Available online at www.sciencedirect.com

International Journal of Mass Spectrometry 232 (2004) 225–229

www.elsevier.com/locate/ijms

Matrix-assisted laser desorption and electrospray ionization mass spectrometry of carminic acid isolated from cochineal

Marta S. Maier*, Sara D. Parera, Alicia M. Seldes[1]

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales,
Universidad de Buenos Aires, Ciudad Universitaria, 1428 Buenos Aires, Argentina

Received 13 October 2003; accepted 1 December 2003

Abstract

Carminic acid, isolated from cochineal, was analyzed by matrix-assisted laser desorption/ionization (MALDI) and electrospray mass spectrometry (ESI-MS). Application of both techniques to the analysis of carminic acid suspended in linseed oil and applied to a piece of canvas, demonstrated the ability of MALDI and ESI-MS to identify this organic dye in a mixture as those used in easel painting.

© 2003 Elsevier B.V. All rights reserved.

Keywords: Carminic acid; Cochineal; Matrix-assisted laser desorption; Electrospray ionization

1. Introduction

Carmine is an organic violet-red colorant obtained from cochineal, the dried bodies of the female scale insect *Coccus cacti* (*Dactylopius coccus* Costa). Cochineal was the main red dye used in Central America before the Spanish conquest and during the 16th century it was imported into Europe and began to replace the domestic kermes as an artist's pigment and for dyeing. Due to its fugitive nature, carmine is now used principally in cosmetics and in student-grade artists' paints [1]. Carminic acid (**1**), an hydroxyanthraquinone linked to a glucose unit (Fig. 1) is the coloring principle of the cochineal extract. This anthraquinone glucoside has been shown to be a potent deterrent to ants [2] and dietary-carminic acid has been used for defense by larvae of insects [3]. Recently, the first synthesis of carminic acid has been reported [4].

Carminic acid has been quantitatively determined by HPLC in foods [5] and identified by reversed-phase HPLC in an extract from silk fibers from an archeological textil [6]. UV-Vis spectrophotometric analysis has shown to be suitable for the identification of this dye in wool fibers [7]. Recently, carmine alum lake has been identified by negative ion laser desorption mass spectrometry in a mixture of the lake suspended in linseed oil and applied to paper [8].

As part of our research on pigments used in South American colonial paintings we have recently reported the identification of a carmine lake in three paintings by hydrolysis of the lake and further TLC of carminic acid on acetylcellulose [9]. These results enabled us to search for new analytical methods that could allow us to identify carminic acid on different supports without previous application of extraction techniques.

In this work, we report the results from MALDI and ESI-MS analysis of carminic acid isolated from cochineal and of an artificially aged sample of carminic acid suspended in linseed oil, applied on a piece of canvas and covered with dammar resin.

2. Experimental

Cochineal *C. cacti* were purchased from Kremer Pigmente, Aichstetten, Allgäu, Federal Republik of Germany. Cochineal was extracted with hot water and carmine lake was precipitated from the aqueous solution by addition of lead acetate. Carminic acid (**1**) was obtained by hydrolysis of the lake in methanolic sulfuric acid solution [10]. Further

---

* Corresponding author. Tel.: +54-11-4576-3346; fax: +54-11-4576-3346.
E-mail address: maier@qo.fcen.uba.ar (M.S. Maier).

[1] Deceased, January 23, 2003.

1387-3806/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2003.12.008

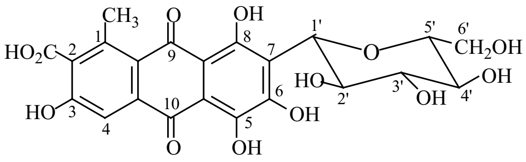

Fig. 1. Chemical structure of carminic acid.

purification by CC (column chromatography) over Sephadex LH 20 afforded pure **1**, identified by comparison with previous IR, ^{1}H and ^{13}C NMR spectral data [4]. TLC was performed on precoated cellulose F_{254} (AcOEt/THF/H_2O (0.6:3.5:4.7 v/v/v)). ^{1}H and ^{13}C NMR spectra were recorded in $(CD_3)_2SO$ in a Bruker ACE-200 spectrometer. The IR spectrum was obtained on a Nicolet Magna-550 FTIR spectrophotometer. LSIMS (positive ion mode) was performed on a VG ZAB-SE magnetic sector mass spectrometer. The primary ion beam was supplied by a CsI gun (10 keV, Cs^+). The sample was dissolved in 3-nitrobenzylalcohol matrix. Matrix-assisted laser desorption/ionization (MALDI, negative ion mode) spectra were performed on a PE Biosystems Voyager DE time-of-flight mass spectrometer. The samples were dissolved in α-cyano-4-hydroxycinnamic acid and run in normal mode (no reflection). Electrospray ionization mass spectrometry was performed on a Micromass Q-TOF Ultima instrument operated at a resolution of 10,000 in reflectron mode. The samples were dissolved in an infusion solution of MeOH/H_2O/AcOH (50:49:1 v/v/v).

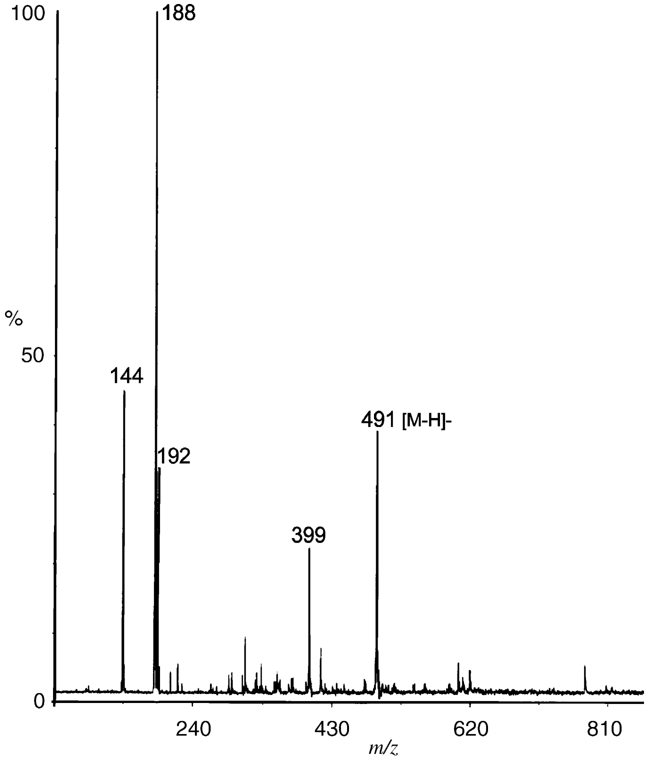

Fig. 2. MALDI mass spectrum (negative ion mode) of carminic acid (**1**).

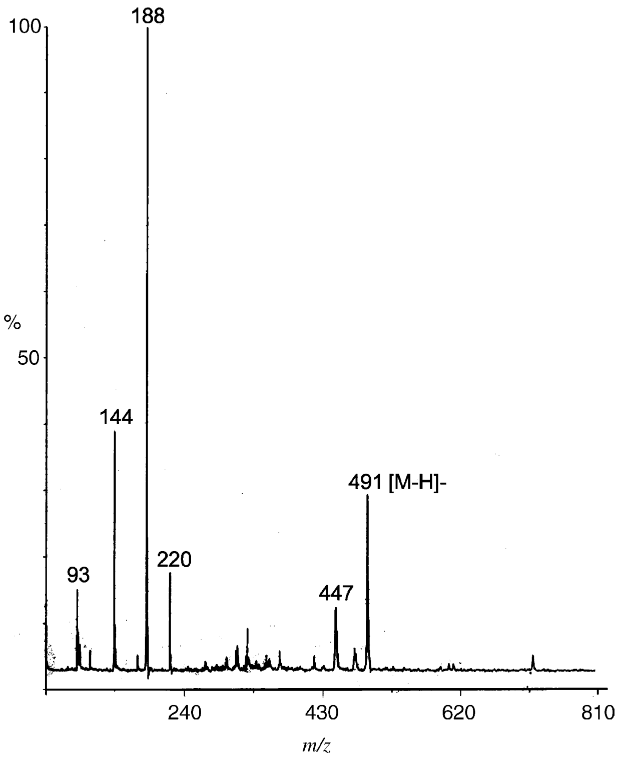

Fig. 3. MALDI mass spectrum (negative ion mode) of **1** applied on a piece of canvas.

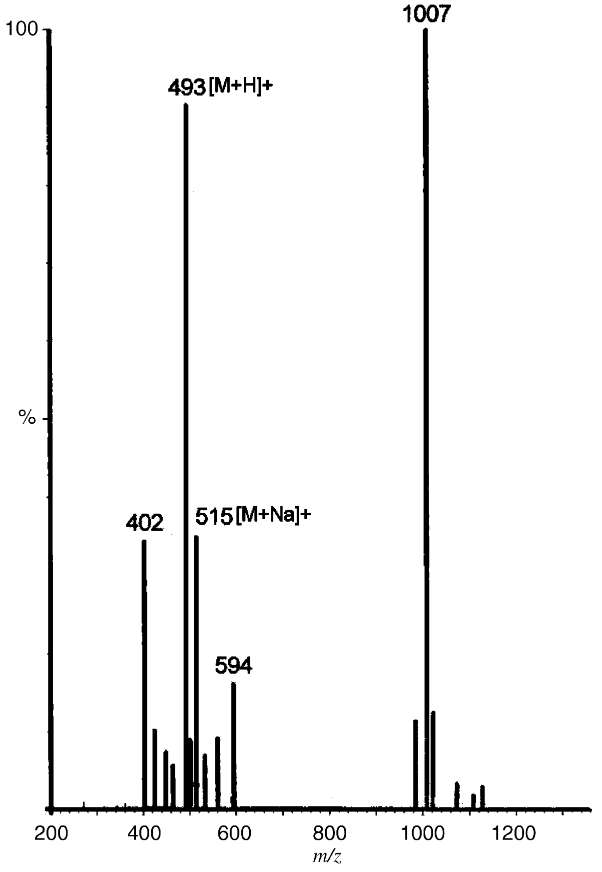

Fig. 4. ESI mass spectrum (positive ion mode) of carminic acid (**1**).

3. Results and discussion

Several modern mass spectrometry techniques have been explored to characterize natural organic pigments and dyes. The chemical identification of very low quantities of these substances traditionally used as dyes in textiles and as lakes in artists' paints is important in the fields of art history, conservation and restoration and can be helpful in their dating. Laser desorption mass spectrometry (LDMS) has shown to be a useful tool for analyzing flavonoids in traditional yellow pigments used as dyes [11] and for the analysis of carmine alum lake on paper [8]. Naphthaquinone pigments, characteristic of *Alkanna tinctoria* Tausch have been identified by MALDI [12]. A mixture of laccaic acids produced by the lac insect *Kerria lacca* Kerr and used as a red pigment in easel painting has been successfully analyzed by HPLC/electrospray mass spectrometry [13].

Carminic acid (**1**) is a polar non-volatile low-mass molecule difficult to be analyzed by the traditional method of electron impact mass spectrometry. Liquid secondary ion mass spectrometry (positive ion mode) of **1** using 3-nitrobenzylalcohol/Li as matrix yielded a weak lithiated pseudomolecular ion at *m/z* 499 $[M + Li]^+$. Therefore, we decided to evaluate whether matrix-assisted laser desorption and electrospray ionization mass spectrometry could be used as rapid and simple techniques to identify carminic acid. Since this pigment has been found in paintings mixed with a medium, we dissolved **1** in linseed oil, applied it on a piece of canvas and covered it with dammar resin. Further exposure to 50 °C [14] for 8 h and daylight for three weeks yielded an artificially aged sample of carminic acid that was analyzed by MALDI and ESI-MS.

Matrix-assisted laser desorption mass spectra of **1** were run in positive and negative ion modes. The most effective mode was shown to be that of negative ion (Fig. 2). The spectrum was characterized by a relatively strong pseudo-molecular ion at *m/z* 491 $[M - H]^-$ together with ions at *m/z* 188 and 144 corresponding to the α-cyano-4-hydroxycinnamic acid used as MALDI matrix. Peak at *m/z* 399 could presumably be due to an impurity. The negative ion MALDI spectrum of an artificially aged sample of carminic acid (**1**) applied on a piece of canvas (Fig. 3) showed the $[M - H]^-$ pseudo-molecular ion of **1** at *m/z* 491 and a peak at *m/z* 447 representing the neutral loss of CO_2 from the deprotonated molecule, allowing the identification of the pigment in the mixture of linseed oil and dammar resin. Ions different from 447 and 491, and those corresponding to the MALDI matrix, could be due to the components of dammar resin and linseed oil.

Electrospray is a soft ionization technique suitable for the detection of very small amounts of polar molecules that contain functional groups capable of charge retention. Analysis of carminic acid (**1**) by electrospray mass spectrometry in positive ion mode gave a spectrum (Fig. 4) that displayed

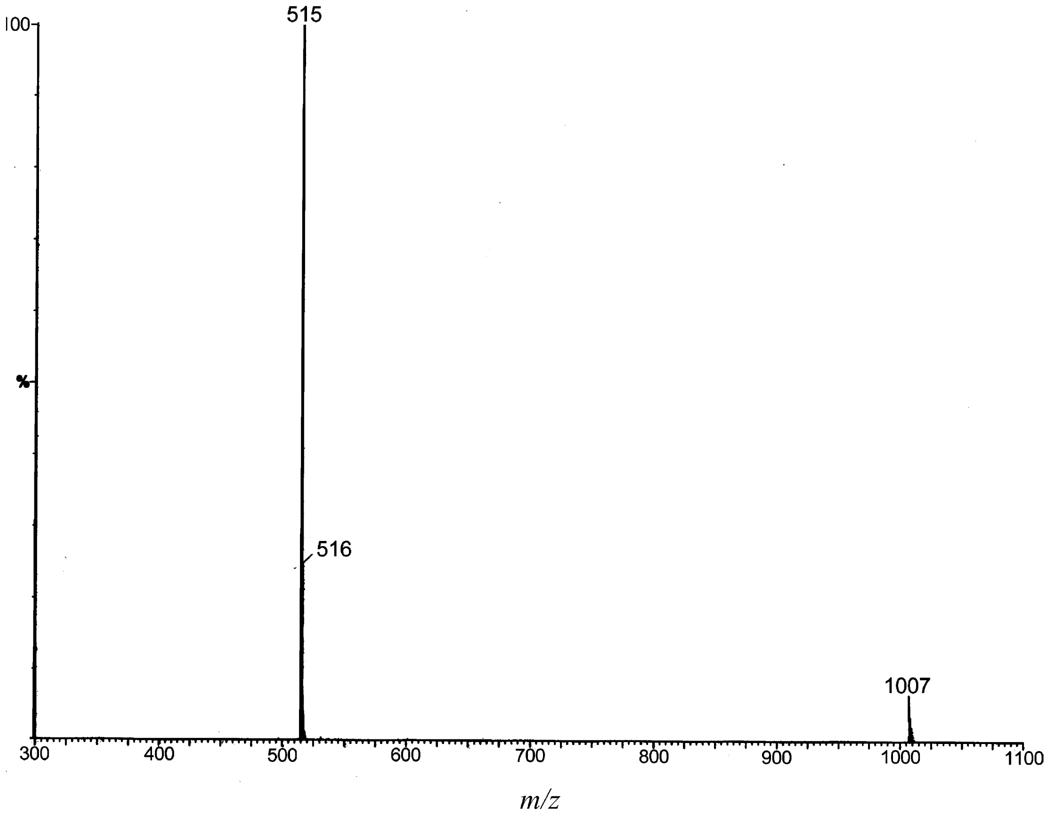

Fig. 5. MS-MS spectrum of sodium cluster dimer at 40 eV.

two pseudo-molecular ions at *m/z* 493 $[M + H]^+$ and 515 $[M + Na]^+$ together with a few fragment ions at *m/z* 402, 424, 559 and 594, and an intense peak at *m/z* 1007. MS-MS experiments of the isolated protonated molecule (*m/z* 493) of **1** at different collisional energies showed that at 20 eV the MS-MS spectrum displayed the peak at *m/z* 493 together with fragment ions due to successive losses of water at *m/z* 475, 457, 439, 427, 397, and a base peak at *m/z* 373 $[M-CO_2H-2CO-H_2O]^+$. At 40 eV, the pseudo-molecular ion at *m/z* 493 was no longer observed and the spectrum displayed fragment ions at *m/z* 421 $[M+H-4H_2O]^+$ and 403 $[M+H-5H_2O]^+$, and a base peak at *m/z* 355 $[M-CO_2H-2CO-2H_2O]^+$. MS-MS experiments of the isolated ion at *m/z* 1007 at 40 eV (Fig. 5) showed the sodiated molecular ion at *m/z* 515 as the predominant peak, confirming that the ion at *m/z* 1007 is a sodiated dimer cluster of **1**. The electrospray ionization mass spectrum in positive ion mode of the artificially aged sample of carminic acid (**1**) (Fig. 6) showed the protonated molecular ion at *m/z* 493 together with a peak at *m/z* 512, presumably due to the presence of the species $[M+2H+H_2O]^+$ and fragment ions at *m/z* 415 $[M+Na-CO_2-2CO]^+$ and 257 $[M+H-162-2CO-H_2O]^+$. The ESI-MS in the negative ion mode of artificially aged **1** gave a very clean spectrum (Fig. 7) displaying a strong pseudo-molecular ion at *m/z* 491 $[M-H]^-$. MS-MS experiment of the isolated pseudo-molecular ion at *m/z* 491 at a collisional energy of 20 eV (Fig. 8) gave this peak together

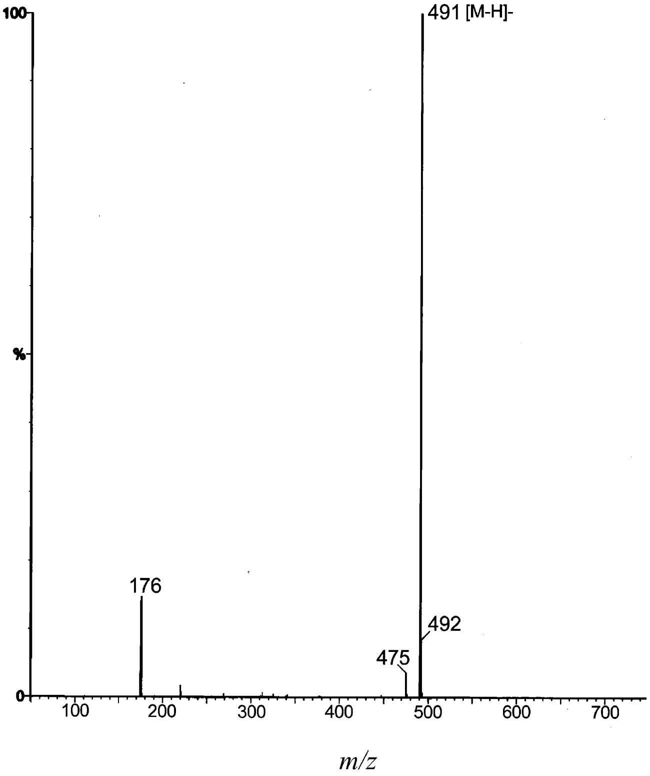

Fig. 7. ESI mass spectrum (negative ion mode) of **1** applied on a piece of canvas.

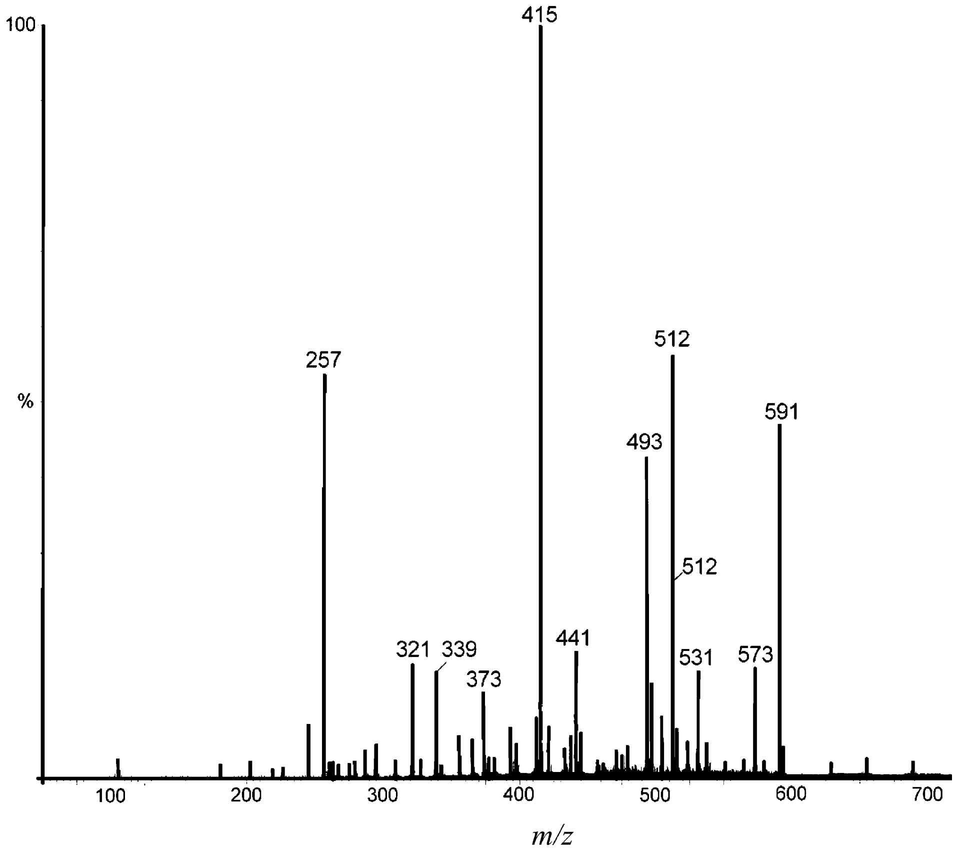

Fig. 6. ESI mass spectrum (positive ion mode) of **1** applied on a piece of canvas.

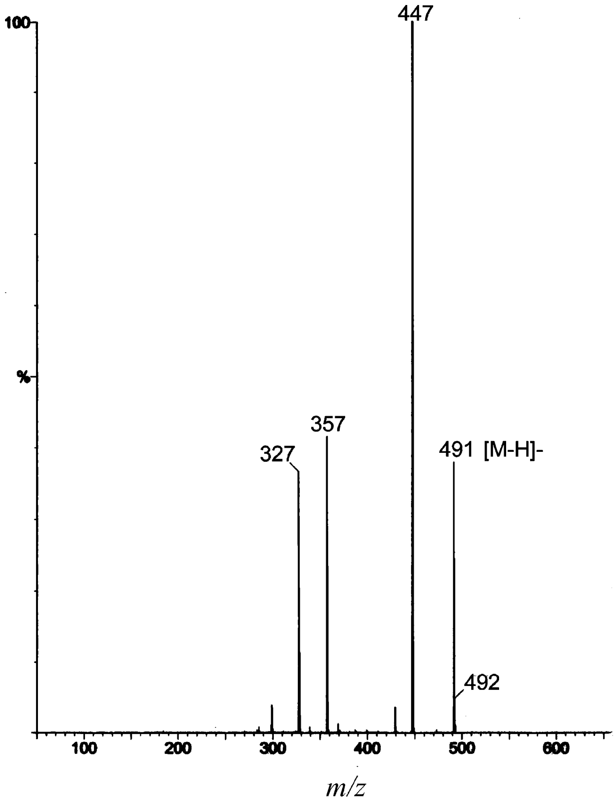

Fig. 8. MS-MS spectrum of isolated pseudo-molecular ion $[M-H]^-$ at 20 eV.

with the fragment ions at *m/z* 447 $[M-H-CO_2]^-$ and 327 $[M-2H-\text{glucose}]^-$, in coincidence with the spectrum obtained by negative ion laser desorption mass spectrometry of carminic acid in linseed oil on paper [8].

4. Conclusions

The results obtained show that soft ionization techniques, as matrix-assisted laser desorption and electrospray ionization mass spectrometry, both in the negative ion mode, are excellent screening methods for confirming the presence of carminic acid in mixtures as those used in easel painting. The presence of other compounds, usually used as binding media, such as triglycerides in linseed oil, do not interfere in the identification of the organic colorant. In this way, both mass spectrometric techniques are powerful tools for the rapid identification of carminic acid in works of art. Related studies with textiles such as wool and silk dyed with cochineal are currently under study.

Acknowledgements

The authors wish to thank the Getty Grant Program for partial financial support. We are indebted to UMYMFOR (CONICET-FCEN, UBA) for NMR spectra. Mass spectrometry was provided by the Washington University Spectrometry Resource with support from the NIH National Center for Research Resources (Grant No. P41RR0954). S.D.P. thanks Fundación Antorchas for a fellowship. M.S.M. is a Research Member of the National Research Council of Argentina (CONICET).

References

[1] H. Schweppe, H. Roosen-Runge, in: R.L. Feller (Ed.), Artists' Pigments: A Handbook of Their History and Characteristics, vol. 1, Oxford University Press, Washington, 1986, p. 255.
[2] T. Eisner, S. Nowicke, M. Goetz, J. Meinwald, Science 208 (1980) 1039.
[3] T. Eisner, R. Ziegler, J.L. McCormick, M. Eisner, E.R. Hoebecke, J. Meinwald, Experientia 50 (1994) 610.
[4] P. Allevi, M. Anastasia, S. Bingham, P. Ciuffreda, A. Fiecchi, G. Cighetti, M. Muir, A. Scala, J. Tyman, J. Chem. Soc. Perkin Trans. 1 (1998) 575.
[5] F.E. Lancaster, J.F. Lawrence, J. Chromatogr. 732 (1996) 394.
[6] J. Orska-Gawrys, I. Surowiec, J. Kehl, H. Rejniak, K. Urbaniak-Walczak, M. Trojanowicz, J. Chromatogr. 989 (2003) 239.
[7] R. Karadag, H. Böhmer, Dyes Hist. Archaeol. 16/17 (2001) 106.
[8] D.M. Grim, J. Allison, Int. J. Mass Spectrom. 222 (2003) 85.
[9] A. Seldes, J.E. Burucúa, G. Siracusano, M.S. Maier, G.E. Abad, J. Am. Inst. Conserv. 41 (2002) 225.
[10] O. Dimroth, Justus Liebig's Annalen der Chemie 399 (1913) 1.
[11] N. Wyplosz, R.M.A. Heeren, G. van Rooij, J.J. Boon, Dyes Hist. Archaeol. 16/17 (2001) 187.
[12] V.P. Papageorgiou, A.S. Mellidis, A.N. Assimopoulou, A. Tsarbopoulos, J. Mass Spectrom. 33 (1998) 89.
[13] R. White, J. Kirby, Dyes Hist. Archaeol. 16/17 (2001) 167.
[14] D. Erhardt, C.S. Tumosa, M.F. Mecklenburg, Polymer Preprints 41 (2000) 1790.