ISSN: 2687-8402

DOI: 10.33552/OAJAA.2022.03.000564

Open Access Journal of Archaeology and Anthropology

Iris Publishers

Research Article

Copyright © All rights are reserved by Yeghis Keheyan

Analysis of Porphyrophora Hamelii Brandt by FTICR and Py/GC/MS Techniques

Yeghis Keheyan*

Department of Chemistry, Smatch- Scientific methodology applied to cultural heritage, University of Rome "La Sapienza", Italy

***Corresponding author:** Yeghis Keheyan, Department of Chemistry, Smatch- Scientific methodology applied to cultural heritage, University of Rome "La Sapienza", Italy

Received Date: June 14, 2022

Published Date: July 18, 2022

Abstract

Natural dyes may be classified by different criteria; the most important of which for analytical purposes is their chemical composition. Most important among these was a red dye, the highly prized "Armenian red", which rivaled royal purple in value. This dye was prepared from the Armenian cochineal insect called vordan in Armenian, late known by its name of kirmiz (Turkish). The insect was found in the Armenian cities of Artashat and Dvin and was particularly renowned for its production. In this paper, the first time we report the examination of Porphyrophora hamelii Brandt performed by pyrolysis gas chromatography-mass spectrometry, and laser desorption FTICR techniques. An experimental sample of dyes was prepared using old recipes from Armenian fonts.

Keywords: Armenian cochineal: Porphyrophora hamelii Brandt: FT-ICR: pyrolysis/GC/MS

Introduction

The red dyes [1] of scale insect origin are of great importance in the history of textiles, manuscripts, paintings etc. The most important insect dyes are as follows: Kerria lacquer Kerr; kermes, from Kermes vermilio Plachon; Polish cochineal, from Porphyrophora Polonica L.; Ararat or Armenian cochineal, from Porphyrophora hamelii Brandt; and cochineal, from Dactylopius coccus Costa. Like madder these dyestuffs are also composed of antraquinone derivatives, and they are applied using mordants, usually alum but sometimes tin or iron salts which give rise to different colours [1 and ref. in]. Red textile dyestuffs and paint pigments produced from female insects of the order Homoptera, suborder Sternorhyncha, superfamily Coccoidea, family Coccidae, have been used on almost every continent [2].

Cochineal insects, replacing purple since Medieval times, were from different species: kermes vermilio (porphyrophora), also called Polish or Armenian Cochineal (kerria), and American Cochineal (dactylopius). Most important among these dyes was a red dye, the highly prized "Armenian red", which rivaled royal purple in value. This dye was prepared from the Armenian cochineal insect called "vordan" in Armenian, late known by its name of "kirmiz" (Turkish) [3-6]. The scientific name for "Vordan Karmir" worm is "Porphyrophora hamelii" and it is known in ancient Latin as "Coccinella Tinctoria" [4]. The insect was found at the foothills of Mt. Ararat, and Arab authors of the Middle Ages tell us that the nearby Armenian cities of Artashat (Artaxata) and Dvin were the main producers of the red-dye making industry. The city of Artashat was known to the Arabs as "Karyat el-Kirmiz", the "City of the Red Dye". Insect dyes have been so important that some insect species were actually domesticated (cochineal), or actively cared for and transplanted onto host trees (Lac, a particular tree that gives a dark red color) [5]. Natural dyes may be classified by different criteria; the most important of which for analytical purposes will be of course their chemical composition. The components of most red

This work is licensed under Creative Commons Attribution 4.0 License

dyes belong to the anthraquinones family. Anthraquinones have a molecular structure based on quinine with two anellated benzene rings on either side. Substitution on the two benzene rings gives particular colour properties to the pigment [7]. The most important representatives of this group coming from the vegetal kingdom are alizarin and purpurin found in the roots of plants from the Rubiaceae family. Anthraquinones do occur well in scale insects (e.g., kermesic acid in kermes). Kermes is the dye from the gravid (egg-bearing) females of "Kermes vermilio" Planchon which lives on a species of oak, Quercus coccifera, growing in countries around the Mediterranean. This is a red dye from an insect that comes out of their red eggs. The structure of kermesic acid shows that it doesn't have ionic bonds and therefore isn't soluble. This would mean that it requires a mordant. The color results in a bright red that is extremely durable. In most natural dyes, several components (e.g., about 10 in Porphyrophora hamelii or Ararat or Armenian cochineal) may contribute to the formation of the color. Moreover, the chemical nature of the components may be very similar, even when from highly different sources. Indeed, anthraquinones and indigoids do occur as well as in plants and animals. The analyses of dyes should be considered as an element in textile analytical studies, which may specifically reveal the biological identity of dye sources, and which may be especially useful in comparative investigations, i.e., studies of textiles which may be considered as a group for historical, geographical, technological, or stylistic reasons. At the beginning of this century, Swedish art critic, F. R. Martin, and Armenian art critic, Armenak Sarkisyan began a great movement on behalf of Armenian rug art history [8]. Armenian rug artwork, based upon historical sources, has been in existence in Armenia since ancient times, many centuries before the Arab invasions of the 7th century. From research of Armenian rugs, it is evident that, in Armenia, the most important and commonly used natural color was red, especially "Vordan Karmir", which is the spirit of the Armenian hues and colors [3].

It is clear that variation in species, extraction and dyeing procedure, crop quality, and atmospheric conditions in which the fibers were preserved will have caused more variation in component distribution.

The standard approaches employed in the identification of dye generally are chromatographic, i.e., TLC [9] and HPLC [2,4,10], and spectroscopic methods such as FTIR [11]. Chromatographic techniques need chemical or thermal degradation of the sample to increase volatility and suitability for conventional analysis. Chromatography and mass spectrometry are certainly the methods of choice for the analysis of organic pigments at molecular level (GC/MS) [12], Py-GC [13], DTMS [14], Py/GC/MS [13,12, 15, 16].

In this paper, we report the examination of Armenian cochineal performed by Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS, afterward FT-MS) and pyrolysis/GC/MS techniques.

About of Cochineal

The "living dye" was known from remote times. The Bible mentions this red dye is derived from the red worm, used by Noah's offspring. In the third century A.D., the king of Persia presented Emperor Aurelian with a woven wool fabric dyed in red, which became a phenomenon because of the brilliance of the color, all the remarkable because the source of that dye was a worm growing in a distant Armenia. Unfortunately, from the 16th century, its use diminished with the appearance of the Mexican cochineal. The insects brought from the New World were smaller, but they had some marked advantages; more brilliant, five generations can be harvested per year, and the cactus on which the insects feed has no fat production, a future that interferes radically in the cultivation of the Ararat insect.

Creation Process

Towards the end of April, the eggs which have wintered come the larvae and wander through the salt marshes until they come across those roots that can nourish them, which are of two kinds here: reed grass (Phragmites australis) and "vordan grass" (Aeluropus littoralis). The wandering stops and the larvae go into the soil, attach themselves to the roots and begin to grow. By August, they will grow several times their original size, light violet but later on deep red. From here on, the growth progresses in two directions simultaneously. The male and female insect differs from each other completely. Toward the middle of August, the females come out onto the surface of the soil. So do the males, with the difference that they are smaller and have not yet developed a full mouth. The entomologists call this the affiancing phase. They continue to crawl and go back into the soil where form a cocoon. In September they come out of their cysts and begin flying around. After mating, they die. The females are ready for fertilization. This phase lasts at least a month and a half. After being fertilized, the females re-enter to the soil, there put their eggs in cocoons formed by waxen threads, after which they die.

In Figure 1 are reported a. Grass root (Aeluropus litoralis) and testa source of carbohydrates, b. the female cochineal, c. the male cochineal photos.

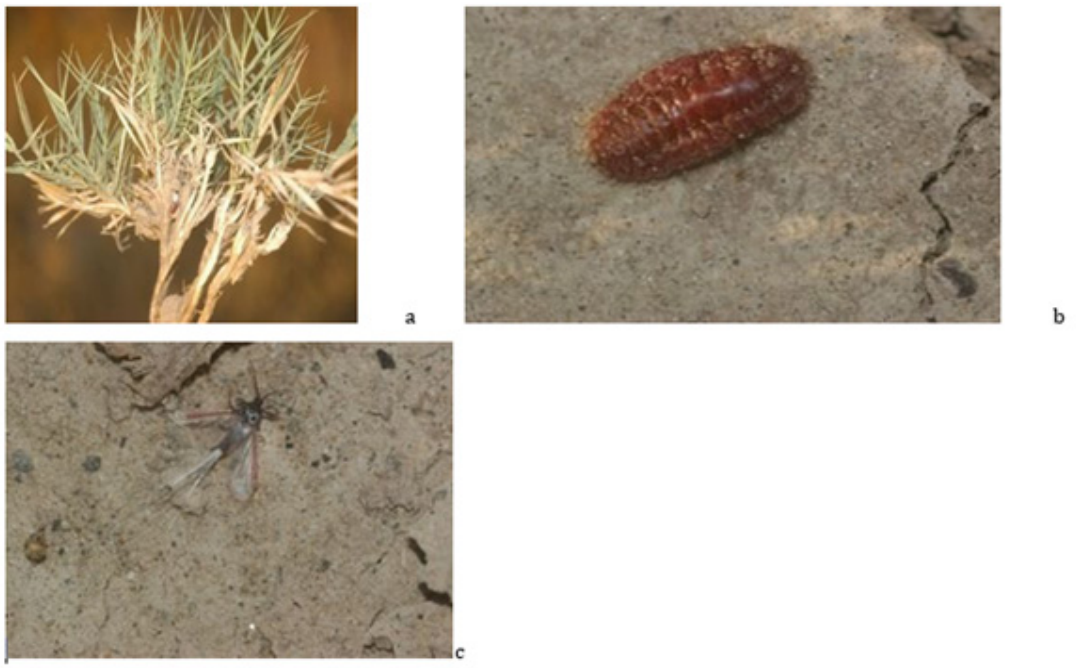


Figure 1: a. Grass root (Aeluropus litoralis) and testa source of carbohydrates, b. female cochineal, c. male cochineal.

Experimental Part

Preparation of pigment of Porphyrophora hamelii Brandt

Porphyrophora hamelii has been prepared by following medieval recipes. Collected Porphyrophora hamelii was boiled in wine and dried up [17]. Then 57g of Porphyrophora hamelii has been crushed and poured into 250 ml of water. Separately 57g of roots of Saponia Officinalis L. has been crushed and cooked in 100 ml of water until to turn yellow the solution [18,19]. Then this mixture has been added to the solution of Porphyrophora Hamelii to boil together [20]. Finally, the solution has been filtered and poured out on flat utensils until a dry pigment formation [20,21].

Methodologies and Instrumentation

The aim of this study was to evaluate whether FT-ICR can be used as a direct and sensitive technique for the identification of small concentrations of colorants and, in particular, madder pigments. This method was first developed and applied to the analysis of synthetic laboratory-made red pigments prepared using alizarin and purpurin with aluminum sulfate. To overcome this difficulty, an alternative and more direct analytical method were developed using laser desorption ionization–mass spectrometry (LDI–MS). It is also a very powerful analytical tool for the analysis of dyes in different fields of application [20-24]. The application of FT-ICR-MS in the field of Cultural Heritage analysis has two principal advantages: the possibility of direct solid sample analysis, without any preliminary "time-consuming" sample treatment and the ionization mode flexibility. In this work has been used an Electron Impact Ionisation mode at 70 eV and 10 mA, at room temperature. LDMS analysis of samples in the condensed phase (solids and liquids) consists of three successive steps:

I. volatilisation,

II. ionisation and

III. analysis of gas-phase ions based on their mass-to-change ratio, gaseous ions are first formed in an ionisation chamber-external to the analyzer- by irradiation with the laser beam.

Subsequently, ions are introduced into the mass analyser for detection. The frequency-triplet output of a Quanta-Ray GCR-II (Spectra Physics Inc) pulsed Nd: YAG laser was used for desorption and ionisation. It produces pulses of ultraviolet laser light with a wavelength of 355 nm (3.49 eV), a tunable pulse-energy of a maximum 60 MJ, and duration of 5 ms. The principal emission wavelength was at 1064nm, and the second harmonic was at 532nm. The spot diameter on the target was measured to be ca 10 μm. Analyses were performed at two different wavelengths: (532-1064 nm). Desorption and ionisation was performed directly (LDI). Measurements were performed in positive mode.

Sample preparation: samples in laser desorption and ionisation (LDI) experiments were deposited as a thick film on a stainless-steel probe.

Pyrolysis was carried out using a SGE's Pyrojector II microfurnace pyrolyser directly connected to the GC/MS system, which consisted of a CLARUS 500 Gas Chromatograph interfaced by direct coupling to the Mass Spectrometer (PERKIN-ELMER). The gas chromatograph was equipped with a 30m x 0.25mm I.D. fused silica column coated with a 0.25µm film of RTXâ5 (Cross bond 5% diphenyl 95% dimethyl polysiloxane). The carrier gas was helium. The velocity of the carrier gas was 37ml/s. The injector and transfer line temperatures were set at 250°C and 220°C. Furnace pyrolysis-GC-MS is a reproducible technique that, in addition, only requires low amounts of material. Samples were injected in the splitless mode, and the oven of the gas chromatograph was programmed as follow: 45°C for 3 min, then 10°C/min to 250°C, hold for 20 min. The operated conditions for the electron impact mass spectrometer consisted of a source temperature 220°C, a filament emission current of 3.7µA, an ionizing voltage of 70eV, and a scan range from m/z 25 to m/z 1200 with a scan time of 0.2s. The mass spectra identifications were carried out by comparison to the NIST 2002 (National Institute of Standard and Technology) as well as to the NBS (N.Y., USA) and Pfleger (Germany) mass spectral libraries. Solid samples were inserted directly into the pyrolyser through a septumless injector, without the need of pre-derivatization.

Results and Discussion

Porphyrophora insects have a relatively high content of body fat: about 30%. This fat may be removed before dyeing; this operation was a real problem, not yet solved or the solution of which had long been forgotten for Porphyrophora hamelii. Methods proposed in the literature include treatment in boiling water; heating the insects in fine sand; digestion in resin-free pine oil or turpentine oil, followed by pressing; a treatment with a soda solution.

The main component of cochineal is carminic acid, which is a C-glycoside, that is to say, the linkage to a sugar molecule is by a carbon-carbon bond rather than through an oxygen linkage. Unlike O-glycosides (in which form many of the anthraquinone dyestuffs may originally be present in the plant and which may be broken down in the process of extraction), C-glycosides are rather stable to be removable by the sugar moiety through acid or enzymatic hydrolysis [25].

The cochineal contains also small percentages of kermesic acid (0.1-1%). $C_{22}H_{20}O_{13}$, mw = 492.38. Structural formulas of red insect dyestuffs are reported in Figure 2.

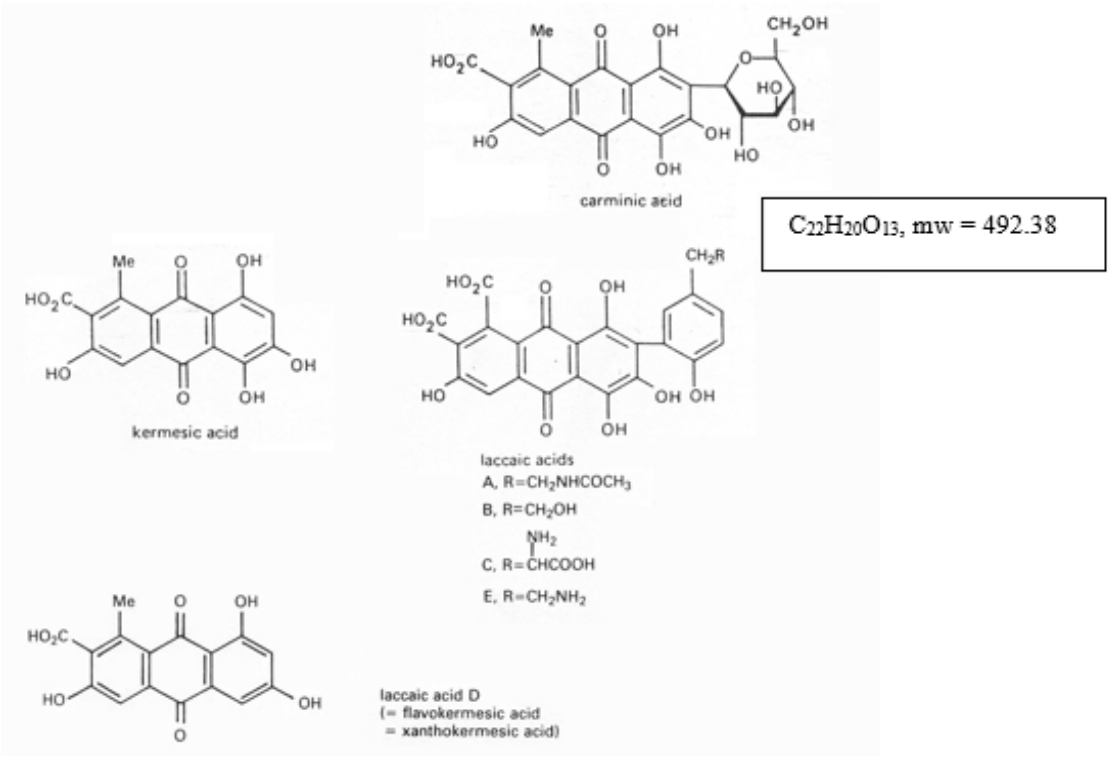


Figure 2: Structural formulas of red insect dyestuffs.

Sample of Porphirophora hamelii is directly pyrolysed in order to obtain the chromatogram of the natural dye. 0.30.5 mg of sample was placed in a microquartz pyrolysis tube. Then 20 μL of a 25% TMAH methylalcohol solution was added. Different pyrolysis trials were performed at 300, 400, 500 and 700°C. Chromatograms at 300°C have acetamide, 2-cyano ($C_3H_4ON_2$), benzaldehyde and pentanoic acid, at 400°C 2-aminopropanol, alanine, cycloheptatriene, p-cresol. At 700°C the major peaks were assigned to PAHs. Chromatogram obtained from the pyrolysis of cochineal with and without TMAH at 500°C are shown in Figure 4 and Figure 5

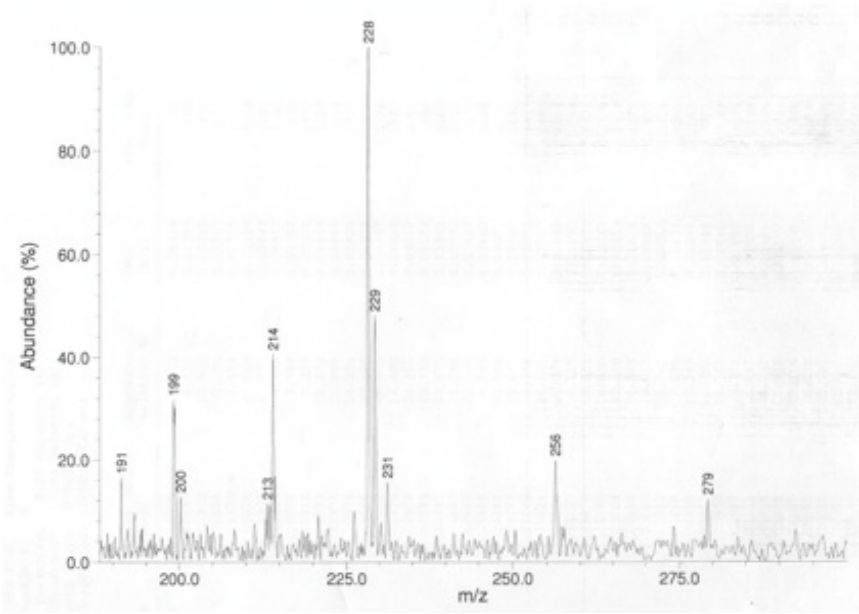


Figure 3: FT-ICR Mass Spectrum of Porphyrophora hamelii.

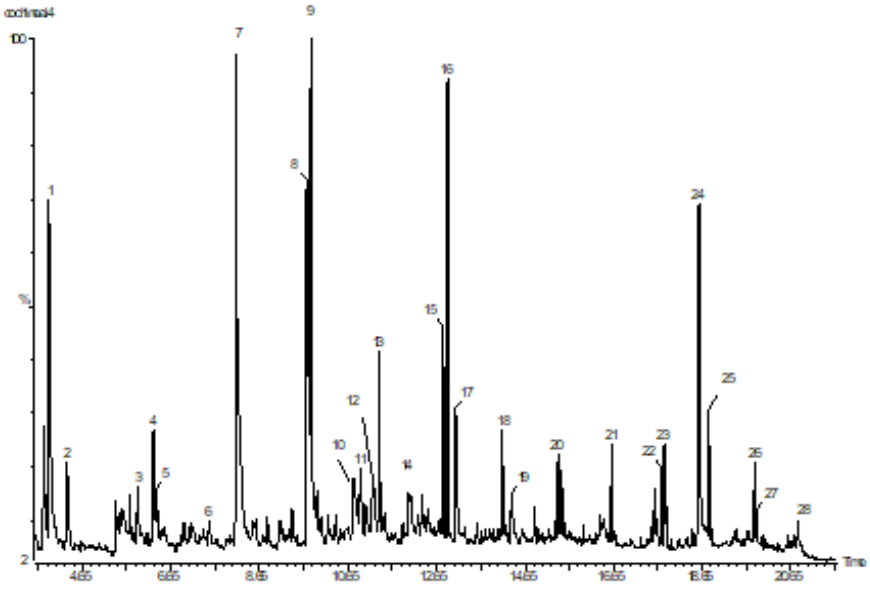


Figure 4: Pyrogram of cochineal at 500°C.

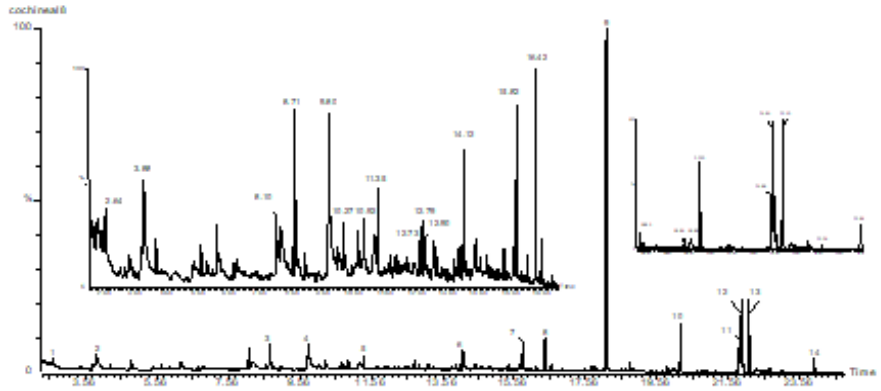


Figure 5: Pyrogram of cochineal at 500°C in the presence of TMAH. Zoom from 2-17 min. and from 18-25min. Peaks, 9-tetradecanoic acid methyl ester,3-benzene, 1-methoxy-4-methyl, 1-H-indole is not changed, 6-benzoic acid, 4-methoxymethylester (14.12min and hexa-o-methyl-mioinositol at 15,63min, 7-14 hexa,hepta, octa, nona and eicosanoic acid methylesthers.

This temperature was selected after a prior series of experiments in which samples of dye were also pyrolysed at 300, 400 and 700°C. In Table 1 are given characteristic peaks from cochineal at 500°C: 7-phenol, 8-4-methylphenol, 9-1-undecene, 15-tridecene, 16-tridecane, 17-indole, 19-methylindole, from 20-24- alkenes, 25-tetradecanoic acid, from 26-29-alkenes (Table 2).

Table 1: Principal products of cochineal pyrolysis.

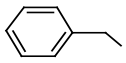
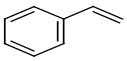
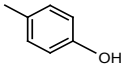
Peak	R/T	Nome	Formula	m/z	Struttura
1	3.90	Toluene	C_7H_8	92	
3	5.90	Ethylbenzene	C_8H_{10}	106	
5	6.32	Styrene	C_8H_8	104	
6	7.51	Benzene,propyl	C_9H_{12}	120	
7	8.12	Phenol	C_6H_6O	94	
8	9.72	Phenol,4-methyl	C_7H_8O	108	
11	10.92	Phenol,2,3,dimethyl	$C_8H_{10}O$	122	
12	11.24	Phenol-4-ethyl	$C_8H_{10}O$	122	
14	12.03	Benzaldehyde,3-methyl	C_8H_8O	120	
25	18.60	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	

Table 2: Principal products of cochineal pyrolysis in the presence of TMAH.

Peak	RT	Name	Formula	m/z	Structure
2	3.90	Toluene	C_7H_8	92	
3	8.71	Benzene,1-methoxy-4-methyl	$C_8H_{10}O$	122	
6	14.12	Benzoic acid, 4-methoxy-methyl ester	$C_9H_{10}O_3$	166	
7	15.82	Dodecanoic acid methyl ester	$C_{13}H_{26}O_2$	214	
8	16.42	Tridecanoic acid methyl ester	$C_{14}H_{28}O_2$	228	
9	18.14	Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$	242	
10	20.20	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	
	20.92	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	284	
11	21.86	9,12,Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	
12	21.91	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	
13	22.13	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	
14	23.04	Nonadecanoic acid methyl ester	$C_{20}H_{40}O_2$	312	

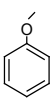
It must be mentioned that several peaks corresponding to TMAH esters of hexadecanoic acid, 9,12-octadecanoic acid and 9-octadecenoic acid were obtained to a low extent. Apart from these fatty acids, benzoic acid, 1,2- and 1,4- benzenedicarboxylic acids were also identified, some of which exhibited important peaks in the chromatogram. High contents of dicarboxylic acids suggest that acyl-lipid material is present. The occurrence of these later compounds could be associated with insufficient alkylation prior to pyrolysis [26,27].

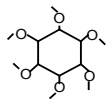
Methoxyphenols and benzoate derivatives were found, which are probably primary pyrolysis product of polysaccharides and relative compounds present in cochineal.

Carminic acid is the most abundant anthraquinoid dye found in cochineal [11]. The chromatogram obtained from TMAH of carminic acid is shown in Figure 6 and tentative structural assignments of the main peaks are reported in Table 1.

(Table 3)

Table 3: Principal products of carminic acid in the presence of TMAH, the peak at 15.63min m/z 162 is a derivative of glucose.

Peak	RT	Name	Formula	M/Z	
1	3.58	isopropanol	C_3H_8O	60	
2	3.89	toluene	C_7H_8	92	
3	5.86	o-xylene	C_8H_{10}	106	
4	6.36	benzaldehyde	C_7H_6O	106	
5	6.91	Benzene,methoxy	C_7H_8O	108	

6	15.69	Myo-inositol,1,2,3,4,5,6-hexa-o-methyl ester	$C_{12}H_{24}O_6$	264	
	15.94	Glucose derivative		162	
7	16.53	Methylated glucose derivative		206	
8	18.17	Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$	242	
9	19.22	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256	
10	20.01	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	
11	20.22	7-Hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	268	
12	21.91	Octadecanoicacid, methyl ester	$C_{19}H_{36}O_2$	296	
13	22.12	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	

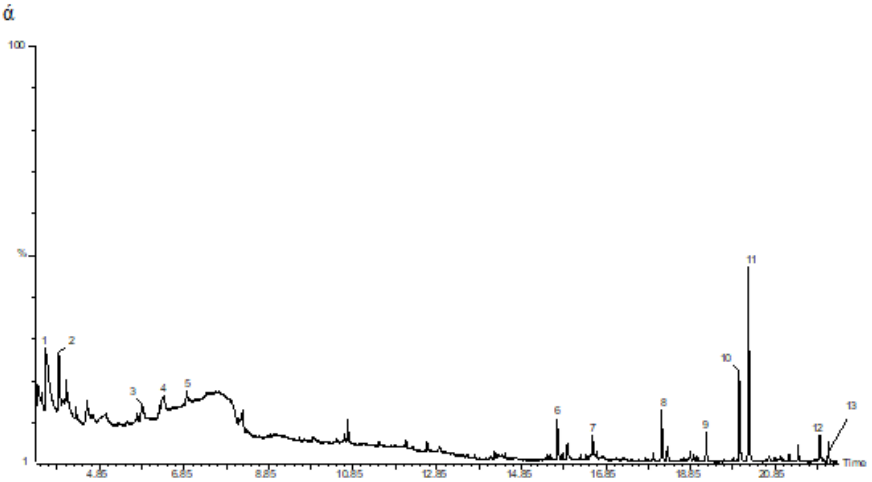


Figure 6: Pyrogram of carminic acid at 500°C in the presence of TMAH. Carminic acid produced a series of methylated fragments deriving from both the substituted anthraquinoid part and the glucosically –C-linked glucose.

Conclusion

LD FT-ICR results are interesting, the detection of m/z 256 that correspond to purpurin is not clear. This is the first detection of cochineal with this technique. The analytical pyrolysis in the presence of TMAH followed by gas chromatographic/mass spectrometric analysis (Py-GC/MS) was found to be a fast and a versatile technique to analyse insect dyes. Currently, vordan karmir (Porphyrophora hamelli) is included in the Red Data Book and faces extinction.

Acknowledgement

None.

Conflict of Interest

No conflict of interest.

References

1. Mills JS, White R (2005) The organic chemistry of museum objects. (2nd Edn.), Butterworth-Heinemann, London.
2. Yardemian PD, Pazmavep (1990) Venezia, S Lazzaro, Venice (eds.), pp. 292-329.
3. Yardemian PD, Pazmavep (1991) Venezia, S Lazzaro, Venice (eds.), pp. 78-125.
4. Wouters J, Verhecken A (1989) The scale insect dyes (Homoptera: Coccoidea). Species recognition by HPLC and diode-array analysis of the dyestuffs. Annales de la Societè Entomologique de France 25(4): 393-410.
5. Verhecken A, Wouters J (1998-9) The coccid insect dyes. Historical, geographical and technical data. Institut Royal du Patrimoine Artistique, Bulletin 22: 207-239.
6. R Janaway, P Wyeth (2004) Zvi Koren in Scientific analyses of ancient and historical textiles.
7. Van den Berg AJJ, Labadie RP (1989) Quinones, Methods in plant biochemistry.
8. In Nemati Parviz (1980) Rugs as an investment. New York, pp.74.
9. Taylor GW (1991) The chemistry of madder dyeing: application of semi-quantitive TLC, 10th annual meeting of DHA.
10. Karapanagiotis I, Chryssoulakis (2006) Investigation of red natural dyes used in historical objects by HPLC-DAD-MS. Annali di Chimica 96(1-2): 75-84.

11. Gillard RD, Hardman SM, Thomas RG, Watkinson DE "The detection of dyes by FT-IR microscopy". Studies in conservation 194(39): 187-192.

12. Learne TJS (1996) The characterization of acrylic painting materials and implications for their use. Conservation and stability, London, University of London.

13. Sonoda N (1999) Characterization of organic azo-pigments by Py/GC/MS. Studies in conservation 44: 195-208.

14. Boon JJ, Pureveen J, Rainford D, Townsend JH (1995) The opening of the Wallahalla, 1842: Studies on the molecular signature of Turner's paint by Direct Temperature-resolved mass spectrometry (DTMS). In Townsend J, Turner's painting techniques in context, London, pp. 35-45.

15. Casas JM, Domenic Carbo MT (2005) Identification of natural dyes used in works of art by pyrolysis-gas chromatography/mass spectrometry combined with in situ trimethylsilylation. Analytical and Bioanalytical Chemistry 382(2): 259-68.

16. Keheyan Y (2008) Py/GC/MS analyses of historical papers. Biores 3(3): 829-837.

17. Harutyunyan A (1941) Paints and inks in ancient Armenian Manuscripts, pp. 18-64.

18. (1650) Matenadarans Manuscripts N. 551: 116-125.

19. Matenadarans Manuscripts N. XVII Century 7251: 475-479.

20. (1694) Matenadarans Manuscripts N. 7322: 2-15.

21. Matenadarans Manuscripts N. XVIII Century 3000: 26-30.

22. Grim DM, Alisson J (2003) Int. J. Mass Spectrom 222: 85-99.

23. Maier MS, Parera SD, Seldes AM (2003) Int J Mass Spectrom 232: 225-229.

24. Siegel J, Allison J, Mohr D, Dunn J (2005) Talanta 67: 425-429.

25. Taylor GW (1983) Detection and identification of dyes on Anglo-Scandinavian textiles. Studies in conservation 28: 153-160.

26. Karapanagiotis I, Chryssoulakis (2006) Investigation of red natural dyes used in historical objects by HPLC-DAD-MS. Annali di Chimica 96(1-2): 75-84.

27. Fabbri D, Chiavari G, He Ling (2000) Analysis of anthraquinoid and indigoid dyes use d in ancient artistic works by thermally assisted hydrolysis and methylation in the presence of TMAH J. Anal. Appl. Pyrolysis 56: 168-178.