The Chemical Processing of Royal Purple Dye: Ancient Descriptions as Elucidated by Modern Science, Part II

R. H. MICHEL AND P. E. McGOVERN
*Museum Applied Science Center for Archaeology (MASCA),
University Museum, University of Pennsylvania, Philadelphia,
Pennsylvania 19104*

ABSTRACT   In Part I of this article (*Archeomaterials* 1.2 [1987]: 135–143; 2.1 [1987]: 93), we described an experimental approach to elucidating the ancient processing of indigoid dyes. We focused specifically on the most renowned dye of antiquity, Royal Purple, whose major constituent is 6,6'-dibromoindigotin (DBI). Allowing for the equivocal and imprecise vocabularies of the ancient texts, including those of Aristotle, Julius Pollux, Plutarch, Pliny the Elder, and several alchemical Egyptian papyri, we stressed that the exegetic task can be advanced by clearly understanding indigoid dye chemistry and then relating specific chemical compounds and reactions to the ancient descriptions. On the basis of recent experiments involving the reduction of DBI, which had not been thoroughly characterized previously, we provide here a more detailed chemical rationale for hypotheses presented in the earlier article, which the interested reader should consult for the pertinent historical texts, scientific experimentation, and an overview of the topic.

INTRODUCTION

The relevant chemical reactions for the formation of DBI and related dyes are summarized in Figure 1 (for references, see Part I). Briefly, the chemical precursors of the dye (compounds I and V in Fig. 1) occur naturally only in the hypobranchial glands of certain marine mollusks. The Mediterranean species include *Murex brandaris, M. trunculus,* and *Purpura haemostoma,* and related species are found throughout the world's oceans (e.g., an important New World species, which was exploited by the ancient Peruvians, is *P. patula*—Baker 1974).

The dye precursors are sulfate esters of indoxyl, 6-bromoindoxyl, and derivatives of these substituted in the 2 position with methylthio or methyl sulfonyl groups. The esters are hydrolyzed to indoxyls by the enzyme purpurase (step 1 in Fig. 1). *Murex trunculus* secretions contain both indoxyls and 6-bromoindoxyls, substituted and unsubstituted in the 2 position. The unsubstituted precursors form indigoids directly by oxidative coupling in air (step 2 in Fig. 1). The precursors substituted in the 2 position oxidatively form the yellowish-green compound (compound VIII; steps 2a and 2b), referred to as tyriverdin for the bromoindoxyl derivative. The compound then converts to the dye by a photochemical process (step 2c) or by heating above 100°C, evolving long observed, odoriferous sulfur compounds (mercaptans). The primary precursors found in the glandular extracts of *M. brandaris* and *P. haemostoma* are 6-bromoindoxyls substituted in the 2 position. Consequently, these species yield exclusively 6,6'-dibromoindigotin, while both indigotin and 6,6'-dibromoindigotin (compound III in Fig. 1) can be produced from *Murex trunculus* extracts.

In the elaborate extraction process for Royal Purple in the *Historia Naturalis* of Pliny the Elder (book IX, sections 60–65, chs. XXXVI–XLI, as translated in the edition of Bailey 1929), most commentators (e.g., Heinisch 1957; Forbes 1964; Bruin 1970; and Reese 1979–1980; contrast Baker 1974) have argued that a true vat dyeing process is being described. This entails reducing the dye to the almost colorless, soluble leuco base (step 3, going from compound III to IV, in Fig. 1), in which form it is absorbed by the textile and reoxidized in air to yield a fast dye. Alternatively, the dyer might simply impregnate a skein of
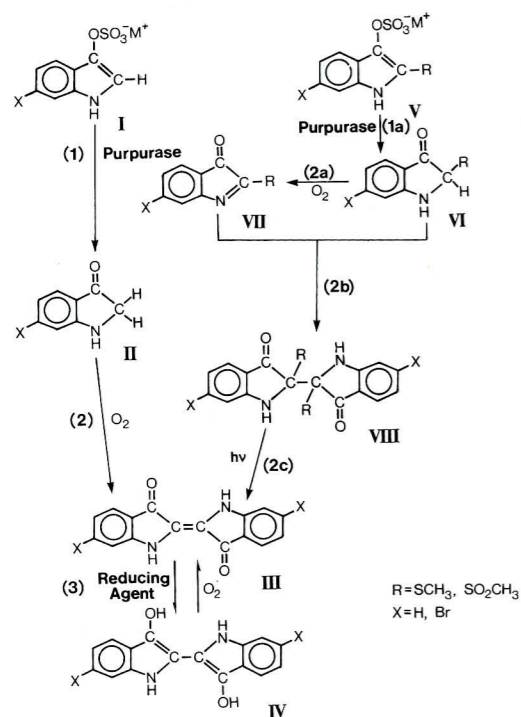
Fig. 1. Production of 6,6'-dibromoindigotin.

fibers directly with the mollusk extract or apply ("paint") it onto a textile surface, as did the ancient Peruvians (Saltzman 1986).

The sequence of operations detailed by Pliny, especially the prolonged heating of the extracts for ten days, indicate that ancient Mediterranean dyers had progressed beyond application of the molluscan extracts. But whether those procedures were intended to produce a true vat dye or were done for some other purpose can be answered only by a detailed consideration of the chemical and textual data. For example, a reducing or antioxidant system might be intended to block the formation of the insoluble dye, until the textile fibers have been impregnated. Heating the extracts might also be a means of deactivating purpurase and inhibiting the formation of the dye (Elsner and Spanier 1985). By carefully tailoring chemical experiments to address ancient textual details of indigoid dye processing, some possibilities can be excluded and the likelihood of others strengthened.

CHEMICAL VAT DYEING EXPERIMENTS

To assess whether Pliny the Elder is describing vat dyeing and/or some other procedure for molluscan indigoid dyes, a series of experiments with different charges and conditions of temperature and time were carried out (Table 1).[1]

One problem in the interpretation of the Pliny text is whether the vessel in which the molluscan extracts were heated for ten days was made of tin or lead. The text reads *plumbum*, but lacks a modifying adjective: if *album* is supplied, "tin" is the proper translation; if *nigrum*, then "lead" is to be understood. We reported previously (*Archeomaterials* 1.2 [1987]: 139) that lead in an alkaline solution (viz., aqueous potash, which had been extracted from charcoal ashes, at a pH of 11.4) did not reduce indigoid dyes, while tin under the same conditions was effective. In the experiments reported upon here, an even more powerful, faster-acting reducing system was established with tin in the more alkaline sodium hydroxide (pH 14) or potassium carbonate (pH 12–12.5) (see Table 1, experiments 1 and 2). Lead was also retested under the more alkaline conditions, and found to reduce DBI as well, though much less vigorously than tin (Table 1, experiments 3 and 4). Unsubstituted indigotin, on the other hand, is reduced effectively by lead in potassium carbonate (Table 1, experiment 5).

The failure of lead in aqueous potash to reduce indigoids may not have been due entirely to the use of potash. Experiment 5 and footnote 1 in Table 1 compare the effect of using a nitrogen bubbler, as done in the recent experiment with potassium carbonate at a higher pH, and simply restricting the air supply by a slotted stopper, as was done in the earlier experiment at a lower pH. The latter procedure gives a much paler blue than the former, implying that reducing conditions were less powerful even though the reducing system (lead/potassium carbonate) was run at a higher pH.

[1] The experimental set-up for each series of tests is detailed in the Appendix: Experimental Procedures.

TABLE 1. *Chemical vat tests: tin, lead, bronze, mercaptan, and dextrose*

Test	Reagents	Temperature (°C)	Time (hr.)	Resulting appearance
1	DBI (50 mg.), 20-mesh tin powder (500 mg.), 1 N NaOH (10 ml.)	90	1	deep purple
2	DBI (50 mg.), 20-mesh tin powder (500 mg.), K_2CO_3 (1.4 g.) in H_2O (10 ml.)	90	2	purple
3	DBI (50 mg.), lead powder (500 mg.), 1 N NaOH (10 ml.)	90	1	lavender
4	DBI (50 mg.), lead powder (500 mg.), K_2CO_3 (1.4 g.) in H_2O (10 ml.)	90	1	pale lavender
5	Indigotin (50 mg.), lead powder (500 mg.), K_2CO_3 (1.4 g.) in H_2O (10 ml.)	90	1	blue[1]
6	DBI (50 mg.), 10% tin-bronze filings (500 mg.), 1 N NaOH (1.4 g.) in H_2O (10 ml.)	90	1	lavender
7	DBI (50 mg.), 1-dodecanethiol (0.2 ml.), 1 N NaOH (5 ml.)	78–88	2	light purple
8	DBI (50 mg.), ground in H_2O (1.7 ml.) with 1 drop of detergent, dextrose (45 mg.), 10% aqueous NaOH (7 ml.)	74–78	0.5	purple
9	DBI (50 mg.), ground in H_2O (1.7 ml.) with 1 drop of detergent, dextrose (45 mg.), 50% aqueous K_2CO_3 (3.2 ml.)	78	0.5	very pale lavender

[1] The result is pale blue if nitrogen is not used and the air supply is limited by using a test tube stopper with a small slot cut out.

While the nitrogen procedure is useful in defining a system's fundamental reducing capability, such means were not available to ancient dyers. It is more reasonable to assume that restricting air supply with a stopper more adequately models ancient dye processing. Although Pliny does not mention any specific steps (e.g., covering the vessel, minimal stirring) that were taken to reduce air supply, he implies that fairly large amounts of extracts were processed in a single vessel and, as such, less air would have reached indigoids in vessels having a lower surface to volume ratio.

Furthermore, Pliny does not explicitly state that an alkali was used, and the alkalinity of the additives (e.g, urine and salt) he does mention would not have been sufficient for reduction to occur. Possibly, this was an oversight in his text, since strong alkalis (potash, soda, and lime) were available to the Romans (Forbes 1965: 181–188, 243). Assuming that moderate alkaline conditions were most likely used, vat dyeing in Roman times probably involved the prolonged heating of the molluscan extracts in a tin or tin-coated vessel, with decreased air supply.

Bronze vessels, while not mentioned in any ancient text, were so ubiquitously used in antiquity that the metal's reductive reactivity is clearly of interest. Comparable experiments with a 10 percent tin-bronze were carried out, and its reactivity in sodium hydroxide was found to be on the same order as that of lead (Table 1, experiment 6).

A vat dyeing process might have been aided by a natural reducing agent present in the molluscan extracts. Elsner and Spanier (1985) have shown that a reducing agent is present in *M. trunculus* extracts, which is effective in a highly alkaline environment. They believe the compound to be methyl mercaptan, a byproduct of tyriverdin photolysis (see Fig. 1, step 2c). We have tested this hypothesis with a more easily handled, higher-boiling synthetic mercaptan, 1-dodecanethiol, and, as experiment 7 in Table 1 indicates, DBI is reduced by the mercaptan in strong alkali at about 80°C.

Recently, in collecting extracts of Mediterranean mollusks at the Arago marine station in Banyuls-sur-Mer, France, we noted that a natural product of *Murex brandaris*, as yet unidentified, was highly effective in reducing indigoids from *M.*

TABLE 2. *Chemical vat tests: iron and urine*

Test sample	Reagents	Temperature (°C)	Time (hr.)	Resulting appearance
1a	DBI (50 mg.), ground with Fe powder (500 mg.) in fermented urine (2 ml.) at ca. pH 9, 2 ml. additional urine	50–60	1	very pale lavender
1b	4 ml. additional urine added to solution 1a after 1 hr.; fresh paper inserted	90	1	very pale lavender
2	DBI (50 mg.), ground with FeO (500 mg.), fermented urine (10 ml.) at pH 8.6–8.8	60–70 / 90	1 / 0.5	very pale lavender
3	DBI (50 mg.), ground with forging slag (500 mg.), fermented urine (10 ml.) at pH 8.6–8.8	50–60 / 90	1 / 0.5	not dyed

trunculus at room temperature, in the absence of any added alkali.

In order to preserve the soluble, reduced form of the dye, the leuco base (compound IV in Fig. 1), Elsner and Spanier added a reducing sugar (glucose from grape juice) to their mixture. The addition of glucose is relevant to a note in Plutarch which states that the very well-preserved purple textiles found by Alexander the Great at the Persian court of Susa were dyed using honey (see the commentary in Blümner 1942). Honey is a mixture of glucose and fructose. One might then ask how efficient honey is as a reducing agent for indigoids. Its reducing capability is known for indigotin (Davidson et al. 1932); tests 8 and 9 are the first evidence that this is also true for DBI. The reduction of the dibromo compound was carried out at about 75°C with potassium carbonate and sodium hydroxide.

The possibility that tin, natural mercaptans, and honey served as reducing agents to form vat dyes of DBI does not exclude their use in other stages of the process. The antioxidant properties of honey, for example, may have allowed the preservation of the molluscan extracts in a soluble, precursor form for a period of time (Bizio 1842).

The possible use of fermented urine and iron filings to prepare a "purple" vat was also discussed in our earlier article, in connection with an excerpt from the alchemical Egyptian Papyrus Leidensis (Berthelot 1887), dating to the third century A.D., with probable roots in much earlier tradition (Reinking 1925; Forbes 1964). We concluded that the "purple" was not DBI but more

probably a mixture of indigotin (blue in color) and a red dye, together giving a purple color, because the reaction mixture of fermented urine and iron filings did reduce indigotin but not DBI. In fact, Reinking had already suggested that the dye in this recipe was the marine lichen orseille, a phenolic phenoxazine derivative used to make litmus, which is a reddish purple dye.

Reinking translated the iron additive mentioned in the Leiden Papyrus from the Greek (σκωρίας σιδήρου) into German as *Eisenhammerschlag*, or "smithing scale" which is a mixture of ferrous and ferric oxides. Lagercrantz (1913: 113) rendered the phrase as *Eisenfeilstaub*, "iron filings," whereas Berthelot (1887: 43) read *scories de fer*, "iron slag." In a recent discussion of the text, Steigerwald (1986: 38–39) prefers Reinking's translation.

Since the translation is debatable, we tested the reduction of DBI with various forms of iron (Table 2)—unoxidized iron filings, ferrous oxide (Pfalz and Bauer), and a pre-Roman (ca. 250–1 B.C.) magnetic forging slag from North Wales, containing Fe_3O_4 (=Fe^{+3} (Fe^{+2} Fe^{+3}) O_4). Thus far, we have not been able to obtain smithing scale for a comparable experiment, but tests 2 and 3 with ferrous oxide and forging slag, with Fe^{+2} potentially acting as the reducing agent in both cases, are probably representative of the results that would be obtained with smithing scale. As shown in Table 2, none of the forms of iron reduced DBI in fermented urine with a pH of about 8.5. Steigerwald (personal communication) suggests that a much higher pH is required.

TABLE 3. *Fermentation vat tests*

Test sample	Reagents	Temperature (°C)	Time (day)	Appearance
1	indigotin (50 mg.), deionized H_2O (50 ml.), woad (1 g.), madder (60 mg.), bran (600 mg.), CaO (20 mg.), $Na_2CO_3 \cdot H_2O$ (36 mg.)[1]	45	6	light blue
2	DBI (50 mg.), deionized H_2O (50 ml.), woad (1 g.), madder (60 mg.), bran (600 mg.), CaO (20 mg.), $Na_2CO_3 \cdot H_2O$ (36 mg.)	45	6	almost colorless with hint of pink
3	Deionized H_2O (50 ml.), woad (1 g.), madder (60 mg.), bran (600 mg.), CaO (20 mg.), $Na_2CO_3 \cdot H_2O$ (36 mg.)	45	6	almost colorless with hint of light brown

[1] See experimental section for complete history of alkali addition and pH control.

While the addition of an alkali might accomplish this, none is mentioned in the text, and the highest pH recorded for fermented urine is 9.5 (Doumet 1980: 39).

FERMENTATION VAT DYEING EXPERIMENTS

The fermentation of woad (*Isatis tinctoria* L.) and of *Indigofera* is an ancient process for producing indigo vats for textile dyeing (compare, e.g., the description in another alchemical Greek text, Papyrus Holmiensis—Lagercrantz 1913). Woad was used primarily in Europe, the Middle East, and North Africa, whereas *Indigofera* was employed in South Asia (Horsefall and Lawrie 1946: 309; Anonymous 1964: 34). Starting with either species, indoxyl is liberated, and indigo is formed by oxidative coupling, and then reduced by a fermentative, enzymatic process to the soluble leuco base. It is of interest that synthetic indigo was still being processed in fermentation vats as recently as the early twentieth century. In addition to woad, these fermentation systems for synthetic indigo also generally utilized madder and bran (Knecht, Rawson, and Lowenthal 1910: 321). The bran nourished the bacteria, which presumably derived from the woad (Wendelstadt and Binz 1906: 1630). Madder (*Rubia tinctoria*) is a plant source for reddish mordant dyes of the alizarin (anthroquinone derivative) family, but probably functions here as a redox intermediate facilitating the reduction of indigo (Friedländer 1911).

Mollusk dyers might have been acquaint-

ed with the fermentation process using indigo plants, and it has been proposed by some investigators that fermentation was involved in processing Royal Purple (e.g., Jensen 1963). In order to test this hypothesis, it was necessary to have a fermentation process which could work on a small scale. Wendelstadt and Binz (1906) had developed such a procedure based on the old commercial fermentation process for synthetic indigo (above), which we were able to duplicate in 250 ml. flasks. In this procedure, the supply of oxygen is controlled, so that the bacteria are neither starved nor are so well supplied that the indigo is not reduced. In a very large commercial vat, the air supply control is a natural consequence of the depth of the vessel in which the process is carried out.

The results of the fermentation tests in vats are given in Table 3. Vats with indigo (test 1) produced a dyed sample reproducibly, with a clear change of color from yellowish-green to light blue. A control (test 3) lacking any dye additive showed no color change, although a hint of a brownish color was noted after washing the sample with water. The vat with DBI (test 2) gave only a hint of pink coloration after washing the sample with water, and the characteristic color change of an indigoid dye from yellowish-green leuco base was not observed.

Apparently, in contrast to indigo, DBI is poorly reduced or not reduced at all using such a fermentation vat. There is still the possibility that different bacteria, perhaps not one found on the woad plants available

available to us, would be able to reduce DBI, and further experimentation is needed to test this possibility.

CONCLUSIONS

The reduction experiments for indigoid dyes described here established several important points regarding the Mediterranean Royal Purple industry, as described in Greek and Latin texts of the Roman period:

(1) If Pliny the Elder's text in *Historia Naturalis* does refer to a vat dyeing process, then the ten day heating of the molluscan extracts was more likely carried out in a tin or tin-coated vessel (*plumbum album*) rather than in a lead vessel (*plumbum nigrum*), under moderate alkaline conditions and with a restricted air supply.

(2) Natural mercaptans, present in the molluscan extracts, might contribute to forming a vat. It is unlikely, however, that sufficient quantities of mercaptans would be available to reduce all the dye to the leuco base and keep it in this form in a vessel open to the air. Other reducing agents, as well as alkalis, would probably be required to carry out vat dyeing.

(3) Glucose, whether in the form of honey, fruit juice, or some other natural product, can also act as a reducing agent and/or antioxidant. Plutarch's reference to very well-preserved textiles at the Susan court might then relate to honey used in a vat dyeing process or as a preservative for the molluscan extract.

(4) A reducing system of fermented urine and various forms of oxidizable iron—filings, ferrous oxide, and forging slag—were tested, and found not to work for DBI. Therefore, the "purple" process described in the Greek alchemical texts probably does not involve Royal Purple.

(5) The hypothesis that a woad/madder fermentation vat was used to process molluscan extracts was not supported by our experiments. This does not rule out the possibility, however, that other strains of bacteria might yield positive results.

The experiments summarized here demonstrate that 6,6'-dibromoindigotin is less easily reduced in a vat dyeing system than indigotin (Elsner and Spanier 1985). Not only was the brominated compound inert to iron/urine reduction and fermentation, but it gave a less intense color in a dextrose, tin, or lead system at lower alkalinity (i.e., in an aqueous potassium carbonate solution).

APPENDIX: EXPERIMENTAL PROCEDURES

Synthetic Royal Purple (6,6'-dibromoindigotin) was prepared from 4-bromo-2-nitrotoluene (Fairfield Chemical Co, Blythwood, SC) via 4-bromo-2-nitro-benzaldehyde (Barber and Stickings 1945). Mass spectrometric analysis of the product confirmed its composition. Synthetic indigotin was purchased from Fisher Scientific.

The experimental procedures described here were developed specifically to distinguish between true dyeing and pigmentation in heterogeneous vat systems. The apparatus used for the chemical vat tests consisted of a 30 ml. test tube with a sidearm and a stopper through which was passed a nitrogen bubbler. The test tube containing the reaction mixture was heated on a hot plate in a magnetically stirred water bath, with a 250 ml. Erlenmeyer flask holding a dial thermometer. The flask was wrapped with aluminum foil to exclude light and prevent debromination of any leuco base formed. The charges, the reaction conditions, and their effectiveness are given in Tables 1 and 2. The products formed in the reaction mixture were tested with a piece of filter paper (0.75 × 3 cm.). This was enveloped in a larger piece of folded filter

paper, which was forced into one end of a 2 cm. length of 7 mm. diameter glass tubing. The test paper, thus protected against dye particulate pigmentation, was suspended in the reaction mixture by a polyester thread.

Successful vat formation results in a yellowish-green solution, the color of the leuco base (compound IV in Fig. 1). The addition of sugar to the reaction mixture yields a brown coloration because of decomposition of the sugar. At the end of the reaction time, the sample holder was removed from the reaction vessel. The wet filter paper, upon removal from its envelope, was yellow if a strong vat had formed. A purple color developed when the sample was held in air 5 to 10 minutes.

Fermentation vats (Table 3) were tested in a 250 ml. Erlenmeyer flask which was equipped with a two-holed stopper holding a thermometer and an exit tube leading to a gas trap. A sample paper was held in the reaction mixture by the same assembly described for the chemical vats. The reaction mixture was warmed either on a hot plate or in an oven. No precautions were taken to exclude air until the reactor had been charged. After charging, sealing, and warming the flask to 40 to 45°C, the pH was approximately 8. About two hours after startup, 8 drops (ca. 0.4 ml.) of a 50 percent potassium carbonate solution were added, raising the pH to 9. Two days later the fermentation was clearly under way, with gas bubbling out through the trap. The pH, which had dropped to about 7, was then raised with the same amount of potassium carbonate. After six days, the sample was removed from the flask and envelope, exposed to air for 10 to 15 minutes, and washed with water. The temperature was held at about 45°C throughout the fermentation.

The preparation of the woad and madder charges was as follows. The leaves of the woad plants were cut off and chopped up. Balls of approximately 5 cm. in diameter were formed and air dried several weeks before use. The madder root was cut off the plant, washed free of soil, air dried, and then chopped and ground into a fine powder.

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