTING SPECTRA OF CHITIN FILMS*

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SUMMARY

The tilting spectra of doubly oriented crab chitin crystallites and blowfly larval cuticle chitin were observed. It has been noted that in addition to the uniaxial orientation, these samples possess different selective uniplanar orientations. For the crab chitin crystallites the 100 crystallographic planes are parallel to the film surface while for the blowfly larval chitin the 001 planes are parallel to the film surface. Based on these orientations and the observed tilting effects, a number of previously made band-assignments were confirmed and new suggestions were made for interpreting the bands at 3106, 2962 and 1619 cm^{-1}.

INTRODUCTION

In the two previous reports of this series[1,2] the general principles for interpreting and the practical procedures for obtaining "tilted" infrared spectra of polymers were discussed as well as the general interpretation of the spectra of chitinous materials. The present study is an examination of the "tilting" effects which were observed in the infrared spectra of two chitin samples: crab chitin crystallites and larval chitin of blowfly. These two samples were chosen because they showed double orientation (*i.e.* one axis of the unit cell oriented in a single direction and one crystallographic plane parallel to the surface) which usually results in pronounced "tilting" effects.

From the tilting spectra of samples with well-defined crystallographic orientation it is frequently possible to obtain confirmation of band assignments which are made more or less empirically. Measurements such as described in the present study can also allow a choice to be made among a number of potential hydrogen bonding sites for a given hydroxyl[3]. Finally the importance of a full interpretation of the chitin spectrum cannot be overemphasized since it is a prototype of numerous biologically important polysaccharides which contain the aminoacetyl group.

EXPERIMENTAL

The preparation of rod-like crystallites from crab chitin has been described[2] previously. The clear transparent films which could be prepared in any thickness by drying

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down a dilute suspension of crystallites or by spreading unidirectionally a concentrated (birefringent gel) suspension[4] of the crystallites proved to be ideal for spectroscopic study.

Purified blowfly larval chitin was kindly supplied by Dr. K. M. RUDALL of Leeds, England. The preparation and the morphological characteristics of this material have been reported[5]. The particular sample studied was 8–10 μ thick, somewhat more than desirable for infrared study. The sample had been stretched 30–40 % to give a moderate degree of uniaxial orientation.

Typical X-ray diagrams shown by the samples used in this investigation are shown in Fig. 1. The degree of uniaxial orientation was sufficient to show good infrared dichroism in the two cases[2]. One would conclude, however, from the similarity of the X-ray orientations and the higher dichroism of the crystallite sample that the blowfly chitin contained more noncrystalline material. This is reasonable in view of the fact that it has not undergone hydrolytic treatment.

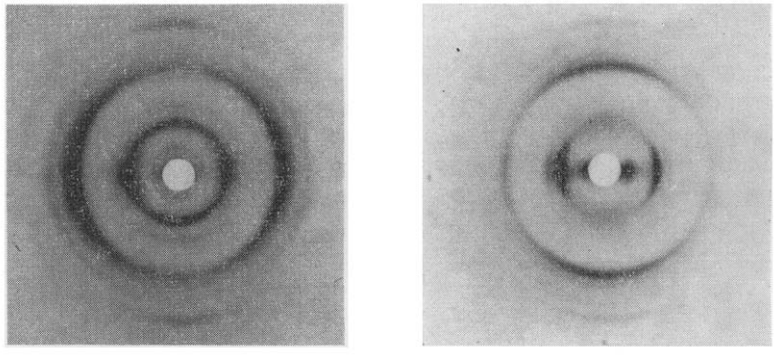


Fig. 1. X-ray diagrams of oriented films of crab chitin crystallites (left) and blowfly larval chitin (right). X-ray beam was parallel to the film surface and surface normal pointing to the equator.

The most significant feature of the X-ray diagrams is the orthogonal relation of the two paratropic reflections (the two most intense ones). For blowfly chitin the 001 (inner of the two) is equatorial but for the crab crystallite sample it is meridional, and *vice versa* for the outer 100 reflection. (The apparent diffuse equatorial maximum of the 001 reflection for the crab crystallites is due to "white" radiation.) This indicates that within the crystallite sample the 100 plane has preferred orientation parallel to the surface plane while the 001 is parallel to the surface of the blowfly chitin sample. These two planes correspond to the mutually perpendicular lateral faces of the chitin unit cell[6] (*cf.* Fig. 2). It should be noted that the double orientation of both samples is far from perfect.

The spectra were recorded in the 3 μ region by means of a Perkin-Elmer model 112 spectrometer using a LiF prism. For the 1800–650 cm^{-1} region a Perkin-Elmer model 21 spectrometer with NaCl optics was used. The settings for recording the tilting spectra have been described previously[1]. The spectrometer slit is used as a vertical reference axis for describing these settings so that the orientation of the fiber axis (f.a.) will be described below as perpendicular or parallel with reference to the slit. The electric vector is kept in a horizontal position, *i.e.* perpendicular to the slit. A tilting spectrum is obtained when the normal to the sample surface is at some angle θ

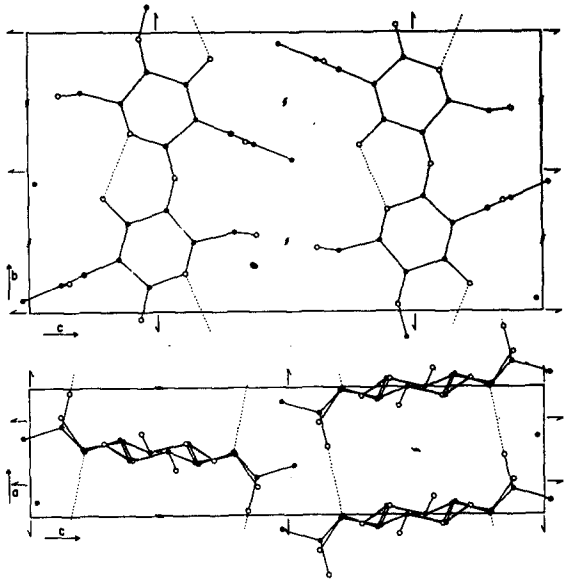


Fig. 2. Unit cell of chitin (after CARLSTRÖM[6]).

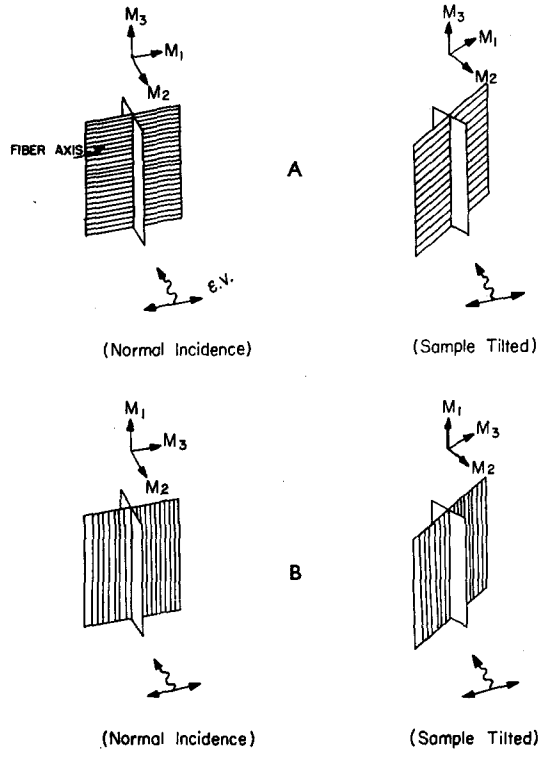


Fig. 3. Tilting effects of doubly oriented films showing settings for observing: A, parallel tilting spectrum; B, perpendicular tilting spectrum.

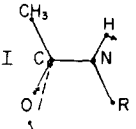
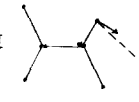
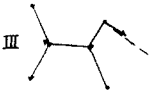
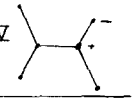
($0° < \theta < 70°$) relative to the incident beam. If the fiber axis is parallel to the slit, a "perpendicular tilting spectrum" is obtained. Conversely if the fiber axis is perpendicular to the slit, a "parallel tilting spectrum" is obtained (*cf.* Fig. 3). If a particular absorption band increases as a result of tilting the result will be referred to as a "positive tilting effect" while a decrease will constitute a "negative tilting effect".

The most striking tilting effects are expected for dipole moment changes which are either parallel to the electric vector or parallel to the incident beam for normal incidence. These dipole moment changes are brought either into, or out of absorbing position by tilting. In general, the transition moment of every vibrational mode can be considered to have components M_1 parallel to the chain axis, M_2 perpendicular to the doubly oriented film surface, and M_3 perpendicular to the chain axis and parallel to the film surface (see Fig. 3). There will always be a positive tilting effect for the component M_2 in both parallel and perpendicular tilting spectra. In the parallel tilting spectrum, there will be a negative tilting effect for the component M_1 while the component M_3 is not in an absorbing position. In the perpendicular tilting spectrum, on the other hand, there will be a negative tilting effect for the component M_3 while the component M_1 is not in an absorbing position. Any transition moment with components M_1, M_2, M_3, none of which is zero, will exhibit all of the foregoing tilting effects. However, it is expected that the tilting effect of the stronger component should be predominant.

Tilting effects for the amide bands

The planar aminoacetyl group, roughly at right angles to the 100 and 001 planes, is expected to show the most pronounced tilting effects, and it will be considered first.

Table I. Amide bands in chitin (700–1700 cm^{-1})

Freq. cm^{-1}	Polarization	Effect of D_2O	Interpretation
1652	⊥	Unchanged	Amide I 
1555	⊥	R.I. Decreased	Amide II 
1310	⊥	R.I. Decreased	Amide III 
730	∥	R.I. Decreased	Amide V 

The absorption bands in chitin which are typical of amides, their polarization and the effect of deuteration are listed in Table I. All the bands in this table have been identified in the chitin spectra[2]. The approximate normal modes for these amide bands are taken from the study of the spectrum of N-methyl acetamide[7]. The broken line in

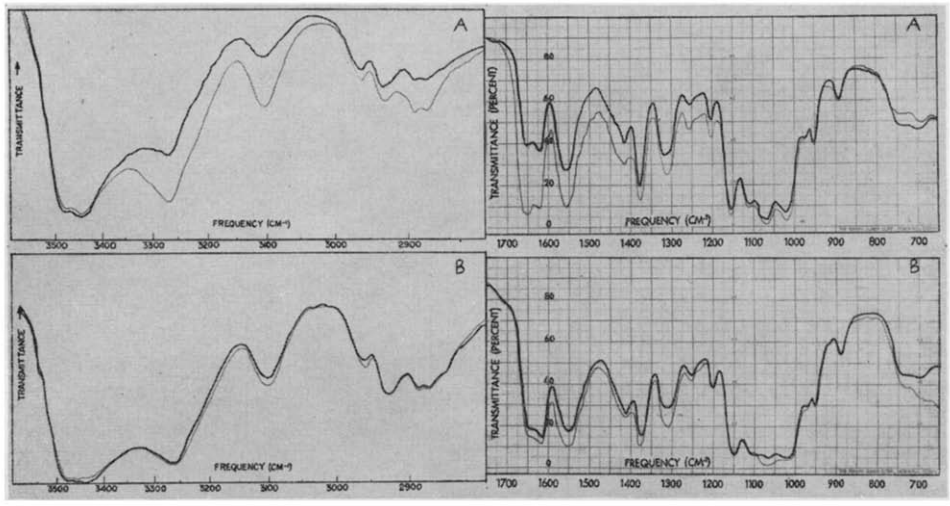


Fig. 4. Tilting spectra for crab chitin crystallites (A) and blowfly larval chitin (B) with electric vector and fiber axis both $\perp$ to slit. (A) ———, normal incidence; ·····, sample tilted 60° (by rotating about a vertical axis parallel to the slit). Film thickness $\simeq 6\ \mu$. (B) ———, normal incidence; ·····, sample tilted 50°. Film thickness $\simeq 8\ \mu$.

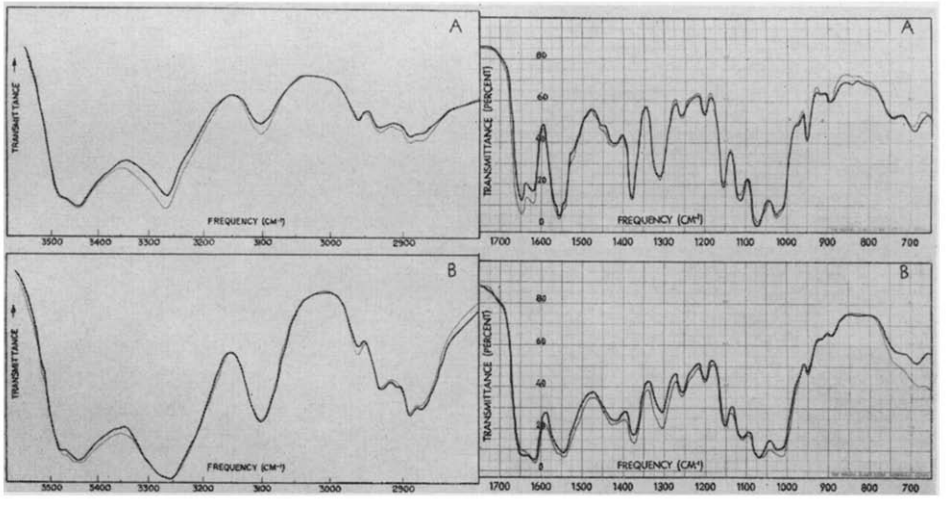


Fig. 5. Tilting spectra for crab chitin crystallites (A) and blowfly larval chitin (B) with electric vector // to slit and fiber axis $\perp$ to slit. (A) ———, normal incidence; ·····, sample tilted 45°. (B) ———, normal incidence; ·····, sample tilted 50°.

the last column of Table I shows the predicted direction of the transition moment vector which has been confirmed experimentally in most cases[8,9].

Referring first of all to Figs. 4 and 5, the effect of opposite selective uniplanar orientation, *per se*, on the infrared spectrum is immediately apparent on comparing the relative intensities of the 1652–1619 cm^{-1} bands *vs.* the 1555 cm^{-1} band for normal incidence. The intensity ratios are reversed for the two samples reflecting their different preferred orientations.

Amide I band

The band at 1652 cm^{-1} almost certainly can be assigned to the amide I mode. The actual tilting effects are apparent in Fig. 4 where one observes that the intensity increase of the 1652 cm^{-1} band of the crab chitin sample is not matched in the blowfly chitin spectrum. A glance at Fig. 2 shows the reason for this. In the former case the C=O bond direction is nearly normal to the film surface and is brought into an absorbing position by tilting. For the latter, the transition moment of the bond in question has a large component in the 001 plane or the film surface and hence one would expect to observe a negative tilting effect. This is the case in Fig. 5B. The observed increase for this band in Fig. 4B then represents the increase in effective thickness after tilting and the imperfect selective uniplanar orientation.

In the perpendicular tilting spectrum one would expect a positive tilting effect for the 1652 cm^{-1} band of the crab sample. This is true as shown in Fig. 5A. However, the effect shown in this figure is much less than that in Fig. 4A. This observation is due to the fact that the crab sample has a high degree of uniaxial orientation but rather poor 100 selective uniplanar orientation.

The interpretation of the 1619 cm^{-1} band is not clear. The tilting effects of this band are noteworthy. The band shows a positive tilting effect in Fig. 4A and Fig. 5A. In Fig. 4A the intensity of this band is greater than that of the 1652 cm^{-1} band for normal incidence. When the sample is tilted, the relative intensities of the two bands change. This observation would mean that the transition moment associated with the 1652 cm^{-1} band is more nearly perpendicular to the chain axis and film surface than that associated with the 1619 cm^{-1} band. In Fig. 5B, opposite tilting effects are observed for the doublet 1652 and 1619 cm^{-1}. This probably means that the transition moment associated with the 1619 cm^{-1} band does not have a large component in the 001 plane. One would conclude from the above observations that if the 1652 and 1619 cm^{-1} bands are both to be associated with C=O stretching then the respective transition moments must have different orientations relative to the 001 plane.

Assignment[2] of the 1619 cm^{-1} absorption band to the C=N stretching mode of the enol form would also account for the observed relative intensity and tilting effects since the direction of the C=O and C=N transition moments relative to the film surface would then differ by about 40°. In this statement the direction of the C=N bond in the enol form is assumed to be the same as the C–N bond in chitin.

Another explanation, which ties in also with chemical data, is that a significant concentration of a rotational isomer of the aminoacetyl group is present. A rotation of 180° about the bond linking the nitrogen atom to the sugar ring gives the most acceptable conformation. The N–H bond direction remains the same but there is a significantly different C=O direction. The perpendicular dichroism of the amide I band would be unchanged but assignment of the 1619 cm^{-1} band to the amide I band of this rotation isomer could explain the difference in behavior of the two bands on tilting. This rotational isomer would necessarily represent some kind of disorder within the crystal. Accordingly, a density measurement was made on the crystalline N-acetylated chitoheptaose which shows the same X-ray diagram and infrared spectrum as native chitin. A value of 1.424 g/ml was obtained which is to be compared with the theoretical value of 1.462 (see ref. 6). The evidence then from the density measurement is not opposed to the hypothesis.

Chemists have frequently referred to the fact that chitin seems to have two kinds

of acetyl groups[10]; one readily saponifiable but the other more resistant. If this is a question of accessibility then the foregoing hypothesis is all the more attractive. In line with this one may cite the fact[2] that the 3106 cm^{-1} N–H stretching band is apparently more readily affected by deuteration than the one at 3264 cm^{-1}. Within the bounds of the rotational isomer hypothesis, the former frequency could be associated with the isomeric form also. Since the direction of the N–H bond is changed by 180° as a result of the rotation, the dichroism of the associated band would be expected to be the same as that of the crystalline band, which is the case[2,5].

Amide II band

The appearance of the amide II′ band at 1470 cm^{-1} on deuteration of the chitin and the near disappearance of the 1555 cm^{-1} band leaves little doubt concerning the assignment of the amide II mode[2]. The direction of this transition moment is about 17° off the line corresponding to the pure N–H in-plane bending[7–9]. The observed positive tilting effects for the crab and blowfly chitin samples shown in Fig. 4 are not unexpected. The transition moment has components in a plane normal to the surface of the films which are brought into an absorbing position by tilting. (Note that the rather large tilting effect of this band in Fig. 4A but not in Fig. 5A is due to high uniaxial but poor selective uniplanar orientations.)

Because the transition moment does not lie directly in the plane of the film or normal to it, the setting used to obtain Fig. 5 is not favorable for tilting effects. From the unit cell one would predict a slight decrease for the crab chitin sample and an increase for the blowfly chitin sample which is in reasonable agreement with observation, taking into account the thickness effect and the imperfect orientation.

Amide III band

This band is observed at 1310 cm^{-1} in chitin and shows a large decrease in intensity on deuteration, in agreement with theoretical analysis[7], which predicts a significant N–H in-plane bending contribution. As shown in Table I the direction of the transition moment is nearly perpendicular to the N–H bond, a situation that should lead to the same "tilting" effects as observed for the amide II band. This is verified by the data in Figs. 4 and 5.

Amide V band

This mode is due entirely to N–H out-of-plane bending, *i.e.*, along the polymer chain axis. Unfortunately, this region of the spectrum is also rich in O–H out-of-plane bending modes which can cause some confusion in interpreting the tilting spectra. For the setting in Fig. 4 a "negative tilting" effect is expected for both samples. In fact, for the crab chitin sample a decrease is observed but the blowfly chitin sample shows an increase. In Fig. 5, similar tilting effects were observed for both samples. Most probably these anomalies are due to the positive contribution of a broad spectrum of O–H bending frequencies in the less-crystalline blowfly chitin. The O–H bands of the cellulose spectrum show positive tilting effects in this region[1].

NH stretching frequencies

Since the NH bond is nearly parallel to the C=O bond, the tilting effects of the NH stretching band should be similar to those of the amide I band. From the spectra

shown in Figs. 4 and 5, it is interesting to note that the tilting effects of the bands at 3264 and 1652 cm^{-1} are very similar. The same is true for the bands at 3106 and 1619 cm^{-1}. These observations would probably support the assignment of both the 3264 and 3106 cm^{-1} bands to N–H stretching modes as discussed above.

Tilting effects for carbon-hydrogen modes

The setting used for Fig. 4A is ideal for observing tilting effects due to the C–H stretching modes which are nearly normal to the 100 plane. The positive tilting effects for the 2878 and 2890 cm^{-1} bands in Fig. 4A confirm their assignment to CH stretching modes. In Fig. 5A a similar intensity increase would be expected if the sample had a perfect selective uniplanar orientation. The very moderate positive tilting effect observed is certainly due to imperfect selective uniplanar but high uniaxial orientation for the crab chitin sample, as already mentioned in the discussion of amide bands.

The blowfly chitin sample shows only a thickness effect in Fig. 4B and a decrease in intensity in Fig. 5B, as expected for a sample with selective orientation of the 001 plane.

The only other carbon-hydrogen stretching frequency showing tilting effects is the 2962 cm^{-1} band which was assigned[2] to the CH_3 antisymmetric stretching mode. The tilting effects, although small, suggest a dipole moment change along the $C-CH_3$ bond which would be characteristic of the symmetric CH_3 stretching mode. The reason for this anomaly is not known. However this band could be assigned to the CH_3 symmetric stretching mode by assuming that the CH_3 antisymmetric stretching mode absorbs at a higher frequency but with very weak intensity in the infrared.

The CH_3 symmetric bending mode assigned to the 1378 cm^{-1} band shows the tilting behavior which is expected from the molecular model. It should be remembered, however, that this band may, in fact, be composite since the C–H bending frequency could occur here also.

Tilting effects offer little help in deciding which of the 1430 and 1420 cm^{-1} bands belongs to the CH_2 symmetric bending mode, since tilting effects were found only in one of the settings. All that can be said is that observations are not contrary to the conformation proposed previously[2] for the hydroxymethyl group.

Tilting effects for hydroxyl modes

Since the hydroxyl stretching frequencies were assigned to bands showing parallel polarizations these bands should show a negative tilting effect for the setting in Fig. 4, as they actually do.

Interpretation of tilting effects for the O–H in-plane and out-of-plane bending modes is less straight-forward because band assignment to the individual hydroxyls has not been made. However, if the conformation of the hydroxymethyl group suggested previously[2] be correct, then the 685 cm^{-1} band is most probably associated with its out-of-plane bending mode. In this case, the transition moment would be oriented at an angle of about 45° to the chain axis and in the 100 plane. This orientation could explain the opposite "tilting" effects observed for the two samples in Figs. 4 and 5.

DISCUSSION

Although imperfect orientation did not always allow the full effect of the orthogonal uniplanar orientations to be observed in the tilted spectra of these chitin samples,

major differences nevertheless were found. These differences were in accord with predictions based on the X-ray unit cell recently proposed by CARLSTRÖM[6]. Since this structure allows for complete N–H···O=C hydrogen bonding the assignment of the 3445 cm^{-1} band to a free N–H group[10] is less acceptable than its assignment to the O–H stretching mode, which has been proposed[2]. However, the apparent presence of two NH stretching and two C=O stretching bands is still not explained satisfactorily. It is noteworthy that the frequency difference between the 1652 and 1619 cm^{-1} bands is about the same as the frequency shift in the $\alpha \rightarrow \beta$ transformation of polypeptides[8,9].

The finding of two chitin samples with different selective planar orientation has its analogue in the cellulose field where samples with 101 and $10\bar{1}$ planar orientation are available[1]. The value of such samples in helping to associate infrared absorption bands with the proper chemical group is clear. From a biophysical point of view it is more important to understand the relation between crystallite shape and planar orientation. Planes rich in hydrogen bonds (001 for chitin) are usually expected to show more rapid growth. Drying stresses may then be expected to orient these larger planes parallel to the membrane surface. Since low angle X-ray diffraction[11] seems to confirm that the widest face of the crystallite is parallel to the film surface the existence of films with the same unit cell but orthogonal planar orientation suggests that factors other than hydrogen bond direction control the crystallite shape.

When hydrolysis is involved, as in the present case, for the preparation of crystallites, the effect of charged groups (such as NH_3^+) in causing cleavage other than that expected crystallographically should not be overlooked. Thus, addition of a proton to the few amino groups which are known to be present in chitin, could bring about interactions which would readily cause cleavage along the 100 plane.

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