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Technological insights into madder pigment production in antiquity

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SUMMARY The root of the madder plant (*Rubia* spp.) has been used for textile dyeing and pigment making for millennia and madder-derived dyes and pigments were widely used in antiquity. Pigments made from madder have been found on objects in the British Museum, particularly those from Greek, Roman, Parthian and Egyptian contexts, and the analyses of a number of these pigments, focusing both on the organic components and the inorganic substrate or support, are presented. Previous analyses of such pigments have also been reviewed and what is striking about these pigments, in addition to their state of preservation, is the strong ultraviolet-induced luminescence that they typically show and the high proportion of (pseudo)purpurin frequently reported. However no clear descriptions of the preparation of madder-based pigments from these periods have been found.

The principal colouring components in madder root are the precursors or decomposition products of alizarin and pseudopurpurin, which usually occur in roughly equal amounts in the root of the cultivated variety of madder (*Rubia tinctorum*). The high proportions of pseudopurpurin, or its decomposition product purpurin, typically found when the colouring components in ancient madder pigment samples are analysed, may indicate that *R. tinctorum* was not being exploited and may suggest the use of wild madder (*R. peregrina*) or another *Rubia* or *Galium* species that has an intrinsically low alizarin content. Alternatively the high proportion of (pseudo) purpurin typically found in early madder pigments may reflect the method of pigment production. To explore this idea further a series of pigments has been prepared based on methods hinted at in documentary sources to investigate how the method of preparation can influence the final composition of the pigment. The solubilities of alizarin, purpurin and pseudopurpurin have been studied in two of the common mixtures used to extract the roots: water and alum solutions. It has been found that pseudopurpurin has a relatively high solubility in 5% alum solution and an alum extraction can be used to prepare a pigment high in pseudopurpurin by selective extraction. Based on the experimental samples and solubility studies, it has been demonstrated that it is possible to obtain pigments with a composition similar to ancient pigments directly from the roots of *R. tinctorum* using materials and technologies that would have been available in the Classical world.

While the exact method of pigment production and the source of the colourant cannot be confirmed, the analytical data presented point to a long-lived and consistent pigment-making technology that extends beyond a Hellenistic/Roman tradition and starts to shed new light on the methods and technology of pigment production.

Introduction

The red colourants extracted from the roots of various species of the genus *Rubia* have been used for textile dyeing and pigment making for millennia. Such madder dyes and pigments were widely employed in antiquity, and a seventh-century BC neo-Babylonian tablet at the British Museum (registration number: 1883,0121.141) is perhaps the earliest written reference to the use of madder for dyeing textiles [1; pp. 4, 113–114 and 119–120]. Dyeing with madder is thought to have been introduced into Egypt as early as the Eighteenth Dynasty (1550–1292 BC), probably from the Levant [1; pp. 119–120 and 123, 2] and was known in Persia, Arabia and Mesopotamia [3]. In Mesopotamia, madder was known by its Akkadian name *hurratu* in texts from the Larsa region of c.1900 BC [1; pp. 119–120].



Figure 1. The upper part of the painted cartonnage mummy case for Tamiris, daughter of Peteminis, showing the use of a characteristically bright pale pink pigment. From Akhmim: first century BC to first century AD. British Museum 1897,0112.1348 (EA29586)

Pigments made from madder have been found on objects in the British Museum (BM) particularly those from Egyptian, Parthian, Greek and Roman contexts, but no clear descriptions of the preparation of madder-based pigments dating to these periods have been located. Despite being based on organic colourants, madder-based pigments from these contexts are often still strongly coloured, having a characteristic bright pale pink colour, Figure 1. What is particularly striking about these pigments is the strong pink-orange coloured luminescence typically seen under ultraviolet or visible radiation with a short wavelength [4] and the molecular profile observed if the dye-stuff composition is analysed, Figure 2. The pigments contain high proportions of the colourants purpurin and/or pseudopurpurin, but little or no alizarin. The colour and properties

of these very early madder pigments closely resemble more recent pigments often described as rose madder pigments, which were industrially produced from the nineteenth century onwards through deliberate modification of the extraction process using chemicals that would not have been available in antiquity [5, 6]. Examples of these more recent pigments include those produced from Kopp's purpurin, Field's rose madder [7] and Winsor & Newton rose madder [8].

Various *Rubia* species have been used in different regions of the world to produce dyes and pigments [1; pp. 107–129, 3, 6, 10]. *Rubia* spp. are members of the Rubiaceae family that includes other plants yielding useful and closely related hydroxyanthraquinone dyestuffs. In Europe, the Mediterranean and Near East, the most important sources of madder dyes are two *Rubia* species, wild madder (*R. peregrina* L.) and the cultivated form (*R. tinctorum* L.) – both of which are native to the eastern Mediterranean, the Middle East and North Africa – but also woodruffs (*Asperula* spp.) and bedstraws (*Galium* spp.), including dyer's woodruff (*A. tinctoria* L.) and lady's bedstraw (*G. verum* L.). It should be noted that it has recently been suggested that *R. peregrina* L. is not currently indigenous to Egypt although how this relates to the situation in antiquity is unclear [11].

Studies of extracts of the plant roots indicate compositional differences between the various madder sources and have identified potential characteristic marker components, but in general the cultivated variety, *R. tinctorum* L., contains more alizarin (or its precursors) than pseudopurpurin or purpurin (or their precursors) when compared to *R. peregrina* L. and *G. verum* L., from which alizarin is often absent or only present in low quantities [1; pp. 112–113, 122–123 and 127, 3, 12–14]. *Rubia peregrina* L. and *G. verum* L. often contain significant quantities of rubiadin, which is not normally a major component in *R. tinctorum* L. [15], but it has been questioned whether rubiadin is a useful species indicator [10].

It is often suggested that the (pseudo)purpurin-rich madder dyes (for example purple hues in Coptic textiles [11, 16, 17]) and pigments associated with the Classical world are evidence for the use of *R. peregrina* L. (or possibly *Galium* spp.) [18–21]. However, other research has questioned this conclusion and highlighted the influence of the cultivation

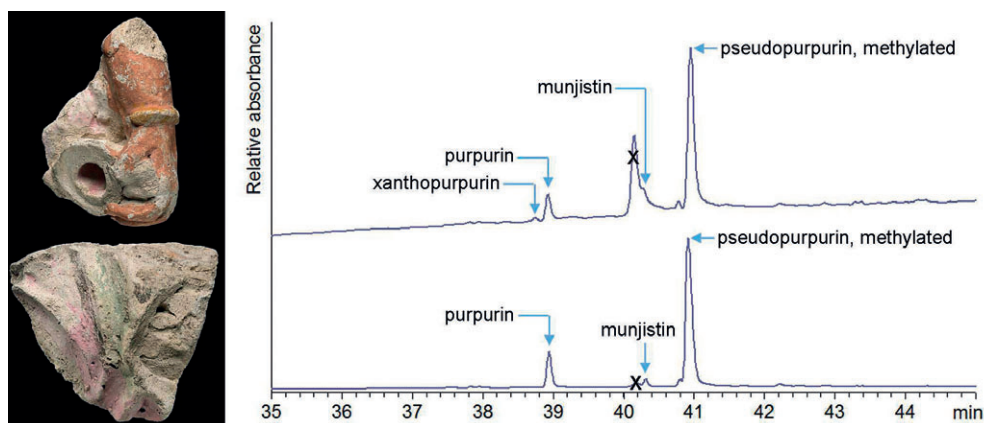


Figure 2. High performance liquid chromatograms of the pink pigments used to colour moulded decorative gypsum plaster architectural friezes from the Parthian House, Uruk, c.150 BC–AD 250 (top: fragment 1856,0903.1164; bottom: fragment 1856,0903.1170). The pigments were extracted using $\text{BF}_3 \cdot \text{MeOH}$, resulting in methylation of pseudopurpurin (for analytical details see [9]). The peak marked with an 'X' corresponds to the formic acid present in the eluent

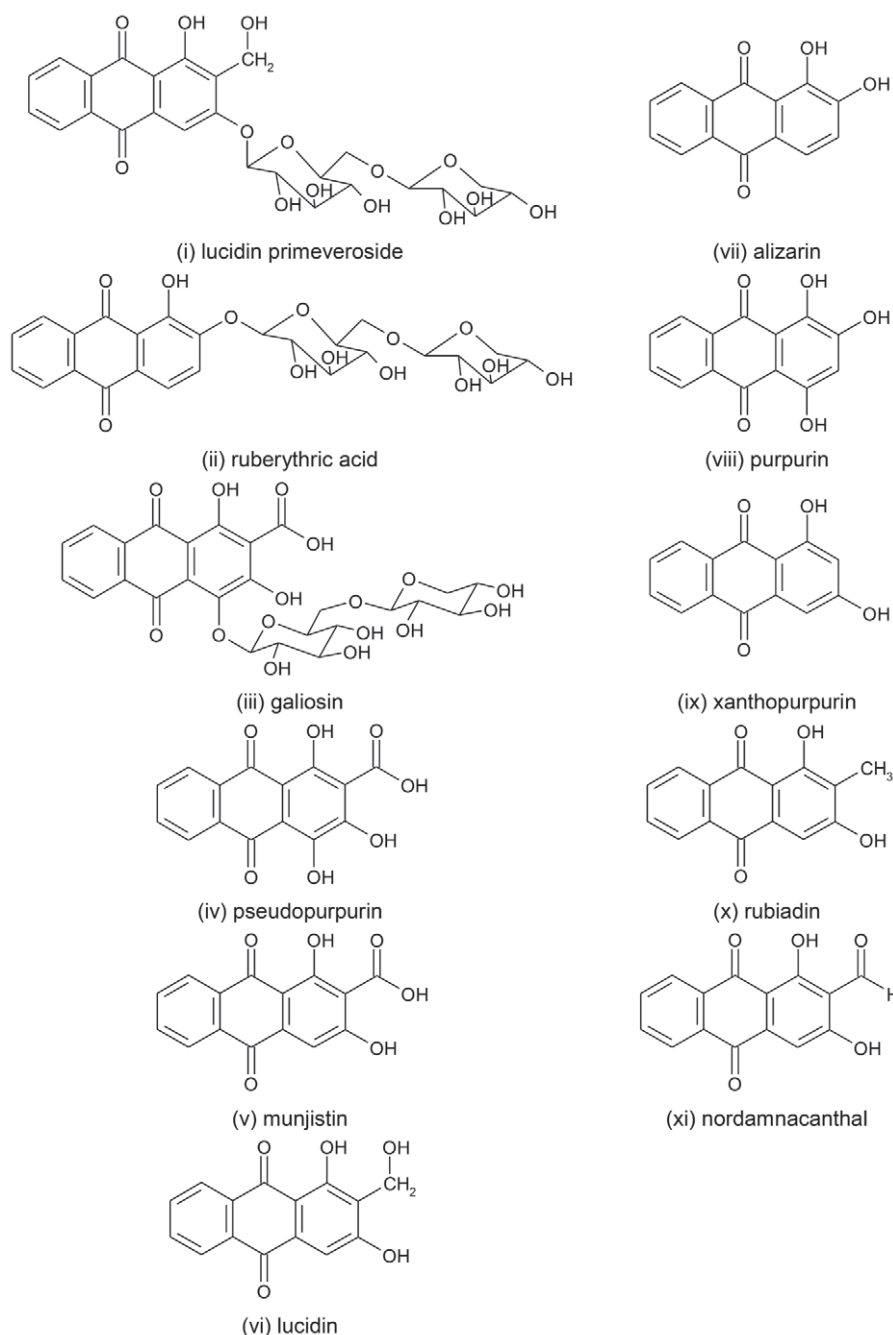


Figure 3. Structures of the principal components present in madder

parameters, harvesting and storage conditions and the age of the plants on the dyestuff composition in the root [5, 22, 23], as well as the significant modifications to the original dyestuff composition that can be brought about in the resulting dye or pigment depending on the method of production [3, 11, 24]. Some authors instead propose the use of the terminology ‘madder A’ to describe dyes rich in alizarin and ‘madder P’ for those that contain mainly purpurin (or its precursors) [16].

In the context of ongoing research at the BM into polychrome decoration in the Classical world, it was of interest to re-examine the assumption that *R. peregrina* L. was the species used in the production of madder lake pigments and to explore whether greater insight could be gained into pigment-making technologies, trade and resource exploitation in antiquity from

detailed examination of surviving artefacts. In order to address these questions, this study has involved three stages:

1. A review of the available contemporaneous descriptions of the production and use of madder-based pigments in antiquity (discussed in the background section below).
2. A review of the published data relating to the analysis of madder-based pigments from antiquity, re-examination of previous analyses undertaken of such material within the BM and new analytical investigations of a number of pigment and paint samples drawn from the BM’s collection and madder roots from a range of sources.
3. Experimental studies to replicate possible pigment production methods and to investigate the solubility of the various colourant components in *Rubia* spp. to determine what the

evidence from ancient pigment samples can reveal about pigment production and resource exploitation.

Background

Composition of madder roots

The roots of *Rubia* spp. are rich in a variety of hydroxy-anthraquinones, of which the most significant are shown in Figure 3 [1; pp. 112–113, 122–123 and 127, 3, 10, 12, 13, 25, 26]. The aglycone forms of the main dye components such as alizarin, purpurin and pseudopurpurin are sparingly soluble in water and within the fresh root are often found as water-soluble sugar derivatives or glycosides. An example is ruberythric acid, in which alizarin is linked to a disaccharide sugar, primeverose, and the relatively unstable glycoside galiosin [27], a primeveroside of pseudopurpurin. The glycosides are readily hydrolyzed to the corresponding aglycone and other components can undergo modification, thus the compounds detected depend on a variety of factors including the degree of enzyme activity within the root and the extraction method (and analytical procedure) chosen [12]. Purpurin is a decomposition product formed by decarboxylation (loss of carbon dioxide) from pseudopurpurin (itself formed from galiosin). As discussed below, because of this ready transformation and the variations induced by the extraction and analysis procedure employed, the term (pseudo)purpurin has been used in this contribution to indicate where the original form in which these two components were present within a plant or pigment sample is unclear.

A number of recent studies have described the colourant composition in different *Rubia* spp. (collected, stored and analysed under various conditions) and in dyes or pigments derived from them [10, 12, 13]. *Rubia tinctorum* L. is typically

rich in ruberythric acid, lucidin primeveroside, alizarin and purpurin (from galiosin) while *R. peregrina* L. is rich in galiosin and lucidin primeveroside and also contains pseudopurpurin, munjistin and variable amounts of purpurin and rubiadin. Following hydrolysis using hydrochloric acid, Cuoco *et al.* reported that their *R. tinctorum* L. root extract contained 47.7% alizarin and 45.1% purpurin [13]. Derksen *et al.* studied the chemical and enzymatic hydrolysis of anthraquinone glycosides and the effect of storage and soaking of madder roots under various conditions [12]. The main anthraquinones found in their freshly dried *R. tinctorum* L. root were lucidin primeveroside, ruberythric acid, pseudopurpurin and munjistin. When heated in an acidic solution, all the pseudopurpurin converted to purpurin, the ruberythric acid to alizarin and the munjistin to xanthopurpurin. Freshly dried madder root was also shown to have sufficient enzyme activity to effect the same transition in less than 24 hours if the madder was soaked in water. At the same time, in the presence of air, lucidin was converted to nordamnacanthal.

High performance liquid chromatographic (HPLC) analysis of water/methanol extracts of the roots of a number of samples of *R. tinctorum* and *R. peregrina* was undertaken as part of this study and the chromatograms obtained for a selection of these samples are shown in Figure 4 (see the materials and methods section and the experimental appendix for details). From these chromatograms, variations in the glycoside to aglycone proportions can be seen. For example, the Iranian madder sample was a younger and fresher material than that from Cornelissen. The sample of *R. tinctorum* root grown in Iran has a relatively low alizarin content (compared to the pseudopurpurin content and the Cornelissen madder) although hydrolysis of the ruberythric acid within the root would significantly increase the proportion in this case. The *R. peregrina* root contains very little alizarin or ruberythric acid.

Alizarin, purpurin and pseudopurpurin differ in colour, with alizarin being a more orange colour, and thus the proportion of the different components influences the colour of the resulting pigment. The ultraviolet-visible (UV-visible) absorption spectra for these three aglycones in toluene solution are shown in Figure 5. It should be noted that the glycosides give closely similar spectra to those of the related aglycones.

The fluorescence behaviour of alizarin, purpurin and pseudopurpurin in solution and as aluminium (Al^{3+}) complexes has also recently been established and microspectrofluorimetry used to characterize the lake pigments [28–31]. Purpurin- Al^{3+} and pseudopurpurin- Al^{3+} complexes are reported to show emission maxima at 551/595 nm and 542/583 nm respectively [30]. Alizarin- Al^{3+} has an emission maximum at *c.*640 nm and a fluorescence quantum yield nearly 50 times lower than the equivalent purpurin complex [28]. A sample of Kopp's purpurin lake prepared following a historic recipe, which should be rich in (pseudo)purpurin with little or no alizarin content [5], was reported to yield an emission maximum at 586 nm (with a shoulder at 610 nm) [30]. The results are quite similar to those obtained from the madder pigments from the 'Treu Head' (BM 1884,0617.1) where the luminescence emission was centred at *c.*608 nm [4], and a Fayum portrait from the Ny Carlsberg Glyptotek in Copenhagen, where the emission maximum was reported at *c.*590 nm [32].

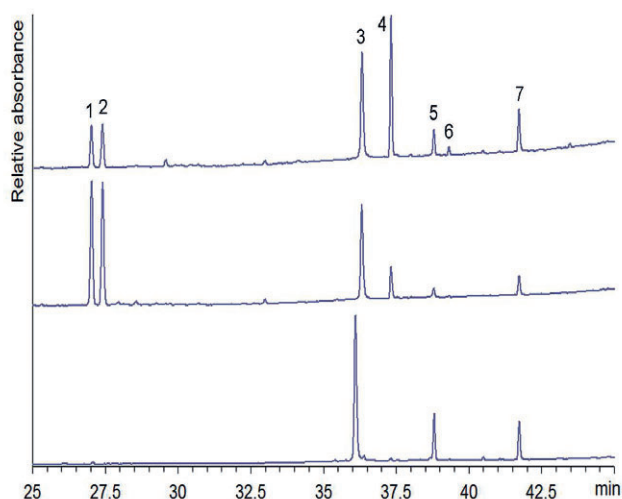


Figure 4. HPLC chromatograms at 254 nm of water/methanol extracts of: (top) dried *R. tinctorum* root from Cornelissen (of unknown Middle Eastern origin); (middle) roots of Iranian *R. tinctorum*; and (bottom) roots of *R. peregrina* from Dentelles de Montmirail, France. Components: 1 – lucidin primeveroside; 2 – ruberythric acid; 3 – pseudopurpurin; 4 – alizarin; 5 – purpurin; 6 – xanthopurpurin; and 7 – nordamnacanthal. Under the analytical conditions used, pseudopurpurin partially co-elutes with munjistin and lucidin but detailed examination of the chromatograms at other wavelengths suggests that the samples contain little munjistin or lucidin and that the peak labelled '3' is predominantly pseudopurpurin

Historical recipes for making madder pigments

While dyeing with madder is well attested it is unfortunate that no documentary evidence referring to the production of madder-containing pigments survives from Classical times. For polychrome decoration, madder extracts may have been used directly or to prepare pigments. If used directly, it is possible that the solution of colouring matter was allowed to evaporate until highly concentrated and was then applied as a wash over a suitable substrate [6, 33, 34]. To prepare a pigment, or for use as a cosmetic [21, 35, 36], the colourant components (and their precursors) within the madder root are extracted into water or an aqueous solution and then adsorbed or complexed with a white inorganic substrate to form an insoluble material suitable for painting [5, 6, 37]. The substrate can either be formed *in situ* so that the colourant components and the substrate co-precipitate [38], or added as a powdered solid to the extract. Examples of materials used in the latter process include: calcium carbonate in the form of chalk, limestone or shell; alum; gypsum; white clays or earths; and other materials that were reportedly employed as white pigments such as various volcanic minerals or *creta anularia* (mixed ground glass and chalk) [6, 21, 35, 39]. It is also possible that a solid pigment was created without the use of a substrate by allowing the colourant solution to evaporate until solids precipitated that could then be used as a pigment.

Pliny, writing in the first century AD, cites the use of madder as a dye and a medicine and although he does not mention the use of madder in pigment making, he describes the manufacture of a desirable purple pigment 'purpurissum' using shellfish-derived colourants by 'dyeing' a white earth substrate (possibly chalk) with purple textiles [40; book 35 XXVI 44]. From Pliny's description it is unclear whether the substrate is dyed alongside the textiles in the same dye bath or whether the dye is obtained from purple-dyed textiles [6, 21, 35]. Vitruvius (c.50 BC–AD 50) describes a similar pigment made by saturating chalk with plant dyes (madder and hyacinth) [41; book 7 XIV]. Diatomite (rock composed predominantly of siliceous microfossils of algae) has been identified as a white pigment and a substrate for madder pigments in wall paintings and pigment pots excavated at Pompeii [6, 21, 39, 42], serving here as an example of the addition of a powdered substrate to a colourant solution.

Alternatively, a lake substrate may be created *in situ* in the colourant solution, for example by combining a solution or suspension of an aluminium salt (usually potash alum – $\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ – although related sulphates or aluminium salts have also been used since antiquity [4]) with an alkaline material such as potassium or calcium carbonate, natron (sodium carbonate) or slaked lime [38]. Pliny describes the creation of a yellow lake pigment using a recipe of this type [40; book 33 XXVII 89] and this approach was certainly used to create madder lake pigments in later periods. The chemistry of the various aluminium oxides and hydroxides is complex and the type of lake substrate thus formed is perhaps best described as an amorphous hydrated alumina that may contain varying amounts of other cations and anions [5]. If calcium carbonate is added to the alum solution in the form of powdered chalk or cuttlefish bone the resulting substrate contains both calcium sulphate (in the form of gypsum) and amorphous hydrated alumina; as discussed below, a number

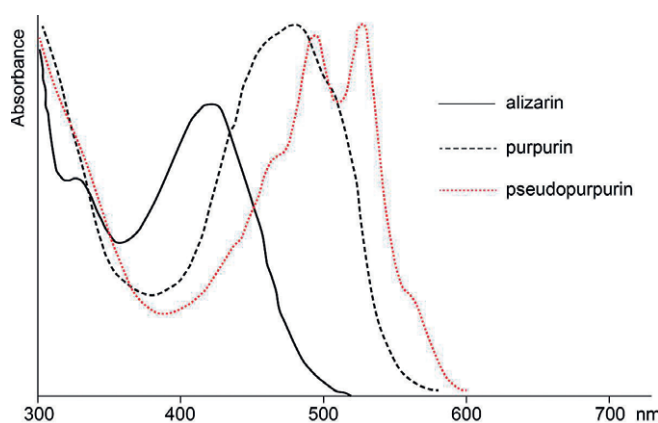


Figure 5. UV-visible absorption spectra of alizarin, purpurin and pseudopurpurin in toluene

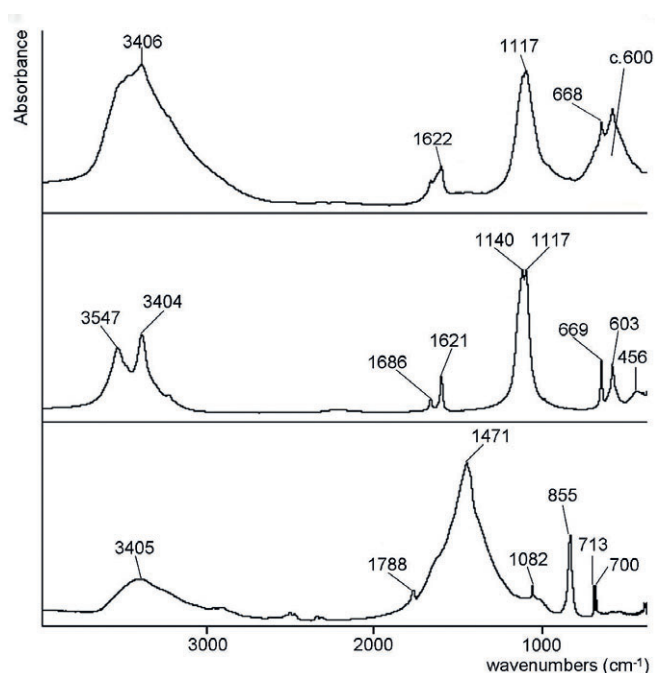


Figure 6. FTIR spectroscopy reveals the formation of amorphous hydrated alumina and hydrated calcium sulphate (principal peaks at c.600 (broad) and 1117 cm^{-1} respectively) on mixing an alum solution with calcium carbonate in the form of cuttlefish bone (top spectrum). The FTIR spectra for reference samples of gypsum (hydrated calcium sulphate: middle) and cuttlefish bone (principally calcium carbonate: bottom) are shown for comparison

of examples of archaeological pigments with substrates of this type have been identified, Figure 6. Pigments could also be produced using combinations of the methods described.

In preparing a workable paint from the resulting madder lake pigment, a range of additional white materials may have been added as extenders or to modify the colour.

Techniques for the characterization of madder-based pigments

To investigate madder pigments, a number of analytical techniques can be used to analyse the organic colourant components and the substrate. Many different methods have been employed over the years in such studies, making direct comparison of the results difficult without at least some understanding of the possibilities and limitations of the various analytical techniques used.

Table 1. Examples of the analysis of madder pigments on British Museum objects from antiquity

Object description	Analytical results and method (dyestuff then substrate) ^a	References
Terracotta figure of a boy seated on a rock (described in the publication as a seated woman): Tanagra, Boeotia, Greece, third century BC (1877,0515.3)	Comments that "The pink colour is probably madder on white inert as shows the expected fluorescence under UV light." Higgins notes that for polychrome decoration of Greek terracottas "The pink, before 330 BC, was a mixture of red ochre and chalk" but that "After 330 BC, it was regularly rose madder, a natural dye made from the root of ... <i>Rubia tinctorum</i> . The colouring agent is mainly purpurin and it gives a brilliant pink with a slightly purplish tinge"	[48]
Pink pigment from Hellenistic terracottas: Canosa di Puglia, 270–200 BC (1846,0925.34, 1846,0925.26.a and 1859,0216.3); Myrina, Turkey, 100 BC (1885,0316.1)	HPLC ^b : the samples were very small but all contain pseudopurpurin, some purpurin and an unknown component, but no alizarin. 1846,0925.26.a also contains some flavokermesic acid	[49]
Painted stucco fragments from Parthian house: Uruk, Iran, 150 BC–AD 250 (1856,0903.1164 and 1856,0903.1170)	HPLC ^c : rich in pseudopurpurin with no alizarin. XRD: gypsum. FTIR: gypsum. SEM-EDX: Ca, S, Si, Al, (Mg, Na, Fe, Cu). It is noted that there may be contamination and not just paint in the sample	[9]
Pink paint from painted stucco fragment of foot: Uruk, Iran, 150 BC–AD 250 (1856,0903.1168: ME 120467)	Luminesces brightly under UV light. HPLC ^c : madder with a high pseudopurpurin content (c.90% pseudopurpurin and purpurin). No alizarin or rubiadin detected (analysis undertaken at the National Gallery, London). FTIR: gypsum	[9]
Roman bowl containing pink paint used by a fresco painter: Hawara tomb, Fayum, Egypt, first century AD (1888,0920.27)	HPLC ^c : rich in pseudopurpurin with no alizarin or rubiadin. c.90% pseudopurpurin/purpurin content (analysis undertaken at the National Gallery, London). Strong luminescence. XRD: gypsum. FTIR (4000–400 cm ⁻¹): gypsum and amorphous hydrated alumina. The pigment was also examined by Russell who concluded that the pigment contained "purpurine" (on the basis of absorption spectroscopy) on a gypsum substrate	[50, 51]
Mummy portraits: Fayum, Egypt, Roman period (EA63394 from er-Rubayat and EA74717 from Hawara)	The identification of madder was based mainly upon its frequent use, distinctive colour and characteristic bright orange fluorescence under UV light. EA63394: Flesh is gypsum (XRD) and also contains madder or ochre while the pink/purple is gypsum (XRD) and also contains madder. EA74717: Purple is Egyptian blue (XRD) and also contains madder	[50, 52]
Pink paint from fragments of painted glass bowls: Fayum, Egypt, Roman period (1903,0219.1 and 1903,0219.2-4)	HPLC ^c : 1903,0219.2-4 is rich in pseudopurpurin with some alizarin and purpurin while 1903,0219.1 contains alizarin, purpurin and pseudopurpurin. XRF: Ca, Al, Pb (S?)	[53]
Pink pigment from mummy case and portrait of Artemidorus: Hawara, Egypt, Roman period, AD c.100–120 (EA 21810)	TLC ^d : purpurin present (plate viewed under UV light). XRF: Ca (did not look for Al). XRD: gypsum, possibly bassanite	[54]
The 'Treu Head': Roman, second century AD (1884,0617.1)	HPLC ^c : pseudopurpurin and some purpurin, no alizarin. SEM-EDX: homogeneous distribution of Al; small amounts of Si and Mg with more heterogeneous distribution, low concentrations of Ca and S in the lake layer. FTIR: gypsum also present. The results suggest an Al salt was used in the colourant preparation and the possible deliberate addition of a clay either to the dyestuff extract to precipitate the colourant or to the resulting pigment or paint. Gypsum is a further minor component that may have been added or formed during lake preparation, a later addition to the pigment or represent an impurity in the substrate	[4]

Notes

- SEM-EDX = scanning electron microscopy with energy dispersive X-ray analysis; FTIR = Fourier transform infrared spectroscopy; XRD = X-ray diffraction; XRF = X-ray fluorescence; TLC = thin layer chromatography; and HPLC = high performance liquid chromatography.
- The colourants were extracted using the method described in the experimental appendix (preserving the glycosides and pseudopurpurin) although here substituting dimethylsulphoxide for dimethylformamide.
- The colourants were extracted using BF₃/MeOH (preserving the glycosides and pseudopurpurin) in the mild extraction method described in Verri *et al.* [4].
- The colourants were extracted in a 2:1 mixture of methanol and 10% sulphuric acid with consequent hydrolysis of glycosides and loss of pseudopurpurin.

To investigate pigment substrates, elemental analysis methods such as X-ray fluorescence (XRF) and scanning electron microscopy with energy dispersive X-ray analysis (SEM-EDX) are often employed. However, XRF analysis may fail to detect elements of low atomic number such as aluminium within a substrate. Microscopic examination of the pigment

morphology can also be very informative in understanding how pigments were produced [39]. Diffraction methods, such as X-ray diffraction (XRD), are also frequently used to analyse pigments including lake pigments, by characterizing their crystalline components; however, pigment substrates are often highly amorphous and unsuited to this approach.

Infrared spectroscopy – including Fourier transform infrared (FTIR) spectroscopy and microscopy – is also widely used, but here again the spectral range for different instruments must be noted as oxide-based substrates may be missed unless low wavenumber regions can be explored (below $c.600\text{ cm}^{-1}$).

When working with samples from archaeological contexts, and particularly with paints that are often applied on stone or clay substrates, it should be borne in mind that it can sometimes be very difficult to distinguish the pigment substrate from contamination by other pigments or extenders in the paint mixture, preparation layers, the underlying support or the (archaeological) environment.

To investigate the organic colourants in lake pigments, a number of colorimetric or spectroscopic methods (including UV-visible absorption, fluorescence [28, 29] and Raman spectroscopy [43]) have been used, although chromatographic methods are preferred in order to undertake detailed analysis of the individual colourant components, with HPLC currently the most commonly employed. Direct mass spectrometric methods have also been used [36].

Despite growing interest in the use of non-invasive *in situ* analytical methods, the majority of the methods used to analyse organic colourants at a molecular level require the colourant components to be extracted into solution. To release the colourant components from the pigment substrate (and possibly a binding medium if working with paint samples), a wide range of extraction methods has been used over the years [44, 45]. The sample extraction procedure can have a profound influence on exactly which colourant components are detected, leading to modification or loss of sensitive components [46]. The extraction procedure widely adopted for the analysis of organic colourants is that developed by Wouters, using aqueous hydrochloric acid (HCl) and methanol [26]. The method is efficient and well tested but results in the loss of acid-sensitive components, hydrolysis of glycosides and decarboxylation reactions, for example the conversion of pseudopurpurin to purpurin. So-called ‘soft’ or multi-step extraction methods, which preserve acid-sensitive or other chemically labile components that may be highly informative of the biological source of the colourant and/or pigment-making or dyeing technologies, are being used increasingly. The choice of the HPLC analytical parameters, particularly the mobile phase, also influences component detection, with readily ionizable molecules such as pseudopurpurin often showing highly variable retention behaviour or being ‘lost’ entirely [10, 25, 46]. Comparison of the chromatographic results obtained using different extraction and analysis methods is consequently often challenging [17, 46].

Materials and methods

As outlined above, there is little contemporaneous evidence of madder pigment manufacture and use in the Classical period, nor about the types and sources of the raw materials used. In the absence of documentary evidence, this study instead focused on the information that can be obtained from detailed examination of surviving pigments and paints. Previous work involving one of the authors (CH) has highlighted the importance of analysing the substrate in addition to the colourant components in order to understand pigment manufacturing technologies

[5], so this study has concentrated on both the substrate and colourant composition. This evidence, combined with an understanding of the materials and technologies that were likely to have been available and experimental work to understand the physical properties of the individual colourant components, informed attempts to replicate pigment production.

Analysis methods

A range of analytical methods was employed to investigate the dye components in the various raw material, pigment and paint samples and to characterize the substrates. Details of the analytical techniques used in this study can be found in the experimental appendix.

As noted above, alizarin, purpurin and pseudopurpurin have distinctive UV-visible spectra so UV-visible spectroscopy can give a qualitative indication of the proportions of the different components (or their glycosides) within a sample and thus provides a useful method to pre-screen samples before the more involved HPLC analysis is undertaken. The extraction method used for UV-visible spectroscopy was based on that developed by Hill and Richter and was applied to both madder root and pigment samples [47]. Samples are soaked in 10% hydrochloric acid and toluene added, forming an immiscible supernatant phase. As the colourant components are released, the aglycones, which are more soluble in toluene, pass directly into the supernatant layer. The water-soluble sugars are hydrolysed in the acid solution, with the resulting aglycones moving into the toluene phase as soon as they form. While pseudopurpurin is normally decarboxylated in acid solution, with this extraction procedure the pseudopurpurin is ‘protected’ from the influence of the acid as it rapidly moves into the toluene layer. Although some decarboxylation of galiosin (the glycoside of pseudopurpurin) may occur while it is in the acidic aqueous phase, significant proportions of pseudopurpurin were preserved in the samples examined in this study. Possible explanations for this observation may include low initial concentrations of galiosin in the samples, a faster rate for the hydrolysis compared to the decarboxylation process, or that the presence of the sugar in galiosin protects the carboxylic acid functional group from decarboxylation.

HPLC-DAD (diode array detection) and UV-visible spectroscopy are both useful methods to explore the (pseudo) purpurin content of madder pigments but it should be noted that the HPLC-DAD extraction method selected preserves the glycoside content while Hill and Richter’s extraction method, which was used for UV-visible spectroscopy, converts all glycosides to their corresponding aglycones.

Materials

To explore the intrinsic variability of colourant components in *R. tinctorum*, various samples of root grown in different geographical regions were acquired and the colourant components in water/methanol extracts analysed by HPLC-DAD as described in the experimental appendix. These samples included madder grown in the UK by one of the authors (VD), dried *R. tinctorum* root of unknown Middle Eastern origin purchased from L. Cornelissen and Son, roots of Iranian *R. tinctorum* from Kouras Samanian and dried Turkish madder

root from Kremer Pigmente. Samples of *R. peregrina* root were obtained from the Botanic Gardens in Brussels and from Dentelles de Montmirail, France for comparison.

Alizarin was obtained from Hopkin and Williams and purpurin from Sigma-Aldrich. Pseudopurpurin is not available commercially and while it may be synthesized [27] or isolated from *R. tinctorum* by one of the methods described by Derksen *et al.* [23], in this study it was isolated from Winsor & Newton's rose madder pigment as this is a readily available material that is much richer in pseudopurpurin than madder root. The extraction method was based on a scaled-up version of the analytical technique developed by Hill and Richter [47].

Details of the archaeological pigment and paint samples analysed during the course of this study, or which have previously been characterized at the British Museum, are noted in Table 1 along with the analytical methods employed.

The experimental lake pigments were prepared using a recipe based on a standardized version of an early nineteenth-century recipe [55], which was developed within the EU FP7 CHARISMA (Cultural Heritage Advanced Research Infrastructures: Synergy for a Multidisciplinary Approach to Conservation/ Restoration) project [56] for use in experimental work and for a workshop on the preparation of historical lake pigments organized at the Doerner Institut in 2011 [57]. The 'standard pigment' sample was prepared by first soaking 10 g ground madder root overnight in a beaker with 300 mL water. After soaking, the solution was then heated to 70°C for 30 minutes to extract the dye. The residual root material was then removed and the solution filtered while still hot. Potash alum (5 g) was then added to the solution which was reheated to 70–80°C. Meanwhile a solution of 1.88 g of K_2CO_3 in 50 mL water was prepared. The dyestuff extract was added gradually to the alkaline solution with constant stirring. The mixture was allowed to settle overnight before the pigment was collected by filtration, washed and dried. Other experimental samples were prepared by modifying this 'standard' recipe.

Solubility studies and the influence of the pigment recipe

To create a madder lake pigment, colourant components must first be extracted from the root and then deposited onto or coprecipitated with a suitable insoluble substrate. For the resulting pigment to be rich in (pseudo)purpurin, either the solution from which the pigment is created must be rich in pseudopurpurin or there must be differential uptake of the various anthraquinones from the solution by the substrate. The use of a *Rubia* species intrinsically rich in pseudopurpurin or its precursors (for example *R. peregrina* or *Galium* spp.) would produce a pigment rich in (pseudo)purpurin. It was, however, also of interest to explore whether, by exploiting the different solubilities and affinities of the various anthraquinones found in cultivated madder, *R. tinctorum* L., a method of pigment preparation using materials that would have been available in antiquity could be found that would result in a pigment deliberately enriched in (pseudo)purpurin. Hofenk de Graaff outlines some of the key parameters in a series of experiments undertaken to understand better the production of red lake pigments in a group of fifteenth-century paintings from the Cologne School [3]. She notes that if the temperature in a madder dyebath is kept low, then alizarin will dye but purpurin

will not, and that the residual dyebath would thus be enriched in purpurin and could be used to create a (pseudo)purpurin-rich pigment. Derksen and van Beek outline how the different distribution and number of carbonyl and hydroxyl groups on hydroxyanthraquinones alter their affinity for mordants [25], while experimental studies that compare the colourant profile in dyebaths and the resulting dyed textiles also indicate differential uptake of components [58]. Hofenk de Graaff also notes that purpurin is more soluble in cold water than alizarin (which is only solubilized in hot water) and so cold water extraction of madder roots should yield a purpurin-enriched extract [3]. This difference in solubility forms the basis of the Leitenberger process to separately isolate alizarin and 'purpurine' from madder [59]; it should be noted that the term 'purpurine' used by Leitenberger may have included pseudopurpurin as the latter was then unknown. Alizarin is also less soluble than purpurin in alum solutions [3, 60; pp. 164 and 175–176]. These parameters might be exploited to alter the ratio of colourant components in a pigment compared with that of the aqueous extract.

A series of experiments, all undertaken using the same sample of *R. tinctorum* L. root (dried roots of unknown Middle Eastern origin obtained from Cornelissen and Son, London), was undertaken to explore these parameters and thus the influence of the recipe.

Solubility of madder aglycones

To understand better the processes occurring during dyeing and pigment manufacture, solubility data for the madder aglycones are needed. As very little such data exist [27], the solubilities were determined experimentally in this study. The frequent detection of aluminium in madder pigments from antiquity suggested that the solubility of the various madder aglycones in alum solution was also of interest. In solution the absorbance of solutes is (for ideal solutions) proportional to their concentration. The relationship is described by the Beer-Lambert law:

$$A = \epsilon c l$$

where A is the absorbance (optical density) of the solution, ϵ the extinction coefficient of the colourant, c the molar concentration of the colourant and l the pathlength in centimetres.

Although the amount of a given compound in solution can be directly related to its absorbance, it is more difficult to compare the proportions of different solutes unless their extinction coefficients are known. This must be borne in mind if attempts are made to compare the areas of HPLC peaks corresponding to different components: if a particular peak is double the size of another this does not necessarily mean that this is also the ratio of the concentrations of the two components in solution. The same problem arises with UV-visible spectra and the visual assessment of solution strength based on depth of colour. Where the extinction coefficients are known, it is possible to determine the exact concentration of the solute present in a given solution.

In this study, the absorbance maxima of the aglycones alizarin, purpurin and pseudopurpurin were compared and solutions of similar absorbance have been assumed to be of approximately equal concentration. Although the extinction

Table 2. Maximum UV-visible absorbance (in the range 190–1100 nm) at room temperature for saturated solutions of madder aglycones in water or a 5% alum solution

Madder aglycone	Solvent	Maximum absorbance
Alizarin	Water	0.11
	5% alum solution	0.12
Purpurin	Water	0.13
	5% alum solution	0.31
Pseudopurpurin	Water	0.13
	5% alum solution	2.20

coefficients for the aglycones in water and alum solutions are unknown (and initial attempts to determine these experimentally failed), this assumption is supported by the similarities in the absorbance maxima and extinction coefficients of the aglycones in either methanol or ethanol quoted by Thomson [61].¹ Experimental work undertaken during this study also suggests that the extinction coefficients in alum solution are similar to those in water and, perhaps somewhat surprisingly, are not influenced by any complex formation.

Relative solubilities were determined by placing an excess of the molecule of interest in water or in a 5% aqueous solution of alum. The mixture was shaken well and left to solubilize for at least seven days at the temperature of interest. The clear saturated supernatant solution was then carefully transferred into a quartz cuvette and the UV-visible spectrum recorded as described in the experimental appendix.

The maximum absorbances recorded at room temperature for alizarin, purpurin and pseudopurpurin in water and 5% alum solution are given in Table 2. Assuming that the aglycones have similar extinction coefficients, these results suggest that pseudopurpurin and purpurin are only marginally more soluble in water at room temperature than alizarin, but the maximum absorbance of the pseudopurpurin in alum is nearly 20 times as high as that for alizarin, and the absorbance of purpurin in the same solvent is about three times as high as alizarin.

These results suggest that pseudopurpurin is significantly more soluble at ambient temperatures in 5% alum solution than either purpurin or alizarin and thus that extraction of madder root with such a solution might be used to obtain an extract enriched in pseudopurpurin that could be used to create a pigment with a high pseudopurpurin content. Figure 7 shows saturated solutions of pseudopurpurin in water and in 5% alum solution alongside a 5% alum solution obtained from powdered *R. tinctorum* root. The madder had previously been soaked in cold water, reducing the residual glycoside content of the sample and allowing hydrolysis of glycosides, and then dried. The 5% alum solutions of pseudopurpurin and of the washed madder are visually alike and have similar UV-visible spectra suggesting pseudopurpurin enrichment of the madder extract is indeed possible. Some enzymatic decarboxylation of the pseudopurpurin during soaking and the experiments cannot be excluded.

Experimental pigments from *R. tinctorum*

Based on the solubility data, which suggested that the extraction of colourants from *R. tinctorum* root using alum solutions



Figure 7. Saturated solutions of: pseudopurpurin in water (left); pseudopurpurin in 5% alum solution (centre); and *R. tinctorum* root (previously soaked in water and dried) in 5% alum solution (right)

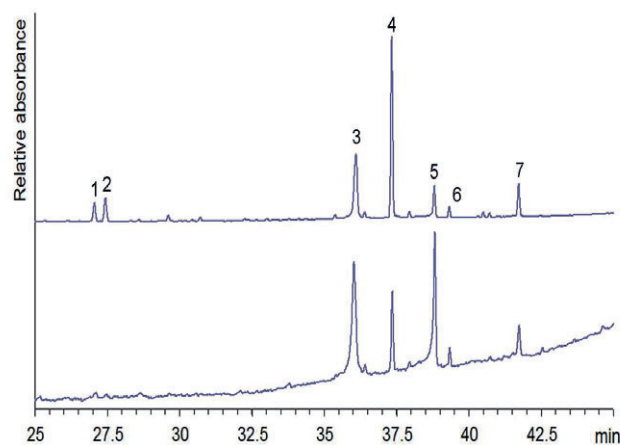


Figure 8. HPLC chromatograms at 254 nm of madder pigments prepared using *R. tinctorum* following the 'standard' recipe (top) and a modified recipe as described in the text to produce a pigment deliberately enriched in pseudopurpurin (bottom) following dimethylformamide/oxalic acid extraction. Peak labelling as for Figure 4

resulted in enrichment of the pseudopurpurin content of the extract, a series of experimental pigments was produced and the proportions of the key aglycones – alizarin, purpurin and pseudopurpurin – in the pigments assessed by UV-visible spectroscopy and HPLC-DAD analysis as described in the experimental appendix.

In these experiments, a lake pigment was produced by combining potassium carbonate (K_2CO_3) with the various alum extracts. The recipe used is similar to that described by Pliny for the production of yellow lake pigments [36, 40; book 33 XXVII 89]. To assess the degree of enrichment, the results for each experimental pigment were compared to the colourant composition in the root (based on a water/methanol extract, Figure 4) and in the 'standard pigment' prepared as described in the materials and methods section. HPLC-DAD analysis of this 'standard pigment' (Figure 8, top) shows that the alizarin content is approximately equal to, or slightly greater than, the combined purpurin and pseudopurpurin content, based on the assumptions noted above.

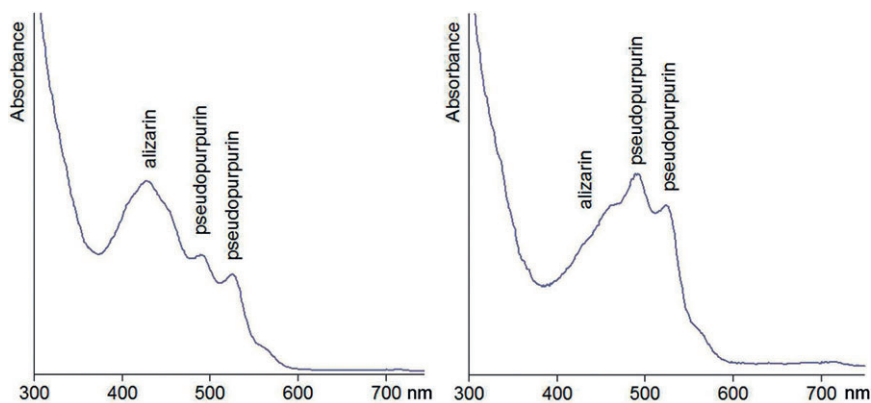


Figure 9. UV-visible spectra (in toluene) of extracts of madder pigments prepared using *R. tinctorum* following the ‘standard’ recipe (left) and the modified recipe described in the text to produce a pigment deliberately enriched in pseudopurpurin (right) following extraction using the Hill and Richter method described in the text (resulting in sample hydrolysis but no decarboxylation of the pseudopurpurin)

In the experiments undertaken in an attempt to produce a pigment deliberately enriched in pseudopurpurin, a series of modifications was made to the ‘standard’ recipe. The preparation was divided into three steps: (1) soaking and washing in water; (2) hydrolysis and extraction using an alum solution; and (3) pigment preparation using K_2CO_3 . The influence of using or omitting the initial pre-wash or soaking step, and its duration, was explored as well as the duration and temperature of the alum extraction step. In all the experiments, a 5% alum solution was used.

Of all the experiments conducted, the recipe that produced a pigment with the highest pseudopurpurin and lowest alizarin content was as follows. Powdered *R. tinctorum* root (5 g) was soaked in water (just sufficient to cover the powder) for one week in a stoppered vessel at ambient temperature. The mixture was filtered and the brown filtrate discarded. The damp powder was then added to 200–400 mL of a 5% alum solution and left for a week. The resulting luminescent orange solution, rich in pseudopurpurin, was filtered before being warmed to 60°C and slowly poured, with stirring, into an equal volume of K_2CO_3 solution (prepared with 1.88 g of K_2CO_3 for every 100 mL of alum solution). The mixture was allowed to stand for a week and the resulting pink pigment was collected by filtration using a Buchner funnel, washed with water and air dried. The method reproducibly yielded pigments with a high pseudopurpurin content and the pigment produced was visually identical in appearance to a Winsor & Newton rose madder pigment that had been shown to contain high levels of pseudopurpurin. The HPLC-DAD analysis of a pigment produced using this recipe is shown in Figure 8 (bottom).

Throughout this experimental work, UV-visible spectroscopy of extracts prepared using the method described by Hill and Richter proved a useful screening method and indicator of the pseudopurpurin content of pigments prepared. The UV-visible spectra for the ‘standard’ and the high pseudopurpurin pigments described above are presented in Figure 9. For the high pseudopurpurin pigment, the spectrum showed the absence of detectable alizarin (absorption maximum at 423 nm). The two main peaks for the pseudopurpurin (493 and 460 nm) in the sample in Figure 9 differ in intensity from those in a pure pseudopurpurin solution (Figure 5), suggesting a contribution to the spectrum from an additional component.

The purpurin (absorption maximum 479 nm) shown to be present by HPLC analysis (Figure 8) would account for the intensity difference noted in the UV-visible spectrum.

Differential uptake of colourant components from solution

In the experiments described above, a lake pigment was produced by adding K_2CO_3 to the various alum extracts. In principle, the alum extracts could also be used to ‘dye’ solid white substrates as described by Vitruvius and Pliny and should still result in a pseudopurpurin-enriched pigment. As the evidence from work on textile dyeing suggests that differential uptake of the colourant components may further alter the colourant proportions in the resulting pigment, it was of interest to explore whether the choice of solid white substrate added to an aqueous extract of madder could alter the colourant composition in the resulting pigment. A preliminary set of experiments has been undertaken in which 10 g powdered madder root was heated with 300 mL water at 60°C for 30 minutes before a selection of possible white substrates (including gypsum, china clay, marble dust, chalk and calcium hydroxide) was added and left in the extract solution for a week at ambient temperature or at 70°C before being collected by filtration. In general the uptake of colour from the solution was quite low, with the extract remaining quite strongly coloured compared to the solution remaining after pigment formation using alum and K_2CO_3 . Further pigment preparations and analytical work to explore the colourant composition of the resulting pigments are planned in order to explore more fully the influence of the substrate.

The relevance of experimental studies of madder pigments to madder compositions found in archaeological objects

There is little documentary evidence relating to the production of madder pigments in antiquity, but preparation seems to have been based on the extraction of colourant directly from the root. This contrasts with subsequent practice (certainly from the later fourteenth to the seventeenth century) where the most common source of colourant seems to have been dyed textiles. The method from antiquity is thus somewhat closer to the methods used in the late eighteenth and nineteenth centuries to prepare madder lakes [5, 24, 57].

Although direct inter-comparison of the results is not always straightforward, a review of previous analyses of early madder pigments from a relatively broad geographical and chronological span (Tables 1 and 3) suggests that many of these madder pigments closely resemble each other: the pigments are frequently a characteristic bright pale pink colour, relatively well preserved, highly luminescent and, where the organic components have been analysed, are rich in (pseudo) purpurin and contain little or no alizarin. As noted by Russell [62], Farnsworth [63] and others, the pigments are quite similar; therefore, to nineteenth- and twentieth-century rose madder pigments.

In Greek terracottas, Higgins suggests that ‘rose madder’ starts to be used from 330 BC [48]. In an Egyptian context, the increasing use of madder-based pigments seems to be linked to changes in the palette from the Ptolemaic period, possibly reflecting Hellenistic and Roman influences or increased exchange with central and eastern Asia at this period [34, 71].

Where the substrate has been characterized the reported results are more variable, but this in part may reflect the choice of analytical method(s) used and the complications of analysing the pigment substrate in the presence of other ‘contamination’ as discussed in the section on techniques for the characterization of madder-based pigments. Laurie suggests that “the lakes described by Pliny are dyed upon an opaque base like gypsum and therefore differ from our modern lakes, precipitated with alum, the receipts for which belong to the fourteenth century” [75].

Farnsworth suggests that Laurie assumed that “the ancients always used a gypsum base” (based on a study of Egyptian pigments) but that her work on earlier samples of pigments from Corinth shows that the Greeks were preparing pigments with an alumina substrate [63]. Linen dyed with madder and an alum mordant from Deir al-Bahri (Twenty-first Dynasty, c.1050 BC) offers clear evidence of the early availability of alum [1; p. 119]. Tables 1 and 3 provide a number of examples of the association of gypsum with madder pigments, but re-examination of the substrate of the madder pigment from Hawara has shown that the substrate is not gypsum alone (as suggested by Russell [62]) but also contains amorphous aluminium-based material. As shown in Figure 6, the use of calcium carbonate as an alkaline ingredient in pigment making may explain the presence of gypsum within the pigment substrate, although the admixture of gypsum and bassanite in some samples has also been interpreted as suggesting the addition of gypsum as an extender [32]. In many of the samples presented in Tables 1 and 3 aluminium has been found quite homogeneously distributed throughout the samples, perhaps pointing to the use of solutions containing aluminium salts during the madder pigment production [38]. Detailed examination of pigment substrates often reveals the admixture of the aluminium-based substrate with more crystalline materials. Tables 1 and 3 present examples where the use of a range of clays, earths and other materials is suggested, these having been employed as ingredients in pigment production (for example as an alkaline material to cause substrate precipitation), solid substrates or extenders. The variability in substrate might argue against a single recipe or simply reflect available resources, but the frequent detection of aluminium may suggest that the pigments were often prepared by starting

with an extraction using an alum (or aluminium-containing) solution. This study has again highlighted the importance of using a range of analytical techniques to characterize lake pigments fully. It has also demonstrated the value of the Hill and Richter extraction method combined with UV-visible absorption spectroscopy as a rapid and simple method to establish the approximate relative proportions of different aglycones in madder pigments.

The experimental pigment production undertaken during this study suggests that through selective extraction, a (pseudo) purpurin-rich pigment can be produced from *R. tinctorum* using materials that would have been available in antiquity. The experiments allow a number of key factors to be identified that promote the production of such pigments. Pre-soaking of the madder root in water was shown to be important. This step may assist the dissolution of the soluble alizarin precursor, ruberythric acid, and the more complete hydrolysis of the glycosides in the root to their respective water-insoluble aglycones [12] or it may help to wash out alizarin glycosides and thus reduce the amount of alizarin (or its precursors) in the root then used to make the pigment. The results shown in Figure 4 suggest that if the madder roots employed are relatively young and fresh, and therefore rich in glycosides, the rinsing away of alizarin precursors could significantly reduce the alizarin content in the resulting pigment. The pre-soaking also seems to help rid the madder root of soluble brown materials that are known otherwise to discolour any pigment produced. The solubilities of most organic compounds increase with temperature. The published solubility data for the various madder aglycones in water and alum solution at different temperatures are somewhat ambiguous but suggest that purpurin and pseudopurpurin are more soluble than alizarin, particularly at lower temperatures [3, 76, 77]. Keeping the temperature low throughout the pigment preparation was important, as experiments using higher temperature for the alum extraction step resulted in a greater solubilization of alizarin.

While there is evidence of cultivation of *R. tinctorum* around Ravenna and Caria in the Roman period [3, 78; book III 160] and in Syria and Anatolia in the Mesopotamian period [79; p. 305], the use of other *Rubia* or *Galium* species cannot be excluded. In the context of dyeing, the seventh-century BC neo-Babylonian tablet mentioned earlier contains two distinct recipes for applying madder after an alum mordant but it is also of interest to note that three types of madder are referred to: *hat-huritu* (used to imitate purple), *huratu* (normal madder) and an imported madder referred to as *inza-huritu* [1; pp. 119–120]. This suggests that different dyeing techniques were known and potentially specific resource selections were being made. A comparison of textile dyeing technologies in Roman and Coptic Egypt over roughly a 1000-year period reveals that the madder colourant composition in purple-coloured dyes differs from that in red, black or any other madder-containing colours and indeed is different to the compositions typical for textiles in Medieval Europe [11, 16]. The lower alizarin content of the madder source used to produce purple suggests that a different dyeing technology was being used for this colour – possibly pointing to an alternative biological source (or controlled cultivation of a particular source) or a different dyeing procedure to enrich the (pseudo)purpurin content and obtain a more purple colour.

Table 3. Comparative examples of the analysis of madder pigments from antiquity

Object description	Analytical results and method ^a	References
Reddish-purple decoration on eighth- and seventh-century BC white-slipped Cypriot pottery		[64]
Deep purple on a fourth-century BC Greek marble basin. J. Paul Getty Museum (85.AA.107)		[18]
Pink pigment on Hellenistic Greek terracotta figurines of the Tanagra type, around late fourth to third century BC. Harvard University Art Museums		[65]
Greek marble throne. Fourth to first century BC		[66]
Pink pigment on Canosa ceramics produced in southeastern Italy, around the fourth to third century BC. J. Paul Getty Museum		[67]
Punic pink cosmetic cones, third century BC. Carthage		[35]
Pink Punic make-up samples of the fourth to first century BC. From archaeological excavation sites or museums in Tunisia		[68]
Lumps of pigment found either in a well (but probably from a workshop or shop from Corinth and dating to c.146 BC) or from inside a bowl from the Agora, Athens (around second century BC)		[63]
Pink pigment at the bottom of a ceramic jar found in terraced house 2, room 17 at Ephesus. First century AD		[69]
Pink paint from Fayum encaustic mummy portraits. Ny Carlsberg Glyptotek, Copenhagen (ÆIN 681: AD 125–150 and ÆIN 682: AD 140–160)		[32]
Encaustic Fayum mummy portraits from the second century AD. Fitzwilliam Museum, Cambridge (2284 and 2285)		[70]
Cartonnage fragments from Hawara dating to around the first century AD. Petrie Museum (UC45948e)		[71]
Pigment on Hellenistic/Roman figurines from tombs in Thessaloniki Hellenistic and Roman pigments from Greece (a Hellenistic pigment from Rhodes and a Roman wall painting pigment from Korinthos of the second century AD)		[20]
Pink colour in a Hellenistic/Roman period tomb painting (possibly on a cartonnage or a wall painting)		[19]
Lee and Quirke [34] cite Russell [62] and describe a "pink colour in a tomb painting (presumably on a wall or ceiling) as madder painted over gypsum". However in the original Russell paper [62] he appears to be describing a pink pigment used on a cartonnage and composed of madder on a gypsum substrate		[34; p. 113, 62]

Violet-pink material (bulk pigment) from the bottom of a goblet and a small Roman amphora from Vicenza	<p>Cyclic voltammetry: alizarin (probably <i>R. tinctorum</i>). XRD: substrate mainly amorphous but with a trace of various minerals. SEM-EDX: amorphous substrate, Al, O, Si and some Fe, Mg and P, Ca, trace Cl and K. FTIR: mainly aluminium hydroxide or hydrated alumina rather than silicate; some calcium sulphate and phosphate. Spectra resemble precipitates formed from an alunite solution on addition of a base. Suggested that the pigment was precipitated from solution by adding an alkaline madder extract (using natron or slaked lime as the alkaline material) to a solution/suspension containing alunite and that salts such as gypsum and calcium phosphate were then added to the resulting pigment</p> <p>Microfluorimetry: emission bands centred in the range 615–640 nm suggest an anthraquinone-based lake of vegetal origin. SERS: madder lake. SEM-EDX: clay substrate – high Si and Al contents in a ratio of about 1:6 (Si:Al)</p>	[38]
Pigments from ceiling of gilded vault of the Domus Aurea in Rome, built after AD 64	<p>SEM-EDX: clay substrate – high Si and Al contents in a ratio of about 1:6 (Si:Al)</p>	[72]
Greco-Roman cosmetics:	SEM-EDX: clay substrate – high Si and Al contents in a ratio of about 1:6 (Si:Al)	[36, 43]
<ol style="list-style-type: none"> 1. Glass bottle (<i>balsamarium</i>) of the first century AD from the Roman cemetery at Wederath, Germany. Rheinisches Landesmuseum, Trier. 2. Glass sphere of the third century AD from the Roman city of Cologne. Römisch-Germanisches Museum, Cologne. 3. Greek 'Pyxide' box of the fourth century BC from the ancient Greek city of Demetrias. Archaeological Museum of Volos <p>Lump of unused pink-purple pigment excavated at Pompeii. National Archaeological Museum, Naples (18107, 18129, 117323)</p>	<ol style="list-style-type: none"> 1. SERS: resembles a reference purpurin lake and no evidence for alizarin. LDI-MS (using MALDI-ToF): purpurin and pseudopurpurin but no alizarin. 2. LDI-MS: purpurin. 3. LC-ESI-HRMS: pseudopurpurin and munjistin <p>SEM-EDX, FTIR, XRD and TEM: substrate is mainly amorphous (containing Si, Al and O) and is suggested to be a silicon-aluminium gel of the allophane clay type. The pigment also contains some crystalline particles of kaolinite and aragonite. HPLC^g: mainly alizarin and purpurin detected, with purpurin predominant over alizarin (c.98% purpurin) suggesting the colourant is not from <i>R. tinctorum</i> but from a wild species, for example <i>R. peregrina</i> or <i>Galium</i> spp. The presence or absence of indigoid dyes (including those derived from shellfish) could not be confirmed Physico-chemical examination and optical microscopy: substrate contained Si, Ca (in the form of calcium carbonate) and small quantities of Fe and other impurities. The presence of diatoms was reported in all the samples suggesting use of diatomite as the substrate for the organic colourant The colourants were identified as 'purpurisum' and to be of shellfish origin PLM: organic red pigment derived from madder on a substrate of aluminium oxide It was concluded that the pale rose-coloured lake contained a dyestuff of vegetable origin, because of "its not affording by decomposition the smell peculiar to animal substances". Davy [74] states that Chaptal [73] regarded the pigment as "analogous to the lake formed by fixing the colouring matter of madder upon alumina". Farnsworth [63] states that Chaptal considered the sample as "a true lake on an alumina base" Described as "a pale rose colour; where it has been exposed to air, it has lost its tint ... but the interior has a lustre approaching to that of carmine" [74; p. 113]. The substrate was composed of "siliceous, aluminous, and calcareous earths" [74; p. 113] (described as "clay" by Farnsworth [63]). Treatment with acid confirmed the presence of calcium carbonate. The organic colourant was not identified but was concluded to be of "vegetable or animal origin" [74; p. 114]</p>	[21]
Violet-coloured samples of unused pigment excavated at Pompeii	SEM-EDX, FTIR, XRD and TEM: substrate is mainly amorphous (containing Si, Al and O) and is suggested to be a silicon-aluminium gel of the allophane clay type. The pigment also contains some crystalline particles of kaolinite and aragonite. HPLC ^g : mainly alizarin and purpurin detected, with purpurin predominant over alizarin (c.98% purpurin) suggesting the colourant is not from <i>R. tinctorum</i> but from a wild species, for example <i>R. peregrina</i> or <i>Galium</i> spp. The presence or absence of indigoid dyes (including those derived from shellfish) could not be confirmed Physico-chemical examination and optical microscopy: substrate contained Si, Ca (in the form of calcium carbonate) and small quantities of Fe and other impurities. The presence of diatoms was reported in all the samples suggesting use of diatomite as the substrate for the organic colourant The colourants were identified as 'purpurisum' and to be of shellfish origin PLM: organic red pigment derived from madder on a substrate of aluminium oxide It was concluded that the pale rose-coloured lake contained a dyestuff of vegetable origin, because of "its not affording by decomposition the smell peculiar to animal substances". Davy [74] states that Chaptal [73] regarded the pigment as "analogous to the lake formed by fixing the colouring matter of madder upon alumina". Farnsworth [63] states that Chaptal considered the sample as "a true lake on an alumina base" Described as "a pale rose colour; where it has been exposed to air, it has lost its tint ... but the interior has a lustre approaching to that of carmine" [74; p. 113]. The substrate was composed of "siliceous, aluminous, and calcareous earths" [74; p. 113] (described as "clay" by Farnsworth [63]). Treatment with acid confirmed the presence of calcium carbonate. The organic colourant was not identified but was concluded to be of "vegetable or animal origin" [74; p. 114]	[42]
Pink pigment from Pompeii	SEM-EDX, FTIR, XRD and TEM: substrate is mainly amorphous (containing Si, Al and O) and is suggested to be a silicon-aluminium gel of the allophane clay type. The pigment also contains some crystalline particles of kaolinite and aragonite. HPLC ^g : mainly alizarin and purpurin detected, with purpurin predominant over alizarin (c.98% purpurin) suggesting the colourant is not from <i>R. tinctorum</i> but from a wild species, for example <i>R. peregrina</i> or <i>Galium</i> spp. The presence or absence of indigoid dyes (including those derived from shellfish) could not be confirmed Physico-chemical examination and optical microscopy: substrate contained Si, Ca (in the form of calcium carbonate) and small quantities of Fe and other impurities. The presence of diatoms was reported in all the samples suggesting use of diatomite as the substrate for the organic colourant The colourants were identified as 'purpurisum' and to be of shellfish origin PLM: organic red pigment derived from madder on a substrate of aluminium oxide It was concluded that the pale rose-coloured lake contained a dyestuff of vegetable origin, because of "its not affording by decomposition the smell peculiar to animal substances". Davy [74] states that Chaptal [73] regarded the pigment as "analogous to the lake formed by fixing the colouring matter of madder upon alumina". Farnsworth [63] states that Chaptal considered the sample as "a true lake on an alumina base" Described as "a pale rose colour; where it has been exposed to air, it has lost its tint ... but the interior has a lustre approaching to that of carmine" [74; p. 113]. The substrate was composed of "siliceous, aluminous, and calcareous earths" [74; p. 113] (described as "clay" by Farnsworth [63]). Treatment with acid confirmed the presence of calcium carbonate. The organic colourant was not identified but was concluded to be of "vegetable or animal origin" [74; p. 114]	[39]
Roman period pink lake pigments from a shop at Pompeii	SEM-EDX, FTIR, XRD and TEM: substrate is mainly amorphous (containing Si, Al and O) and is suggested to be a silicon-aluminium gel of the allophane clay type. The pigment also contains some crystalline particles of kaolinite and aragonite. HPLC ^g : mainly alizarin and purpurin detected, with purpurin predominant over alizarin (c.98% purpurin) suggesting the colourant is not from <i>R. tinctorum</i> but from a wild species, for example <i>R. peregrina</i> or <i>Galium</i> spp. The presence or absence of indigoid dyes (including those derived from shellfish) could not be confirmed Physico-chemical examination and optical microscopy: substrate contained Si, Ca (in the form of calcium carbonate) and small quantities of Fe and other impurities. The presence of diatoms was reported in all the samples suggesting use of diatomite as the substrate for the organic colourant The colourants were identified as 'purpurisum' and to be of shellfish origin PLM: organic red pigment derived from madder on a substrate of aluminium oxide It was concluded that the pale rose-coloured lake contained a dyestuff of vegetable origin, because of "its not affording by decomposition the smell peculiar to animal substances". Davy [74] states that Chaptal [73] regarded the pigment as "analogous to the lake formed by fixing the colouring matter of madder upon alumina". Farnsworth [63] states that Chaptal considered the sample as "a true lake on an alumina base" Described as "a pale rose colour; where it has been exposed to air, it has lost its tint ... but the interior has a lustre approaching to that of carmine" [74; p. 113]. The substrate was composed of "siliceous, aluminous, and calcareous earths" [74; p. 113] (described as "clay" by Farnsworth [63]). Treatment with acid confirmed the presence of calcium carbonate. The organic colourant was not identified but was concluded to be of "vegetable or animal origin" [74; p. 114]	[63, 73, 74]
Roman period rose pink pigment in a broken vase found at the baths of Titus, Rome	SEM-EDX, FTIR, XRD and TEM: substrate is mainly amorphous (containing Si, Al and O) and is suggested to be a silicon-aluminium gel of the allophane clay type. The pigment also contains some crystalline particles of kaolinite and aragonite. HPLC ^g : mainly alizarin and purpurin detected, with purpurin predominant over alizarin (c.98% purpurin) suggesting the colourant is not from <i>R. tinctorum</i> but from a wild species, for example <i>R. peregrina</i> or <i>Galium</i> spp. The presence or absence of indigoid dyes (including those derived from shellfish) could not be confirmed Physico-chemical examination and optical microscopy: substrate contained Si, Ca (in the form of calcium carbonate) and small quantities of Fe and other impurities. The presence of diatoms was reported in all the samples suggesting use of diatomite as the substrate for the organic colourant The colourants were identified as 'purpurisum' and to be of shellfish origin PLM: organic red pigment derived from madder on a substrate of aluminium oxide It was concluded that the pale rose-coloured lake contained a dyestuff of vegetable origin, because of "its not affording by decomposition the smell peculiar to animal substances". Davy [74] states that Chaptal [73] regarded the pigment as "analogous to the lake formed by fixing the colouring matter of madder upon alumina". Farnsworth [63] states that Chaptal considered the sample as "a true lake on an alumina base" Described as "a pale rose colour; where it has been exposed to air, it has lost its tint ... but the interior has a lustre approaching to that of carmine" [74; p. 113]. The substrate was composed of "siliceous, aluminous, and calcareous earths" [74; p. 113] (described as "clay" by Farnsworth [63]). Treatment with acid confirmed the presence of calcium carbonate. The organic colourant was not identified but was concluded to be of "vegetable or animal origin" [74; p. 114]	[63, 74; pp. 113–116]
Notes	<p>a. PLM = polarized light microscopy; SEM-EDX = scanning electron microscopy with energy dispersive X-ray analysis; TEM = transmission electron microscopy; UV-vis = ultraviolet-visible; FTIR = Fourier transform infrared spectroscopy; SERS = surface enhanced Raman spectroscopy; XRD = X-ray diffraction; XRF = X-ray fluorescence; TLC = thin layer chromatography; HPLC = high performance liquid chromatography; GC-MS = gas chromatography-mass spectrometry; LC-ESI-HRMS = high performance liquid chromatography coupled to electrospray ionization with high resolution mass spectrometry; LDI-MS = laser desorption ionization-mass spectrometry; MALDI = matrix assisted laser desorption ionization; and ToF = time of flight b. Colourant extracted using concentrated H₂SO₄ with the expected loss of glycosides and pseudopurpurin. c. Colourant extracted using HCl with the expected loss of glycosides and pseudopurpurin. d. Mild colourant extraction using HF to preserve the glycosides and pseudopurpurin. e. Mild colourant extraction using BF₃·MeOH to preserve the glycosides and pseudopurpurin. f. Semi-mild extraction using a combination of HCl/MeOH treatment followed by ethyl acetate extraction, with the expected loss of the glycosides but with the pseudopurpurin protected from the acid in the solvent extract. g. Method of extraction not reported but assumed to be based on the use of HCl with the expected loss of glycosides and pseudopurpurin.</p>	

Unfortunately there is less analytical information available on pigment technologies, but the evidence from textiles points to the transmission of specific skills and traditions over long periods. The rather consistent use of (pseudo)purpurin-rich madders highlighted in this study may reflect the selection of a particular madder source and/or the control of the production technology in pigment manufacture. As with the textiles, in the post-Byzantine [80] and Medieval periods the pigment technology appears to change and more alizarin-rich pigments tend to be found, but more data are required to understand why and when this change occurred. Additional work is planned to determine whether the addition of a solid substrate to a colourant extract and the choice of such a substrate can result in preferential uptake of colourant components and modifications to the colourant composition. Further studies are also being developed to explore the relative stabilities of alizarin, purpurin and pseudopurpurin (and their precursors) and to investigate the good preservation of colour observed for many examples of early madder lake pigments.

Conclusions

Based on experimental pigment production and the evidence from the analytical study of early madder pigments, this article presents technological insights into madder pigment production in antiquity in the absence of direct documentary evidence. As such pigments are typically rich in (pseudo)purpurin and contain little or no alizarin, it has been suggested that *R. tinctorum*, which tends to yield pigments containing significant proportions of alizarin, was not the colourant source employed to create madder pigments at this period. While the exploitation of other dye plant species, including *R. peregrina* – which is intrinsically rich in (pseudo)purpurin – or the controlled cultivation of *R. tinctorum* is possible, this study has demonstrated that early pigment makers could have developed a process of selective extraction of colourants from *R. tinctorum* based on the use of soaking, alum extraction and temperature adjustment that would have given a higher (pseudo)purpurin content (and more purple colour) using materials and technologies available at the time.

Although variations in the pigment substrate, additives and extenders have been noted in this study, the madder pigment technology in terms of the colourant composition appears – in common with madder dyeing technologies – to have been quite consistent over a long period and wide geographical region but to be quite distinct from the later technologies in use in Medieval Europe.

Experimental appendix

HPLC-DAD analysis

All pure analytical standards were dissolved in a 1:1 (v/v) mixture of water and methanol at room temperature. The madder root samples were ground to a powder and then a few milligrams were extracted in 1 mL of the same solvent at 100°C for 30 minutes. After 5 minutes of centrifugation the extract was transferred in a HPLC vial. For the archaeological and replica pigment samples, c.1 mg pigment powder was extracted in 200 µL of a 2:1 (v/v) mixture of dimethylformamide (DMF) and methanol at 80°C for 10 minutes. The extract

was transferred to a HPLC vial and 200 µL of a mixture of methanol, acetone, water and 0.5M oxalic acid (30:30:40:1 by volume) was added to the remaining pigment and the sample heated at 80°C for 15 minutes. The solution was evaporated under nitrogen and the residue was reconstituted with 200 µL of a 2:1 (v/v) mixture of water and methanol and the solution added to the HPLC vial containing the DMF extract. The combined solution was then analysed by HPLC by injecting 10 µL samples.

Analyses were carried out using an Agilent HPLC HP1100 system comprising a vacuum solvent degasser, a binary pump, an autosampler and a column oven. The column used for the separation was a Phenomenex Luna C18(2) 100 Å, 150 × 2.0 mm, 3 µm particle size, held at 40°C in a column oven. Detection was performed using an HP1100 DAD with a 500 nL flow cell and using detection wavelengths from 200 to 700 nm. Two solvents were used as eluent: (A) 99.9% water with 0.1% trifluoroacetic acid (v/v); and (B) 99.9% acetonitrile with 0.1% trifluoroacetic acid (v/v). The elution programme provided a linear increase in the ratio of A to B from 95:5 to 70:30 over a period of 25 minutes followed by a second linear gradient to 0:100 over the next 15 minutes. After eluting with pure B for 10 minutes a third linear gradient was used to return to the initial composition (95% A to 5% B) in 15 minutes before eluting with this solvent mixture for a further 10 minutes to stabilize the system. The flow rate was fixed throughout at 0.2 mL per minute, creating a system back pressure of about 118 bars (11.8 MPa).

UV-visible spectroscopy

A quantity of root or pigment was soaked in a vessel containing 10% hydrochloric acid. Toluene was poured over this aqueous phase and formed an immiscible supernatant layer. The mixture was left to stand overnight and then heated to 100°C for 30 minutes. In this study the soaking period was increased to seven days for convenience.

The toluene solution was placed in a quartz cuvette and UV-visible spectra were acquired using a Thermo-Fisher Evolution 220 UV-visible spectrophotometer with a double-beam geometry and a wavelength range of 190 to 1100 nm.

By scaling up the quantities involved this extraction method was also used to isolate a small quantity of pseudopurpurin from Winsor & Newton rose madder pigment (known to be rich in pseudopurpurin). The toluene extract was allowed to evaporate to yield a solid sample of pseudopurpurin for use in the solubility experiments.

FTIR spectroscopy

Samples for FTIR analysis were prepared in the form of pressed potassium bromide (KBr) pellets and analysed in transmission mode using a Nicolet 6700 spectrometer with a DLaTGS detector. The spectra were acquired over the range 4000–400 cm⁻¹ using 32 scans at a resolution of 4 cm⁻¹ and automatic gain.

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Note

1. Alizarin (molecular weight 240) – λ_{\max} (EtOH) 247, 278, 330 and 434 nm; $\log \epsilon$ 4.45, 4.13, 3.46 and 3.70. Purpurin (molecular weight 256) – λ_{\max} (MeOH) 255, 290(sh), 457(sh), 485, 518 and 542(sh) nm; $\log \epsilon$ 4.41, 4.09, 3.79, 3.96, 3.86, 3.58. Pseudopurpurin (molecular weight 300) – λ_{\max} (EtOH) 256, 285 (sh), 487 and 520 nm; $\log \epsilon$ 4.52, 4.10, 3.96 and 3.85 [61; p. 407].