

Isolation and identification of 1,2-dihydroxy-9,10-anthraquinone (Alizarin) from the roots of Maddar plant (*Rubia tinctorum*)

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(Received: March 02, 2007; Accepted: April 03, 2007)

ABSTRACT

The roots of madder (*Rubia tinctorum*) are source of anthraquinone dyes with alizarin being the main component. Free alizarin (I) is present in madder root in only small quantities, most of it is present as its glycoside i.e. ruberythric acid(III) On the basis of the importance of alizarin, in this research, it was isolated, purified and its structure was confirmed by various spectra. Maceration of the powdered madder roots in various polar and non-polar solvents, as well as Soxhlet extraction resulted in the formation of a reddish solid material (5.2% and 14.2% yield, respectively). Successive TLC and column chromatography of the solid material on silica gel with methanol as the mobile phase, gave three fractions with $R_f = 0.21, 0.48$ and 0.68 . The fraction with $R_f = 0.68$ was the major one. ¹HNMR, ¹³CNMR, IR, UV and Mass spectra of this fraction were taken. On the basis of the results obtained from the spectra, the chemical structure of alizarin was determined and confirmed.

Keywords: Alizarin, 1,2-dihydroxy-9,10-anthraquinone, 1,2-dihydroxy-9,10-anthracenedione, *Rubia tinctorum*, madder roots, ruberythric acid.

INTRODUCTION

Madder plant is a perennial plant which grows from 60 to 100 cm high. The pencil-thick rhizome creeps widely underground. The stem is quadrangular with backward turning prickles at the edges. The stems are at times so thin that they are more decumbent than erect. The medicinal part of this plant is its dried root. The plant is indigenous to Southern Europe, Western Asia and North Africa and is cultivated elsewhere. The roots of this plant have been used as antiseptic, diuretic, laxative that stimulates the liver and uterus and relaxes spasms. It is used medicinally for the treatment of kidney and bladder stones (internally) and for wounds (externally). Alizarin, [1,2-dihydroxyanthraquinone or 1,2-dihydroxy-9,10-anthracenedione, mordant red, Turkey red, mordant red 11, alizarin B, alizarin red] (I) is the red dye originally derived from the root of the madder plant. The word alizarin ultimately derives from the Arabic **al-usara**, juice.¹In 1869 it became the first natural pigment to be duplicated

synthetically. Madder has been cultivated as a dyestuff since antiquity in central Asia and Egypt, where it was grown as early as 1500 BC. Roots of the madder plant are dried, crushed, hulled, boiled in weak acid to dissolve the dye, and fermented to hydrolyze anthraquinones from the glycosides. The extracted dye is made into a pigment by dissolving the dye in hot alum (aluminum potassium sulphate; $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) solution, and precipitating pigment with soda or borax. Alizarin lakes are prepared by reaction of alizarin with aluminum hydroxide.² By 1804, the English dye maker George Field had developed a technique to lake madder by treating it with alum. This turned the water-soluble madder extract into a solid, insoluble pigment. The resulting madder lake had a longer-lasting colour, and could have more versatility, for example, by blending it into a paint. The term lake is a bit confusing for those unfamiliar with painting terminology. A lake is a pigment made by precipitation of a soluble dye with a metallic salt or acid complex with a base.³ Over the following years,

it was found that other metal salts, including those containing iron, tin, and chromium, could be used in place of alum to give madder-based pigments of various other colours. Alizarin was used for dyeing in Asia and Egypt in ancient times. Later, it began to be used for painting.¹ Alizarin is the main colourant found in the madder plant *Rubia tinctorum*. Alizarin red is also known as Rubia, Rezza, Alizarin crimson, crimson madder and Rose madder.³

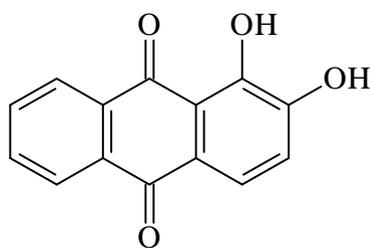
In 1826, the French chemists Colin and Pierre-Jean Robiquet found there were two colourants in madder root, the red alizarin and the more rapidly fading purpurin.^{1,4} Therefore, in the madder root, there are two colouring agents, one is the permanent alizarin (I) and the other rapidly fading purpurin (II). The alizarin component became the first natural dye to be synthetically duplicated in 1868 when the German chemists Carl Graebe and Carl Liebermann, working for BASF, found a way to produce it from anthracene. About the same time, the English dye chemist William Perkin independently discovered the same synthesis, although the BASF group filed their patent before Perkin by only one day. The synthesis of alizarin caused rapid decline and almost total disappearance of the madder-growing industry. As

in the natural madder lake aluminum hydrate (alum) is used as a substrate for the synthetic variety, most commonly known as alizarin crimson. Although alizarin crimson had superior permanence over the madder lake because of the absence of purpurin, both madder lakes were used in oil and watercolour painting.⁴

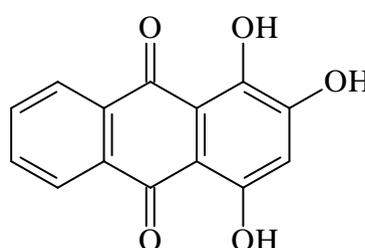
The synthetic alizarin could be produced at less than half the cost of the natural product, and the market for madder collapsed virtually overnight. Alizarin itself has been in turn largely replaced today by the more light-resistant quinacridone pigments developed at DuPont in 1958. Madder was formerly used in large quantities for dyeing textiles and is still the colour for French military cloth.⁵

EXPERIMENT

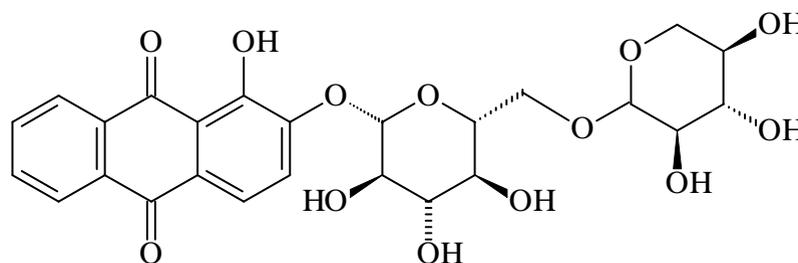
NMR spectra were recorded on Varian FT-NMR spectrophotometer 500 MHz (¹H) and 125 MHz (¹³C) using TMS as internal standard. Mass spectra were measured on a QP-1000 Shimadzu (Japan) mass spectrometer. Infra-red spectra were recorded using a JASCO, IR700 Infrared spectrophotometer. UV-Visible spectra were recorded using a JASCO, 810-UV spectrophotometer. All the chemicals were purchased from Merck.



(I)



(II)



(III)

1. Supplying the madder roots

Two kilograms of the dried madder roots was purchased from Isfahan Goldarou Company. The plant was identified as *Rubia tinctorum* by the faculties of agriculture and sciences of Shahid Chamran University, Ahwaz, Iran. The roots of the plant were crushed then ground by an electrical mill.

2. Extraction of the dried ground roots by maceration method

Maceration of 100 g of the dried roots in 350 mL of each of the individual polar and non-polar solvents (methylene chloride, benzene, toluene, ethanol, water and methanol) was carried out. The mixture was shaken by an electrical shaker at room temperature for 48 hours. After that time, the mixture was filtered and the solvent was removed on a rotary evaporator. The results are given in table 1.

3. Soxhlet extraction of the dried ground roots

Soxhlet extraction of 30 g of the dried ground roots in 300 mL of each of the individual polar and non-polar solvents (methylene chloride, benzene, toluene, ethanol, water and methanol) for 8 hours followed by removal of the solvent on rotary evaporator gave a red-orange solid material. The results are given in Table 1.

4. Thin Layer Chromatography analysis of the extracted solid material

Tlc on silica gel with methanol as the mobile phase was carried out. The results showed three distinct coloured spots with $R_f = 0.21, 0.48,$ and 0.68 .

5. Column Chromatography analysis of the extracted solid material

1 g of the solid extract was dissolved in 3 mL of methanol and then column chromatographed on silica gel (60 mesh) with methanol as the mobile phase. Finally one major fraction with $R_f = 0.68$ was separated and characterized. IR, UV-Visible, ^1H NMR, ^{13}C NMR, and MS spectra of the fraction was taken. This process was repeated several times and the fractions with $R_f = 0.68$ were added together.

6. Characterization of the component with $R_f = 0.68$

Its IR (mull in Nujol) had ν (cm^{-1}): 3372 (OH, br, s); 2922, 2854 (CH, s), 1663 (C=O, m), 1631 (C=O, m), 1587, 1455 (C=C aromatic, s), 1296 (C-O, s); its UV spectrum in with methanol showed $\lambda_{\text{max}} = 430.5, 278.0, 248.0$ nm; its ^1H NMR (d_6 -DMSO, 500 MHz) had δ (ppm): 7.12, 7.13 (d, H-3), 7.51, 7.53 (d, H-4), 7.81 (br, s, H-6 + H-7), 8.02, 8.08 (dd, H-5 + H-8), 10.875 (br, s, OH), 12.445 (br, s, OH); its ^{13}C NMR (d_6 -DMSO, 125 MHz) had δ (ppm):

Table - 1: Results obtained from the extraction of the ground Madder roots

No.	Method of extraction	Ground roots used (g)	Solvent used	Extraction time (hrs)	Crude extract (g)	Yield (%)
1	Soxhlet	30	Methanol	8	4.26	14.2
2	Soxhlet	30	Ethanol 80%	8	3.96	13.2
3	Soxhlet	30	CH_2Cl_2	8	3.72	12.4
4	Soxhlet	30	Benzene	8	3.70	12.3
5	Soxhlet	30	Toluene	8	3.66	12.2
6	Soxhlet	30	Water	8	2.13	7.1
7	Maceration	100	Methanol	48	5.20	5.2
8	Maceration	100	Ethanol 80%	48	4.30	4.3
9	Maceration	100	CH_2Cl_2	48	4.20	4.2
10	Maceration	100	Benzene	48	3.50	3.5
11	Maceration	100	Toluene	48	3.40	3.4
12	Maceration	100	Water	48	2.80	2.8

121.19 (C-3), 121.50 (C-4), 124.10 (C-9a), 126.79 (C-7/C-6), 127.05 (C-6/C-7), 133.12 (C-8), 133.88 (C-5), 134.33 (C-10a/C-8a), 135.42 (C-8a/C-10a), 151.18 (C-1), 153.15 (C-2), 180.77 (C=O), and 189.08 (C=O); its MS (EI) showed m/z: 240 [M⁺, 100%], 212 [(M - CO)⁺, 27%], 184[(M - 2 × CO)⁺, 20%]. On the basis of these results, this fraction was identified as 1,2-dihydroxy-9,10-anthraquinone, Alizarin(I).

DISCUSSION

The roots of *Rubia tinctorum* L. (madder) are the source of a natural dye. The dye components are anthraquinones and contain rubian, rubiadin, ruberythric acid (III), purpurin (II), tannin, sugar and especially alizarin (I). Alizarin is present as its glycoside ruberythric acid. Fermentation of this root yields alizarin. When the dead roots ferment, the glycosides hydrolysed and yield free anthraquinones. This process causes the powder particles to stick together and form a solid mass (a lake). Alizarin is one of the two main dyes, the other being purpurin, that come from the root of the madder plant. Artists extract alizarin dye to make a red lake pigment which they can use in paints. Alizarin occurs as orange-red crystals, almost insoluble in water, but readily soluble in alcohol, ether, the fixed oils and alkaline solutions.⁶

Regarding the chemically importance of alizarin, it was decided to carry out the following objectives in this research: (i) isolation of alizarin from the madder roots by maceration and Soxhlet

extraction methods using various polar and non-polar solvents and finally (ii) confirmation of the chemical structure of alizarin by spectroscopic methods. We tried to isolate alizarin without direct hydrolysis in acidic, basic or under fermentation conditions. The plant was identified as *R. tinctorum*. Maceration of the powdered roots in various polar and non-polar solvents (methylene chloride, benzene, toluene, ethanol, methanol and water) were carried out at room temperature for 48 hrs. The best solvent was found to be methanol (Table 1). Soxhlet extraction of the powdered roots was carried out in the same solvents mentioned above for 8 hours. In this method too, the best solvent was found to be methanol (Table 1). However, the yield from Soxhlet extraction method was much higher than that from the maceration method in the same solvent (14.2% and 5.2% respectively) and the isolation time was much shorter (8 hours versus 48 hours) as well. Successive TLC and column chromatography of the extracted red-orange solid material resulted in the separation of three fractions with R_f = 0.21, 0.48, and 0.68 with the last one as the major fraction. IR, UV-Visible, ¹HNMR, ¹³CNMR, and MS spectra of the fraction with R_f = 0.68 were taken. It had m.p. 291 °C (literature 290 °C). The IR and UV spectra were compared with the corresponding standard spectra, they were almost identical. On the basis of the spectroscopic results, especially the mass spectra fragmentation pattern and melting point, it was confirmed that the fraction with R_f = 0.68 is pure alizarin.

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