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Archaeo-chemical analysis of Royal Purple on a Darius I stone jar

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Abstract. High-performance liquid chromatography (HPLC) coupled with photodiode array (PDA) detection was used for the microchemical analysis of a purple residue on the surface of a 2500-year old stone jar. This 30×37 cm pear-shaped marble vessel contains carved inscriptions praising the Persian king Darius I and is unique due to the use of quadrilingual writings on a vessel of that king. The major colorants identified in the purple pigment are 6,6′-dibromoindigo, 6-monobromoindigo, and 6,6′-dibromoindirubin, with negligible contributions by indigo and 6-bromoisatin. This analysis establishes that a marine mollusk was the source of the purple pigment, which is the famous Royal Purple or Tyrian Purple of the ancients. A comparison with the relative dye compositions of various Muricidae species (*Hexaplex trunculus*, *Bolinus brandaris*, and *Stramonita haemastoma*), and with their newly formulated Di-Mono Index values, suggests that the biological provenance of this ancient pigment was probably an indigo-deficient *Hexaplex trunculus* sea snail. The entire exterior of the vessel – including its base – was originally painted purple by using a fresco-type technique. This is only the second chromatographic finding of a molluskan purple colorant in use as an ancient paint pigment and not as a textile dye, and the only example yet discovered where it is the sole paint pigment on such a large royal art object.

Keywords: HPLC; Tyrian Purple; *Hexaplex trunculus*; *Bolinus brandaris*; *Stramonita haemastoma*; bromoindigo; bromoindirubin; bromoisatin; Di-Mono Index; King Darius I

Royal Purple is a designation used to describe those regal textiles dyed with the purple pigment extracted from the hypobranchial glands of certain Muricidae sea snails inhabiting the Mediterranean. These purple or violet garments conferred upon the wearer an aura of power and sacredness and, thus, these dressed only sovereigns, military generals, eminent officials, and high priests.

In the past two decades, this molluskan pigment has been the focus of accelerated research as it is one of the most mystifyingly complex of all the natural colorants used in antiquity. Much has been written on its general history, initially by classical authors, such as the 4th century BCE (Before the Common Era) Greek philosopher Aristotle [1] and the 1st century CE (Common Era) Roman historian Pliny [2], and more recently by Cardon [3, 4] and Haubrichs [5, 6]. Its chemistry has been reviewed [7, 8], and analytical methods have been developed for multicomponent identifications of Muricidae pigments via liquid chromatography [9–16].

The three main molluskan species that have been associated with purple dyeings in the Mediterranean

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region are *Hexaplex trunculus* (synonyms: *Murex trunculus*, *Phyllonotus trunculus*), *Bolinus brandaris* (synonyms: *Murex brandaris, Phyllonotus brandaris*), and *Stramonita haemastoma* (synonyms: *Purpura haemastoma*, *Thais haemastoma*) [3–5, 17, 18]. The biochemical procedure of extraction of the dye from the living animal and the subsequent textile dyeing process was one of the most complex of crafts practiced by the ancients. The pigment is not present in the gland of the living animal, but is spontaneously produced once the gland and its contents are excised from the snail or it expires due to other causes. Enzymatic hydrolysis of the dye precursors followed by photochemical oxidative processes occur to produce the purple or violet pigment.

The discoverers of this purple pigment were probably the ancient Minoans in the Aegean from at least the 18th century BCE [19, 20]. However, the earliest direct archaeological and chemical evidence for the use of the purple molluskan pigment in dyeing is from Phoenician sites at Sarepta, modern Sarafand, Lebanon, in the 13th century BCE [21, 22], and at Tel Akko in today's northern Israel, in the 13th–12th centuries BCE [23, 24]. At these and other Phoenician sites, potsherds with molluskan purple residues were found. These fragments broke off from large clay vats that could contain several hundred liters of dye solution in which textile dyeing was performed. Hence, this pigment has also been called Tyrian Purple after Tyre, one of the capital cities – and a purple production center – of the Phoenicians.

The vatting process, necessary for dyeing textiles with this colorant, required the dissolution of the purple pigment via reduction to a leuco-form. After the textile was immersed in the dye solution and then removed from it, the dye underwent air-oxidation back to its insoluble oxidized form in the textile fibers. The all-natural fermentative reduction that would have conceivably been performed in antiquity has recently been independently rediscovered by John Edmonds [25], Inge Boesken Kanold [26], and by this author [18].

In the current study, the chromatographic method is applied to the analysis of purple stains from the surface of a stone jar belonging to the Achaemenid king Darius I (also known as Darius the Great) who reigned in ancient Persia from 521 – 486/485 BCE [27, 28]. Purple pigments extracted from the three molluskan species mentioned above have also been analyzed in order to determine the biological provenance of the purple residue from the 2500-year old Darius vessel.

Description of the stone jar

The pear-shaped stone jar investigated in this study (Fig. 1) is exhibited at the Bible Lands Museum in Jerusalem, catalog # BLMJ 1979, and briefly described in the guide to the museum [29] well as in the museum's internet site [30]. The 30 cm (maximum width) by 37 cm (height) vessel has a rounded bottom, a thick everted rim, and several horizontal natural bands encircling it. In its current state, the jar consists of various pieces that have been glued together in the restoration process, but some of the sections from the body of the vessel are missing. It contains carved inscriptions extolling King Darius I in four languages: Egyptian hieroglyphs (containing a dedication to his 36th regnal year, which was his last, in 486/485 BCE) and wedge-shaped cuneiform scripts in Old Persian, Elamite, and Akkadian [27]. This is a rare find, as this jar is the only vessel of any kind yet discovered belonging to Darius I that has multilingual inscriptions.

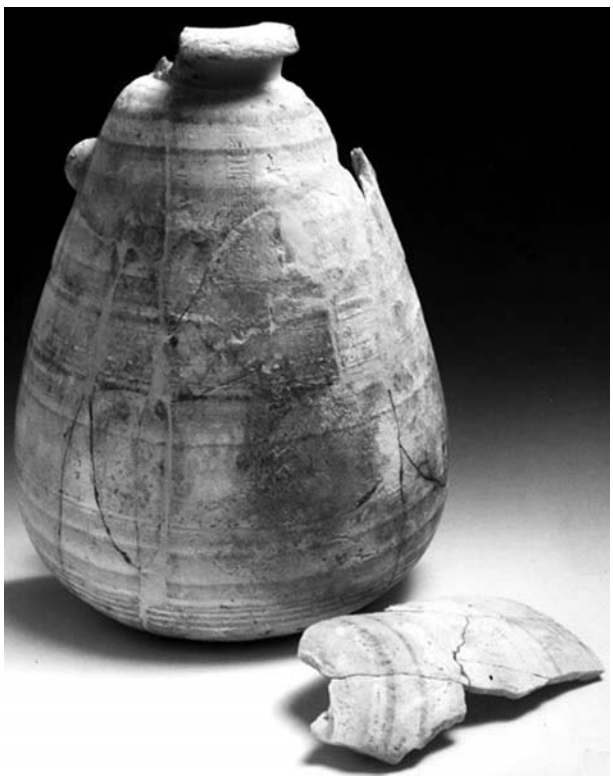


Fig. 1. Darius I stone jar (486/485 BCE) measuring 30 cm at maximum width by 37 cm high (Courtesy of the Bible Lands Museum, Jerusalem)

This author's close inspection of the residual material encompassing the Darius jar revealed that the entire outside of the stone vessel was first plastered with a white chalky material and then painted with the purple pigment in a fresco-type technique. This conclusion is further supported by the surprising fact that even the base of the vessel was painted purple, as residual plaster and purple pigment adhering to it are still visible. The brown stains on the vessel are a result of fouling and soiling of the vessel and are not part of the original coloration.

The royal jar's title-holder, Darius I (*Darayavaush* in Old Persian) has a colorful history. He is related to two other kings of the Achaemenid dynasty, Cyrus the Great and Xerxes I (the Great), and these three are probably the most famous of Persian rulers. Darius I (the Great) was the son-in-law of Cyrus (*Kurush*), and the latter's reign (550-530 BCE) began the Achaemenid dynasty [28]. In the Bible, the name *Daryavesh* (''Darius'') appears in Prophets (Hagai and Zecharia) and in Scriptures (Daniel, Ezra, and Nehemia). Furthermore, the biblical Book of Esther provides colorful descriptions of the Persian palace at the capital of *Shushan* (Susa) that was festooned with purple and violet cloths, which according to their Hebrew etymology were produced from molluskan pigments. That biblical narrative describes how Queen Esther married the Persian king *Akhashverosh*, probably Xerxes (*Khashayarsha*) is intended, who was the son of Darius the Great.

An analysis of the elemental composition of the stone jar was performed by Dr. S. Ilani of The Geological Survey of Israel, Jerusalem [27]. The method was based on scanning electron microscopy (SEM) with an energy dispersive system (EDS). It was reported that the jar's material is made of Ca, C, and O, and that it reacts with HCl (5%), which is indicative of calcite ($CaCO_3$) [27], and signifying that the stone jar's material is marble. The white chalky coat onto which the purple pigment adhered was also analyzed in that same laboratory with the result that the white material consisted of Al, Si, and O, with the Al and Si contents almost equal (about 15%), a com-

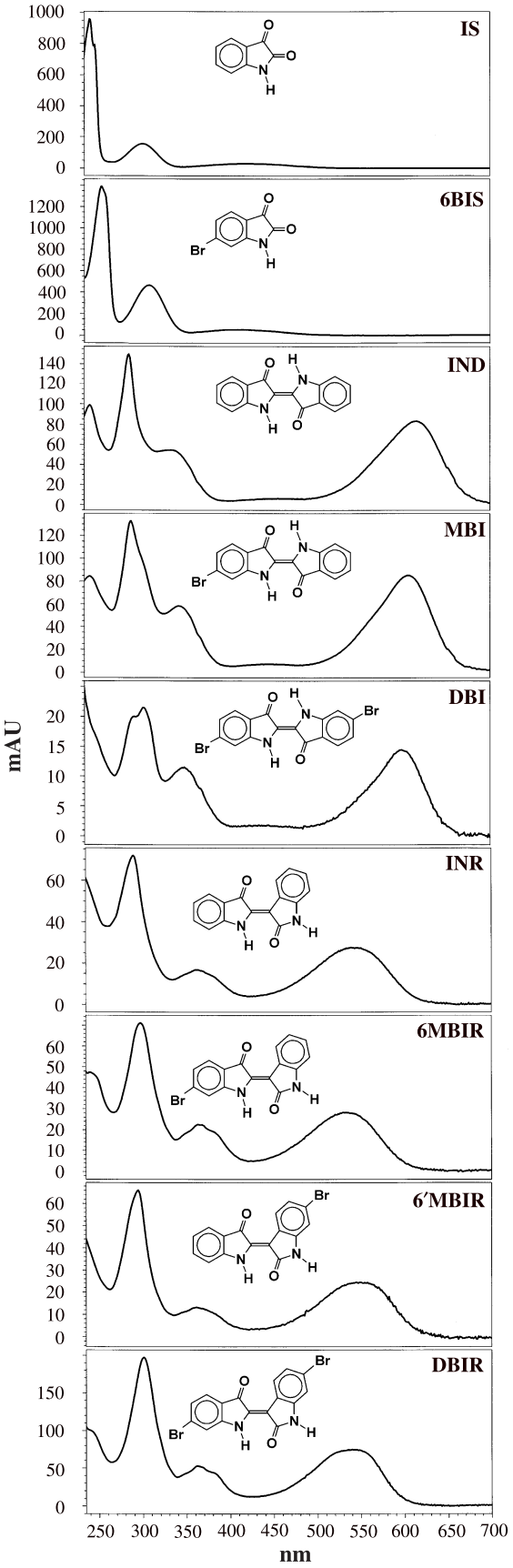


Fig. 2. Molecular structures, PDA-produced UV/Vis spectra, and abbreviated names of the standard molluskan dyes: isatinoids (*IS* isatin, *6BIS* 6-bromoisatin), indigoids (*IND* indigo, *MBI* 6-monobromoindigo, *DBI* 6,6′-dibromoindigo), and indirubinoids (*INR* indirubin, *6MBIR* 6-monobromoindirubin, *6′MBIR* 6′-monobromoindirubin, *DBIR* 6,6′-dibromoindirubin)

position indicative of kaolinite ($Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$) [27].

Although the museum's website [30] describes the residual pigment on the Darius jar as ''Purple Dye (Murex)'', the pigment has never been scientifically identified as such prior to this current work. This description was simply based on a visual assessment of the hue of the pigment. Recently, this purple stain was mineralogically analyzed, but with an ambiguous determination that the reddish colorant might be due to red ochre, hematite, Fe_2O_3 [27].

Flora and fauna sources for purple and pink pigments from other than molluskan origins are varied. For example, purple dyes (or pigments) can be produced by mixing the blue indigo dye (extracted from the leaves of the indigo or woad plants) together with a red dyestuff (extracted, for example, from the roots of the madder plant, or from scale insects) [31]. Further, pink and violet mineral pigments were found in an artist's palette and on the frescoed walls at the 1st century BCE King Herod's palace at Jericho. In those examples, the pink pigment originated from heating white kaolinite containing a small amount of yellow goethite to 850 °C, thereby transforming goethite to red hematite, which fused with the kaolinite, and the violet pigment was a mixture of that pink colorant with Egyptian blue [32]. Similarly, Vitruvius, the 1st century BCE Roman architect and engineer, described imitation purple pigments for wall paintings [33].

The importance of this current work was therefore to determine the nature and source of the ancient Darius purple pigment by microchemical means.

Experimental

Reagents and dyes

The extracting solvent, dimethyl sulfoxide (DMSO), was spectrophotometric grade and supplied by Mallinckrodt (Paris, Kentucky, USA; www.mallinckrodt.com). The HPLC-grade eluents consisted of methanol and water, both supplied by J. T. Baker (Deventer, Holland; www.jtbaker.com), and 85% ortho-phosphoric acid by Fluka (Buchs, Switzerland; www.fluka.com). The concentrated acid was diluted with HPLC-grade water to provide a 5% w/v solution with a pH of 1.50 at 25 °C.

The standard reference dyes were synthesized and kindly provided by Dr. Chris Cooksey (University College London, UK) [7, 8], except for indigo, which was obtained as ''indigo rein'' from BASF (Germany; www.basf.com). The common and abbreviated names and the molecular structures of the nine dyes analyzed are given in Fig. 2.

HPLC system

The ambient-temperature reverse-phase chromatographic system manufactured by Waters (Milford, MA, USA; www.waters.com) consisted of a 600E Controller pump and a 996 PDA detector, each controlled by the Millennium-32 software. The stationary phase consisted of a 3.0×150 mm C_{18} Symmetry column (Waters Part No. WAT054200) with 5 μm and 100 Å particle and pore diameters, respectively. A 20-μL sample loop was used. The ternary mobile phase system consisted of water, methanol, and 5% w/v H_3PO_4. Two linear gradient elution methods were utilized, and these are shown in Table 1: Method 1 consists of a constant flow rate of 0.8 mL min^{-1}, while Method 2 uses an increasing flow rate towards the end of the run. Both methods are identical up to 21 min of elution.

Sample preparation for HPLC analysis

The extractions of the archaeological and snail pigments and their subsequent filtrations were performed under subdued lighting conditions in order to prevent the photo-debromination and thus degradation of any brominated colorant that may be present in the extracted solution. Each solid micro-sample analyzed (about a milligram) was treated with 200 μL of dimethyl sulfoxide (DMSO) and heated for 5 min at 100 °C. The resulting mixture was allowed to cool to room temperature for 15 min, and then filtered in a 0.45-μ micro-spin polypropylene centrifuge tube with nylon filter (Alltech, Part No. 2490; www.alltechweb.com/product) and immediately injected into the HPLC system.

Archaeological sample analyzed

The stone jar consisted of many outer areas containing residues with a large variety of purple hues. For the current study, minute samples were scraped off the stone jar from two areas. Each archaeological sample analyzed consisted of a mostly white powdery material

Table 1. Linear HPLC gradient elution methods for the analysis of molluskan dyes: constant (Method 1) and increasing (Method 2) flow rate methods

Method	Time (min)	Flow rate (mL min^{-1})	Methanol (%)	Water (%)	5% H_3PO_4 (pH = 1.50) (%)
Methods 1 and 2	0–3	0.8	30–75	60–15	10
	3–20	0.8	75	15	10
	20–21	0.8	75–100	15–0	10–0
Method 1	21–30	0.8	100	0	0
Method 2	21–24	0.8–1.4	100	0	0
	24–25	1.4–2.1	100	0	0
	25–27	2.1	100	0	0

Table 2. Description of the Muricidae snail samples used in this study

Snail species	Provenance	Sample abbreviation used in the figures	Sample description
Hexaplex trunculus	Akhziv, northern Israel	*Tr.* A.	The glands from 20 snails were excised on the beach of Akhziv, Israel, on an overcast morning of Friday, May 14, 1993 Three DMSO extractions from this sample mixture were prepared for analysis
Hexaplex trunculus	Spain	*Tr.* S.	The excision and drying of the glands were performed in Spain and obtained from J. Guberman Ten DMSO extractions from this sample mixture were prepared for analysis
Bolinus brandaris	Fiumicino, Italy	*Br.* F.	The glandular contents were smeared as a stain on a filter paper and obtained from C. Porter Two DMSO extractions from this sample mixture were prepared for analysis
Stramonita haemastoma	Israel	*Hm.* I.	The snails were collected along the coast of Israel and placed in the author's lab aquarium. The glands were excised on Tuesday, June 1, 1993 and placed in a Petri dish. The color was very slow to develop in the lab and the final purple color appeared after a number of days Three DMSO extractions from this sample mixture were prepared for analysis

mixed with some purple pigment. Under an optical microscope, it was seen that some of the pigment particles adhered to the white plaster particles while others were seemingly pure. Three extracts of the archaeological samples were analyzed.

Preparation of the molluskan pigments

The shells of *Hexaplex trunculus*, *Bolinus brandaris*, and *Stramonita haemastoma* sea snails were strategically broken with a hammer blow to expose each snail's hypobranchial gland, which was then excised from the animal and exposed to the natural light and temperature of the surroundings. The pigment produced from the glandular fluids, whose final color was purple, was allowed to naturally dry. Each such sample consisted of remnants of the glandular meat and the pigment itself. The snails used for this study are described in Table 2. Several DMSO-extractions were performed from each sample and the exact numbers are given in the table.

Results and discussion

HPLC analyses

The constant flow-rate elution method (Table 1) utilized in this study was previously successfully used for the production of a calibration chromatogram for the detection of ten reference dyes composed of indigoid, indirubinoid, and isatinoid colorants [13]. In the current study, a second method was also used. These two methods are identical for the elution of the first nine dyes that may be present in molluskan purple pigments, but in order to hasten the exit of the last component (DBIR) the flow rate was increased for the second method at a rate that would yield column head pressures below 4000 PSI.

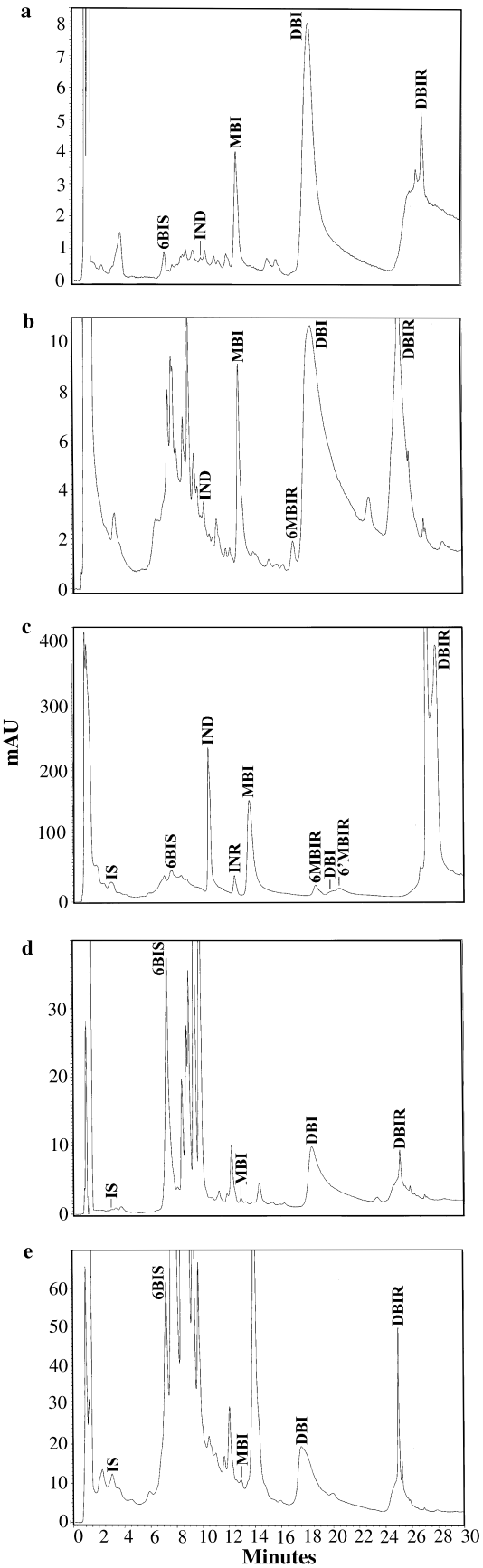
The structures of the standard dyes and their accompanying UV/Vis spectra produced by the PDA detector are shown in Fig. 2. As expected, dyes in the same chemical group show similar visible wavelengths at maximum absorption, λ_{max}, which for the two isatinoids is in the range of 410–418 nm, for the three indigoids 598–615 nm, and for the four indirubinoids 530–544 nm.

The chromatograms for the archaeological and modern snail pigments are shown in Fig. 3 and are discussed below. The respective UV wavelengths chosen for displaying these chromatograms are the ones that clearly show all the detectable colorants. The other peaks appearing in the chromatograms are only UV-absorbing substances and thus are non-colorants.

Chromatographic properties, such as retention times, t_R, and absolute and relative integrated peak areas for the dyes in the archaeological and molluskan samples were calculated at three relevant wavelengths and are shown in Table 3. The wavelengths chosen for each dye correspond to a standard uniform 288 nm, at which all the dyes have significant absorption, and to the UV and visible λ_{max} for the specific dye. Though the areas are not actual concentrations, semi-quantitative comparisons can nevertheless be made with them by using a standard wavelength of 288 nm, for example, for all peak areas.

HPLC analyses of the archaeological sample

The chromatogram of the extracted Darius pigment is shown in Fig. 3a and the corresponding chro-



matographic properties of each identified dye in that pigment are given in Table 3. The major dyes that were detected in extracts from the archaeological purple residue were DBI, MBI, and DBIR with UV-Vis absorption spectra and retention times clearly matching the respective standard dyes. Traces of IND and 6BIS were also detected. The identification of DBI in such a sample is definite evidence that the purple used in painting the stone vessel was the Tyrian Purple or Royal Purple of molluskan origin. The DBI dye is a chemical biomarker for such a marine pigment.

The finding of archaeological fibers dyed with a real molluskan purple pigment is a rare event by itself as these textiles were reserved for the most powerful and holy. In the current study, the unique use of this colorant as a painting pigment – and not as a textile dye – is a major discovery. The only other definitive chromatographic detection of the use of purple as a paint pigment is from the Late Bronze Age (17th century BCE) wall paintings of Akrotiri (the ancient name of the Greek island of Santorini) at Thera [14, 16, 34]. These purple paint pigments may be the purpurissum, a processed mixture consisting of a chalky substance mixed with a molluskan pigment, and described many centuries later by Pliny [2, 16].

HPLC analyses of the molluskan pigment samples

Archaeometric analyses often involve elemental analyses of archaeological objects such as clayware, glass, ceramics, etc. in order to determine the geographical provenance of the object. In this current work, an attempt was made to determine the biological provenance of the Darius purple pigment by analyzing various purple colorants from different Muricidae species that were listed in Table 2. The corresponding chromatograms of extracts from these snails are shown in Figs. 3b–e, and the relevant chromatographic properties given in Table 3. For clarity of comparisons, the percent areas measured at 288 nm for all samples analyzed are also plotted in Fig. 4.

Fig. 3. HPLC chromatograms of the DMSO-extracted purple pigments from: (a) the Darius jar (Method 1 of Table 1) at 300 nm; (b) *Hexaplex trunculus* snails collected at Akhziv (Method 2) at 290 nm; (c) *H. trunculus* snails collected in Spain (Method 1) at 300 nm; (d) *Bolinus brandaris* collected in Fiumicino, Italy (Method 2) at 288 nm; (e) *Stramonita haemastoma* collected in Israel (Method 2) at 288 nm

Table 3. Integrated peak areas and retention times at a standard wavelength (288 nm) and at the UV and Vis λ_{max} values for each dye for the Darius and snail pigments (see also Table 2)

Dye	λ (nm)	Darius pigment			*H. trunculus* (Akhziv)			*H. trunculus* (Spain)			*B. brandaris* (Fiumicino)			*S. haemastoma* (Israel)		
		t_R* (min)	Absolute area (PDA units)	% area, 288 nm	t_R** (min)	Absolute area (PDA units)	% area, 288 nm	t_R* (min)	Absolute area (PDA units)	% area, 288 nm	t_R** (min)	Absolute area (PDA units)	% area, 288 nm	t_R** (min)	Absolute area (PDA units)	% area, 288 nm
IS	288		0	0		0	0	3.00	166,013	0.87	2.95	1,045	0.08	3.02	69,633	2.80
	241		0			0			1,894,860			20,704			76,631	
	418		0			0			28,015			754			9,662	
6BIS	288	7.12	1,624	0.21		0	0	7.42	111,922	0.59	7.28	506,784	37.45	7.19	513,509	20.65
	255		11,901			0			436,537			3,051,471			4,640,068	
	410		958			0			23,438			117,834			179,169	
IND	288	9.94	1,441	0.19	10.10	7,585	0.35	10.29	7,406,725	38.91		0	0		0	0
	286		1,480			9,648			8,098,088			0			0	
	615		2,711			7,254			5,090,386			0			0	
INR	288		0	0		0	0	12.24	608,002	3.19		0	0		0	0
	290		0			0			612,170			0			0	
	540		0			0			225,819			0			0	
MBI	288	12.60	114,682	14.81	12.75	160,281	7.36	13.37	7,517,966	39.49	12.88	11,508	0.85	12.94	16,372	0.66
	607		70,942			101,620			6,279,090			6,976			5,322	
6MBIR	288		0	0	16.95	15,944	0.73	18.37	350,777	1.84		0	0		0	0
	298		0			19,542			436,200			0			0	
	530		0			10,460			209,274			0			0	
DBI	288	18.14	638,231	82.43	18.31	1,479,373	67.89	19.89	773,655	4.06	18.35	801,977	59.27	17.52	1,627,617	65.44
	303		640,405			1,411,713			684,167			839,431			1,662,425	
	598		480,404			1,096,127			667,782			647,431			1,270,390	
6′MBIR	288		0	0		0	0	20.18	217,697	1.14		0	0		0	0
	294		0			0			237,972			0			0	
	544		0			0			85,908			0			0	
DBIR	288	26.93	18,264	2.36	25.07	516,030	23.68	27.58	1,883,797	9.90	25.09	31,744	2.35	24.97	260,184	10.46
	300		26,456			745,782			4,982,726			43,929			368,338	
	540		10,293			275,715			127,947			14,109			136,329	

* Retention times based on the constant flow rate method, Method 1, of Table 1.

** Retention times based on the increasing flow rate method, Method 2, of Table 1.

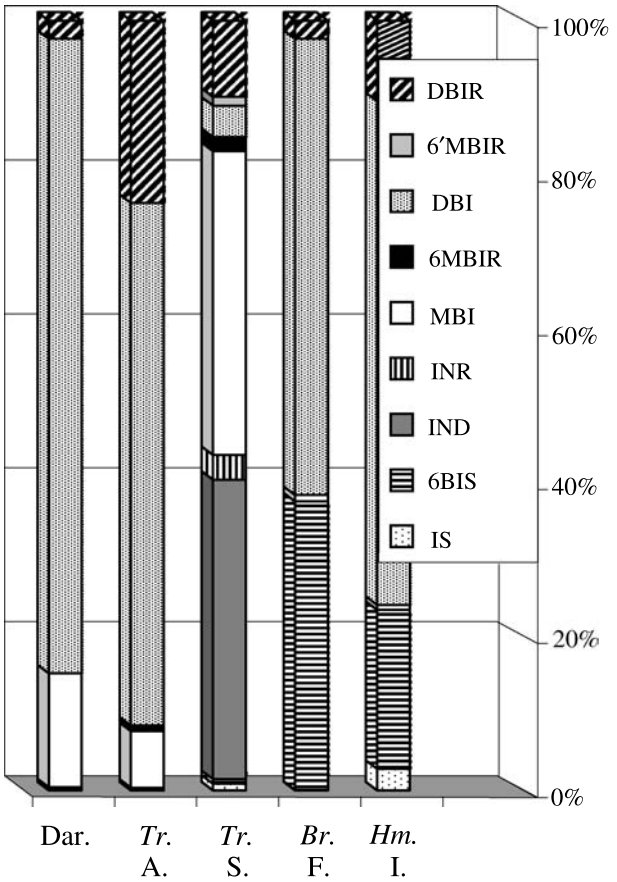


Fig. 4. Comparative plot of the percent peak areas measured at 288 nm for nine possible dyes from the Darius (''Dar.'') and snail samples (see Table 2 for an explanation of the snail abbreviations)

The striking results for several snails of *H. trunculus* of different geographical origin analyzed show that the adage of ''not all *H. trunculus* snails are created equal'' [13] is again quite apparent in this study. *H. trunculus* snail pigments can have radically different compositions and thus hues – from reddish purple to a bluish purple (or violet), depending on the relative quantities of redder or bluer colorants constituting the pigment. There is a misconception regarding *H. trunculus* pigments in that it is often thought that they all tend to have bluer (violet) tones as compared with other species. However, the results for the purple *H. trunculus* pigment from Akhziv in northern Israel, for example, show that this is not always the case.

The *H. trunculus* pigment from Akhziv, Fig. 3b, contains five of the nine dyes that were studied, whereas the Spanish *H. trunculus* pigment, Fig. 3c, has all of them. The two brominated indirubin isomers and the two isatinoids were first detected in pigments of *H. trunculus* snails in 2001 [13, 35]. However, the two most glaring differences between these snails is that the Akhziv sample has a negligible quantity of IND (<0.4% peak area at 288 nm), whereas the Spanish mollusk shows nearly 40% peak area (Table 3). On the other hand, the relative DBI compositions of the two snails are quite the opposite with Akhziv at almost 70% peak area and the Spanish at only 4%.

Both the Akhziv and Spanish *H. trunculus* pigments contain significant levels of MBI, with the Akhziv sample at about 7% and the Spanish sample at nearly 40%. Other studies have also shown that *H. trunculus* snails contain significant amounts of MBI [9, 10, 12, 13, 15].

The first HPLC detection of DBIR in *H. trunculus* snails was in 1995 [12] and the relative quantity of this dye is perhaps an unexpected result. The Spanish sample shows about 10% peak area whereas the Akhziv sample shows about 24%.

These results indicate that the common denominator for all *H. trunculus* snails studied so far is the existence of significant levels of MBI. However there are indigo-rich (DBI-poor) and indigo-poor (DBI-rich) *H. trunculus* snails. The indigo-rich mollusks, such as the ones from Spain of this investigation and from Tel Dor in north-central Israel [12], as well as the snails previously studied [9, 10], produce bluer purple (violet) pigments. The pigment produced from indigo-poor *H. trunculus* snails, as the ones from Akhziv, is a redder purple.

The differences between *B. brandaris*, Fig. 3d, and *S. haemastoma*, Fig. 3e, snails are only slight. They both do not contain any IND, and negligible levels of MBI. As expected, their DBI levels are high with both at about 60% peak area, and they contain some DBIR from 2 to 10%, respectively (Table 3). This general trend is also corroborated by other researchers [9, 10, 15]. However, the Spanish *H. trunculus* sample and the *S. haemastoma* snail have nearly the same relative peak area for DBIR at about 10% each. The most dramatic and surprising aspect of the dye analysis is that both the *B. brandaris* and *S. haemastoma* samples show large quantities of 6BIS, almost 40% peak area for the *B. brandaris* to about 20% for the *S. haemastoma*. This is the first time that this dye has been found in these species, certainly at such high levels, and is in sharp contrast to the *H. trunculus* snails, which have virtually none. Thus, the significant presence or lack of this 6BIS dye can also be used to

differentiate the *B. brandaris* and *S. haemastoma* snails from *H. trunculus* ones.

The findings regarding the relative dye compositions of Muricidae snails can be applied to determine the biological provenance of the purple pigment used in the painting of the Darius stone jar. However, such a comparison needs to be cautiously applied, as it is nearly an impossible statistical mission to analyze all purple-producing snail species in a standardized manner. There are innumerable parameters to control in order to qualitatively and quantitatively chemically fingerprint these snail pigments. These variables include the geographical location of the snails, the biological and chemical environmental conditions of their habitat including possible man-made pollution, the sizes and ages of the snails, their sex, the method of extraction of the pigment from the gland and the accompanying lighting conditions, etc. From Table 3 and the dye profiles of Fig. 4, it is apparent that (at 288 nm) the Darius pigment has only two major dye components, DBI and MBI, at a total of >97% peak area. As only *H. trunc.* snails (from any region) possess a significant amount of MBI (>7%), it appears that the most probable candidate for producing the purple colorant on the Darius jar is the pigment produced from indigo-poor (and DBI-rich) *H. trunculus* snails, similar to the ones from Akhziv.

The results of the relative peak areas that were clearly reported at specific wavelengths by other HPLC studies of Muricidae snails and archaeological pigments were combined with those from this study and are given in Table 4. These include a purple pigment on a potsherd from the 7th century BCE Phoenician site at Tel Kabri in northern Israel [12] and the paint pigment from Akrotiri (Santorini), Thera [14, 16, 34]. The analyses of the Kabri pigment however should be used cautiously as it is from a dyeing vat and thus this pigment has undergone vatting (reduction and oxidation) for the dyeing procedure, and is a residue from that processing. In some of the analyses performed by other researchers, only four components were reported on, IND, MBI, DBI, and DBIR, and sometimes a fifth (INR) was also evaluated. A comparison among all these values is rather risky as they correspond to different extraction procedures as well as different chromatographic conditions (mobile phase, stationary phase, and elution method). Though the results would be more accurate if all analyses were performed similarly and also better amenable to interpretation if more components were

Table 4. Relative peak areas as a percentage of five dyes at 288 nm for archaeological and modern snail pigments (values from this study except where noted)

	Archaeological samples and abbreviated names			Modern snail samples and abbreviated names							
Dye	Darius	Thera[a]	Kabri[b]	*H. trunculus*[b] (Akhziv)	*H. trunculus* (Spain)	*H. trunculus*[c]	*B. brandaris* (Fiumicino)	*B. brandaris*[c]	*B. brandaris*[d]	*S. haemastoma* (Israel)	*S. haemastoma*[c]
	Dar.	Th. [14]	Kb. [12]	*Tr.* [12]	*Tr.* A.	*Tr.* S.	*Tr.* [10]	*Br.* F.	*Br.* [10]	*Br.* [15]	*Hm.* I.	*Hm.* [10]
IND	0.19	0.71	3.93	4.05	0.35	40.72	55	0	0	0–1	0	0
INR	0			0	0	3.34	7	0	0		0	0
MBI	14.84	25.90	24.07	17.79	7.41	41.33	35	1.36	3	1–2	0.86	3
DBI	82.61	66.29	54.75	60.00	68.39	4.25	3	94.88	83	97–98	85.48	91
DBIR	2.36	7.10	17.25	18.16	23.85	10.36	0	3.76	14	1	13.66	6

[a] Karapanagiotis, Ref. [14] and personal communication.
[b] Koren, Ref. [12], converted from the original 600 nm values by means of the relative absorptivities of these dyes as calculated from their UV/Vis spectra.
[c] Wouters, Ref. [10].
[d] Karapanagiotis et al., Ref. [15] and personal communication.

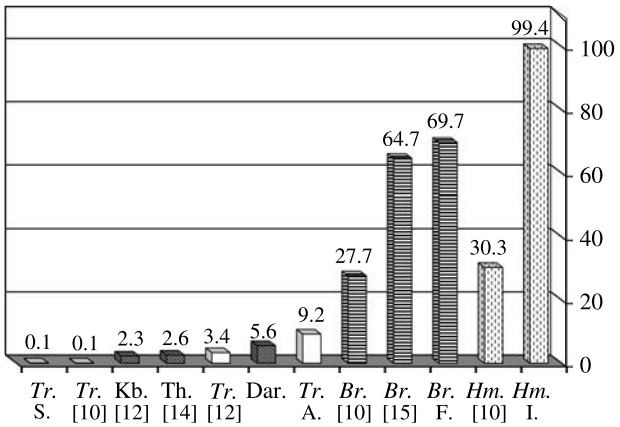


Fig. 5. Di-Mono Index (D.M.I.) values at 288 nm for pigments from Muricidae snails and on archaeological objects (see Tables 2 and 4 for explanations of the sample abbreviations; values obtained from this study, except where noted)

reported upon, nevertheless a pattern can be spotted in these cases. As was the case with the Darius jar, the Thera and Kabri pigments have a considerable amount of MBI, which is negligible in the *B. brandaris* and *S. haemastoma* samples. Thus, it is most probable that the archaeological Thera and Kabri pigments were produced from similar indigo-poor *H. trunculus* snails, as was the case with the Darius jar.

This study suggests that only a few important dyes can help determine the zoological source of the dye, at least in the case of *H. trunculus*. Thus, a simple predictive index to determine whether *H. trunculus* snails were used for the production of a particular pigment may be the ratio of peak areas for the di- and monobrominated indigoids, A_{DBI}/A_{MBI}, at 288 nm or another wavelength with significant absorptions for both. This ''Di-Mono Index'' (D.M.I.) for each pigment is depicted in Fig. 5. This simple model needs to be tested further of course with more snails analyzed. However, the trend in values appears to be that pigments from *H. trunculus* species have D.M.I.'s in the range of less than 1 and up to 10, with the indigo-rich snails at the low end of the scale and the indigo-poor ones at the higher end. The range of D.M.I. values for *B. brandaris* are from about 30–70, and for the *S. haemastoma* about 30–100. Thus, there are clear differences between the values for *H. trunculus* and those for the other two species. Based on the respective D.M.I. values, it can be deduced that the three archaeological pigments mentioned above were probably produced from similar indigo-poor *H. trunculus* snails, which reaffirms the previous conclusion.

Provenance and function of the stone jar

The hieroglyphic inscriptions carved on the stone jar are indicative that these – and the vessel itself – were probably produced in Egypt, which was at the time part of the Achaemenid Empire and virtually the only place where the use of Egyptian hieroglyphs had practical meaning [27]. However, part of the jar's processing was almost certainly produced in an area other than Egypt, an argument that can be deduced from the nature of the paint pigment – molluskan purple. There is no known association of molluskan purple pigment in Pharaonic Egypt [36], neither as a textile dye nor as a paint pigment. The purple craft probably did not enter Egypt before the Greek Macedonian conquest of Egypt by Alexander the Great and the establishment of the Hellenistic Ptolemaic dynasty at the end of the 4th century BCE after the demise of Alexander. Hence, it is also most probable that the plastering and painting of the vessel were performed in a different part of the Persian Empire. The painting would have been performed at a geographical location where the expertise in extracting the purple pigment from Murex snails existed. Finding such a site is not difficult as during the reign of Darius I the Persian Empire controlled much of the Mediterranean basin [28], home to the purple-producing Muricidae snails.

An obvious conjecture regarding the use of this stone jar is that it was somehow associated with containing the purple pigment – either for storage to be used later or as a dyeing vessel. These assumptions, however, can be convincingly disproved for a number of reasons. The interior walls of this jar are relatively clear and do not show any residual signs that the vessel contained any purple (or other) pigment inside it, unlike the ancient dyeing vat potsherds that were found in the Levant [12]. This clean-look does not appear to be a result of any deliberate cleaning process in antiquity or by modern-day conservators. Furthermore, due to the delicate nature of the externally painted jar, there would be no sensible reason to use this vessel as a dye vat. Heating of the purple vat was described by Pliny [2], and its use can also be deduced from the char marks still visible on the outsides of archaeological potsherds [12]. Heat was necessary for the effective dissolution via reduction of the pigment in the vat's liquid mixture and would have obviously damaged the royal purple painting. It also does not seem reasonable to suppose that the container held cosmetics, for instance, as smaller containers

were used for that purpose as can be seen from the images of servants carrying small vessels at the Darius palace in Persepolis.

It is probable that this vessel was never intended to be used as a container to contain anything. Using it as such entailed handling of it, which would result in abrasion of the fragile purple paint on the jar. The obvious detrimental effects of manhandling this vessel would have been known to the maker of the finished object.

A study of other inscriptions from the Achaemenid dynasty and the locations of where these vessels have been found may hold the key to the jar's function. The similarity of inscriptions on various vessels from the Darius, Xerxes, and Artaxerxes reigns indicates that they were produced according to a uniform standard at the behest of the monarchy. Further, a number of these objects were found at various sites outside of and far from central Persia, such as Uruk (modern Iraq), Zippori (Israel), Halicarnassus (Turkey), and Orsk (Russia) [27]. These indicate that rather than being presents to the king, the vessels were royal gifts from the king. This majestic purple painted marble jar – a magnificent royally prized objet d'art – was then probably commissioned by Darius and bestowed to an individual who was held in high esteem by him. This idea is also reflected in the sixth chapter of the biblical Book of Esther, a Persian queen in the Achaemenid court:

"*In this manner shall be done to the man whom the king desires to honor.*"

Conclusions

Archaeological and comparative chromatographic and spectrometric microchemical analyses were performed in order to deduce a more complete picture of the historical significance of the unique Darius I marble jar and of its purple paint. This object's singularity is two-fold: Its use of quadrilingual extollations of this Achaemenian king, and the use of the precious molluskan purple pigment extracted from *Hexaplex trunculus* snails, not as a dye for textiles but as the sole pigment for the painting of the entire stone jar. Indeed this was a royal piece de resistance fit for King Darius the Great.

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