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Supporting Information

A study in Mauve Unveiling Perkin's Dye in Historic Samples

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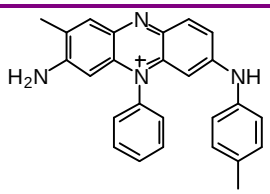
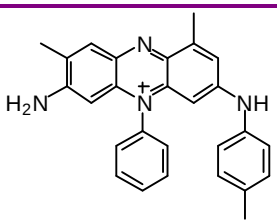
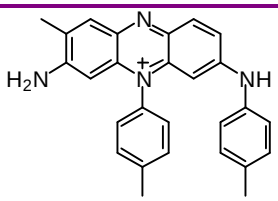
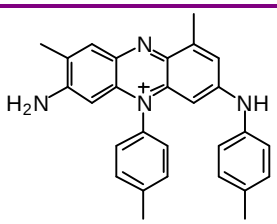
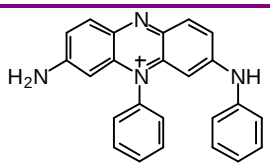
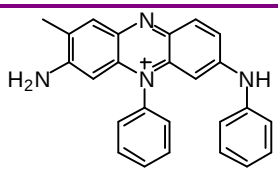
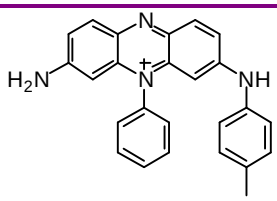
Supporting Text

1- Data Summary

Table S1. Structures and summarized spectral characteristics of mauveine compounds isolated from different historical samples.

	mauveine A	**mauveine B**	**mauveine B2**	**mauveine C**
FDMS *m/z*	391.2	405.3	405.3	419.3
FTICR *m/z*	391.19172 (calc. for $C_{26}H_{23}N_4^+$: 391.19226)	405.20737 (calc. for $C_{27}H_{25}N_4^+$: 405.20791)	405.20737 (calc. for $C_{27}H_{25}N_4^+$: 405.20791)	419.22302 (calc. for $C_{28}H_{27}N_4^+$: 419.22356)
structure (^{1}H NMR)				
HPLC-DAD				
Tr (min)	16.57	21.10	16.85	22.88
$\lambda_{máx}$(nm)	549	548	550	549

	pseudo-mauveine	**mauveine C_{25}a**	**mauveine C_{25}b**	**Mauveine C_{25}c**
FDMS *m/z*	364.17	378.47	378.47	378.47
FTICR *m/z*	363.16042	377.17607	377.17607	377.17607
	(calc. for $C_{24}H_{19}N_4^+$: 363.16096)	(calc. for $C_{25}H_{21}N_4^+$: 377.17661)	(calc. for $C_{25}H_{21}N_4^+$: 377.17661)	(calc. for $C_{25}H_{21}N_4^+$: 377.17661)
structure (^{1}H NMR)				-
HPLC-DAD				
Tr/min	11.83	14.12	14.12	16.08
$\lambda_{máx}$/nm	547	548	548	548

	Mauveine B3	**Mauveine B4**	**Mauveine C1**	**Mauveine D**	**Mauveine E**
FDMS *m/z*	405.3	405.3	419.3	433.2	447.1
FTICR *m/z*	405.20737	405.20737	419.22302	433.23867	447.25432
	(calc. for $C_{27}H_{25}N_4^+$: 405.20791)	(calc. for $C_{27}H_{25}N_4^+$: 405.20791)	(calc. for $C_{28}H_{27}N_4^+$: 419.22356)	(calc. for $C_{29}H_{29}N_4^+$: 433.23921)	(calc. for $C_{30}H_{31}N_4^+$: 447.25486)
HPLC-DAD					
Tr/min	17.70	18.12	22.23	23.67	24.40
$\lambda_{máx}$/nm	544	544	541	545	540

2- HPLC-DAD/LC-MS characterization of historical mauveine samples

Mauveine salts

The HPLC-DAD chromatograms of mauveine salts acquired at 550nm are presented in Fig. S1.

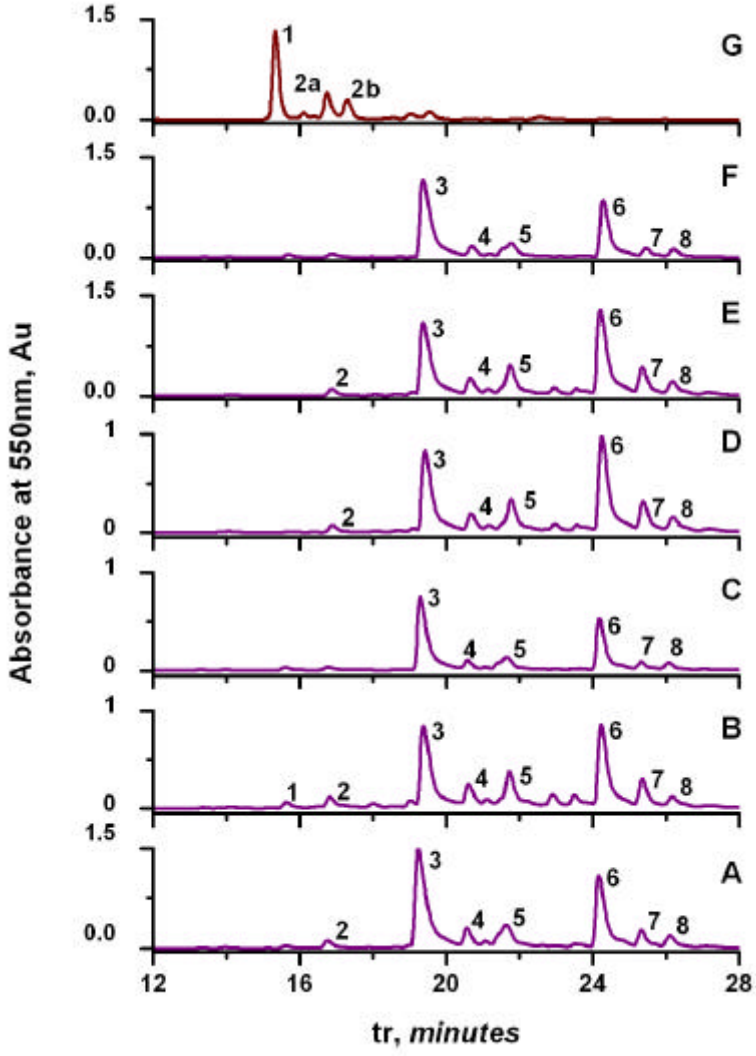


Fig. S1. Mauveine salts HPLC-DAD chromatograms obtained at λ = 551nm for A: Science Museum 1; B: Science Museum 2; C: Science Museum 3; D: Science Museum 4, E: Museum SI Manchester 1; F: Chandler Museum; G: Museum SI Manchester 2 (This sample was analyzed with the Polaris C18-A column (150mm × 2mm) in order to separate successfully the mauveine C_{25} isomers). All the samples were dissolved in methanol. The major compounds identified correspond to the numbered peaks: 1- pseudo-mauveine; 2- two C_{25} isomers; 3- mauveine A; 4- mauveines B3 + B4; 5- mauveine B2; 6- mauveine B; 7- mauveine C1; 8 - mauveine C. For more details see text and Table S1 for structures.

The names for the compounds given in **Table S1** are in accordance with those introduced by Meth-Cohn and Smith in 1994,[1] and more recently by some of us in ref. [2]. The logics assisting these names is based on the number of methyl groups around the 7-amino-5-phenyl-3-(phenylamino)phenazin-5-ium core (pseudo-mauveine) common to all mauveine compounds: two methyl groups - mauveines A; three methyl groups - mauveines B, four methyl groups - mauveines C. Peaks number **3** and **6** correspond, respectively, to the mauveine A and the mauveine B described by Meth-Cohn and Smith in 1994;[1] peaks **5** and **8** correspond, respectively, to mauveines B2 and C, recently described[2]; peak number **1** was identified as pseudo-mauveine; the compounds corresponding to peak **4** were designated as mauveine B3 and mauveine B4 since they are isomers of mauveine B; the compound corresponding to peak **7** was named mauveine C1 since it is an isomer of mauveine C; finally, the compounds corresponding to peak 2 are two C_{25} isomers containing each one methyl group and were designated as mauveine C_{25a} and mauveine C_{25b}.

The mass determination of some mauveine minor compounds, namely those corresponding to peaks 4 and 8, was performed with LC-MS in *Science Museum 1* and in a mauve-dyed textile sample *Science Museum F6*, see **Figs S2** and **S3**, respectively. The *Museum SI Manchester 2* sample was also analysed with LC-MS, see **Fig. S4.**

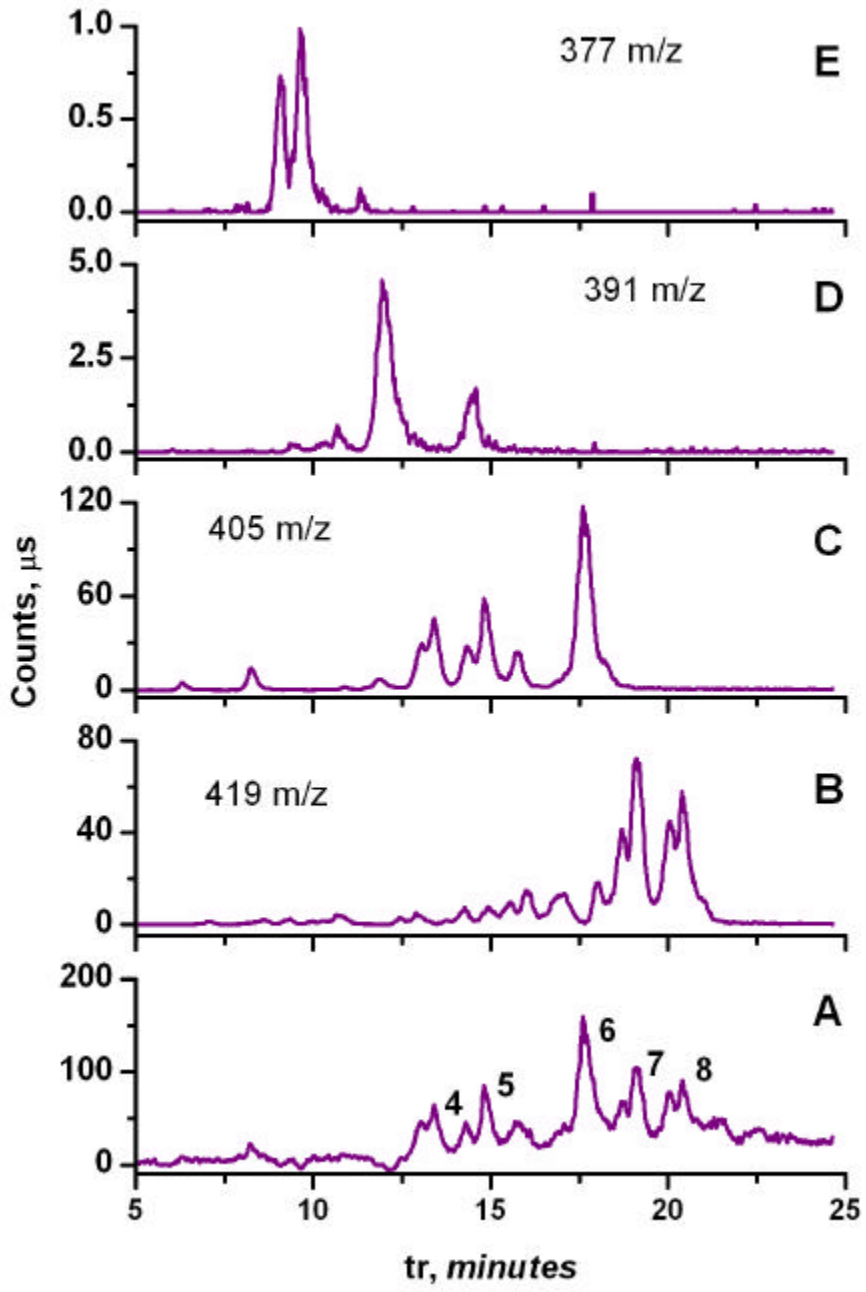


Fig. S2. HPLC-MS total ion chromatogram (TIC) of A) Science Museum 1 salt sample; B) 419 m/z compounds; C) 405 m/z compounds; D) 391 m/z compounds, E) 377 m/z compounds. The compounds identified in TIC correspond to the numbered peaks: 4- mauveines B3 + B4; 5- mauveine B2; 6- mauveine B, 7- mauveine C1, 8- mauveine C.

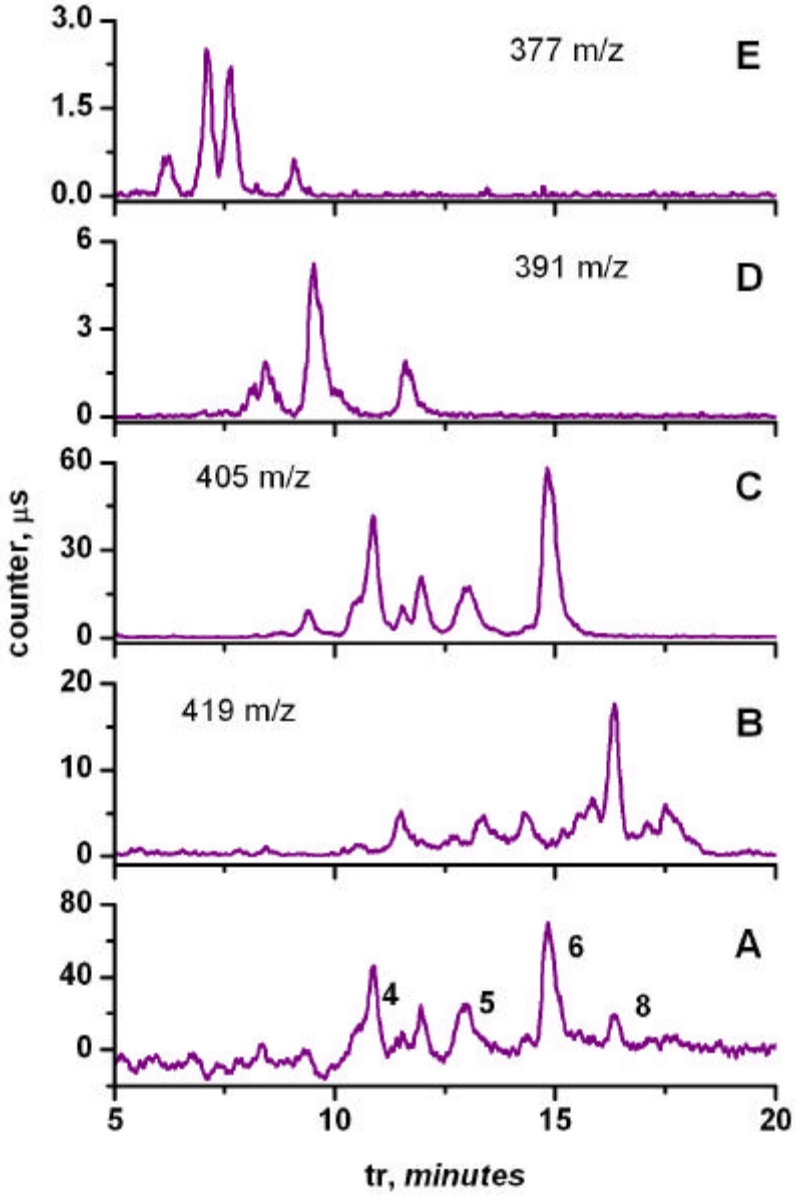


Fig. S3. HPLC-MS total ion chromatogram (TIC) of A) Science Museum F6 mauve dyed shawl; B) 419 m/z compounds; C) 405 m/z compounds; D) 391 m/z compounds, E) 377 m/z compounds. The compounds identified in TIC correspond to the numbered peaks: 4- mauveines B3 + B4; 5- mauveine B2; 6- mauveine B, 8- mauveine C.

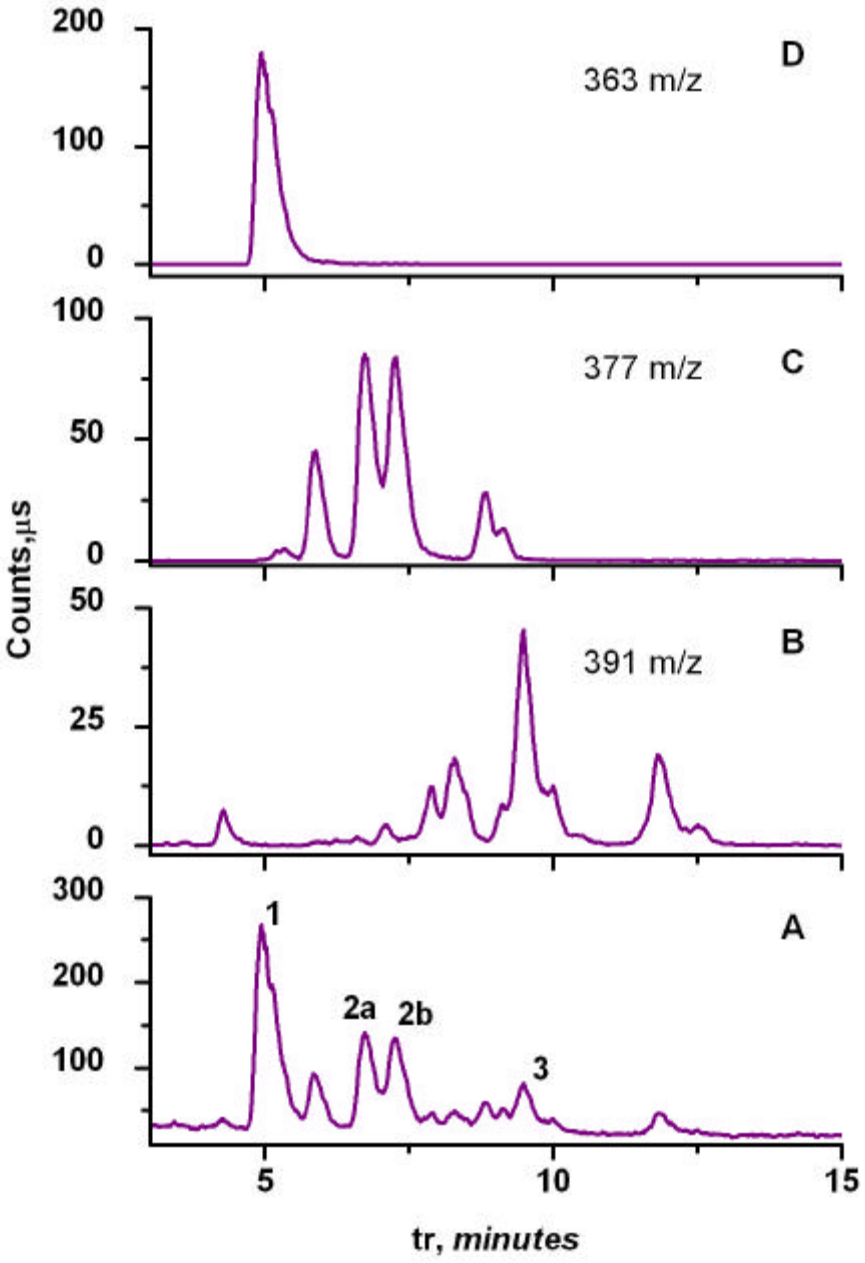


Fig. S4. HPLC-MS total ion chromatogram (TIC) of a) Museum SI Manchester 2 salt sample; b) 391 m/z compounds; c) 377 m/z compounds; d) 363 m/z compounds. The compounds identified in TIC correspond to the numbered peaks: 1- pseudo-mauveine; 2- mauveines C_{25a} and C_{25b}; 3- mauveine A.

Mauve-dyed textiles

Six extraction procedures to enable the recovery of all mauveine chromophores prior to HPLC-DAD analysis were tested in textile historical reconstructions, this is, silk textiles dyed with *Science Museum 1* according to Perkin's recipes[3]: ***extraction 1*****:** MeOH; ***extraction 2*****:** MeOH / H_2O (25:75, v/v); ***extraction 3*****:** MeOH / HCOOH 98 % (95:5, v/v); ***extraction 4*****:** 0.2 M oxalic acid / MeOH / acetone / water (1:3:3:4, v/v/v/v); ***extraction 5*****:** MeOH + 1 drop of 0.01 M HCl / H_2O (pH = 2); ***extraction 6*****:** MeOH + 1 drop of NaOH / H_2O (pH = 10).

The extraction procedures were always carried out as follows: a small sample of thread (around 0.1mg) was extracted with 400μL of the solution mixture in 1.5ml eppendorfs for 30 min at 60ºC (water bath) under constant stirring.[4] After extraction, each extract was dried in a vacuum system, where the resulting dry residues were reconstituted with 50μL of methanol and 25 μL were analysed in HPLC-DAD.

From the results obtained it was possible to conclude that the methods 1, 3 and 5 are the more efficient methods, since methods 4 and 6 promote some degradation of the mauveine dye and method 2 does not allow full extraction of the mauveine chromophores. Furthermore, the methods 1, 3 and 5 allowed the extraction of the different mauveine chromophores in equal amount. All the mauve-dyed samples were extracted with method 5 which was the most efficient method. When there was enough sample amount, methods 3 and 1 were also applied, and standard deviation values calculated.

The HPLC-DAD chromatograms for the dyed textile samples are shown in **Fig. S5.** The structures are identical to those found in the chemical samples and can be found in **Table S1.**

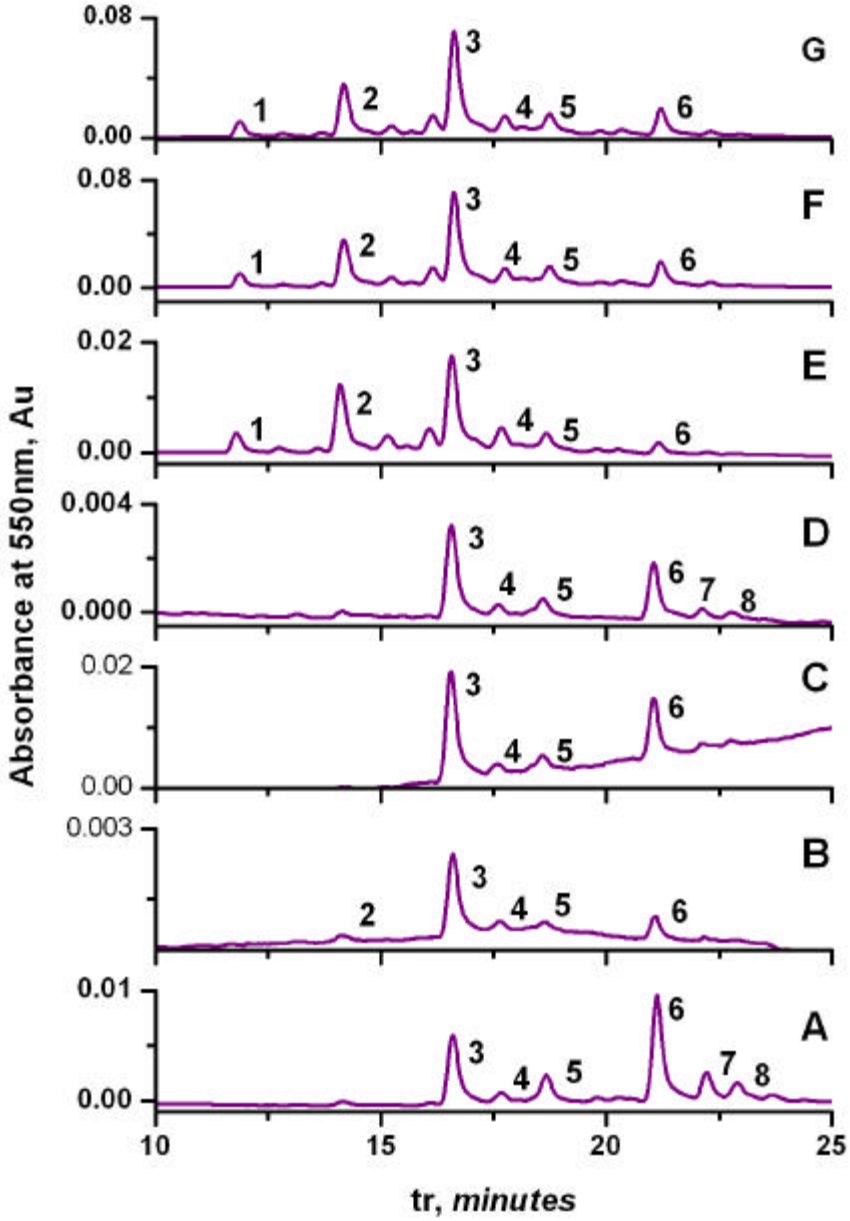


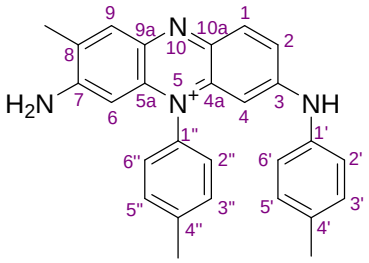
Fig. S5. Mauve-dyed textiles HPLC-DAD chromatograms obtained at λ = 551nm for A: Science Museum F1; B: Science Museum F2; C: Science Museum F3; D: Science Museum F4, E: Science Museum F5; F: Perth Museum; G: Science Museum F6. All the samples were extracted with MeOH + 1 drop of HCL/H_2O (pH=2), for more details see extraction methods described below. The major compounds identified correspond to the numbered peaks: 1- pseudo-mauveine; 2- mauveines C_{25a} + C_{25b}; 3- mauveine A; 4- mauveines B3 + B4; 5- mauveine B2; 6- mauveine B; 7- mauveine C1; 8 - Mauveine C.

3- NMR characterization of the compounds isolated by HPLC

Compounds isolated from HPLC were lyophilized and further dried under vacuum at room temperature. All compounds were dissolved in CD_3OD, and the residual solvent peak was used as a reference to calibrate spectra.

Tables S2-S6. ^{1}H- and ^{13}C-NMR data for the isolated mauveine compounds. Spectra were run at 298.0 K, in CD_3OD, at 400.13 Hz (1H) and 100.00 Hz (^{13}C) for pseudo-mauveine and mauveines B2 and C and at 600.13 Hz (1H) and 150.91 Hz (^{13}C) for isomeric mauveines C_{25}a and C_{25}b. HMBC data refers to correlations of each hydrogen atom to the indicated carbon atoms.

Table S2. ^{1}H- and ^{13}C-NMR data for the isolated mauveine B2.

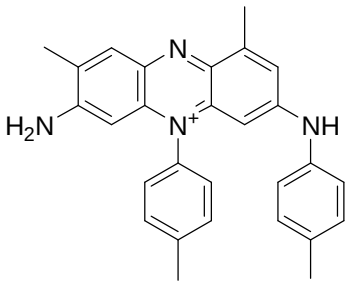


mauveine B2

7-amino-8-methyl-5-p-tolyl-3-(p-tolylamino)phenazin-5-ium

Position	**^{1}H** d/**ppm (J/Hz)**	**^{13}C** d/**ppm**	**HMBC**
1	8.01 (d, 9.3)	134.36	3, 4a
2	7.41 (dd, 9.3, 2.2)	121.57	
3	-	153.91	
4	6.33 (d, 2.2)	95.12	2, 10a
4a	-	137.88	
5a	-	137.60	
6	6.08 (s)	95.27	8, 9a
7	-	159.10	
8	-	131.92	
9	7.92 (s)	133.68	5a, 7, 8-CH_3
9a	-	139.01	
10a	-	137.70	
1’	-	137.36	
2’,6’	7.03 (d, 8.4)	123.45	2’,6’, 4’
3’,5’	7.12 (d, 8.4)	131.05	1’, 3’,5’, 4’-CH_3
4’	-	136.77	
1’’	-	135.23	
2’’,6’’	7.36 (d, 8.1)	128.51	2’’,6’’, 4’’
3’’,5’’	7.61 (d, 8.1)	133.00	1’’, 3’’,5’’, 4’’-CH_3
4’’	-	142.82	
8-CH_3	2.37 (s)	20.96	7, 8, 9
4’-CH_3	2.30 (s)	17.54	2’, 3’, 4’
4’’-CH_3	2.52 (s)	21.31	3’’, 4’’

Table S3. ^{1}H- and ^{13}C-NMR data for the isolated mauveine C.

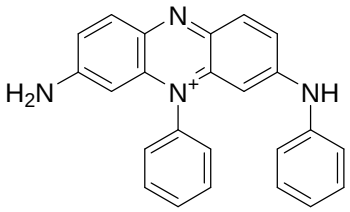


mauveine C

7-amino-1,8-dimethyl-5-p-tolyl-3-(p-tolylamino)phenazin-5-ium

Position	**^{1}H** d/**ppm (J/Hz)**	**^{13}C** d/**ppm**	**HMBC**
1	-	138.99	-
2	7.25 (br. s)	121.02	
3	-	153.70	-
4	6.17 (d, 2.0)	93.78	2, 10a
4a	-	143.83	-
5a	-	137.3	-
6	6.11 (s)	95.09	8, 9a
7	-	158.67	-
8	-	131.17	-
9	7.89 (s)	133.90	5a, 7, 8-CH_3
9a	-	137.3	-
10a	-	137.61	-
1’	-	137.3	-
2’,6’	7.01 (d, 8.2)	123.58	2’,6’, 4’
3’,5’	7.11 (d, 8.2)	131.01	1’, 3’,5’, 4’-CH_3
4’	-	136.73	-
1’’	-	135.55	-
2’’,6’’	7.33 (d, 8.1)	128.52	1’’, 2’’,6’’, 4’’
3’’,5’’	7.60 (d, 8.1)	132.95	1’’, 3’’,5’’, 4’’-CH_3
4’’	-	142.70	-
1-CH_3	2.77 (s)	17.88	1, 2, 10a
8-CH_3	2.37 (s)	17.53	7, 8, 9
4’-CH_3	2.30 (s)	20.95	3’, 4’
4’’-CH_3	2.52 (s)	21.30	3’’, 4’’

Table S4. ^{1}H- and ^{13}C-NMR data for the isolated pseudo-mauveine.

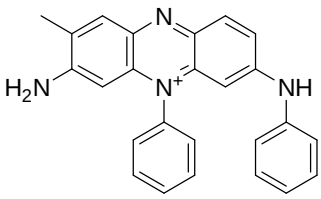


pseudo-mauveine

7-amino-5-phenyl-3-(phenylamino)phenazin-5-ium

Position	**^{1}H** d/**ppm (J/Hz)**	**^{13}C** d/**ppm**	**HMBC**	**NOESY**
1	7.95 (d, 9.2)	134.75	3, 4a	
2	7.43 (m)	121.77	10a	
3	-	154.11	-	
4	6.33 (d, 1.3)	95.45	2, 10a	4 ↔ 2’,6’, 2’’,6’’
4a	-	138.08	-	
5a	-	138.89	-	
6	6.02 (d, 1.4)	94.88	8, 9a	6 ↔ 2’’,6’’
7	-	159.70	-	
8	7.28 (dd, 8.0, 1.3)	123.36	9a	
9	8.02 (d, 9.2)	135.43	5a, 7	
9a	-	139.17	-	
10a	-	137.66	-	
1’	-	139.90	-	
2’,6’	7.14 (m)	123.30	2’,6’, 4’	
3’,5’	7.30 (m)	130.61	1’, 3’,5’	
4’	7.13 (m)	126.72	2’,6’	
1’’	-	137.74	-	
2’’,6’’	7.51 (d, 7.4)	128.81	2’’,6’’, 4’’	
3’’,5’’	7.81 (m)	132.63	1’’, 3’’,5’’	
4’’	7.75 (m)	126.72	2’’,6’’	

Table S5. ^{1}H- and ^{13}C-NMR data for the isolated mauveine C_{25a}.

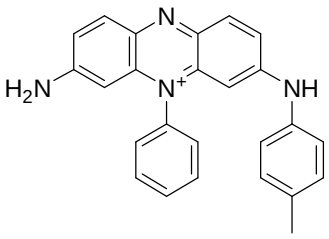


mauveine C_{25a}

7-amino-8-methyl-5-phenyl-3-(phenylamino)phenazin-5-ium

Position	**^{1}H** d/**ppm (J/Hz)**	**^{13}C** d/**ppm**	**HMBC**
1	8.04 (d, 9.4)	134.74	3, 4a
2	7.44 (dd, 9.4, 2.0)	121.63	10a
3	-	153.52	-
4	6.38 (d, 2.0)	95.38	2, 3, 10a
4a	-	137.53	-
5a	-	137.74	-
6	6.11 (s)	94.98	5a, 7, 8, 8-CH_3, 9a
7	-	159.36	-
8	-	132.24	-
9	7.87 (d, 1)	133.79	5a, 7, 8-CH_3
9a	-	139.38	-
10a	-	137.66	-
1’	-	140.06	-
2’,6’	7.14 (m)	123.13	1’, 2’,6’, 3’,5’, 4’
3’,5’	7.29 (dd, 8, 8)	130.60	1’, 2’,6’, 3’,5’
4’	7.13 (m)	126.50	2’,6’
1’’	-	137.77	-
2’’,6’’	7.52 (d, 7)	128.83	1’’, 2’’,6’’, 4’’
3’’,5’’	7.81 (dd, 8, 8)	132.62	1’’, 2’’,6’’, 4’’
4’’	7.75 (t, 8)	132.19	1’’, 2’’,6’’, 3’’,5’’
8-CH_3	2.38	17.58	7, 8, 9, 9a

Table S6. ^{1}H- and ^{13}C-NMR data for the isolated mauveine C_{25b}.



mauveine C_{25b}

7-amino-5-phenyl-3-(p-tolylamino)phenazin-5-ium

Position	^{1}H d/ppm (*J*/Hz)	^{13}C (d/ppm)	HMBC
1	8.01 (d, 9.2)	134.46	3, 4a
2	7.41 (dd, 9.2, 2.0)	121.83	10a
3	-	154.32	-
4	6.29 (d, 2.0)	95.08	2, 3, 10a
4a	-	138.23	-
5a	-	138.86	-
6	6.01 (d, 2.0)	94.87	7, 8, 9a
7	-	159.56	-
8	7.27 (dd, 9.2, 2.0)	123.02	9a
9	7.95 (d, 9.4)	135.37	5a, 7
9a	-	138.86	-
10a	-	137.80	-
1’	-	137.18	-
2’,6’	7.02 (d, 8.0)	123.38	2’,6’, 3’,5’, 4’
3’,5’	7.12 (m)	131.08	1’, 2’,6’, 3’,5’, 4’-CH_3
4’	-	136.92	-
1’’	-	137.85	-
2’’,6’’	7.51 (d, 7)	129.08	1’’, 2’’,6’’, 4’’
3’’,5’’	7.81 (dd, 8, 8)	132.65	1’’, 2’’,6’’, 3’’,5’’
4’’	7.75 (t, 8)	132.19	2’’,6’’, 1’’
4’-CH_3	2.29	20.97	2’,6’, 3’,5’, 4’

4- Counter ion analysis

The counter-ions of salt samples were identified in a Dionex ICS 3000 CR-TC, RFIC HPLC anion exchange chromatography system with an Ion Pack® CG 16 column with 5x50mm guard column, using 37.5mM KOH as eluent.

The HPLC anion exchange chromatography of counter ions are presented in **Fig. S6**. The mauveine salts chromatograms were compared with ion standards presented in **Table S7**.

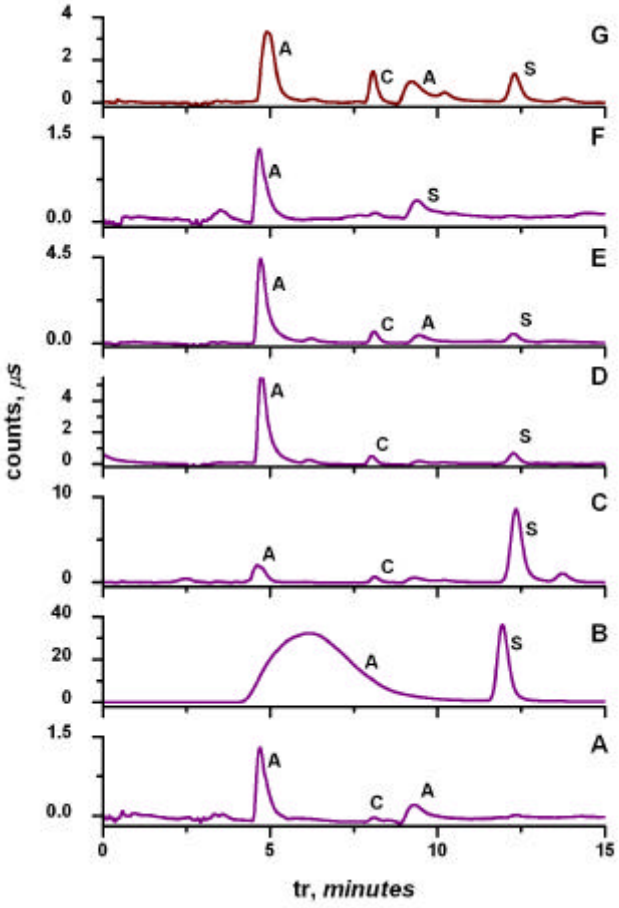


Fig. S6. Mauveine salts HPLC-AEC chromatograms obtained for A: Science Museum 1; B: Science Museum 2; C: Science Museum 3; D: Science Museum 4, E: Museum SI Manchester 1; F: Chandler Museum; G: Museum SI Manchester 2. All the mauveine salts were dissolved in water, with exception of Science Museum 2 sample which did not dissolve in water and thus methanol had to be added*. The major compounds identified correspond to the signalled peaks: A - acetate; C - chloride; S - sulphate. For more details see text and Table S8 with relative areas.

* decreasing order of solubility in water: Science Museum 1, Science Museum 4, Chandler Museum, Museum SI Manchester 1 >> Museum SI Manchester 2 > Science Museum 3 >>> Science Museum 2. The sample's solubility is clearly related to the increase in the percentage of the sulphate counter-ion.

Table S7. Ion standards and respective retention times (t_r) in water.*

Standards (M)	**t_r (min)**
Acetate 5×10^{-5}	4.8±0.1
	9.5±0.3
Chloride 5×10^{-4}	8.1±0.2
Sulphate 5×10^{-4}	12.4±0.4
Nitrate 5×10^{-4}	21.7±0.3

* the standards were dissolved in water since with methanol the acetate standard precipitates giving rise to a broad band, masking the identification of other ion standards.

Table S8. Counter-ions of the mauveine analysed samples.

	counter-ions		
	acetate (%)	chloride (%)	sulfate (%)
Science Museum 1	97	3	0
*Science Museum 2**	2	0	98
Science Museum 3	19	4	67
Science Museum 4	82	6	9
Museum SI Manchester 1	86	7	6
Museum SI Manchester 2	68	13	17
Chandler Museum 2	100	0	0

* For the *Science Museum 2* sample which did not dissolve in water, the value for the acetate percentage in this sample was obtained by comparison with the acetate standard in methanol.

5- Mordent analysis

Fe, Al, and Sn mordent identification was performed in three mauve-dyed textile samples (*Science Museum F4*, *Science Museum F6* and *Perth_Museum*) using ICP-AES (Inductively Coupled Plasma- Atomic Emission Spectroscopy). Prior to mordent quantification, the textiles were identified with optical microscopy through longitudinal view. The mordents analyses were performed in a Jobin-Yvon Ultima ICP-AES, with a RF 40, 68MHz generator and a Czerny-Turner 1,00m monochromator. The conditions used were: 1000kW potency; 12L/min of argon flow, Meinhard nebulisator with 3 bar pressure; pump velocity of 20rpm; 10ml/min of sample flow debit with three analyses for each sample. Before the ICP-AES injection, calibration curves were constructed with ICP standards and the correlation coefficients for the calibration curves were 0.99 for the range studied (0,2-1 ppm for iron and copper; 0.01-0.35ppm for aluminium). The results are summarized in **Table S9**.

Table S9. Mordent analysis of three mauve-dyed textile samples.

Sample/textile	Iron (mg) / textile (g)	Tin (mg)/ textile (g)	Aluminium (mg) / textile (g)
Science Museum F4 / cotton	1.20	10.06	-
Science Museum F6 / wool	5.59	-	5.89
Perth Museum / silk	1.06	-	-

The *Science Museum F4* sample displays tin as mordant, as expected, since in 1857 Perkin and Mr. Pullar started using tin to fix the mauveine dye in cellulose based textiles. With this method the mauveine dye would stand to the action of soap, contrary to the other dyeing recipes used for protein based textiles[3].

6- Mauveine from other sources: mauve-dyed textiles

Mauveine dyes from two books, namely a purple sample with blue shade (b*= -30.64±0.01) from 1926[5] (*JCE* 1926) and a purple sample from 2001[6] (DHA 2001) were extracted as described in **2- HPLC-DAD/LC-MS characterization of historical mauveine samples** prior to HPLC-DAD analysis, see **Table S10**. The HPLC-DAD chromatograms acquired at 550nm are presented in **Fig. S7**. In the *DHA 2001* sample, the major compound is Mauve A (38%); whereas in the tissue taken from *JCE 1926* volume the major compounds are mauveine C_{25}a + C_{25}b isomers (80%).

Table S10. Mauve-dyed textiles from two books (1926 and 2001) and respective colours according to CIE L* a* b* colour classification system.

Sample	Picture	Color		
		L*	a*	b*
JCE 1926[5]		31.24±0.03	24.69±0.00	-30.64±0.01
DHA 2001[6]		45.4±0.2	19.19±0.07	-20.92±0.06

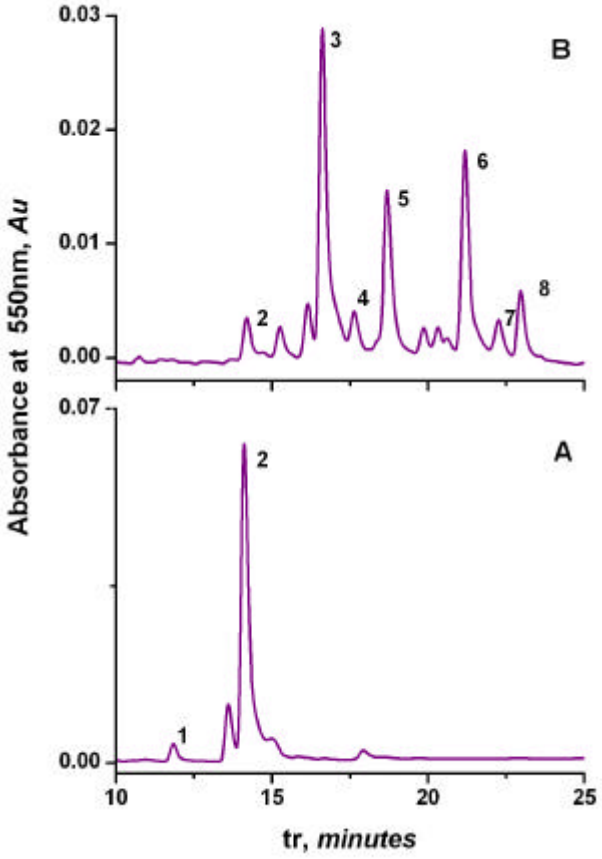


Figure S7. - Mauve-dyed textiles HPLC-DAD chromatograms obtained at λ≓ 551nm for **A**: JCE 1926, ref. [5]; **B**: DHA 2001, ref. [6]. All the samples were extracted with methanol with one drop of HCl (for more details see extraction methods described above). The major compounds identified correspond to the numbered peaks: **1**- pseudo-mauveine; **2**- mauveines C_{25a} + C_{25b}; **3**- mauveine A; **4**- mauveines B3 + B4; **5**- mauveine B2; **6**- mauveine B; **7**- mauveine C1; **8** - mauveine C. For more details see text and **Table S1** for structures.

Together with the mauveine salt from Schunk's collection, the fibre dyed with mauve from the 1926 library volume of the *Journal of Chemical Education* is the only sample where mauveine C_{25} compounds are present as major chromophores. The availability of this volume in numerous libraries makes it a standard for the analysis of mauveine-like compounds.

References

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