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IDENTIFICATION OF COCHINEAL AND OTHER DYES IN BYZANTINE TEXTILES OF THE 14TH CENTURY FROM MOUNT ATHOS

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ABSTRACT

Samples from ecclesiastical textiles (epitaphioi) which date in the byzantine period (14th century) and belong to monasteries of Mount Athos, are investigated using High Performance Liquid Chromatography coupled to a Photodiode Array Detector (HPLC-PDA). Prior to HPLC analysis, the historic samples are treated following a two step method, which was recently devised for dyestuff extraction: samples are first treated with hot dimethyl sulfoxide (DMSO) followed by mild acid (trifluoroacetic acid, TFA) hydrolysis.
For the first time the use of cochineal in a byzantine textile is reported herein. According to the HPLC semi-quantitative results the cochineal dye was originated probably from *Porphyrophora polonica* L. (Polish cochineal) insects. Weld (*Reseda luteola* L.) is identified in the historic samples by detecting aglycone and glycoside components of the yellow dye, thus demonstrating that compounds which are destroyed under harsh acidic conditions (glycosides) can be recovered by the suggested two step dyestuff extraction method. Finally, kermes (*Kermes vermilio* Planchon), indigoid dyes which can be either indigo (*Indigofera tinctoria* L. and others) or woad (*Isatis tinctoria* L.), and tannins are found in the samples.

KEYWORDS: cochineal, weld, indigo, textile, byzantine, Mount Athos, *Porphyrophora polonica L.*

1. INTRODUCTION

Textiles which date in the byzantine period, that is before the conquest of Constantinople by the Ottomans (1453 AD), are very rare. Some byzantine textiles have been preserved in the monasteries of Mount Athos which is the eastern peninsula of the area of Halkidiki, Greece. Mount Athos has been an important religious spiritual centre of the Eastern Church since 1054 and has enjoyed an autonomous status since byzantine times. Today, the "Holy Mountain" is still a major centre of the Christian religion with over 20 large, occupied and active monasteries, which according to UNESCO are considered to have outstanding universal value.

The vast majority of the religious textiles, which can be found in the monasteries of Mount Athos, date in the postbyzantine period, ranged from the middle of the 15th century onwards. Forty six (46) ecclesiastical and historic postbyzantine textiles from Mount Athos (Karydis. 2010; Karydis, 2014) have been recently subjected to dyestuff analysis using High Performance Liquid Chromatography coupled to a Photodiode Array Detector (HPLC-PDA) (Karapanagiotis and Karadag 2015; Karapanagiotis et al. 2008; Karapanagiotis et al. 2011; Mantzouris et al. 2011). HPLC is the most reliable and widely used method for the analysis and identification of natural dyes (Hofenk de Graaff 2004). However, none of the rare byzantine textiles of Mount Athos was previously investigated by HPLC.

For the first time, the biological origins of the colouring materials contained in Athonian textiles of the byzantine period are revealed, herein. Samples, removed from three epitaphioi which are liturgical textiles used in the Good Friday service according to the tradition of the Eastern Church, are investigated using HPLC-PDA. The three objects are shown in Figure 1. They are of the 14th century and are among the most precious objects of Mount Athos (Karydis 2014). Interestingly, the unexpected identification of cochineal, which was probably originated from *Porphyrophora polonica* L. insects, is reported in the HPLC results of the historic samples.

Dyestuff extraction is achieved using a newly devised two step method (van Bommel 2014): the sample is treated with warm dimethyl sulfoxide (DMSO) to extract direct and vat dyes. The remaining solid is then separated and subjected to hydrolysis under mild acidic conditions, induced by trifluoroacetic acid (TFA), to extract mordant dyes. As reported previously, DMSO provides good yields in the extraction of Tyrian purple (Karapanagiotis et al. 2013b), indigo and safflower (Mantzouris et al. 2014). TFA is a good reagent for the extraction of mordant dyes as is provides good yields preserving simultaneously the flavonoid glycosides (Valianou et al. 2009). Extraction of dyes using TFA from historic textile samples has been previously reported (Karapanagiotis et al. 2011; Mantzouris et al. 2011; Valianou et al. 2009). Treatment of the sample using a mild acid is important to preserve the glycosides (Marques et al. 2009; Valianou et al. 2009; Zhang and Laursen 2005) which are destroyed when harsh acidic conditions, such as HCl (Wouters 1985), are applied for dyestuff extraction. Treatment of the sample with DMSO prior to acid hydrolysis is a safe route to extract direct and vat dyes which could be sensitive to acidic conditions (Degano et al. 2011; Joosten and van Bommel 2008; Serrano et al. 2015). The applied method was recently developed and evaluated using dyed yarns which were prepared following traditional recipes (van Bommel 2014). The method is used herein for the analysis of the historic samples.

Figure 1. Epitaphios from the Vatopediou monastery (object A), Xeropotamou monastery (object B) and Stavronikita monastery (object C), Mount Athos.

2. EXPERIMENTAL

2.1. Objects and samples

The three epitaphioi included in the study are shown in Figure 1 and are preserved in the monasteries of Vatopediou (object A), Xeropotamou (object B) and Stavronikita (object C) which are located in Mount Athos. The three religious textiles date in the 14th century, although object C was subjected to later interventions (15th/16th century). Nine samples were removed from already damaged areas (ground fabric and embroidery decoration) of the original objects and studied using HPLC-PDA. Microphotographs of three samples are provided in Figure 2 as examples.

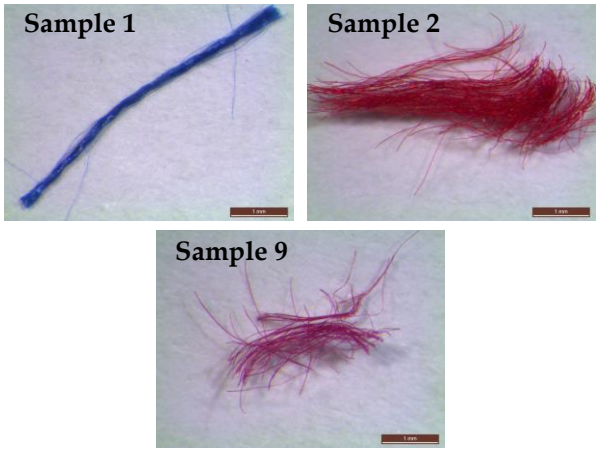

Figure 2. Microphotographs of three samples labelled later samples 1, 2 and 9. The scale bar is 1 mm.

2.2. Materials

The following solvents and chemicals were used for chromatography and sample treatment process: HPLC-grade water (Chem-Lab NV, Belgium), HPLC-grade acetonitrile and methanol (CH_3CN and MeOH) (J.T. Baker, USA), HPLC-grade dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA) and trifluoroacetic acid (TFA) (Riedel-de Haën, Germany) of 99% purity.

2.3. Sample treatment method

Samples were treated according to a recently devised two step dyestuff extraction method (van Bommel 2014) as follows:

Step 1: Sample was first immersed in a DMSO bath (200μl) heated at 80°C for 10 min. These conditions were previously optimized for the extraction of vat and direct dyes such as Tyrian purple (Karapanagiotis et al. 2013b), indigo and safflower (Mantzouris et al. 2014). The liquid phase was then transferred to another vial.

Step 2: 200 μl of H_2O:MeOH:0.5M TFA (1:1:2, v/v/v) were added to the remaining sample. The acidic bath with the remaining sample was introduced in a preheated bath at 65°C and left it for 5 min. The liquid phase was then evaporated under gentle nitrogen flow until the sample was dried completely. Different conditions, such acid concentration and treatment temperature, were used in the original TFA dyestuff extraction method (Valianou et al. 2009).

Finally, the DMSO extract obtained in step 1 was added to the dried sample (step 2). A mixture of 200 μl of H_2O:MeOH (1:1 v/v) was added, centrifuged and subjected to HPLC.

2.4. HPLC instrumentation

The HPLC–PDA system (Thermoquest, USA) consisted of a 4000 quaternary HPLC pump, a SCM 3000 vacuum degasser, an AS3000 autosampler with a column oven, a Rheodyne 7725i injector with a 20 μl sample loop, and a UV 6000LP photodiode array detector. Analyses were carried out with an Alltima C18 (Alltech Associates, USA) column (5 μm particle size, 250 mm x 3.0 mm) at a stable temperature of 35°C. For gradient elution, two solvents were used, consisting of (A) 0.1% (v/v) TFA in water and (B) 0.1% (v/v) TFA in acetonitrile (Karapanagiotis et al. 2008). Data were received and analysed using the Xcalibur software (Thermoquest).

3. RESULTS AND DISCUSSION

The compounds detected in the sample extracts are described in Table 1. The UV-Vis absorbance maxima of the compounds used for identification purposes are included in Table 1. These compounds were identified in the historic samples, according to the results summarised in Table 2.

Table 1. UV-Vis absorbance maxima of compounds which were detected in the historic samples by HPLC. The 2-C-α/β-glucofuranoside of kermesic acid correspond to the widely known dcIV and dcVII cochineal components, respectively (Stathopoulou et al. 2013).

Compound	Absorbance maxima (nm)
Apigenin	221, 267, 337
Apigenin-7-O-glucoside	221, 267, 337
Carminic acid	223, 275, 309, 493
2-C-α-glucofuranoside of kermesic acid	225, 275, 311, 487
2-C-β-glucofuranoside of kermesic acid	223, 277, 309, 487
Ellagic acid	213, 253, 367
Flavokermesic acid	223, 283, 343, 431
Indigotin	215, 241, 285, 330, 605
Indirubin	217, 239, 289, 363, 539
Kermesic acid	223, 273, 307, 489
Luteolin	223, 253, 265, 345
Luteolin-7-O-glucoside	221, 253, 265, 345, 353
Luteolin-3',7-di-O-glucoside	223, 239, 267, 341

Table 2. Compounds detected in the historic samples by HPLC and identified dyes.

Sample No	Sample description	Compounds detected by HPLC	Identified dyes
Epitaphios from the Vatopediou monastery, Mount Athos (object A)			
1	Blue	Indigotin, Indirubin	Indigo/Woad
2	Red	Keremesic acid, Flavokermesic acid, Indigotin	Kermes, Indigo/Woad
3	Green	Luteolin-3',7-di-O glucoside, Luteolin-7-O-glucoside, Apigenin-7-O-glucoside, Luteolin, Apigenin, Indigotin, Indirubin	Weld, Indigo/Woad
Epitaphios from the Xeropotamou monastery, Mount Athos (object B)			
4	Red	Kermesic acid, Flavokermesic acid, Indigotin (trace)	Kermes, Indigo/Woad
5	Blue	Indigotin, Indirubin	Indigo/Woad
6	Orange	Luteolin-3',7-di-O glucoside (trace), Luteolin-7-O-glucoside (trace), Apigenin-7-O-glucoside (trace), Indigotin, Indirubin	Weld, Indigo/Woad
7	Red	Kermesic acid, Flavokermesic acid, Indigotin (trace)	Kermes, Indigo/Woad
Epitaphios from the Stavronikita monastery, Mount Athos (object C)			
8	Yellow	Luteolin-3',7-di-O glucoside, Luteolin-7-O-glucoside, Apigenin-7-O-glucoside, Luteolin, Apigenin	Weld
9	Red	Unknown, Carminic acid, Ellagic acid*, 2-C-α-glucofuranoside of kermesic acid, 2-C-β-glucofuranoside of kermesic acid, Kermesic acid, Flavokermesic acid	Cochineal

*Ellagic acid is contained in tannins.

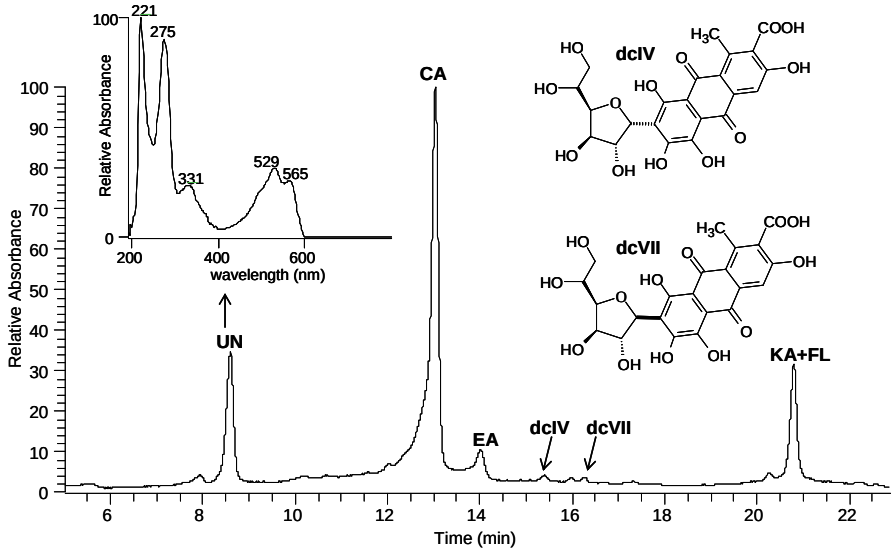

Figure 3. Chromatogram acquired at 275 nm for sample 9. Carminic acid (CA), the marker compound for the identification of cochineal, is detected. Kermesic (KA) and flavokermesic (FL) acids co-elute in our chromatographic system. The relative peak areas which are measured in the graph are: CA (80%) and KA+FL (20%). Other components of cochineal are 2-C-α-glucofuranoside of kermesic acid (dcIV), 2-C-β-glucofuranoside of kermesic acid (dcVII) and an unknown (UN) compound. The structures of the two glucofuranosides and the absorption spectrum of the UN are included. Finally, ellagic acid (EA) is detected in the chromatogram.

Coccid dyes obtained from scale insects are included in the results of Table 2. In particular, kermes (*Kermes vermilio* Planchon) was identified in samples 2, 4 and 7 removed from textiles A and B and cochineal was found in sample 9 which was part of object C. In the byzantine period, ranged up to the conquest of Constantinople by the Ottomans (1453 AD) kermes was the most important coccid dye. After the discovery of the New World (1492 AD) kermes was almost entirely replaced by Mexican cochineal (*Dactylopius coccus* Costa and other) which was imported in the European continent in large quantities (Cardon 2007). The earliest date for the cochineal's introduction into Europe is given as 1518 (Schweppe and Roosen-Runge 1997). Indeed, kermes was not detected in any of the 126 samples which were removed

from 46 postbyzantine (16th – early 20th) textiles from Mount Athos and were previously analysed by HPLC (Karapanagiotis and Karadag 2015; Karapanagiotis et al. 2008; Karapanagiotis et al. 2011; Mantzouris et al. 2011). Likewise, s sharp chronological boundary for the use of kermes was assessed for icons of the Cretan School of iconography (Karapanagiotis et al. 2013a). It was shown that kermes was used exclusively only in icons which date before the first half of the 16th century (Karapanagiotis et al. 2013a). According to the above, the identification of kermes in the two byzantine textiles (A and B) is supported by the previously published historical and chemical reports.

Consequently, the identification of cochineal in sample 9 (Table 2) removed from object C is a very interesting result. The HPLC chromatogram collected at 275 nm for sample 9 is shown in Figure 3. This is the first chemical report which proves the use of cochineal in an object which dates in the late byzantine period. Apparently, the coccid dye must have been prepared with cochineal scale insects that are native to Eurasia, such as *Porphyrophora hamelii* Brandt (Armenian cochineal) and *Porphyrophora polonica* L. (Polish cochineal). The source of a Eurasian cochineal dye can be identified if we take into account the HPLC peak areas of 2-C-glucopyranoside of flavokermesic acid, carminic acid, kermesic acid and flavokermesic acid, according to the results summarised in Table 3 (Wouters and Verhecken 1989).

Table 3. Characterisation of Armenian and Polish cochineal according to HPLC (Wouters and Verhecken 1989). The 2-C-glucopyranoside of flavokermesic acid corresponds to the widely known dcII cochineal component (Stathopoulou et al. 2013).

Cochineal	Relative (%) composition measured by the integrated HPLC peak areas at 275 nm
Armenian cochineal	2-C-glucopyranoside of flavokermesic acid (0.1-1.2) Carminic acid (95-99) Kermesic+Flavokermesic acid (1.0-4.2)
Polish cochineal	2-C-glucopyranoside of flavokermesic acid (trace) Carminic acid (62-88) Kermesic+Flavokermesic acid (12-38)

Figure 3 shows that the 2-C-glucopyranoside of flavokermesic acid was not detected in the chromatogram of sample 9. Moreover, relatively high amount of kermesic and flavokermesic acid is revealed compared to that of carminic acid. Based on the HPLC profile of Figure 3 it is measured that the % HPLC peak areas for the three aforementioned components of the coccid dye are as follows: kermesic+flavokermesic acid corresponds to 20 and carminic acid to 80. These results suggest that sample 9 must have been prepared with Polish cochineal (*Porphyrophora polonica* L.), according to the data of Table 3.

It should be noted, however, that the discussion becomes too complicated if we consider the possibility of having a mixture of coccid dyes in sample 9. For example, a mixture of kermes (rich in kermesic and flavokermesic acid) and Armenian cochineal (rich in carminic acid) in appropriate proportions may result in the relative composition reported in the chromatogram of Figure 3. The possibility of having a mixture of coccid dyes in sample 9 cannot be excluded but, in principle, is less likely than having a single insect source for dyeing purposes.

In the chromatogram of Figure 3 two more components of cochineal, which are the dcIV and dcVII compounds, are detected. For decades their structures have been unknown. NMR and LC-MS studies revealed recently that dcIV and dcVII are the 2-C-α/β-glucofuranosides of kermesic acid (Stathopoulou et al. 2013), as shown in Figure 3.

An unknown compound (labelled UN) is recorded in the graph of Figure 3. The compound gives a very characteristic absorption spectrum which is included in Figure 3. Interestingly, the same compound was reported in a recently published HPLC study where Armenian cochineal was found in Hellenistic funeral figurines (Fostiridou et al. 2015; Mantzouris and Karapanagiotis 2015). The UN compound could have originated from either within the insect source or it might have been formed during the dyeing process.

Finally, the detection of ellagic acid in sample 9 (Figure 3) suggests the use of a tannin product during dyeing. Tannins have been used both as dyes to induce dark-black hues in textiles and as dyestuff adhesives (Cardon 2007).

According to the results of Table 2, weld (*Reseda luteola* L.) was found in samples 3, 6 and 8 which were removed from the three objects A, B and C, respectively. Figure 4 shows the chromatogram for sample 3. It is shown that the two step method, used to treat the historic samples, is successful in extracting both aglycones and glycosides. Interestingly, luteolin and apigenin (aglycones) are recorded in the graph of Figure 4 in smaller quantities than the corresponding glycosides, which are luteolin-3',7-di-O-glucoside, luteolin-7-O-glucoside and apigenin-7-O-glucoside. For this reason, the aglycone compounds were not detected in sample 6 where the glycosides were detected in trace amounts.

Weld components, described above, are not the only compounds detected in the chromatogram of Figure 4. Moreover, indigotin and indirubin were detected thus suggesting the presence of an indigoid

dye which can be either indigo (*Indigofera tinctoria* L. and others) or woad (*Isatis tinctoria* L.). Indigo and woad cannot be distinguished by HPLC. Woad has been known since antiquity worldwide, while indigo has been imported to the Mediterranean area from India since antiquity (Hofenk de Graaff 2004). Consequently, the biological sources of the indigoid dyes used in sample 9 and in several other samples of Table 2 cannot be identified based on chemical or historical evidence.

4. CONCLUSIONS

The major finding from this work is the identification of cochineal in a byzantine (14th century) ecclesiastical textile (epitaphios) from Mount Athos. The results of the HPLC analysis suggest that the cochineal dye was prepared using probably *Porphyrophora polonica* L. (Polish cochineal) insects. Moreover, it was shown that the two step TFA method devised recently (van Bommel 2014), enables successful extraction of weld components including aglycones (luteolin, apigenin) and glycosides (luteolin-3',7-di-O-glucoside, luteolin-7-O-glucoside, apigenin-7-O-glucoside) from historic textile samples. This result offers support to a previous report (Valianou et al. 2009) where the use of TFA was suggested for dyestuff extraction. Finally, kermes, indigoid dyes (either indigo or woad) and tannins were found in the historic samples which were removed from the three ecclesiastical byzantine textiles (epitaphioi) included in the study.

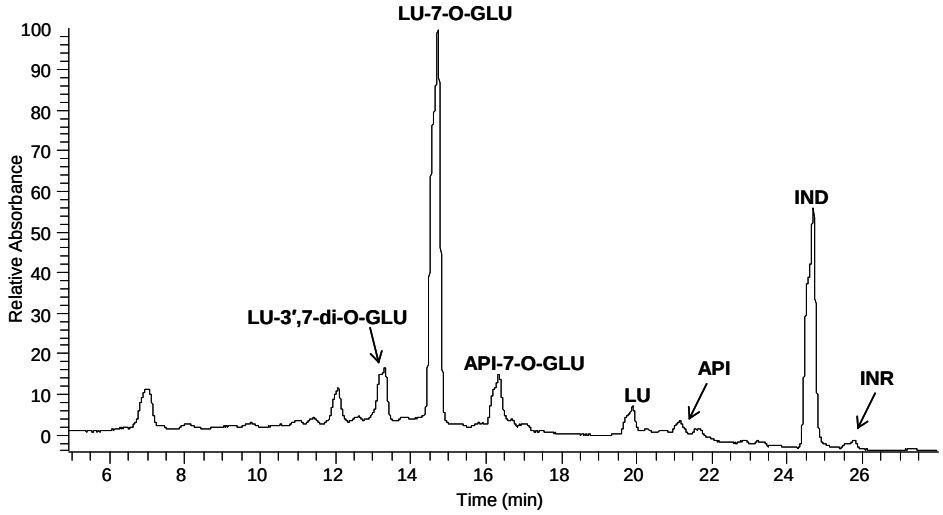

Figure 4. Chromatogram acquired at 350 nm for sample 3. The following components of weld are detected: luteolin-3',7-di-O-glucoside (LU-3',7-di-O-GLU), luteolin-7-O-glucoside (LU-7-O-GLU), apigenin-7-O-glucoside (API-7-O-GLU), luteolin (LU) and apigenin (API). Moreover, components of an indigoid dye, that are indigotin (IND) and indirubin (INR), are detected in the chromatogram.

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