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Diene conformation in the naturally occurring tricarboxylic acid, telfairic acid, by Raman spectroscopy

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Abstract

The conformational geometry of the two C=C bonds in a naturally occurring dienetricarboxylic acid, telfairic acid, isolated from *Xylaria telfairii*, has been determined by Raman spectroscopy. This application of analytical Raman spectroscopy is important because infrared, UV and NMR spectroscopic techniques have failed to provide sufficient information for the determination of the diene geometry. Several specially synthesised model dienecarboxylic acids with known diene conformations were used to calibrate the Raman data. The natural extract from *X. telfairii* was shown to be a mixture of both *cis* (*Z*) and *trans* (*E*) forms whereas the purified extract was predominantly the *trans* isomer. Raman data also suggest that the established method for the preparation of a *trans–trans* sorbic acid is open to debate. Further applications of the Raman technique for the determination of fungal metabolite extracts are proposed.

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1. Introduction

Previous vibrational spectroscopic work on the conformational geometry of C=C bonds in polybutadienes has demonstrated the application of Raman spectroscopy in particular for the qualitative and quantitative estimation of (1,4)-cis and (1,4)-trans structures and of the presence of (1,2)pendant vinyl units in the polymer skeletal structure [1,2]. Whereas, all three conformers exhibit Raman spectroscopic activity in the 1600–1700 cm^{-1} region of the spectrum, the situation is very different for infrared spectroscopy since the (1,4)-trans conformer has no dipole moment and is therefore infrared inactive in this region [3]. In a critical comparison of infrared, Raman and NMR structural analyses of polybutadiene conformers synthesised using anionic polymerisation methods [4], the infrared inactivity of the *trans* component resulted in a significant dissimilarity being observed between the infrared data from conformational mixtures and Raman and NMR data from the same systems. The advantage of Raman techniques over NMR spectroscopy for the rapid analysis of conformational diene compositions of the polybutadienes was further noted for the simplicity of the quantitative assessment and the ability to record the Raman data directly from the solid polymer specimens without prior mechanical or chemical treatment. In addition, the NMR spectroscopic differentiation [5] between the *cis* and *trans* diene conformations was achieved by the observation of their coupling constants *J*, namely 6–15 and 11–18 Hz, respectively, for the *cis* and *trans* forms. However, a requirement for the NMR analysis of these coupling constants is the presence of adjacent C–H bonds at carbons 1–4 of the –C=C–C=C– unit.

Ultraviolet spectroscopic absorptions of dienecarboxylic acids are also not sufficiently discriminating for the identification of the *cis* and *trans* geometric isomers since, the *cis–cis*, *cis–trans* and *trans–trans* forms all absorb at 262 ± 3 nm with extinction coefficients in the range 24,000–36,000.

Following the recent isolation [6] of telfairic acid, a new unsaturated diene tricarboxylic acid from *Xylaria telfairii*, an interesting structural problem has been realised; because of the absence of a proton on carbon 4 of the (1,4)-diene system the NMR spectra cannot provide values of the *J* coupling constants for the (3,4)-unsaturation on -C=C-, although that of the corresponding (1,2)-alkene moiety is still accessible. Since, the infrared spectral activity of a *trans*-conformational geometry at this (3,4)-C=C- unit is also forbidden by the selection rules, it appears that Raman spectroscopy could uniquely afford the opportunity to determine unequivocally the molecular

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conformational structure of the geometry of the substituted alkene at this location in the diene.

To demonstrate the reliability and potential effectiveness of the Raman spectroscopic analytical approach for this novel natural product, several model compounds of (1,2) and (3,4) unsaturated dienecarboxylic acids of reliably known alkene bond conformational geometries were studied. From these results, it was possible to monitor and evaluate the analytical Raman spectroscopic data and to determine for the first time the conformational geometry of the naturally occurring diene tricarboxylic acid, telfairic acid.

2. Experimental

2.1. Specimens

X. telfairii is a tropical fungus species which is found growing on dead wood; the specimens for the current analyses were collected in Fazenda Intervales, Sao Paulo State, Brazil, from dead dicot wood and were cultured for 8 weeks on a 3% malt extract medium in Thompson bottles ($2 L \times 20$), each containing 1 L of culture medium [6]. The fungus produced a thick white mycelium supporting small white stromata, approximately 3 mm long. Twenty litres of medium was extracted three times with ethyl acetate; the extracts were them dried and solidified to yield some 2.9 g of crude material. Following trituration with ethyl acetate, 0.8 g of a colourless solid was recrystallised from nitromethane and ethyl acetate to give 0.58 g of telfairic acid, 127–134 °C.

A work-up of the filtrate from the isolation of the telfairic acid produced above by evaporation to dryness, extraction with toluene/ethyl acetate/acetic acid (50/49/1) and elution on a silica gel column gave two fractions, one of which was identified as a 2,3-didehydrotelfairic acid and the other a colourless solid which on recrystallisation from ethyl acetate gave identical infrared and UV spectra to the telfairic acid isolated above, indicating that they were both telfairic acid [6]. Small changes in the ¹³C and ¹H NMR spectra of the two isolates confirmed that they were both telfairic acid, but with the *threo*-and *erythro*- conformations, *a* and *b*, respectively, as shown below.

The significant difference between these two telfairic acids is the conformation at the optically active site at carbon atom 2; this has no effect on the IR and UV spectra but does produce subtle changes in the NMR spectra as indicated above. It is clear, however, that the local conformation of the diene units have not been derived in the analyses, and this forms the objective of the current study.

Samples of telfairic acid, isolated from cultures of *X. telfairii* as described previously [6], including the sample which has been recrystallised from ethyl acetate, and having the proposed molecular structure shown in a above were analysed using FT-Raman spectroscopy. Model compounds based on a diene carboxylic acid system and having known structural C=C conformational geometries were also prepared according to literature methods as described below. The

molecular structures of telfairic acid and of the selected diene carboxylic acids are given below.

2.1.1. Buta-1,3-diene-1,4-dioic acid (muconic acid) CH(COOH)=CHCH=CH(COOH)

Cis–cis muconic acid, 2(*Z*), 4(*Z*)-muconic acid, was prepared by the oxidation of phenol with peracetic acid under carefully controlled conditions, avoiding the use of heat and maintaining the reactants in a darkened environment [7,8]. The *cis–cis* acid crystallised from boiling ethanol and remained unaffected during subsequent acidification and isolation from solution. The solid *cis–cis* acid was stable for several hours at 100 °C and was unchanged on exposure to daylight for up to 5 h. However, exposure of the ethanolic solution of the *cis–cis* acid resulted in conversion into the *trans–trans* isomer.

2.1.2. Buta-1,3-diene-1,4-dimethyl-1,4-dioic acid (dimethylmuconic acid)

CH₃C(COOH)=CHCH=C(CH₃)COOH

A similar synthetic procedure to that outlined above was undertaken using 2,5-dimethyl phenol (*para*-xylenol) and peracetic acid at room temperature for several days followed by recrystallisation from ethanol produced a specimen of *ciscis* dimethylmuconic acid.

2.1.3. Hexadieneoic acid (sorbic acid) CH₃CH=CHCH=CHCOOH

Sorbic acid was amongst the first diene carboxylic acids to be isolated, obtained by Hofmann [9] by the action of alkalis or mineral acids on rowanberry oil. The synthesis of sorbic acid from the ring opening of a methyl pentenyl lactone with boiling aqueous sodium hydroxide solution or methanolic sodium methoxide which produced the *cis–cis* and *cis–trans* isomers, respectively. Here, a classic Doebner [10] reaction between crotonaldehyde and malonic acid in dry pyridine, refluxed on a steam bath for 3 h followed by recrystallisation from boiling water gave colourless crystals of the *trans–trans* sorbic acid.

2.1.4. 5-Phenylpentadienoic acid

C₆H₅CH=CHCH=CHCOOH

Decarboxylation of a phenyl divinylmalonic acid, 2-carboxy-5-phenylpentadienoic acid (cinnamylidenemalonic acid), in pyridine solution [11] produced the *cis–cis* dienoic acid; sustained heating of the acid in quinoline at 130 °C for several hours produced isomerisation to the *cis–trans* isomer, specifically the 2(Z) 4(E) geometric isomer. Further thermolysis gave a mixture of the 2(Z) 4(E) and 2(E) 4(E) forms in approximately a 60:40 ratio, from which the 2(E) 4(E) isomer is obtained preferentially by recrystallisation.

2.1.5. Telfairic acid

CH₃CH₂C(COOH)=CHCH=C(COOH)CH(CH₃)COOH

The specimens of telfairic acid subjected to analysis were obtained from cultures [6] of *X. telfairii*, with a sample that had been recrystallised from ethyl acetate.

2.2. Raman spectroscopy

Fourier transform Raman spectra of telfairic acid and the model diene carboxylic acids were obtained using a Bruker IFS66/FRA 106 spectrometer with Nd³⁺/YAG laser excitation at 1064 nm and a liquid nitrogen cooled InGaAs detector. Spectra were recorded in a macroscopic mode with a spectral footprint of 100 microns using a spectral resolution of 4 cm⁻¹ over a wavenumber range of 100-3750 cm⁻¹; this range covered the skeletal modes of the diene as well as the CH stretching and bending modes. Spectral accumulation of 500 scans over a time of about 10 min was necessary to achieve improved signal-to-noise ratios for spectral comparisons between the extracts and the model compounds. Strong, sharp spectral bands were accurate to better than ± 1 cm⁻¹. The spectra shown in the figures have not been subjected to smoothing.

3. Results and discussion

Previous Raman spectroscopic studies [1,2,4] of polybutadienes revealed that the three possible diene geometrical confirmations, namely (1,4)-*trans*, (1,4)-*cis* and (1,2)-vinyl pendant, were identified through characteristic bands at 1655, 1640 and 1624 cm⁻¹, respectively. From controlled anionic polymerisation syntheses of di-block copolymers involving two or more of the conformers, it was demonstrated [2] conclusively that the diene geometrical conformers could be estimated quantitatively with a precision of about 1%.

In Fig. 1a, the FT-Raman spectrum of the muconic acid specimen synthesised in the present work is shown over the whole wavenumber range for C=C characterisation, 100– 3600 cm^{-1} . A single symmetric Raman band at 1642 cm⁻¹ (Fig. 1b) confirms the exclusively *trans-trans* (2Z, 4Z) geometrical arrangement of the diene double bonds in this compound.

Fig. 2 shows the Raman spectrum of the specimen of dimethylmuconic acid, which again exhibits a single strong band in the diene stretching region at 1625 cm^{-1} ; this is characteristic of a cis-cis conformational arrangement of the two C=C bonds. In both Figs. 1 and 2, there is no evidence for the presence of a *cis* or a *trans* conformer, respectively, in the predominantly *trans-trans* and *cis-cis* structures. Fig. 2 is also interesting in that the large molecular scattering cross-section of the C=C moiety is seen; the C=C is clearly the strongest band in the Raman spectrum and previous quantitative measurements of C=C conformational composition in dienes can be made to 1% or better. The C=C band is 500% stronger in intensity than the group of bands characteristic of CH stretching near 3000 cm^{-1} . The acid C=O functionality, which occurs strongly in the infrared and which can interfere with the determination of the infrared active diene modes is not visible at all in the Raman spectrum of the dienedioic acid in Fig. 2. There are other subsidiary features in the Raman spectra which are indicative of the additional methyl groups in the dimethyl muconic acid such as the more complex CH stretching region near 2950 cm^{-1} , CH



Fig. 1. FT-Raman spectrum, 1064 nm excitation, of *trans-trans*-muconic acid, with a C=C stretching wavenumber of 1641 cm⁻¹. Spectrum (a), 100–3400 cm⁻¹. Spectrum (b), 1575–1700 cm⁻¹, showing the singlet Raman band characteristic of the all *-trans* diene conformation.

deformations in the range $1350-1460 \text{ cm}^{-1}$ and C–C bond stretching at 780 cm⁻¹.

Fig. 3a shows the Raman spectrum of the sorbic acid specimen synthesised according to the definitive literature recipe in this work; however, the Raman spectrum of the 1580–1700 cm⁻¹ region (Fig. 3b) which is definitive for the C=C stretching conformation geometry reveals that the specimen has two bands, at 1627 and 1641 cm⁻¹, which is indicative of both *cis* and *trans* components being present. It is not clear from the data whether the sample is a *cis–trans* (2E, 4Z) or a mixture of *cis–cis* (2E, 4E) and *trans –trans* (2Z, 4Z) isomers. Nevertheless, it appears that the literature experiment for the preparation of a pure *trans –trans* specimen



Fig. 2. FT-Raman spectrum, 1064 nm excitation, of dimethylmuconic acid with a *cis–cis*-diene conformational arrangement and a C=C stretching wavenumber of 1625 cm⁻¹, wavenumber region 100–3400 cm⁻¹.



Fig. 3. FT-Raman spectrum, 1064 nm excitation, of an apparently *cis–cis-*diene conformation specimen of sorbic acid; (a), wavenumber range, $100-3400 \text{ cm}^{-1}$, showing a doublet band in the C=C stretching region, $1600-1650 \text{ cm}^{-1}$; (b), wavenumber range, $1580-1700 \text{ cm}^{-1}$, confirming the presence of both *cis* and *trans* conformational components at 1627 and 1641 cm^{-1} , respectively.

of sorbic acid needs to be questioned on the evidence of our Raman data. It is possible that there is some dependence on recrystallisation temperature or time that has not been realised hitherto but this can only be checked by an exhaustive range of repetitive experiments.

In the preceding Raman spectra, it has been noted that the *trans* and *cis* wavenumbers are at about 1642 and 1627 cm⁻¹, respectively, which are displaced some -13 cm^{-1} from their analogous positions in the unsubstituted butadienes [1,2,4]; this can be attributed to the influence of the electron-withdrawing carboxylic acid groups attached to the C=C units in the diene dicarboxylic acids.

This electron-withdrawing effect is even greater for the phenyl-substituted diene carboxylic acid specimen and Fig. 4 shows the Raman spectrum of the trans-trans isomer with two bands at 1629 and 1595 cm⁻¹; the former is the C=C stretching band of the trans-trans diene unit and the latter is the quadrant CCH stretching mode of the aromatic ring. In contrast, the specimen of 5-phenylpentadienoic acid which gave the Raman spectrum in Fig. 5 shows three bands at 1629, 1615 and 1595 cm⁻¹; here, the additional band at 1615 cm⁻¹ is now assigned to the cis geometric isomer, but it is not possible to say that this originates from a *cis-cis* or a *cis-trans* moiety in this compound or a mixture of these isomers. It is clear from the bands shown in Figs. 4 and 5 that the further reduction in band wavenumber caused by the presence of the phenyl group substituent in this dienoic acid is about -24 cm^{-1} based on the unsubstituted dienes. The effect of



Fig. 4. Detail of the $1580-1650 \text{ cm}^{-1}$ region of the FT-Raman spectrum of phenylpentadienoic acid, showing the presence of an all *trans–trans* conformational arrangement of the C=C bonds with a characteristic stretching mode at 1629 cm^{-1} , with the quadrant CCH aromatic ring stretching band at 1595 cm^{-1} .

the aromatic ring substituent in the dieneoic acid is mirrored in styrene monomer, a *trans*-alkene, where the Raman bands occur in this region at 1631 and 1604 cm⁻¹.

The Raman spectra of the isolated specimens of telfairic acid, namely the crude extract from X. telfairii (a, upper spectrum) and a recrystallised compound (b, lower spectrum) are shown in Figs. 6 and 7 where the two wavenumber regions $2500-3400 \text{ cm}^{-1}$ and $100-1800 \text{ cm}^{-1}$, respectively, are presented; the former indicates the CH stretching region with a C=CH band at 3020 cm^{-1} and saturated aliphatic CH stretching below 3000 cm⁻¹. The latter region shows the C=C stretching activity for the crude extract is based on a mixture of cis and trans geometries with bands at 1627 and 1642 $\rm cm^{-1}$, respectively, whereas the recrystallised compound obtained from boiling ethyl acetate has just one band at 1642 cm^{-1} indicative of the trans component solely. There is a slight asymmetry noted on the low wavenumber side of the 1642 cm^{-1} band in the lower spectrum which could indicate the presence of a small amount of residual cis isomer.

In common with the literature preparations of *trans* carboxylic acids from *cis–cis* and *cis–trans* isomers, the heating effected (and possible increased exposure to light) during the



Fig. 5. Detail of the 1580–1650 cm⁻¹ region of the FT-Raman spectrum of a phenylpantadienoic acid specimen which shows a *cis–trans*-conformational arrangement with C=C bands at 1629 and 1615 cm⁻¹, respectively, for the *cis* and *trans* C=C components. The quadrant CChH aromatic ring stretching band occurs at 1595 cm⁻¹.



Fig. 6. FT-Raman spectral stackplot of (a), telfairic acid specimen extracted from *Xylaria telfairii*, and (b), specimen recrystallised from boiling ethyl acetate; 1064 nm excitation, wavenumber region, $250-3400 \text{ cm}^{-1}$.

recrystallisation process has converted the *cis-trans* mixture almost completely to an all-*trans* isomeric arrangement.

This is the first time that a definitive statement can be made concerning the geometric arrangement of the diene C=C bonds in this natural product.

3.1. Raman band intensities

Throughout this current study, it is apparent that the Raman bands which are characteristics of the *cis* and *trans* diene isomeric arrangements do not have the same intensities and it is important to attempt an explanation of this. There are essentially two possible factors that could contribute to the different band intensities observed for the *cis* and *trans* C=C modes in these Raman spectra:

• The molar scattering coefficients of the *cis* and *trans* isomers are not identical; this is not the case for unsubstituted butadienes reported in the literature, where Raman band intensities of the three isomeric components were used to derive estimates of specimen composition which were accurate to about 1% when correlated with data



Fig. 7. FT-Raman spectral stackplot of (a), telfairic acid specimen extracted from *Xylaria telfairii*, and (b), specimen recrystallised from boiling ethyl acetate; 1064 nm excitation, wavenumber region, $100-1800 \text{ cm}^{-1}$.

obtained from a variety of other analytical techniques [2,3]. The assumption made in the previous Raman spectroscopic work that the molar scattering coefficients for the *cis*-, *trans*- and pendant vinyl components in butadienes was the same therefore seemed to be correct to within 1%.

• The presence of *cis-cis*, *cis-trans* and *trans-trans* geometric structures in our specimens are not completely differentiable in the Raman spectra. Hence, although it is clear that it is possible to discriminate unequivocally between a totally *cis* and a totally *trans* component in a mixture, the influence of the mixed *cis-trans* component in the same molecular structure has not been defined. A possible test of this would be effected if the Raman spectrum of a well-characterised *cis-trans* molecule could be obtained. The doubt already cast upon apparently well-established literature methods of preparation of these materials by the work carried out here means that the selection of such an apparently well-defined molecular geometry is not simple.

We believe that the band intensity differences between the Raman spectra of the geometric isomers studied here represents a real indicator of contributions from mixed component species rather that a fundamental difference between the molar scattering coefficients of the individual isomeric molecular forms which is not displayed elsewhere. On the basis of the assumption of similar molar scattering coefficients for the cis-cis, cis-trans and trans-trans isomers and the spectra recorded here, we can state that a 20% cisisomer excess initially created from the extraction of telfairic acid from X. telfairii is reduced upon recystallisation from boiling ethyl actetate solution to a predominantly trans-isomer with perhaps only a residual content of about 3% ciscomponent, i.e. the recrystallisation has resulted in the production of a 97% geometric isomeric purification into the trans-trans form.

4. Conclusions

The results of this study demonstrate the viability of Raman spectroscopy for the determination and identification of the diene geometries in a natural product dienetricarboxylic acid, telfairic acid. The opportunities for using Raman spectroscopy for monitoring the production of dienecarboxylic acids in biotechnological processes is also now presented, for example the ortho-cleavage of para-xylene by Nocardia corallina results in good yields of alpha, alpha-dimethyl-muconic acid which is believed to be the cis-cis form [12]. Benzene can be oxidised microbiologically (Pseudomonas sp.) to catechol and hence to cis-cis- muconic acid [13,14], which is one of the model type compounds used in the present analyses. Stanier[15] has stated that the production bacterially of *cis*trans geometric isomers of substituted muconic acids from benzene derivatives needs to be considered. Evidence is also now being presented for the production of difunctionalised dienes [16], including dienoic acids, from enzymatic degradation of chlorophenyl aromatic compounds. As the scope for

adoption of bacterial microorganisms in biotechnological reactions grows, the use of a technique for the analytical monitoring of the chemical species is needed and it is here that some possibilities are offered by on-line Raman spectroscopic probes. For this reason, the acquisition of spectroscopic data which enable the assignment of key marker bands in the spectra which can define the geometric structures of isomers is a real requirement and the present study addresses this.

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