1

History of Natural Dyes in the Ancient Mediterranean Civilization

Maria J. Melo

LAQV/REQUIMTE, Department of Conservation and Restoration;
Faculty of Sciences and Technology, Universidade NOVA de Lisboa,
Monte de Caparica, Portugal

COPYRIGHTED MATERIAL

The colors used on textiles and artifacts, their social significance and the scope of their trade are part and parcel of a people's overall history.

Jenny Balfour-Paul, in *Indigo*

1.1 Introduction

1.1.1 Ancient Mediterranean World

The buildup of *Mare Nostrum* probably began much earlier than the 6th–5th millennium BC, and there is material evidence pointing to such activity as early as the 12th–11th millennium BC [1]. *Mare Nostrum,* the Roman name for the Mediterranean Sea, was to become the home of a global market that expanded beyond its natural borders in the first millennium BC. The Phoenicians, the Etruscans, the Greeks and finally the Romans shaped *Mare Nostrum*; a geographic as well as a cultural domain. It was also home to the first global dye, Tyrian purple, traded by the ingenious and industrious Phoenicians. The purple of Tyre was famous, as were the luxury textiles dyed and produced by the Phoenicians [2, 3]. It is said that the Greeks named the Phoenicians after *phoinikes*, the ancient Greek word for "red color," probably as a result of their famous purple trade.

By the time of the founding of the Mediterranean civilizations, what we would consider the classical palette for natural dyes had already been established, and the most valued colors were indigo for the blues, anthraquinone-based chromophores for the reds and 6,6'-dibromoindigo for purple. These colors were traded all over the Mediterranean, regardless of the distance to be

Handbook of Natural Colorants, Second Edition. Edited by Thomas Bechtold, Avinash P. Manian and Tung Pham.
© 2023 John Wiley & Sons Ltd. Published 2023 by John Wiley & Sons Ltd.

traveled or the price to be paid. The natural sources for yellows were much more diverse, so yellows could generally be obtained locally. For dyeing, with the exception of some browns, all other colors and shades, including green and orange, could be obtained with these blue, red, purple and yellow dyes. This classical palette was preserved over centuries, if not millennia. The first adjustment resulted from the loss of Tyrian purple after the fall of Constantinople and the subsequent collapse of the Roman social and commercial web. This was followed by a new entry, cochineal red, brought by the Spanish from the New World [4]. However, even with the introduction of cochineal the chemical nature of the classical palette was maintained, as carminic acid is still a substituted 1,2-dihydroxy anthraquinone. This classical palette was only challenged by the audacity of chemists, who created new molecules, and colors never seen before, from the mid-19th century on [5].

1.1.2 Dyes from Antiquity

Natural dyes, discovered through the ingenuity and persistence of our ancestors, can resist brightly for centuries or millennia and may be found hidden in such diverse places as the roots of a plant, a parasitic insect and the secretions of a sea snail. By contrast, the bright colors that we see in the green of a valley, the red of a poppy, the purple of mauve or the blue of cornflower are less stable. Natural dyes were used to color a fiber or to paint. It is useful to distinguish between dyes and pigments based on their solubility in the media used to apply the color; dyes are generally organic compounds that are soluble in a solvent, whereas pigments, used in painting, are usually inorganic compounds or minerals that are insoluble in the paint medium (oil, water, etc.), and are dispersed in the matrix. A pigment lake is formed by precipitating a dye onto the surface of an inorganic substance, namely by complexation with a metal ion.

Dyeing, in red, blue, purple or yellow, is a complex task that requires skill and knowledge [6]; this is true now and has been for several millennia. Color is obtained by a chemical compound called a chromophore or chromogen—that which brings or creates color. To be used as a textile dye, the chromophore must also be captured as strongly as possible into the fibers, that is, it must be resistant to washing. Dyes can bind to the surface of the fiber or be trapped within. They are often bound to textiles with the aid of metal ions known as mordants, which can also play a significant role in the final color obtained (Box 1.1). As a source of aluminum ion, alum is an important historical mordant and was widely used in the past. Other important mordants used in the past were iron, copper and tin ions [6, 7]. Dyes, such as indigo, which are trapped in the fibers due to an oxidation–reduction reaction, without the aid of a mordant, are known as vat dyes.

As pigments, natural dyes have been widely applied in painting. For example, anthraquinones have been used as red dyes and pigment lakes from prehistoric times, and we can find written accounts of the use of anthraquinone reds and purples as dyes in ancient Egypt [7, 8]; anthraquinone lake pigments (e.g., madder red) were also very popular with Impressionist painters, including Vincent van Gogh [9b]. Red, purple and yellow lake pigments can be prepared by precipitating the dye extract with aluminum or other inorganic salts, such as alum [9] (Box 1.1). Pure dyes such as indigo have also been used as painting materials, for example, in medieval illuminations (Figure 1.1).

These *eternal* colors will be described in more detail in the following sections, after a brief account of the analytical techniques used to reveal the secrets of these ancient materials. The natural colorants will be organized according to the color: first, the anthraquinone reds, followed by the blues and purples, where indigo and its bromo derivatives play a major role. Yellows will close this overview. More information about other important ancient dyes is provided in EuroWeb Digital Atlas [10].



Figure 1.1 *Medieval Portuguese illumination, dated from 12–13th-century, Lorvão 15 f. 50 (National Archive of Torre do Tombo, ANTT, Lisbon, Portugal). Dark blues were painted with indigo, whereas the inorganic pigment lapis lazuli was used for the background.*

Box 1.1 Natural dyes as lake pigments

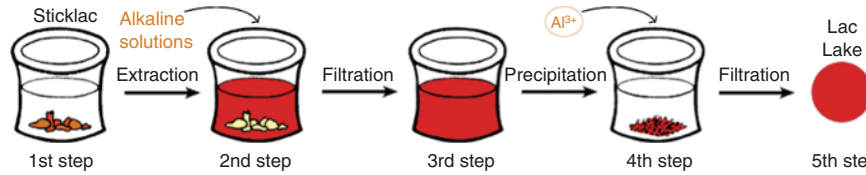


Figure 1 (Box 1.1) *Preparing a lake pigment using lac dye as raw material. Design by Rita Carvalho.*

A lake pigment is a metal complex, insoluble in water, prepared by adding a metal ion such as Al^{3+} to an aqueous solution of a dye extracted from a natural source. The complex will precipitate when a neutral molecule is formed, usually between a pH 6 and 7. In ancient times, the dye was extracted in acidic media (in the presence of Al^{3+}), in neutral (in urine) or

(*Continued*)

basic solutions (prepared with the ashes of plants, in which the oxides of alkaline metals are transformed into the corresponding hydroxides by reaction with water). The main steps for preparing a lac dye pigment are represented in Figure 1 (Box 1.1). Important lake pigments



Figure 2 (Box 1.1) *Anthraquinone reds can resist for centuries or millennia, as shown in the precious Andean textiles (embroidered mantle with shaman figures, mfa 31.501, 0- A.N.D. 100; Museum of Fine Arts, Boston, USA). Lac dye, also based on alizarin core, was found on a Persian carpet (17th century, Museum of Fine Arts, MNAA, Lisbon, Portugal) and applied as a lake pigment in manuscript illuminations dating from the 12th century. As for any alizarin-based chromophore, it can take many shades of red, purple and pink, as on the background used to highlight the dragon, symbol of the struggle between light and darkness, but also of renaissance, painted in the monumental Bible Santa Cruz 1 (Municipal Library of Porto, BPMP, Porto, Portugal).*

used in medieval manuscript illuminations were based on lac dye and brazilwood extracts, Figure 2 (Box 1.1).

The same kind of metal complex was used to bind the dye to a textile fiber; again aluminum ion was one of the most important metal ions used in the past, as illustrated in the following text. These metal ions, also known as mordants, were used to capture the dye to be resistant to washing. Other important mordants were iron, copper and tin ions. The structures of these dye–metal colors are complex, and there is still much we do not know about them. However, we are getting closer to the answers with computational quantum mechanical modeling (such as DFT, density functional theory).

1.1.3 Unveiling the Secrets of Ancient Dyes with Modern Science

Identifying the dyes and dye sources used in the past has only been possible with the development, in the past two decades or so, of sensitive new microanalytical techniques [11–16]. Chromophores are extracted, then separated chromatographically and characterized by UV–VIS spectrophotometry or mass spectrometry; whenever possible, comparison with authentic references is performed. Currently, the use of high-performance liquid chromatography with diode array detection (HPLC-DAD) enables dyestuff characterization from as little as 0.1 mg of thread, by comparison with reference compounds. For the identification of unknown components, analysis by high-resolution mass spectrometry (HRMS/MS) is required, coupled to an HPLC or an ultra-high performance liquid chromatography (UHPLC) system—HPLC–HRMS/MS or UHPLC–HRMS/MS [17–19]. Concomitantly, the development of mild extraction methods as alternatives to extraction in strongly acidic media was essential for the identification of the natural sources [13, 14, 20]. These new extraction procedures preserve the integrity of the glycoside fingerprint, avoiding decomposition to their parent aglycons. The glycoside fingerprint is particularly important for discussing the sources of yellow and red dyes. In 2011, within the European project CHARISMA (Cultural Heritage Advanced Research Infrastructures), the literature on the extraction of organic colorants in cultural heritage objects for HPLC analysis was reviewed [17]. The final aim was to propose the most appropriate extraction procedure for any kind of sample.

Mordant analysis can also provide relevant information about the dyeing method or process used. The metal ions can be quantified by inductively coupled plasma separation with atomic emission spectrometric (ICP-AES) or mass spectrometric (ICP-MS) detection of samples (approximately 0.25 mg textile strands) previously digested with nitric acid solutions [15, 16, 21]. Before the sample analysis, calibration curves must be constructed using standards. Concentration linearity in the range of ppb to ppm (or higher) can be achieved.

1.2 Ancient Reds

1.2.1 Anthraquinone Reds

The most stable reds used in antiquity are based on the 1,2-dihydroxy anthraquinone chromophore (Figure 1.2), also known as alizarin. Dyes containing anthraquinone and its derivatives are among the most resistant to light-induced fading [7]. These dyes were obtained from parasitic insects, such as the famous *Kermes vermilio*, or from the roots of plants belonging to the *Rubiaceae* or madder family and were among the reds that dominated the dye markets of Europe [6, 22]. Alizarin and purpurin are the main chromophores in *Rubia tinctorum*, the most important species of the family Rubiaceae. In Persia and India, other red dyes—of animal origin—were also used. These dyes were imported or sometimes

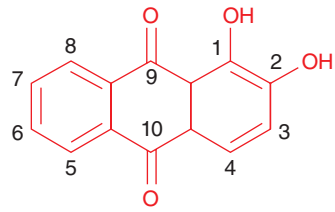


Figure 1.2 *Alizarin (1,2-dihydroxy anthraquinone).*

found locally, and were considered luxury goods. Well-known examples are the reds based on the laccaic acids, kermesic acid and carminic acid (Table 1.1) from the scale insects, lac, kermes and cochineal, respectively [6, 7, 23–24]. The female lac insect secretes a red resin, stick lac, from which both lac dye and shellac resin are obtained. Common or Indian lac such as *Kerria lacca* (= *Laccifer lacca*, *Carteria lacca*, *Tachardia lacca* and *Lakshadia lacca*) and *Kerria chinensis* are examples of species that have been widely exploited [7]. In both cochineal (*Dactylopius coccus*) and kermes (*K. vermilio*), the red dye is obtained from the eggs of the female insect, and therefore the harvest seasons corresponded to the stage when the female was full of unlaid eggs. It was at this stage that the female insects, alive or dead, were collected. *K. vermilio* is probably the most famous of the European insect parasites and produced a dye "brighter than madder and faster than cochineal" [6, 9a], as already described in the works of Theophrastus and Pliny. The word for worm in many Latin languages is based on *verme* (or *vermiculu*, meaning "small worm") and thus is the root of the word for red in several modern European languages, namely, vermejo, vermelho, vermeil, and vermilion in Spanish, Portuguese, French, and English, respectively [24].

Box 1.2: Armenian cochineal (*Porphyrophora hamelii*)

In the Armenian language, cochineal is known by the name *vordan karmir* (**որդան կարմիր**), which literally means *the worm's red* (*vord* = worm, *karmir* = red), and *vordan* can be broadly applied to an insect or worm, red color or dyestuff. The etymology of *karmir* is explained by an ancient root *kirm* (Pahlavi) or *kṛmi* (Sanskrit), meaning the *worm* [25].

P. hamelii species is endemic to the Ararat Valley, between Anatolia, the Armenian Highlands and the Iranian Plateau. In Armenia, the semi-desert vegetation areas are their habitats. Common host plants are coastal light-loving *Aeluropus littoralis* and common water-loving *Phragmites australis*, named in Armenian sources as *sēz* (grass), *vordnaxot* (worm's grass) or *ełeg* (reed).

The females of this species are up to 12 mm long and 6 mm wide, oval-shaped, flat, without wings, with six small legs, small eyes and antennae and dark red coloration, resembling a worm. Males are smaller, with black body, transparent and purple-edged wings, big eyes, long legs and long filiform tail, resembling a fly. They grow attached to the roots of the host plant throughout a year. In August–September, both the females and males emerge from the ground and seek mating. This period lasts about six weeks, but only one day for each insect. Afterward, the males die, while the females bury themselves in the ground and snuggle up to the roots of the host plant or nearby, preparing the waxy pockets for egg-laying. Later, the females also die. In April–May, the newborn larvae go out from the egg pockets and move



The field at the foot of Mount Ararat in the early morning. Aeluropus littoralis, the host plant of Armenian cochineal and the emerging cochineal. Photography courtesy of Hermine Grigoryan, Universidade NOVA de Lisboa.

toward the plant roots, if they are not already there. They feed on the roots and rhizomes of the host plant until becoming mature in the next year [25].

Since ancient times, Armenian cochineal has been used as a dyestuff. According to Łazar Pʿarpecʿi (5th century AD) "*De même la gracieuse plaine d'Ararat produit. . .des cochenilles pour la fabrication des couleurs vermeilles qui donnent du profit et du luxe aux gens intéressés*" [25]. This insect was profusely used in medieval Armenian workshops. From the 16th century onward, Armenian cochineal was gradually replaced by the American species [25]. In the words of a 19th-century traveler, Armenian cochineal was everywhere, "*In some places*

(*Continued*)



The female (left) and the male (right) of P. hamelii. Photography courtesy of Hermine Grigoryan, Universidade NOVA de Lisboa.

it propagates in the quantities that the land seems a carpet with red ornaments. The animals grazing here seem to have red-coloured legs, of having trampled the cochineal" [25].

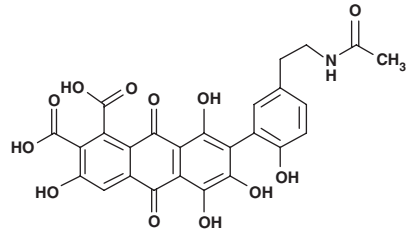
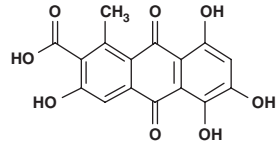
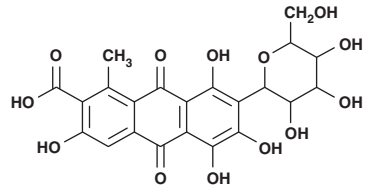
From 1930s to 1970s, attempts were made to recover the cochineal production in Soviet Armenia, but it remained at limited levels. In 1987, the natural state reserve *Vordan Karmir* was established, to ensure the protection of the saline ecosystems of the Ararat Valley, and its flora and fauna. However, in the following decades, the insect was endangered due to the human factor. Agricultural interventions and animal pasture have reduced dramatically the population. Nowadays, around 220, formerly 11 000, hectares of state reserve are protected areas of *P. hamelii*, and the insect is included in the Red Book of endangered species of the Republic of Armenia.

Other important historical insect sources of red or scarlet dyes derived from species of *Porphyrophora*, for example, Polish cochineal (*Porphyrophora polonica*) and Armenian cochineal (*Porphyrophora hamelii*). Know more about Armenian cochineal in Box 1.2 [25]. In Europe, most of these sources were replaced in the 16th century by the American cochineal (*D. coccus*), commercialized by the Spanish empire, which had earlier been carefully domesticated and cultivated by the New World's indigenous peoples [4]. Although the various species of *Porphyrophora* also contain carminic acid, dried specimens of *D. coccus* have a much higher content (15–20%) of the dye, compared with only 0.8% and 0.6% for the Armenian and Polish specimens, respectively [7].

1.2.2 Redwoods

Redwoods, also known in antiquity as brasil, were used as sources of dyes and pigment lakes [7, 9a]. It is said that the country Brazil was named after those redwoods, this possibly being the only country in the world named after a tree. The name the Portuguese had in mind was *Terra da Vera Cruz* (land of the Holy Cross). The reds obtained from the bark of the tree are not as stable as the previously described anthraquinone reds, but they were important pigments in medieval miniature paintings, particularly in books of hours produced in French and Flemish workshops [26a, 27, 28]. They were also used in cosmetics [9a, 26a]. In his book, Cennino Cennini recommends the use of a good *verzino* (brazilwood) to highlight the reddish-blue tone of purified lapis lazuli [26a]. This good *verzino* should be obtained from a young lady, who, using it for lipstick and other cosmetics, would take care that she had obtained a well-prepared product of a nice color.

Table 1.1 *Chemical structures for anthraquinone reds, produced by scale insects.*

Anthraquinone reds	
Lac dye *Kerria lacca*	
Kermes *Kermes vermilio*	
Cochineals *Dactylopius coccus* *Porphyrophora* spp.	

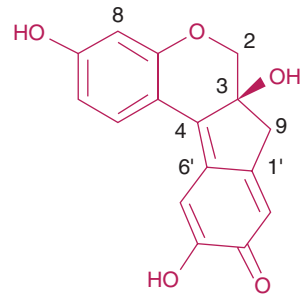


Figure 1.3 *Brazilein; hematein is 8-hydroxy brazilein.*

Since antiquity, brazilwood was used for pink and red colors in ancient Asian civilizations, and in the early Middle Ages, it began to be imported into Europe from Sri Lanka, India, and Southeast Asia [7, 29]. At that time, the Asian sappanwood, *Caesalpinia sappan*, was the only known brazilwood species. At the beginning of the 16th century, the Portuguese imported from Brazil to Europe new redwoods belonging to different species, replacing to some extent the Asian sappanwood. These new redwoods included *Caesalpinia echinata*, *Caesalpinia brasiliensis*, *Caesalpinia violacea*, *Caesalpinia crista*, and *Haematoxylum brasiletto* [29]. Some are still important today, not so much as a source of red dye, but for the medicinal properties of their components (*C. sappan*) or because their wood is considered ideal for making violin bows (*C. echinata*) [29]. For dyeing, only the colored heartwood of the trunk and larger branches of the trees were used, which could be cut up into chips and ground into powder [7, 29].

The main dye in *C. brasiliensis*, *C. echinate, C. sappan, C. violacea,* and *H. brasiletto* is brazilin. In *Haematoxylum campechianum*, the main dye is hematoxylin. Through oxidation, brazilin and hematoxylin are converted to the darker red compounds, brazilein and hematein, respectively (Figure 1.3) [30].

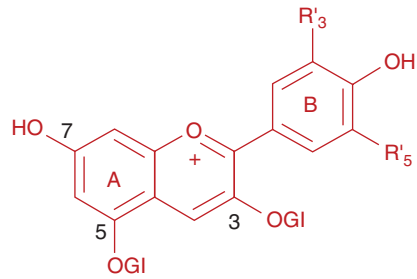


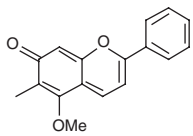
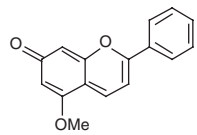
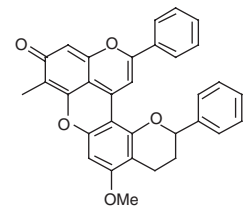
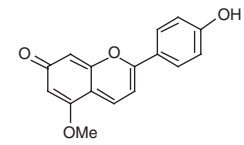
Figure 1.4 *In the basic structure of anthocyanins, a hydroxyl group is present at positions 4′ and 7, and a sugar at position 3 (monoglycoside) or positions 3 and 5 (diglycoside).*

1.2.3 Flavylium/Anthocyanin Reds

Anthocyanins are ubiquitous colorants responsible for the impressive red and blue colors of flowers and fruits (Figure 1.4). Natural flavylium reds can be considered their aglycone ancestors [31]. Anthocyanins have been found in a wide range of applications, from the millenary paints described by the Roman architect Vitruvius to food additives, combining color and antioxidant effects, and even as light absorbers in solar cells [31b,c, 32]. They were used as watercolors and textile dyes or as substitutes for unavailable inorganic pigments. Vitruvius described a color for replacing Tyrian purple in mural paintings; it was prepared by encapsulating the blueberry's juice in a clay [31b, 32]. Watercolors are described in written sources on illumination painting, including *The Strasbourg Manuscript, De Arte Illuminandi, the book on how to make all the color paints* [33, 34]. Flavylium, as a textile dye, was reported in Indian communities in Central and South America, and dragon's blood resins were found in artworks [31b, 35].

The color domain ranges from red to blue with anthocyanins, but with natural flavylium dyes it is limited to yellow-red. One of the most famous examples of a natural flavylium red can be found in dragon's blood resins. Dragon's blood is a natural resin with a deep, rich red color obtained from various trees, namely, *Dracaena draco* and *Dracaena cinnabari*, which belong to the *Liliaceae* family. The resin appears in injured areas of the plant, and it has been used for centuries for diverse medical and artistic purposes [36]. These resins contain not only the red chromophores but also additional flavonoids and steroids; dragon's blood resins have been used in traditional Chinese medicine. The molecules responsible for the red color of the resin obtained from the palm tree *Daemonorops draco* (*Calamus draco* was used in the past) have been characterized by Brockmann *et al*. and named dracorubin and dracorhodin (Table 1.2) [37]. Brockman *et al*. concluded that dracorhodin is a natural flavylium chromophore belonging to the anthocyanin family. More recently,

Table 1.2 *Chemical structures responsible for the red color in dragon's blood resins. The structures correspond to the quinoid bases (A).*

dracorhodin	nordracorhodin	dracorubin	dracoflavylium
			

other natural flavylium chromophores, such as dracoflavylium, have been identified in *D. draco*, *D. cinnabari* and other *Dracaenaceae* (Table 1.2) [36].

1.2.3.1 Equilibria in solution

As stated in the earlier text, dracorhodin and dracoflavylium are natural flavylium reds related to anthocyanins. Anthocyanins are characterized by a hydroxyl group in positions 4' and 7, and a sugar in position 3 (monoglycosides) or 3 and 5 (diglycosides). On the other hand, in anthocyanidins, the hydroxyl groups take the positions of the glycosides, leading to unstable structures in solution. By contrast, the so-called deoxyanthocyanidins correspond to "anthocyanidins" lacking the hydroxyl in position 3 (but bearing a hydroxyl in position 5) and are quite stable in solution [31, 32].

In the 1970s, it was established by Dubois and Brouillard (anthocyanins) [38a] and McClelland (synthetic flavylium salts) [38b] that both families of compounds undergo multiple structural transformations in aqueous solution following the same basic mechanisms [31, 39] (Figure 1.5). The flavylium cation (AH^+) is the dominant species in very acidic solutions, but with increasing pH a series of more or less reversible chemical reactions take place: (i) proton transfer leading to the quinoidal base (A), (ii) hydration of the flavylium cation giving rise to the colorless hemicetal (B), (iii) a tautomerization reaction responsible for ring opening to give the pale yellow Z-chalcone, form (C_c), and finally, (iv) *cis–trans* isomerization to form the pale yellow E-chalcone (C_t). Furthermore, at higher pH, and depending on the number of hydroxyl groups, further deprotonated species are found, such as C_t^{n-} and A^{n-}. The relevant contributors to color are AH^+ and the quinoid bases A and A^-. The red color of dragon's blood resin was found to be due mainly to the red quinoid base A [36].

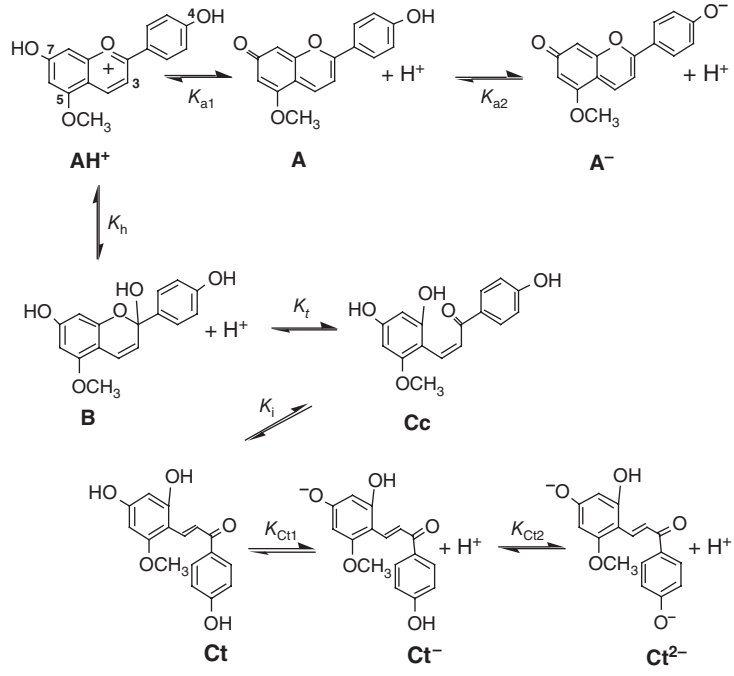


Figure 1.5 *Scheme of chemical reactions for dracoflavylium. Reprinted with permission from [36], Melo, M. J. et al., Chem. Eur. J., 13, 1417–1422 (2007) © 2007, Wiley-VCH.*

1.3 Ancient Blues

1.3.1 Indigo Blues

Indigo blue was one of the earliest and most popular dyestuffs known to man (Figure 1.6). It is still a universally used color, as the worldwide use of indigo-dyed blue jeans can attest. Indigo, as the name implies, has its origins in ancient India. In the great civilizations of Egypt, Greece and Rome, it was prized for its quality as a dye even though transportation costs meant it was very expensive [40]. Indigo sources are found all over the world, and several plants were most likely used in antiquity. In his *Commentarii de Bello Gallico*, Julius Caesar describes the warrior skin paintings of his Gaulish adversaries, as being obtained from a blue juice; the warriors' faces were frightening, and they believed that dyeing the skin would protect them, making them invulnerable.

Isatis tinctoria was grown in Europe, but it is known that indigo from *Indigofera tinctoria* was traded to Europeans, namely from Persia to Muslim Spain, and from there distributed to other European countries [41]. Species of *Indigofera* produced high-quality indigo.

Indigo is also one of the most light-stable organic dyes, a characteristic that explains not only its wide use in antiquity and the premodern era, but also its longevity as a colorant [42–44].

The stability of indigo is also the reason why it was used in medieval illuminations and by the great masters of the 17th and 18th centuries, such as Rubens [42, 44, 45] (Figure 1.1). Usually, with the exception of some necessary and almost irreplaceable color lakes, organic compounds were avoided in oil painting, as it was known that they were less stable to light than the inorganic available pigments. Highly pure indigo was the exception.

In 1865, Bayer started his work on indigo, and some years later, he proposed the chemical structure and a possible synthesis of indigo. At the time, indigo was an important molecule due to its commercial value as a dye; synthetic indigo paved the way not only for the development of the "big" German chemical and pharmaceutical industries but also helped to end the colonial production of indigo, which until then had come from natural sources in British, French and Iberian colonies. This particularly affected the British Indian colony, which was the major producer at that time. Even at the beginning of the 21st century, indigo is still a surprisingly important dye. Since indigo can be obtained from natural sources using microbial fermentation, its production using "green" chemistry is of considerable interest [46].

The precursors of indigo (= indigotin) can be extracted entirely from plants. The extract, a solution of the water-soluble indoxyl glucoside, indican, is hydrolyzed via fermentation to give indoxyl, which will further react with another indoxyl molecule producing indigo or with isatin, resulting in indirubin, a reddish dye [7, 46] (Figure 1.7). The indigo derivatives are generally known as vat dyes and, in their oxidized forms, are insoluble in water [7].

A common characteristic of vat dyes is the presence of one or more carbonyl groups, which, when treated with a reducing agent in the presence of an alkali, form a water-soluble dye known as

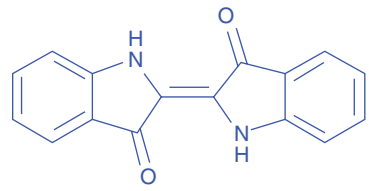


Figure 1.6 *Indigo (= indigotin).*

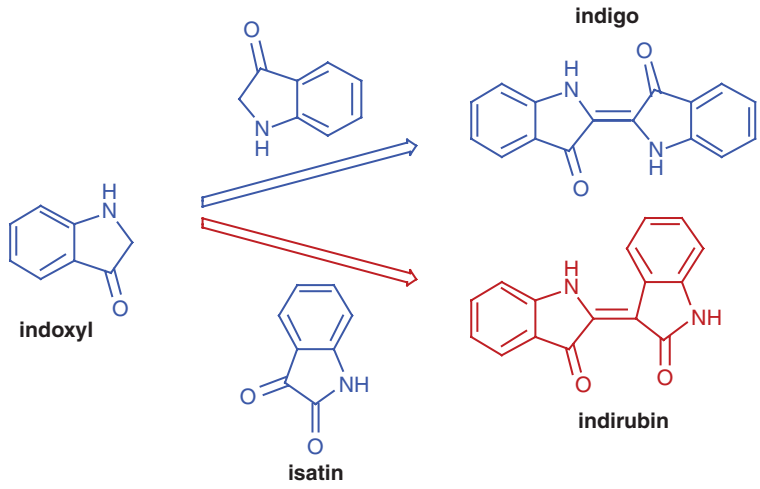


Figure 1.7 *Indigo dye bath: colorless precursors and pigments formed—indigo blue and indirubin red.*

the leuco species. The process of dyeing a textile with these species involves the use of the vat dye in a reducing media leading to its leuco form [7, 47]. The explanation of the blue color of indigo and its derivatives has also been an intriguing and fascinating subject, and was explored in great detail and ingenuity during the 1970s and 1980s [48]. It has been discovered that the fundamental chromophore of the indigo dyes includes the central double bond (connecting the two rings) together with the nitrogen and carbonyl groups [44, 48a–c]. Substitution in different ring positions of indigo is likely to promote significant shifts in both the visible long-wavelength and UV bands [48d].

Indirubin can be formed during the dyeing with indigo, namely in the interface where more oxygen is available, which is the reason why it is also known as the red shade of indigo [49]; as was previously mentioned, indirubin is formed through the reaction of indoxyl anion with isatin, a side reaction in the reaction of indoxyl anion with itself to give indigo (Figure 1.7). It can also be present in the Muricidae (see Section 1.4.1—Tyrian Purple: Real Purple from Sea Snails). Indirubin was identified in the blues used in pre-Columbian textiles, such as the magnificent Andean textiles from the Museum of Fine Arts (MFA, Boston) collection [43b] (Box 1.1). For dragon's blood, its renaissance has been a consequence of its pharmaceutical activity [49].

1.3.2 Anthocyanin Blues

As reported in Section 1.2.3, there are several ancient references to the use of anthocyanin watercolors for painting, particularly for illuminations, for example, as described in the Strasbourg Manuscript [33a]. More recently, the use of *Commelina communis* for blue in Aztec manuscripts was proposed [50].

In the past, extracting and capturing an anthocyanin blue were not the easy tasks; these blues are complex, self-assembled, supramolecular structures, bound by noncovalent interactions. The overall supramolecular complex must be preserved, not just the monomeric subunits; otherwise, the color is lost. For example, to obtain a "cornflower" blue, the supramolecular assembly must be maintained within a certain pH range. Apparently, this was achieved by following the recipes

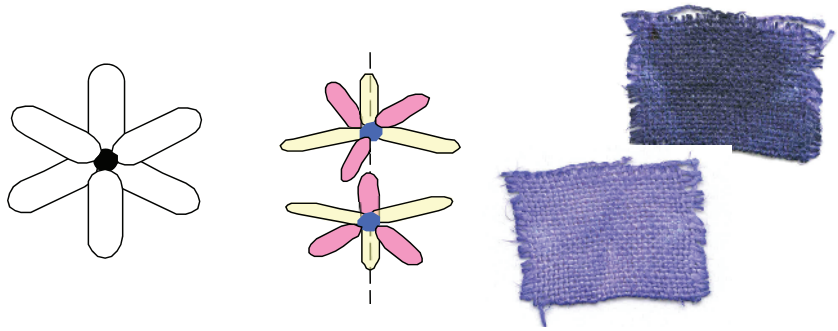


Figure 1.8 *Schematic representation of the blue supramolecular complex identified in Commelina communis. The two metal ions, Mg^{2+}, are the center of two planes; a top view of one of these planes is represented on the left. Each metal ion (in blue) coordinates three anthocyanin chromophores (pink); three flavones (yellow) are intercalated with the three anthocyanins, filling the void spaces. These blues applied as watercolors were recently identified in pre-Columbian codices [50b]. In medieval times, watercolors were preserved as clothlets (pezzuole in Italian), as depicted on the left using another anthocyanin blue, extracted from Centaurea cyanus.*

described in medieval treatises such as the Strasbourg Manuscript [33a]. The structure of the self-assembled supramolecular pigment from the blue cornflower (*Centaurea cyanus)* was elucidated by Kondo *et al*. [51a]; the supramolecular components are succinylcyanin (Sucy), a cyanidin chromophore with a sugar in position 3, malonyl flavone (Mafl) a flavone with a sugar in position 3, and the metal ions Mg^{2+} and Fe^{3+}, the exact composition being [$Sucy_6Mafl_6Fe^{3+}Mg^{2+}$]. These authors proposed that the supramolecular complex should be similar to that of commelinin (Figure 1.8). The metal center organizes the geometry of the complex, which is held together by weak hydrophobic interactions, such as π–π stacking of the aromatic rings and hydrogen bonding through the sugar moieties [51]. Cornflower and commelinin blues are only stable in concentrated solutions. When diluted, the solution quickly becomes colorless due to dissociation of the components of the complex.

1.4 Ancient Purples

1.4.1 Tyrian Purple: Real Purple from Sea Snails

The high status of the wearer of purple-dyed textiles in the past was never surpassed by any other color, natural or synthetic. Purple has a commercial and economic history, but also a cultural history, with profound political and religious impact [7, 52–55]. Purple was a status symbol for the Roman emperors as well as for the powerful representatives of the emergent power, the Catholic Church. The Levantine Phoenicians probably discovered the method by which these pigments could be dissolved for the dyeing of textiles for sovereigns, high-ranking military personnel, and high priests [56a]. Imperial or divine purple was used for powerful emotional moments, where authority was conveyed, in rituals of high visual impact. Purple was also the color of the mantle Christ used in his sacrifice, becoming a symbol of his sacrifice for humankind, and, as such, of his glory [52]. With it, purple achieved the status of a sacred color. The original color was the most expensive dye on the market, but even its imitations (as purple obtained by a combination of indigo with madder or other similar red) could maintain its status symbol [54]. It will be difficult to find an example that can convey the impact of this purple in antiquity in our present times.

True purple dye, Tyrian purple, was obtained from Mediterranean shellfish of the genus *Purpura* (Figure 1.9) [55, 56]. The Mediterranean purple mollusks *Murex brandaris*, *Murex*

trunculus, and *Purpura haemastoma* have recently been renamed *Bolinus brandaris*, *Hexaplex trunculus*, and *Stramonita haemastoma*, respectively, with the result that *Murex* and *Purpura* had disappeared from the name, and with it the memory of their importance as historic dyes was canceled [53]. As already mentioned in the introduction, it was one of the luxury products traded by the Phoenicians, hence the common name, "Tyrian purple," named after the Phoenician city, Tyre. This purple dye was produced by extraction of the secretions of the hypobranchial glands of Mediterranean gastropod mollusks from at least 1800 BC [2]. Piles of purple-yielding mollusk shells on Crete and along the Levantine coast and elsewhere in the Mediterranean have provided the primary archaeological evidence for the extent of this industry [55b]. Pigments from these invertebrates were used in the Akrotiri frescoes, on Santorini in Greece, dating back to the 17th century BC [57].

The precursors of Tyrian purple and derivatives such as mono-bromoindigo are stored in the hypobranchial glands of live muricids and can be released after death or when the mollusk is pressed and rubbed. The entire biosynthetic pathway for the production of dye precursors has yet to be elucidated, but the compounds generated in the pathway are biologically active and include choline esters that may be employed as muscle relaxants and indole derivatives with cytotoxic (anticancer activities) and microbial properties [58]. Tyrian purple and its derivatives also occur naturally in the egg masses of the Muricidae.

Figure 1.9 depicts the colored compounds that can be obtained as final products from the glandular secretions: true purple (6,6'-dibromoindigotin), monobrominated indigo derivatives (6 and 6'-bromoindigotin), brominated indirubin derivatives (6,6'-dibromoindirubin, 6- and 6'-bromoindirubins), indirubin, and indigo. It is worth pointing out that indigo is blue, whereas its

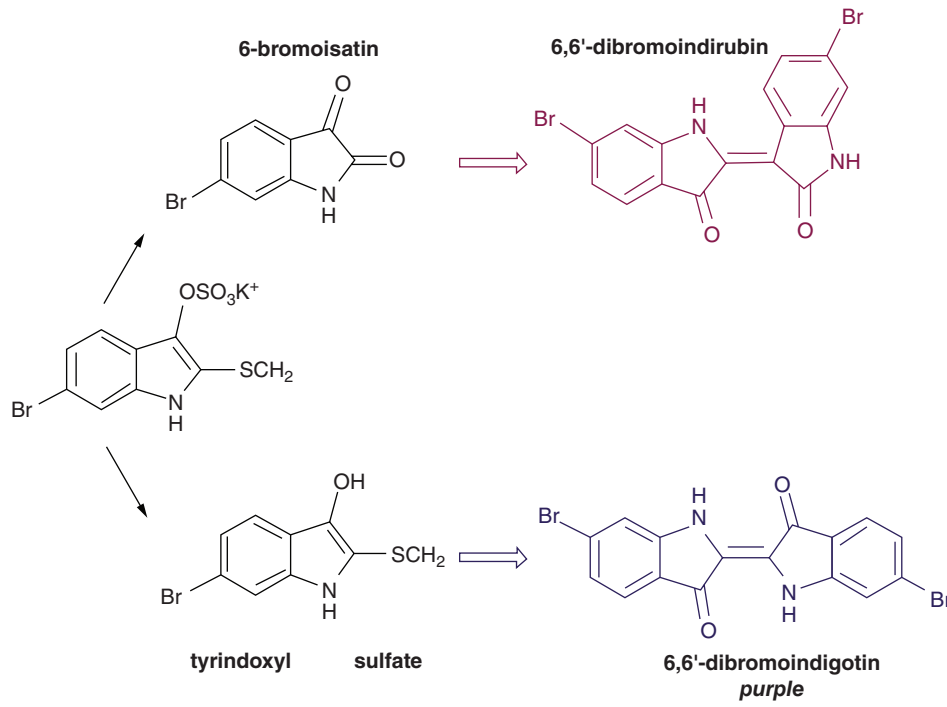


Figure 1.9 *Synthesis of purple, 6,6′-dibromoindigotin. Precursors and schematic pathways for the chromophores obtained from the muricids. 6,6′-dibromoindirubin, other indirubin monobromo derivatives, as well as indigo and indirubin can also be produced (see text).*

brominated derivatives are violet and indirubin is reddish. The shades of purple obtained depend on the species of mollusk and the dyeing process used. McGovern and Michel reported the chemical distribution of these insoluble dyes to be the following*: M. brandaris* and *P. haemastoma* produce mainly 6,6'-dibromoindigotin (purple), while *M. trunculus* also gives indigotin (blue), that is, indigo in addition to the purple. The first steps of the synthesis are believed to involve an enzymatic process resulting in the production of tyrindoxyl sulfate (Figure 1.9), which is followed by chemical processes involving light and oxygen. Because the hypobranchial glands of the species that produces indigo, *M. trunculus*, contain a more efficient enzyme, *M. trunculus* was mixed with other species for purple vat dyeing in the Mediterranean. Hence, the final color obtained ranged between reddish purple and bluish violet [47c, 55b, 56].

The story of purple was to be continued in the 19th century with the discovery of mauveine by Perkin and the rebirth of the ancient *purple mania* as *mauve mania* [59, 60]. The synthesis of mauveine in 1856—subsequently known as aniline purple (1857), Tyrian purple (1858), and mauve dye (from 1859)—is a story that demonstrates how a well-prepared mind can change history. The synthesis of the iconic mauveine is a major landmark in the history of science and technology, as it led to the establishment of the synthetic dye industry, opening a new era for chemistry.

1.4.2 Orchil Purples

Orchil purples were produced from lichens (such as the *Roccella*, *Lasallia*, *Lecanora*, and *Variolaria* species) found all over the world, from mountains to the coasts of subtropical or tropical regions in Europe, Asia, Africa, or South America. After extensive use during antiquity and the early Middle Ages, its production was lost in Europe. In the 13th century, Frederigo Rocellai returned from the Levant to Italy with the formula for dyeing with orchil. The novelty of this purple dyeing made the Florentine family famous. This fame and fortune were such that in 1763, Linnaeus named a lichen species in their honor, *Roccella tinctoria* [6, 61].

The main chromophores in *Roccella* and *Lasallia* spp. are phenoxazones, namely, amino-orceins, hydroxy-orceins, and amino-orceimines (Figure 1.10). Amino derivatives generally have a bluish hue. These chemical structures were discovered by Musso and Matthies [61, 62]. In 1957, Hans Musso and Hans-Georg Matthies provided a full comparative analysis of the main chromophores isolated from orcein lichens based on infrared and UV–Visible spectra [62a]. This work would be further expanded in the 1961 *Angewandte Chemie* paper [62b], which appeared in an English translation in 2003 [62c]. Orcein chromophores can thus be classified as oxazine dyes, which belong to the larger family of azine cationic dyes [63]. This family also includes phenazine dyes, such as mauveine, the landmark purple color discovered by Perkin in 1856, which was the first synthetic dye produced on an industrial scale [63]. Orcein purples have been identified in cultural heritage, in Renaissance tapestries [64a], and in medieval codices such as *The Book of Kells* dated from approximately 800 AD [64b] and *Codex Brixianus* from the 6th century [65a]. It was also used to dye parchment in late antiquity manuscripts. This process of purple dyeing was recreated and compared with the Vienna Genesis and other manuscripts from the 6th and 8th centuries [65b].

1.4.3 Folium

Folium was extracted from the fruits of the plant *Chrozophora tinctoria* (previously *Croton tinctorium*). Over the centuries, it was variously titled solsequium, tournesol (or turnsole), morella (maurella), and *Heliotropium tricoccum*. *C. tinctoria* and its uses were well known in antiquity and medieval times (Figure 1.11). However, the practice of creating the blue color fell out of use and was lost in the 19th century. Its medicinal properties were first described by Dioscorides (*De Materia Medica*,

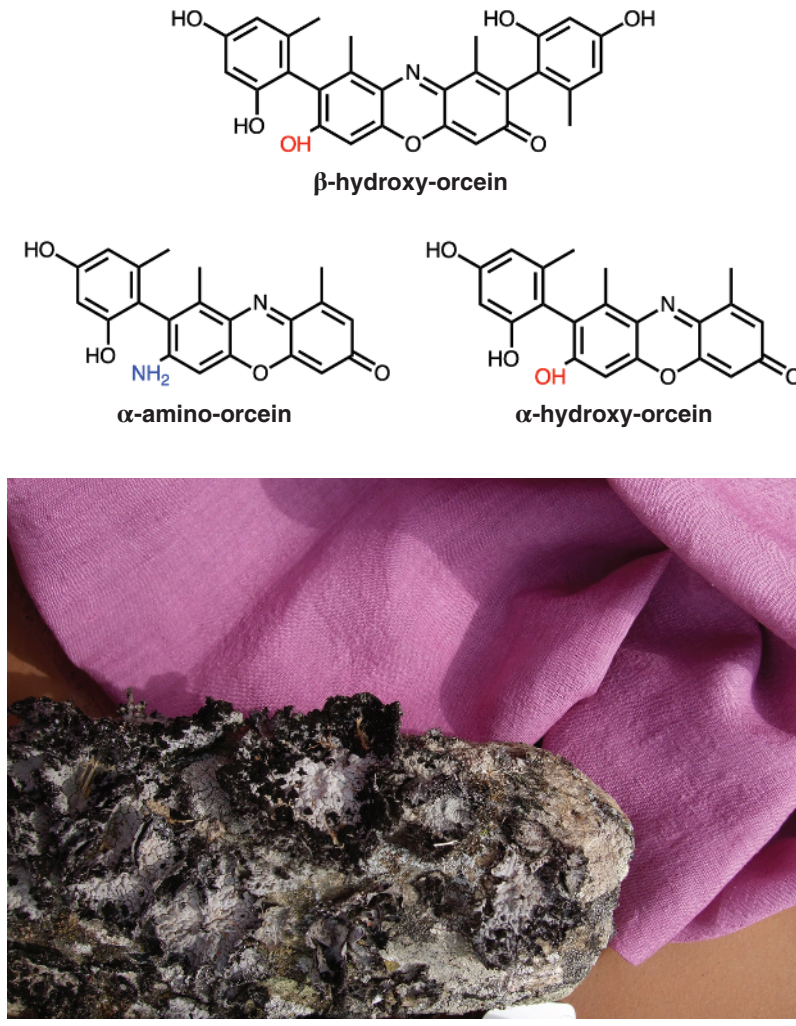


Figure 1.10 *Molecular structures for the main chromophores in orcein samples: α-orceins are the major compounds in silk dyed with Lasallia pustulata, and β-orcein is the major compound in synthetic orcein. Photography courtesy of Isabella Whitworth, fiber artist, who also dyed the fabric.*

1st century) and were also mentioned in medieval pharmacopoeia texts, and studies focusing on its anti-inflammatory properties have been published recently [66].

Until the 18th century, it was still extensively produced in Montpellier, to be exported to Germany and Holland to give color to wines, liquors, cheese, and confitures [66]. The blue/purple solution extracted from the fruits was stored, after adsorption of the juice onto linen clothlets. These would be stored until required. The paint thus obtained was named *folium* or *tornasol* (turnsole); this latter designation is common to the blue/purple watercolors obtained from lichens (e.g., *R. tinctoria*, *Lasallia pustulata*) and, consequently, *C. tinctoria* dyes remained forever interlinked to these lichens.

The molecular structure of this medieval watercolor was finally solved in the 21st century by a multidisciplinary team [66]. The interdisciplinary approach was crucial to produce extracts that were prepared following medieval instructions and revealed the blue/purple chromophore as the

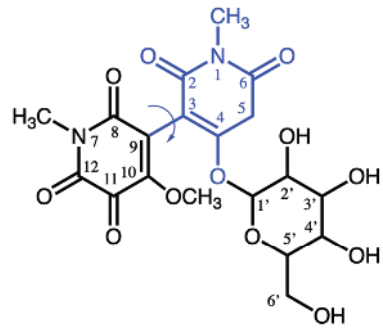


Figure 1.11 *Chrozophoridin is the main dye in Chrozophora tinctoria fruits (shell). It is a derivative of hermidin, shown in blue.*

major dye in *C. tinctoria* fruits. The results demonstrated that it corresponds to a hermidin derivative, which was named *chrozophoridin*. Chrozophoridin is neither an anthocyanin nor indigo; it turns out to be in a class of its own [66].

1.5 Ancient Yellows

1.5.1 Flavonoid Yellows

The sources for yellow dyes are enormous. Knowledge of many of these natural sources for yellow and how to dye with them has been lost, and therefore many of these species are not described or well documented in the literature.

The yellow chromophores based on the flavone chromophore can be divided into two large groups, those with and those without a hydroxyl group in the C3 position (Figure 1.12 and Table 1.3) [67, 68]. The first group includes important chromophores such as apigenin and luteolin. These appear in the plant as glycosides, that is, with sugar residues attached to one or more of the hydroxyl positions shown in Figure 1.12. Luteolin and its sugar derivatives are considered to be among the most stable yellows and thus were widely used for dyeing. Perhaps the most commonly used plant source for luteolin derivatives was weld, *Reseda luteola* (*lírio-dos-tintureiros* in Portuguese, *arzica* in Italian, and *gaude* in French) (Table 1.3) [7, 9a].

The second group, the flavonols (3-hydroxyflavones), contains flavonoids widely known for their antioxidant properties, for example, quercetin, kaempferol, rhamnetin, and also morin, myrecetin, and fisetin. These yellows are found in a variety of plants, including Persian berries (*Rhamnus* spp.), young fustic (*Cotinus coggygria*), old fustic (*Chlorophora tinctoria*), and yellow

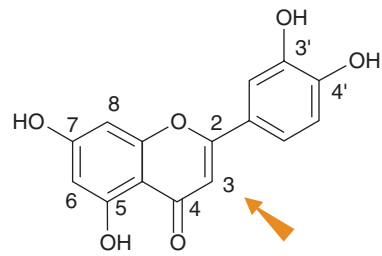
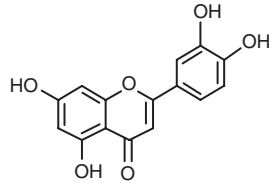
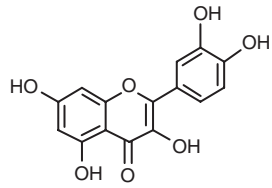
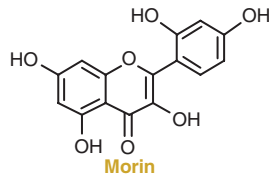


Figure 1.12 *Luteolin (flavone).*

Table 1.3 *Chemical structures for flavone and flavonol yellows extracted from plants and berries.*

Flavone and flavonol yellows	
Weld *Reseda luteola* **Dyer's broom** *Genista tinctoria*	 **Luteolin** 5, 7, 3', 4'-OH-flavone
Persian berries *Rhamnus* spp.	 **Quercitin** 3, 5, 7, 3', 4'-OH-flavone
Old fustic *Maclura tinctoria*	 **Morin** 3, 5, 7, 2', 4'-OH-flavone

wood (*Solidago virgaurea*). Other sources are onion skins (*Allium cepa*), saw-wort (*Serratula tinctoria*), dyer's greenweed (*Genista tinctoria*), marigold (*Chrysanthemum* spp.), dyer's chamomile (*Anthemis tinctoria*), flax-leaved daphne (*Daphne gnidium*), etc. [7].

Yellow dyes are usually considered less resistant to fading [11, 69, 70] than the red or blue colors, and therefore, it can be expected that the actual yellow colors observed today are different from the original. This conviction has been partially challenged by the recent investigation into the stability of flavonoid yellows used in cultural heritage [67]. A scale to accurately measure the stability of flavonoid yellows was provided: Lut-7-*O*-glu > Que-3-*O*-glu > Kaem-3-*O*-glu ≈ luteolin >> quercetin ≥ kaempferol. Somewhat unexpected was the finding that the stability of luteolin and the 3-*O*-glycosides quercetin and kaempferol can be compared to the values obtained for indigo in stable environments [44, 67].

1.5.2 Carotenoid Yellows

Carotenoids are present in our everyday diet, in the red of a tomato, the orange of a carrot, or the golden yellow of "Risotto alla Milanese," and in many Persian rice dishes. The traditional yellow used in these rice recipes is obtained from the stigmas of saffron, *Crocus sativus*, a beautiful flower, 10-cm high, used for dyeing since antiquity.

The main chromophore is the aglycone (crocetin), which can be found in the form of the glycoside (crocin) (Figure 1.13) or with other substituted glycoside patterns. Other carotenoid yellows were used in the past, but the most famous was golden saffron.

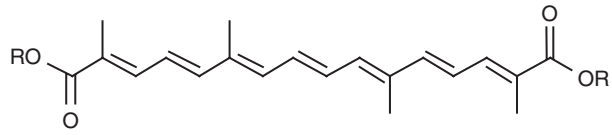


Figure 1.13 *Crocetin (R=H) and crocin (R=β-D-gentiobiosyl, a diglycoside), are all-trans carotenoids found in saffron.*

1.5.3 Chalcone and Aurone Yellows

Similar to saffron, these chromophores absorb light at longer wavelengths and have golden/orange hues.

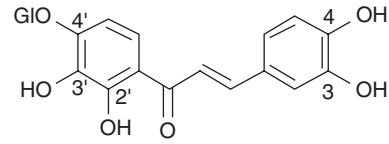


Figure 1.14 *Marein, okanin-4′-O-glucoside (chalcone).*

Chalcone yellows have been recently identified in ancient Andean textiles, as marein and other glycosides of okanin (2',3',4',3'4-pentahydroxychalcone), possibly obtained from a *Coreopsis* species (Figure 1.14) [71, 72].

Acknowledgements

The swift revision of the manuscript by Vanessa Otero and Paula Nabais is greatly appreciated. Funding by Portuguese Science Foundation [Fundação para a Ciência e Tecnologia, Ministério da Educação e da Ciência (FCT/MCTES)] through the projects "Polyphenols in Art: Chemistry and biology hand in hand with conservation of cultural heritage" and "From singing to writing – survey on material production and routes of Galician-Portuguese Lyric" is also acknowledged (PTDC/QUI-OUT/29925/2017; PTDC/LLT-EGL/30984/2017).

References

1. F. Braudel, *The Mediterranean in the Ancient World,* Penguin Books, 2002.
2. C. López-Ruiz, *Phoenicians and the Making of the Mediterranean*, Harvard University Press, Cambridge (Massachusetts), 2021.
3. A. Parrot, M. H. Chéhab and S. Moscati, *Les Phéniciens*, Gallimard, Sabadell (Spain), 2007.
4. (a) R. A. Donkin, Spanish red. An ethnogeographical study of cochineal and the Opuntia Cactus, *Trans. Am. Philos. Soc.*, New Ser., **67** (5), 1–84 (1977). (b) R. A. Donkin, The insect dyes of western and west-central Asia, *Anthropos,* **72**, 647–680 (1977).
5. P. Ball, *Bright Earth: Art and the Invention of Color*, Farrar, Straus and Giroux, New York, 2002.
6. F. Brunello, *L'arte della tintura nella storia dell'umanità,* Neri Pozza, Vicenza, 1968.
7. (a) D. Cardon, *Le Monde des Teintures Naturelles*, Éditions Belin, Paris, 2003. (b) D. Cardon, *Natural Dyes*. Sources*, Tradition, Technology and Science*, Archetype Publications, 2007.
8. R. Halleux (ed.), *Les Alchimistes Grecs: Papyrus de Leyde, Papyrus de Stockholm,* Recettes, Les Belles Lettres, Paris, 2002.

9. (a) J. Kirby, *Paints, pigments, dyes*, in T. Glick, S. J. Livesey and F. Wallis (eds), *Medieval Science, Technology, and Medicine-an Encyclopedia*, Routledge, 2005. (b) A. Burnstock, I. Lanfear, K. J. van der Berg, L. Carlyle, M. Clarke, E. Hendriks and J. Kirby, Comparison of the fading and surface deterioration of red lake pigments in six paintings by Vincent van Gogh with artificially aged paint reconstructions, *Preprints of the 14th Triennial Meeting of the ICOM Committee for Conservation*, **1**, 459–466 (2005).
10. Europe through textiles, https://euroweb.uw.edu.pl/
11. E. Ferreira, A. Hulme, H. McNab and A. Quye, The natural constituents of historical textile dyes, *Chem. Soc. Rev.*, **33**, 329–336 (2004).
12. J. Wouters and A. Verhecken, The coccid insect dyes: HPLC and computerized diode-array analysis of dyed yarns, *Stud. Conserv.*, **34**, 189–200 (1989). J. Wouters and A. Verhecken, The scale insect dyes (*Homoptera:* Coccoidea). Species recognition by HPLC and dyode-array analysis of the dyestuffs, *Ann. Soc. Entomol. Fr.*, **25** (4), 393–410 (1989).
13. X. Zhang and R. A. Laursen, Development of mild extraction methods for the analysis of natural dyes in textiles of historical interest using LC-diode array detector-MS, *Anal. Chem.,* **77** (7), 2022–2025 (2005).
14. P. Guinot, A. Gargadennec, G. Valette, A. Fruchier and C. Andary, Primary flavonoids in marigold dye: extraction, structure and involvement in the dyeing process, *Phytochem. Anal*., **19** (1), 46–51 (2008).
15. D. A. Peggie, *The Development and Application of Analytical Methods for the Identification of Dyes on Historical Textiles*, PhD dissertation, The University of Edinburgh, 2006.
16. M. Sousa, M. J. Melo, A. Aguiar-Ricardo and P. Cruz, *A green approach to antique textile cleaning, Preprints 14th Triennial Meeting ICOM-CC,* **2***, 944–954*, James & James, 2005.
17. T. Devièse, C. Higgitt, I. Karapanagiotis, J. Kirby, M. van Bommel and I. Vanden Berghe, Review of extraction methods for the characterisation by HPLC of organic colorants in textiles and pigments in cultural heritage objects. Within European project CHARISMA: Cultural Heritage Advanced Research Infrastructures: Synergy for a Multidisciplinary Approach to Conservation/Restoration.
18. I. Petroviciu, F. Albu and A. Medvedovici, LC/MS and LC/MS/MS based protocol for identification of dyes in historic textiles, *Microchem. J.*, **95**, 247–254 (2010).
19. A. Serrano, M. Van Bommel and J. Hallett, Evaluation between ultrahigh pressure liquid chromatography and high-performance liquid chromatography analytical methods for characterizing natural dyestuffs, *J. Chromatogr*. **A 1318**, 102–111 (2013).
20. C. Mouri, V. Mozaffarian, X. Zhang and R. Laursen, Characterization of flavonols in plants used for textile dyeing and the significance of flavonol conjugates, *Dyes Pigm.*, **100**, 135–141 (2014).
21. R. Marques, M. M. Sousa, M. C. Oliveira and M. J. Melo, Characterization of weld (*Reseda luteola* L.) and spurge flax (*Daphne gnidium* L.) by high-performance liquid chromatography-diode array detection-mass spectrometry in Arraiolos historical textiles, *J. Chromatogr. A*, **1216**, 1395–1402 (2009).
22. R. Chenciner, *Madder Red*. A History of Luxury and Trade, Curzon Press, 2000.
23. (a) R. Castro, F. Pozzi, M. Leona and M. J. Melo, Combining SERS and microspectrofluorimetry with historically accurate reconstructions for the characterization of lac dye paints in medieval manuscript illuminations, *J. Raman Spectrosc.*, **45,** 1172–1179 (2014). (b) R. Castro, *The Book of Birds in Portuguese Scriptoria: Preservation and Access*, PhD dissertation, New University of Lisbon, 2016. http://hdl.handle.net/10362/21481.
24. H. Schweppe and H. Roosen-Runge, Carmine-cochineal carmine and kermes carmine, in R. L. Feller (ed.), *Artists' Pigments – A Handbook of their History and Characteristics*, Volume 1, Cambridge University Press, Cambridge, 1986.
25. H. Grigoryan, A. Sahakyan and M. J. Melo, Vordan Karmir: an attempt to unravel the mystery of Armenian cochineal recipes for paints and inks used in manuscripts, in J. Kirby (ed.), *Dyes in History and Archaeology*, Archetype, London, forthcoming publication.
26. (a) F. Brunello (ed.), *Cennino Cennini*, *Il Libbro dell'Arte*, Neri Pozza Editore, Vicenza, 1997. (b) Clothlets, translated from the Italian "pezzuole", small pieces of cloth imbibed with the dye, that would be applied as an water colour.
27. P. Roger, I. Villela-Petit and S. Vandroy, Les laques de brésil dans l'enluminure médiévale: reconstituition à partir de recettes anciennes, *Stud. Conserv.*, **48**, 155–170 (2003).
28. M. J. Melo, V. Otero, T. Vitorino, R. Araújo, V. S. F. Muralha, A. Lemos and M. Picollo, A spectroscopic study of brazilwood paints in medieval books of hours, *App. Spec.*, **68**, 434–444 (2014).
29. T. Vitorino, M. J. Melo, L. Carlyle and V. Otero, New insights into brazilwood lake pigments manufacture through the use of historically accurate reconstructions, *Stud. Conservat*., **61**, 255–273 (2016).
30. D. Cardon with C. Higgitt, Appendix: Chemical structures of the dyestuff groups *in* [7b].

31. (a) F. Pina, A. Alejo-Armijo, A. Clemente, J. Mendoza, A. Seco, N. Basílio and A. J. Parola, Evolution of flavylium-based color systems in plants: what physical chemistry can tell us, *Int. J. Mol. Sci*., **22**, 3833–3851 (2021). (b) F. Pina, M. J. Melo, C. A. T. Laia, A. J. Parola and J. C. Lima, Chemistry and applications of flavylium compounds: a handful of colours, *Chem. Soc. Rev*., **41**, 869–908 (2012). (c) L. Cruz, N. Basilio, N. Mateus, V. de Freitas and F. Pina, Natural and synthetic Flavylium-based dyes: The chemistry behind the color, *Chem. Rev.*, **122**, 1416–1481 (2022).
32. M. J. Melo, Missal blue: anthocyanins in nature and art, in J. Kirby (ed.), *Dyes in History and Archaeology 21,* Archetype Publications, 2008.
33. (a) V. Borradaile and R. Borradaile, *The Strasburg Manuscript: A Medieval Painters' Handbook*, Transatlantic Arts, New York, 1966. (b) S. Blondheim, An old Portuguese work on manuscript illumination, *JQR*, **XIX**, 97–135 (1928). (c) F. Brunello, *De Arte Illuminandi e altri Trattati sulla Miniatura Medievale*, Neri Pozza Editore, Vicenza, 1992.
34. M. J. Melo, R. Castro, P. Nabais and T. Vitorino, The book on how to make all the colour paints for illuminating books: unravelling a Portuguese Hebrew illuminators' manual, *Herit. Sci*., **6**, 1–8 (2018).
35. A. Roquero, *Tintes y Tintoreros de America*, Ministerio de Cultura, Madrid, 2006.
36. M. J. Melo, M. Sousa, A. J. Parola, J. S. Seixas de Melo, F. Catarino, J. Marçalo and F. Pina, Identification of 7,4' -dihydroxy-5-methoxyflavylium in "Dragon's blood". To be or not to be an anthocyanin, *Chem. Eur. J*., **13**, 1417–1422 (2007).
37. (a) H. Brockmann and H. Junge, Die Konstitution des Dracorhodins, eines neuen Farbstoffes aus dem "Drachenblut" *Ber. Dtsch. Chem. Ges. B*, **76**, 751–763 (1943); (b) H. Brockmann and R. Haase, Über Dracorubin, den roten Farbstoff des "Drachenblutes", *Ber. Dtsch. Chem. Ges. B*, **69**, 1950–1954 (1936).
38. (a) J. R. Brouillard and J. E. Dubois, Mechanism of structural transformations of anthocyanins in acidic media, *J. Am. Chem. Soc.*, **99**, 1359 (1977); (b) R. A. McClelland and S. Gedge, Hydration of the flavylium ion, *J. Am. Chem. Soc.*, **102**, 5838–5848 (1980).
39. (a) F. Pina, Thermodynamics and kinetics of flavylium salts – Malvin revisited, *Chem. Soc., Faraday Trans.*, **94**, 2109–2116 (1998). (b) F. Pina, M. Maestri and V. Balzani, in H. S. Nalwa (ed.), *Handbook of Photochemistry and Photobiology, Vol. 3: Supramolecular Photochemistry*, American Scientific Publishers, 2003.
40. J. Balfour-Paul, *Indigo*, British Museum Press, 2000.
41. O. R. Constable, *Trade and Traders in Muslim Spain*, Cambridge University Press, Cambridge. 2003.
42. M. Clarke, Anglo-Saxon manuscript pigments, *Stud. Conserv.*, **49**, 231–244 (2004).
43. (a) J. Seixas de Melo, A. P. Moura and M. J. Melo, Photophysical and spectroscopic studies of indigo derivatives in their keto and leuco forms, *J. Phys. Chem. A.*, **108**, 6975–6981 (2004). (b) M. M. Sousa, C. Miguel, I. Rodrigues, A. J. Parola, F. Pina, J. S. Seixas de Melo and M. J. Melo, A photochemical study on the blue dye indigo: from solution to ancient Andean textiles, *Photochem. Photobiol. Sci.,* **7**, 1353–1359 (2008).
44. C. Miliani, L. Monico, S. Fantacci, A. Romani, M. J. Melo, E. M. Angelin and K. Janssens, Recent insights into the photochemistry of artists' pigments and dyes: towards better understanding and prevention of colour change in works of art (minireview), *Angew. Chem. Int. Ed*., 57, 7324–7334 (2018).
45. M. van Eikema Hommes, *Discoloration in Renaissance and Baroque Oil Paintings*, Archetype Publications, 2004.
46. (a) A. Berry, T. C. Dodge, M. Pepsin and W. Weyler, Application of metabolic engineering to improve both the production and use biotech indigo *J. Ind. Microbiol. Biot.*, **28**, 127–133 (2002). b) A. N. Padden, V. M. Dillon, P. John, J. Edmonds, M. D. Collins and N. Alvarez, Clostridium used in mediaeval dyeing, *Nature*, **396**, 225–225 (1998).
47. (a) R. J. H. Clark, C. J. Cooksey, M. A. M. Daniels and R. Withnall, Indigo-red, white and blue, *Educ. Chem.* 16–19 (1996). b) C. J. Cooksey, Tyrian purple: 6, 6'-dibromoindigo and related compounds, *Molecules*, **6**, 736 (2001). (c) C. Cooksey, Tyrian purple: the first four thousand years, *Science Progress*, **96** (2), 171–186 (2013).
48. (a) M. Klessinger, Captodative substituent effects and the chromophoric system of indigo, *Angew. Chem. Int. Ed. Engl.*, **19**, 908–909 (1980). (b) E. Wille, W. Lüttke, 4,4,4',4'-Tetramethyl-⊗2,2'-bipyrrolidine-3,3'-dione, a compound having the basic chromophore system of indigo, *Angew. Chem. Int. Ed. Engl.*, **10**, 803–804 (1971). (c) G. Pfeifer, W. Otting and H. Bauer, Octahydroindigo, *Angew. Chem. Int. Ed. Engl.*, **15**, 52 (1976). (d) P. W. Sadler, Absorption spectra of indigoid dyes, *J. Org. Chem.*, **21**, 316–319 (1956). (e) G. M. Wyman, Reminiscences of an accidental Photochemist, *EPA Newsletter*, **50**, 9–13 (1994).
49. L. Meijer, N. Guyard, L. A. Skaltsounis, G. Eisenbrand (eds), *Indirubin, the Red Shade of Indigo*, *Life in Progress*, Roscoff, France, 2006.
50. (a) Grazia, D. Buti, A. Amat, F. Rosi, A. Romani, D. Domenici, A. Sgamellotti and C. Miliani, "Shades of blue: non-invasive spectroscopic investigations of Maya blue pigments. From laboratory mock-ups to Mesoamerican

codices", *Herit. Sci.* (2020). DOI 10.1186/s40494-019-0345-z; (b) F. Pottier A. Michelin, A. Genachte–Le Bail, A. Tournié, C. Andraud, F. Goubard, A. Histace and B. Lavédrine, Preliminary investigation on the Codex Borbonicus, in E. Dupey García and M. L. Vázquez de Ágredos Pascual (eds), *Painting the Skin: Pigments on Bodies and Codices in Pre-Columbian Mesoamerica*, University of Arizona Press, 2019.

51. (a) T. Kondo, M. Ueda, M. Isobe and T. Goto, A new molecular mechanism of blue color development with protocyanin, a supramolecular pigment from cornflower, *Centaurea cyanus, Tetrahedron Lett.*, **39**, 8307–8310 (1998). b) T. Kondo, K. Yoshida, A. Nakagawa, T. Kawai, H. Tamura and T. Goto, Structural basis of a blue-colour development in flower petals from *Commelina communis*, *Nature,* **358**, 515–518 (1992). (c) T. Goto and T. Kondo, Structure and molecular stacking of anthocyanins- flower color variation, *Angewandte Chemie Int. Ed. Engl.,* **30** (1), 17–33 (1991).
52. Guglielmo Cavallo, La porpora tra scienze e culture. Una introduzione, in O. Longo (ed.), *La porpora, realtà e immaginario di un colore simbolico, Istituto Veneto di Scienze*, Lettere ed Arti, Venezia 1998.
53. R. Haubrichs, Natural history and iconography of purple shells, in L. Meijer, N. Guyard, L. A. Skaltsounis and G. Eisenbrand (eds), *Indirubin, the Red Shade of indigo*, Life in Progress, Roscoff, France, 2006.
54. *Teintures Précieuses de la Méditerranée: Poupre, Kermès*, Pastel, D. Cardon (ed.), *Musée des Beaux-Arts de Carcassonne – Centre de Documentació I Museu Tèxtil de Terrassa*, Carcassonne, 1999.
55. (a) P. E. McGovern and R. H. Michel, Royal purple dye: the chemical reconstruction of the ancient Mediterranean industry, *Acc. Chem. Res.*, **23**, 152–158 (1990). (b) P. E. McGovern and R. H. Michel, Royal purple and the pre-Phoenician dye industry of Lebanon, *Masca J.*, **3**, 67–70 (1984).
56. (a) Z. C. Koren, Archaeological shades of purple from flora and fauna from the ancient near east, in M. V. Orna and S. C. Rasmussen (eds), *Archaeological Chemistry: A Multidisciplinary Analysis of the Past*, Cambridge scholars publishing, 2020. (b) Z. C. Koren, High-performance liquid-chromatographic analysis of an ancient tyrian-purple dyeing vat from Israel Is. *J. Chem*., **35**, 117–124 (1995).
57. (a) S. Sotiropoulou, I. Karapanagiotis, K. S. Andrikopoulos, T. Marketou, K. Birtacha and M. Marthari, Review and new evidence on the molluscan purple pigment used in the early late bronze age Aegean wall paintings, *Heritage*, **4**, 171–187 (2021). (b) I. Karapanagiotis, A review on the archaeological chemistry of shellfish purple, *Sustainability,* **11**, 1–23 (2019).
58. (a) C. B. Westley, K. L. Vine and K. Benkendorff, A proposed functional role for indole derivatives in reproduction and defense Muricidae (Neogastropoda: Mollusca), in L. Meijer, N. Guyard, L. A. Skaltsounis and G. Eisenbrand (eds), *Indirubin, the Red Shade of Indigo*, Life in Progress, Roscoff, France, 2006. (b) K. Benkendorff, Natural product research in the Australian marine invertebrate *Dicathais orbita*, *Mar. Drugs*, **11**, 1370–1398 (2013).
59. (a) A. S. Travis, *The Rainbow Makers: The Origins of the Synthetic Dyestuffs Industry in Western Europe*, Lehigh University Press, Bethlehem, 1993.(b) S. Garfield, MAUVE. *How One Man Invented a Color That Changed the World*, W.W. Norton & Company, 2002.
60. (a) M. M. Sousa, M. J. Melo, A. J. Parola, P. J. T. Morris, H. S. Rzepa and J. S. Seixas de Melo, A study in mauve. Unveiling Perkin's dye in historic samples, *Chem. Eur. J.*, **14**, 8507–8513 (2008). (b) J. Seixas de Melo, S. Takato, M. Sousa, M. J. Melo and A. J. Parola, Revisiting Perkin's Dye(s): The spectroscopy and photophysics of two new mauveine compounds (B2 and C), *Chem. Comm.*, 2624–2626 (2007).
61. M. J. Melo, P. Nabais, M. Guimarães, R. Araújo, R. Castro, M. C. Oliveira and I. Whitworth, Organic dyes in illuminated manuscripts: a unique cultural and historic record, *Phil. Trans. R. Soc. A*., **374**, 20160050:1–20 (2016); theme issue "Raman Spectroscopy at the Arts/Science Interface: Applications in Art and Archaeological Analysis.
62. (a) H. Musso, H. G. Matthies, IR- und UV-Spektren hydroxy- und amino-substituierter phenoxazone, *Chem. Ber.* 90, 1814–1827 (1957). (b) H. Beecken, E. M. Gottschalk, U. V. Gizycki, H. Kramer, D. Maasen, H. G. Matthies, H. Musso, C. Rathjen and U. I. Záhorszky, Orcein und lackmus, *Angew. Chem.,* **20**, 665–688 (1961). (c) H. Beecken, E. M. Gottschalk, U. V. Gizycki, H. Kramer, D. Maasen, H. G. Matthies, H. Musso, C. Rathjen C. and U. I. Záhorszky, Orcein and litmus, *Biotech. Histochem*., **78**, 289–302 (2003).
63. K. Hunger, *Industrial Dyes: Chemistry, Properties, Applications*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2003.
64. (a) B. Doherty, A. Daveri, C. Clementi, A. Romani, S. Bioletti, B. Brunetti, A. Sgamellotti and C. Miliani, The Book of Kells: a non-invasive MOLAB investigation by complementary spectroscopic techniques, *Spectrochim. Acta Part A*, **115**, 330–336 (2013). (b) F. Rosi, C. Clementi, M. Paolantoni, A. Romani, R. Pellegrino, B. Brunetti, W. Nowik and C. Miliani, Study of Raman scattering and luminescence properties of orchil dye for its nondestructive identification on artworks, *J. Raman Spectrosc*., **44**, 1451–1456 (2013).

65. (a) M. Aceto, A. Idone , A. Agostino, G. Fenoglio, M. Gulmini, P. Baraldi and F. Crivello, Noninvasive investigation on a VI century purple codex from Brescia, Italy, *Spectrochim. Acta Part A*, **117**, 34–41 (2014). (b) C. Hofmann, J. Vnouček, S. Rabitsch, Ju. Sonderegger, S. Fiddyment, M. Collins, A. Quandt, A.Malissa, K. Uhlir, M. Griesser, K. Hradil, R. Erlach, M. Aceto, and M. J. Melo, The Vienna Genesis: an example of Late Antique purple parchment, *Restaurator*, 2022.
66. P. Nabais, J. Oliveira, F. Pina, N. Teixeira, V. de Freitas, N. F. Brás, A. Clemente, M. Rangel, A. M. S. Silva and M. J. Melo, A 1000-year-old mystery solved: unlocking the molecular structure for the medieval blue from *Chrozophora tinctoria*, also known as folium, *Sci. Adv.*, **6** (2020); DOI: 10.1126/sciadv.aaz7772
67. S. Sharif, P. Nabais, M. J. Melo, F. Pina and M. C. Oliveira, Photoreactivity and stability of flavonoid yellows used in cultural heritage, *Dyes Pigm*., **199**, 110051 (2022).
68. (a) E. Haslam, *Practical Polyphenols*. From Structure to Molecular Recognition and Physiological Action, Cambridge University Press, Cambridge, 1998; (b) J. B. Harbone, *The Flavonoids – Advances in Research since 1986*, Chapman & Hall, London, 1994.
69. (a) M. Heitor, M. Sousa, M. J. Melo, J. Hallett and M. C. Oliveira, The colours of the carpets, in T. Pacheco Pereira and J. Hallett (ed.), *The Oriental Carpet in Portugal. Carpets and Paintings, 15th-18th Centuries*, Museu Nacional de Arte Antiga, 2007. (b) J. S. de Melo, M. J. Melo and A. Claro, As moléculas da cor na arte e na natureza, *Química-Boletim da Sociedade Portuguesa de Química*, **101**, 44–55 (2006).
70. H. Böhmer, *Koekboya, Natural Dyes and Textiles: A Colour Journey from Turkey to India and Beyond*, Remhob-Verlag, Ganderkesee, 2002.
71. X. Zhang, R. Boytner, J. Cabrera and R. Laursen, Identification of yellow dye types in pre-Columbian Andean textiles, *Anal. Chem.*, **79**, 1575–1582 (2007).
72. I. Santos Rodrigues, *A Cor dos Têxteis Andinos – Análise da cor em têxteis da cultura milenar de Paracas, Master dissertation*, New University of Lisbon, 2008. http://hdl.handle.net/10362/7829.