High prestige Royal Purple dyed textiles from the Bronze Age royal tomb at Qatna, Syria

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During the ongoing excavations in the palace of the famous Qatna complex, the excavators noted patches of brown staining on the floor of a high status tomb. Chemical extraction revealed the presence of brominated derivatives of indigo and indirubin, and more detailed characterisation showed that it likely came from Hexaplex trunculus. *In short, this was none other than the renowned Tyrian or Royal Purple mentioned by Pliny, which was to have such an influential career colouring the clothing of the powerful. Furthermore, it was associated in the tomb with ghosts of high quality textiles preserved in gypsum.*

Keywords: Qatna, Syria, royal tomb, sediments, biomarkers, indigoids, indirubinoids, shellfish purple, textiles, gypsum, chemical mapping, excavation technique

Method

Introduction

One of the most significant tombs discovered in recent times emerged during excavations in 2002 of the Bronze Age royal palace of ancient Qatna, Tell Mishrife, Syria (Pfälzner 2004; Lange 2005; Figure 1). The tomb complex (Figures 1 and 2) comprises four chambers cut into the rock of the cliff face, all of which had remained sealed since destruction of the overlying palace in 1340 BC by the invading Hittites. More than 2000 individual artefacts have been found within the tomb, including numerous ceramic and stone vessels, human and animal bones, metal objects, jewellery and decorative items fashioned from gold, amber and precious stones (Mukherjee *et al*. 2007). It is estimated that the tomb was used for

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Received: 19 June 2008; Accepted: 1 September 2008; Revised: 29 April 2009

ANTIQUITY 83 (2009): 1109–1118

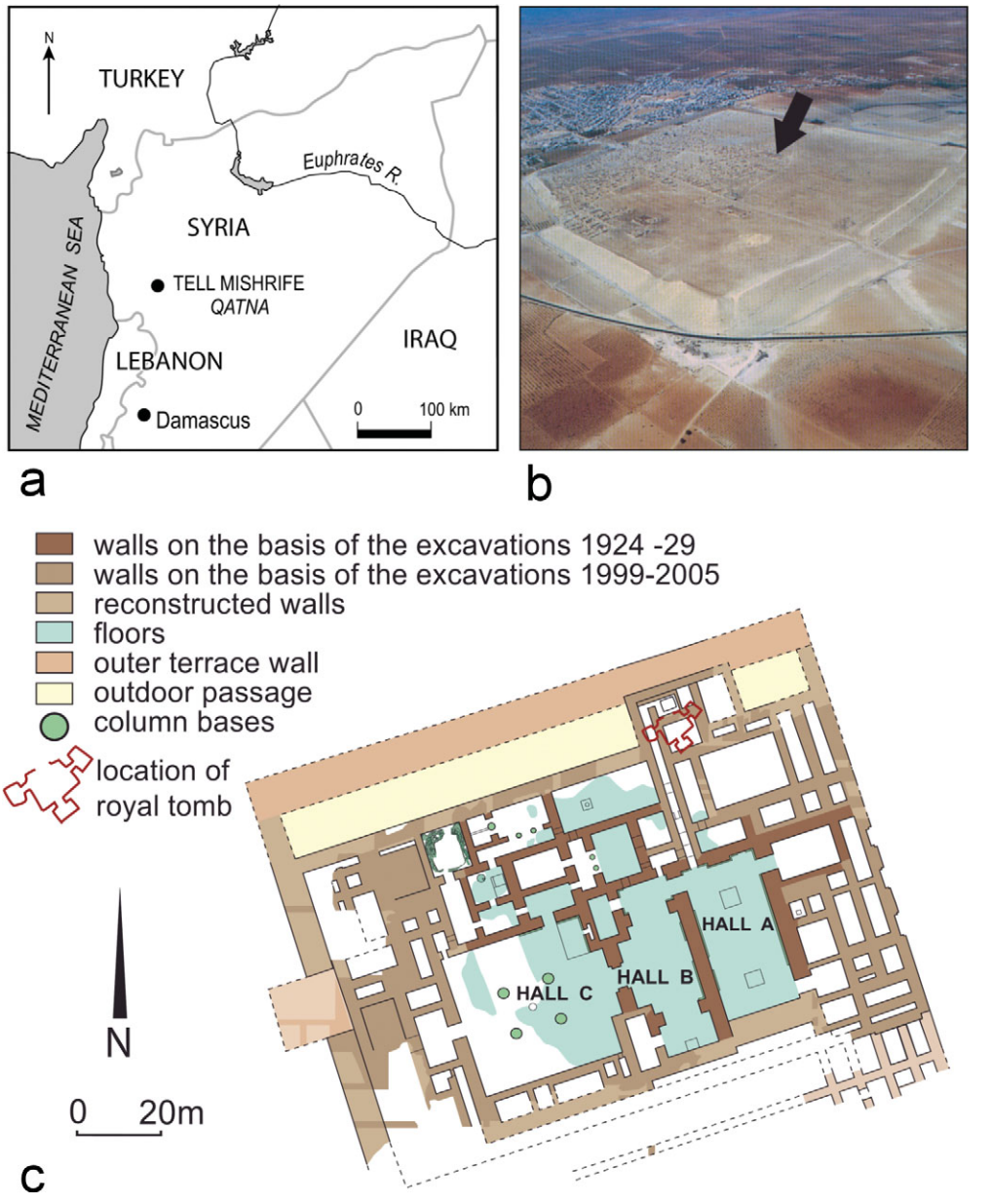

Figure 1. a) Location of ancient Qatna, Syria; b) view looking to the north-west across modern Tell Mishrife indicating the position of the palace complex; c) location of the Royal Tomb within the palace complex.

300-400 years for the burial of a number of the royal elite, prior to the destruction of the palace complex.

In addition to the extensive range of artefacts, a layer of sediment up to 150mm deep covered the tomb floor, with several loci characterised by substantial areas of dark brown

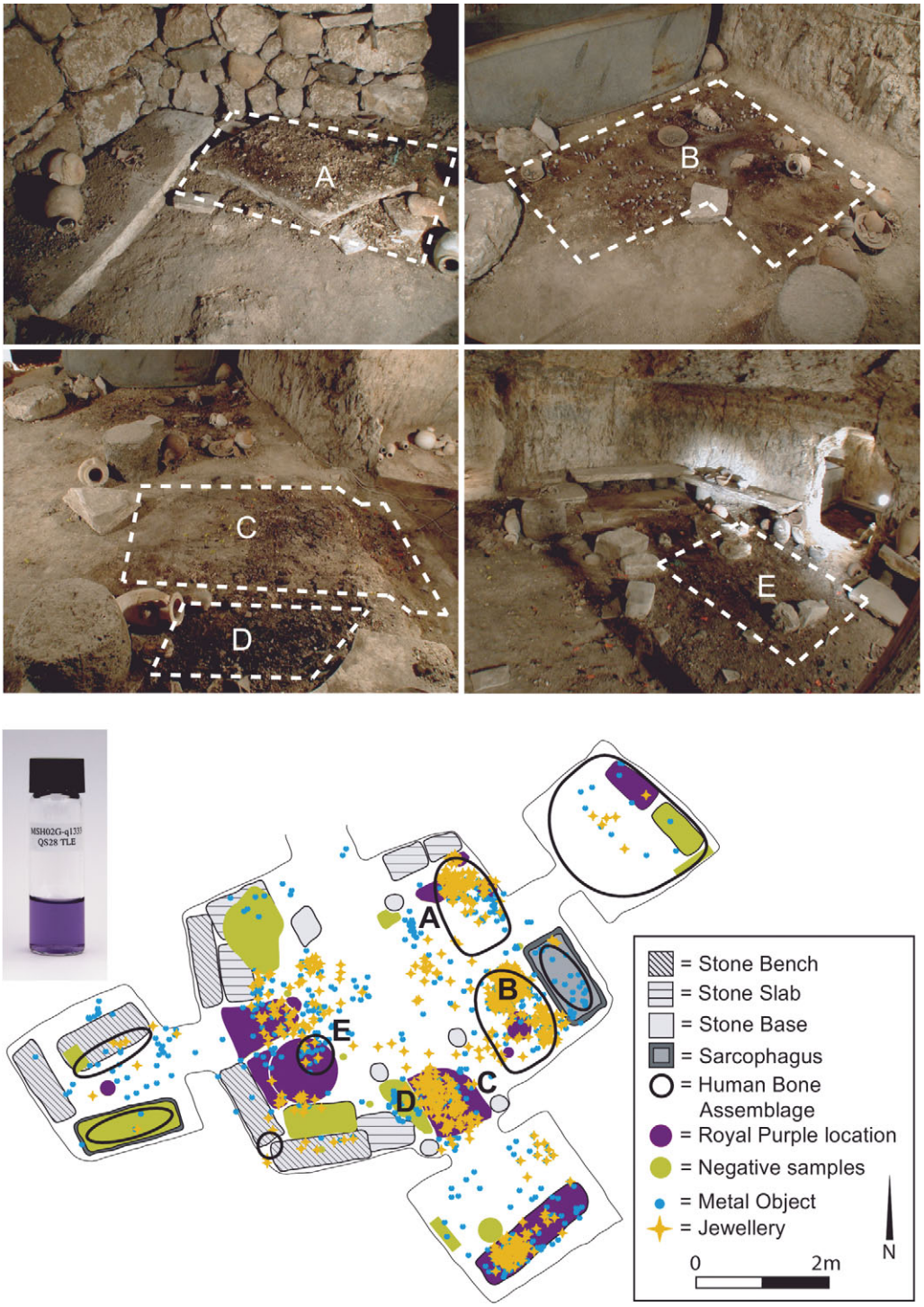

Figure 2. The upper four photographs show examples of discoloured sediments in the Royal Tomb. The letters (A-E) on the photographs correspond to those on the tomb plan below which also shows all areas from which purple coloured solvent extracts (see inset solvent extract of sediment sample MSHO2G-q1333) were obtained.

staining (Figure 2). Since the tomb is rock-cut, these sediments are entirely anthropogenic in nature, having formed as a result of ceremonial/ritual activities and the decay of funerary paraphernalia, offerings and corpses. Due to the unusual nature of these sediments and the poor morphological preservation of organic materials within the tomb, investigations were initiated that aimed at mapping funerary activities by means of sediment chemistry.

Detection and characterisation of Royal Purple in the tomb sediments

Fifty-two sediment samples were taken. Organic elemental analyses (C,H,N) confirmed that darkly stained areas were higher in organic matter (%C 0.6 to 7.6; %N 0.05 to 1.02) than those from other parts of the tomb complex (%C 0.1 to 0.5; %N 0 to 0.02). On extraction with solvents, a number of the darkly coloured sediments yielded vivid purple extracts, which were subsequently determined by a combination of high performance liquid chromatography (HPLC), mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR) to contain brominated derivatives of indigo and indirubin (see Technical Note, below).

Whilst indigo occurs in a number of plants and invertebrates, its brominated derivatives, together with brominated indirubins, are unique to molluscan purple dyes (McGovern *et al*. 1990; Koren 1995; Cooksey 2001; Karapanagiotis *et al*. 2006). The presence of these compounds in the sediment extracts is therefore unequivocal evidence for the use of this dyestuff in the royal tomb at Qatna. Tyrian or Royal Purple can be obtained from numerous species of mollusc throughout the world, although *Hexaplex trunculus*, *Bolinus brandaris* and *Stramonita haemastoma* were the principal species exploited in antiquity in the Mediterranean region (Robinson 1971; Spanier & Karmon 1987; McGovern & Michel 1990; Stieglitz 1994). The principal component of fresh dye from the majority of species is 6,6'-dibromoindigo (Cooksey 2001), however, only *H. trunculus* is known to yield both brominated and non-brominated components (Wouters & Verhecken 1991; Cooksey & Withnall 2001; Sotiropoulou & Karapanagiotis 2006; see also Technical Note).

Discovery of purple dyed woven fabrics

The loci of several of the purple coloured extracts were associated with the presence of precious artefacts, including jewellery and gold beads, likely to have decorated garments or fabrics that adorned corpses, now apparently completely decayed. The obvious conclusion is that the pigments present in the sediment extracts were remnants of the Royal Purple used to dye those fabrics. With this in mind, we performed microscopic hand sorting of the sediments collected from the tomb floor and from a stone table, revealing several thousand millimetre-sized fragments of textile, identifiable from weave patterns, e.g. Figure 3a-c. More significantly, a number of textile fragments exhibited distinctive traces of colour on their surface or within their cross sections, clearly suggesting the presence of the dyestuff. The majority of the fragments are woven in plain weave, although one exhibited a

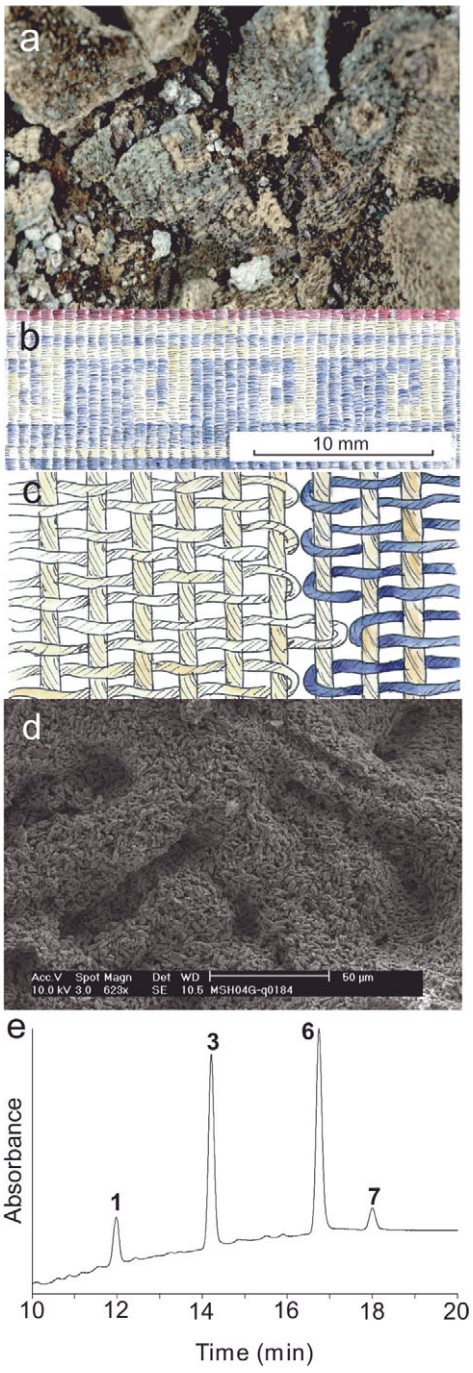

Figure 3. Details of textile fragments found in the tomb complex showing: a) patterned textile fragment from stone table in chamber 4 exhibiting interwoven ornamental pattern in blue; b) reconstruction of the ornamental pattern; c) reconstruction of kilim weaving technique identifiable from examination of a; d) electron micrograph of fossilised textile showing gypsum crystals; e) HPLC analysis of extract of fragment of textile in a; peak identities are as in Figure 4.

remarkable coloured tapestry segment, woven with a kilim technique (Figure 3c). The exceptionally fine weave of the textiles (up to 16×70-80 threads per cm^2) is comparable to contemporaneous patterned linen textiles recovered from Pharaonic tombs (Barber 1991). To obtain such fine woven fabrics, extremely fine spun yarns would have been necessary (diameter *c*. 0.07-0.1mm), requiring the use of a raw material of very high quality. This implies exceptional technical proficiency and value of these fabrics. Fabrics of this quality were without any doubt exclusively used by the upper social stratum and served as a kind of prestige object, a marker for the elite of society.

X-ray crystallography and electron microscopy showed the textile fragments are preserved as fossilised gypsum replicas, with the crystal dimensions, *c*. $2 \times 1 \mu$m (Figure 3d). Chemical (HPLC) analysis of 15 individually pigmented fossilised textile fragments showed the same mixtures of indigoid and indirubinoid components identified in the sediments (Figure 3e). The survival of the dye components indicates a remarkable resistance to decay of the indigoid and indirubinoid structures relative to the original biopolymers comprising the textile fabric that are entirely fossilised. While the fossilised nature of the textile fibres might appear to preclude its identification, the fact that cotton and silk can be excluded, based on the specific morphology of the fabric, makes wool the only reasonable candidate (Völling 2008).

Royal Purple in prehistory

The colour purple was a symbol of economic and social status in the ancient Mediterranean world (Leggett 1944; Reinhold 1970), with the best known purple dye, the Tyrian or Royal Purple obtained from Gastropodes of the *Muricidae*, discussed in Near Eastern ancient texts (Pliny 9.60-64; Rendsburg 1991; Stieglitz 1994). Evidence for a purple dyeing industry exists in the form of shell deposits and putative dye vats along the Levantine coast dating from the mid-second millenium BC (Jensen 1963; Karmon & Spanier 1987; Stieglitz 1994; Koren 1995). The perfection of Royal Purple dyeing is attributed to the sea-faring Phoenicians, although the practice is currently believed to originate from Minoan Crete around 1700-1600 BC (Ziderman 1990; Stieglitz 1994; Koren 2005; Sotiropoulou & Karapanagiotis 2006). The earliest textual evidence for the use of the colour purple in the Near East is from Akkadian tablets from Nuzi, Mesopotamia, dating to 1425 BC (Rendsburg 1991). The Armana letters from the fourteenth century BC record purple textiles sent from the Mittani to the pharaoh Amenophis III, whilst Ugaritic and Hittite documents record their inherent value (Rheinhold 1970). The first use of the term 'Royal Purple' occurs in the thirteenth century BC, recorded in Linear B clay tablets from Knossos (Sotiropoulou & Karapanagiotis 2006), somewhat later than the date of the Qatna tomb. It has been shown that 12 000 individual snails are required to produce just 1.4g of dye (Friedländer 1909) which, along with great demand for its unusual and desirable colour and its exceptional colour fastness, established purple dyed textiles as one of antiquity's most valuable commodities.

In summary, the characterisation of Royal Purple dye on the Qatna textiles provides the earliest direct evidence of purple dyed textiles and sets a precedent for its symbolic significance amongst the Bronze Age royal elite, with the value of the dyestuff correlating with the very high quality of the textiles. Our recovery of identifiable dyed textiles from deposits of the Bronze Age Mediterranean region and period is quite unique and affords one of the earliest identifications of tapestry in the ancient Near East, while the only other example appears to be from Kaman-Kalehöyük, Turkey, dating to the eighteenth–nineteenth century BC (Fairbairn 2004 as discussed in Völling 2008). Indeed, Neo-Assyrian images depicting such elaborately interwoven garments do not appear in the archaeological record until more than 500 years later, in the early Iron Age (Völling 2008).

Technical Note

Method

The sediments were extracted in a solution of dichloromethane:acetone (9:1 *v*/*v*). Despite the intensity of colour, the complexity of the UV/visible spectra recorded for crude extracts precluded meaningful interpretations. However, analysis of a pigment fraction by Fourier transform ion cyclotron resonance mass spectrometry (FTICRMS) yielded a complex spectrum within which a 1:2:1 triplet molecular anion cluster was recognised, centred at m/z 418.8853. Accurate mass determinations yielded an elemental composition of $C_{16}N_2O_2H_7Br_2$ for each ion, with the possible combinations of two $^{79/81}$Br atoms giving rise to the major ions. Significantly, the masses of these ions correspond to the calculated $[M\text{-}H]^-$ anions of the isobaric species 6,6′-dibromoindigo and 6,6′-dibromo-indirubin, indicating that one or both of these species were present in the extract. Both are components of the ancient dye Royal Purple, with 6,6′-dibromoindigo being primarily responsible

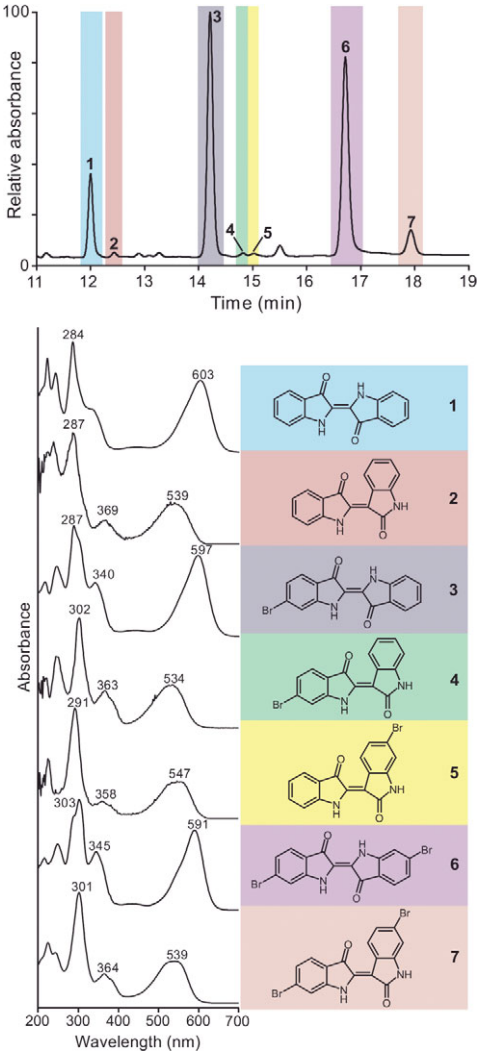

Figure 4. HPLC PDA chromatogram of solvent extract from sample MSH02G-q1333. Structures correspond to: 1) indigo; 2) indirubin; 3) 6-monobromoindigo; 4) 6-monobromoindirubin; 5) 6′-monobromoindirubin; 6) 6,6′-dibromoindigo; 7) 6,6′-dibromoindirubin, all shown with their corresponding UV-Vis absorption spectra. Identifications were made based on retention times relative to authentic standards, UV-Vis, MS and NMR data and comparison with published data.

for its coveted purple colour (McGovern & Michel 1990; Cooksey 2001; Sotiropoulou & Karapanagiotis 2006).

HPLC analysis (Figure 4) of extracts with photodiode array (PDA) detection revealed the presence of three major and four minor coloured components. Structural confirmation of the eluting compounds was sought through a combination of approaches including NMR and MS. The low concentrations (ng to μg g^{-1}) of individual compounds recoverable from the Qatna deposits precluded the use of conventional NMR techniques (Voss 2000). However, recent developments in NMR probe technology enable spectra to be recorded from sub-microgram quantities of material. Hence, semi-preparative HPLC was used to isolate 1800ng of component 3 and 800ng of component 7, sufficient to enable 1H 1D and 2D homonuclear TOCSY NMR spectra to be recorded using a ^{1}H observe salt tolerant cryo-probe at 600 MHz. From the 1D spectra and high quality 2D spectra, the aromatic substitution pattern of 6-monobromoindigo (component 3) was clearly discernable, confirming the presence of a non-spin active nucleus (Br) at C-6 of the benzene ring. The spectrum of component 7 confirmed substitution at the C-6 position of both aromatic rings, consistent with the assignment of 6,6′-dibromoindirubin. Subsequent nanospray-FTICRMS of the HPLC purified 6,6′-dibromoindirubin confirmed its molecular weight and elemental composition. Interestingly, the purified 6-monobromoindigo failed to ionise using nanospray-FTICRMS, however, prominent 1:1 doublet ion pairs corresponding to $[M]^-$ and $[M\text{-}H]^-$ was observed using matrix-assisted laser desorption/ionisation-time-of-flight (MALDI-TOF) MS with a novel graphite-based matrix.

Comparison of HPLC retention times and UV-visible absorption spectra (Figure 4) to those of authentic standards confirmed the identities of indigo (1), indirubin (2), 6-monobromoindigo (3) and 6,6′-dibromoindigo (6), which exhibited absorption maxima in the visible region at 603nm, 537nm, 597nm and 591nm, respectively. Interestingly, although blue in solution, the absorption maximum of 6,6′-dibromoindigo has been shown to shift markedly to 520nm in the solid state, giving rise to the purple colour (Clark & Cooksey 1999; Cooksey 2001). Components 4 and 5 were assigned based on published elution orders and UV-vis spectra (Karapanagiotis 2006; Karapanagiotis *et al.* 2006). Extracts from different areas of the tomb exhibited similar distributions of dye components to those shown in Figure 4, suggesting a common source.

Fresh dye from *H. trunculus* contains significant amounts of 6-monobromoindigo and indigo, along with lower concentrations of indirubin derivatives (e.g. Wouters & Verhecken 1991; Cooksey 2001; Karapanagiotis *et al*. 2006). Hence, the distribution of compounds in Figure 4 points to a *H. trunculus* source, which is consistent with archaeological evidence for the exploitation of this species along the Levantine coast (Jensen 1963; Herzog 1987; Karmon & Spanier 1987). However, it has been demonstrated that photodebromination may be induced upon exposure of the reduced form of brominated indigoids to UV radiation. Due to uncertainties surrounding the exact dyeing process utilised in antiquity, the production of a mixture of both brominated and non brominated compounds by exposure of a dyebath containing solely brominated derivatives to light cannot be unequivocally excluded.

Equipment and reagents

Elemental analysis: C:H:N determinations were made using a Carlo Erba CHN EA1108 elemental analyser.

Soxhlet extraction: sediment samples (*c*. 200g) were sieved using a 2mm sieve and ground using a pestle and mortar. Ground samples (5g) were placed in pre-extracted cellulose thimbles and reflux extracted with DCM:acetone (9:1 *v*/*v*, 250 mL, 24 h). The majority of solvent was removed by rotary evaporation and the extracts transferred to glass vials. The remaining solvent was removed under a steady stream of nitrogen and the dry extracts stored under nitrogen at −5°C.

Isolation of dye fraction: each solvent extract was suspended in hexane by ultrasonication and transferred to a silica column pre-conditioned with hexane. The column was eluted with hexane (3 × 1mL) and DCM (3 × 1mL) to remove neutral components. A purple band was eluted with DCM:MeOH (49:1 *v*/*v*, 6 × 1mL) and collected. Solvent was removed under nitrogen. The resulting residue was then washed with methanol (3 × 1mL) and redisolved in DCM:methanol (2:1 *v*/*v*).

Electrospray (ESI) mass spectrometry: ESI MS was performed using a Bruker Daltronics Apex 4, 7.0 Tesla FTICR, using an Advion Biosciences Nanomate 100 'chip-based' nanospray system coupled with an Apollo ESI source, operated in the negative ion mode. The carrier solvent was DCM:MeOH (1:1 *v*/*v*).

Matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry: MALDI-TOF MS was performed using an Applied Biosystems 4700 Proteomics Analyser (tandem time-of-flight instrument) using graphite matrix deposited from artist grade medium wash 4B charcoal pencil (Derwent).

Dimethyl formamide extraction: ground samples (0.5g) were extracted with hot dimethyl formamide (3 × 1mL, 80°C) in culture tubes. The extracts were then immediately analysed by HPLC.

High performance liquid chromatography (HPLC): HPLC was performed on a ThermoFinnigan Surveyor LC system comprising autosampler, LC pump and PDA detector. Chromatographic separation was carried out using a ZORBAX SB-C18 5μm 4.6 ×150mm HPLC column (Agilent Technologies). A gradient elution program was used according to a previously published method (Karapanagiotis *et al*. 2006). Preparative chromatography was carried out by transferring the eluent to furnaced glass vials and the solvent removed under a steady stream of nitrogen. Prepared fractions were then stored in the dark under nitrogen in a dessicator.

Nuclear Magnetic Resonance (NMR) spectroscopy: all NMR spectra were recorded on a salt tolerant ^{13}C enhanced ^{1}H observe cryo-probe at the Varian Applications laboratory, Darmstadt. Samples were dissolved in 70μL of DMSO and transferred to a 3mm Shigemi tube matched to the magnetic susceptibility of DMSO. ^{1}H NMR spectra were recorded with 10000 scans. 2D homonuclear ^{1}H TOCSY experiments with zero quantum filtering were recorded using an 80ms spin-lock at 7kHz and 256 complex points in F_1. 32 and 80 scans per increment were acquired for compounds 3 and 7, respectively.

X-ray crystallography: a fragment of textile was ground to a fine powder using an agate pestle and mortar and applied to a silicon wafer sample holder. X-ray spectra were recorded on a Bruker D8 Advance diffractometer.

Electron microscopy: textile samples were coated with gold using an EMITECH low voltage cool sputter coater K 550der and examined using a Phillips XL-40 scanning electron microscope with an accelerating voltage of 10 kV and working distance of 10.5mm.

Acknowledgements

We thank Dr I.D. Bull and Dr R. Berstan for their technical assistance and the Natural Environment Research Council (NERC) for funding the Bristol node of the Life Sciences Mass Spectrometry Facility. Eberhard Hoffmann and Varian Deutschland GmbH are thanked for obtaining NMR data. Dr Paul Bellendorf, Institut für Restaurierungswissenschaften der Uni Bamberg is thanked for technical assistance with SEM. Elisa Roßberger and Dr Carsten Witzel are thanked for the provision of distribution data for archaeological artefacts. The Engineering and Physical Sciences Research Council (EPSRC) and the Wellcome Trust are thanked for providing a PhD studentship for M.A.J. and a Bioarchaeology Fellowship for A.J.M., respectively. The German Research Foundation (DFG) is thanked for continuous funding of the German component of the excavations at Tell Mishrife, ancient Qatna. Particular thanks are due to the Director General of Antiquities and Museums of Syria, Dr Bassam Jamous, and the Director of Excavations in the Directorate General of Antiquities and Museums of Syria, Dr Michel Maqdissi, who is also co-director with P.F. of the Syrian-German Archaeological Mission at Tell Mishrife/Qatna. Figure 1b is reproduced by kind permission of the Archaeology Mission of the University of Udine to Mishrifeh.

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