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Degradation of lucidin: New insights into the fate of this natural pigment present in Dyer's madder (*Rubia tinctorum* L.) during the extraction of textile artefacts

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ABSTRACT

The complex mixtures of colorants present in different madder species can provide significant information about which plant species or technique was used to dye the fibres of historical textile artefacts, hence, when extracting and analysing colorants from textile artefacts as much of this information as possible should be preserved. Historical textiles are most commonly extracted with 37% hydrochloric acid: methanol: water (2:1:1, *v/v/v*), but this solvent system hydrolyses dye glycosides and may also induce chemical reactions. One of the primary components in Dyers' madder (*Rubia tinctorum* L.) is lucidin primeveroside, but it is rarely seen in artefacts, nor is the corresponding aglycon lucidin. It has been demonstrated that the hydrochloric acid method causes hydrolysis of anthraquinone glycosides to their aglycon counterpart. Herein it is demonstrated that lucidin is not stable in such acidic conditions and degrades rapidly to xanthopurpurin. This is confirmed by HPLC, LC-MS and ^{1}H NMR, which also provide evidence of the mechanism of degradation being a retro-aldol process.

1. Introduction

Natural colorants are complex mixtures of many different molecules and plant dyes are often a mixture of aglycons of the parent colorant moiety and their glycosidic counterparts. The ratio of the abundance of these molecules can provide significant information about which plant species was used to dye the fibres or the technique used for the dyeing process. In the context of historical textiles, this information is of paramount importance for conservation and restoration purposes, as well as the generation of information on the ethnographic origins of the artefacts.

Colorants obtained from the roots of Dyers' madder (*Rubia tinctorum* L.), are grouped collectively in the Colour Index as C. I. Natural Red 8, and have been used as a red dyestuff for centuries. Over 35 anthraquinonoid compounds have been reported to be extractable from madder roots [1], however, many of these compounds are artefacts of inherent reactivity during analytical extraction methods and are suspected as not being not present *in planta*; for example, anthraquinones that contain a 2-methoxymethyl- or a 2-ethoxyethyl group are formed during extraction with hot methanol or ethanol, respectively [1,2]. When extracting and analysing colorants from textile artefacts as much information should be preserved as possible in order to gain better insight on how they were dyed and the plant species from which the dye originated, hence, it is important to limit the damage to the colorant molecule in the extraction process. However, extraction of artefacts is not straightforward as the dyes are strongly bound to the substrate *via* a mordant metal (typically Al^{3+}); the most common literature extraction procedure uses a 37% hydrochloric acid: methanol: water (2:1:1, *v/v/v*) mixture [3–8], as the strong acid enables displacement of the dye molecules from their mordant metal complex [9]. However, such conditions may also induce a chemical reaction, hence, it is vital that fundamental understanding of the reactivity of such natural dyes is developed alongside the analysis of the components within the mixture. If the conditions of extraction and analysis of these dyed textiles changes the ratios of the compounds present, or modifies their structure, then valuable information on that artefact will be lost or potentially misinterpreted.

Only relatively recently has there been significant evidence confirming the primary anthraquinone components in *Rubia tinctorum* roots as the glycosides ruberythric acid (**1**) and lucidin primeveroside (**3**) [10–14]; the majority of literature has pointed to alizarin (**2**) as the major anthraquinone present, and whilst it does occur in the plant, it is in much lower concentrations than its glycoside [11,12,14]. We have previously suggested [15] that acidic conditions used in extraction and

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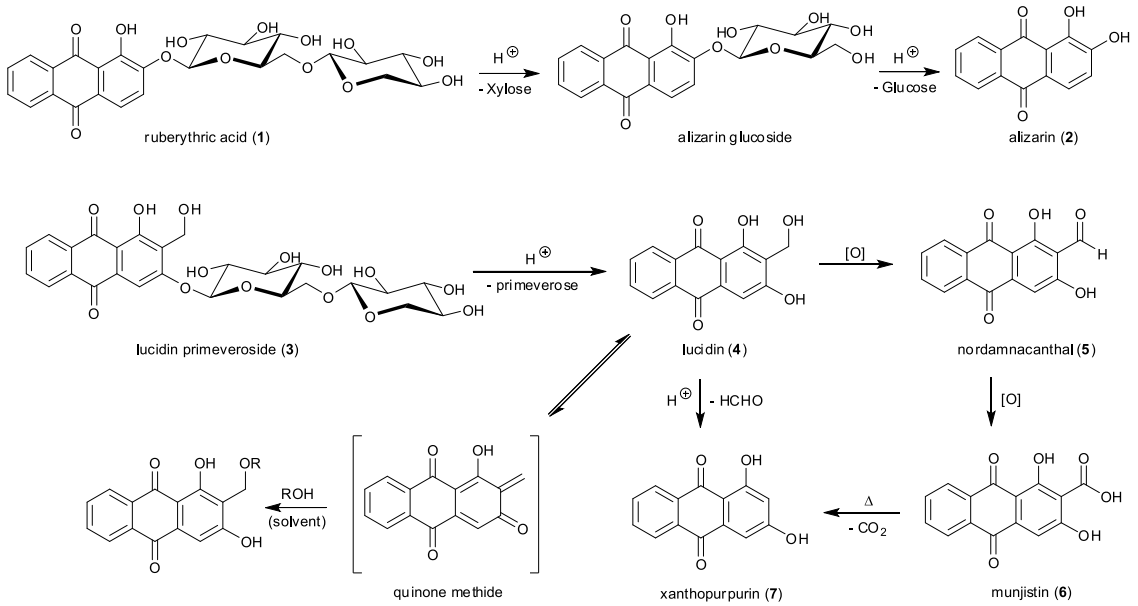

Fig. 1. Possible inter-relationships between anthraquinone compounds found in *Rubia tinctorum* based on chemical or biochemical interconversion.

analysis of dyes in previous studies may have led to observations that alizarin was the primary component, it being the product of ruberythric acid hydrolysis (**1** → **2**). However, despite high concentrations of lucidin primeveroside (**3**) in *Rubia tinctorum* roots [11,12,14], the aglycon lucidin (**4**) is rarely detected (and then only in low and trace concentrations *in planta* and in textile artefacts [10–12,16]) even when acidic conditions are used that would promote hydrolysis (**3** → **4**); it is suspected that the reactive nature of lucidin means that it is readily converted to other compounds. As Fig. 1 shows, lucidin (**4**) can be oxidised to nordamnacanthal (**5**), and studies have suggested this is catalysed by endogenous oxidase enzymes in the plant [17,18]. It is possible that nordamnacanthal (**5**) can form munjistin by the action of endogenous oxidase; subsequently xanthopurpurin (**7**) may be formed through decarboxylation of munjistin (**6**). It has also been proposed [19] that another enzymatic reaction can occur that converts lucidin into the quinone methide (Fig. 1). It is thought this intermediate may be able to be formed by acidic conditions, but the actual intermediate is too difficult to isolate due to it being a very strong electrophile and addition at the double bond by any nucleophile is highly likely [12].

However, these enzymes are probably denatured in the dyeing process and hence this mode of degradation probably is not responsible for these compounds in historic artefacts. Mouri & Laursen [12] recently confirmed that, unless *R. tinctorum* roots were "warmed in water" for prolonged periods (hence, providing enzymatic incubation conditions), significant concentrations of anthraquinone glycosides were present in the dyebath and on dyed wool fibre. They demonstrated that steaming madder roots or boiling them in water for 30 s was sufficient to deactivate the hydrolytic enzymes. An initial extraction process by boiling the madder root was typically performed in the Japanese *Kusaki-zome* dyeing method and typical European madder dyeing processes historically involved heating the dyebath to 75–80 °C, which would most probably also denature the endogenous enzymes present [20].

We have previously suggested [15] that xanthopurpurin may also be formed directly from lucidin (**4**) through an acid (or base)-catalysed loss of formaldehyde through a retro-aldol type process (Fig. 1), but there is no literature to support this proposal. The absence of lucidin in the analysis of artefacts dyed with madder is rarely acknowledged, or it is stated that lucidin is degraded into unknown products [9]. Lucidin is the only commonly reported anthraquinone detected in the roots of *Rubia tinctorum* to contain a primary alcohol, which could make its degradation unique. As described in our previous research [15], use of the 37% hydrochloric acid: methanol: water (2:1:1, *v/v/v*) solvent system causes hydrolysis of anthraquinone glycosides present in madder root, with the result that only aglycons are detected in back extraction experiments.

Herein it is suggested that when such acidic methods of extraction are used to solvate the dye compounds, degradation of the aglycon lucidin may also occur. The purpose of the research described is to study the degradation of lucidin under the conditions of extraction involved with the common 37% hydrochloric acid: methanol: water (2:1:1, *v/v/v*) solvent method.

2. Materials and methods

2.1. Materials and solvents

All chemicals were purchased from Sigma-Aldrich. All solvents used were of HPLC grade and also purchased from Sigma-Aldrich. HPLC grade water was obtained by distillation on site.

2.2. General procedures and instrumentation

Nuclear magnetic resonance (NMR) spectra recorded for ^{1}H NMR at 300.13 MHz and 500.21 MHz and ^{13}C at 75.45 MHz on a Bruker *DPX300* and *DRX500* spectrometer. Chemical shifts are given in parts per million (ppm) downfield of tetramethylsilane (singlet at 0 ppm) for proton resonances. The proton coupling constants are corrected and given in Hz and expressed, *e.g.* as multiplicities, singlet (s), broad singlet (bs), doublet (d), double doublet (dd), triplet (t) and quartet (q). High resolution electrospray (ESI+) mass spectrometry was performed on a Bruker MaXis Impact spectrometer, *m/z* values are reported in Daltons to four decimal places. Liquid Chromatography with Mass Spectrometry (LC-MS) was carried out for analysis synthetic references. LC analyses were carried out at room temperature on a Phenomenex Hyperclone C_{18} column, 5 µm particle size, 250 × 4.6 mm I.D. column equipped with a pre-column. Chromatography was carried out using two solvents: (A) water and 0.1% formic acid solution and (B) acetonitrile and 0.1% formic acid solution. A linear gradient programme was applied: of 0–3 min 0–100% increase of solvent B. The flow rate during the experiment was 1.0 ml min^{-1}. Injections were made by a Basic Marathon autosampler equipped with a 20 µl loop. The method