International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.4, pp 1941-1944, Oct-Dec 2011

Isolation and characterization of 2-hydroxy-1,4-naphthoquinone (lawsone) from the powdered leaves of henna plant marketed in Ahwaz city of Iran

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Abstract: Regarding the therapeutic effects and traditional applications of henna, it was tried to isolate and purify Lawsone from the leaves of henna marketed in Ahwaz city of Iran by various methods of maceration and Soxhlet extraction techniques in different polar and non-polar solvents. Maceration of the powdered leaves in hot water with stirring for around 6 hours was carried out followed by addition of solid $NaHCO_3$.The suspension was filtered by gravity. The filtrates were combined and acidified to pH 3 by addition of 0.12 M HCl. The filtrate was extracted with diethyl ether. The combined ethereal phases were washed with water and dried over anhydrous $MgSO_4$. After the removal of ether on a rotary evaporator, a reddish solid material was obtained as crude product. The progress of the isolation of Lawsone was monitored by IR spectroscopy and TLC on silica gel. Column chromatography on silica gel 60 with a mixture of EtOH:EtOAc (1:2 v/v) as the mobile phase resulted in the formation of different colour zones. The crude product (0.5 g) was dissolved in 10 mL of the eluent and placed at the top of the column and the elution was started. Fractions of 10 mL were taken. Almost 50 fractions were collected. The compositions of all fractions were monitored by TLC. Fractions 25 -40 had the same R_f and contained Lawsone. The yield was very low (around 30 mg) (m.p. 191-194 °C).
Keywords: Henna, lawsone, lawsonia, 2-hydroxy-1,4-naphthoquinone.

Introduction

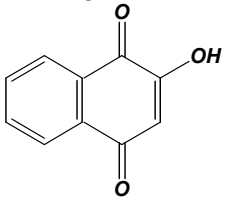
Henna (Lawsonia inermis, also called mignonette tree)[1] is a flowering plant which has been used since the Bronze Age to dye skin (including body art), hair, fingernails, leather, silk and wool. In several parts of the world it is traditionally used in various festivals and celebrations. The name is also used for dye preparations derived from the plant. (Scientific classification: Kingdom: Plantae; Division: Magnoliophyta; Class: Magnoliopsida; Order: Myrtales; Family: Lythraceae; Genus: Lawsonia; Species: L. inermis; Binomial name; Lawsonia inermis L). The trivial name of this compound was hennotannic acid. Interesting from the chemists' point of view are the redox properties of naphthoquinones such as lawsone.

The henna plant is a tall flowering shrub or tree about 5 m in height, native to tropical and subtropical regions of Africa, southern Asia, and northern Australaia in semi-arid zone and oases in the Sahara. Henna shrubs need light and warmth and are regarded as rather pest resistant. It is glabrous, multibranched with spine tipped branchlets. Leaves are opposite, entire, glabrous, sub-sessile, elliptical, and broadly lanceolate (1.5–5.0 cm x 0.5–2 cm), acuminate, having depressed veins on the dorsal surface.

Henna is commercially cultivated in UAE, Morocco, Yemen, Tunisia, Libya, Saudi Arabia, Egypt, western India, Iran, Pakistan, Bangladesh, Afghanistan, Turkey, Somalia and Sudan. Presently the Pali district of Rajasthan is the most heavily cultivated henna production area in India, with over 100 henna processors operating in Sojat City. Use of henna for body art has enjoyed a recent renaissance due to improvements in cultivation, processing, and the emigration of people from traditional henna-using regions.[2]

For skin dyeing, a paste of ground henna (either prepared from a dried powder or from fresh ground leaves) is placed in contact with the skin from a few hours to overnight. Henna stains can last a few days to a month depending on the quality of the paste, individual skin type, and how long the paste is allowed to stay on the skin. Henna also acts as an anti-fungal [3] and a preservative for leather and cloth. Henna flowers have been used to create perfume since ancient times, and henna perfume is experiencing a resurgence. Henna repels some insect pests and mildew.

Henna's coloring properties are due to lawsone, a burgundy organic compound that has an affinity for bonding with protein. Lawsone is primarily concentrated in the leaves, especially in the petioles of the leaf. Lawsone content in leaves is negatively correlated with the number of seeds in the fruits.[4] Whole, unbroken henna leaves will not stain the skin. Henna will not stain skin until the lawsone molecules are made available (released) from the henna leaf. Fresh henna leaves will stain the skin if they are smashed with a mildly acidic liquid. The lawsone **(I)** will gradually migrate from the henna paste into the outer layer of the skin and bind to the proteins in it, creating a fast stain.



(I)

Henna stains are orange soon after application, but darken over the following three days to a reddish brown. Soles and palms have the thickest layer of skin and so take up the most lawsone, and take it to the greatest depth, so that hands and feet will have the darkest and most long-lasting stains. Steaming or warming the henna pattern will darken the stain, either during the time the paste is still on the skin or after the paste has been removed. Chlorinated water and soaps may spoil the darkening process: alkaline products may hasten the darkening process.

Experimental

Reagents and solvents (reagent grade) were purchased from Aldrich, Fluka and Merck and used without further purification. Evaporation and concentration in vacuo were performed at water aspirator pressure. Column chromatography (CC) was carried out with SiO2 60 (particle size 0.040- 0.063 mm, 230-400 mesh; Merck) and commercially available solvents. Melting points (m.p.) were measured on a Buchi B-540 melting-point apparatus in open capillaries and are uncorrected. ^{1}HNMR spectra were recorded in $CDCl_3$ and d_6-DMSO 400 MHz Brucker instruments at 20 °C. Chemical shifts are reported in ppm relative to the signal of Me_4Si. Low resolution electron impact (E.I.) mass spectra were recorded on A.E.I. MS30 instrument.

1. **Maceration in H_2O at 75 °C:** powder of dried henna leaves (80 g) was placed in a 5 litre Erlenmyer flask containing a magnetic bar and distilled water (4000 mL) was added. The suspension was stirred on a magnetic stirrer with heating while the temperature was kept around 75 °C. After around 60 minutes, the colour of the green suspension turned brown. After around 6 hrs, solid $NaHCO_3$ (17 g) was added. The suspension was filtered by gravity over several large glass funnels with filter paper. Suction filtration was avoided, because the colloidal particles would rapidly plug the pores of the filter paper. The filtrates were combined and acidified to pH 3 by addition of 0.12 M HCl. At this stage, the brown extract turned slightly cloudy. The swollen plant material was discarded. The filtrate was extracted with diethyl ether (4 × 400 mL). The combined ethereal phases were washed with water (3 × 100 mL) and dried over anhydrous $MgSO_4$. After the removal of ether on a rotary evaporator, a reddish solid material was obtained as crude product (1.1 g). The progress of the isolation of Lawsone was monitored by IR spectroscopy and TLC on silica gel. Column

chromatography on silica gel 60 with a mixture of EtOH:EtOAc (1:2 v/v) as the mobile phase resulted in the formation of different colour zones. The crude product (0.5 g) was dissolved in 10 mL of the eluent and placed at the top of the column and the elution was started. Fractions of 10 mL were taken. Almost 50 fractions were collected. The compositions of all fractions were monitored by TLC. Fractions 25 -40 had the same R_f . The yield was very low (around 30 mg) (m.p. 191-194 °C). The IR spectrum (mull in nujol) showed (cm^{-1}): OH_{str} (around 3400 - 3300, br), C=O (1680 and 1645, s), C=C (1590, 1575, m, sharp, aromatic), and C-O (1280-1218, s); its UV spectrum in EtOH showed λ_{max}^{EtOH} (nm) 214, 233 and 270 absorptions; its ^{1}HNMR (d_6-DMSO, 400 MHz) showed (δ): 6.15 (s, H-3), 7.72, 7.69 (dt, H-6+H-7), 7.8, 7.77 (d, H-5) and 7.85, 7.84 (d, H-8); its ^{13}CNMR ((d_6-DMSO, 100 MHz) showed (ppm):110.12 (C-3), 123.52 (C-7/C-6), 124.10 (C-6/C-7), 131.51 (C-8), 132.43 (C-5), 128.22 (C4-a/C-8a), 130.18 (C-8a/C-4a), 158.24 (C-2), 180.12 (C=O, C-4), and 182.46 (C=O, C-1); its MS (EI) showed m/z: 174 (M^{+}, 100%), 146 [$(M\text{-}CO)^{+}$, 16%], 105 [$(146\text{-}C{\equiv}CO)^{+}$, 51%], 77 [$(105\text{-}CO)^{+}$, 19%].

2. **Soxhlet extraction in various solvents (toluene, chloroform, methylene chloride and water):** powder of dried henna leaves (20 g) was placed in a 1 litre round bottomed flask and 600 mL of the solvent was added (each individual solvent in a separate experiment). The flask was equipped with a Soxhlet extracting funnel and a condenser. The extraction was carried out for 8 hours. Then, the solvent was removed on a rotary evaporator. The IR spectrum of each of the residues were taken, the results were unsatisfactory and sometimes did not show the functional groups of Lawsone **(I)**.

3. **Tommasi's procedur:** powder of dried henna leaves (500 g) was placed in a 5 litre Erlenmyer flask containing a magnetic bar and distilled water (2500 mL) was added. The suspension was stirred on a magnetic stirrer with heating while the temperature was kept around 80 °C. After around 2 – 3 hours, the colour of the green suspension turned brown. The suspension was left overnight. After that lime water was added until the suspension turned alkaline. The suspension was filtered by gravity over several large glass funnels with filter paper. The filtrates were combined and acidified to pH 3 by addition of 0.12 M HCl. The filtrate was extracted with diethyl ether (3 × 1000 mL). The combined ethereal phases were washed with water (3 × 1000 mL) and dried over anhydrous $MgSO_4$. After the removal of ether on a rotary evaporator, more or less a reddish solid material was obtained as crude product (3.25 g). The progress of the isolation of Lawsone was monitored by IR spectroscopy and TLC on silica gel. The purification and characterization of the crude product were more or less the same as procedure mentioned in 1 (Maceration in H_2O at 75 °C).

Discussion

Lawsone occurs in the henna plant leaves in the form of glycosidic precursors that have to be cleaved prior to isolation. It is a kind of miracle that the dried and powdered leaves do contain an intact glycosidase even after years, able to split the glycosidic bond, when brought into contact with hot water. Therefore, the henna leaf powder suspension is stirred for several hours in water at 70 °C.[5]

Henna penetrates the dead cell of the horny outermost layer of the skin. To obtain a paste with colouring properties requires that dried and ground henna leaves are treated in a special manner including a warm and aqueous medium that ensures deglycosylation and aglycone (lawsone) formation. How long the henna paste affect the skin is decided by the depth of the reddish brown coloration and how long it will be visible. Usually, 6 – 8 hours are required to achieve a satisfactory result. Since ancient times, henna leaves have been used in traditional medicine as an astringent, antiseptic and antipyretic. Henna was used in ancient times also to treat serious diseases (leprosy, smallpox, chickenpox, tumours) by Islamic doctors.

More recently, henna has been investigated and some physiological effects have been confirmed, e.g. bactericidal and fungicidal action by its tanning effect.[5] Henna itself is not an allegen, nor could rumours be proved that it might be a carcinogen. The wound healing process is supported by a henna leaf extract.[6] Recently, lawsone was found to be suitable as a reagent for the detection of latent fingermarks on paper, which is still an extremely important requirement in criminology as contact evidence. Lawsone, in this context, could serve as a substitute for ninhyfrin, used hitherto.[7]

Regarding the therapeutic effects and traditional applications of henna, it was decided to carry out the following objectives in this research: (i) isolation and purification of Lawsone from the leaves of henna marketed in Ahwaz city of Iran (ii) identification and determination of the chemical structure of the isolated

and purified Lawsone by various spectroscopic techniques.

Based upon the fact that the leaves contain a small amount of Lawsone (0.5 – 1.5%), a comprehensive effort was made to use various methods of maceration and Soxhlet extraction techniques in different polar and non-polar solvents such as EtOAc, $C_6H_5CH_3$,EtOH, $CHCl_3$, CH_2Cl_2 , Et_2O, H_2O and n-C_6H_{14}.

Because lawsone was not readily soluble in water and it is acidic (pK_a 4), we had to add $NaHCO_3$ to make the aqueous phase weakly basic (pH 7.5) and bring any lawsone into solution before the suspension was filtered. The filtrate was then acidified and lawsone was extracted into diethyl ether. From the solid crude product remaining after removal of the ether, Lawsone was separated in a highly pure form by means of column chromatography.

Maceration of 100 g of the powdered henna leaves in 1000 mL of each of the above mentioned solvents individually, at either room temperature or 80 °C and also Soxhlet extraction of 20 g of the powdered leaves of henna in 300 -600 mL of the solvents were performed. The results showed the functional groups of Lawsone, but other impurities were present too. Soxhlet extraction techniques in different polar and non-polar solvents all failed to give lawsone, obviously the glycosidic precursors must be cleaved prior to any isolation.

Tommasi's procedure, i.e., maceration in aqueous $Ca(OH)_2$ on ca 2 kg scale in 10 liters of water by adding $Ca(OH)_2$ at room temperature for 72 hours was performed, too. After successive filtration, acidification and extraction with diethyl ether, finally, Lawsone was isolated. Purification was achieved through successive TLC (on silica gel) and column chromatography. Progress of the separation was monitored by IR spectroscopy.

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