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QUALITATIVE HPLC DETERMINATION OF MAIN ANTHRAQUINONE AND LAKE PIGMENT CONTENTS FROM *Dactylopius coccus* DYE INSECT

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Anthraquinone, flavonoid, and indigotin compounds present in plants (buckthorn, madder, weld, indigo, etc.) and insects (cochineal, kermes, lac, etc.) are used in several fields as dye and pigment. They are known as natural dyes [1–5]. Since prehistoric times, natural dyes have been used for many purposes such as the coloring of the natural fibers wool, cotton, and silk as well as fur and leather [6]. Metal-flavonoid or metal-anthraquinone complexes contain metals like tin(II) [$SnCl_2 \cdot 2H_2O$], aluminum(III) [$KAl(SO_4)_2 \cdot 12H_2O$], and iron(II) [$FeSO_4 \cdot 7H_2O$]. Al(III), Fe(II), and Sn(II) metal cations are called as mordant metals [7]. The complex dyes are known as lake pigments [8]. HPLC techniques provide high-resolution separation for identification of plant and insect dyes [9].

The goal of our work was to generate the lake pigments with mordant metals from Mexican cochineal (*Dactylopius coccus* Costa) dye insects and to identify their content with HPLC.

The most common method for chromatographic determination of flavonoid or anthraquinone is the HPLC method [10]. In this paper, High-performance liquid chromatography (HPLC) with diode-array detection (DAD) was utilized for the identification of the lake pigments. According to the results of HPLC analysis, it was determined that carminic acid present in the lake pigments was precipitated by Al(III) and Fe(II). Also, the carminic acid from Mexican cochineal dye insects was identified by HPLC using MeOH–H_2O (2:1; v/v) solution. Retention times of the identified dyes are given in Table 1.

We used distilled water to extract carminic acid as the main dye from Mexican cochineal (*Dactylopius coccus* Costa) insects. Carminic acid is an anthraquinone dye. In the case of precipitation, the main chemical process involved in the lake pigment formation is complexation between the dye molecules and a metal cation called a mordant [7]. This complex was precipitated by K_2CO_3 solution. The carminic acid was determined in the aluminum-cochineal and iron-cochineal lake pigments after acid hydrolysis of the lake pigments. The extraction of dyes from the lake pigments was carried out with HCl–MeOH–H_2O (2:1:1; v/v/v) solution.

Insect, Standard Natural Dye, and Chemicals. Mexican cochineal (*Dactylopius coccus* Costa) insects were obtained from the Laboratory for Natural Dyes, Faculty of Fine Arts, Marmara University. Standard natural dye (carminic acid) was provided by Sigma. HCl, CH_3OH, $SnCl_2 \cdot 2H_2O$, $FeSO_4 \cdot 7H_2O$, and K_2CO_3 were provided by Merck (Darmstadt, Germany). $KAl(SO_4)_2 \cdot 12H_2O$ was provided by Pancreac. High-purity water was purified by passing though a Milli-Q treatment system (Millipore, Bedford, MA, USA), and the HPLC mobile phase was prepared using Milli-Q water.

TABLE 1. Retention Times of the Standard Dye, Dye Insect, and Identified Dyes Carminic Acid from Lake Pigments

Sample	Retention time, min	Sample	Retention time, min
Iron-cochineal lake pigment	16.421	Mexican cochineal dye insect	14.138
Aluminum-cochineal lake pigment	16.048	Standard dye (carminic acid)	15.939

Carminic acid in tin-cochineal lake pigment was not identified.

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Apparatus. Heraeus D-6450 Hanay (Merck, Germany), WiseStir MSH-20A Daihan Scientific Co., Shimadzu AEX-200G, and Gesellshaft fur Labortechnik (GFL) were used.

HPLC Analysis. Chromatographic experiments were performed using an Agilent 1100 series system (Agilent Technologies, Hewlett-Packard, Germany), including a model G1311A quaternary HPLC pump with a 50 μL loop and Rheodyne valve (7725i sample injector). A G1315A diode-array detection was performed by scanning from 191 to 799 nm with a resolution of 2 nm, and chromatographic peaks were monitored at 255, 268, 276, 350, and 491 nm. A G1322A vacuum degasser and a G1316A thermostatted column compartment were used, and the data station was an Agilent Chemstation. A Nova-Pak C18 analytical column (3.9 × 150 mm, 4 μm, Part No. WAT086344, Waters) protected by a guard column filled with the same material was used. Analytical and guard columns were maintained at 30°C. Chromatographic separation of the hydrolyzed sample was carried out using a gradient elution program that utilizes two solvents: solvent A: H_2O – 0.1% TFA and solvent B: CH_3CN – 0.1% TFA:

Time, min	*A*	*B*	*Time, min*	*A*	*B*
0.0	95.0	5.0	28.0	40.0	60.0
1.0	95.0	5.0	33.0	5.0	95.0
20.0	70.0	30.0	35.0	5.0	95.0
25.0	40.0	60.0	45.0	95.0	5.0.

The flow rate was 0.5 mL/min, and a sequential elution program was applied.

Dye Extraction. 2 g of ground Mexican cochineal (*Dactylopius coccus* Costa) was transferred to a beaker, and 1000 mL distilled water was then added. The mixture of Mexican cochineal was heated with a magnet mixer. This process was continued up to 100°C, and this temperature was held for 20 min for the Mexican cochineal. Then mixture was filtered to obtain the Mexican cochineal extract.

Aluminum-Cochineal Lake Pigment Formation. A 15% $KAl(SO_4)_2 \cdot 12H_2O$ (alum) solution and 50 mL Mexican cochineal extract were heated separately to 90°C and 60°C respectively. 50 mL Mexican cochineal extract at 60°C was added to 30 mL alum solution at 90°C. Then 1 M K_2CO_3 solution was added to neutralize the mixture (pH 6–7). The mixture was cooled to room temperature to precipitate the lake pigment. After settling, the mixture was filtered. The dye residue was dried on filter paper at 101°C for 30 min. The dried lake pigments were powdered. All these processes were repeated to obtain lake pigments with cochineal extract from 3% $FeSO_4 \cdot 7H_2O$ and 3% $SnCl_2 \cdot 2H_2O$ solutions too (30 mL metal solution with 50 mL Mexican cochineal extract for iron-cochineal lake pigment, and 50 mL metal solution with 75 mL Mexican cochineal extract for tin-cochineal lake pigment).

Dye Characterization. The prepared lake pigments were hydrolyzed with H_2O–MeOH–37% HCl (1:1:2; v/v/v) in glass conical tubes for precisely 8 min in a water bath at 100°C to extract the organic dyes. The solution was cooled rapidly and evaporated to dryness in a water bath at 65°C under a gentle stream of nitrogen. The dry residue was dissolved in MeOH–H_2O (2:1; v/v) for analysis. The resulting hydrolysates of iron-cochineal (5 μL), tin-cochineal (30 μL), and aluminum-cochineal (30 μL) lake pigments were analyzed. The HPLC analysis showed the presence of the main anthraquinone compound. The bibliographic data, the UV/Vis spectra, and the retention times permitted the identification of the anthraquinone: carminic acid for iron-cochineal lake pigment and aluminum-cochineal lake pigment.

REFERENCES

1. J. Sanyova and J. Reisse, *J. Cult. Herit.*, **7**, 229 (2006).
2. J. Wouters and N. R. Chirinos, *J. Am. Inst. Conserv.*, **31**, 237 (1992).
3. K. G. Gilbert and D. T. Cooke, *Plant Growth Regul.*, **34**, 57 (2001).
4. J. K. Kumar and A. K. Sinha, *Nat. Prod. Lett.*, **18**, 59 (2004).
5. I. Surowiec, A. Quye, and M. Trojanowicz, *J. Chromatogr. A*, **1112**, 209 (2006).
6. D. Cristea and G. Vilarem, *Dyes Pigm.*, **70**, 238 (2006).
7. C. Clementi, B. Doherty, P. L. Gentili, C. Miliani, A. Romani, B. G. Brunetti, and A. Sgamellotti, *Appl. Phys. A*, **92**, 25 (2008).
8. H. Schweppe, *J. Am. Inst. Conserv.*, **19**, 14 (1979).
9. X. Zhang and R. A. Laursen, *Anal. Chem.*, **77**, 2022 (2005).
10. D. Cristea, I. Bareau, and G. Vilarem, *Dyes Pigm.*, **57**, 267 (2003).