

THEORETICAL STUDY OF THE C=C STRETCHING VIBRATIONS IN LINKED POLYENE CHAINS: NYSTATIN [☆]

Francesco ZERBETTO ¹ and Marek Z. ZGIERSKI

Division of Chemistry, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6

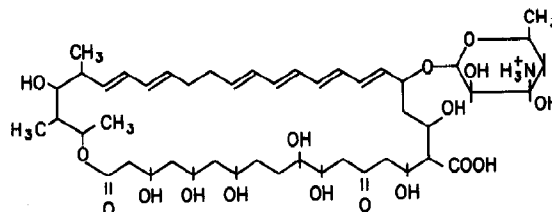
Received 27 October 1987; in final form 6 January 1988

It is shown from a simple model and by quantum-chemical calculations that the observed Raman fundamentals of nystatin in the range of 1550–1650 cm^{-1} belong to four C=C stretching vibrations of mixed a_g and b_u symmetry of the octatetraene fragment of the molecule. A simple explanation of the variation of the relative intensities of these fundamentals in different environments is presented which does not require the assumption of an unusually strong participation of the C=O stretching vibration in the Raman spectrum.

1. Introduction

Polyenes attract extensive experimental and theoretical work due to the role they play in biological systems as chromophores [1,2], and due to the unusual electric and magnetic properties of pure and doped polyene chains such as polyacetylenes [3]. Polyenic fragments appear also in a number of antibiotic compounds, e.g. amphotericin B with a heptaene chain [4,5] and nystatin containing tetraene and diene chains connected by an ethane bridge [6,7].

In recent papers we were concerned with the absorption, emission and Raman spectra of polyenes and polyacetylenes [8–13]. The first two spectra belong to the $1A_g \rightarrow 1B_u$ and $1A_g \rightarrow 2A_g$ (C_{2v} symmetry) electronic transitions in which the C=C stretch and C–C stretch a_g vibrations form well-developed Franck–Condon (FC) progressions. The FC activity of the a_g modes in polyenes of different chain length has been studied by us with the use of quantum-chemical methods. Although there are $\frac{1}{2}n(+\frac{1}{2})$ totally symmetric C=C stretches in a polyene with n conjugated double bonds, only one C=C stretch is strongly FC active. This result of theoretical calculations is in perfect agreement with the experimental



Nystatin: C₄₇ H₇₅ O₁₇ N

Scheme 1.

data (see, e.g. ref. [2]). Molecules with a single polyene chain show a correlation between the frequency of the FC active C=C stretch mode and the energy of the $1A_g \rightarrow 1B_u$ transition [14]. This correlation was rationalized by us in terms of vibronic coupling between the $1A_g$ and $2A_g$ electronic states via the FC active C=C stretch model [12].

In this paper we address the problem of FC activity of the C=C stretch modes in nystatin (see scheme 1), whose chromophore part consists of an octatetraene chain connected via an ethane bridge with a butadiene chain. Special attention is paid to the mixing of different C=C stretches of the tetraene chain resulting from the interaction between the two polyene fragments.

The Raman spectrum of nystatin exhibits two strong fundamentals in the region of the C=C stretch frequencies: 1610 and 1557 cm^{-1} [15]. Iqbal and

[☆] Issued as NRCC No. 28377.

¹ Research Associate.

Weidekamm [15] assigned these fundamentals to a C=O stretch (1610 cm^{-1}) and a C=C stretch mode (1557 cm^{-1}). They noticed that the relative intensities of these two Raman bands vary appreciably with the nystatin environment. This assignment poses, however, a serious problem of explaining how the C=O stretch mode can be strongly Raman active in the preresonance region of an electronic transition localized in the tetraene unit of the molecule. Such strong activity of the C=O mode is absent in retinal [16] which possesses a configuration more favourable to strong mixing of the C=C and C=O stretches.

An alternative assignment of these bands was proposed recently by Colline et al. [17]. They attributed the 1610 cm^{-1} band to a C=C stretch mode and the 1557 cm^{-1} band to an impurity with a heptaene unit. The C=O stretch was reassigned to a weaker Raman line at 1632 cm^{-1} . Colline et al. [17] supported this assignment with two observations:

(i) Chromatographical purification of nystatin lowers the intensity of the weak electronic transition located at $\approx 24000\text{ cm}^{-1}$ by a factor of 10 identifying it as an impurity band. It also reduces the intensity of the 1557 cm^{-1} Raman fundamental by a factor of $\approx 3-4$. This weak electronic transition was previously ascribed to the $1A_g \rightarrow 2A_g$ transition in the tetraene unit [15] and is almost in resonance with the exciting laser wavelength (454.5 nm).

(ii) Only the 1610 cm^{-1} fundamental is visible under 304.5 nm laser excitation (between the 0-1 and 0-2 vibronic bands of the $1A_g \rightarrow 1B_u$ transition).

The assignment of the 1557 cm^{-1} band to an impurity is however not conclusively demonstrated by these observations. Firstly, the decrease of the impurity absorption by a factor of 10 should lead to a corresponding decrease in the (near) resonance Raman impurity band by a factor of 100. This is not observed for the 1557 cm^{-1} band. Secondly, the low intensity of one fundamental in the resonance region at one particular wavelength does not prove that this fundamental has low intensity in the whole resonance region since resonance Raman excitation profiles are generally highly structured [18]. Thus, although the 1557 cm^{-1} band is most probably contaminated by an impurity, it appears that it has also a component inherent to nystatin.

Elementary argument suggests that the lowest electronic excitation will be strongly localized in the te-

traene unit of the chain. On this basis one would expect strong activity of a single C=C stretch mode in the absorption and Raman spectra. However, we expect that the C=C stretch modes in the tetraene unit will be perturbed by similar modes in the same frequency range associated with the diene unit. We assume that this perturbation will mix the tetraene modes and redistribute their intensity. Therefore we investigate the possibility that the strong bands at 1610 and 1557 cm^{-1} both represent C=C stretching modes in the tetraene unit of nystatin. The investigation is carried out in three steps. First we study a simple model based on a tetraene chain in which the stretching modes are coupled by a weak external perturbation, using the coupling strength as an adjustable parameter. Then we carry out quantum-chemical calculations to check whether this model can be quantitatively justified. Finally, we use the quantum-chemical results to obtain a more detailed picture of the interaction between two unequal polyene chains linked by an ethane bridge.

2. Simple coupling model

Excitation of a π electron in the tetraene unit of nystatin leads to an excited state that corresponds to the $1B_u$ state of octatetraene. The "vertical" excitation energy of this state is $\approx 32800\text{ cm}^{-1}$ [15,17]. From the correlation curve discussed in ref. [12] we obtain the value of $\omega_{10} \approx 1598\text{ cm}^{-1}$ for the frequency of the FC active C=C stretch. This value lies between the two observed FC and Raman active mode frequencies of nystatin. There are two a_g C=C stretches in tetraene, one is an in-phase stretch, the other is an out-of-phase stretch with two nodes. We postulate that these two modes mix in nystatin due to interaction between the tetraene and diene fragments. Denoting by ω_{i0} and Q_{i0} ($i=1, 2$) the frequencies and mass-weighted normal coordinates of these two modes in the unperturbed octatetraene, we write the vibrational potential describing these modes in nystatin as

$$V = \frac{1}{2}(\omega_{10}^2 Q_{10}^2 + \omega_{20}^2 Q_{20}^2) + 2k(\omega_{10}\omega_{20})^{1/2} Q_{10} Q_{20}, \quad (1)$$

where k is the strength of mixing which is equal to the interaction between the unperturbed $|1_1, 0_2\rangle$ and $|0_1, 1_2\rangle$ levels of octatetraene. The normal coordinates Q_i of nystatin are now expressed in terms of coordinates Q_{i0} as [19]

$$\begin{pmatrix} Q_1 \\ Q_2 \end{pmatrix} = \begin{pmatrix} \cos \phi & \sin \phi \\ -\sin \phi & \cos \phi \end{pmatrix} \begin{pmatrix} Q_{10} \\ Q_{20} \end{pmatrix}, \quad (2)$$

where

$$\tan 2\phi = 4k(\omega_{10}\omega_{20})^{1/2}/(\omega_{10}^2 - \omega_{20}^2). \quad (3)$$

The vibrational frequencies of nystatin are given by

$$\omega_1 = [\omega_{10}^2 + 2k(\omega_{10}\omega_{20})^{1/2} \tan \phi]^{1/2}, \quad (4a)$$

$$\omega_2 = [\omega_{20}^2 - 2k(\omega_{10}\omega_{20})^{1/2} \tan \phi]^{1/2}. \quad (4b)$$

Setting $\omega_1 = 1610 \text{ cm}^{-1}$, $\omega_2 = 1557 \text{ cm}^{-1}$ and $\omega_{10} = 1598 \text{ cm}^{-1}$ we obtain $\omega_{20} = 1569 \text{ cm}^{-1}$, $k = 22 \text{ cm}^{-1}$ and $\phi = 28.61^\circ$.

The intensity of the Raman fundamental of a totally symmetric mode in the preresonance region of a strong electronic transition is proportional to the square of the displacement parameter of this mode [18]. The dimensionless displacement parameters B_i of nystatin modes expressed in terms of similar displacement parameters B_{i0} of octatetraene now read:

$$B_1 = (\omega_{10}/\omega_1)^{3/2} B_{10} \cos \phi + (\omega_{20}/\omega_1)^{3/2} B_{20} \sin \phi, \quad (5a)$$

$$B_2 = (\omega_{10}/\omega_2)^{3/2} B_{10} \sin \phi + (\omega_{20}/\omega_2)^{3/2} B_{20} \cos \phi, \quad (5b)$$

and the ratio of intensities of the two Raman lines is approximately given by

$$I = (B_2/B_1)^2 = \left(\frac{(\omega_{20}/\omega_2)^{3/2} \rho \cos \phi - (\omega_{10}/\omega_2)^{3/2} \sin \phi}{(\omega_{20}/\omega_1)^{3/2} \rho \sin \phi + (\omega_{10}/\omega_1)^{3/2} \cos \phi} \right)^2, \quad (6)$$

where $\rho = B_{20}/B_{10}$. Setting $I = 0.9$ (nystatin powder [15]) we get $\rho = -0.245$.

As can be seen from eq. (6), a small change in the value of ρ produces quite large changes in the relative intensities of the two Raman lines. We obtain $I = 1.1$ for $\rho = -0.3$ (nystatin in methanol [15]),

$I = 1.7$ for $\rho = -0.42$ (nystatin in dimyristoyl phosphatidylcholine multilayers (DML) [15]), $I = 2.1$ for $\rho = 0.49$ (nystatin in buffered cholesterol [15]), and $I = 2.5$ for $\rho = -0.54$ (nystatin in cholesterol + DML [15]). Thus the small variation of the displacement parameters of the "zero-order" modes of the octatetraene unit in the range of values, where only one "zero-order" mode is strongly Raman active, produces the relative Raman intensities of the two nystatin fundamentals that vary in the broad range of experimentally observed values. We notice that the other factor that can change these intensities as well as the frequencies of the modes is the strength of the mode mixing parameter k . Both k and ρ can be affected by the environment of the nystatin molecule. Thus increasing k to 24.75 cm^{-1} and setting $\rho = -0.275$, while keeping the value of ω_{10} and ω_{20} the same as above, we get $I = 1.1$, $\omega_1 = 1612 \text{ cm}^{-1}$ and $\omega_2 = 1555 \text{ cm}^{-1}$.

The simple model presented above explains the appearance of the two strongly Raman active C=C stretches in nystatin as a result of the mixing of the two a_g C=C stretches of octatetraene of which only one is strongly Raman active. This mixing is thought to be caused by the interaction between the tetraene and diene chains in nystatin. Wide variations of the relative intensities of the two Raman active vibrations of nystatin in different environments are then explained as caused by relatively small variations of the displacement parameter of the weakly Raman active mode in the octatetraene unit.

3. Quantum-chemical calculations

To verify the validity of the proposed model we perform quantum-chemical calculations of the displacement parameters of nystatin modes for the electronic transition observed in the absorption spectrum. For this purpose we select the QCFF/PI method [20,21] which has been shown to be reliable for the normal mode analysis of a number of polyatomic molecules [12,13,22-30]. This semiempirical π -electron method includes σ -bonds and non-bonded interactions through empirical potential functions combines ground and excited state conformational analysis and normal mode analysis in a consistent way [20,22]. The program of ref. [21] was

slightly modified as described in ref. [13]. Since our study is limited to the electronic ground state and the excited state that corresponds to the $1B_u$ state of a polyene with C_{2v} symmetry, we model nystatin with all-trans-1,3,7,9,11,13-tetradecahexaene (TDH), i.e. a polyene built of tetraene and diene chains connected by an ethane bridge. To obtain the "zero-order" frequencies ω_{i0} and displacement parameters B_{i0} of the tetraene unit we perform calculations for all-trans-1,3,5,7-octatetraene.

We start with optimizing the geometry of the ground state S_0 , and of the first excited state S_B with a strong transition dipole moment ($1B_u$). Then we calculate the ground state vibrational frequencies and normal coordinates. The calculated S_0 and S_B geometries and normal coordinates allow calculation of the displacement vector A :

$$A = [X(S_B) - X(S_0)] \mu^{1/2} \mathcal{L}(S_0), \quad (7)$$

where $X(\Gamma)$ is the $3N$ -dimensional vector of the Cartesian coordinates of the N atoms in the Γ electronic state, \mathcal{L} is the $3N \times (3N - 6)$ matrix of normal coordinates and μ is the $3N \times 3N$ diagonal matrix of atomic masses. The component A_i of the vector A is proportional to the dimensionless displacement parameter B_i of the i th mode

$$B_i = 0.176 \omega_i^{1/2} A_i, \quad (8)$$

where ω_i is the vibrational frequency in cm^{-1} and A_i is given in $\text{\AA} \text{amu}^{1/2}$ ($\text{amu} \equiv$ atomic mass unit). The parameter B_i determines the FC activity of the i th mode in the $S_0 \rightarrow S_B$ transition and the preresonance Raman intensity of the fundamental of this mode measured below the origin of that transition. In our calculations we use only singly excited configurations since we are interested in B_u -type excited states which are well described in this configuration interaction scheme [2]. The A_g -type excited states, which contain a large admixture of doubly excited configurations, carry little or no intensity and therefore do not contribute significantly to absorption or Raman scattering.

Table 1 lists the calculated frequencies and displacement parameters of the four C=C bond stretching vibrations of the unperturbed tetraene unit - 1,3,5,7-octatetraene. Two of these vibrations have b_u symmetry and thus zero displacement parameters. The two others have a_g symmetry and are identified

Table 1

C=C stretch modes in all-trans-1,3,5,7-octatetraene and their displacement parameters, B_i , in the $1B_u$ state relative to the $1A_g$ ground state obtained by QCFF/PI calculations

Frequency (cm^{-1})	Symmetry	B_i
1646	b_u	0
1642	a_g	1.16
1618	a_g	-0.55
1579	b_u	0

as in-phase and out-of-phase totally symmetric stretching modes. The ratio ρ defined after eq. (6) is equal to -0.474 ; its sign is the same as that given by the simple model, but it is larger in absolute value by nearly a factor of 2. The separation between the two frequencies ω_{10} and ω_{20} is 24 cm^{-1} which should be compared to the value of 29 cm^{-1} used in the model of section 2 to fit the experimental data. The semi-quantitative agreement of the quantum-chemical calculations with the result of the simple model supports the physical validity of this model. To verify the quality of the calculations, we note that the calculated displacement parameters give the overall intensity of the 0-1 (C=C) transitions relative to the 0-0 transition as 0.86. This compares very well with the value of ≈ 0.9 observed in the absorption spectrum of octatetraene in 3-methylpentane (fig. 5 of ref. [2]). Also the calculated excitation energy of 32630 cm^{-1} agrees well with the position of the center of gravity of the absorption spectrum ($\approx 33900 \text{ cm}^{-1}$ [2]). The amplitudes of the motion of individual atoms in the two a_g C=C stretches of octatetraene are given in fig. 1.

Table 2 lists the carbon-carbon bond lengths in the S_0 and S_B states of TDH resulting from our calculations. Comparing the two columns one notices that upon electronic excitation only the bonds belonging to the tetraene unit of TDH change in length, supporting the notion that the S_B state has an electronic excitation strongly localized on this unit. The calculated energy of this state is 32530 cm^{-1} which agrees well with the position of the center of gravity of the absorption band corresponding to the $S_0 \rightarrow S_B$ transition of nystatin ($\approx 32800 \text{ cm}^{-1}$ [15]). The calculated oscillator strength for this transition is 1.95. The experimental spectrum [15] shows a sec-

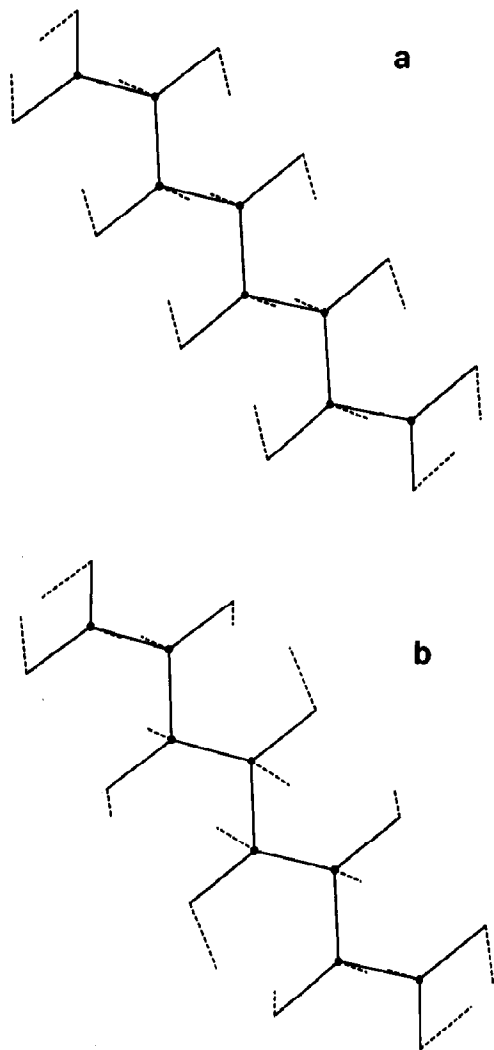


Fig. 1. The zero-point amplitudes of atomic motions (dashed lines) for two a_g C=C stretches of all-trans-1,3,5,7-octatetraene. (a) 1642 cm^{-1} , (b) 1618 cm^{-1} .

ond electronic transition centered at $\approx 43650\text{ cm}^{-1}$ with an oscillator strength smaller than that of the first transition. This can be compared with the calculated energy of 43515 cm^{-1} and oscillator strength 0.65 of the second strong electronic transition in TDH. The final state of this transition is identified as the perturbed $1B_u$ state of the diene chain. Table 3 lists the calculated frequencies, displacement parameters and relative Raman intensities of the FC active modes of TDH. They are compared with the frequencies and relative Raman intensities of the

Table 2

Bond lengths (in Å) in the S_0 ($1A_g$) and S_B ($1B_u$) states of TDH obtained by QCFF/PI calculations

Bond	S_0	S_B
C ₁ -C ₂	1.342	1.342
C ₂ -C ₃	1.474	1.473
C ₃ -C ₄	1.345	1.346
C ₄ -C ₅	1.483	1.483
C ₅ -C ₆	1.542	1.542
C ₆ -C ₇	1.484	1.481
C ₇ -C ₈	1.347	1.373
C ₈ -C ₉	1.470	1.430
C ₉ -C ₁₀	1.355	1.411
C ₁₀ -C ₁₁	1.466	1.414
C ₁₁ -C ₁₂	1.355	1.410
C ₁₂ -C ₁₃	1.469	1.431
C ₁₃ -C ₁₄	1.343	1.368

modes of nystatin as a powder and in methanol solution. The calculations predict four C=C stretch FC active modes in TDH. They can be described as perturbed a_g and b_u C=C stretches of the tetraene unit (see section 4). Since the TDH molecule does not have any symmetry elements, the a_g and b_u modes of tetraene can mix freely. The two most strongly FC active C=C stretches (1644 and 1623 cm^{-1}) are identified with the two modes of the simple model of section 2. The highest frequency C=C stretch mode of TDH (1660 cm^{-1}), which corresponds to a perturbed b_u mode of octatetraene, is identified with the

Table 3

Calculated ground state frequencies ω_i (in cm^{-1}) and displacement parameters B_i of the C=C and C-C stretches of TDH compared to those of nystatin [15]

ω_i	$ B_i $ ^{a)}	I_i ^{b)}	ω_i (nystatin)	I_i (nystatin) ^{c)}
1660	0.49	0.27	1633 ^{d)} (1635) ^{e)}	0.4 ^{d)} (0.20) ^{e)}
1644	0.95	1.0	1610 (1612)	1.0 (1.0)
1625	0.20	0.04		
1623	0.74	0.61	1557 (1556)	0.9 (1.1)
1251	0.18	0.04		
1188	0.29	0.09	1190	0.12
1187	0.72	0.57	1140 (1140)	0.30 (0.12)
1162	0.11	0.01	1159 (1158)	0.48 (0.36)
1142	0.10	0.01		
1133	0.17	0.03		

^{a)} Only modes with $|B_i| \geq 0.1$ are listed. ^{b)} $I_i = B_i^2 / B_{1644}^2$.

^{c)} I_i is determined as the relative Raman intensity at the excitation wavelength of 4579 nm .

^{d)} Solid. ^{e)} Methanol solution.

1633 cm^{-1} mode of nystatin. The latter mode was assigned previously to the C=O stretch vibration [15,17]. According to our calculations all Raman fundamentals of nystatin seen in the range of 1550–1650 cm^{-1} belong to the C=C stretching vibrations localized on the tetraene chain. The vibrational amplitudes of individual atoms in the 1644 and 1623 cm^{-1} modes of TDH (representatives of the 1610 and 1557 cm^{-1} modes of nystatin) are depicted in figs. 2a and 2b, respectively. We see that the latter mode has a small contribution from a diene chain; this contribution is negligible for the 1644 cm^{-1} mode. Comparing fig. 2 with fig. 1 we notice that the identity of the a_g C=C stretch normal modes of octatetraene is preserved to a large degree in the TDH molecule.

The calculations predict three Raman active modes in the range of the C–C stretch vibrations (1251, 1188 and 1187 cm^{-1}). The last two vibrations with very close frequencies are expected to mix easily under the influence of any weak internal perturbations imposed on the TDH molecule. These vibrations can be identified with the 1190, 1140 and 1159 cm^{-1} modes of nystatin of which the last two exhibit large variations of Raman intensity in different solvents (see table 3).

4. Vibrational analysis in terms of molecular fragments

In sections 2 and 3 we have demonstrated that the normal modes of different polyenic fragments of TDH preserve the identity of the unperturbed modes of isolated polyenes to a large degree. This suggests that many normal modes of molecules consisting of interacting polyenic chains can be analyzed in terms of normal modes of individual terms. It is therefore interesting to study the ground state force field of TDH from the point of view of the normal modes of its fragments (butadiene, ethane and octatetraene). For this purpose we use the Cartesian coordinate framework. The normal coordinate matrix \mathcal{L} of TDH satisfies the equation

$$\mu^{1/2} \mathbf{F} \mu^{1/2} \mathcal{L} = \mathcal{L} \mathbf{E}, \quad (9)$$

where \mathbf{F} is the force-field matrix and \mathbf{E} is the $(3N-6)$ -dimensional vector containing the vibra-

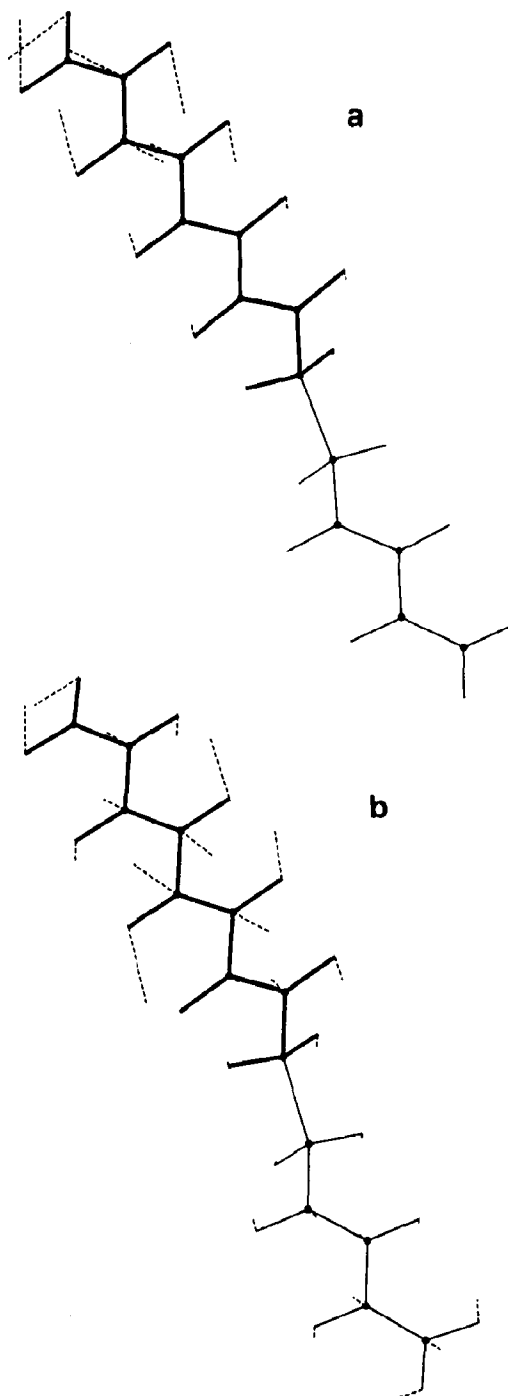


Fig. 2. The zero-point amplitudes of atomic motions (dashed lines) of the two strongly Raman active C=C stretches of TDH. The octatetraene fragment is depicted by a heavy line. (a) 1644 cm^{-1} ; (b) 1623 cm^{-1} .

tional frequencies. We calculate the normal mode coordinates of butadiene, ethane and octatetraene using the QCFF/PI method and construct the fragment mode matrix \mathcal{L}_P :

$$\mathcal{L}_P = \begin{pmatrix} \mathcal{L}_B & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathcal{L}_E & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathcal{L}_O \end{pmatrix}, \quad (10)$$

where $\mathbf{0}$ are rectangular matrices with all elements equal to 0 and \mathcal{L}_P is a partitioned, rectangular, non-unitary matrix, whose quasisdiagonal blocks are defined as follows:

(i) \mathcal{L}_B is a 27×24 matrix of normal modes of butadiene without the Cartesian components of the hydrogen atom replacing the ethane bridge.

(ii) \mathcal{L}_E is a 18×18 matrix of normal modes of ethane without the Cartesian components of the two hydrogen atoms replacing the diene and tetraene chains.

(iii) \mathcal{L}_O is a 51×48 matrix of normal modes of octatetraene without the hydrogen atom replacing the ethane bridge.

With the help of \mathcal{L}_P we obtain the block-diagonal interaction matrix \mathcal{V} whose off-diagonal elements inside each block are a measure of the interaction between modes localized on a given fragment. This matrix is calculated from

$$\mathcal{V} = \mathcal{L}_P^{-1} \boldsymbol{\mu}^{1/2} \mathbf{F} \boldsymbol{\mu}^{1/2} \mathcal{L}_P, \quad (11)$$

where \mathcal{L}_P^{-1} is a generalized inverse matrix of \mathcal{L}_P :

$$\mathcal{L}_P^{-1} = (\mathcal{L}_P^+ \mathcal{L}_P)^{-1} \mathcal{L}_P^+. \quad (12)$$

Examination of the interaction matrix \mathcal{V} shows that couplings between the modes of the same polyene fragment are much stronger than couplings between modes of different polyene fragments, indicating that the ethane bridge keeps diene and tetraene normal modes effectively localized, in agreement with the assumptions underlying the simple model of section 2.

The submatrix of \mathcal{V} for the C=C stretch modes of the octatetraene unit takes the form

$$\mathcal{V}_0(\text{C=C}) = \begin{pmatrix} 1635.8 & \cdot & \cdot & \cdot \\ -10.2 & 1633.5 & \cdot & \cdot \\ -11.6 & -10.9 & 1606.9 & \cdot \\ 13.2 & 12.5 & 13.7 & 1565.1 \end{pmatrix} \begin{matrix} b_u \\ a_g \\ a_g \\ b_u \end{matrix}, \quad (13)$$

where the elements are expressed in cm^{-1} , and the original symmetries of the C=C stretches of octatetraene are indicated on the right-hand side of the matrix. The off-diagonal elements are about $\frac{1}{2}$ the value of the "effective" mode mixing parameter k of the simple model in section 2. The matrix (13) demonstrates that all C=C stretches of the octatetraene unit mix equally strongly irrespective of their original parity. Replacing the diagonal values of (13) by the experimental frequencies of the C=C a_g and b_u stretches of octatetraene [31], we obtain the following frequencies for TDH: 1646, 1630, 1615 and 1587 cm^{-1} , whereas the eigenvalues of the matrix (13) are: 1645, 1635, 1607 and 1554 cm^{-1} . Both these sets agree very well with the Raman fundamentals of nystatin in the region of C=C stretch frequencies [15]: 1652, 1633, 1610 and 1557 cm^{-1} , confirming that these fundamentals belong to the C=C stretching modes of the octatetraene unit with little, if any, participation of the C=O stretching mode located at the hydrophylic end of the nystatin molecule.

Acknowledgement

We thank Willem Siebrand and Giorgio Orlandi for their critical remarks.

References

- [1] R. Birge, *Ann. Rev. Biophys. Bioeng.* 10 (1981) 315.
- [2] B.S. Hudson, B.E. Kohler and K. Schulten, in: *Excited states*, Vol. 6, ed. E.C. Lim (Academic Press, New York, 1982) p. 1.
- [3] M. Andretta, R. Serra, G. Zanarini and K. Pendergast, *Advan. Chem. Phys.* 63 (1985) 1.
- [4] A.C. Cope, V. Axen, E.P. Burrows and J. Weinlick, *J. Am. Chem. Soc.* 88 (1966) 4228.
- [5] P. Ganis, G. Avitabile, W. Mechlinski and C.P. Schaffner, *J. Am. Chem. Soc.* 93 (1971) 4560.
- [6] B.T. Golding, R.W. Rickards, W.E. Meyer, J.P. Patrick and M. Barber, *Tetrahedron Letters* (1966) 3551.

- [7] E. Borowski, J. Zielinski, L. Falkowski, T. Ziminske, J. Golik, P. Kolodziejczyk, E. Jereczek, M. Gdulewicz, Yu. Shenin and T. Kotienko, *Tetrahedron Letters* (1971) 685.
- [8] W.H. Henneker, W. Siebrand and M.Z. Zgierski, *J. Chem. Phys.* 79 (1983) 2495.
- [9] W. Siebrand and M.Z. Zgierski, *J. Chem. Phys.* 81 (1984) 185.
- [10] G. Orlandi and F. Zerbetto, *Chem. Phys.* 108 (1986) 187.
- [11] F. Zerbetto, M.Z. Zgierski, G. Orlandi and G. Marconi, *J. Chem. Phys.* 87 (1987) 2505.
- [12] F. Zerbetto, M.Z. Zgierski and G. Orlandi, *Chem. Phys. Letters* 141 (1987) 138.
- [13] F. Zerbetto and M.Z. Zgierski, *Chem. Phys. Letters* 143 (1988) 153.
- [14] L. Rimai, M.E. Heyde and D. Gill, *J. Am. Chem. Soc.* 95 (1973) 4493.
- [15] Z. Iqbal and E. Weidekamm, *Biochim. Biophys. Acta* 555 (1979) 426.
- [16] Y. Koyama, Y. Mukai, J. Umemura, M. Ito and K. Tsukida, *J. Raman Spectry.* 15 (1984) 300.
- [17] A. Colline, J. Bolard, L. Chinsky, J.-R. Fang and K.L. Rinehart Jr., *J. Antib.* 38 (1985) 181.
- [18] W. Siebrand and M.Z. Zgierski, in: *Excited states*, Vol. 4, ed. E.C. Lim (Academic Press, New York, 1979) p. 1.
- [19] W. Siebrand and M.Z. Zgierski, *Chem. Phys. Letters* 62 (1979) 3.
- [20] A. Warshel and M. Karplus, *J. Am. Chem. Soc.* 94 (1971) 5612.
- [21] A. Warshel and M. Levitt, QCPE 247, Indiana University (1974).
- [22] A. Warshel, in: *Modern theoretical chemistry*, Vol. 7, Part A, ed. G.A. Segal (Plenum Press, New York, 1977) p. 133.
- [23] B.S. Hudson and J. Andrews, *Chem. Phys. Letters* 63 (1979) 493.
- [24] A.C. Lasaga, R.J. Arni and M. Karplus, *J. Chem. Phys.* 73 (1980) 5230.
- [25] G. Orlandi, G. Poggi and F. Zerbetto, *Chem. Phys. Letters* 115 (1985) 253.
- [26] G. Orlandi and F. Zerbetto, *Chem. Phys. Letters* 120 (1985) 140.
- [27] R.J. Heneley, B.R. Brooks and M. Karplus, *J. Chem. Phys.* 85 (1986) 6550.
- [28] F. Zerbetto and M.Z. Zgierski, *Chem. Phys.* 110 (1986) 421.
- [29] S.O. Smith, J.A. Pardoan, J. Lugtenburg and R.A. Mathies, *J. Phys. Chem.* 91 (1987) 804.
- [30] F. Zerbetto, M.Z. Zgierski and G. Orlandi, *Chem. Phys. Letters* 139 (1987) 401.
- [31] E.R. Lippincott, W.R. Feairheller Jr. and C.E. White, *J. Am. Chem. Soc.* 81 (1959) 1316.