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The analysis of indigoid dyes by mass spectrometry

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High-resolution mass spectrometry provides an expeditious technique for positively identifying indigoid dyes of molluscan origin. Indigoids on modern and ancient textile fibres, as well as indigoid mixtures produced by reactions, are discussed. Steam treatment of the fibre or isolation of the dye by solvent extraction permits the analysis by mass spectrometry of dyes which bind more tightly to fibres, such as 6,6'-dibromoindigotin (C.I. 75800, Natural Vat Dye). The demonstration of the stepwise photolytic debromination of the leuco base of 6,6'-dibromoindigotin may be of more far-reaching significance in distinguishing between natural and intentional mixtures of indigotin and its dibromo derivative.

INTRODUCTION

Natural blue indigotin (structure 4a in Figure 1; C.I. Natural Blue 1) and its 6,6'-dibromo derivative (structure 1; C.I. 75800, Natural Vat Dye), which is purple in colour, are among the most ancient and renowned dyes known to man [1]. The plant sources of indigo are the various species of *Indigofera* [2] and *Isatis tinctoria* (woad) [3]. The former are native to India and other equatorial countries. The latter is widely distributed throughout Europe, Asia and northen Africa. The plants were exploited for dyes as early as the second millennium BC and were used well into the 20th century, when they were displaced by synthetic indigo. Whether natural or synthetic, indigo is applied to a textile fibre as the leuco base. The reducing medium used by ancient dyers was a fermentation vat.

Precursors of the 6,6'-dibromo derivative, which convert to the dye in air and light, are found in nature in the hypobranchial secretions of certain marine molluscs. The molluscs occur world-wide, and are found in the Pacific Ocean, the Atlantic Ocean, and the Mediterranean Sea. The chemistry of the dye precursors has been shown to be similar in many respects for the various species [4]. The glandular secretions of one Mediterranean species, *Murex trunculus*, appear to be unique in containing the precursors of both indigo and the dibromo derivative, the relative amounts of each depending upon the sex of the individual specimen and possibly the season of the year [5].

The Mediterranean industry of producing blues, purples, and violets (combining blue and purple) from molluscan extracts was probably first developed by the ancient Canaanites and Minoans in the mid-second millennium BC [6–8]. The use of molluscan indigoid dyes apparently developed independently at a later date in East Asia (Japan and China) and the New World (Central America and Peru) [9]. In the Mediterranean region large-scale dyeing operations ceased with the Ottoman invasions in the 15th century [10]. Since then there has been little commercial interest in the natural dye. Even after the synthesis of 6,6'-dibromoindigotin in the early 20th century [11], this dye has not been produced on an industrial level.

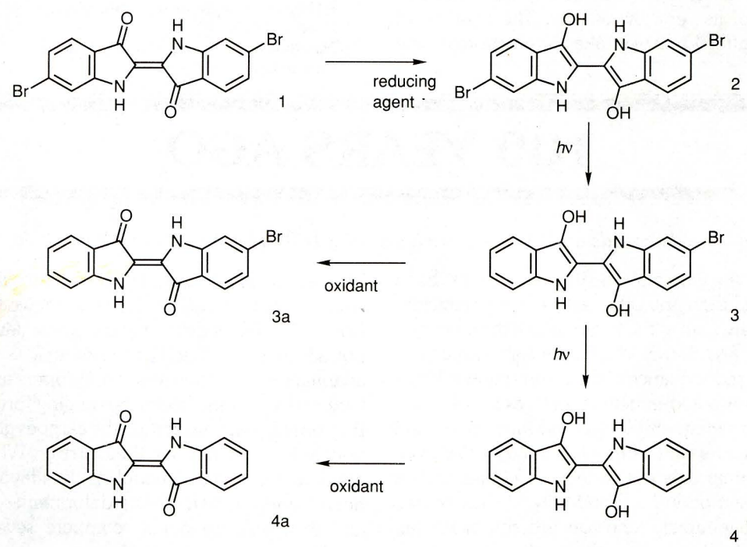

Figure 1 – Indigoid chemistry, showing photolysis of the leuco base of 6,6'-dibromoindigotin to 6-bromoindigotin and indigotin

Despite the decline in commercial and industrial importance, the molluscan indigoids are still used sparingly today by Central American and Japanese dyers, who directly apply the secretions to fibres of wool and cotton, followed by exposure to sun and air, rather than employ the more sophisticated vat dyeing process. Moreover, because of the political and religious importance of the natural dibromo derivative to many cultures of the world, attempts have recently been made to establish and replicate the ancient dyeing processes [5,8].

EXPERIMENTAL

Ancient and modern materials

Only New World textiles dyed with indigoids have been analysed. The ancient samples are from Peru (University Museum Nos. 39-11-1; SA3530; SA3531), and belong to the Paracas (approx. 900–200 BC) and Nazca (approx. 200 BC–600 AD) cultures. A modern yarn of Mexican cotton dyed with the extracts of *Pupura patula pansa*, provided by Dr Saltzman of the Laboratory of Historical Colorants at UCLA, was also analysed.

Synthetic 6,6'-dibromoindigotin was prepared from 4-bromo-2-nitrotoluene (Fairchild Chemical Co.) via 4-bromo-2-nitrobenzaldehyde [12,13].

Sample preparation for mass spectrometry

The textile fibres that required steam treatment were held in a stream of 104°C steam for 6 h, after which they were pushed into the bottom of a capillary with a fine needle. A control sample of steam-treated synthetic 6,6'-dibromoindigotin was prepared by mounting a 2.5 cm long melting point tube containing the dye on a section of cork stopper and wedging this into the steamline exit, with the end of the tube facing into the steam flow. The treatment was carried out for 24 h.

Quinoline solutions of dye extracted from fibres were transferred while hot to a tube, using a hypodermic needle. The solvent was then evaporated in a vacuum oven at progressively higher temperatures (75–160°C over a week).

In preparation for the photolysis of the leuco base of 6,6'-dibromoindigotin by ultra-violet (u.v.) light, 25 mg of the indigoid compound was rapidly dissolved in a solution of 75 mg sodium hydrosulphite and 6 ml 0.05 mol/l sodium hydroxide at 60°C, contained in a 30 ml pyrex test tube equipped with a side arm and a stopper through which a nitrogen bubbler was inserted. The solution was cooled to room temperature under nitrogen. It was protected against accidental exposure to light at all times by an aluminium foil wrapper. The cooled solution was clamped about 7 mm from, and parallel to, a fluorescent u.v. lamp (Fisher 11-984-42 lamp in a model DE holder, 365 nm cut-off, UV Products), and exposed for 10 s. Other solutions were exposed for durations of 30 s, 90 s and 30 min each. (The dye used in the 30 min exposure had been isolated after a previous 1 min exposure.)

After exposure 15 ml of 3% hydrogen peroxide solution was added. The mixture was transferred to a beaker, and the reactor rinsed into this with an additional 10 ml of hydrogen peroxide. The product was isolated by filtration, water washed, and air dried for mass spectrometry.

Mass spectrometric procedure

The high-resolution mass spectra were determined with a VG-Instrument Co. ZAB-2F mass spectrometer. Ionisation was by electron impact at 70 eV (1.12×10^{-17} J). The ion source temperature was 200°C. The samples were loaded in melting point capillary tubes, which were inserted into the probe, and vaporised at temperatures up to 400°C. Dibromoindigotin, monobromoindigotin and indigo were positively identified by accurate mass measurement at a resolving power of $M/\Delta M$ = 10 000 (10% valley). Thereafter these species were identified by low-resolution mass spectrometry.

RESULTS AND DISCUSSION

Fibre analysis

The analysis by mass spectrometry of textile fibres dyed with indigotin has been reported by Quinlivan [14]. However, de Wong [15] was not able to detect 6,6'-dibromoindigotin by direct analysis, but only after it had been separated by reductive extraction with sodium hydrosulphite.

Binz and Mandowsky [16] observed a decline in the rub fastness of cotton dyed in a indigo vat when steam treated. This suggested that steam pretreatment of fibres dyed with 6,6'-dibromoindigotin might make it possible to identify the latter compound by mass spectrometry. Reductive extraction has the disadvantage that it is difficult to prevent debromination of the leuco base by photolysis. Prior steam treatment of the fibres, however, does not result in debromination. A comparison of the essentially identical spectra (Figure 2(a and b)) of synthetic 6,6'-dibromoindigotin before and after steam treatment convincingly demonstrates this fact. Both the spectra have triplet ion clusters centred at an M/Z (mass/charge) of 420 (i.e. 418, 420 and 422), representing the three isotope combinations of the dibromo compound. Neither spectrum shows any evidence of monobromoindigo (M/Z 340 and 342) or of indigo (M/Z 262), while other minor components of these samples are the same. Whether the effect of the steam treatment on indigoid volatility is due to a change in the dye's secondary bonding to the substrate and/or in its physical state is uncertain and currently under investigation.

Direct analysis of the Mexican cotton fibre dyed with 6,6'-dibromoindigotin from *Purpura patula pansa* predictably gave no evidence of the dye. After steam treatment the mass spectrometric spectrum showed a peak at M/Z 420. The latter could be resolved into the characteristic 6,6'-dibromoindigotin triplet.

This triplet was even stronger in the residue from a quinoline extract of the same fibre. A sample of synthetic 6,6'-dibromoindigotin, recovered after having been dissolved in quinoline, confirmed that this procedure does not affect its mass spectrum.

The black dyes in fibres of ancient Peruvian origin (Paracas and Nazca cultures) gave strong peaks for indigo at M/Z 262 by direct analysis. The presence of this dye in

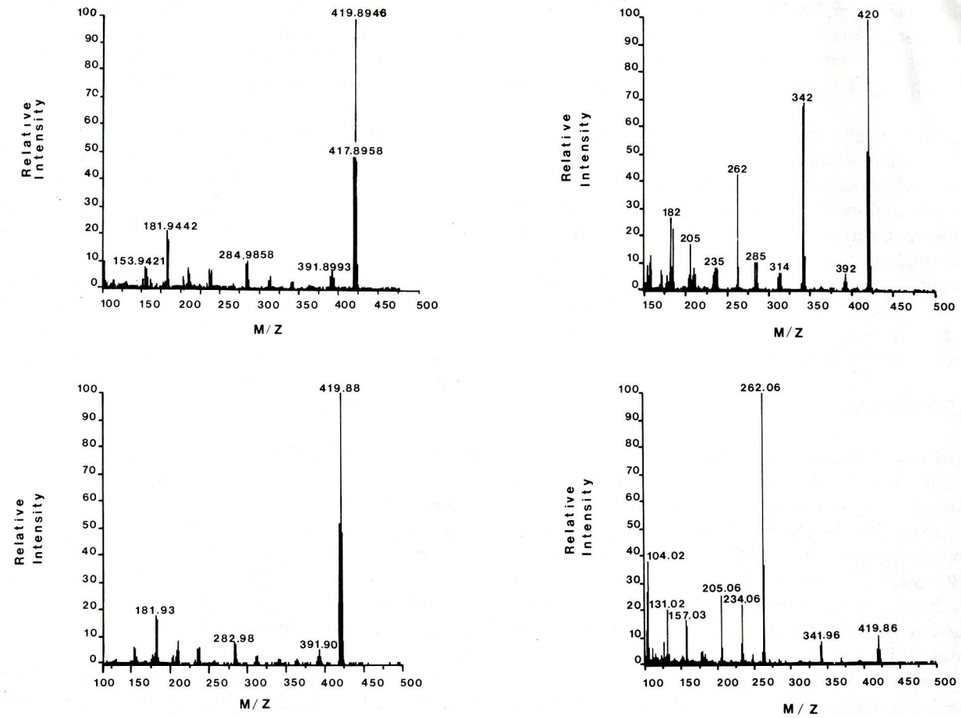

Figure 2 – Mass spectrum of (top) synthetic 6,6'-dibromoindigotin and (bottom) steam-treated synthetic 6,6'-dibromoindigotin

Figure 3 – Mass spectrum of (top) the product from synthetic 6,6'-dibromoindigotin, after u.v. irradiation for 1.5 min, and (bottom) the product from synthetic 6,6'-dibromoindigotin, after u.v. irradiation for 30 min

Paracas black is well documented [1].

Questions regarding the presence of dibromoindirubin, an isomer of 6,6'-dibromoindigotin and a possible by-product in the formation of molluscan purple dye, cannot be answered by mass spectrometry. The separation of the two compounds is made difficult by their similar physical behaviour, e.g. solubility and probably sublimation, since the unsubstituted indirubin sublimes. The indirubin, however, is generally a minor end product and, because it is not as fast, its concentration in washed and sun dried textiles is further decreased.

Photolysis of 6,6'-dibromoindigotin

Driessen [17] and van Alphen [18] have reported the debromination of the leuco base of 6,6'-dibromoindigotin (structure 2 in Figure 1) by sunlight, to give indigotin. Elsner and Spanier [5] showed that a mixture of dyes could be produced at a lower level of irradiation, to yield a range of more or less bluish colours. The analytical capability of mass spectrometry allows the intermediate stages of photodebromination to be more precisely defined.

Figure 3(a) is a typical spectrum after 90 s u.v. irradiation. The presence of indigotin, 6-bromoindigotin, and the starting material 6,6'-dibromoindigotin (structures 4a, 3a, and 1 respectively in Figure 1) are clearly shown by the single peak at *M/Z* 262, the double peak at *M/Z* 340 and 342, and the triple peak at *M/Z* 418, 420 and 422. Figures 2(a) and 3(b) respectively show the starting material and the almost completely photodebrominated 6,6'-dibromoindigotin after 30 min exposure. The presence of the monobromo compound and indigotin in the less exposed samples and the disappearance of nearly all but indigotin in the most exposed sample proves stepwise debromination of the dibromo compound.

Naturally occurring and process-dependent mixtures of indigoid dyes

The major components of the natural dye produced from the hypobranchial gland secretions of *Murex trunculus* are reported to be indigotin and 6,6'-dibromoindigotin. Were this to be confirmed, then any monobromoindigo present in a dye mixture could only be the result of photodebromination of 6,6'-dibromoindigotin as the leuco base. On the other hand, if the natural product does contain monobromoindigo, then a dye composition of only 6,6'-dibromoindigotin and indigotin might be an intentional mixture of the two dyes, using either a plant or animal source for indigotin or a totally photodebrominated material. The presence of monobromoindigo in the natural product has not been reported in the literature. However, when processing the secretions from *M trunculus*, which have not been segregated by sex, the natural indigoids form by the coupling of bromo substituted and unsubstituted indoxyl derivatives in the same reaction mixture. Consequently the formation of the monobromo derivative is a definite possibility.

CONCLUSIONS

The full potential of mass spectrometry in dye analysis is only beginning to be realised. It has been shown here that this technique provides a very expeditious and exact means of identifying indigoid dyes on ancient and modern fibres. The complicating reactions of reductive extraction are bypassed by steam treatment or solvent extraction. By precise discrimination of the different indigoids, mass spectrometry can also shed light on the processing of the dye materials. The next logical step in our investigations is to identify the indigoids formed from the glandular extracts of Mediterranean and New World mollusc species by sex and season. This will provide the crucial points of reference for judging whether an ancient dye mixture is natural or the result of processing.

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Ethylenediamine tetra(methylene phosphonic) acid as a carrier for the disperse dye Palanil Violet 3B

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Ethylenediamine tetra(methylene phosphonic) acid in an acidic medium is able to attach itself to the dye molecules while being adsorbed by polyester fibres. Hydrogen bonds as well as electrostatic attractive forces are involved in both processes, leading to an increased dyeing rate.

INTRODUCTION

Polyester fibre, by virtue of its hydrophobic nature, its tightly packed molecular structure and its lack of reactive groups, is unreceptive to most dye molecules. Disperse dyes were found to be suitable for dyeing polyester, with the dyeing process taking place through hydrogen bond formation between amino groups in the dye molecule and carbonyl groups in the fibre polymer, or through electrostatic attraction between the dye molecule and the fibre. Disperse dyes can be applied to polyester fibres at temperatures below 100°C by the aid of 'carriers' added to the dyebath. Some disperse dyes can be applied at higher temperatures and under pressure higher than atmospheric.

Several theories (varying in credibility) have been put forward to explain the mode of action of such carriers. At one time it was supposed that dye molecules became attached to the carrier and were drawn into the fibre with it as a result of the affinity of the fibre for the carrier. According to another view the carrier forms a film covering the surface of the fibre, the dye dissolves in it and is thus more easily transferred to the hydrophobic fibre than from an aqueous medium [1].

There is evidence that most if not all commercial carriers cause swelling of polyester fibres, and it is widely believed that these function by causing the polymer chains to move apart so that dye molecules are more easily accommodated [2,3]. However, it was concluded that the swelling action is not their sole function.

Organic phosphonates resemble polyamine carboxylates in their structure. In acidic media the nitrogen atoms of phosphonates become protonated while their hydroxyl