FTIR spectroscopy of synthesized racemic nonacosan-10-ol: a model compound for plant epicuticular waxes

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Abstract As there are no published graphically presented, detailed IR spectra of nonacosan-10-ol (occurring naturally and widely in plant epicuticular waxes of nanotube form), near IR FTIR spectroscopy (fundamentals, overtones and combinations) has been performed on laboratory synthesized racemic nonacosan-10-ol, as a crystalline solid on Mylar, and polypropylene substrates. Room temperature, *in vacuo* data are presented graphically, in full, and show evidence of extensive hydrogen bonding, an orthorhombic perpendicular subcell, a methylene wagging progression, diagnostic of all-*trans* conformational order, and Fermi resonance. Moderate or stronger anharmonicity is confirmed. Detailed discussion, quantitative in parts, is given of the observed spectral features, especially as to how they inform crystal structure and molecular conformation, and assignments given for some of the features. The results will serve as a reference for future IR studies of the natural epicuticular wax nanotube form of (S)-nonacosan-10-ol.

Keywords FTIR spectra · Hydrogen bonding · Orthorhombic perpendicular subcell · Racemic nonacosan-10-ol · Fermi resonance · Secondary alcohol

Abbreviations

FR Fermi resonance

1 Introduction

(S)-nonacosan-10-ol, also termed ginnol, is known to be the dominant, if not the sole, component of crystalline epicuticular wax nanotubes. They are found throughout much of the plant kingdom, especially in coniferous taxa, but there is no rigorous experimental evidence as to their molecular architecture [1–8].

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Nonacosan-10-ol is widely occurring, and numerous *in vivo* functions are attributed to its natural form as nanotubes. It therefore merits persistent investigation in the hope of unravelling the secrets of molecular architecture and structure–function relationships.

The chiral molecule has very low symmetry, possessing only the identity symmetry element, and is comprised of 26 methylene groups (CH_2), two methyl groups (CH_3), one methine group (CH) and one hydroxyl (OH) group. It belongs to the point group C_1 , in which there is only one symmetry species. It follows that there are no restrictions, based on symmetry considerations, on any particular normal mode of vibration coupling with any other, or interacting through Fermi resonance (FR).

The energetically most favourable and most commonly occurring conformation for a long-chain molecule in the solid phase is the extended all-*trans* conformation.

There are published absorption spectral data for nonacosan-10-ol (C_{29} -10-ol hereafter):

1. extracted and purified from natural sources [2, 9–16];
 - (a) as above, but in the molten state [17];
2. synthesized, racemic, in KBr [14];
3. synthesized (S)-enantiomer [18, 19].

The original form of the C_{29} -10-ol, as nanotubes on the plant surface, is of course destroyed by extraction and purification processes. None of the above published absorption data is graphically presented, and only that of Berkenbusch and Bruckner [19] was obtained using a Fourier Transform instrument.

In general, the various features are reported by the above authors as “bands”, e.g. the CH stretch feature is not resolved into its four components (except in Theuns et al. [17]). Some of these report very rudimentary data (e.g. [2, 11]), giving no more than one or three values for absorption peaks. In only a few instances are assignments made.

FR has been reported for some long-chain organic compounds (details and references can be found in Section 4). We show, in discussion, how the CH_2 wagging progression is a significant feature. As none of the above authors address these two issues, we herein give them careful and detailed consideration.

The National Institute of Advanced Industrial Science and Technology, Japan, IR spectral database [20] holds an entry, which is graphically presented, for nonacosan-10-ol, (SDBS 25971). The spectrum was obtained by that organisation using a KBr pellet in a FTIR instrument. No particulars as to sample source are given.

C_{29} -10-ol is not found in another major IR spectroscopy database, NIST (National Institute of Standards and Technology, USA). There are no published reports of spectra of the natural nanotube form of C_{29} -10-ol. Secondary alcohols, as a class, are poorly represented in the published IR spectral data. Of the few reports of IR spectra of unbranched secondary alcohols C_{10} - 31, none have been found with sufficient detail to be of use in comparison with the present data.

The purpose of this work is to provide detailed IR absorption spectral data on synthesized, racemic nonacosan-10-ol, focussing especially on those features which inform crystal structure and molecular conformation. It is hoped that these data can be used as a model system, for comparison with future work on natural C_{29} -10-ol in various forms, especially the naturally occurring crystalline nanotube form.

Nonacosan-10-ol can be expected to show common features with and be broadly similar to *n*-alkanes and polymethylene, differing from those by only a single oxygen atom.

Fig. 1 Prepared sample (*left*); low power magnification (30 $\times$) of low density region (*centre*); higher magnification (275 $\times$) of low density region (*right*)

2 Experimental

2.1 Sample preparation

Racemic nonacosan-10-ol, mp 79.5°C, was synthesized and supplied by the School of Chemistry, Cardiff University. A 10-mg sample was placed in the centre of a thin substrate membrane (0.9 μm Mylar film, or 3.3 μm polypropylene film) tautly mounted on a 45 mm diameter metal ring. The sample and substrate were then placed on a preheated thick metal plate in an oven and held at 88°C for 20 min. The oven, remaining unopened, was allowed to cool slowly overnight, to encourage natural crystallisation of the sample material. The prepared sample was typically found to have regions of varying density of structured material, representatively illustrated by Fig. 1. Several separately prepared samples were measured, for each substrate.

2.2 FTS measurement

The samples were measured spectrally, at room temperature over the range 600 to 6000 cm^{-1} using a BOMEM DA-8 Fourier Transform Spectrometer (FTS) in rapid scan mode. The BOMEM was configured with a potassium bromide crystal beam divider, a Globar source that emits as a blackbody at an approximate temperature of 1000 K, and a mercury cadmium telluride (MCT) photoconductive detector cooled to 77 K. Interferograms were recorded over an optical path difference of 0.665 cm and were sampled at 0.32 μm intervals, to yield, after Fourier transformation, data encoded every 1.174 cm^{-1} . To reduce detector noise, 10 interferometric scans were averaged to provide a single high signal-to-noise interferogram. In addition, phase errors were reduced by recording and Fourier transforming a minimum of three such averaged interferograms for each sample.

Measurements were made of (a) the spectral throughput of the instrument, as a reference (b) the substrate and (c) the prepared sample, positioned so that the beam covered, in turn, different density regions. The individual data sets were then Fourier transformed and the resulting three spectra from each measurement (a–c) were averaged. To reveal the position of the substrate features data set b is ratioed against data set a, the background. The sample data set c were then ratioed against the substrate data set b, to remove the substrate features from the sample spectrum. Although this was reasonably effective, incomplete cancellation of the substrate features was still seen occasionally, e.g. Fig. 2, at 1252 and 1717 cm^{-1} . The reason for this is that the nonacosan-10-ol wax has a refractive index of approximately 1.45

and acts as an antireflection coating on one side of the substrate film, effectively reducing the measured absorption. The ratio of c to b thus shows an apparent enhancement near the centres of the strong substrate absorption features. Fortunately, the features of interest are, in general, sufficiently well separated from these spectral artefacts to not affect the results presented here.

However, to rigorously exclude the possibility of the 720 cm^{-1} doublet being an artefact arising from the coincident feature in Mylar's absorption, the entire spectra was remeasured several times, using polypropylene (which does not absorb in this wavenumber region) as the substrate, and using the preparation protocol described above. There is not a parallel situation for the 1470 doublet, the measurements on Mylar being completely free of this complicating artefact.

2.3 Molecular modelling

In parallel with experimental work, molecular modelling software, Gaussian 98W (Rev A.9) [21], has been used to perform *ab initio* geometry optimisation using the density functional B3LYP method with a 6-31G(d) basis set (the minimum for reliable frequency prediction [22]) followed by vibrational normal mode calculation, upon a single isolated $\text{C}_{29}\text{-10-ol}$ molecule, in each of its three stable OH- group internal rotational conformers. These results are used to identify the relative relationships between calculated energy, conformer, and OH stretch vibrational frequency, and are not here reported in full.

3 Results

A full, detailed transmission spectrum 600 to 6000 cm^{-1} for the synthesized racemic compound was obtained, and is presented, Fig. 2, as the principal results data. Selected areas are also shown expanded (in Section 4), to clarify the detail they contain. The FTS instrument did provide measurement data up to 6000 cm^{-1} , but beyond 4600 cm^{-1} the data deteriorated into noise, containing no meaningful information, and is not shown.

The raw result data consisted of a number of spectra, differing in density of the sample material at the particular local area being measured. As no one single spectrum shows all the features to best effect, two spectra are given, as being a full presentation of the result data.

Significant features seen include:

- doublet features at $719/729$ and $1462/1472\text{ cm}^{-1}$, with the components having similar magnitude and 10 cm^{-1} separation;
- a progression of weak bands 1180 to 1325 cm^{-1} ;
- a broad hydrogen bonding feature at 3337 cm^{-1} , which resolves into two peaks, 3243 and 3337 cm^{-1} in the lower density area of the sample (however, the relative intensities of the two components are reversed in the two spectra);
- two features at 3603 and 3622 cm^{-1} , in the region normally occupied by the OH stretch fundamentals;
- a cluster of features for the CH stretches: 2848 to 2953 cm^{-1} ;
- peaks at 4111 , 4251 and 4325 cm^{-1} , where combination/overtone features normally occur (Table 1).

Fig. 2 FTIR spectra of synthesized racemic nonacosan-10-ol, *lower trace*; a low density region of the same sample, *upper trace*. The full spectrum is shown split, at 1600 cm^{-1} , into two sections, with different x -axis scales. The *inset* fragment is nonacosan-10-ol on polypropylene substrate, illustrating the validity of the doublet. The upper fragment trace is polypropylene itself, showing freedom from the coincident absorption given by Mylar. Both traces of the inset fragment are shown with arbitrary transmission scale, but actual wavenumber. Those features referred to in Table 1 and in the text, whose identity is not clear and obvious, are indicated. The *two arrowed shoulders* are discussed in Section 4.8

Table 1 Summary of the principal observed absorptions and assignments, for solid racemic C₂₉-10-ol

Wavenumber (cm ⁻¹)	Proposed assignment (details and references given in Section 4)
719/729	CH ₂ rocking
1134	C–OH stretch
1350	C–H deformation (tentative assignment)
1376	CH ₃ symmetric deformation ('umbrella')
1412 sh / 1435 sh	CH ₂ rocking 1st overtone
1452 sh	C–O–H in-plane angle-bending (tentative assignment)
1462/1472	CH ₂ scissors
2848	CH ₂ scissors (doublet) 1st overtone
Shoulder on lower side of 2916	
2871	CH ₃ symmetric stretch
2916	CH ₂ asymmetric stretch
2953/2963	CH ₃ asymmetric stretch
3243	Hydrogen bonding
3337	Hydrogen bonding
3603	OH (<i>anti</i> -) free stretch
3622	OH (<i>gauche</i>) free stretch
4251	Sum combination: CH ₂ symmetric stretch fundamental and scissors (doublet) fundamental
Shoulder on upper side of 4325	
4325	CH ₂ scissors (doublet) 2nd overtone
	Sum combination: CH ₂ asymmetric stretch fundamental and CH ₂ scissors (doublet) fundamental

sh = shoulder

4 Discussion

The results represent the experimental manifestation of the wide gamut of possible intra-molecular vibrations of the nonacosan-10-ol molecule in a crystalline environment, which we discuss here in turn, broadly ordered according to type and atom combination. Only the chain backbone/skeletal modes and hindered internal rotation of the OH are absent, lying, as they do, in the far IR.

We allow in every instance for anharmonicity, which is not necessarily uniform, by, for example, using non-integer multipliers and non-equality in sum/difference combinations.

4.1 OH stretch doublet

The two absorptions, at 3603 and 3622 cm⁻¹, the latter not explicitly reported by any of the authors above, we take to be the OH free stretch fundamentals (of a 2° alcohol, [23–25]) arising from the three internal rotational conformations of the OH group [26, 27].

This interpretation is supported by the report of Emmeluth et al. [28], who state, in respect of ethanol, "... in the monomer where the accepted experimental gas phase t/g splitting (refs 15 & 32 therein) amounts to 16–17 cm^{-1} ."

Using the Gaussian result, we can assign the absorption at 3622 cm^{-1} to the combined absorbance of both *gauche* conformers, where the OH is in a staggered relation to the hydrogen on carbon 10, and at approximately 120° to the molecule's long axis, and that at 3603 cm^{-1} to the lower energy (i.e. molecular potential energy) *anti*- conformer, where the OH is in a staggered relation to the hydrogen on carbon 10 and at approximately 90° to the molecule's long axis.

4.2 Hydrogen bonding

Hydrogen bonding is responsible for the large feature centred on 3337 cm^{-1} [24, 29]. The high integrated absorption seen here, taken together with the much lower absorption of the two OH fundamentals, indicates that most of the OH groups are participants in hydrogen bonding.

The wavelength value of the features indicates an O...H-O distance, i.e. between the two oxygen atoms, of about 2.72 Å, as estimated from the graph of correlations between vibrational frequency and O...O distance given by Jeffrey [29] (taken from Novak 1974). Hydrogen bonds of these lengths are categorised as 'strong' by Desiraju and Steiner [30], and as 'weak' (borderline, almost intermediate) by Jeffrey [29]. 'Intermediate' might be an acceptable compromise between these two author's scales, differing significantly in their relative terminology and rather poor agreement.

The separation of this broad feature into two well-resolved components (3243 and 3337 cm^{-1}), seen clearly in the result for the sample area of lower density, suggests chains of hydrogen bonds, with different dimensional details and/or multiple geometries within individual chains, and hence more than one set of O...H-O parameters.

Much stronger evidence for chains lies in the ratio of observed integrated intensities of the hydrogen bonded OH and the free OH, the vast majority being participants in hydrogen bonding. Thus, the intensity ratio can be explained by lengthy chains with a single free OH at one end.

It is conceivable that all the hydrogen bonds could exist as mutual pairings of molecules of opposite chirality in the racemic sample. However, this would imply no free OH, contrary to what is observed.

No firm conclusions can be drawn from the results as to the direction(s) which the hydrogen bond chains might lie in relation to the major molecular axis.

The *existence* of hydrogen bonding is almost certainly independent of conformation, but it is entirely plausible for its geometric parameters to be functions of conformation.

The reversal in the intensity of the two components of the overall hydrogen bond feature, as seen in the spectra of the two areas of contrasting density of the sample, could be due to non-uniform crystallisation dynamics (during the slow-cooling phase of sample preparation), resulting in variations in the crystallite structure and hydrogen bonding pattern in different regions of the sample, e.g. homochiral vs. racemic crystallites, and very small vs. larger crystallites [31], in turn influencing the balance and distribution of absorption frequency over the whole of the hydrogen bonding systems.

We propose that the reversal observed in our sample could be explained by polymorphism originating in this way, and where hydrogen bonded chains may exist in more than one pattern, and in more than one direction in relation to molecular axes and

the crystallographic subcell, and where small differences in the exact hydrogen bond dimensional parameters give rise to the observed multiple peak values.

The dominance of a particular polymorph could result from temporal and spatial local variation within the sample during cooling and crystallisation.

This is broadly in line with the explanations given by Marikhin et al. [32] for a very similar situation of multiple peaks of inconsistent dominance, which they found in long-chain α,ω -diols. These authors give a detailed discussion of the issue of multiple peaks within the very commonly observed hydrogen-bonding envelope, and of the alternation and inconsistency of intensity.

It is interesting that the AIST database spectrum [20] shows three clear peaks (in contrast to the present data, and that of Theuns et al. [17] and Marikhin et al. [32], and none of which are within 40 cm^{-1} of the values reported herein), but no further comment can be made without knowing details of the preparation and state of the AIST sample.

As a class of chemical bonding, hydrogen bonding is weaker than covalent bonding, but stronger than Van der Waals forces, and almost certainly plays a major role in the crystalline architecture of nanotubes, especially when existing as extensive, cooperative chains. Its powerful capacity for influencing and defining structure and properties is well illustrated by, for example, hydrogen bonding in the various forms of ice, its being responsible for the maximum density of water occurring at 4°C and the elegant structure of the DNA double-strand.

4.3 C-H stretches

The four grouped features for the methylene and methyl C-H stretches (2848 to 2953 cm^{-1}), Fig. 3a, are in agreement with the well-known traditional interpretation [24, 33] of spectra of long-chain aliphatic molecules, and with the CH_2 and CH_3 intensities in ratios appropriate to a chain molecule 29 carbons long. The wavenumber values are in very good agreement with those given by Theuns et al. [17], but also recognising that his results were obtained with different phase and temperature of the sample.

However, there are reports, particularly in the physical chemistry and spectroscopy disciplines of the literature, which reveal the traditional interpretation and assignments, in parts, to be too simplistic, and flawed, as we shall show.

- CH_2 (symmetric and asymmetric)

The peak at 2848 cm^{-1} has long been, and widely, ascribed to the CH_2 symmetric stretch. However, some authors report the occurrence, effects and details of FR involving the CH_2 symmetric stretch fundamental and the 1st overtone of the CH_2 scissors mode and collectively argue and present a consensus that FR is a standard feature of hydrocarbon chain vibrational spectra [34–38]. These two modes are of the same symmetry species, A, and can thus interact through FR [39].

Greene and Bain [40] describe a similar FR in the total internal reflection Raman spectra of “barley leaf epicuticular wax” (where β -diketones are known to be a dominant component [7]). However, there seems some confusion in their description in

1. there being conflict in assignment and attributing of peaks involved in the FR
2. what is stated in the text does not appear to be supported by the data presented graphically.

Fig. 3 **a–c** Expansion of selected parts of the full spectra of nonacosan-10-ol on Mylar substrate, showing features in detail, and indicating FR. **a** Low density area of sample, CH₂ stretches region; **b** full density area of sample, CH₂ wag progression region, with assigned k numbers; **c** medium (*top trace*) and full (*lower trace*) density areas, combinations/overtone region. Further details given in Section 4

Further, they make no mention of symmetry species, an essential element of FR. Thus, although we mention it here in our discussion of FR, as being the published report most similar in context to the present case, we cannot accept it as convincing evidence of a genuine instance of FR.

In light of these reports and the above discussion, we make a significant revision of the traditional interpretation and assign the 2848 cm^{-1} band and the shoulder on the lower side of the 2916 cm^{-1} band as being in FR, the lower component being the 1st overtone of the CH_2 scissors, the upper the CH_2 symmetric stretch fundamental (Fig. 3a).

For the peak at 2916 cm^{-1} , we retain the well-known standard assignment of CH_2 asymmetric stretch, but can give no reliable explanation for the shoulder seen on the upper side at 2936 cm^{-1} .

There may be still further complexity in this area arising from e.g. the influence of the doublet nature of the fundamental, and from the parallel FR with the minority methyl group. Lavalley and Sheppard [41] aptly state “The presence of additional bands of considerable intensity originating in 2σ (CH_3) complicates the appearance, and makes more difficult the interpretation, of spectra in the 2900 cm^{-1} region.”

Unfortunately, the present experimental instrumentation and technique afford neither the resolution of subtle complexities nor a rigorous quantitative analysis of the FR.

It appears that FR is a common, perhaps even general, feature (although not always correctly recognised or assigned) in methylene chain molecules, which are widely represented amongst biological molecules.

Gibbs [42], in respect of insect cuticular waxes, discusses the behaviour of the frequency of the CH_2 symmetric stretch as a function of *trans-cis* conformation, stating that “Adjacent methylene groups are predominantly in the *trans* configuration and absorb strongly at $\sim 2849\text{ cm}^{-1}$.”, and that a change to predominantly *cis* conformation, brought about by thermal mobility of the molecule, i.e. folded/kinked chains, shifts the absorbance to $\sim 2854\text{ cm}^{-1}$. That he assumes the band to be the CH_2 symmetric stretch does not, we believe, invalidate his assertion of indication of chain conformation. Thus, we enlist his argument, when applied to our observed value of 2848 cm^{-1} , as initial evidence of all-*trans* conformation in our sample, as would be expected for a wax in the crystalline state.

- CH_3 asymmetric

The band peak at 2953 cm^{-1} is consistent with the well-known standard assignment for CH_3 asymmetric stretch. Additionally, however, a weak shoulder appears on the upper side of the band, at 2963 cm^{-1} , in all our measurements.

Snyder et al. [34] report the CH_3 asymmetric stretch in polymethylene as having two components (2953 and 2962 cm^{-1}) of similar intensity. Our data are very consistent with this in terms of wavenumber, but the 2963 cm^{-1} component is at much lower intensity, just discernable as a slight shoulder on the blue side (Fig. 3a).

Durie and Harrisson [43] report the CH_3 asymmetric stretch as being “distinctly split” in triclinic (i.e. crystalline) *n*-dodecane (C12) and *n*-docosane (C22), but not other homologues, and mention a similar doublet associated with triclinic *n*-heptadecanoic acid (C17).

As the wavenumber values we have measured are in very close agreement with these, we also conclude that, in spite of clear evidence for orthorhombicity, our samples may have a small percentage of triclinic crystalline inclusions.

- CH₃ symmetric

The CH₃ symmetric stretch at 2871 cm⁻¹ appears a straightforward and reliable assignment, and appears within 3 cm⁻¹ or less of the values given for solid-state *n*-alkanes [24, 33] and within 2 cm⁻¹ of that given for polymethylene by Snyder et al. [34].

The methine C-H stretch, expected, as a weak feature, to occur at about 2890 cm⁻¹ [24] is almost certainly masked by the stronger CH₂ absorptions in this area.

As noted above, there may be further subtleties in combinations and/or FR in this area, which the present data do not permit the elucidation of. Future use of more sophisticated techniques, such as 2DIR, may contribute to further understanding of these complexities.

4.4 CH₂ deformations

The doublet features at 720 and 1470 cm⁻¹, with 10 cm⁻¹ splitting, very clearly seen in the sample low density area, are the 1st and principal member of the CH₂ rocking progression, and the CH₂ scissors normal mode respectively [44], and are conclusive evidence of an orthorhombic perpendicular subcell [32, 44–47]. (It is important to note that the splitting is an intermolecular effect, not an inherent characteristic of the single isolated molecule.) The similar magnitude of the two split components indicates near 90° chain zigzag plane relationships [44]. These two findings further confirm crystallinity and are important and definitive evidence of the type of molecular packing existing in the sample. Ulubelen [10] alone reports the two values of the 720 cm⁻¹ doublet, but only the single value of 1460 cm⁻¹.

That the 720 cm⁻¹ doublet is not an artefact arising from Mylar's coincident absorption is shown by the inset of this feature, measured on polypropylene (Fig. 2).

The 1st overtone of the rocking fundamental doublet is discussed in Section 4.8.

Another CH₂ deformation, wagging, we discuss separately in the next section.

4.5 The CH₂ wagging progression

There is a series of evenly spaced weak absorptions, in the region where progressions due to coupling of the methylene wagging oscillators have been described [33, 48–50]. Crucially, the progression only occurs where the chain or chain segment has all-*trans* conformation. The frequencies and spacing are a function of chain length, and the individual members of the series are identified with a *k* integer [33, 48].

The occurrence of such a progression is widely accepted as being diagnostic of all-*trans* conformational order, and is used for that purpose [51, 52].

Having established that the sample has all-*trans* conformation, there then follows the question: how long is the all-*trans* unit/segment, i.e. how many methylene units participate in the coupled set of oscillators?

We consider several possibilities, but 1st mention, for clarity, that the nonacosan-10-ol molecule consists, notionally, of 27 methylene units (the coupled oscillators) and two methyl end groups.

It is conceivable that the C29 backbone is broken or divided into two smaller parts by the molecule being folded upon itself, at some point along its length — a tight fold, as occurs in cycloalkanes and crystalline polyethylene, where it is sometimes termed ‘adjacent re-entry’ [53].

The full linear set of coupled oscillators can similarly be broken into smaller subunits by another possible instance of disorder, one or more random *gauche* bonds/conformations. However, this would be much less consistent with crystalline order and so, unlikely.

If either of these were the case, the lowest member of the progression, $k = 1$, would be observed above wavenumber approximately 1215 cm^{-1} .

What appears to be the lowest member of the progression is observed at 1181 cm^{-1} . This is too low for a segment significantly shorter than the full C29.

Could the O / O-H effectively split the coupled set of 27 into two smaller independent subsets, but with the full length of the molecule still in linear registry? By the same argument in respect of the position of $k = 1$, no.

When compared with the *n*-alkanes [33, 48], the observed progression, in both its position and spacings, is more similar to that of *n*-nonacosane than any other *n*-alkane in the range C10-30. The slight irregularity observed for $k = 1$ and $k = 5$ could be an effect of the alteration of one of the methylene oscillators in the coupled set.

The values of φ_k , the phase difference between adjacent methylene oscillators, calculated, for each k (Table 2), using the formula [33, 48, 49, 54]

$$\varphi_k = \frac{k 180}{(m + 1)} \quad (k = 1, 2, 3 \dots m) \quad (1)$$

(where m = number of coupled methylene oscillators)

when placed on the dispersion plot [33, 48], fall on the common curve for the *n*-alkanes. This is evidence for correctness in our assignments for the k values. The anomalies, mentioned above, of $k = 1$ and $k = 5$ are manifested in their respective points falling not quite on the curve.

It could conceivably be argued that $m = 26$ should be used, because the oscillator at C10 is not strictly a methylene unit and interrupts the uniformity/continuity of the coupled set. When a dispersion plot is again made using this value for m , the plotted points, as a set, move away from the curve, indicating $m = 26$ to be a less valid choice.

Thus, we argue that although the oscillator at C10 is not technically a methylene, it can still be treated as such for the purposes of the formula and dispersion plot. This implies that

Table 2 CH₂ Wagging progression: assignments and calculated phase angles; and published wavenumbers for *n*-nonacosane

k	Wavenumber ^a	Calculated phase angle, in degrees	<i>n</i> -Nonacosane wavenumber ^b
1	1186	6.42	
2		12.9	1192
3	1208	19.3	1206
4		25.7	1218
5	1236	32.1	1230
6		38.6	1242
7	1255	45.0	1254
9	1281	57.9	1278
11		70.7	1301
13	1322	83.6	1323
15		96.4	1342
17		109.2	1357

^avacancies, for the sample, are either ambiguous or unobserved

^bmeasurements made at -180°C and reported by Snyder [48]

the substitution of the 16-fold heavier oxygen atom does not greatly affect the frequencies associated with the coupled system.

We make k -value assignments, based on and verified by the particulars discussed above, for all the observed members of the progression, and give these in Table 2, where the calculated phase angles are also included.

An artefact arising from incomplete cancellation of a Mylar feature (see Results, Section 3) coincides with the $k = 7$ absorption, but that peak is still clearly seen within the larger envelope due to the artefact. We assert that $k = 7$ is still a valid absorption, in spite of this circumstance.

In the n -alkane homologous series, all k values are observed for odd numbered chains, whereas only $k = \text{odd}$ values are observed for even numbered chains [33]. The present data, where only $k = \text{odd}$ values are seen, is not consistent with the pattern seen in the chains without the oxygen substitution, i.e. n -alkanes. Snyder and Schachtschneider [33] state that, for C odd chains, the k even are less intense than k odd. This could explain the discrepancy, in the k even being less intense to the point of disappearance. Alternatively, or perhaps additionally, it could again be an effect of the presence of the oxygen atom.

Although theoretically predicted, the higher members of the progression are seldom observed, as the present data illustrate.

We acknowledge that much of the above discussion relies on the assumption that the characteristics of the wag progression in secondary alcohols will be similar to that in the n -alkanes. We believe there to be no significant theoretical argument or experimental evidence against such a similarity, and so believe it a reasonable assumption.

Thus, the observation, in nonacosan-10-ol, of a methylene wagging progression is diagnostic for the all-*trans* conformation, with both the position of $k = 1$ and the spacing between members forming rigorous evidence for the entire length of the molecule being in the fully extended all-*trans* conformation.

The CH₂ rocking/twisting normal modes also form progressions, but that area also contains C-C stretch vibrations making interpretation more difficult [48]. As these rocking/twisting progressions are not so readily useful in giving an indication of the degree of chain order/disorder, we do not give them the detailed treatment that we have done for the wag progression.

4.6 C-OH stretch and angle bend; CH₃ deformations

Theuns et al. [17] assign the C-OH stretch to 1132 cm⁻¹. The clear absorbance at 1134 cm⁻¹ in this work is in good agreement with this and the (unassigned) 1133 cm⁻¹ absorbance reported by Kuo and Chen [15].

Hahn [14] alone reports the absorbance at 1380 cm⁻¹ and assigns it to the “O-H deformation”, i.e. the C-O-H angle bend. However, Avram and Mateescu [24] point out that its frequency is increased by participation in hydrogen bonding (e.g. in liquid methanol it is 1420 cm⁻¹) and that in more complex structures it cannot readily be identified. It is here suggested that this C-O-H in-plane angle-bending normal mode may account for the shoulder at 1452 cm⁻¹, seen on the lower side of the strong CH₂ scissors feature at 1462/72 cm⁻¹ (Fig. 2).

The absorbance found at 1376 cm⁻¹ can instead be assigned to the CH₃ symmetric deformation [24]. The CH₃ asymmetric deformation at 1460 cm⁻¹ [24] is normally masked by the strong CH₂ feature at 1462/72 cm⁻¹.

4.7 1350 cm⁻¹: a C-H deformation?

A moderately strong absorption appears at 1350 cm⁻¹. Absorption at this frequency has been ascribed to *gauche* conformations in the chain, but this seems inconsistent with the clear evidence for all-*trans* conformation and crystallinity. We propose an alternative, a deformation of the lone hydrogen at carbon 10. The observed intensity seems very high for an absorption originating essentially from a single hydrogen atom. We suggest the intensity is augmented by the strongly electronegative oxygen atom inductively withdrawing electron density towards carbon 10, increasing the magnitude of the C-H bond dipole, which in turn increases absorption, and by the fact of the change in the dipole moment being one of direction, relative to an external coordinate reference frame.

There is a direct parallel for this mechanism of intensity increase in the hydrogen cyanide molecule, where the analogous lone hydrogen normal mode is the strongest [39].

The participation of the oxygen in hydrogen bonding might also be a contributing factor. In unpublished data for some secondary alcohols, this band is sometimes present, sometimes not. Accounting for its origin does not seem straightforward, so we make no more than a provisional assignment.

4.8 Combinations and overtones

The features seen above 3800 cm⁻¹ are necessarily combinations and/or overtones of the fundamental modes. The observed features, between 4000 and 4400 cm⁻¹, are strongly similar to the data, analysis and interpretation given by Ricard-Lespade et al. [55] in respect of short *n*-alkanes and hexatriacontane (C36) in the solid state, wherein this whole group of features is termed the $\Delta\nu = 3/2$ polyad. Thus, the present data is described as being:

1. a combination of the CH₂ asymmetric stretch fundamental with the CH₂ scissors (doublet) fundamental, giving the observed dominant peak around 4325 cm⁻¹
2. a combination of the CH₂ symmetric stretch fundamental and the scissors (doublet) fundamental, which is in FR with the scissors 2nd overtone. The FR displaced combination band appears at 4251 cm⁻¹, and the FR displaced 2nd overtone as a shoulder on the upper side of the 4325 cm⁻¹ feature. A 2nd overtone is normally very weak, but here can gain intensity through the FR mechanism.

These details are illustrated in Fig. 3c.

A 2nd overtone will itself be a more widely spaced doublet, which could either lie entirely on the upper side, or straddling the main peak. The present data do not allow a resolution between these two locations, which will depend on the exact degree of anharmonicity present.

The wavenumbers of the lower two of the three shoulders on the lower side of 1470 (Fig. 2) can be related to those of the rocking fundamental doublet

$$719 \times 1.9638 = 1412 \quad (2a)$$

$$729 \times 1.9684 = 1435 \quad (2b)$$

clearly indicating them to be the 1st overtone of the fundamental. The members of the doublet will not necessarily have identical anharmonicity, accounting for the very small difference seen in the multiplication factors. A 2nd overtone, expected at approximately 2056/2084 cm⁻¹, is not observed.

Similarly, for the absorbance at 2660 cm^{-1} and the fundamental at 1350 cm^{-1}

$$1350 \times 1.9703 = 2660 \quad (3)$$

indicating 2660 cm^{-1} to be the 1st overtone of the fundamental provisionally assigned as the C-H deformation. A 2nd overtone, expected at approximately 3892 cm^{-1} , is not observed.

Further, for the absorbance at 2732 cm^{-1} and the CH_3 “umbrella” fundamental at 1376 cm^{-1}

$$1376 \times 1.9855 = 2732 \quad (4)$$

indicating a 1st overtone of the fundamental. The multiplication factor being closer to exactly double indicates weaker anharmonicity for this vibration. There is an extremely weak peak at 3984 cm^{-1} , which is exactly where a 2nd overtone would be expected, but it cannot be reliably differentiated from noise.

The C-OH stretch fundamental (1134 cm^{-1}), being a strong absorbance, and involving the electronegative oxygen atom, might be expected to show overtone(s). However, of the three weak absorbances in the vicinity of the expected value of a 1st overtone, 2229 cm^{-1} , none is a suitable multiple, even allowing for possible stronger anharmonicity.

Overtone of the strong CH_2 scissors are discussed in Section 4.3 (the 1st), and above (the 2nd). Overtone of the various C-H stretches occur in a region beyond the obtained data.

Combinations and overtones are often subject, in their activity, to symmetry-based/derived selection rules. However, this generalisation does not, theoretically, apply to nonacosanol, because of its complete lack of any symmetry.

We have given detailed consideration to other possible permutations of combinations/overtone of the observed fundamentals, but none were found having better merit for explaining the data than the above.

Numerous other permutations of combinations are theoretically possible, but none can be reliably identified in the present data.

4.9 Anharmonicity

Analysis of the observed absorptions permits comment to be made upon the anharmonicity of the underlying normal vibrations.

Simple harmonic motion is never more than an approximation, when applied to real molecules, which always display some degree of anharmonicity, and where the Morse potential becomes a more accurate description.

Anharmonicity causes the breakdown of the selection rule $\Delta v = \pm 1$, and it is confirmed in the nonacosanol sample by the observance of overtones, combinations, FR, hotband(s) and couplings between otherwise independent normal modes.

The degree of anharmonicity is indicated qualitatively by the number and strength of overtones, magnitude of separation in the components of FR, and extent of couplings. The deviation of the fundamental/overtone wavenumber ratios from the integer value of the harmonic approximation, and the magnitude of the red shift of hotband(s) can give quantitative indications of the strength of anharmonicity.

That the various spectral indicators of anharmonicity are observed for some of the molecule's vibrations indicates moderate or stronger anharmonicity in the vibrations of the atoms responsible for the relevant normal modes.

A few of these can be treated somewhat more quantitatively in being ordered according to degree of anharmonicity, i.e. in approximate order of decreasing strength, based on the values of fundamental/ 1st overtone wavenumber ratio:

CH₂ rocking 719/29 cm⁻¹, 1.9638/1.9684; C-H deformation 1350 cm⁻¹, 1.9703; CH₃ symmetric deformation 1376 cm⁻¹, 1.9855.

These ratios are all lower than 20 fundamental/ 1st overtone wavenumber ratios (average 1.982) taken from Herzberg's [39] extensive tables of IR data and assignments for common molecules, suggesting, again, moderate and stronger anharmonicity.

The 1st and 2nd overtone of the CH₂ scissors vibration cannot be treated semi-quantitatively, as the above have been, because of their being simultaneously involved in FR. However, three factors point strongly to this vibration nonetheless having strong anharmonicity:

1. simultaneous occurrence of the 1st overtone and a FR with it
2. involvement of the scissors fundamental in a combination with the CH₂ asymmetric stretch fundamental
3. simultaneous occurrence of the 2nd overtone and a FR with it. Strong anharmonicity would seem entirely consistent with even a basic "classical" consideration of the physical nature of the scissors vibration.

The other vibrations showing anharmonicity are CH₂ symmetric stretch (FR) and CH₂ asymmetric stretch (combination).

The remaining vibrations, those not named anywhere above, do not show unambiguously any evidence of anharmonicity, the C-OH stretch for example. That overtones are not observed does not mean absence of anharmonicity — anharmonicity is never completely absent in real molecules.

In some cases, degree of anharmonicity will be a function of conformation and/or local environment, i.e. crystalline architecture/ relationship to neighbouring molecules, hence the relevance of the detailed analysis made here.

The CH₂ rocking vibration (719/729 cm⁻¹), being a mode with stronger anharmonicity, could be expected to have a hot band in the 668 cm⁻¹ area. That one is not seen is almost certainly because the frequency and ambient temperature are not sufficiently favourable for higher vibrational levels to be significantly populated, according to the Boltzmann distribution.

The observance of multiple transitions enables, at least in principle, the calculation of anharmonicity constants, and ultimately force constants, but are beyond the scope of this study.

The experimental method does not permit an analysis of the contributions of electrical and mechanical anharmonicity to the total anharmonicity. We are limited to applying, to the present case, the generalisation that anharmonicity is usually mostly mechanical in origin.

4.10 General discussion

The results seen here are generally in good agreement with previous works, although splitting at 720 and 1470 cm⁻¹ is not mentioned by Theuns et al. [17] (their sample was not in the solid state), or Hahn [14], or seen in the AIST database spectrum [20]. In the case of these latter two, this could mean no more than that the sample was in the alternative triclinic form. Hahn [14] does not report, for either of the two forms she examined, the weaker

methyl stretch absorptions, observed in this work at 2953 and 2871 cm^{-1} . The combination and overtone features, and Fermi resonances have not previously been reported.

It can be expected that some features will show a sensitivity, through subtle changes in anharmonicity, to molecular conformation and/or crystalline environment [55]. The detailed values presented here may be of use in comparison with future data in informing or giving indications as to conformation.

Whereas the CH_2 symmetric stretch frequency gives a reasonable qualitative indication of the degree of chain order/disorder, the wag progression has much greater sensitivity, and can be and is used quantitatively [51].

As might be expected, there is no obvious evidence of absorbance at 700, 1344 and 1442 cm^{-1} [56] or at 1298, 1345 and 1369 cm^{-1} [53], which would indicate a tight fold in the chain, i.e. the molecules are in the all-*trans*, extended chain conformation. Although most of these values, ascribed to a fold, coincide with the prominent feature observed here at 1350 cm^{-1} , it is smooth-sided, with no trace of the shoulders which would have been caused by the above absorbances.

When examinations of other forms of C29 are made, an important consideration will be the determining of the type of subcell. We have shown how the CH_2 wagging progression can be a valuable and appropriate analytic tool.

Although there is no obvious evidence in the spectra for the presence of water in the sample, there remains the theoretical possibility of water molecules being present in the crystalline lattice, and participating in the hydrogen bonding system.

A common technique in the preparation and mounting of samples for IR is as a thin film cast from a volatile solvent, or a drop of molten sample placed on/between salt plate(s). In both cases the resulting sample is likely to be in a glassy state. In contrast, the controlled, slow-cooling method we used maximises the likelihood of the solid sample being truly crystalline, as evidenced by the results.

We have not here attempted the assignment of every feature seen in the results, e.g. all the members of the rocking/twisting progressions, which, in a chain 29 carbons long, are many, and whose analysis is further complicated by the couplings between them and the skeletal normal modes, in this the so-called fingerprint region of the molecule's spectrum. Rather, the making of assignments has concentrated on those features for which this can be reliably done, and which hopefully will include those features which inform crystal structure and molecular conformation.

The advantages of a spectroscopic study of C₂₉-10-ol in the solid state are twofold. Firstly, it frees it from the interpretative complications arising from a solvent, and, from this point of view, validates any future direct spectroscopic comparison with the intact natural nanotube form of (S)-nonacosan-10-ol. Secondly, the measurement is rendered simpler than in other phases by virtue of the greater number of molecular conformations that usually exist in the liquid. In a solid there will be a minimum of different conformers, perhaps just those which arise from the OH rotation.

4.11 IR spectroscopy and natural epicuticular wax nanotubes

The puzzle of the molecular architecture in nanotubes is a difficult and long-standing one. What spectral phenomena might we expect from epicuticular wax nanotubes, the natural form of nonacosan-10-ol?

Structural gradation, in, for example, bond angles, hydrogen bonding geometry, and degree of steric hindrance, arising from the curvature present (i.e. differing degree of

curvature at any particular radius in the nanotube wall) could be manifest as broadening or blurring of spectral features.

Subtle changes in FR and small alterations of wavenumber could all inform nanotube molecular architecture.

Of the range of spectral features and manifestations revealed in this study, hydrogen bonding, the precise shape of the absorption features, the methylene wag progression and FR are those most likely to be useful in furthering our understanding of the molecular architecture of nanotubes.

Evidence will be needed in respect of type of subcell, molecular packing, hydrogen bonding parameters, and molecular conformation, i.e. straight, bent, kinked, folded, or curved in some way.

Of considerable importance will be the determination of the direction(s) of hydrogen bond chain direction in relation to the nanotube long axis. Possibilities include, at least, parallel, radial, and tangential. If polarised IR study of oriented, isolated nanotubes proves possible, some evidence may be gained on this point.

It is interesting to speculate upon whether the crystalline aggregation of homochiral nonacosan-10-ol in a nanotube form (itself an unusual crystal phenomenon) might give rise to any unexpected or novel absorption phenomena.

Another major class of nanotubular epicuticular wax features β -diketone as the dominant component substance [7]. This certainly has the long chain molecular structure motif in common with nonacosan-10-ol, but is not an alcohol. The presence of the two ketone groups, in the close mutual positional relationship designated β , can be expected to have very different IR absorption characteristics to nonacosanol. We could not condone the wholesale application of these results to β -diketone molecules. The utility of the present results, as a model, must be limited to nonacosanol, but use of selected aspects could be made, e.g. the diagnostic capacity and value of the methylene wag progression, hydrogen bonding, FR, overtones and anharmonicity.

Thus, FTIR study of the racemic substance, which could be viewed as a simpler form, where any complications or phenomena arising from homochirality are absent, establishes some clear basic absorption data — a model compound furnishing a benchmark which future work can draw upon.

5 Conclusions

The results presented here are consistent with a C29 secondary alcohol and are of sufficient quality and detail to conclude that:

- there is a regular crystalline structure, with orthorhombic perpendicular molecular packing and subcell
- the molecules are in the all-*trans*, extended chain conformation
- there are extensive chains of moderately strong intermolecular hydrogen bonding
- FR is a complicating feature of the methylene stretch ensemble, and in the overtone region
- anharmonicity is confirmed for some of the molecule's vibrations, and shown to be moderate or stronger.

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References

1. Jeffree, C.E.: Some aspects of the fine structure of the cuticle and epicuticular waxes of Sitka spruce, *Picea sitchensis*. Bong., Carr., Ph.D. Thesis, Aberdeen University (1974)
2. Franich, R.A., Wells, L.G., Holland, P.T.: Epicuticular wax of *Pinus radiata* needles. *Phytochemistry* **17**, 1617–1623 (1978)
3. Berg, V.S.: Physical, chemical and spectral properties of leaf surfaces of *Berberis aquifolium* (Pursh.). *Plant Cell Environ.* **8**, 631–638 (1985)
4. Jetter, R., Riederer, M.: Epicuticular crystals of nonacosan-10-ol: in-vitro reconstitution and factors influencing crystal habits. *Planta* **195**, 257–270 (1994)
5. Kusumi, T., Takahashi, H., Hashimoto, T., Kan, Y., Asakawa, Y.: Determination of the absolute configuration of ginnol, a long-chain aliphatic alcohol, by use of a new chiral anisotropic reagent. *Chem. Lett.* **1994**, 1093–1094 (1994)
6. Barthlott, W., Neinhuis, C., Cutler, D., Ditsch, F., Meusel, I., Theisen, I., Wilhelm, H.: Classification and terminology of plant epicuticular waxes. *Bot. J. Linn. Soc.* **126**, 237–260 (1998)
7. Jeffree, C.E.: The fine structure of the plant cuticle. In: Riederer, M., Müller, C. (eds.) *Biology of the Plant Cuticle*, pp. 11–125. Blackwell Publishing, Oxford (2006)
8. Koch, K., Ensikat, H.J.: The hydrophobic coatings of plant surfaces: Epicuticular wax crystals and their morphologies, crystallinity and molecular self-assembly. *Micron* **39**, 759–772 (2008)
9. Beri, R.M., Lemon, H.W.: Chemical examination of the wax from needles of black spruce (*Picea mariana*) and balsam fir (*Abies balsamea*). *Can. J. Chem.* **48**, 67–69 (1970)
10. Ulubelen, A.: Constituents of leaves and stems of *Clematis vitalba*. *Phytochemistry* **9**, 233–234 (1970)
11. Lu, S.T., Lun, C.N., Wu, T.S.: Studies on alkaloids of Formosan *Corydalis* species 3. Alkaloids of *Corydalis tashiroi* Makino. *J. Chin. Chem. Soc.* **19**, 41–44 (1972)
12. Luo, S., Gong, X., Gao, Z., Tan, J.: Studies on the chemical constituents of *Corydalis pallida* var. *speaose* Kom. *Yunnan Zhiwu Yanjiu* **5**, 315–316 (1983)
13. Ahmad, V.U., Mohammad, F.V., Rasheed, T.: Hirsudiol, a triterpenoid from *Cocculus hirsutus*. *Phytochemistry* **26**, 793–794 (1987)
14. Hahn, A.: Aggregation of (S)-nonacosan-10-ol, supramolecularly organized natural product, isolated from *Picea pungens* and its synthesized racemic form. *Korean Biochem. J.* **27**, 499–504 (1994)
15. Kuo, Y-H., Chen, W-C.: Chemical constituents of the pericarp of *Platycladus orientalis*. *J. Chin. Chem. Soc.* **46**, 819–824 (1999)
16. Kouda, K., Kusumi, T., Ping, X., Kan, Y., Hashimoto, T., Asakawa, Y.: 2-Anthrylmethoxyacetic acid, a new chiral anisotropic reagent for elucidating the absolute configuration of acyclic alcohols. *Tetrahedron Lett.* **37**, 4541–4544 (1996)
17. Theuns, H.G., Janssen, R.H.A.M., Biessels, H.W.A., Salemink, C.A.: Constituents of *Papaver bracteatum*: o-methyl- α -thebaol and 10-n-nonacosanol. Lanthanide-induced chemical shifts in ^1H NMR and ^{13}C NMR. *Phytochemistry* **24**, 163–169 (1985)
18. Langer, F., Schwink, L., Devasagayaram, A., Chavant, P.-Y., Knochel, P.: Preparation of functionalized dialkylzincs via a boron-zinc exchange. Reactivity and catalytic asymmetric addition to aldehydes. *J. Org. Chem.* **61**, 8229–8243 (1996)
19. Berkenbusch, T., Bruckner, R.: Synthesis of (+)-Ginnol, a type R(long)—CH(OH)—R'(long) alcohol, by an asymmetric beta, gamma-unsaturated ester $\rightarrow$ gamma-butyrolactone conversion. *Tetrahedron* **54**, 11471–11480 (1998)
20. AIST 2009 SDBSWeb: <http://riodb01.ibase.aist.go.jp/sdbs/> (National Institute of Advanced Industrial Science and Technology) Cited 27 Apr 2010
21. Gaussian 98 (Revision A.9), Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Zakrzewski, V.G., Montgomery, J.A. Jr., Stratmann, R.E., Burant, J.C., Dapprich, S., Millam, J.M., Daniels, A.D., Kudin, K.N., Strain, M.C., Farkas, O., Tomasi, J., Barone, V., Cossi, M., Cammi, R., Mennucci, B., Pomelli, C., Adamo, C., Clifford, S., Ochterski, J., Petersson, G.A., Ayala, P.Y., Cui, Q., Morokuma, K., Malick, D.K., Rabuck, A.D., Raghavachari, K., Foresman, J.B., Cioslowski, J., Ortiz, J.V., Baboul, A.G., Stefanov, B.B., Liu, G., Liashenko, A., Piskorz, P., Komaromi,

- I., Gomperts, R., Martin, R.L., Fox, D.J., Keith, T., Al-Laham, M.A., Peng, C.Y., Nanayakkara, A., Gonzalez, C., Challacombe, M., Gill, P.M.W., Johnson, B.G., Chen, W., Wong, M.W., Andres, J.L., Head-Gordon, M., Replogle E.S., Pople, J.A., Gaussian, Inc., Pittsburgh P.A. (1998)
22. Foresman, J.B., Frisch, A.: Exploring Chemistry with Electronic Structure Methods: A Guide to Using Gaussian, 2nd ed. Gaussian Inc., Pittsburgh, USA (1996)
23. Piccolini, R., Winstein, S.: Doublet character of O-H absorption in saturated alcohols. *Tetrahedron Lett.* **1**, 4–10 (1959)
24. Avram, G., Mateescu, G.D.: *Infrared Spectroscopy, Applications in Organic Chemistry*. John Wiley and Sons (1972)
25. van der Maas, J.H., Lutz, E.T.G.: Structural information from OH stretching frequencies monohydric saturated alcohols. *Spectrochim. Acta, Part A*. **30A**, 2005–2019 (1974)
26. Fruwert, J., Hanschma, G., Geiseler, G.: Zur Dublettstruktur Der Oh-Valenzschwingungsbande. *Z. Phys. Chem.* **228**, 277–279 (1965)
27. Bakke, J.M.: Rotational isomerism around the C-O bond in alcohols. Assignments of the OH IR bands. *Acta Chem. Scand.* **B40**, 407–410 (1986)
28. Emmeluth, C., Dyczmons, V., Kinzel, T., Botschwina, P., Suhm, M.A., Yanez, M.: Combined jet relaxation and quantum chemical study of the pairing preferences of ethanol. *Phys. Chem. Chem. Phys.* **7**, 991–997 (2005)
29. Jeffrey, G.A.: *An Introduction to Hydrogen Bonding*. Oxford University Press, Oxford (1997)
30. Desiraju, G.R., Steiner, T.: *The Weak Hydrogen Bond: in Structural Chemistry and Biology*. Oxford University Press, Oxford (2001)
31. Jacques, J., Collet, A., Wilen, S.H.: *Enantiomers, Racemates, and Resolutions*. Wiley, Chichester (1981)
32. Marikhin, V.A., Radovanova, E.I., Ivan'kova, E.M., Myasnikova, L.P., Volchek, B.Z., Medvedeva, D.A., Vlasova, E.N.: IR spectra of long-chain alpha,omega-alkanediols: 1,22-docosanediol and 1,44-tetradecanediol. *Polym. Sci., Ser. A*. **50**, 403–410 (2008)
33. Snyder, R.G., Schachtschneider, J.H.: Vibrational analysis of the n-Paraffins - I Assignments of infrared bands in the spectra of C₃H₈ through n-C₁₉H₄₀. *Spectrochim. Acta*. **19**, 85–116 (1963)
34. Snyder, R.G., Hsu, S.L., Krimm, S.: Vibrational-spectra in C-H stretching region and structure of polymethylene chain. *Spectrochim. Acta, Part A*. **34A**, 395–406 (1978)
35. Snyder, R.G., Scherer, J.R.: Band-structure in the C-H stretching region of the Raman spectrum of the extended polymethylene chain: influence of Fermi resonance. *J. Chem. Phys.* **71**, 3221–3228 (1979)
36. Abbate, S., Zerbi, G., Wunder, S.L.: Fermi resonances and vibrational-spectra of crystalline and amorphous polymethylene chains. *J. Phys. Chem.* **86**, 3140–3149 (1982)
37. Donaldson, P.M., Guo, R., Fournier, F., Gardner, E.M., Barter, L.M.C., Barnett, C.J., Gould, I.R., Klug, D.R., Palmer, D.J., Willison, K.R.: Direct identification and decongestion of Fermi resonances by control of pulse time ordering in two-dimensional IR spectroscopy. *J. Chem. Phys.* **127**, 114513 (2007)
38. Donaldson, P.M., Guo, R., Fournier, F., Gardner, E.M., Gould, I.R., Klug, D.R.: Decongestion of methylene spectra in biological and non-biological systems using picosecond 2DIR spectroscopy measuring electron-vibration-vibration coupling. *Chem. Phys.* **350**, 201–211 (2008)
39. Herzberg, G.: *Molecular Spectra and Molecular Structure. 2, Infrared and Raman spectra of polyatomic molecules*. Van Nostrand Reinhold, New York (1945)
40. Greene, P.R., Bain, C.D.: Total internal reflection Raman spectroscopy of barley leaf epicuticular waxes in vivo. *Colloid. Surf., B*. **45**, 174–180 (2005)
41. Lavalley, J.C., Sheppard, N.: Anharmonicity of CH₃ deformation vibrations and Fermi resonance between symmetrical CH₃ stretching mode and overtones of CH₃ deformation vibrations. *Spectrochim. Acta, Part A*. **28A**, 2091–2101 (1972)
42. Gibbs, A.G.: Lipid melting and cuticular permeability: new insights into an old problem. *J. Insect Physiol.* **48**, 391–400 (2002)
43. Durie, R.A., Harrison, R.J.: Effects of urea-adduct formation and physical state on the infrared spectra of n-paraffin hydrocarbons. *Spectrochim. Acta*. **18**, 1505–1514 (1962)
44. Li, H-W., Strauss, H.L., Snyder, R.G.: Differences in the IR methylene rocking bands between the crystalline fatty acids and n-alkanes: Frequencies, intensities, and correlation splitting. *J. Phys. Chem. A* **108**, 6629–6642 (2004)
45. Stein, R.S.: Interaction of methylene deformation frequencies in paraffin crystals. II. A theoretical treatment of the interaction of vibrations. *J. Chem. Phys.* **23**, 734–736 (1955)
46. Snyder, R.G.: Vibrational spectra of crystalline n-paraffins. II. Intermolecular effects. *J. Mol. Spectrosc.* **7**, 116–144 (1961)
47. Snyder, R.G.: Vibrational correlation splitting and chain packing for the crystalline n-alkanes. *J. Chem. Phys.* **71**, 3229–3235 (1979)

48. Snyder, R.G.: Vibrational spectra of crystalline n-paraffins. Methylene rocking and wagging modes. *J. Mol. Spectrosc.* **4**, 411–434 (1960)
49. Senak, L., Moore, D., Mendelsohn, R.: CH₂ Wagging progressions as IR probes of slightly disordered phospholipid acyl chain states. *J. Phys. Chem.* **96**(6), 2749–2754 (1992)
50. Jones, R.N., McKay, A.F., Sinclair, R.G.: Band progressions in the infrared spectra of fatty acids and related compounds. *J. Am. Chem. Soc.* **74**, 2575–8 (1952)
51. Moore, D.J., Wyrwa, M., Reboulleau, C.P., Mendelsohn, R.: Quantitative IR studies of acyl-chain conformational order in fatty-acid homogeneous membranes of live cells of *Acholeplasma laidlawii* B. *Biochemistry* **32**(24), 6281–6287 (1993)
52. Venkataraman, N.V., Bhagyalakshmi, S., Vasudevan, S., Seshadri, R.: Conformation and orientation of alkyl chains in the layered organic-inorganic hybrids: (C_nH_{2n+1}NH₃)₂PbI₄ (n = 12,16,18). *Phys. Chem. Chem. Phys.* **4**(18), 4533–4538 (2002)
53. Ungar, G., Organ, S.J.: Infra-red spectrum of tight chain folds in crystals of long linear paraffins. *Polym. Comm.* **28**(8), 232–235 (1987)
54. Chia, N.C., Mendelsohn, R.: CH₂ wagging modes of unsaturated acyl chains as IR probes of conformational order in methyl alkenoates and phospholipid bilayers. *J. Phys. Chem.* **96**(25), 10543–10547 (1992)
55. Ricard-Lespade, L., Longhi, G., Abbate, S.: The 1st overtone of CH stretchings in polymethylene chains: a conformationally dependent spectrum. *Chem. Phys.* **142**, 245–259 (1990)
56. Grossmann, H.P., Arnold, R., Buerkle, K.R.: IR-spectroscopic investigations of cycloalkanes. *Polym. Bull. (Berlin, Ger.)* **3**, 135–142 (1980)