Synthesis and analysis by liquid chromatography-mass spectrometry of a mauveine composition similar to museum stored mauveine

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Key words: mauve, mauveine, liquid chromatography-mass spectrometry, silica gel

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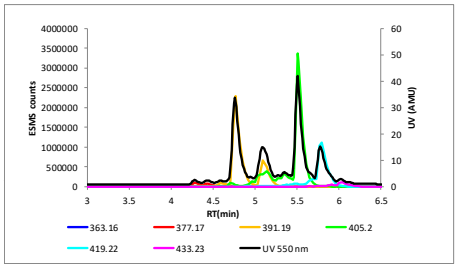
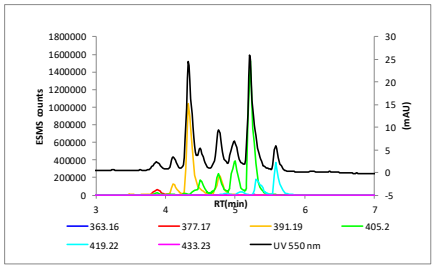
Mauveine similar to museum stored mauveine has been made by an improved method of purification.



Museum stored mauveine



New mauveine

Abstract

Our proposal that museum stored mauveine was made by a modified method compared to mauveine made by the William Henry Perkin (WHP) patented method of 1856 is exemplified. An improved method of purification allows greater resolution and separation of the different mauveine chromophores. LC-MS charts for museum stored mauveine are compared to the new mauveine reported here. The formation of deprotected mauveine chromophores and *N*-tert-butylated mauveine chromophores is discussed.

Introduction

Bottles of mauveine with provenance relating them to the WHP family are stored in the Science Museum,[1] Blythe House,[2] the Manchester Museum of Science and Industry,[3] the Bradford Colour Experience Museum,[4] Brent Museum and Archives[5] and the Chandler Museum, New York.[6] These samples are all very similar in composition containing a high proportion of mauveine A and B (Figure 1).[7] In addition to these samples there is a bottle of mauveine in the Manchester Museum of Science and Industry known as Schunck's mauveine and one in the Deutsche Museum in Germany which is known as Caro's mauveine.[8] These two samples are similar but different from the others and contain a high propotion of either pseudomauveine or a monomethylpseudomauveine chromophore.[9-10] There are two original patented methods for making mauveine. The WHP method of 1856 used $K_2Cr_2O_7$ as oxidant and the Caro method of 1860 used $CuCl_2$ as oxidant.[11-12] The purity or composition of the aromatic amines used in the reaction mixture can explain these differences.[7] WHP has used a toluidine rich mixture of aniline whereas Caro has used a purer grade of aniline. Both of these important figues in the aniline dye industry made mauveine as their first synthetic dye. Their

preserved samples of mauveine in museums came to light with pioneering analytical studies some time later.[13-15]

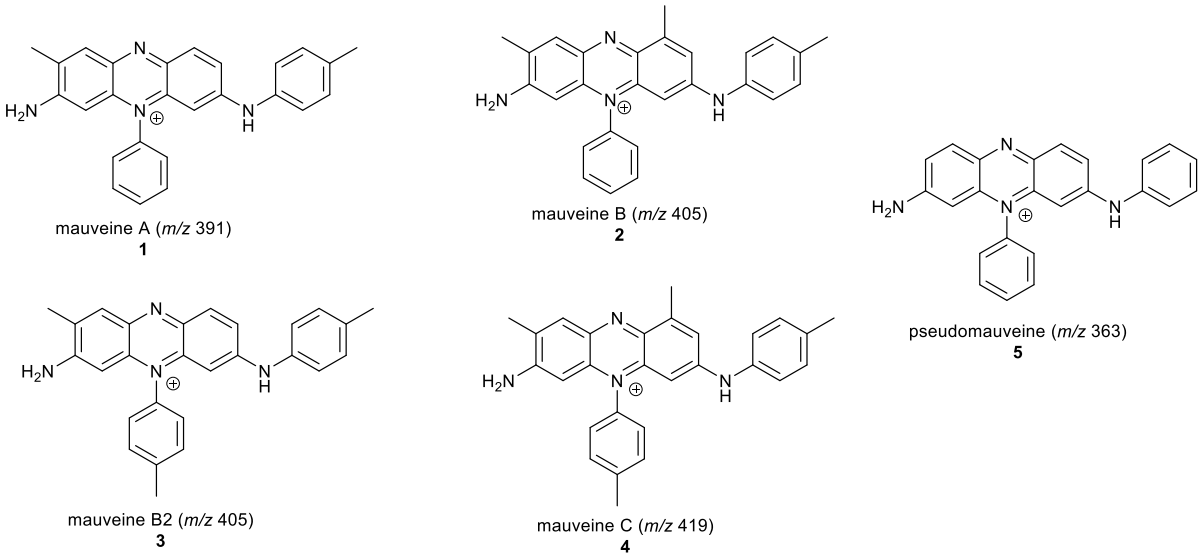


Figure 1 Chemical structures of mauveine A, B, B2, C and pseudomauveine

Figure 2 shows the liquid chromatography-mass spectrometry (LC-MS) chart for Manchester mauveine and Table 1 shows the corresponding table of retention times and molecular masses.[7] In the supplementary section Figures S1-S3 show an expanded chart and table for Manchester mauveine, a chart and table for Bradford mauveine and a chart and table for Sudbury mauveine, respectively.[7] The composition data arise from the peak areas in the LC-MS charts. The numbers by the short lines are the molecular weights of the mauveine chromophores which have been separated down a reversed-phase column. The wavelength of detection is 550 nm and mAU stands for milli absorption units.

The charts are useful for comparison and illustrate that the composition is rich in mauveine A and B with a much lower amount of other mauveine chromophores present. This has always surprised us because the chart in Figure 3 shows a typical mauveine composition which is obtained by making mauveine by the WHP patented method of 1856.[7] Rather than being rich in mauveine A and B, as would have been expected, it is rich in mauveine A, B2, B and C-four chromophores rather than two.[14] As we found it impossible to convert the former to the latter by removing two chromophores B2 and C from the mixture, we have always maintained that the WHP mauveine was made by a modified method of synthesis which WHP did not reveal.[16-18] Our studies showed that replacing *p*-toluidine with *N*-*tert*-butyl-*p*-toluidine, and subsequently deprotecting the mauveine chromophores from the *tert*-butyl group, gave a mixture of mauveine chromophores rich in mauveine A and B.[16-18] Our studies were reported with HPLC charts and were explained by assuming that capping the *p*-toluidine group blocks it from competing with aniline to form either mauveine B2 or mauveine C.[18] Hence only mauveine A and B dominate. A recent paper reported a synthesis of mauveine identical with the WHP 1856 Science Museum bottle of mauveine and the Chandler Museum mauveine from a *p*-toluidine rich mixture of arormatic amines.[19] At about the same time our paper was also published which showed using LC-MS that using a *p*-toluidine rich mixture of amines to make mauveine did not do this but instead gave a mauveine composition with no

mauveine A and B present but only mauveine B2 and C.[7] It appears that *p*-toluidine competes with aniline so instead of mauveine A, only mauveine B2 forms, and instead of mauveine B, only mauveine C forms. It suggests that a common trimeric intermediate is involved for each pathway.[7]

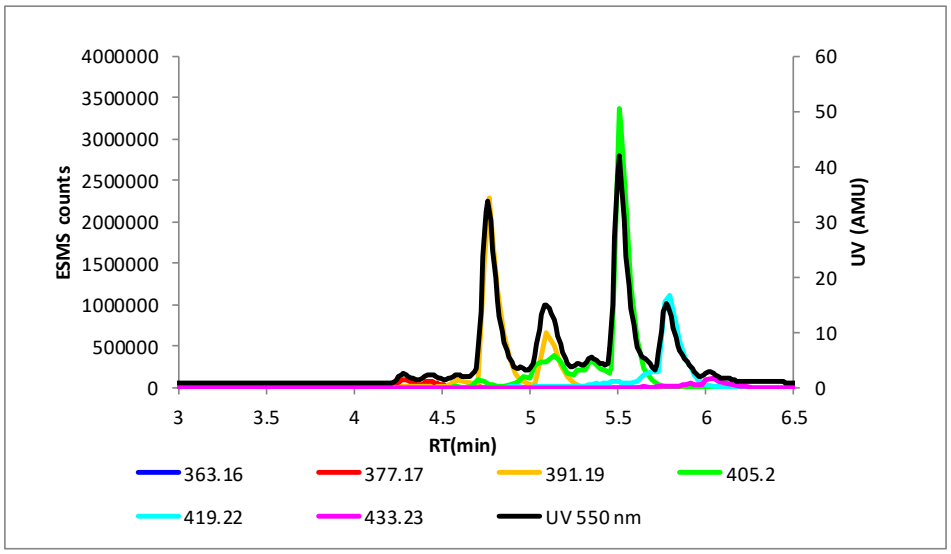


Figure 2 Mauveine from the Manchester Museum of Science and Industry. UV at 550 nm and extracted ion chromatograms

min	*m/z* 363	*m/z* 377	*m/z* 391	*m/z* 405	*m/z* 419	*m/z* 433
4.1						
4.3		1.0				
4.4						
4.7			1.2			
4.8			24.9	1.0		
5.1			9.0	3.8		
5.2				4.5		
5.4				3.9		
5.5				31.9	1.2	
5.7					2.2	
5.8					13.8	
5.9						
6.0						1.6

Table 1 Retention time and molecular mass for Figure 2.
m/z 377 is mauveine C25[15-16] and *m/z* 433 is mauveine D.[15]
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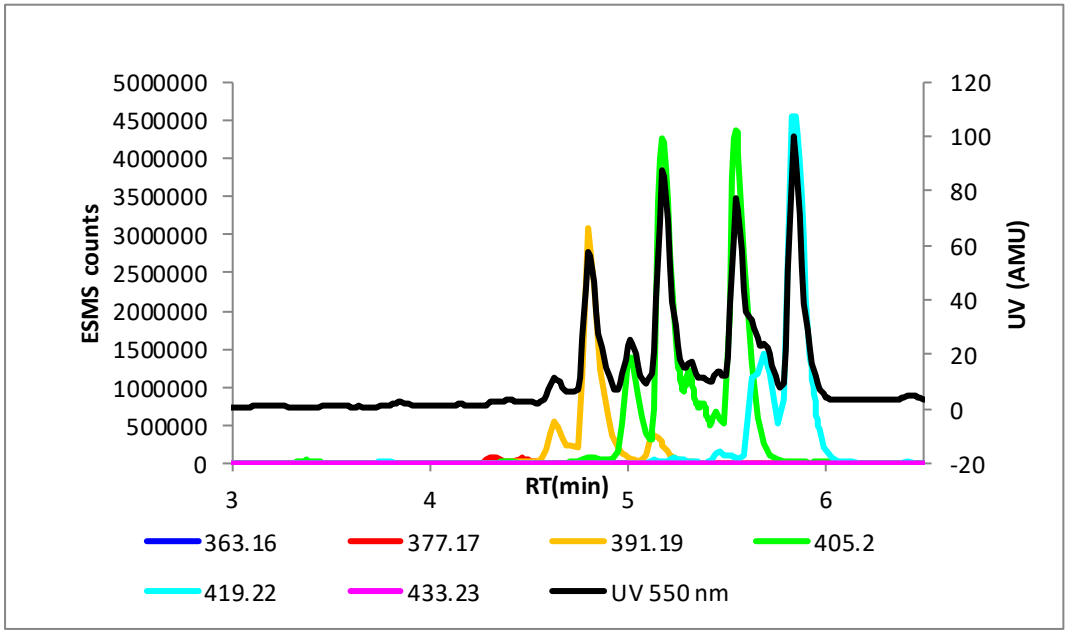

Figure 3 Mauveine made by the WHP method of 1856.[11] UV at 550 nm and extracted ion chromatograms.

min	*m/z* 363	*m/z* 377	*m/z* 391	*m/z* 405	*m/z* 419	*m/z* 433
4.6			3.4			
4.8			12.9			
5.0				6.3		
5.1			1.7	20.0		
5.3				3.2		
5.4				1.0		
5.5				1.5		
5.5				19.1		
5.6						
5.7					8.0	
5.8					22.9	

Table 2 Retention time and molecular mass for Figure 3.

Discussion

Figure 4 shows the structure of *N-tert*-butylmauveine A **6**, *N-tert*-butylmauveine B **7** and *N-tert*-butyl-*p*-toluidine hydrochloride **8** used in these studies.[16-17,20]

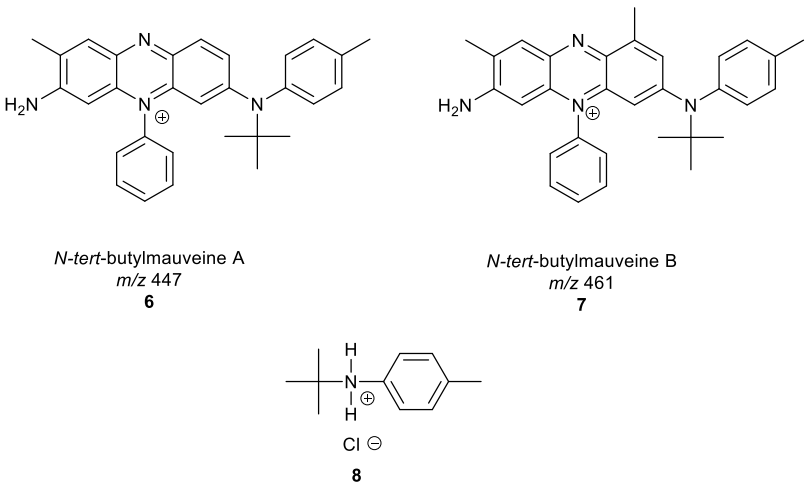

Figure 4 Chemical structures of *N*-*tert*-butylmauveine A **6**, *N*-*tert*-butylmauveine B **7** and *N*-*tert*-butyl-*p*-toluidine hydrochloride **8**.

Our general methods used for making mauveine have been reported previously.[16-18,20-21] Figure 5 shows a typical LC-MS chart for mauveine made using a modification of the WHP method in which *p*-toluidine was replaced with *N*-*tert*-butyl-*p*-toluidine hydrochloride **8** and the *tert*-butylated chromophores were deprotected. A typical composition is *N*-*tert*-butyl-*p*-toluidine hydrochloride **8**, aniline, o-toluidine (1.0:1.5:1.5) with $K_2Cr_2O_7$ as oxidant in dilute aqH_2SO_4. The crude precipitated product was purified by chromatography with aqNH_3/MeOH (20:80), evaporated to dryness, then treated with cHCl/MeOH (1.0:1.0) and evaporated to dryness. The chart shows the dominance of mauveine A and B as for museum stored mauveine. However, some differences remain which puzzled us. There are two peaks eluting before mauveine A, a monomethylpseudomauveine and a mauveine A isomer, which were always present along with a higher background of other minor chromophores. These appear to be much lower in the Bradford and Sudbury mauveines (Figure S2 and S3 in the supplementary section).[7] The intensity of the two peaks eluting before mauveine A has been partially attentuated by selectively removing some of the first fractions of mauveine from the chromatography column but it is difficult not to loose mauveine A this way and no colour changes in the fractions occur to help. Examination of the expanded chart of Manchester mauveine (Figure S1 in the supplementary section) reveals two peaks that look similar to these that have the same molecular weights and retention times. This provenance might be evidence of a similar method of synthesis although their identity is not proven in these mauveines.

After many experiments producing LC-MS charts similar to the chart shown in Figure 5, and failing to selectively get rid of minor chromophores, the solvent system of aqNH_3/MeOH (20:80) was changed to aqNH_3/EtOH (20:80). Figure 6 and 7 show the LC-MS of the mauveine made the same way as above. Firstly care was taken not to overload the column. The product splits into two bands on the column. Firstly, a purple band of deprotected mauveine chromophores elute first followed by a more polar red band of *tert*-butylated mauveine chromophores. The two coloured bands were striking after so many columns had been run with aqNH_3/MeOH as eluent where no separation like this occurs. Secondly, the first fractions off the column are redder and less clean and are easily identified and separated. By chromatography two easy points are available to influence the overall composition of the mixture from combining the bands, the beginning and in the middle when the red band begins

to elute. The separation occurs easily because of the difference in polarity of the *N-tert*-butylated chromophores compared with that of deprotected mauveine chromophores.

The supplementary section shows some typical photographs (Figure S4-S9) of the column. The LC-MS charts in Figures 6 and 7 are a better match to museum stored mauveine as the two peaks eluting before mauveine A are now very weak. Compared to Manchester mauveine only one weak B group peak, at a retention time of 5.0 min, is slightly higher in intensity.

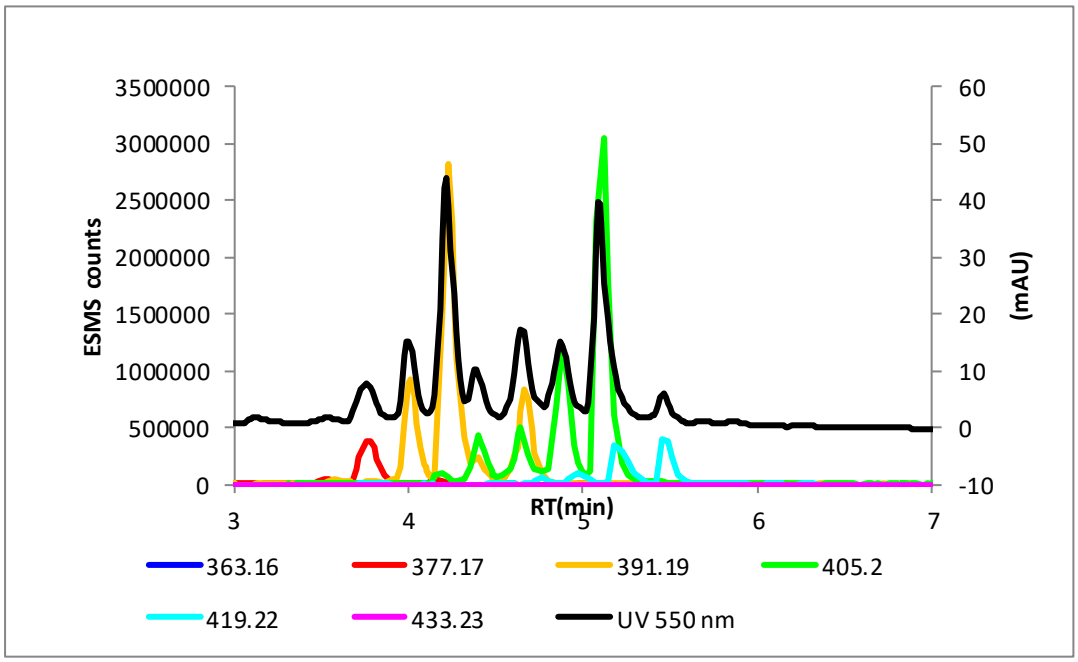

Figure 5 Mauveine made using a modification of the WHP method[11] in which *p*-toluidine is replaced with *N-tert*-butyl-*p*-toluidine hydrochloride **8**. The reaction precipitate was chromatographed with aqNH_3/MeOH prior to deprotection. UV at 550 nm and extracted ion chromatograms

min	*m/z* 363	*m/z* 377	*m/z* 391	*m/z* 405	*m/z* 419	*m/z* 433
3.8		4.3				
4.0			8.1			
4.2			21.9	1.1		
4.4			3.1	5.0		
4.6				5.5		
4.7			6.8			
4.9				10.1		
5.0					1.0	

5.1				26.1		
5.2					3.5	
5.5					3.5	

Table 3 Retention time and molecular mass for Figure 5.

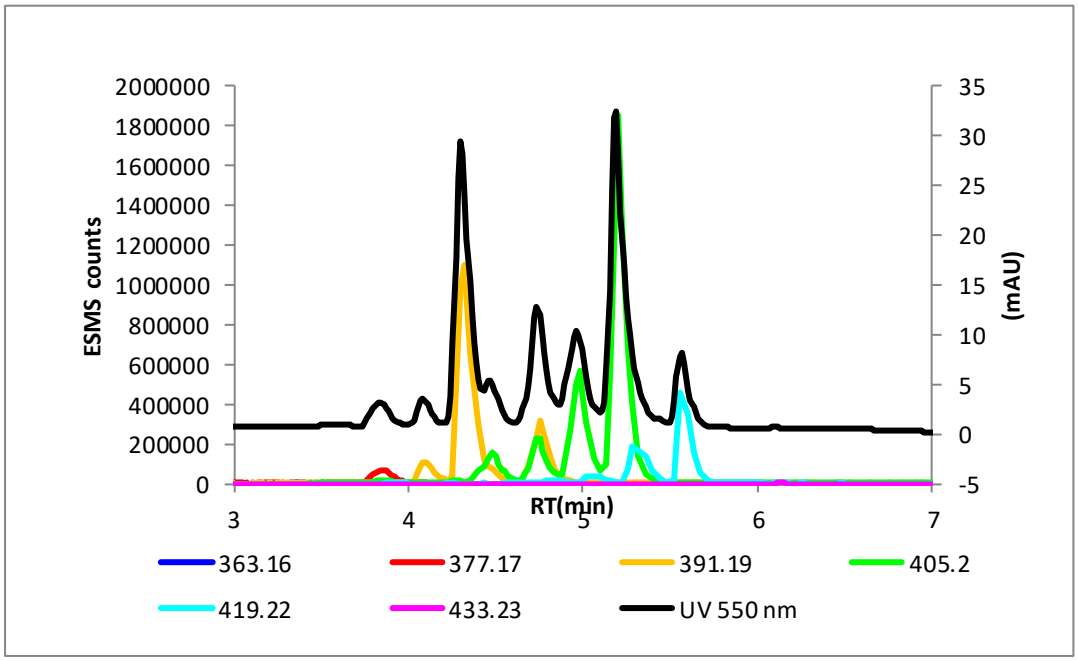

Figure 6 Mauveine made using a modification of the WHP method[11] in which *p*-toluidine is replaced with *N*-*tert*-butyl-*p*-toluidine hydrochloride **8**. The reaction precipitate was chromatographed with aqNH_3/EtOH prior to deprotection. UV at 550 nm and extracted ion chromatograms

min	*m/z* 363	*m/z* 377	*m/z* 391	*m/z* 405	*m/z* 419	*m/z* 433
3.8		1.9				
4.1			2.3			
4.3			22.9			
4.5				3.3		
4.7				5.1		
4.8			6.7			
5.0				9.1	1.1	
5.2				32.4		
5.3					4.3	
5.6					6.7	

Table 4 Retention time and molecular mass for Figure 6.

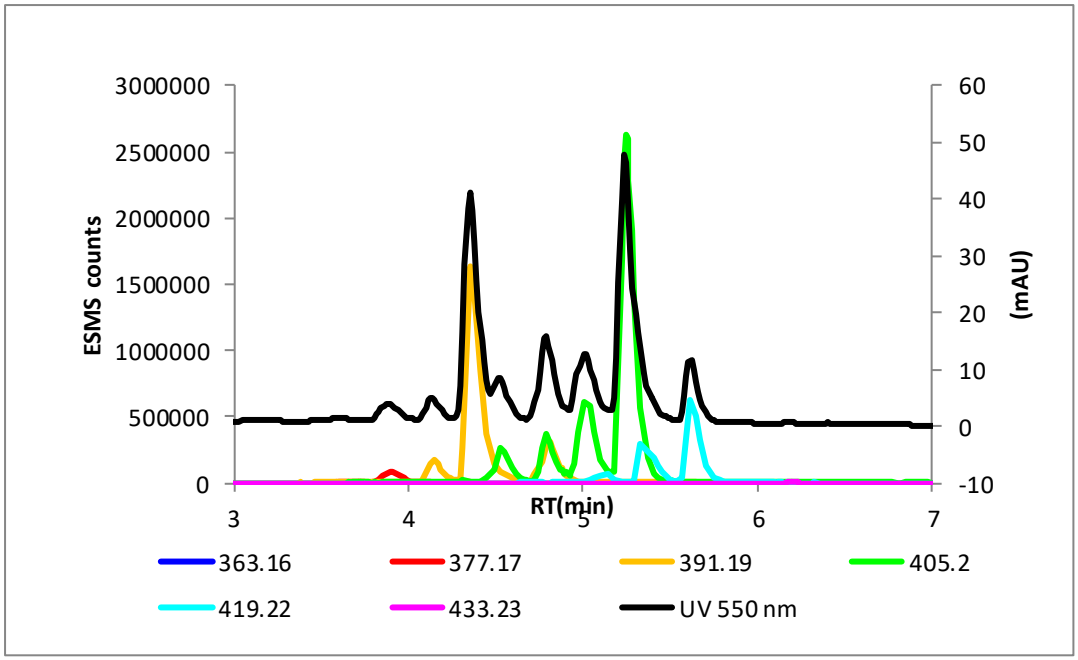

Figure 7 Mauveine made using a modification of the WHP method[11] in which *p*-toluidine is replaced with *N*-*tert*-butyl-*p*-toluidine hydrochloride **8**. The reaction precipitate was chromatographed with aqNH_3/EtOH prior to deprotection. UV at 550 nm and extracted ion chromatograms

min	*m/z* 363	*m/z* 377	*m/z* 391	*m/z* 405	*m/z* 419	*m/z* 433
3.9		1.6				
4.1			2.4			
4.4			22.5			
4.5				3.7		
4.8			4.7	5.4		
5.0				9.8		
5.1					1.1	
5.2				35.9		
5.3					4.9	
5.6					8.0	

Table 5 Retention time and molecular mass for Figure 7.

The purple and red bands, separated using aqNH_3/EtOH eluent on a silica gel column, were also collected as separate fractions and analysed by LC-MS. Figure 8 shows the LC-MS chart for the more polar red band. It is as expected largely *N*-*tert*-butylated mauveine chromophores. *N*-*tert*-butylmauveine B (*m/z* 461) **7** is the most dominant then *N*-*tert*-butylmauveine A (*m/z* 447) **6**. This region of 5.8-7.0 min is the correct region for these compounds to elute in whereas mauveine chromphores usually elute in the region 4.0-5.6

min. Previously we characterised these compounds with difficulty having to run multiple numbers of columns and collecting fractions to get good NMR spectra.[20] Figure 9 shows a typical LC-MS chart which is obtained after treating the mixture of *N*-*tert*-butylmauveine chromophores with cHCl/MeOH. As expected mauveine A (*m/z* 391) **1** is quite weak and mauveine B (*m/z* 405) **2** dominates. Figure 10 shows the purple band before acid treatment although it is essentially unchanged after acid treatment. Mauveine A **1** is the major deprotected chromophore but some of mauveine B **2** and others are also present.

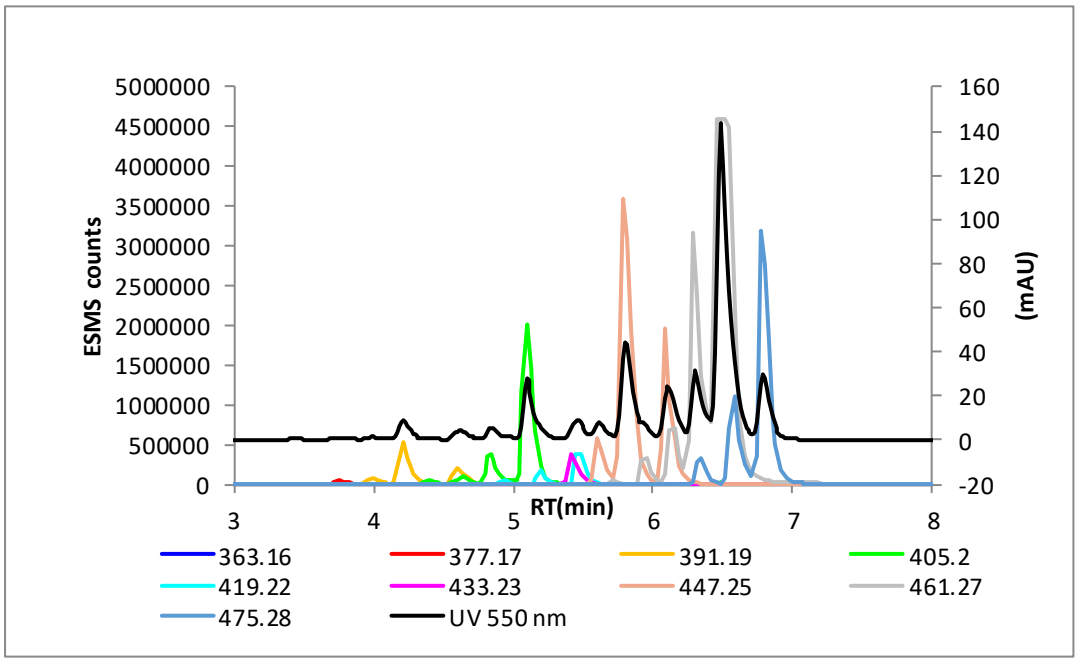

Figure 8 Mauveine made using a modification of the WHP method[11] in which *p*-toluidine is replaced with *N*-*tert*-butyl-*p*-toluidine hydrochloride **8**. The reaction precipitate was chromatographed with aqNH_3/EtOH giving a purple and a red band. The more polar red band shown here primarily consists of *N*-*tert*-butylated mauveine chromophores. UV at 550 nm and extracted ion chromatograms.

min	*m/z* 363	*m/z* 377	*m/z* 391	*m/z* 405	*m/z* 419	*m/z* 433	*m/z* 447	*m/z* 461	*m/z* 475
4.2			2.0						
4.6			0.9						
4.8				1.5					
5.1				7.4					
5.4						1.3			
5.5					1.6				
5.6							2.0		
5.8							13.6		
5.9								1.3	
6.1							6.3	2.8	
6.3								9.8	1.1

6.5								32.8	
6.6									4.3
6.8									11.3

Table 6 Retention time and molecular mass for Figure 8.

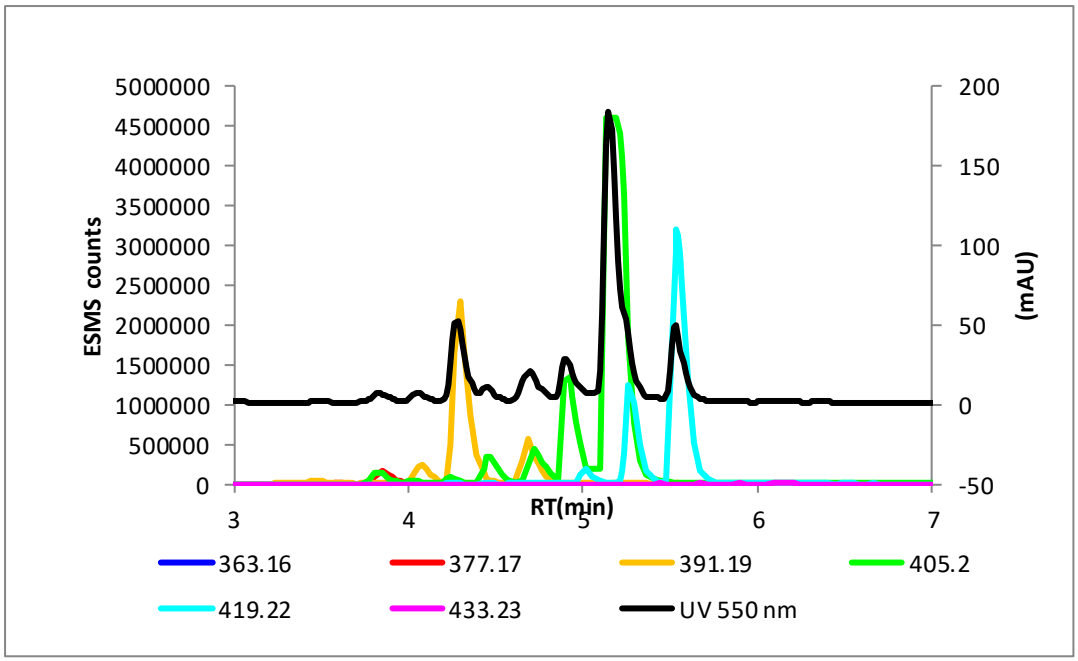

Figure 9 Mauveine made using a modification of the WHP method[11] in which *p*-toluidine is replaced with *N*-*tert*-butyl-*p*-toluidine hydrochloride **8**. The reaction precipitate was chromatographed with aqNH_3/EtOH allowing separation of a red band primarily consisting of *N*-*tert*-butylated mauveine chromophores which were deprotected and are shown here. Compare to Figure 10. UV at 550 nm and extracted ion chromatograms

min	*m/z* 363	*m/z* 377	*m/z* 391	*m/z* 405	*m/z* 419	*m/z* 433
3.8		1.1				
4.1			1.5			
4.3			14.4			
4.5				2.0		
4.7			3.4	2.8		
4.9				7.7		
5.0					1.0	
5.2				44.3		
5.3					6.4	
5.5					16.7	

Table 7 Retention time and molecular mass for Figure 9.

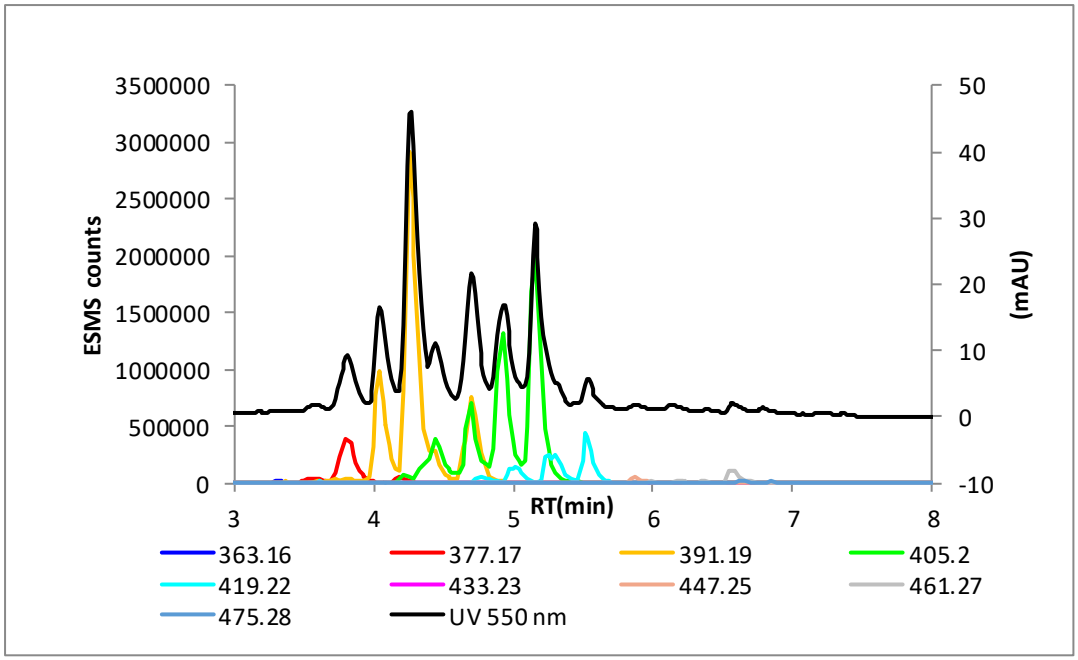

Figure 10 Mauveine made using a modification of the WHP method[11] in which *p*-toludine is replaced with *N*-*tert*-butyl-*p*-toluidine hydrochloride **8**. The reaction precipitate was chromatographed with aqNH_3/EtOH firstly eluting a purple band primarily consisting of deprotected mauveine chromophores which are shown here. UV at 550 nm and extracted ion chromatograms

min	*m/z* 363	*m/z* 377	*m/z* 391	*m/z* 405	*m/z* 419	*m/z* 433	*m/z* 447	*m/z* 461	*m/z* 475
3.8		4.5							
4.0			9.2						
4.3			26.1	1.9					
4.4			1.5	4.4					
4.7			7.4	6.3					
4.9				12.4					
5.0					1.5				
5.1				17.6					
5.3					3.1				
5.5					3.1				
6.6								1.0	

Table 8 Retention time and molecular mass for Figure 10.

The greater quantity of *N*-*tert*-butylmauveine B **7** in the red band might occur if an intermediate that leads to *N*-*tert*-butylmauveine A **6** is less stable than an intermediate which can lead to *N*-*tert*-butylmauveine B **7**. Figure 11 proposes two such intermediates. The extra methyl group in intermediate **10** will help to stabilise the molecule to loss of the *tert*-butyl group by inductively stabilising the positive charge. Resonance stabilisation of the charge is shown in resonance form **11**. At the bottom a scheme is drawn showing how the *tert*-butyl group might fragment from intermediate **9** to give compound **12** and the *tert*-butyl cation **13**. Compound **12** is an oxidised form of a similar precursor we used to make pseudomauveine **5**[21] so would be expected to continue mauveine synthesis. The use of the *tert*-butyl group in the starting material *N*-*tert*-butyl-*p*-toluidine hydrochloride **8** was not wasted because *N*-alkyl-*p*-toluidines are more electron rich than *p*-toluidine and oxidise faster.[22]

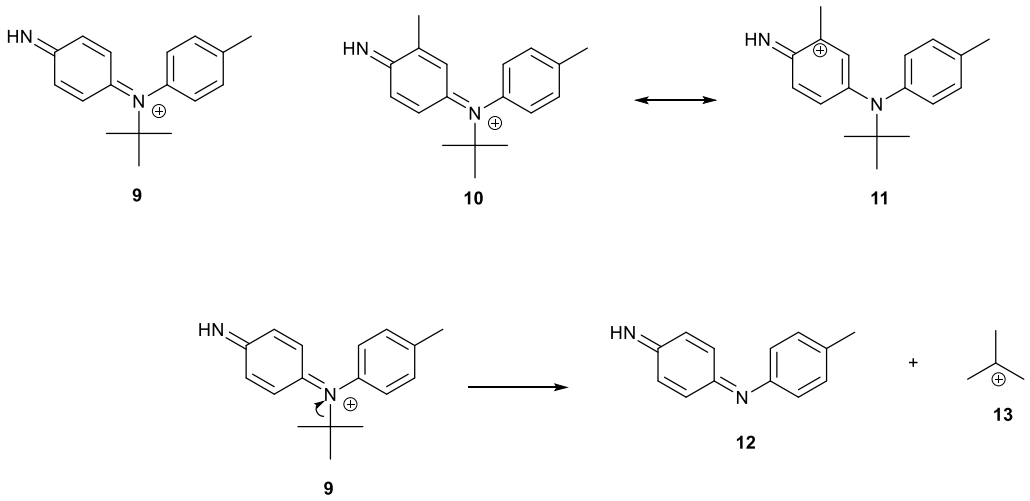

Figure 11 Top: Chemical structures of two possible intermediates **9** and **10** which might deprotect loosing a *tert*-butyl group. Bottom: A scheme showing how the *tert*-butyl group might be fragmented from compound **9**.

The possible use of silica gel to purify mixtures of protected and deprotected mauveine chromophores in the WHP Greenford factory should not be ruled out because of these results and because silica gel was known in the 17th century and earlier.[23]

Conclusion

The purification of crude mauveine has been improved by changing the eluent of silica gel chromatography from aqNH_3/MeOH to aqNH_3/EtOH. EtOH is less polar and the mauveine chromophores elute more slowly. Mauveine chromophores, deprotected in the reaction, are separated from *N*-*tert*-butylmauveine chromophores. This chromatography allows two minor deprotected chromophores, running ahead of mauveine A, to be largely separated. The molecular weights indicate these are a monomethylpseudomauveine chromophore and an unknown mauveine A isomer.[24] The final mauveine is a good match to museum stored mauveine since it is rich in mauveine A and B and lacks these two minor chromophores in it in any appreciable quantity (Figure 6 and 7).

Experimental

LC-MS

For analytical separation an Agilent 1290 Infinity HPLC system consisting of a quaternary HPLC pump, cooled auto sampler compartment, column compartment and diode-array UV-VIS detector was used. A Gold C-18 column (2.1 x 150 mm, Thermo Scientific, UK) was used for separation with a water/methanol gradient (both 0.1% v/v formic acid) from 40% MeOH to 100% in 7 min. The flow rate was 0.5 mL min–1, column temperature 40 ºC and sample volume 5 µL. The mass spectrometer (ES-MS) used was a MAXIS II UHR-TOF LC-MS System (Bruker UK Ltd) with ESI source connected to the UV-VIS detector by a short length of Peek-tubing. The ES-MS was operated in positive ion mode with a capillary voltage of 4.5 kV using sodium formate clusters for calibration and methyl stearate as lock-mass. Mass spectra were recorded automatically.

General procedure The general methods used here have been reported previously.[16-18,20] The change of eluent from aqNH_3/MeOH to aqNH_3/EtOH slows down the column and improves the separation. When handling conc. aqNH_3 ALWAYS hold the winchester close to the entrance of the fume hood which must be switched on. cH_2SO_4 is always dispensed dropwise from 15 cm Fisherbrand pasteur pipettes as it is a simple and quick method for small scale work and is reliable.

Typical synthesis *N*-*tert*-Butyl-*p*-toluidine hydrochloride **8** (187 mg, 0.94 mmol), aniline (131 mg, 1.4 mmol) and *o*-toluidine (150 mg, 1.4 mmol) [1.0:1.5:1.5] were dissolved in water (100 ml) at 60 ºC acidulated with 7 drops of cH_2SO_4. $K_2Cr_2O_7$ (500 mg, 1.7 mmol) [1.7 eq] was then added and the mixture stirred for 5 h. After cooling it was filtered through a fine pore sinter, washed with water (200 ml) then extracted with MeOH (60 ml x5) in the sinter. The combined extracts were evaporated to dryness then purified by chromatography on silica gel. Typically part of the mixture, from a half to a quarter, is applied to the column in MeOH and initially eluted with MeOH to remove much of the precipitate by-product. The products were then eluted with aqNH_3/EtOH (20:80). A purple band elutes first which was shown by LC-MS to be deprotected mauveine chromophores followed by a red/pink band which was shown by LC-MS to be *tert*-butylated mauveine chromophores. The LC-MS charts are discussed in the text and appear in Figures **6** and **7**. Discarding some early fractions of the first purple band, approximately 50 ml, helps to improve the purity of the final mauveine by largely removing the first two deprotected mauveine chromophores which run before mauveine A in the charts. This is done carefully and is monitored by eye. Much of the mauveine is more polar as it is *tert*-butylated which helps the separation. For the purple band the supplementary section has pictures showing the colour changes which occur when the first two minor chromphores running ahead of mauveine A are removed and discarded. The two bands (purple and red) were combined and deprotected with cHCl/MeOH (50:50) by heating to dryness. The final LC-MS charts are presented in Figures **6** and **7**. The purple and red fractions can be separated and dried then treated with acid to deprotect them, although the purple band contains very little *tert*-butylated mauveine chromophore. Yields depend upon the purity from precipitate by-product and are typically in the range 2-4%. Here we have not done base precipitation cycles but that can be done to remove inorganics and baseline. For a cycle the product is treated with boiling water, then KOH to precipitate product, filtration, extraction of the filtered material with MeOH then acidify with a few drops of cHCl and evaporate.

Electronic Supplementary Information

Three LC-MS charts (Fig. S1-S3**)**, three tables of mauveine peak integrations (Tab. S1-S3) and six photographs illustrating the separation of coloured mauveine chromophores (Fig. S4-S9).

Acknowledgement
We are grateful to the EPSRC National Mass Spectrometry Foundation (NMSF) for mass spectra.

References

1. Science Museum, Exhibition Road, South Kensington, London SW7 2DD
2. Blythe House Archive, 23 Blythe Rd, London, W14 0QX
3. Manchester Museum of Science and Industry, Liverpool Rd, Manchester M3 4FP
4. Colour Experience Museum, Providence St, Bradford, BD1 2PW
5. Brent Museum and Archives, Willesden Library Centre, 95 High Rd, London, NW10 2SF
6. Chandler Museum, Columbia University, Department of Chemistry, Havemeyer Hall, New York, 10027
7. M. J. Plater and A. Raab, *J. Chem. Res*., 2017, **41**, 441.
8. Deutsche Museum, Museumsinsel 1, 80538, München, Germany
9. M. C. Oliveira, A. Dias, P. Douglas and J. S. S. Melo, *Chem. Eur*., 2014, **20**, 1808.
10. M. J. Plater and A. Raab, *J. Chem. Res*., 2015, **39**, 180.
11. W. H. Perkin, G. B. Patent 1984, issued 26th August 1856.
12. J. Dale and H. Caro, G. B. Patent 1307, issued 26th May 1860
13. O. Meth-Cohn and M. Smith, *J. Chem. Soc., Perkin Trans.*, I 1994, 5.
14. J. Seixas de Melo, S. Takato, M. Sousa, M. J. Melo and A. J. Parola, *Chem. Commun.*, 2007, 2624.
15. M. M. Sousa, M. J. Melo, A. J. Parola, P. J. T. Morris, H. S. Rzepa and J. Sergio Seixas de Melo, *Chem. Eur. J*., 2008, **14**, 8507
16. M. J. Plater, *J. Chem. Res*., 2014, **38**, 163.
17. M. J. Plater and W. T. A. Harrison, *J. Chem. Res*., 2014, **38**, 651.
18. M. J. Plater, *J. Chem. Res*., 2015, **39**, 180.
19. T. F. Cova, A. A. Pais and J. S. S. Melo, *Scientific Rep*. 2017, **7**, 6806.
20. M. J. Plater, W. T. A. Harrison and H. S. Rzepa, *J. Chem. Res*., 2015, **39**, 711.
21. M.J. Plater, *J. Chem. Res*., 2011, 304.
22. M.J. Plater, *J. Chem. Res*., 2013, **37**, 427.
23. M. Feldman and P. Desrochers, *Industry and Innovation*, 2003, **10**, 5. (pp 18).
24. M. J. Plater and A. Raab, *J. Chem. Res*, 2016, **40**, 648.