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Reviews

Bartolomeo Bizio and the rediscovery of Tyrian purple

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Abstract. The origin and chemical properties of Tyrian purple were first described in 1833 by Bartolomeo Bizio, a Venetian chemist. The work of Bizio on the red-purple dye is reported in a series of papers covering the period from 1832 to 1860.

Bizio's description of the nature and properties of the dye was not only remarkably accurate, but clearly predates what were claimed as original discoveries by later authors. It is astonishing that a man who lived and worked in the early nineteenth century anticipated so many aspects of the chemistry of Tyrian purple that would be confirmed many years later.

Key words. Tyrian purple; red-purple.

Introduction

Tyrian purple disappeared from commercial use during the Middle Ages, and occasional references to it are by authors who had knowledge of it through Greek and Latin writers.

The first work to mark the revival of interest in the chemical nature and properties of Tyrian purple was published in 1833 by Bartolomeo Bizio, an Italian chemist who lived in Venice. His first paper appeared in the *Annali delle Scienze del Regno Lombardo-Veneto* and was entitled 'Scoperta del principio purpureo dei due *Murex trunculus* e *Murex brandaris* (Linn.) e studio delle sue properietà' (On the discovery of the purple agent of *Murex brandaris* and *Murex trunculus* (Linn.) and the study of its properties)[7]. The author published all his subsequent papers on this subject in the same journal.

Bartolomeo Bizio and his early chemical works

Bartolomeo Bizio was born in 1791 in a village near Vicenza. His father was a tailor, who tried to make his son one too, but the boy insisted on doing an apprenticeship in a local chemist's shop[33]. Years later, Bizio got a job in Padua, where he succeeded in obtaining the degree of apothecary from the University in 1820. This gave him the possibility of indulging his fascination with physical chemistry and pursuing his investigations of the composition of natural substances to satisfy his curiosity.

During the course of his life, Bizio published more than one hundred papers, which appeared in *Atti e memorie dell'Istituto Lombardo-Veneto* and in other journals. In 1827 many of these papers were reprinted in Venice in a volume entitled *Opuscoli chimico-fisici del farmacista Bartolomeo Bizio*. Among them are accounts of the chemical composition of wheat and corn, of coffee, bile, cuttlefish ink, of fig latex, of pomegranate fruit, of rape oil and others. Most of Bizio's experimental work falls within the field of organic chemistry, more properly in that branch of it which is today called the chemistry of naturally occurring substances.

In the early 1800s organic chemistry was in its infancy. Natural substances had been classified as organic or inorganic, and Lavoisier had demonstrated that organic compounds are made of carbon, oxygen, hydrogen, nitrogen, sulphur and phosphorus. Organic chemistry, however, was still an empirical and almost entirely descriptive science. Wöhler synthesized urea in 1828 but Berzelius in 1833 still denied the possibility of synthesizing organic substances from simple inorganic compounds. The belief that life is generated and sustained by a vital principle was an insuperable barrier between the worlds of inorganic and organic chemistry. Organic chemistry would develop thanks to Jean Baptiste Dumas and to Justus von Liebig who were to build, in France and Germany respectively, the most prestigious international schools. In Italy Stanislao Cannizzaro would present his *Lezione sulla teoria atomica* around 1850 and Raffaele Piria (to mention two of the most famous Italian chemists) would write his *Trattato di Chimica Organica* in 1860.

Bartolomeo Bizio happened to live and work at the beginning of the nineteenth century and he had, therefore, to rely on the limited knowledge of the time. He was, however, gifted with penetrating powers of observation and the ability to interpret these observations critically. Bizio, in fact, anticipated many discoveries that were claimed later by people who, perhaps unaware of his work, made similar experiments with the same results.

Around 1818 an event occurred near Vicenza that caused amazement and animated discussions among the local population. Red spots started to appear on grain, specially on corn-meal mush, which was the most common food. What were they made of? Where did they come from? How did they grow and become larger and larger? Many explanations were advanced, some of them highly fantastic.

Bizio studied the phenomenon and reached the conclusion that the agent responsible for the red spots was something like a mould. In 1819 he announced that the spots were 'di natura vegetabile' (of biological nature) and were made by a mould he called *Serratia marcescens*[5].

These pioneering results were published in the most widely known Italian scientific journals of the time. Yet when, twenty-four years later, in 1844, similar red spots were observed on grain in Paris, the phenomenon was described as 'new and peculiar' and as such was studied by Dumas, Pelouze and Payen who came to the same conclusions as Bizio. Today, textbooks of microbiology classify the agent of the red spots in the family Enterobacteriaceae and the genus is that given by the Italian chemist, Bizio, in 1819: *Serratia*[32].

The subject that kept Bizio busy for several years, and that gave him his reputation, was the purple dye, also called 'the purple of antiquity' or Tyrian purple. In 1832 in Venice, Bizio published a monograph entitled *La porpora degli antichi rivocata entro i confini del rosso.* Dissertazione critica[6]. This is not an experimental work but a critical review that demonstrated, on the basis of historical records, that the chemical agent that in antiquity was obtained from certain marine animals, is chemically a single substance and not a mixture of substances of different colours as asserted by contemporary authors. According to Bizio the 'changing property' of the purple dye, that nuance of colours which had made the material so precious in the past and which was considered proof that it was a mixture of different colours, is due to the diffraction of light caused by ripples and cracks on the surface of the coloured fibres. Bizio's conclusions in this dissertation are also a clear indication of his future research: 'We shall investigate the purple dye which flourished in antiquity; the little we know about it must encourage us in this work'. The following year he presented the first experimental results on the origin, preparation and characterization of the purple dye.

The chemistry of indigo blue and Tyrian purple

Tyrian purple is a bromine derivative of indigo. As is well known, indigo is a dye obtained from plants of the genus *Indigofera* which are also called indigo plants. Species that give large amounts of dye come originally from India and China but are cultivated also in other countries. Plants of the genera *Isatis*, *Polygonum* and *Marsdenia* give smaller quantities of dye. Most contain a colourless precursor, indican, which is the β-glucoside of indoxyl: 3-hydroxyindole, a compound chemically related to the amino acid tryptophan. By hydrolysis, indican gives indoxyl, which is oxidized in air and transformed into indigo (fig. 1). The chemistry of indigo was studied by J. B. Dumas in 1857 and its structure was elucidated by A. von Baeyer in 1887.

When the branches and the leaves of the plants are macerated in water, indican dissolves and is decomposed into indoxyl and glucose by a hydrolytic enzyme from the plant (reaction 1, fig. 1). As soon as the liquid is made alkaline by the addition of NaOH and is stirred in air, the indoxyl, which is colourless and soluble in water, oxidizes and transforms into indigo, which is blue and insoluble in water (reaction 2, fig. 1). Indigo precipitates as blue flakes from the solution.

The insoluble dye is then reduced with $Na_2S_2O_4$ in the presence of NaOH (reaction 3, fig. 1). The leuco form, being soluble in water, can be used for dying. When exposed to air, the leuco form is transformed again into indigo blue which, being insoluble, remains tightly attached to the material being treated.

Several halogenated derivatives of indigo blue are known. Among them is the bromine derivative, which is the red-blue compound known in ancient times as Tyrian purple. Whereas indigo blue is of plant origin, the purple is found only in a few animals: all are marine Gastropods (Mollusca) of the genera *Murex*, *Purpura* and *Mitra* which are able to synthesize it. It is present in the secretion of the hypobranchial gland as a colourless compound which, under the action of light, becomes green and then red-purple. The dye was isolated and crystallized by Edward Schunck in 1879 and its chemistry was finally elucidated by P. Friedländer in 1907. It is 6,6′-dibromoindigo (fig. 2)[26].

Tyrian purple is a very interesting example of the biochemical relationships between plants and animals. Fundamental organic substances such as carbohydrates, lipids, proteins and nucleic acids (also called primary metabolites) are common to both plants and animals. There is, however, a great number of organic compounds (known as secondary metabolites) which apparently occur only in plants. Very surprisingly, some are also found in a few groups of invertebrates and vertebrates which are able to synthesize them. This ability of animals to synthesize compounds normally found in plants is a fascinating puzzle for evolution.

Indigo and red-purple are undoubtedly secondary metabolites. Yet, whereas indigo is a specific vegetable substance common in several genera of plants, red-purple, which chemically is a derivative of indigo, is absent in plants and occurs only in a few species of marine gastropods. These are apparently the only animals to have retained the biochemical machinery for synthesizing indigo and for transforming it into red-purple.

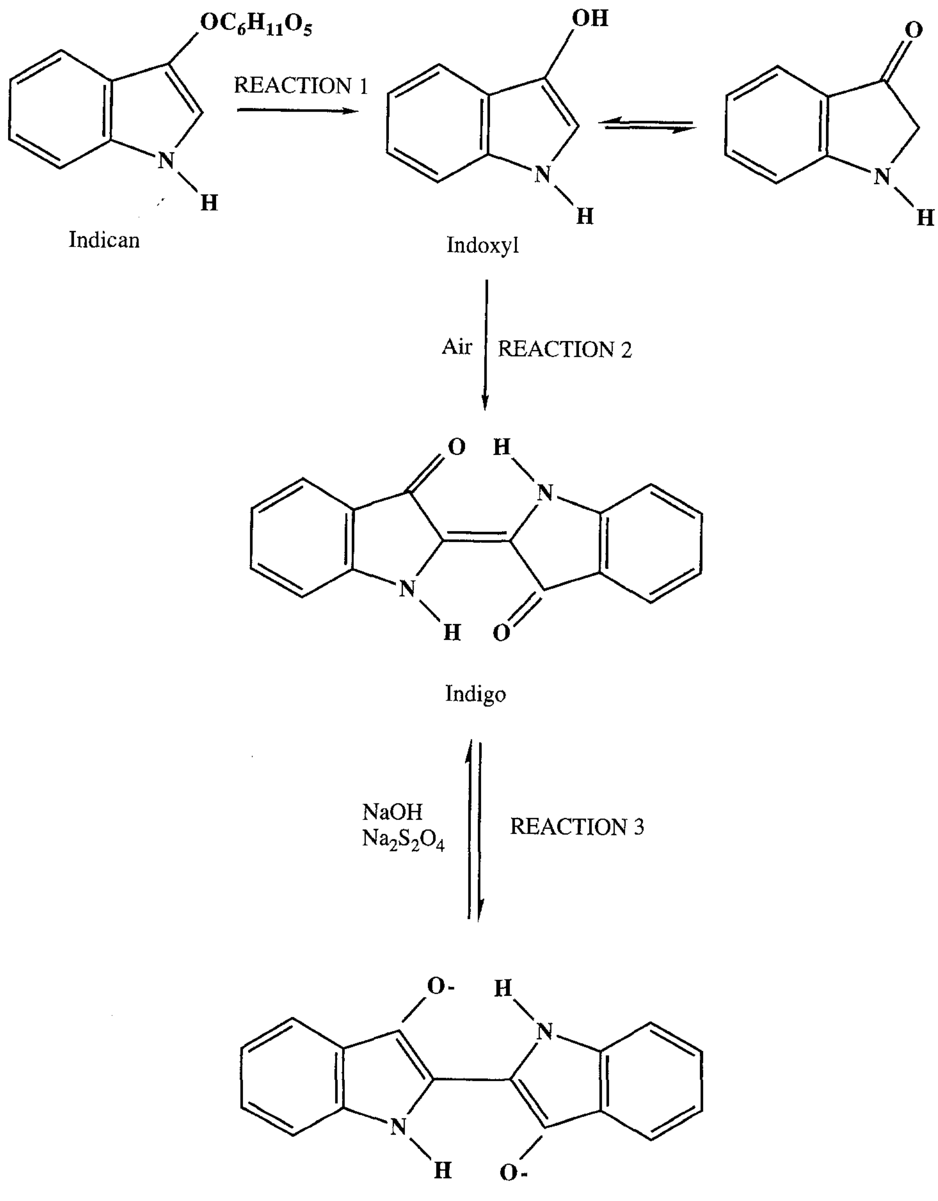

Figure 1. Reaction 1: Indican dissolves in water and is decomposed into indoxyl and glucose. Reaction 2: When the liquid is made alkaline and stirred in air, the indoxyl oxidizes and transforms into indigo. The indigo precipitates as blue flakes from the solution. Reaction 3: Indigo is reduced with $Na_2S_2O_4$ to make a leuco form which is soluble in water and can be used for dying.

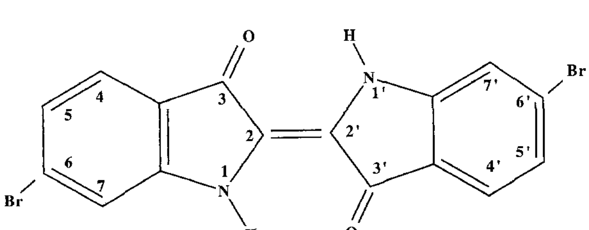

Figure 2. 6,6′-dibromoindigo.

The rediscovery of Tyrian purple

When initiating his investigations on purple, Bizio's intentions were exclusively practical. In all his papers he makes it clear that his aim is to find something useful or suitable for practical applications. For instance: when extracting from the bark of pomegranate fruit the substance he called 'austerogeno', Bizio thinks of using it for tanning[4]; when analyzing the black secretion squirted out by cuttlefish, he plans to make a printer's ink of it[3]; when discovering a dye in coffee, he wonders whether by the action of oxygen, it could be turned into a 'beautiful lacquer'[2]. The same is true of the purple dye. Along with his plans for discovering the source and chemical nature of Tyrian purple there is, as Bizio explicitly says: 'the strong desire to bring back to life those magnificent robes the ancients used to wear'. The knowledge available, however, was very scanty. 'Cosicchè, per rimettere in vita quell'arte, è mestieri riedificare tutto intero il sontuoso edificio del quale, dopo la caduta, non restano che pochi e informi avanzi, e tali che bastano appena a testimoniarne la perduta esistenza' (It is necessary, therefore, to reconstruct the sumptuous building upon the scanty and shapeless ruins which barely witness to its former existence).

Bizio published his first experimental work on the purple in 1833[7]. This long paper is exhaustive but several other papers were to follow, at first frequently, later at longer intervals until almost the end of his life[10-13].

Bizio wanted to bring Tyrian purple back to life. If the dye was a mixture of different compounds, he would admit failure and stop trying, but he felt intuitively that the purple was made of a single substance whose 'changing colours' can be explained on physical grounds. No doubt of it. However, where to look for the material?

It was reported that the ancients extracted the purple from the marine molluscs *Murex*, *Purpura* and *Buccinum*, but not everybody agreed with this. Giuseppe Olivi in his famous *Zoologia Adriatica*[32] explicitly denies that live shellfish are able to produce anything like a dye. He states precisely: 'Two shellfish species, *Murex trunculus* and *Murex brandaris*, very common in the Adriatic Sea, are believed to have been used in the past for preparing purple. However, I have never seen any coloured substance in them with the exception of a pale yellowish colour which originates from a coelenterate attached to the shell' (p. 157).

Giuseppe Olivi was an authoritative zoologist. Bizio, however, when beginning his experimental studies on the purple, looked for the material precisely in these controversial gastropod species. He discovered that 'the substance which is transformed into purple is present in the colourless fluid secreted by a peculiar organ situated in the interior part of the body in both the shellfish species'. This organ is what we call today the hypobranchial gland and comprises two elongated bodies situated near the gills. Besides the purple, the secretion contains mucus, proteins and toxins.

When collecting the glandular secretion, Bizio made a very important observation that is the keystone of the synthetic pathway of the purple dye. 'As soon as the colourless fluid is exposed to light, even a very faint one, it becomes immediately yellow and then greenish; soon afterwards, passing through all possible shades, it turns, in order, into deep emerald green, blue, deep blue and finally reaches the lovely purple red colour'.

Bizio carried out a number of experiments with the aim of confirming the necessity of light for the formation of the purple. He also wondered whether the increase of temperature that follows lighting could have something to do with the phenomenon. He concluded that 'the rapid colour change is only due to a peculiar effect of light'.

Besides the action of light, Bizio became aware of another very important fact: the need for oxygen. During the development of the colour, an oxidative reaction occurs. 'No doubt,' he concluded, 'that oxygen is necessary for the production of the purple'. Moreover he reported that 'during the reaction a nauseating smell is emitted, a smell of garlic or asafoetida'. Indican, as was found later, is indeed a sulphur compound of indoxyl[25]. In the above mentioned *Dissertazione critica*[6], even though stating that the purple dye is made by a single chemical compound, Bizio did not exclude 'certain differences' between purples from different sources. Now he became convinced of this. 'Whereas the purple of *Murex brandaris* has a genuine deep red colour, the purple of *Murex trunculus* is also coloured red but tends towards blue, reminding one of the amethystin purple described by Pliny *alium (colorem) in amethysto*'. However, whereas according to Pliny the difference is ecological and linked to the habitat in which the animal lives, Bizio quite correctly says that the difference is species-specific. 'As the animals all came from the same place and were all collected during the same season, the colour differences of the purple must be accounted for by an intrinsic character of the animal species'.

When considering its practical applications, Bizio was very concerned about the resistance of the purple dye to washing in water, with or without soaps or detergents. He recalled what happened to him when collecting the purple glands from thousands of animal specimens. After crushing the shell, Bizio removed the gland from the body with his hands. 'After a while,' he says, 'my fingers became grennish and then, passing little by little through all the different colours, the skin and the nails appeared covered by large red purple coloured spots. The spots did not disappear even after repeated washings with detergents; they were removed later by abrasion and scaling of the epidermal tissues'.

The conclusions reached by Bizio in this first paper on the purple are worth reporting verbatim. 'Among the common dyes, only indigo is similar in its chemical properties to red-purple. Indigo is present as a colourless compound in *Indigofera tinctoria*, *I. dispersa*, *I. argentea*, *I. coerulea* and other plants. As soon as this compound is oxidized by exposure to air, a green colour appears which, going through different shades, finally becomes deep blue. This is exactly what happens during the formation of red-purple'.

Bizio repeatedly analyzed the ashes of the entire body and of the separate organs of the animals used for these studies. It was during these analyses that he noticed that the ashes contained unexpectedly large amounts of copper. Copper is, in fact, a toxic metal and occurs only in trace amounts in living animals. Bizio was very surprised by this finding and hurried to announce it in an appendix to his first paper of 1833 on the purple[7]; later he devoted several specific papers to this problem[8,9]. It is interesting to recall that these studies on copper were the starting point of a line of research that was to lead to the discovery of haemocyanin, the first known copper protein, which carries oxygen in the blood of several invertebrates[27].

In another long paper, published in 1835[10], Bizio reported an impressive number of experiments comparing the purple of *Murex brandaris* with that of *Murex trunculus*. This comparison brought him to a conclusion that is truly remarkable considering the complete lack of knowledge at that time of the purple's chemical composition. Bizio says that, although the purple of both species is a single compound, that of *Murex trunculus* once known as amethystin, is actually a mixture of red and blue dyes. The red is present in much larger amounts but its colouring capacity is largely surpassed by the blue dye. Even in small quantities, this is able to transform the purple colour 'in that delicate variation which has the nuance and the original freshness of a violet flower'.

The rediscovery of the purple made by Bartolomeo Bizio can be summarized in the following points:

1) The ancients who lived in the Mediterranean coasts used to extract the purple dye from two marine gastropod species: *Murex trunculus* and *Murex brandaris*. These animals secrete the substance from a glandular body located near the gills.

2) The fluid made by the gland is colourless and contains large amounts of mucus. The colour appears as soon as the liquid is exposed to light in the presence of air; the purple derives from the oxidation of a colourless compound.

3) During the oxidation reaction the production of a sulphur compound is detectable by its smell.

4) Light is necessary for the formation of the purple. Any incidental warming from the light source is ineffective.

5) The colour difference between the purple of *Murex trunculus* and that of *Murex brandaris* is species-specific and is not related to ecology.

6) The purple of *Murex trunculus*, the 'amethystin' of Pliny, is a mixture of two dyes, one red-purple and the other blue.

7) In its chemical properties red-purple is similar to indigo, the blue dye produced by indigo plants.

All these points were subsequently confirmed and substantiated by later authors, who would claim to have 'discovered' them themselves.

The purple dye after Bartolomeo Bizio

Nowadays most English authors ascribe the rediscovery of Tyrian purple to William Cole in 1684 (see Clark et al.[17]). The report of this author, however, is very vague and similar to popular knowledge that had been transmitted orally from ancient times.

Most French authors, for their part, have celebrated Lacaze-Duthiers as the 're-discoverer', for having described the properites of Tyrian purple in 1859 in a paper entitled: *Mêmoir sur la pourpre*[28]. This paper is still largely quoted in the current literature[1,30].

Henry Lacaze-Duthiers (1821–1901) was Professor of Zoology in the University of Lille and then of Paris. He is well known for his studies in marine biology and for having founded the Zoological Marine Stations of Roscoff and of Banyuls-sur-Mer. In his paper, when summarizing the literature on the purple, he mentions Bartolomeo Bizio but, inexplicably, he quotes only his paper of 1835, which he mentions 'en passant'. It is difficult to explain this apparently restricted knowledge in such an author as Lacaze-Duthiers, who was interested in the biology of marine organisms, and specifically in the problem of Tyrian purple. In fact the 'discovery' of the purple by Lacaze-Dutheirs twenty-six years after Bizio's first publication mirrors the 'discovery' of copper in marine molluscs by Emil Harless in Germany fourteen years after Bizio!

Tyrian purple has fascinated many authors. In 1875 the brothers Antonio and Giovanni De Negri[18], chemists in Genoa, published the first detailed spectroscopic studies on the purple dye and confirmed Bizio's observations on the appearance and the formation of the colours as well as his conclusions about the presence of indigo in the purple of *Murex trunculus*.

Letellier in 1889[29], Dubois in 1890[21–24], and Derrien in 1910[19,20] all re-described the properties of purple with the addition, obviously, of some new details, thanks to the availability of improved techniques. As mentioned above, Friedländer in 1911 found that the purple of *Murex brandaris* is chemically a bromine derivative of indigo, 6,6'-dibromoindigo. Much more recent is the work of Jean Roche on the biosynthesis of the purple[14–16]. Thanks to the newly-discovered chromatographic methods of analysis, Roche was able to recognize the pro-chromogen of the purple and to reconstruct, step by step, the biosynthesis of the dye. In *Murex trunculus* there are two different pro-chromogens, I and II. *Murex brandaris* has only pro-chromogen II. Both substances have a sulphur radical but only pro-chromogen II is brominated. This clearly explains the formation of 6,6'-dibromoindigo alone in *Murex brandaris* and the presence in *Murex trunculus* of indigo in addition to red-purple. As predicted by Bizio, 'amethystin' is a mixture of indigo and red-purple.

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