Dyes and Pigments 118 (2015) 129–136

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

HPLC-DAD-MS analysis of colorant and resinous components of lac-dye: A comparison between *Kerria* and *Paratachardina* genera

Raquel Santos [a], Jessica Hallett [a], M. Conceição Oliveira [b, *], Micaela M. Sousa [c], Jorge Sarraguça [d], M.S.J. Simmonds [e], M. Nesbitt [e]

[a] CHAM (Centre for Overseas History), Faculdade de Ciências Sociais e Humanas, Universidade Nova de Lisboa and Universidade dos Açores, 1069-061 Lisboa, Portugal
[b] Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal
[c] REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Monte da Caparica, Portugal
[d] REQUIMTE, Laboratório de Química Aplicada, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, 4050-313 Porto, Portugal
[e] Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK

ARTICLE INFO

Article history:
Received 22 June 2014
Received in revised form
27 November 2014
Accepted 25 February 2015
Available online 12 March 2015

Keywords:
Dyes/pigments
Liquid chromatography
Mass spectrometry
Lac-dye
Paratachardina spp.
Kerria spp.

ABSTRACT

A database using high performance liquid chromatography with diode array detection was created for lac-dye insects (*Kerria* and *Paratachardina* genera) in order to identify the red dye used in historical textiles. Lac from *Kerria* and *Paratachardina* species was easily distinguished by liquid chromatography coupled to negative electrospray ionization tandem mass spectrometry. Seventy-six historical lac-dye specimens and seven reference insect specimens were analyzed and compared with 41 historical carpet samples. Using this approach, the presence of *Kerria* spp. was identified in three carpets, pointing to an Iranian provenance and corroborating a 16th/17th-century date for them, obtained previously with C14 analysis.

Multivariate data analysis was applied to differentiate sources of *Kerria* and improve the lac-dye database. PCA indicates that discrimination can be obtained according to composition. Information regarding pigment and resin concentrations is recovered separately in first and second principal components, respectively, which offers a basis of separation for this type of data.

The development of this lac-dye database is an important step towards improving research on lac-dye species, which can contribute to more accurate identification of the provenance of historical textiles. This work reinforces the importance of using taxonomically-verified specimens of *Kerria*, previously submitted to molecular taxonomic studies, to construct a more reliable lac-dye database capable of identifying the lac species present in historical textiles, as well as the provenance of the insect used.

1. Introduction

High performance liquid chromatography (HPLC) combined with sensitive and selective detection techniques such as diode array detector (DAD) and more recently mass spectrometry (MS) are widely applied to identify the colorants used in works of art [1,2]. The identification of natural dyes in historical textiles is a challenging task owing to factors such as small sample size as well as the complexity of dye degradation processes [3–5].

Extraction procedures have a direct impact on the results obtained by HPLC-DAD-MS^n and other related analytical techniques [6,7]. Extraction of dyes from a textile fiber was usually carried out with 3 M hydrochloric acid-methanol (1:1, v/v) at boiling point. In the past few years, this traditional aggressive acidic extraction method [8,9] has been replaced by new soft extraction methods that enable the sugar derivatives of the colorants to be extracted intact [6,7,10,11]. These extracts contain a greater diversity of compounds and therefore more complete information for differentiating samples and identifying dye sources. Indeed, sample preparation has proven to affect both the quality and quantity of the isolated colorants [7], something which is of fundamental importance for studying precious historical textiles.

* Corresponding author.
E-mail addresses: conceicao.oliveira@tecnico.ulisboa.pt, ana.rsantos@fcsh.unl.pt (M.C. Oliveira).

http://dx.doi.org/10.1016/j.dyepig.2015.02.024
0143-7208/

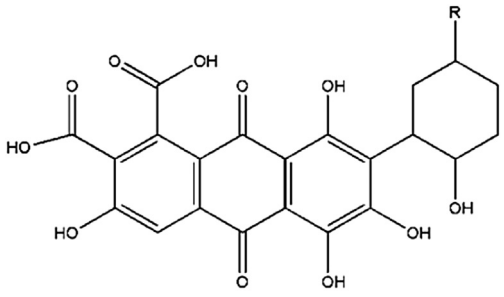
Lac-dye is a natural red dye used in works of art and obtained from lac-insects. These insects are part of the Kerriidae family, comprising nine genera and approximately 90 described species [12]. Lac-dye is obtained from female insects of two genera: *Kerria* and *Paratachardina*. However, the diversity of insects used to make lac has not been well studied and molecular taxonomic studies for confirmative species identification are only available for *Paratachardina* [13]. This genera comprises nine species, mainly from China, India, Sri Lanka, Philippines and Papua New Guinea [13], while *Kerria* comprises about 26 species, found primarily in India, China, Taiwan, Sri Lanka, Australia and Pakistan [14]. The secretions of *Kerria* insects are commonly used in commercial products, namely resins, waxes and dyes [15,16] for use in the manufacture of thermoplastics, adhesives, sealants, insulating and coating materials, such as industrial materials, medicine and food ingredients [17]. *Kerria lacca* is the most common species reported to be the source of lac-dye in historical textiles [15].

Lac comes onto the market as a raw product in many forms; among the most common are *sticklac* (the lac-coated branches of trees), *grain/seed lac* (produced by crushing and washing of sticklac), and *shellac* (a pale lac produced by filtering molten lac) [15,18,19]. Stick lack is composed of wax (6–7%), red coloring matter (4–8%), resin (70–80%) and other extraneous matter. According to the literature, this composition is very constant, although quantitatively the proportion of some components may depend on the host trees on which the insect feeds [21,23].

The red coloring matter of sticklac is soluble in water and thus removed during the initial washing. It can be concentrated by evaporation into cakes of lac-dye [20]. Ethanolic extractions of the sticklac product will remove only the resinous matter, which is composed mainly of terpenic acids (jalaric and laccijalaric acids) [18], aleuritic acid, and several minor fatty acids [17–19,21,22]. In order to obtain lac-dye, the pure deep red coloring matter suitable for dyeing purposes, it is necessary to perform a simple extraction with 100% water [24]. The water extracts the principal chromophores of lac-dye, namely laccaic acid A, B, C and E (Fig. 1) [24,25].

Red dyes are important sources of information for determining the provenance and date of historical textiles and carpets [26–29]. In 2007, when three 'Salting' carpets were discovered in the Palace of the Dukes of Braganza in Guimarães (Portugal), identifying the red dye used in their knotted-wool pile became the focus of intense research in order to resolve questions concerning their history and preservation [30]. For more than a hundred years, the origin and date of the 'Salting' group have been the source of considerable debate [31] with possibilities ranging from Turkey to Iran, and from the 16th to the 19th centuries. It is well known that, in general, the reds in 16th-century Turkish carpets reflect the presence of madder, while in classical Persian carpets, they were obtained using the more expensive dye extracted from lac insects [32,33]. In the 19th century, both of these natural dyes were replaced by synthetic dyestuffs [34]. Hence, characterizing the precise dyestuff used in the Guimarães carpets could offer important evidence for associating the carpets with a specific region and date of production. Although historical documents record the use of lac-dye in Asian textiles from at least the 4th century BC [15,35], no study to date has used HPLC-DAD to differentiate lac-insect species to identify locations of dye production as has occurred, for example, for cochineal, dragon's blood, madder or flavonols [27,37–39]. Indeed, for lac species of Asian origin belonging to the *Kerria* genus (some 26 species) [14], only *Kerria lacca* has been characterized using HPLC-DAD [40,41,44].

Thus, this study aims to compare the chemical diversity of lac-insects with dyed historical textile samples taken from the Guimarães carpets. For the first time, taxonomically verified species of *Kerria* and *Paratachardina* as well as unverified specimens, from different regions and different host plants, were analyzed using HPLC-DAD and Principal Components Analysis (PCA). This methodology represents an important tool for fundamental research on lac-dye species, to establish the precise sources of this red dye and consequently identify the provenance of historical textiles.



Laccaic acid A R=$CH_2CH_2NHCOCH_3$
Laccaic acid B R=CH_2CH_2OH
Laccaic acid C R=$CH_2CH(NH)_2COOH$
Laccaic acid D R=$CH_2CH_2NH_2$

Fig. 1. Chemical structures of the laccaic acids, the principal chromophores of lac-dye.

2. Methods and materials

2.1. Chemicals and reagents

All reagents and solvents used were of analytical grade. The aqueous solutions for making the extracts were prepared with ultrapure water from Millipore Simplicity® *Simpak* 2, $R = 18.2$ MΩ cm (Millipore, Bedford, MA, USA), methanol (99.9%) and hydrochloric acid (37%) from *Panreac* (Barcelona, Spain), perchloric acid, formic acid and trifluoroacetic acid (TFA) from *Riedel-de-Haën* (Seelze, Germany), acetone from *Aga* (Prior Velho, Portugal), and oxalic acid from *BDH* (Poole, England). HPLC and LC-MS analyses were performed with LC-MS grade methanol and acetonitrile grade from *Fisher Scientific* (Loughborough, Leicestershire, UK) and formic acid from *Agros Organics* (Geel, Belgium). A standard of laccaic acid A from *Wako Pure Chemicak Industries Ltd* (Osaka, Japan) was used to optimize the LC-MS conditions.

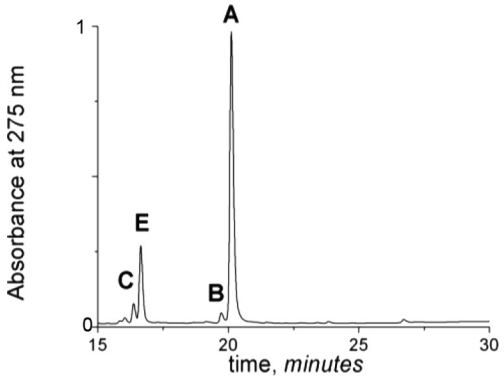
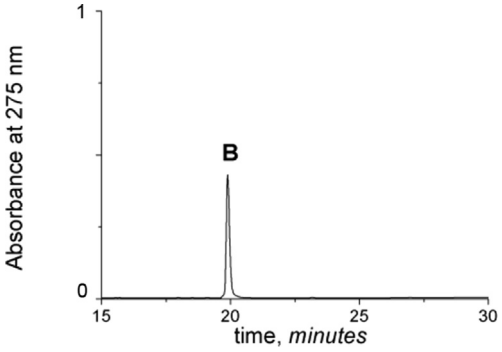
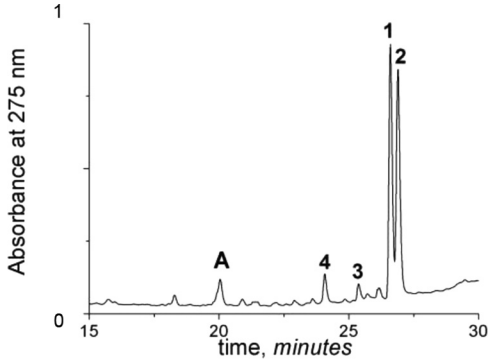
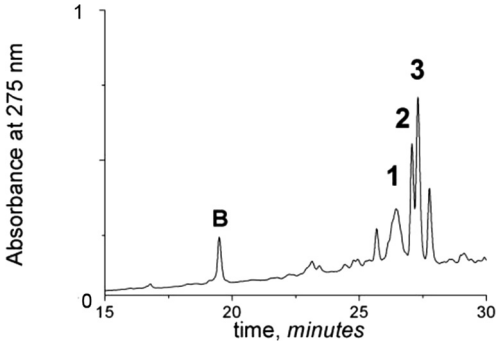
2.2. Samples

The samples analyzed included seven insect specimens from two genera *Kerria* and *Paratachardina* acquired from two entomologists; 76 unidentified historical lac-dye specimens from the 19th and 20th centuries, representing different regions (India, Australia, Taiwan, Vietnam, Laos, Singapore, Pakistan, Bangladesh and Sri Lanka), as well as different host plants obtained from the Economic Botany Collection (EBC) of the Royal Botanic Gardens, Kew (Table 2); 30 reference textiles dyed according to historical recipes [15] with *Kerria* spp., supplied by *Kremer-Pigmente* (Aichstetten, Germany), and 41 red wool fibers obtained from the three historical 'Salting' carpets (Palace of the Dukes of Braganza, Guimarães, Portugal).

2.3. Samples preparation

The lac-dye was extracted from the specimens (83) with 400 μl water for 10 min, in a 60 °C water-bath, with constant mechanical agitation [10,36]. Each insect provided three aliquots of the extract for HPLC-DAD analysis. The resin samples were extracted with 400 μl MeOH, and whenever necessary the dye extracts were filtered. Four extraction solutions (400 μl) were tested on the dyed

Table 1
HPLC-DAD chromatogram obtained at 275 nm and MS data for *Kerria* sp. and *Paratachardina* sp. insects from India. Both insects were extracted either with 100% water or 100% methanol.

Extraction	*Kerria* sp.	*Paratachardina* sp.
H_2O extraction		
Compounds identified by LC-MS analysis	**A**: R_t = 20.1 min, λ_{max} = 496 nm, $[M-H]^-$ = 536 (laccaic acid A), **B:** R_t = 19.7 min, λ_{max} = 490 nm, $[M-H]^-$ = 495 (laccaic acid B), **C:** R_t = 16.3 min, λ_{max} = 494 nm, $[M-H]^-$ = 538 (laccaic acid C) and **E:** R_t = 16.6 min, λ_{max} = 494 nm, $[M-H]^-$ = 494 (laccaic acid E).	**B:** R_t = 20.0 min, λ_{max} = 490 nm, $[M-H]^-$ = 495 (laccaic acid B)
MeOH		
Compounds identified by LC-MS analysis	**A**: R_t = 20.1 min, λ_{max} = 496 nm, $[M-H]^-$ = 536 (laccaic acid A) **1:** R_t = 25.4 min, λ_{max} = 437 nm, $[M-H]^-$ = 827 and **2:** R_t = 26.9 min, λ_{max} = 461 nm, $[M-H]^-$ = 827 (assigned as a mixture of two terpenic acids and one aleuritic acid). Other minor peaks **3** and **4** were also identified which correspond to soft resins, namely peaks at *m/z* 565 and 581. These peaks are consistent with aleuritic acid linked to a jalaric acid or a shellolic acid, respectively, as reported in literature [17].	**B:** R_t = 19.7 min, λ_{max} = 490 nm, $[M-H]^-$ = 495 (laccaic acid B) **2:** R_t = 26.2 min λ_{max} = 310 nm, $[M-H]^-$ = 567 and **3:** R_t = 26,8 min λ_{max} = 320 nm, $[M-H]^-$ = 567 attributed to the soft resin composed by laksholic and aleuritic acids. It was also identified a minor peak **1**, which is assigned to aleuritic with laccilaksholic acid (*m/z* 551).

reference textiles (ca. 0.2 mg per sample) to optimize the method for extracting the dye from the historical textiles: (a) Formic acid: MeOH (5: 95, v/v) [5,10,43]; (b) (HCl) 37%: MeOH: H_2O (2:1:1, v/v/v) [8,9]; (c) Oxalic acid 0.2 M: acetone: MeOH: H_2O (0.1:3:3:4, v/v/v/v) [11] and (d) TFA 2 M [6]. After the extraction (30 min at 60 °C, with constant mechanical agitation), the dye extracts were dried in a vacuum system and the final dye residue was dissolved using H_2O: MeOH: $H_2O/HClO_4$ (50:20:30, v/v/v) [27]. The HPLC-DAD analyses were performed on six replicates for each extraction method.

2.4. Apparatus

The insect and historical lac-dye specimens, as well as the textile samples, were analyzed using a Thermofinnigan, Surveyor PDA 5 HPLC-DAD system (Thermofinnigan, USA), with a reversed-phase column (Zorbax Elipse Plus C18, 250 mm × 4.6 mm, 5 μm) with a flow rate of 0.5 ml/min at 35 °C constant temperature. The samples were injected onto the column via a Rheodyne injector with a 25 μl *loop* [11]. A solvent gradient of A-pure methanol and B-0.3% (v/v) aqueous perchloric acid (v/v) adapted from Ref. [11] was applied to the separation of colorants in the insect extracts and textiles 0–2 min 7A:93B isocratic, 8 min 15A:85B linear, 25 min 75A:25B linear, 27 min 80A:20B linear, 29 min 95A:5B linear, and 33 min 7A:93B isocratic.

A 500-MS Ion Trap LC mass spectrometer with an electrospray ion source and controlled by a Varian MS Control 6.9.3 Workstation software (Varian Inc. Palo Alto, CA, USA) was used to analyze six specimens of *Kerria* and *Paratachardina*, as well as two historical textiles samples in order to characterize and confirm the structures of the lac-dye chromophores present in the extracts. Chromatographic separation conditions used in these analyses were analogous to those for HPLC-DAD described above, using a solvent gradient with A – methanol and B – aqueous formic acid (0.3%, v/v). ESI negative ionization mode was used to identify and characterize the laccaic acids and the resinous compounds. The experimental conditions were optimized for the deprotonated molecule of laccaic acid A. The ESI voltage was −4.8 kV; the capillary voltage was 40 V and RF loading at 90%. Nitrogen was used as the

Table 2
Lac dye samples analyzed by HPLC-DAD and submitted to PCA analysis.

EBC[a]	EBC[a] description	Plant species (host plant)	Geography (country/location)	Composition type
43042	Lac	MORACEAE *Ficus benghalensis* L.	India/Rajputana (Rajasthan)	Mixture of *Lac dye* and resin
43050	Lac	MORACEAE *Ficus benghalensis* L.	India/Thallawar (Rajasthan)	Resin
43061	Lac	MORACEAE *Ficus rumphii* Blume	India/Kamrup (Assam)	Mixture of *Lac dye* and resin
43087	Lac	MORACEAE *Ficus religiosa* L.	India/Rajasthan	Mixture of *Lac dye* and resin
43108	Lac	MORACEAE *Ficus religiosa* L.	India/Ahmedabad (Gujarat)	Resin
43144	Twigs with lac exuding from them	MORACEAE *Ficus* sp.	Australia/Shark Bay	Resin
43148	Lac	MORACEAE	Laos	Mixture of *Lac dye* and resin
43149	Shellac	MORACEAE	?	Resin
43150	Lac Dye	MORACEAE	Indian subcontinent	Resin
43151	Shellac	MORACEAE	India/Mirzapur (Uttar Pradesh)	Resin
43152	Lac	MORACEAE	Bangladesh/Sylhet	Mixture of *Lac dye* and resin
43153	Shellac	MORACEAE	India/Mirzapur (Uttar Pradesh)	Resin
43154	Ground lac	MORACEAE	Indian subcontinent	Resin
43155	Lac Dye	MORACEAE	?	*Lac dye*
43160	Stick lac	MORACEAE *Ficus* sp.	Vietnam/Tonkin	Resin
43161	Stick Lac	MORACEAE *Ficus* sp.	India/Mysore (Karnataka)	Mixture of *Lac dye* and resin
43162	“Lac Lora” Stick Lac	MORACEAE *Ficus* sp.	India/Raipur (Chhattisgarh)	Mixture of *Lac dye* and resin
43164	Lac	MORACEAE *Ficus* sp.	Vietnam/Tonkin	Mixture of *Lac dye* and resin
43165	Stick Lac	MORACEAE *Ficus* sp.	Vietnam/Tonkin, Hanoi	Resin
43166	Stick Lac	MORACEAE *Ficus* sp.	Thailand	Mixture of *Lac dye* and resin
43167	Stick Lac	MORACEAE *Ficus* sp.	Vietnam/Tonkin	Mixture of *Lac dye* and resin
43172	Stick Lac	MORACEAE *Ficus* sp.	India/Assam	Mixture of *Lac dye* and resin
43173	Grain Lac	MORACEAE *Ficus* sp.	India/Calcutta (West Bengal)	Resin
43174	Seed Lac	MORACEAE *Ficus* sp.	Indian subcontinent	Resin
43175	Garnet Lac	MORACEAE *Ficus* sp.	India/Calcutta (West Bengal)	Resin
43179	Orange Stick Lac	MORACEAE *Ficus* sp.	India/Birbhum (West Bengal)	Resin
43180	Lac Dye	MORACEAE *Ficus* sp.	?	Mixture of *Lac dye* and resin
43181	Dark Seed Lac	MORACEAE *Ficus* sp.	?	Resin
43182	White lac	MORACEAE *Ficus* sp.	?	Resin
43183	Finest White Shellac	MORACEAE *Ficus* sp.	?	Resin
43185	Button Lac	MORACEAE *Ficus* sp.	India/Beerbhoom [Birbhum?], Elambagon District	Resin
43188	Seed Lac	MORACEAE *Ficus* sp.	India/Raipur (Chhattisgarh)	Resin
43189	Grain Lac	MORACEAE *Ficus* sp.	India/Visakhapatnam (Andhra Pradesh)	Resin
43191	Stick Lac	MORACEAE *Ficus* sp.	India/Raipur (Chhattisgarh)	Resin
43195	Finest stick lac	MORACEAE *Ficus* sp.	Singapore	Resin
43196	Lac Dye	MORACEAE *Ficus* sp.	Indian subcontinent	Mixture of *Lac dye* and resin
43218	Lac Dye	MORACEAE *Ficus* sp.	India/Malwa (Madhya Pradesh)	*Lac dye*
43230	Lac Dye	MORACEAE *Ficus* sp.	Indian subcontinent	Mixture of *Lac dye* and resin
60073	Crude Lac	FABACEAE *Butea monosperma* (Lam.) Taub.	India/Saharanpur (Uttar Pradesh)	Resin
60074	Lac	FABACEAE *Butea monosperma* (Lam.) Taub.	India/Lucknow (Uttar Pradesh)	Mixture of *Lac dye* and resin
60079	Lac	FABACEAE *Butea monosperma* (Lam.) Taub.	Indian subcontinent	Mixture of *Lac dye* and resin
60087	Stick lac	FABACEAE *Butea monosperma* (Lam.) Taub.	Indian subcontinent	Mixture of *Lac dye* and resin
62429	Stick Lac	SAPINDACEAE *Schleichera oleosa* (Lour.) Oken	Indian subcontinent	Resin
62433	Lac on branches	SAPINDACEAE *Schleichera oleosa* (Lour.) Oken	Sri Lanka	Resin
62437	Sticklac	SAPINDACEAE *Schleichera oleosa* (Lour.) Oken	Indian subcontinent	Mixture of *Lac dye* and resin
62462	Seed Lac	SAPINDACEAE	East Indies	Resin
62628	Lac	RHAMNACEAE *Ziziphus jujuba* Mill. var. *jujuba*	Pakistan/Karachi	*Lac dye*
62655	Lac formed on branches	RHAMNACEAE ?*Ziziphus* sp.	Indian subcontinent	Mixture of *Lac dye* and resin
67729	Lac sticks	FABACEAE *Butea monosperma* (Lam.) Taub.	Indian subcontinent	Mixture of *Lac dye* and resin
67730	Stick lac	SAPINDACEAE *Schleichera oleosa* (Lour.) Oken	Indian subcontinent	Mixture of *Lac dye* and resin
Phoenix	?	?	?	

[a] Economic Botany Collection, Royal Botanic Gardens, Kew (London).

nebulizing and drying gas at pressure of 45 and 15 psi, respectively; drying gas temperature 300 °C. The spectra were recorded in the range 100–1200 Da. Spectra typically correspond to the average of 35–40 scans. The MS/MS spectra were obtained with an isolation window of 2.0 Da, excitation energy values between 0.9 and 2.2 V, and an excitation time of 10 ms.

2.5. Multivariate analysis

The chromatograms obtained from the HPLC experiments on the Economic Botany Collection (EBC) samples (see Table 2) were analyzed by Principal Components Analysis (PCA). Each of the referenced EBC samples in the table corresponded to 3 samples that may vary in composition. The chromatograms were the result of HPLC analysis with UV detection at a fixed wavelength (275 nm), and only the retention time region $tr = 15–29$ min was considered to be used as variables for PCA, as the relevant chromophore peaks occur here. The data was mean-centered prior to analysis, with no further pre-processing being used for the model presented. Calculations were carried out using Matlab version 7.4 release 2007a (Mathworks, Natick, MA) and the PLS toolbox version 4.2.1 (Eigenvector Research, Manson, WA).

3. Results and discussions

3.1. Kerria and Paratarchadina genera – reference insect specimens

The oxalic acid method was selected for the extraction of lac-dye as it revealed better extraction results not only for lac dye but also for other related red insect dyes (cochineal) [27]. This is the first

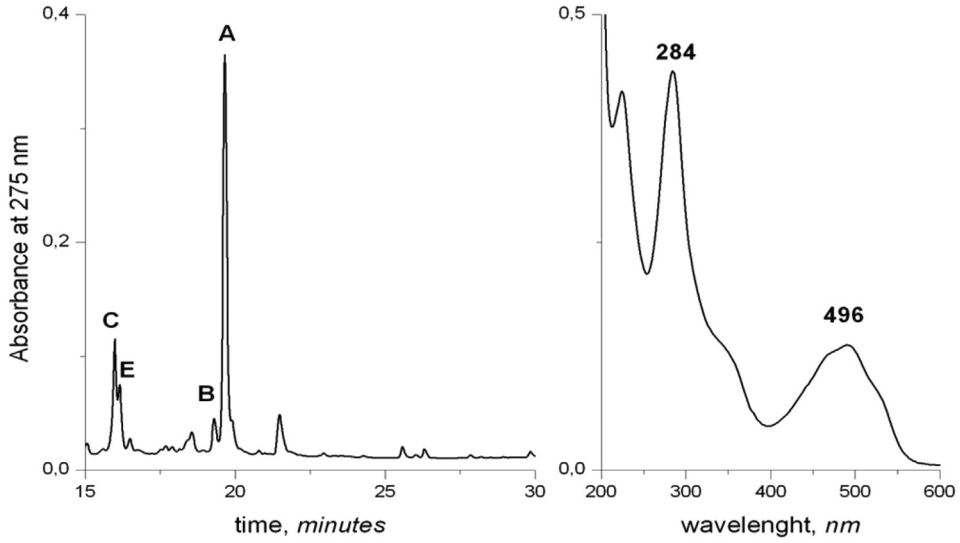


Fig. 2. HPLC-DAD chromatogram acquired at 275 nm of a red historical textile sample from PD77, were it is possible to identify: A) laccaic acid A (R_t = 19.63 min, λ_{max} = 496 nm); B) laccaic acid B (R_t = 19.28 min, λ_{max} = 490 nm); C) laccaic acid C (R_t = 15.97 min, λ_{max} = 493 nm) and E) laccaic acid E (R_t = 16.13 min, λ_{max} = 494 nm). All the red and pink samples analyzed have a similar elution profile to this chromatogram.

time, HPLC-DAD analysis has been conducted on specimens of *Paratachardina*, but previous data are available for *Kerria* [40,42,44]. The two genera exhibit distinct elution profiles when extracted with water, or with methanol (Table 1). LC-MS analysis of the water extracts from specimens of *Paratachardina* identified laccaic acid B as the major red chromophore. The tandem mass spectra obtained for the deprotonated molecule of this laccaic acid are in agreement with literature [25,44].

The red chromophores detected in the extracts of samples of *Kerria* were identified as laccaic acid A (R_t = 20.10 min, λ_{max} = 496 nm, $[M-H]^-$ = 536), laccaic acid B (R_t = 19.72 λ_{max} = 490 nm, $[M-H]^-$ = 495), laccaic acid C (R_t = 16.37 min, λ_{max} = 494 nm, $[M-H]^-$ = 538) and laccaic acid E (R_t = 16.64 min, λ_{max} = 494 nm, $[M-H]^-$ = 494) [44].

The presence of the red laccaic acids A, C and E can be used to differentiate the two sources as they are only detected in extracts of *Kerria*. Hence, only *Kerria* has sufficient red-dye chromophores to be used successfully as a dye for textiles. Furthermore, methanolic extracts of *Kerria* and *Parathacardina* insect specimens revealed different HPLC-DAD chromatograms, enabling their distinction. The LC-MS analysis of resins from insect specimens from both genera revealed the presence of aleuritic acid and sesquiterpenic acid units as reported in the literature [18,19,22,25]. However, *Kerria* specimens contained compounds with *m/z* 827 (peaks **1** and **2**), whereas in the *Paratachardina* specimens the major compound (peaks **2** and **3**) had a deprotonated ion with *m/z* 567 (Table 1). The extracted ion chromatograms and the tandem mass spectra of the major peaks identified in the *Kerria* and *Paratachardina* methanolic extracts, respectively Figures S2 and S3, are given as supporting information.

The mass spectrometric data for compounds **1** and **2** in *Kerria* resins (Figure S2) pointed to a mixture of an aleuritic acid unit with two terpenic acids (jalaric and laccijalaric units) bound by ester linkages. The MS^2 spectrum of compound **1** contained two peaks at *m/z* 565 and 303, respectively, formed through elimination of a residue of one jalaric acid (262 u) and a residue of one laccijalaric acid linked to a jalaric acid unit (524 u). The MS^2 spectrum of compound **2** showed two fairly intense peaks at *m/z* 565 and 549 indicating that each terpenic acid unit is connected to the aleuretic acid by ester bonds. The minor peaks **3** and **4** were assigned to a unit of aleuretic acid linked to a jalaric acid (*m/z* 565) or to a shellolic acid (*m/z* 581), respectively.

The extracted ion chromatogram of *m/z* 567 from *Paratachardina* resins (Figure S3) presented two peaks (**2** and **3**), indicating the presence of isomeric structures. The MS^2 spectrum of compound **2** yielded a base peak at *m/z* 281, whereas compound **3** produced *m/z* 303, as a major product ion. These results were assigned to laksholic and aleuritic acid units with different positions for their ester linkages. The peak **1** was attributed to an aleuretic acid linked to a laccilaksholic unit.

These results indicate that the major sesquiterpenic components of the *Kerria* soft resin are jalaric, laccijalaric and shellolic acids, whereas those of *Paratachardina* are laksholic and laccilaksholic acid units. The results reported herein are in agreement with those reported for fresh and aged shellac from *Kerria* genera fully characterized by GC–MS procedures [17,18]. However, further studies using high-resolution mass measurements and comparison with pure compounds, when available, will provide a more conclusive characterization of both resins by LC-MS methodologies.

3.2. Lac-dye reference database – historical insect specimens

Although the extracts of the 76 historical lac-dye specimens from the Economic Botany Collection (Table 2) displayed heterogeneous chromatographic profiles it was possible to separate the samples in three groups according to their composition after HPLC-DAD and PCA analysis. A PCA model using two principal components, captured approximately 89% of the total variance (82.75% and 6.81% corresponding to PC1 and PC2, respectively), and the principal components were observed to be able to capture the information corresponding to pigment and resin separately. The concentration of pigment and resin was observed to nearly be linearly independent for the dataset analyzed. No clear separation was obtained to distinguish groups according to differences in sample composition. This was not due to a lack of ability of PCA to discriminate compositions, but directly related to the sample compositions themselves. The pigment and resin samples have very disperse concentration distributions which inhibit clear separation, although some higher density scores are observed both for PC1 and PC2. (For further details about PCA, as well as PC1 vs PC2 and loadings plots, please see Supplementary Material). Group I is comprised of samples rich in laccaic acids that were designated as lac-dye (30%); Group II samples contained a mixture of laccaic acids

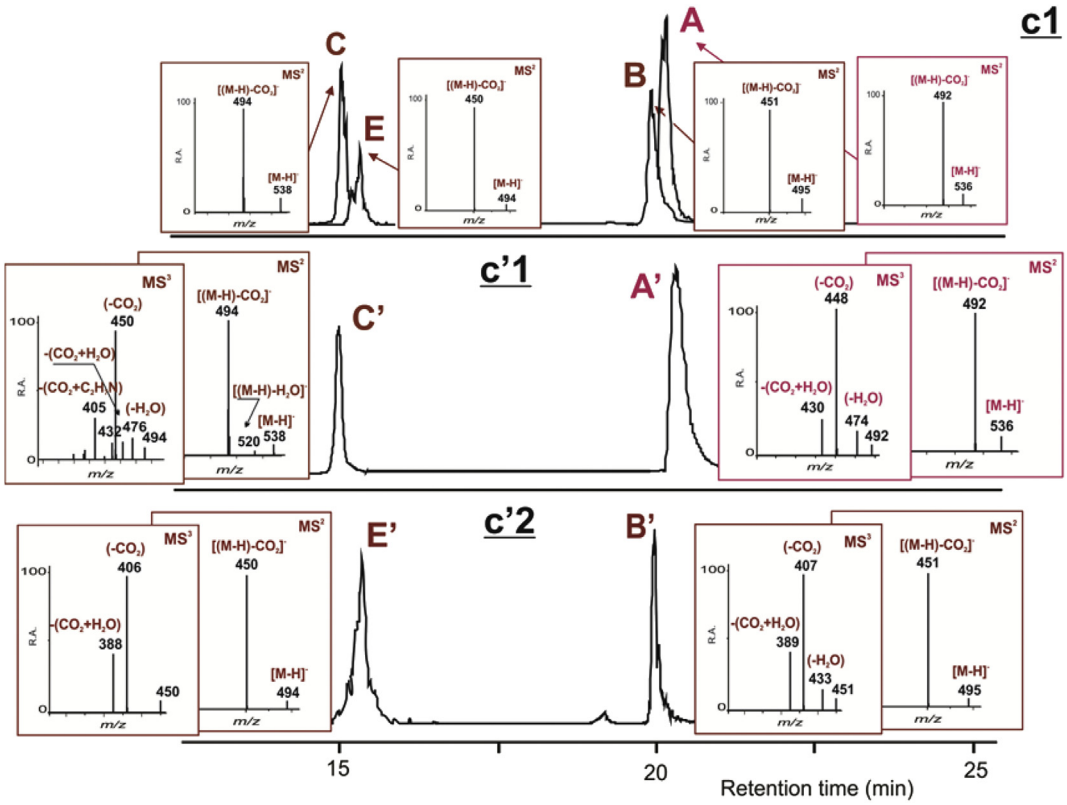


Fig. 3. Analysis of a red-dye extract from PD77 and comparison with an extract of *Kerria* sp. insect. (c1) HPLC-ESI/MS^2 chromatograms of the red extract from PD77 and product ion spectra of the *m/z* 536 (A) 495 (B), 538 (C) and 495 (E) ions. (c'1) and (c'2) HPLC-ESI/MS^n chromatograms of the *Kerria* sp. insect and product ion mass spectra of *m/z* 536, 538, 495 and 494 ions of the laccaic acid A, laccaic acid C, laccaic acid B and laccaic acid E, respectively.

and terpenic acids from the resinous lac matter (referred to here as a mixture of lac-dye and resin) (29%); and Group III samples contained the yellowish terpenic acid compounds from the resinous lac matter (referred to as resin) (41%). The groupings of samples resulting from the PCA analysis of the chemical data have been added to the column labeled 'composition type' in Table 2.

This information would appear to indicate that the historical descriptions of the samples in the Economic Botany Collection (*sticklac*, 34%; *shellac*, 12%; *grain/seed lac*, 14%; *lac*, 12% and other types, 20%) are correct, as it was possible to identify the principal components of lac-dye, or the compounds of resinous lac, in all the samples analyzed.

However, it was not possible to discriminate the samples by geographic region or host plant. No molecular taxonomic studies exist for *Kerria* species, in contrast to *Paratachardina* [13]. If this data was available it would be possible to develop a more sophisticated database capable of characterizing the precise insect species used in historical textiles (and their provenance) as has occurred, for example, with cochineal red dyes [27].

3.3. Identification of lac-dye in historical carpets

LC-MS analyses of samples from the three Guimarães carpets revealed the presence of laccaic acids (Fig. 2), the main chromophores of lac-dye. Besides laccaic acid A (R_t = 20.10 min, λ_{max} = 496 nm, $[M-H]^-$ = 536), usually the major compound, laccaic acids B (R_t = 19.72 min, λ_{max} = 490 nm, $[M-H]^-$ = 495), C (R_t = 16.37 min, λ_{max} = 494 nm, $[M-H]^-$ = 538) and E (R_t = 16.64 min, λ_{max} = 494 nm, $[M-H]^-$ = 494) were also identified by comparison of HPLC-ESI-MS^2 patterns with those of the corresponding laccaic acids present in *Kerria* spp. [44], as shown in Fig. 3.

The use of lac-dye in the reds and pinks is consistent with other results for carpets associated with Iran in Portuguese collections [45,46]. Indeed, the characteristic molecular ions of alizarin (*m/z* 240) and purpurin (*m/z* 256) associated with madder [47], and usually present in the reds of Turkish carpets, have only been found in extracts from the orange samples taken from the three Guimarães rugs and other Iranian carpets [28,45]. Together, these results suggest that this palette (lac-dye for reds and pinks, and madder with a yellow dye for oranges) is a standard characteristic of classical Iranian dyeing practices and can serve as an indication of the provenance of precious historical textiles.

Although, it was not possible to discriminate the insect and historical lac-dye specimens according to their species or regional provenance to make a more precise attribution of the dye source, this work reinforces the importance of using taxonomical-verified specimens of *Kerria* previously submitted to molecular taxonomic studies to build a sophisticated lac-dye database in order to identify the species of lac in historical textiles, as well as the provenance of the insect used [27].

4. Conclusions

The reference database confirmed that reds and pinks in the Guimarães carpets were achieved using lac-dye; reinforcing the notion that lac-dye (and not madder) typically belongs to classical Iranian (and not Turkish) dyeing practices. Together with previous data on carpets associated with Iran in Portuguese collections and historical documentation, these results represent a significant contribution to questions concerning the origin and date of the 'Salting' group.

The results obtained in this work indicate that the two most important genera that produce lac-dye: *Kerria* and *Paratachardina*,

are easily distinguished by HPLC-DAD-MS analysis; *Paratachardina* specimens have a very low content of red laccaic acids, which explains their exclusion as a commercial source of red dye for textiles. These results also confirmed the composition of historical lac-dye specimens from the Economic Botany Collection, Royal Botanic Gardens, Kew. The PCA score plot suggests that these specimens can be discriminated according to composition. The pigment and resin components are essentially separated in the two first principal components, which allows for a separation basis for this type of data. The concentration of both components is observed to be essentially uncorrelated in the dataset available. A more comprehensive dataset, with a larger number of samples for each reference would allow us to further pursue a clear differentiation of samples by origin.

Further development of the database obtained in this work is required to establish more rigorous comparisons between historical textiles and lac-insect sources. Hence, for *Kerria* the next phase will require the collaboration of entomologists, and taxonomical identification of reliable *Kerria* insect specimens with well-documented information concerning their precise origin as well as their precise host plants.

Acknowledgments

We would like to thank Paula Cruz and Cátia Frade from DGPC – Laboratório José de Figueiredo (Lisbon) for granting access to the Guimarães carpets; Douglas Miller (Agricultural Research Service, Systematic Entomology Laboratory, Maryland), Dominique Cardon (CIHAM/UMR, Lyon), Penny Gullan (University of Florida) for kindly providing insect specimens for analysis. The Fundação para a Ciência e Tecnologia (SFRH/BD/72882/2010) and FEDER (POCI/QUI/099388/2008, REM 2013 and PEst-OE/QUI/UI0100/2013) provided funding for this research. J. Sarraguça would also like to acknowledge FCT for the grant SFRH/BPD/46319/2008 co-financed by QREN-POPH.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dyepig.2015.02.024.

References

[1] Ferreira ES, Hulme AN, McNab H, Quye A. The natural constituents of historical textiles dyes. Chem Sci Rev 2004;33:329–36.
[2] Rosenberg E. Characterisation of historical organic dyestuffs by liquid chromatography-mass spectrometry. Anal Bioanal Chem 2008;391:33–57.
[3] Petroviciu I, Albu F, Medvedovici A. LC/MS and LC/MS/MS based protocol for identification of dyes in historic textiles. Microchem J 2010;95:7–254.
[4] Colombini MP, Andreotti A, Baraldi C, Degano I, Lucejko JJ. Colour fading in textiles: a model study on the decomposition of natural dyes. Microchem J 2007;85:174–82.
[5] Gulmini M, Idone A, Diana E, Gastaldi D, Vaudan D, Aceto M. Identification of dyestuff in historical textiles: strong and weak points of a non-invasive approach. Dyes Pigments 2013;98:136–45.
[6] Valianou L, Karapanagiotis I, Chryssoulakis Y. Comparison of extraction methods for the analysis of natural dyes in historical textiles by high performance liquid chromatography. Anal Bioanal Chem 2009;395:2175–89.
[7] Lech K, Jarosz M. Novel methodology for the extraction and identification of natural dyestuffs in historical textiles by HPLC–UV–Vis–ESI MS. Case study: chasubles from the Wawel Cathedral collection. Anal Bioanal Chem 2011;399:31–3251.
[8] Wouters J. High performance liquid chromatography of antraquinones: analysis of plant and insect extracts and dyed textiles. Stud Conserv 1985;30:119–28.
[9] Wouters J, Verhecken A. The coccid insect dyes: HPLC and computerized diode-array analysis. Stud Conserv 1989;34:189–200.
[10] Zhang X, Laursen R. Development of mild extraction methods for the analysis of natural dyes in textiles of historical interest using LC-diode array detector-MS. Anal Chem 2005;77:2022–5.
[11] Marques R, Sousa MM, Oliveira MC, Melo MJ. Characterization of weld (*Reseda Luteola* L.) and spurge flax (*Daphne gnidium* L.) by high performance liquid chromatography-diode array detection-mass spectrometry in Arraiolos historical textiles. J Chrom A 2009;1216:1395–402.
[12] Gullan PJ, Kondo T. The morphology of lac insects (Hemiptera: Coccoidea: *Kerriidae*). In: Proceedings of the XI international symposium of scale insect studies; 2007. p. 63–70.
[13] Kondo T, Gullan P. Taxonomic review of the lac insect genus *Paratachardina* Balachowsky (Hemiptera: Coccoidea: *Kerriidae*), with a revised key to genera of *Kerriidae* and description of two new species. Zootaxa 2007:1–41.
[14] United States Department of Agriculture – Systematic Entomology Laboratory. http://www.sel.barc.usda.gov/catalogs/kerriida/KerriaAll.htm. [accessed 22.08.13].
[15] Cardon D. Natural dyes: sources, tradition, technology and science. London: Archetype Publications; 2007.
[16] Sharma KK, Jaiswal AK, Kumar KK. Role of lac culture in biodiversity conservation: issues at stake and conservation strategy. Curr Sci 2006;91:894–8.
[17] Wang L, Ishida Y, Ohtani H, Tsuge S. Characterization of natural resin shellac by reactive pyrolysis – gas chromatography in the presence of organic alkali. Anal Chem 1999;71:1316–22.
[18] Colombini MP, Bonadue I, Gautier G. Molecular pattern recognition of fresh and aged shellac. Chromagraphie 2003;58:357–64.
[19] Khurana RG, Singh AN, Upadhye AB, Mhaskar VV, Dev S. Chemistry of lac resin III: an integrated procedure for their isolation from hard resin; chromatography characteristics and quantitative determination. Tetrahedron 1970;26:4167–75.
[20] Watt G. The commercial products of India. London: Hazell Watson and Valey Ltd; 1908.
[21] Khurana RG, Singh AN, Upadhye AB, Mhaskar VV, Dev S. Chemistry of lac resin I: butolic, jalaric and laksholic acids. Tetrahedron 1969;25:3841–54.
[22] Khurana RG, Singh AN, Upadhye AB, Mhaskar VV, Dev S. Chemistry of lac resin IV: isolation and quantitative determination of constituent acids. Tetrahedron 1970;26:4177–87.
[23] Brown KS. The chemistry of aphids and scale insects. Chem Soc Rev 1975;4:263–88.
[24] Hofenk de Graaff JH. The colourful past: the origins, chemistry and identification of natural dyestuffs. London: Abbeg-Stiftung & Archetype Publications Ltd; 2004.
[25] Oka H, Ito Y, Yamada S, Kagami T, Hayakawa J, Harada K, et al. Separation of lac dye components by high-speed counter-current chromatography. J Chrom A 1998;813:71–7.
[26] Phipps E, Cochineal Red. The art history of a color. The metropolitan museum of art. New York: Yale University Press; 2010. New Haven and London.
[27] Serrano A, Sousa MM, Hallett J, Lopes JA, Oliveira MC. A new methodology for the analysis of natural red dyes (Cochineal) in textiles of historical importance using high performance liquid chromatography with diode array detector and multivariate data analysis. Anal Bioanal Chem 2011;401:735–43.
[28] Santos R. Three 'Salting' carpets discovered in the Palace of the Dukes of Braganza. Master's Thesis. Lisbon: Faculdade de Ciências e Tecnologias, Universidade Nova de Lisboa; 2011.
[29] Petroviciu I, Cretu I, Vanden Berghe I, Wouters J, Medvedovici A, Albu F, et al. A discussion on the red anthraquinone dyes detected in historic textiles from Romanian Collections. e-PS 2012;9:90–6.
[30] Frade C, Cruz P, Lopes E, Sousa MM, Hallett J, Santos R, et al. Cleaning classical Persian carpets with silk and precious metal thread: conservation and ethical considerations. In: Proceedings of ICOM-CC 16th Triennial Conference, Lisbon; 2011. p. 283–92.
[31] Franses M. Salting carpets. California: oriental carpet and textile studies. 1999.
[32] Enez N. Dye research on the prayer rugs of Topkapi collection. California; oriental carpet and textile studies. 1993.
[33] Böhmer H, Enez N. A Topkapi prayer rug: arguments for a Persian origin. Hali 1988;41:11–3.
[34] Sousa MM, Melo MJ, Parola AJ. A study in mauve: unveiling Perkin's dye in historic samples. Chem Eur J 2008;14:8507–13.
[35] Donkin RA. The insect dyes of Western and West-Central Asia. Anthropos Int Rev Ethnol Linguist 1977;72:864–5.
[36] Wouters J, Verhecken A. Potential taxonomic applications of HPLC analyses of Coccoidea pigments (Homoptera: Sternorhyncha). Belg J Zool 1991;121:211–25.
[37] Sousa MM, Melo MJ, Parola AJ, Melo JS, Catarino F, Pina F, et al. Flavylium chromophores as species markers for Dragon's Blood resins from *Dracaena* and *Daemonorops* Trees. J Chrom A 2008;1209:153–61.
[38] Mouri C, Laursen R. Identification of anthraquinone markers in textiles and fibers dyed with madder *(Rubia spp.)* using liquid chromatography—tandem photodiode array and mass spectrometric detection. Microchim Acta 2012;179:105–13.
[39] Mouri C, Mozaffarian V, Zhang X, Laursen R. Characterization of flavonols in plants used for textile dyeing and the significance of flavonol conjugates. Dyes Pigments 2014;100:135–41.
[40] Wouters J, Verhecken A. The scale insect (Homoptera: Coccoidea) species recognition by HPLC and diode-array analysis of the dyestuffs. Ann Soc Ent Fr 1989;25:393–410.
[41] Halpine SM. An improve dye and lake pigment analysis method for high-performance liquid chromatography and diode-array detector. Stud Conserv 1996;41:76–94.
[42] Saito M, Hayashi A, Kojima M. Identification of six natural red dyes by high-performance liquid chromatography. Dyes Hist Archaeol 2003;19:79–89.
[43] Zhang X. Identification of yellow dye types in pre-colombian Andean textiles. Anal Chem 2007;79:1575–82.

[44] Szostek B, Orska-Gawrys J, Surowiec I, Trojanowicz M. Investigation of natural dyes occurring in historical Coptic textiles by high-performance liquid chromatography with UV-VIs and mass spectrometric detection. J Chrom A 2003;1012:179–92.

[45] Armindo E, Sousa MM, Hallett J, Melo MJ. A Persian carpet's paradise garden: discovering historical and technical aspects through carpet conservation and restoration. In: Proceedings of ICOM-CC 15th Triennial Conference, New Delhi; 2008. p. 824–31.

[46] Heitor M, Sousa M, Melo MJ, Hallett J, Oliveira MC. The colours of the carpets. In: Hallett J, Pereira TP, editors. The Oriental Carpet in Portugal: carpets and paintings 15th–18th centuries, exhibition catalogue. Lisbon: Museu Nacional de Arte Antiga; 2007. p. 161–8.

[47] Manhita A, Ferreira V, Vargas H, Ribeiro I, Candeias A, Teixeira D, et al. Enlightening the influence of mordant, dyeing technique and photodegradation on the colour hue of textiles dyed with madder – a chromatographic and spectrometric approach. Microchem J 2011;98:82–90.