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Citation for published version:
Peggie, D, Kirby, J, Poulin, J, Genuit, W, Romanuka, J, Wills, DF, De Simone, A & Hulme, AN 2018, 'Historical mystery solved: A multi-analytical approach to the identification of a key marker for the historical use of brazilwood (Caesalpinia spp.) in paintings and textiles', Analytical methods. https://doi.org/10.1039/C7AY02626A

Digital Object Identifier (DOI):
10.1039/C7AY02626A

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Analytical methods

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Historical mystery solved: A multi-analytical approach to the identification of a key marker for the historical use of brazilwood (*Caesalpinia* spp.) in paintings and textiles

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Dyes derived from brazilwood (*Caesalpinia* spp.) are known to have been used in a diverse range of objects, from Medieval European textiles to North American First Nations objects, while pigments made from brazilwood feature in the palette of a number of painters, including Rembrandt and Van Gogh. For almost two decades, an unknown marker has been used to detect brazilwood colourants in historical objects. Limited sampling opportunities mean that the identification of the chemical structure of this marker has eluded scientists to date. Using a combination of synthesis, UPLC-ESI-MS/MS, HPLC, NMR and GC-MS, the identity of this unknown marker was confirmed as the benzochromenone, urolithin C. Structural identification provides a reliable reference compound for use across a range of analytical techniques employed in the cultural heritage sector and will enable the future development of non-destructive techniques for its identification on high-status objects.

1. Introduction

Brazilwood (mainly *Caesalpinia* spp.) was an important natural source for the production of red dyes and pigments from ancient times through to the advent of synthetic colourants in the nineteenth century. The historical importance of the use of this raw material as a dye source, together with the different species of soluble redwoods used to obtain the colouring material, is detailed elsewhere. The heartwood could produce a dye or pigment ranging in hue from pink to purple, depending on the chosen recipe, but although initially strongly coloured, they are particularly light sensitive and prone to fading. Despite this, brazilwood-derived dyes are known to have been used in a diverse range of objects, from Medieval European textiles to North American First Nations objects, and brazilwood-derived pigments feature in the palette of a number of painters active at different periods and in different places, for example, Raphael, Pietro da Cortona, Rembrandt and Van Gogh (Figure 1). The accurate identification of the biological source of the colouring material used in historical objects often requires that a small sample is taken and the organic dye components are extracted from this before being separated chromatographically. Many dye sources have the same major constituent, so identification of this major component may not uniquely define the origin of the dye. In such cases, the presence of characteristic minor constituents can act as marker compounds to allow species identification. This biological source identification is usually performed using high performance liquid chromatography (HPLC) with photodiode array (PDA) detection, but more recently UPLC-MS/MS and GC-MS techniques have been employed. Although studies have identified various components from the heartwood, the principal colourant in brazilwood species is obtained from brazilein (1), which when oxidised produces the chromophore brazillein (2).

In practice, when analysing historical objects the colour has often faded and consequently the amount of brazilein (2) or related homoisoflavonoids detected in the extracts of brazilwood-containing samples is generally very small. This is presumably largely due to photo-degradation reactions and in many cases the degradation has been so severe that the samples may contain no observable levels of brazilein (2). No other minor constituents are generally observed in historical samples.
except for a minor unidentified brazilwood marker component (Figure 2).

This constituent has a characteristic retention time and UV-Vis spectrum and seems to be a general marker for the use of brazilwood, rather than a marker for a distinct species within this group. Since the evidence for the use of a brazilwood-derived colouring material in historical objects often hinges solely on the detection of this marker, it is essential that it is characterised. This will not only help to confirm that it is indeed a marker for brazilwood species, but will also aid more fundamental studies exploring the origin and/or generation of the component and will be useful when designing strategies to improve the identification methods for dye sources in historical objects. This paper presents a comprehensive study of the identification of the structure of this marker component.

2. Experimental

2.1 Materials and Chemicals

2.1.1 Reference lake pigment. The reference pigment sample (NGLake4) was made at the National Gallery, London in 1989 from Caesalpinia echinata (Lam.) (Pernambuco wood, recently reclassified as Paubrasilia echinata (Lam.) Gagnon, H.C. Lima & G.P. Lewis). This was supplied by Ashill Colour Studio, Church Street, Alcombe, Minehead, Somerset TA24 6BL UK (now subsumed under P&M Woolcraft, Pindon End, Hanslope, Milton Keynes. MK19 7HN) following a modified historical recipe based on one cited by P.F. Tingry in the nineteenth century. The wood shavings were placed in water (ca. 500 mL) and boiled until ca. 200 mL water remained. The solution was filtered and Al2(SO4)3 (ca. 16 g) added to the filtrate before addition of Na2CO3 (ca. 8 g) until an approximately neutral pH was obtained (no further effervescence). The precipitate was filtered, washed and dried to give a crimson red-coloured lake pigment which was stored in the dark. The pigment degraded to a brown colour in a relatively short period of time (ca. 2 years) and analysis (by HPLC) confirmed that it contained a large amount of the marker component. The reasons for the extent of degradation in this particular sample are presently unknown, but it was noted that other pigments made using slightly different recipes have degraded to different extents and that the presence of a large amount of alkali in the recipe appears to encourage this change.

2.1.2 Standards. Xanthone (4) was synthesised using a Friedel-Crafts acylation of 3,4-dimethoxyphenol with 2,6-dimethoxybenzoyl to give the corresponding benzophenone. Base-mediated closure of the central ring established the xanthone core and subsequent boron tribromide-mediated demethylation gave the desired tri-hydroxylated xanthone (4).

(See SI file for experimental protocols and spectroscopic data). Urolithin C (6) was purchased from Dalton Research Molecules, 349 Wildcat Road, Toronto, Ontario, M3J 2S3, Canada (purity greater than 95% by HPLC). Dyestuff from the Forbes Pigment Collection was also tested by GC-MS, including heartwood from Paubrasilia echinata (Lam.) Gagnon, H.C. Lima & G.P. Lewis (Forbes 6.03.44a). A dried reference yarn utilised for the GC-MS segment of this research was obtained from a workshop presented by Helmut Schewepe at the Smithsonian Institute.

2.2 Extraction Protocols

2.2.1 HPLC (Reference lake pigment, standards and paint samples). The samples (ca. 5-10 mg) were extracted using 4% BF3 in MeOH (ca. 10 μL). The solution was left for between 4 and 24 hours, depending on the nature of the sample, before being centrifuged for 1 minute at 3,000 rpm and the supernatant collected and analysed.

2.2.2 UPLC-ESI-MS/MS (Reference lake pigment). The reference lake pigment (ca. 5-10 mg) was extracted using a mixture of 37% HCl:H2O:MeOH [400 μL 2:1:1 (v/v/v)]. The solution was agitation then centrifuged and the supernatant (now red in colour) removed with a pipette and placed in a reactor. This was repeated until the supernatant was no longer coloured and the combined aliquots evaporated to dryness (50 °C; dry N2). The dry residue was reconstituted with H2O:MeOH [400 μL 1:1 (v/v)] centrifuged and the supernatant transferred to glass injection vials. For the preparative studies the reconstituted residues of approximately 10 extractions were combined and again evaporated to dryness before reconstituting with 0.5% aqueous formic acid:acetonitrile [4 mL 1:1 (v/v)].

2.2.3 GC-MS (roxilithin C, Forbes brazilwood reference material, sample from dyed objects). The samples were extracted using m-(trifluoromethyl)phenyltrimethylammonium hydroxide, TMTFTH (5–10 μL 0.2 N in MeOH) and an equal volume of toluene. The volume of reagents added varied with the sample size. For the urolithin C standard, approximately 10 μg was extracted in a total volume of 20 μL, while for the dyed deer hair sample and Forbes brazilwood reference material approximately 300 μg was extracted in a total volume of 20 μL. As per dried historical samples, the hair was heated (60 °C for 1 hour) then centrifuged at 1500 rpm for 1 minute before being left at room temperature overnight. The urolithin C and the Forbes brazilwood reference material were extracted at room temperature and analysed after one hour and 24 hours.

2.3 Analytical Techniques

2.3.1 HPLC. The samples were analysed using a standard protocol developed at the National Gallery for the analysis of lake pigments extracted from historical paint samples. An Agilent Technologies High Performance Liquid Chromatography system, comprising a 1200 series capillary pump and vacuum degasser and an HP 1100 series column oven and Photo Diode Array (PDA) detector was used. The samples were injected manually via a Cheminert Valco low dispersion 6-port injector with a 2 μL sample loop. HP Chemstation software (revision B.04.03[106]) was used to control the equipment and process the data. The flow cell path length was 10 mm (0.5 μL volume) and the bandwidth (resolution) of the detector was 8 nm. The method used a Phenomenex LUNA C18(2) reverse phase column, 5 μm particle size, 150 × 0.5 mm (length × i.d.). The total run time was 80 minutes (+ 15 minutes post-run at initial conditions) at a
flow rate of 10 µL min⁻¹ and the column was maintained at a temperature of 40 °C. A binary solvent system was used; A = 0.1% aqueous trifluoroacetic acid, B = 94.9% acetonitrile, 5% methanol, 0.1% trifluoroacetic acid. The elution program was a linear gradient from 95A:5B to 70A:30B (0 to 25 mins), followed by a linear gradient to 5A:95B (65 mins). Isocratic conditions were maintained (75 mins) before a linear return to the starting conditions, 95A:5B (80 mins).

2.3.2 UPLC-ESI-MS/MS. The samples were analysed using a protocol specifically developed for the fast separation of the compound of interest. A Waters Acquity H-class UPLC system equipped with a Photo Diode Array UV absorption detector (PDA) and coupled to a Waters Synapt G2-Si quadrupole - time-of-flight (Q-Tof) tandem mass spectrometer was used. The column was a Phenomenex Luna Omega C18 reverse phase column, 100 × 2.1 mm (length × i.d.), 1.6 µm particle size. The column was maintained at a temperature of 40 °C. A binary solvent system was used: A = 0.1% aqueous formic acid, B = 94.9% acetonitrile, 5% methanol, 0.1% formic acid. The elution program was a linear gradient from 90A:10B to 70A:30B (0 to 5 min) followed by a step change to 10A:90B (5 to 7 min) and another step change back to initial conditions (7 to 11 min). Total cycle time was 11 minutes at a flow rate of 500 µL min⁻¹. The injection volume was 0.2 µL. Both positive and negative electrospray ionization was applied as the ionization method. Prior to the measurements, in both polarity modes the m/z scale of the time-of-flight mass spectrometer was calibrated in the conventional way using a series of sodium formate clusters. The mass resolution of the instrument, set in ‘resolution mode’, was around 20,000. During the LC-MS runs, the m/z scale was locked every 30 seconds to the mass of the pseudo-molecular ion of Leucine-Enkephalin (556.2771 Da in positive and 554.2615 Da in negative ion mode, respectively) which was infused via a separate ‘lock’ spray. MS/MS collisional dissociation studies were performed using argon as collision gas and applying a collision energy ramp of 25-50 eV. H/D exchange analysis was made by post-column addition of D₂O at a flow rate of 100 µL min⁻¹.

2.3.3 NMR spectroscopy. Samples for NMR analysis were prepared using the UPLC system given in section 2.3.2 with simplified solvent and elution conditions; A binary solvent system was used: A = 0.5% aqueous formic acid, B = acetonitrile, while the elution program was a linear gradient from 90A:10B to 40A:60B (0 to 7 min) followed by isocratic conditions for 1 minute before a linear gradient back to the initial conditions (8 to 11 min). Two fractions were generated by collecting the eluent from two retention windows (4.0 - 5.5 min and 5.5 – 7.0 min) corresponding to a reference fraction (eluting before the peak of interest) and the fraction containing the marker component. The respective fractions from 60 successive 25 µL injections of the NGLake4 extract were combined and evaporated to dryness under nitrogen at 60 °C. The NMR samples were prepared by re-dissolving the dry residue in D₂O and the experiments conducted at a temperature of 25 °C on a 400 MHz Agilent spectrometer: ¹H spectra were collected for both fractions while a ¹³C TOCSY was acquired for the fraction containing the compound of interest.

2.3.4 GC-MS. The samples were analysed using a standard protocol developed at the Canadian Conservation Institute (CCI) for the analysis of natural dyes. An Agilent 7890 GC with an Agilent 5975 quadrupole MS was used. Separation was achieved using a Phenomenex ZB-SMSi (5%- phenyl-95%-dimethylpolysiloxane) column (30 m length × 0.25 mm i.d., 0.25 mm film thickness). The GC was run in splitless mode with an inlet temperature of 250 °C, MS interface temperature 280°C. Ultra-high purity helium was used as the carrier gas with a constant linear flow of 1.2 mL min⁻¹. The GC oven temperature was programmed with a ramp of 10 °C min⁻¹ from 50 to 200 °C and then with a ramp of 6 °C min⁻¹ from 200 to 300 °C, with the final temperature held for 15 minutes. The MS was run in scan mode from 50 to 750 amu, with the source and quadrupole set at 230 and 150 °C, respectively. The MS was operated in electron impact (EI) positive ion mode (70 eV). Data acquisition was achieved using Agilent MSD Chemstation D.02.00.275 software, while data processing was performed using both Chemstation and NIST AMDIS 32 software.

3. Results and Discussion

3.1 Assessment of homoisoflavonoids as the structural class of the marker component

The origin of the marker component is not entirely clear, but a photo-degradation product based on the homoisoflavonoids (Figure 3, Class 1) might be expected if it was produced directly from ageing. In order to obtain enough material to characterise the marker component by NMR, a semi-preparative LC method was devised to extract the reference lake sample NGLake 4 and 60 fractions containing the marker component collected (Section 2.3.3). In an effort to identify the peaks stemming from the marker component, the ¹H spectra of the background reference and marker component fractions were compared. Four peaks belonging to the component of interest were identified, with chemical shifts suggesting they are aromatic in nature. However, obtaining a ¹³C NMR spectrum for the component was problematic and identification of the marker by this means proved unsuccessful. Initial studies using UPLC-ESI-MS in negative mode provided an accurate mass for the pseudo molecular ion [M-H]⁻ of the marker component (Figure 3; C₁₃H₁₀O₅; calculated mass, 243.0293).

![Chemical structures of the marker components](image)

**Class 1: homoisoflavonoids**

**Class 2: xanthenes**

**Class 3: benzochromenones**

Figure 3: The braziwood marker component observed in historical samples and reference materials is most likely to belong to one of three structural classes; homoisoflavonoids, xanthenes or benzochromenones.
However, no obvious degradation products corresponding to the parent molecular formula $C_{13}H_{18}O_6$ could be proposed to arise from either brazilen (1; $C_{16}H_{12}O_6$) or brazilin (2; $C_{16}H_{12}O_6$). Further negative ion ESI-MS fragmentation studies of the marker component in either $H_2O$ or $D_2O$ (Section 3.4.1) revealed a maximum number of two exchangeable sites were present in the pseudo molecular ion, suggesting a structure with three hydroxyl groups in the neutral compound. A structure based on the homisoflavonoids was therefore ruled out and it was postulated that the marker component was most likely based on one of two further chemical classes; xanthones or benzochromenones (Figure 3, Class 2 and Class 3 respectively). Compounds based on both of these structures have been isolated from the heartwood of *Caesalpinia sappan* L. (sappanwood, reclassified as *Biancaea sappan* (L. 1753) Tod. 1875),\(^{16,17}\) although only the dihydroxyxanthone, euxanthone (3; $C_{13}H_{18}O_6$) has so far been reported from this chemical class.\(^ {16}\)

### 3.2 Assessment of xanthones as the structural class of the marker component

Previous studies have shown that the marker component would itself dye onto a metal-mordanted fibre,\(^ {18}\) indicating that it is likely to contain an alpha-hydroxy ketone or similar functionality as found in euxanthone (3). A synthetic route which might give access to two representative trihydroxyxanthone compounds (4) and (5) was therefore devised (see SI). Compound (4) was synthesised and was shown by negative ion ESI-MS to have the correct mass for the pseudo molecular ion ([M-H]$, m/z = 243$). However, chromatographic analysis indicated that the retention time of compound (4) was far longer than that of the marker component (40.4 min cf. 28.2 min by HPLC). Furthermore, the UV-Vis spectrum of (4) was so unlike that obtained from the marker component ($\lambda_{\text{max}}$ 250 nm with a shoulder at 270 nm and broad bands at 294 nm and 374 nm cf. $\lambda_{\text{max}}$ 258 nm, and broad bands at 306 and 338 nm) that minor variations to the position of the hydroxyl group were unlikely to produce a compound with the required spectrum. Thus compound (5) and, by analogy, all other trihydroxyxanthones, were ruled out as possible structures for the marker component.

### 3.3 Assessment of benzochromenones as the structural class of the marker component

The remaining structural class likely to produce a candidate for the marker component is the benzochromenones (Figure 3, Class 3). The urolithins belong to this group and are hydroxyl-substituted dibenzopyran-6-one derivatives. They are not particularly common in nature, but have been reported in plants rich in ellagitannins.\(^ {19}\) Urolithin C (6) is a trihydroxy-substituted compound of the correct molecular formula (as suggested by the accurate mass results from the marker compound) which has only relatively recently been isolated from the heartwood of *Biancaea sappan* (L. 1753) Tod. 1875).\(^ {16}\) A sample of urolithin C was therefore obtained and its retention time and UV-Vis spectrum matched extremely favourably to those obtained from the marker component. Urolithins have particularly characteristic UV spectra related to the hydroxyl-substitution patterns on the urolithin nucleus (two main absorption bands; 250–280 nm and 330–380 nm and in most cases a third band, 280–320 nm).\(^ {20,21}\) Two components with closely matching spectra would therefore suggest the same substitution pattern and, in this case, indicate that urolithin C is indeed the brazilwood marker component. Furthermore, a recent review on the isolation of such compounds from plants, fungi, lichens and animal waste has shown that urolithin C has so far been isolated from only two plant species, *Biancaea sappan* (L. 1753) Tod. 1875) and *Acacia mearnsii* L., a tree not known to have been used for dyeing purposes.\(^ {22}\) The fact that this marker compound also appears to be present in other *Caesalpinia* species,\(^ {2}\) suggests urolithin C is likely to be a highly specific marker for the use of brazilwood colourants on historical objects.

### 3.4 Confirmation of the identity of the marker component in lake pigments and dyes

The benzochromone, urolithin C, was confirmed as the marker component using a variety of analytical techniques. Different extraction protocols and derivatising agents were selected depending on the nature of the sample to be analysed (a powdered lake pigment, paint fragment or dyed textile for example) and the analytical technique chosen (HPLC, UPLC or GC-MS) and reflect procedures already in place within the field of cultural heritage. The boron trifluoride solution used to solubilise paint samples before HPLC analysis performs two separate functions. It breaks up the paint film via methylation, releasing the lake pigment from the binder, while also disrupting the linkage between the dye molecules and the metal ions of the lake substrate, helping to solubilise the compounds of interest. The main drawback with this method is that any dye (or dye-related) components containing a carboxylic acid functionality will also methylate, however, this is not important when considering urolithin C. The primary function of the TMTFTH methylating reagent used in the GC-MS analysis of dyed textile samples is the derivatisation of polar functional groups to improve chromatographic behaviour. However, it similarly aids the extraction of the components of interest, with the alkaline pH of the reagent helping to strip the dye compounds from the substrate. In the case of urolithin C, an artefact from this derivatisation process is also observed in the chromatogram (Section 3.4.2) and this must also be considered when attempting to determine the presence of urolithin C in a sample.

### 3.4.1 UPLC-ESI-MS/MS

Analysis of the urolithin C reference standard using UPLC-ESI-MS/MS in both positive and negative ion modes confirmed that urolithin C had an identical retention time, pseudo molecular ion and MS/MS spectra to the brazilwood marker component observed in the extracts from the reference lake pigment (NGLake4) (see Figure 4 and SI Table S1). The low-energy collision induced dissociation (CID) mass spectrum in the positive ion mode contains a distinct base peak at $m/z$ 155, arising from cleavage of the lactone ring.\(^ {23}\) However, the negative ion mode CID spectrum contains a large number of hydrogen and skeletal rearrangements. This has resulted in characteristic groupings of molecular species, with each group containing ions with the same number of carbon and oxygen atoms and differing only in the number of hydrogens. This is often the case in low-energy CID spectra and means the spectra cannot be explained in terms of specific dissociation reaction mechanisms.\(^ {24}\) The hydrogen-deuterium exchange in the pseudo molecular ion was studied using $D_2O$ and a comparison of the measured and calculated isotope patterns in the negative ion mode confirmed that there are a maximum number of two exchangeable sites present in the pseudo molecular ion (see SI Table S2).
3.4.2 GC-MS. Analysis of the urolithin C reference standard by GC-MS with in situ methylation using TMTFTH resulted in a component with an identical retention time, molecular ion and fragmentation pattern to that observed in similarly treated extracts of brazilwood reference standards (see SI Figure S5). The component has a molecular ion (M⁺) of m/z 286 and was identified as the trimethyl analogue of urolithin C (7) by comparison to the NIST mass spectral library.²⁵

A second component is observed if the urolithin C reference standard is extracted in TMTFTH and left to stand for one hour at room temperature before analysis (8). This has been tentatively identified as methyl 2”,4”,4’,5”-tetramethoxy-1,1”-biphenyl-2-carboxylate (8) and is thought to originate from the hydrolytic cleavage of the urolithin C cyclic ester functional group and methylation of all hydroxyl and acid functional groups. A previous study has noted that certain classes of dyes (including flavonols and depsidones) are vulnerable to hydrolytic cleavage by TMTFTH.³ Supporting this hypothesis, it was noted that if left under the hydrolytic conditions for 24 hours the urolithin C reference standard underwent complete hydrolytic cleavage to compound 8, leaving no intact methylated urolithin C (7) in the resultant chromatogram. Surprisingly, complete hydrolytic cleavage to compound 8 is not generally observed in the extracts from historical objects dyed with Caesalpinia spp., even after extended extraction periods as illustrated for the First Nations garter pendant after 24 h extraction (Figure 6). This is possibly due to the effects of substrate and mordant interactions with the in situ dye compounds, which may protect the cyclic ester group and lend resistance to hydrolytic cleavage. Similar observations have been noted for other dye compounds extracted with TMTFTH.³ For different historical samples, the relative ratio of compounds 7 and 8 is likely to vary depending on the composition of the substrate, the presence or absence of a metal mordant, and the length of extraction time (SI Figure S6). Therefore, both compounds 7 and 8 should be used as markers to determine the presence of degraded brazilwood dye using this method.

3.5 Historical context to the detection of urolithin C in samples

The identification of urolithin C as the brazilwood marker component leads naturally to the question of its origin within sample extracts. Although the presence of urolithin C in the heartwood of brazilwood, Biancaea sappan (L. 1753) Tod. 1875), has been confirmed,¹⁶,¹⁷,²² a number of additional factors are important when considering pigments or dyestuffs made from a brazilwood source. These include the particular species
used; the conditions employed during the original extraction of the dyestuff from the wood chips; the subsequent preparation of the lake pigment or the dyeing of the fibre; and even the possibility that urolithin C might also be generated from other precursors during ageing. For historical objects the details of the preparation of the dye or pigment are rarely known,\textsuperscript{26} however, initial extraction studies performed at the National Gallery, London, using \textit{Biancaea sappan} (L. 1753) Tod. 1875, showed that an increase in the amount of urolithin C was observed when the colouring components were extracted from the wood chips in an alkaline solution at an elevated temperature (unpublished results). These conditions would also accelerate the oxidation of brazill to brazilein,\textsuperscript{2} conceivably linking the presence of urolithin C to the efficient extraction of the colouring material from brazillwood. In addition, historical recipes for the preparation of brazillwood-derived lake pigments can utilise either acidic or alkaline conditions, resulting in pigments with a range of different colours. The colouring components for the reference lake used in this study (NGLake4) were extracted from brazillwood using boiling water. The end-point of the lake precipitation reaction, assessed by the absence of effervescence, is difficult to establish unequivocally and as such a slight excess of alkali is likely to have been added. The extent to which the manufacturing process may have altered the amount of urolithin C present in the extracts of this pigment is not known. However, it is interesting to note that reference pigments made with slightly different recipes have degraded to different extents over the same amount of time. The reference lake used in this study appears to be one of the most severely degraded, despite being protected from light, and also seems to contain a particularly large amount of urolithin C in the extracts, suggesting there may be a link between alkalinity, pigment degradation and the presence of urolithin C. The main colouring components of brazillwood are known to be extremely light-fugitive and the importance of urolithin C as a brazillwood marker stems from the fact that it may be the only observable component when extracts from a historical sample are analysed using techniques such as HPLC.

4. Conclusions

Whilst understanding the potential influence of the manufacturing process or the subsequent degradation of the pigment on the amount of urolithin C present in the extract of a historical sample of brazillwood (\textit{Caesalpinia} spp.) is clearly important, the fact that the identity of the marker component is now known and that it can be linked unequivocally to brazillwood makes urolithin C an extremely important marker for this colouring source. Structural identification provides a reliable reference compound for use across a range of analytical techniques and will enable the future development of non-destructive techniques for the identification of this molecule on high-status objects.

Acknowledgements

We thank Shell UK for their sponsorship of the exhibition, ‘Rembrandt: The Late Works’ at the National Gallery, London from 15\textsuperscript{th} October 2014 – 18\textsuperscript{th} January 2015 and the scientific partnership which this facilitated. We would also like to thank Raymond White, formerly of the National Gallery, London, for useful discussions in the early stages of this project.

Notes and references

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\textsuperscript{b} formerly (now retired) of National Gallery London, Scientific Department, Trafalgar Square, London WC2N 5DN, UK.
\textsuperscript{c} Canadian Conservation Institute, Department of Canadian Heritage / Government of Canada 1030 Innes Road, Ottawa ON K1B 4S7, Canada.
\textsuperscript{d} Shell Global Solutions International B.V. Grasweg 31, 1031 HW Amsterdam, The Netherlands.
\textsuperscript{e} EaStCHEM School of Chemistry, Joseph Black Building, David Brewster Road, Edinburgh, EH9 3FJ, UK.

Electronic Supplementary Information (ESI) available: HPLC Methods for Synthesis; Preparation of an authentic sample of 2,3,8-trihydroxyxanthone 4; LC trace for synthtic 2,3,8-trihydroxyxanthone 4 (Figure S1); \textsuperscript{1}H and \textsuperscript{13}C NMR spectra of 2,3,8-trihydroxyxanthone 4 (Figure S2); \textsuperscript{1}H and \textsuperscript{13}C NMR spectra of 3,8,9-trihydroxy-6H-benzof[\textit{c}]chromen-6-one (urolithin C) 6 (Figure S3); PDA spectra for 2,3,8-trihydroxyxanthone 4 and urolithin C 6 (Figure S4); MS/MS fragmentation studies of urolithin C 6 reference material (Table S1) and H/D exchange of urolithin C 6 in negative ion electrospray mass spectrometry (Table S2); GC-MS chromatogram from reaction of urolithin C 6 with TMTFTH after one hour and 24 hour extraction times and mass spectra of compounds 7 and 8, formed from urolithin C 6 reactions with TMTFTH (Figure S5); GC-MS chromatogram of the TMTFTH extraction of a brazillwood dyed reference yarn obtained from a workshop presented by Helmut Schweppe at the Smithsonian Institute and from the Forbes Pigment Collection (Figure S6).

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