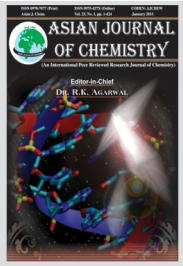
Asian Journal of Chemistry; Vol. 23, No. 10 (2011), 4403-4406

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Formation and HPLC Analysis of the Natural Lake Pigments Obtained from Weld (*Reseda luteola L.*)

RECEP KARADAG[1,*], EKREM CUCEN[1] and YUSUF YILDIZ[2]

[1]Laboratory of Natural Dyes, Faculty of Fine Arts, Marmara University, 34718, Acibadem, Kadikoy, Istanbul, Turkey
[2]Center for Mass Spectrometry, Department of Chemistry and Biology, Stevens Institute of Technology, Hoboken, New Jersey 07030, USA

*Corresponding author: Fax: +90 216 3621499; Tel: +90 216 3262667/176; E-mail: rkaradag@marmara.edu.tr

(*Received*: 2 November 2010; *Accepted*: 22 June 2011) AJC-10058

In this work, the weld lake pigments, aluminium-weld and tin-weld were obtained by the reaction of $KAl(SO_4)_2.12H_2O$ and $SnCl_2.2H_2O$ solutions with weld. The high performance liquid chromatography (HPLC) with diode-array detection method was utilized for the identification of the lake pigments. Reversed-phase HPLC with diode-array UV-VIS spectrophotometric detection has been used in this identification. The extraction of dyestuffs from the lake pigments were carried out with HCl/methanol /water (2:1:1; v/v/v) solution. From the results of the HPLC analysis of the weld lake pigments, it was determined that the luteolin and apigenin present in the lake pigments was precipitated by Al(III) and Sn(II).

Key Words: Weld, Dyestuff, Pigment, Luteolin, Apigenin, HPLC.

INTRODUCTION

From prehistoric times humans have left their mark on their environment in the form of painted images, whether in the form of simple handprints, works of fine art or spray-can graffiti. It seems that people have an underlying conscious or subconscious urge to mark their passing. It may be that primitive man-made marks by scratching trees or rocks with stones as a way of marking a track, indicating a source of food or water or even marking territory. At some stage, however it was discovered that some materials worked more effectively when mixed with a medium such as water or saliva and painting was born[1] and afterwards organic natural pigments were used in the some paintings.

Weld and madder lakes in ancient paintings are mainly the complexes of flavonoids and anthraquinones with aluminium cations, adsorbed on amorphous alumina. Their composition as determined today depends not only on the plant species and origin but also on the procedures used for the extraction from plants and on the method used for the preparation of the lakes. Last but not least, their composition is influenced by ageing processes. Alizarin, purpurin and anthraquinones are commonly found as components of these lakes. The commonly used methods for the analysis of natural dyes in micro-samples of aluminium-containing lake pigments involve sample treatment under rather extreme acidic conditions, followed by extraction into a suitable solvent and analysis of the extract by TLC, UV-VIS spectrophotometry or HPLC equipped with a UV or UV-DAD detector.[2-11] These methods, originally developed for the analysis of colourants on textile fibers, have the disadvantage of promoting the hydrolysis of a series of molecules less resistant than alizarin and purpurin such as pseudopurpurin, munjistin and the dyes' glycosidic precursors.[11]

Anthraquinones, present in the madder roots (roots and rootstocks of *Rubia tinctorum* L.), have been used for dyeing textile fibres especially to give a red colour and they have also been used as a pigment rarely since ancient times. [2-7] Later on, several anthraquinone derivatives were proved to exert different biological activities, such as antioxidant, antimicrobial, antifungal, cytotoxic, larvicidal, antiviral and unfortunately also genotoxic activities. Excellent compilations are available which deal with anthraquinones of the *Rubia sp*.[12]

Metal flavonoid or metal anthraquinone complexes formed with metals like aluminium(III) [$KAl(SO_4)_2.12H_2O$], iron(II) [$Fe(SO_4).7H_2O$] and tin(II) [$SnCl_2.2H_2O$] from these dyestuffs are known as natural lake pigments.[9,13] In alkaline solution, the pigments precipitate as insoluble metal-dyestuff complexes.[10] The composition of the pigments depends not only on the plant species and origin but also on the procedures used for the extraction from the plants and on the method used for pigments preparation. Moreover, their composition is influenced by ageing processes.[11]

The identification of pigments and dyes is one of the most important targets aimed for in the scientific examination of

paintings, textiles, illuminated manuscripts and other historic and archaeological materials. Thus, several analytical techniques have been used, for example gas chromatography/mass spectrometry, UV-visible spectrophotometry, thin layer chromatography, high performance liquid chromatography[14], reversed phase liquid chromatography[15] and capillary electrophoresis with electrospray mass spectrometric detection, FT-IR spectroscopy and Raman spectroscopy.[16] Of these techniques, high performance liquid chromatography using a diode-array detection is ideally suited to the identification of dyes and lake pigments sampled from museum collections especially.[17]

Colour of a pigment is the result of three combined factors: The spectrum of the light source, the spectral reflectivity of the pigment and the spectral sensitivity of the eye. The CIELAB (1976)-system was introduced to describe colour as a result of these three factors. This system is a three-dimensional space, with coordinate axes L^*, a^* and b^*. L^* denotes the brightness of the colour (L^*= 0: black, L^* = 100: white), a^* represents the green-red axis (a^* negative: green, a^* positive: red) and b^* represents the blue-yellow axis (b^* negative: blue, b^* positive: yellow). Each pigment colour can be represented as a set of values for L^* a^* and b^* and consequently as a point in this colour space[18].

EXPERIMENTAL

Plant, standard natural dyes and chemicals: All reagents were analytical grade, unless stated otherwise. HCl, CH_3OH, $SnCl_2.2H_2O$, $KAl(SO_4)_2.12H_2O$ and K_2CO_3 were from Merck (Darmstadt, Germany) and, luteolin and apigenin were from Carl Roth (Karlsruhe, Germany).

Weld (*Reseda luteola L.*) plant was obtained from the Laboratory for Natural Dyes, Faculty of Fine Arts, Marmara University, Istanbul. 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.

Hanna instruments HI 8314 membrane pH meter, Heraeus D-6450 Hanau Oven, WiseStir MSH-20A Daihan Scientific Co. Stirrer, Shimadzu AEX-200G, Gesellschaft für Labortechnik (GFL), GretagMacbeth SpectroEye spectrophotometer were used.

HPLC equipment: Chromatographic experiments were performed using an Agilent 1200 series system (Agilent Technologies, Hewlett-Packard, Germany) including a model G1311A quaternary HPLC pump, G1315A diode-array detector (chromatograms were obtained by scanning the sample 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 and the data were analyzed using an Agilent chemstation. A Nova-Pak C_{18} analytical column (3,9 × 150 mm, 4 µm, Part No WAT 086344, Waters) protected by a guard column filled with the same material, was used. Analytical and guard columns were maintained at 30 ºC. 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. The flow rate was 0.5 mL/min and following elution program was applied (Table-1).

TABLE-1
PARAMETERS OF GRADIENT ELUTION PROGRAM

Time (min)	H_2O+0.1 %, TFA (%)	CH_3CN+0.1 %, TFA (%)
0.0	95.0	5.0
1.0	95.0	5.0
20.0	70.0	30.0
25.0	40.0	60.0
28.0	40.0	60.0
33.0	5.0	95.0
35.0	5.0	95.0
45.0	95.0	5.0

Extraction: 5 g of dried and ground weld aerial parts were transferred to a beaker. 375 mL distilled water was then added. The mixture of weld was heated with a magnet mixer. This process was continued up to 100 ºC and this temperature was held for 1 h. Then the mixture was filtered to obtain the weld extract.

Formation of lake pigments: 15 % $KAl(SO_4)_2.12H_2O$ (alum) solution and 375 mL weld extract were heated separately to 90 and 60 ºC, respectively. 25 mL from alum solution at 90 ºC was added to weld extract at 60 ºC. Afterwards, 0.1 M K_2CO_3 solution was added to neutralize the mixture. The mixture was cooled to room temperature to precipitate the lake pigment. After settling down, the mixture was filtered and the precipitate was washed with distilled water. The residue was dried on filter paper at 101 ºC for 0.5 min. The dried lake pigments were powdered. The same process was repeated using 25, 50, 75, 100 and, 125 mL of alum solution to each part of 375 mL of weld extract. All these processes were repeated to obtain lake pigments from 3 % $SnCl_2.2H_2O$ solutions too.

HPLC analysis: Weld extract and the prepared pigments were hydrolyzed using 37 % HCl-CH_3OH-H_2O (2:1:1; v/v/v) mixture before chromatographic analysis. The aqueous mixture was evaporated on a hot water-bath. The solid residue was dissolved in CH_3OH-H_2O (2:1; v/v) for analysis. The chromatograms and spectra relating to the acid hydrolyzed weld extract, lake pigments and standard dyestuffs are given in Figs. 1-10.

Colour measurement of lake pigments: L^*, a^* and b^* values of pigments were measured with Gretag macbeth spectro eye spectralphotometer. CIELAB graphs of the pigments were drawn by using of the measured values of lake pigments (Figs. 11 and 12).

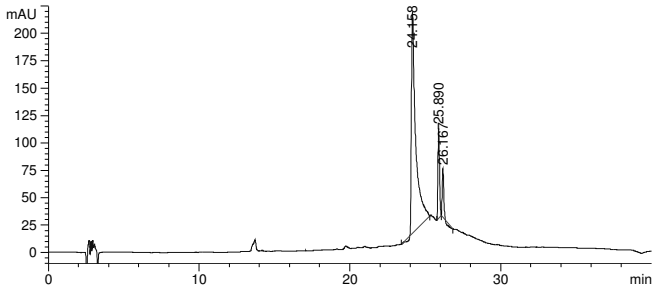


Fig. 1. HPLC chromatogram of acid hydrolyzed weld extract. Luteolin (24.1 min) and apigenin (25.8 min) are identified.

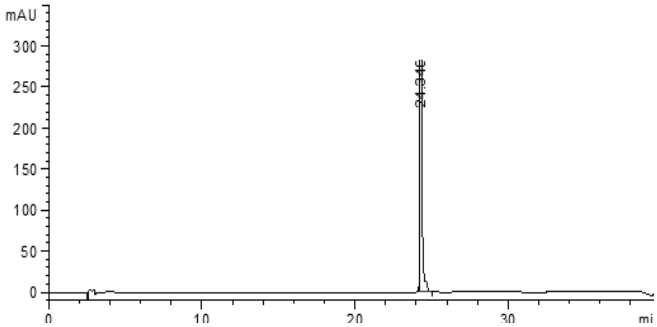


Fig. 2. HPLC chromatogram of luteolin standard compound.

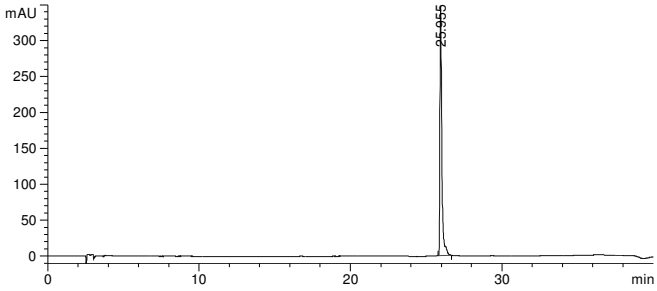


Fig. 3. HPLC chromatogram of apigenin standard compound.

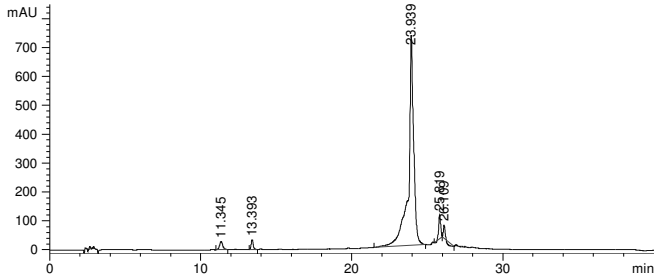


Fig. 4. HPLC chromatogram of acid hydrolyzed aluminium-weld lake pigment. Luteolin (23.9 min) and apigenin (25.8 min) are identified.

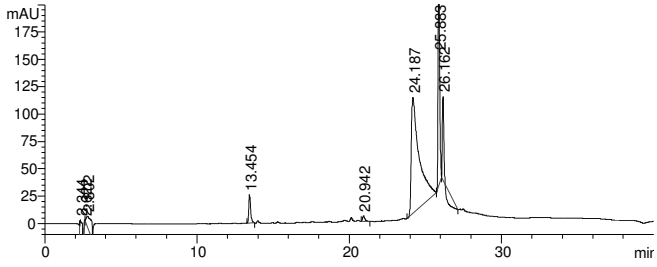


Fig. 5. HPLC chromatogram of acid hydrolyzed tin-weld lake pigment. Luteolin (24.1 min) and apigenin (25.8 min) are identified.

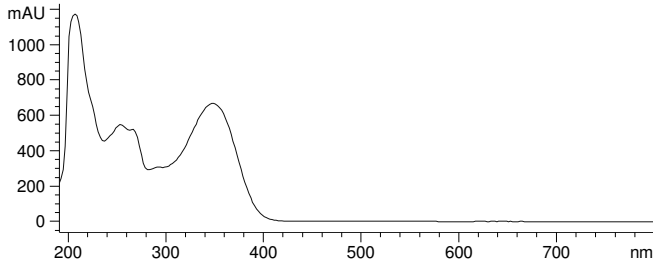


Fig. 6. Photodiode array spectrum of acid hydrolyzed weld extract.

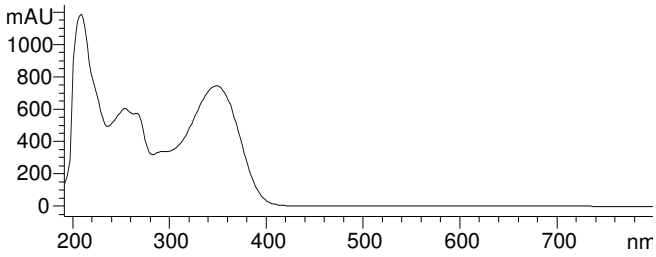


Fig. 7. Photodiode array spectrum of acid hydrolyzed aluminium-weld lake pigment.

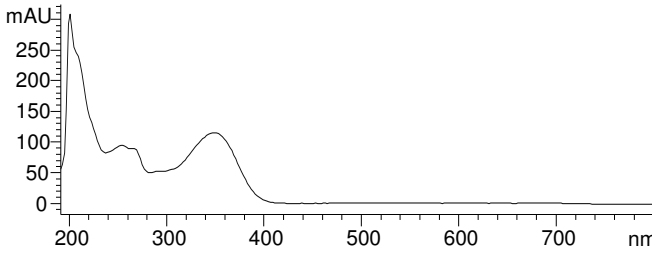


Fig. 8. Photodiode array spectrum of acid hydrolyzed tin-weld lake pigment.

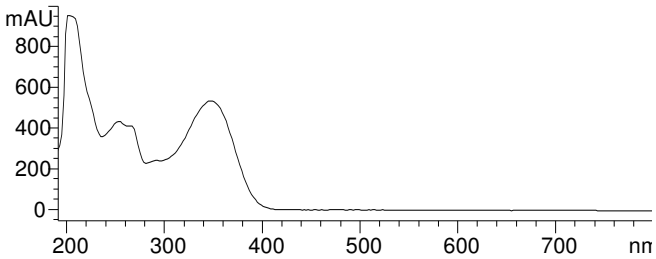


Fig. 9. Photodiode array spectrum of luteolin standard compound.

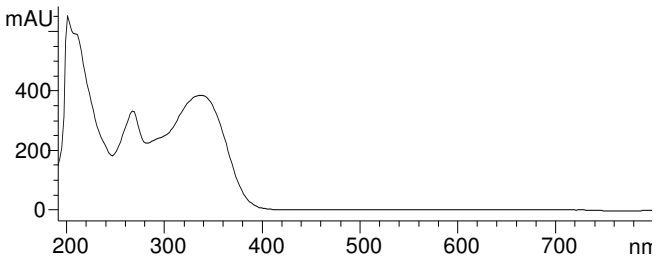


Fig. 10. Photodiode array spectrum of apigenin standard compound.

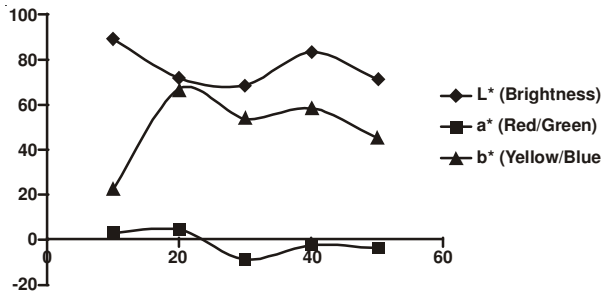


Fig. 11. CIE LAB graph of aluminium-weld lake pigment.

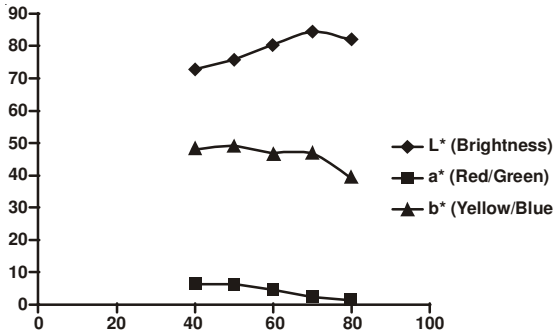


Fig. 12. CIE LAB graph of tin-weld lake pigment.

RESULTS AND DISCUSSION

In the present study, complexes formed with weld and aluminium(III) and tin(II) were obtained as lake pigments. The solution of each one metal; 25, 50, 75, 100 and 125 mL were added to the extract obtained from weld and the lake pigments were formed. These lake pigments were analyzed quatitatively by a reversed phase high performance liquid chromatography (RP-HPLC). The composition was determined by comparison with standard dyestuffs. HPLC analysis shows that luteolin and apigenin were determined in the acid hydrolyzed weld extract, aluminium-weld and iron-weld lake pigments. It was obtained that the quantity of luteolin dyestuff present in the acid hydrolyzed weld extract and the aluminium-weld lake pigment to be less compared to apigenin dyestuff. Otherwise, it was determined that the tin-weld lake pigment present more apigenin to luteolin dyestuff. The brightness and colour values of aluminium-weld and tin-weld lake pigments were determined by CIELAB colour space system. The best values for aluminium-weld and tin-weld lake pigments were observed in samples that prepared by using 40 and 70 mL of the solution of metal salts, respectively.

Conclusion

In this study, the reaction with aluminium(III) and tin(II) of the dyestuffs present in weld (*Reseda luteola*) has been used to prepare natural lake pigments. Results from the HPLC analysis of the acid hydrolyzed weld extract, the aluminium-weld lake pigment and the tin-weld lake pigment show that luteolin and apigenin are present. The effect of different volumes of metal solutions on the colouring scale of the lake pigments were investigated (Figs. 11 and 12).

ACKNOWLEDGEMENTS

This work supported by the Marmara University, FEN-D-0300609-0231 BAPKO project is gratefully acknowledged. The authors also thanks Dr. Turkan Yurdun for HPLC analysis.

REFERENCES

1. J.R. Barnett, S. Miller and E. Pearce, *Optics Laser Technol.*, **38**, 445 (2006).
2. R. Karadag, Dogal Boyamacilik, Geleneksel El Sanatlari ve Magazalar Isletme Müdürlügü Yayinlari (2007).
3. H. Itokawa, Y. Qiao and K. Takeya, *Phytochemistry,* **30**, 637 (1991).
4. R. Singh, R. Geetanjali and S.M.S. Chauhan, *Chem. Biodiver.*, **1**, 1241 (2004).
5. N. Orbán, I. Boldizsár, Z. Szucs and B. Dános, *Dyes Pigments,* **77**, 249 (2008).
6. D. De Santis and M. Moresi, *Ind. Crops Prod.*, **26**, 151 (2007).
7. R. Karadag and E. Dölen, *Ann. Chim.*, **97**, 583 (2007).
8. H. Schweppe, Handbuch Der Naturfarbstoff, Landsberg:Ecomed (1992).
9. H. Schweppe, *J. Am. Inst. Conservat.*, **19**, 14 (1979).
10. C. Clementi, D. Doherty, P.L. Gentili, C. Miliani, A. Romani, B.G. Brunetti and A. Sgamellotti, *Appl. Phys. A*, **92**, 25 (2008).
11. J. Sanyova and J. Reisse, *J. Cult. Herit.*, **7**, 229 (2006).
12. I. Boldizsár, Z. Szucs, Z.S. Füzfai and I. Molnár-Perl, *J. Chromatogr. A,* **1133**, 259 (2006).
13. O. Deveoglu, R. Karadag and T. Yurdun, *Jordan J. Chem.*, **4**, 377 (2009).
14. A. Romani, C. Zuccaccia and C. Clementi, *Dyes Pigments*, **71**, 218 (2006).
15. O. Deveoglu, E. Torgan, T. Taskopru and R. Karadag, SEM-EDX/HPLC Analysis and Production of Natural Pigments from Quercus Ithaburensis with Al^{3+}, Fe^{2+} and Sn^{2+} metals, Proceeding 6th Conference on Medicinal and Aromatic Plants of Southeast European Countries, Turkey, 2010.
16. C.E. Silva, L.P. Silva and H.G.M. Edwards, and L.F.C. De Oliveria, *Anal. Bioanal. Chem.*, **386**, 2183 (2006).
17. S.M. Halpine, *Stud. Conservat.*, **41**, 76 (1996).
18. J.B. Krikken, J.R. Zijp and M.C. Huysmans, *J. Dentistry,* **36**, 731 (2008).