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Vibrational Spectroscopy 28 (2002) 243–249

VIBRATIONAL SPECTROSCOPY

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# Vibrational spectroscopic study of brazilin and brazilein, the main constituents of brazilwood from Brazil

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Received 19 June 2001; received in revised form 11 October 2001; accepted 17 October 2001

**Abstract**

In this work, the vibrational spectra (FT-Raman and infrared spectra) of brazilin, the major component of brazilwood *Caesalpinia echinata* (from Bahia, Brazil), and brazilein, the oxidised pigment, are investigated. The FT-Raman spectra of the compounds show different patterns in the carbonyl stretching region, where brazilein presents a Raman feature at 1697 $cm^{-1}$ that is tentatively assigned to a coupled vibrational mode described by C=O and aromatic C=C stretching. Infrared measurements are used to support this assignment. The spectral region between 1700 and 1500 $cm^{-1}$ is also proposed as a fingerprint for brazilin and brazilein. Comparisons with some quinones and polyalcohols as parent molecules and other deep red resin pigments such as ''dragon's blood'' are undertaken to assist the vibrational assignment. As a test of the spectroscopic protocol for the identification of these pigments in natural brazilwoods, an 80-year-old archival specimen of *Caesalpinia echinata* was analysed non-destructively and the feature of brazilein shown from the Raman spectrum. 

*Keywords:* Brazilin; Brazilein; Brazilwood; FT-Raman spectroscopy

## 1. Introduction

In 1500, when Portuguese navigators arrived in South America, on what is today Brazil's coast, they found a very dense forest that was rich in a tree used by the Indians as a source of red pigment. The Portuguese realised that the tree was very similar to one they were used to carrying from Asia, named brazilwood (''pau brasil'', in Portuguese, meaning literally blazing wood). Since then, the name has been adopted by the country and there is a general agreement between historians that this provides a unique case where the name of a country was derived from that of a tree. There is an historical connection between the discovery of the new land and the growth of a dying industry from wood [1]. There are several varieties of brazilwood, and all of them can be employed as dyestuffs [2], but there is confusion over the species. *Caesalpinia sappan* (also known as *Biancaea sappan*), named sappanwood, is native to South India and Asia, and was the main source of brazilwood dye before the discovery of the Brazilian source. Pernambuco wood

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0924-2031/02/$ – see front matter 
PII: S0924-2031(01)00138-2

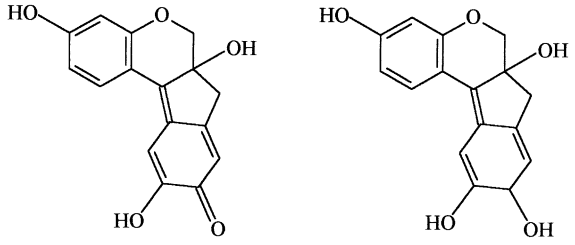


Fig. 1. Structural formulae of brazilin and brazilein.

is a product of *Caesalpinia echinata* (also named *Guilandina echinata*), native to the Brazilian Atlantic coast, it was cultivated but is now threatened. Other brazilwood are: *Caesalpinia crista* (also *Caesalpinia brazilienses* or *Caesalpinia violacea*), *Haematoxylum brasiletto* (or *Haematoxylum boreale*) and *Haematoxylum campechianum*. The species derived from *Caesalpinia* have the same red pigment, whose main constituent is brazilin, but brazilein is also present, and the species from *Haematoxylum* are the source of hematoxylin, that is used as dye for biological staining. The structural formulae of brazilin and brazilein are depicted in Fig. 1. It is interesting to note that brazilin is the major constituent in the crude dye, and brazilein can be isolated in large quantities when the organic extract is exposed to air and light, resulting in the oxidation of the hydroxyl of brazilin to a carbonyl group. Both compounds are tetracyclic, with two aromatic rings, one pyrone and one five membered ring. The aromatic ring bonded to the pyrone ring should have its origin in the acetate pathway, and the aromatic ring bonded to the five membered ring originates in the shikimic acid pathway. These substances should have a mixed biosynthetic route, with a predominance of the hydroxyl form in the plant structure. Since the discovery of Brazil, the brazilwood was highly valued for its timber and as a source of red pigment, and for its acoustical properties [3], and was logged almost to extinction [1]. There have been relatively few structural studies of brazilin and brazilein; the most recent is a $^{1}H$ and $^{13}C$ NMR investigation of brazilein [4]. The powdered wood of *Caesalpinia sappan* has been used in ancient traditional Chinese medicine as an analgesic and anti-inflammatory agent, and several recent studies have been directed toward this [5–12].

In this work, we present for the first time a vibrational spectroscopic study of brazilin and brazilein isolated from *Caesalpinia echinata* obtained from Bahia, Brazil, using FT-Raman and infrared techniques. Attempts to obtain the Raman spectra with visible excitation were unsuccessful due to fluorescence emission. The motivation for this study is provided by the identification of key molecular signatures for the main components of brazilwood, brazilin and brazilein, and their use in the non-destructive analysis of important and valuable archival materials. It is imperative for the characterisation of archival materials that no chemical or mechanical treatment is effected on the specimen; in this, the viability of the Raman technique is demonstrated here.

## 2. Experimental

A small piece (about 50 g) of heartwood of *Caesalpinia echinata* was ground and extracted with methanol (three times, at room temperature). The methanol extract was concentrated under reduced pressure and partitioned between water and methanol (3:1 v/v, 100 ml) and washed three times with 50 ml aliquots of ethyl acetate. The ethyl acetate extract was concentrated and separated in a silica gel column, eluting with chloroform–methanol (varying from 15:1 to 5:1 v/v), according the procedure described by Kim et al. [4]. The crude red portion was again chromatographed on silica gel, using chloroform–methanol–water (10:3:1 v/v/v). The separated fractions were allowed to stand overnight at room temperature, and two fractions, identified as PB3 and PB5 were investigated.

$^{1}H$ (270 MHz) and $^{13}C$ (270 MHz) NMR measurements were performed using a Jeol JMN-GX270 FT-NMR spectrometer, using deuterated acetone as a solvent. FT-Raman spectra were excited at 1064 nm using a Nd:YAG laser and a Bruker IFS66 spectrometer with FRA 106 Raman module, using 180° scattering geometry, and liquid-nitrogen-cooled germanium detector. Laser power was set at ca. 80 mW and 2000 scans were accumulated with a spectral resolution of 4 $cm^{-1}$. Infrared spectra were obtained with a Nicolet FT-IR instrument in the range 4000–4000 $cm^{-1}$, with 4 $cm^{-1}$ spectral resolution and 64 scan accumulations. Infrared absorption spectra were obtained with KBr pellets.

## 3. Results and discussions

The $^{13}C$ and proton NMR spectra of the two compounds were obtained in order to confirm their structures. In this sense, the PB5 sample (brazilin) showed in the $^{13}C$ spectrum a signal at 144.6 ppm, assigned to C9 atom bonded to a hydroxyl group, as described by Fuke et al. [13]. This signal is absent in the spectrum of brazilein (PB3 sample), but a new signal occurs at 179 ppm, assigned to a carbonyl group in the C9 position [4]. The remaining signals were almost identical in the two compounds. The proton spectrum of brazilin also shows a very good correlation with the literature [13], with a group of aromatic signals at the range between 7.15 and 6.50 ppm, and the carbinolic protons at 4.12 and 4.39 ppm.

The electronic spectrum of brazilein shows maximum absorptions at 445 and 556 nm [4], red-shifted when compared with brazilin, due to the increasing electronic de-localisation caused by the presence of the carbonyl group. The molar absorption coefficients of these bands are 64,500 and 36,500 l $mol^{-1}$ $cm^{-1}$, respectively [4]. In the brazilein structure, only the C6a and the pyrone oxygen atoms are $sp^3$ hybrids, and this part of the molecule is not planar, but the rest of molecule must participate in the electronic de-localisation process. Similarly, brazilin, which has another $sp^3$ carbon atom at C9, also does not have planarity in this part of the molecule. These structural features give rise to the colour difference between the two species, with brazilin having its electronic transitions blue-shifted when compared with brazilein.

The Raman spectra of brazilin and brazilein are displayed in Fig. 2, in the 2000–200 $cm^{-1}$ range (the inset shows the spectrum in the region 3500–2500 $cm^{-1}$), and the Raman and infrared spectra of both compounds are displayed in Figs. 3 and 4, in the 1800–600 $cm^{-1}$ range. Table 1 shows the vibrational wavenumbers of both compounds, together with a tentative assignment, based on literature of the parent molecules.

In the high wavenumber region, the Raman and infrared spectra of brazilin and brazilein are very similar, showing the vibrational modes related to the stretching of the different CH groups present in their structures.

The spectral region between 1500 and 1700 $cm^{-1}$ in both compounds exhibits the most prominent differences. As can be observed in Fig. 2, brazilein shows a band at 1564 $cm^{-1}$ that is not present in the spectrum of brazilin. At first sight, this Raman feature could be assigned to a vibrational mode related to the carbonyl group of brazilein. Comparison with the model compounds 1,4-benzoquinone (the prototype quinone molecule) [14–18], 1,3,5-benzenetriol [19] or 2,5-dihydroxy-1,4-benzoquinone [20] show that the

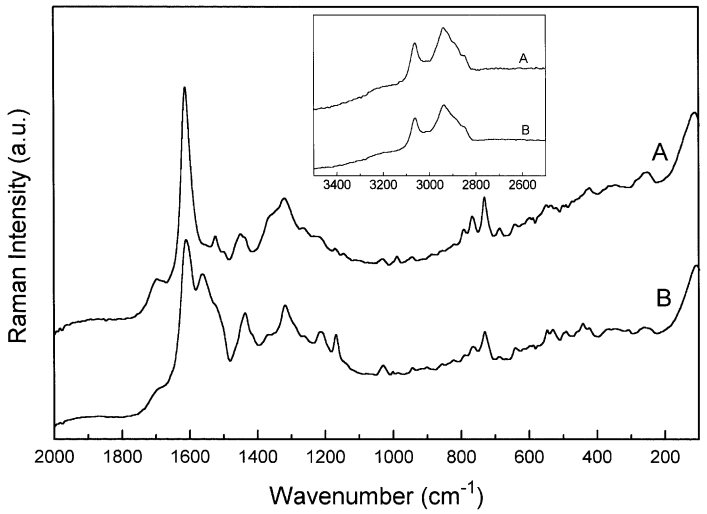


Fig. 2. FT-Raman spectra of pure solids brazilin (A) and brazilein (B), in the 20,000–100 $cm^{-1}$ region. The inset shows the Raman spectra in the region 3500–2500 $cm^{-1}$.

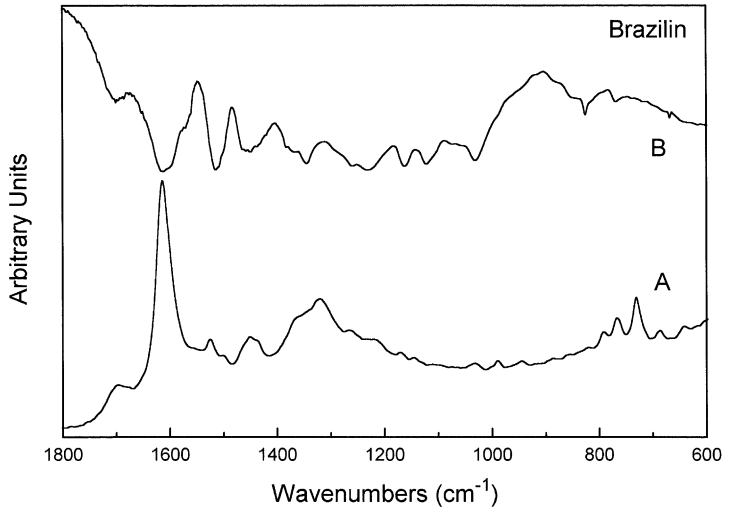


Fig. 3. FT-Raman (A) and FT-infrared spectra (B) (in KBr pellets) of brazilin, in the 1800–600 $cm^{-1}$ region.

carbonyl vibrational band might be expected at higher wavenumbers. For example, in *p*-benzoquinone the carbonyl group is present in the bands at 1666, 1639 and 1616 $cm^{-1}$, with different degrees of coupling with the C=C bond. In the case of brazilein, there is a band at 1697 $cm^{-1}$ that is not present in the spectrum of brazilin, and we could assign to the presence of the carbonyl group, along with some other bands present in the same region, that also could be assigned as coupled modes with the C=C bond. However, this comparison is not straightforward since the brazilein molecule has only one carbonyl group instead of the two groups present in the benzoquinone structure. In fact, the presence of only one carbonyl group could lead to a very small degree of de-localisation in the brazilein structure, which is reinforced by the Raman feature assigned to the C=O vibration. In a very recent paper, Zhan et al. [18] determined a generalised

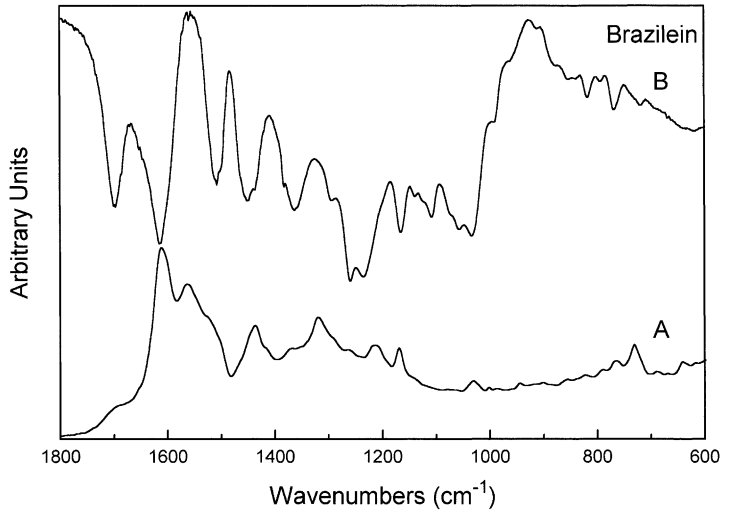


Fig. 4. FT-Raman (A) and FT-infrared spectra (B) (in KBr pellets) of brazilein, in the 1800–600 $cm^{-1}$ region.

Table 1
Raman wavenumber values (in $cm^{-1}$, excited at 1064 nm) and band assignments of brazilein and brazilin

| Brazilin | Brazilein | Tentative assignment |
|---|---|---|
| 252 vw | 261 vw | Ring deformation |
| 423 vw | 442 w | $\delta$(ring) + $\delta$(CO) |
| 473 vw | | $\delta$(ring) |
| 490 vw | 492 w | $\delta$(ring) |
| 501 vw | | $\delta$(ring) |
| | 531 w | $\delta$(ring) |
| 549 vw | 547 | $\delta$(ring) |
| 642 vw | 641 vw | $\gamma$(CH) |
| 687 vw | | $\gamma$(CH) + $\delta$(CC=O) + $\delta$(CC–O) |
| 732 mw | 731 mw | $\gamma$(CO) + $\gamma$(CH) |
| 767 w | 765 w | $\gamma$(CO) + $\gamma$(CH) |
| 792 vw | | $\gamma$(CO) + $\gamma$(CH) |
| 945 vw | | $\rho(CH_2)$ |
| 990 vw | | $\nu$(C–C) + $\nu$(C–O) |
| 1032 vw | 1031 vw | Ring stretching |
| 1172 vw | 1169 m | $\delta$(CCH) + $\nu$(C–C) |
| 1230 sh | 1214 m | $\nu$(C–O) + $\nu$(C–C) |
| 1260 sh | | $\nu$(C–O) + $\nu$(C–C) + $\delta(CH_2)$ |
| 1320 m | 1320 ms | $\nu$(C–O) + $\delta$(OCC) + $\delta(CH_2)$ |
| | 1365 sh | $\nu$[C=C(=O)–C=C] |
| 1451 w | 1437 ms | $\nu$(C=C) + $\delta$(ring) + $\delta$(COH) |
| 1525 w | | $\nu$(C=C) |
| | 1564 s | $\nu$(C=C) |
| | 1612 vs | $\nu$(C=C) + $\nu$(C=O) |
| 1614 s | | $\nu$(C=C) |
| | 1697 w | $\nu$(C=O) + $\nu$(C=C) |
| 2858 w | 2850 sh | $\nu$(CH) |
| 2900 sh | | $\nu(CH_2)$ |
| 2940 m | 2938 m | $\nu(CH_2)$ |
| 3060 mw | 3062 mw | $\nu$(CH) aromatic |

relationship to elucidate C=O bond stretching vibrational frequencies based on the maximum bond order of the C=O group, using quantum chemical calculations. They showed that a change in the s character is the most important factor for the determination of the position of the vibrational frequency, whose values may vary over a range of almost 100 $cm^{-1}$, from ca. 1680 to 1780 $cm^{-1}$. In molecules where the C=O group is directly linked to the benzene ring, like benzoquinone or 2,5-dihydroxy-benzoquinone, the C=O stretching is to be expected below this value, in the range 1620–1630 $cm^{-1}$. Hence, the wavenumber of the coupled stretching mode of C=C and C=O in benzoquinone (where it has electronic de-localisation over the whole molecule) can be observed at 1619–1639 $cm^{-1}$ [16]. The analogous mode is tentatively assigned here at 1697 $cm^{-1}$, and can be explained on the assumption that in brazilein there is a more explicit s character in the C=O bond. Even in the case of 1,4-benzoquinone, the prototype quinone molecule, there is some controversy in the literature about the exact potential energy distribution in the $\nu_2$(C=C bond) and $\nu_3$(C=O bond), and more experimental work must be undertaken for this system [16]. Keeping this in mind, all the vibrational assignments made here for brazilein and brazilin are necessarily tentative, and it must be expected that most of the vibrational bands must be coupled.

It is generally observed that Raman bands of molecules containing C=O groups are rather weak in intensity, but these occur with stronger intensity in the infrared. A comparison of the Raman and infrared spectra of brazilin and brazilein in the range 1800–600 $cm^{-1}$ is shown in Figs. 3 and 4, respectively, for which the presence of a strong band centred at 1697 $cm^{-1}$ in the infrared spectrum of brazilein can be observed. A weak feature at almost the same frequency is seen in the brazilin spectrum, assigned as the combination band (1230 + 473 $cm^{-1}$).

The molecular structures of brazilein and brazilin (Fig. 1) are very similar, and this fact is reflected in the vibrational spectra of both compounds. A detail that could also be used in the differentiation of both molecules is the Raman band that shows up as a shoulder in the spectrum of brazilein, at ca. 1365 $cm^{-1}$, and can also be seen in the infrared spectrum, at 1363 $cm^{-1}$, as a medium intensity feature. This band is assigned here as a stretching vibration involving the conjugated system present in brazilein and related to a C=C(=O)–C=C system. A comparison with the benzoquinone system is of interest, since we have single and double carbon–carbon bonds, together a carbon–oxygen double bond, but with less electronic conjugation over the C=O bond in the brazilein. From the work of Zhan et al. [18] on benzoquinone, it can be suggested that the C=O bond in brazilein has a more effective s character than in benzoquinone molecule. Brazilin does not show this vibrational band at 1365 $cm^{-1}$ because of the absence of the C=O bond.

Structurally, there is a great similarity between brazilin, brazilein and other natural red resin pigments, such as ''dragon's blood''. As discussed in a very recent paper [21], there are several different types of ''dragon's blood'' dyes, but it is believed that the

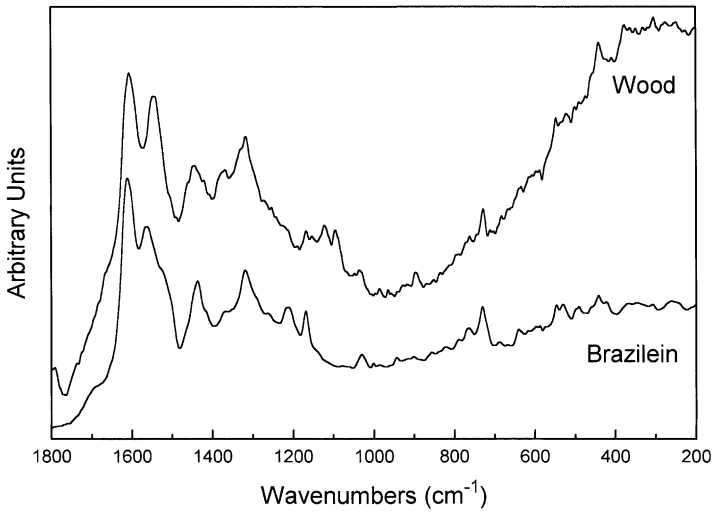


Fig. 5. FT-Raman spectra of brazilein (A) and 80-year-old wood sample (B) from the archives of Royal Botanic Gardens at Kew, UK.

original source in antiquity was obtained from the fruit of *Dracaena draco*, and the major components are dracorubin and dracorhodin, which have a similar chemical groupings to the quinone portion of brazilein. The Raman spectrum of *Dracaena draco* "dragon's blood" also shows the prominent bands in the 1600–1500 $cm^{-1}$ region, that have been assigned as a contribution of C=C and C=O stretching modes, in the same way as brazilein.

In ancient times, the crude powder of brazilwood was used by the Indians to dye ceramics and arrows, e.g. and was also used by the Europeans to dye clothes. Brazilin is the major component of the crude wood powder, but exposure to light and air lead to the formation of the brazilein compound, and so both compounds are responsible for the colouring properties of the dye. As a test of the identification of brazilin and brazilein in natural wood, we have recorded the FT-Raman spectrum of an archival sample of *Caesalpinia echinata* which had been collected in an expedition to Brazil (W.E. Hill & Sons) in 1920. This specimen is valuable and was obtained from the Royal Botanic Gardens Collection at Kew, UK. The non-destructive spectroscopic analysis of the specimen was imperative and the sample could not be subjected to any form of mechanical or chemical treatment—hence, powdering and extraction of the organic colorants was not possible. Fig. 5 shows a stack-plot of the Raman spectra of the 80-year-old archival specimen of brazilwwod, together the one from brazilein. The spectrum of the wood shows many similarities, including the strong features in the 1500–1700 $cm^{-1}$ region, characteristic of brazilein. It is interesting also to note that there is little evidence on the natural wooden specimen for the brazilin component; this we can attribute to oxidation to brazilein over time.

## 4. Conclusions

The FT-Raman and infrared spectra of brazilin and brazilein obtained as extracts from *Caesalpinia echinata* show several similarities. Characteristic spectroscopic differences in the C=C and C=O region, specially the vibrational band at 1564 $cm^{-1}$ in the Raman and the band at 1695 $cm^{-1}$ in the infrared spectra for brazilein, are not present in the brazilin spectra. Therefore, these bands can be assigned as the fingerprints of the brazilein compound. Comparison with other natural dyes as "dragon's blood" shows a common pattern in the Raman spectrum, and so the technique is proposed to be used as a non-destructive tool to identify these compounds. As a test of the spectroscopic procedure, we have examined a valuable archival sample of brazilwood using the molecular markers for brazilein, and have confirmed the presence of brazilein in the 80-year-old wood. In a future

study, this investigation will be extended to the non-destructive analysis of other species of brazilwood as weel as substitutes.

## Acknowledgements

LFCO thanks to CAPES (Brazil) for a research fellowship during which part of this work was carried out. The authors thank also to Mr. D.W. Farwell for obtaining the NMR data.

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