High-performance liquid chromatography of blue and purple indigoid natural dyes

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Four major components of blue and purple natural dyes of the indigoid class were separated and spectrally characterised by high-performance liquid chromatography: indigotin, indirubin, 6-monobromoindigotin and 6,6′-dibromoindigotin. It has been shown for the first time that a dyeing with a hypobranchial glandular secretion of *Murex trunculus* L contains indigotin and both its mono- and di-substituted brominated derivative. The analytical features presented may be used to study the composition of old dyes on yarns.

INTRODUCTION

Recently indigoid natural dyes, and especially these of animal origin (Tyrian purple from various species of mollusc), have been the subject of renewed investigations in diverse scientific disciplines: an evaluation of the hue of genuine Tyrian purple based on biochemical, historical and linguistic data [1], a historical description and some remarks about the constitution and identification of the dyes in purple parchments of codices [2], dyeing experiments with preparations from freshly gathered *Murex brandaris* L, *Murex trunculus* L and *Thais haemastoma* L [3], a review on the occurrence and biosynthesis of indole pigments in mollusca [4] and the mass spectrometric detection of dibromoindigotin on yarns [5].

It may be expected that the detection and relative quantification of the components present in natural indigoid dyes may help in researching their biological origin. This kind of research is of great importance in characterising the dyes on old yarns, and it might also put an end to recent controversies regarding the nature of antique blue and purple dyes [6,7].

A method is reported in the present paper for separating and identifying the major dye components present in blue and purple indigoid dyes. Further investigation regarding etymological and historical matters will be published shortly after.

EXPERIMENTAL

The separation of vegetable and animal indigoid dye components by high-performance liquid chromatography (h.p.l.c.) was accomplished according to the procedure outlined previously [8]. This procedure was adopted in our laboratory for detecting indigotin (I) and indirubin (II) in pre-Columbian Peruvian textiles [9], using a separation method developed for mordant dyes [10,11]. Extraction of dyes from a dye powder or from a yarn was done in pyridine, at 100°C. The chromatogaphic conditions were as follows: column Rosil C18, 3 µm, 50×4.6 mm (RSL, Eke, Belgium); pump HP-1050 (Hewlett-Packard, USA); detector 990+ photodiode-array detector (Waters-Millipore, USA); data handling NEC APCIII computer and Waters 990 printer plotter (Waters-Millipore, USA); 20 µl sample loop, pyridine extract injected without dilution; flow rate:1.0 ml/min; analytical wavelength 288 nm; flow scheme (A = methanol, B = water, C = 50 g/l phosphoric acid in water) (30A/60B/10C), 1 min, linear gradient to 60A/30B/10C, 2 min, linear gradient to 90A/10C, 12 min, 90A/10C 2 min.

RESULTS AND DISCUSSION

By analysing a limited number of samples it was possible to identify the major blue and purple indigoid dye components. The large peak obtained at the start of each chromatogram represented pyridine. Only peaks that show absorption in the visible region of the electromagnetic spectrum and that may thus contribute to the formation of colour are characterised in the present paper, by a roman numeral, referring to the spectral and structural data given.

Sample 1

Cotton yarn, dyed with synthetically prepared 6,6′-dibromoindigotin (IV), supplied by Dr M Saltzman, Los Angeles, USA. The pyridine extract only contained IV, as shown by Figure 1.

Sample 2

Generated from sample 1 by irradiating with u.v. radiation (254 nm) in an alkaline reducing medium for 60 min. It had been shown previously that this treatment causes photolytic debromination of IV to 6-monobromoindigotin (III) and, by the same photolytic action on this latter component, to indigotin (I) [5]. The chromatogram of the extract of the irradiated sample is given in Figure 2. Obviously IV has partially persisted during the photolytic debromination, but also appreciable amounts of two other products were generated: the first was I, so that the second one must be III.

Sample 3

An indigo powder from the Leeds Collection (University

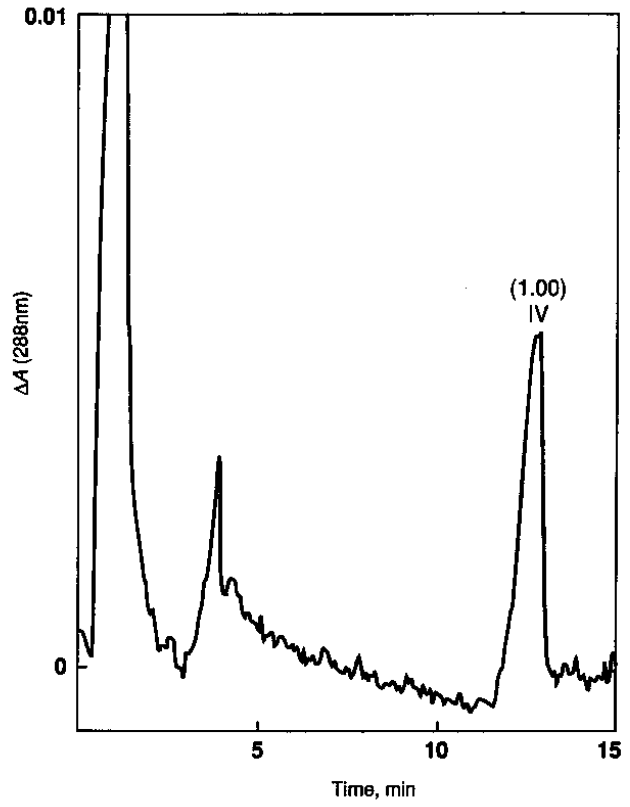

Figure 1 H.p.l.c. of a pyridine extract of cotton yarn, dyed with synthetic 6,6'-dibromoindigotin (IV)

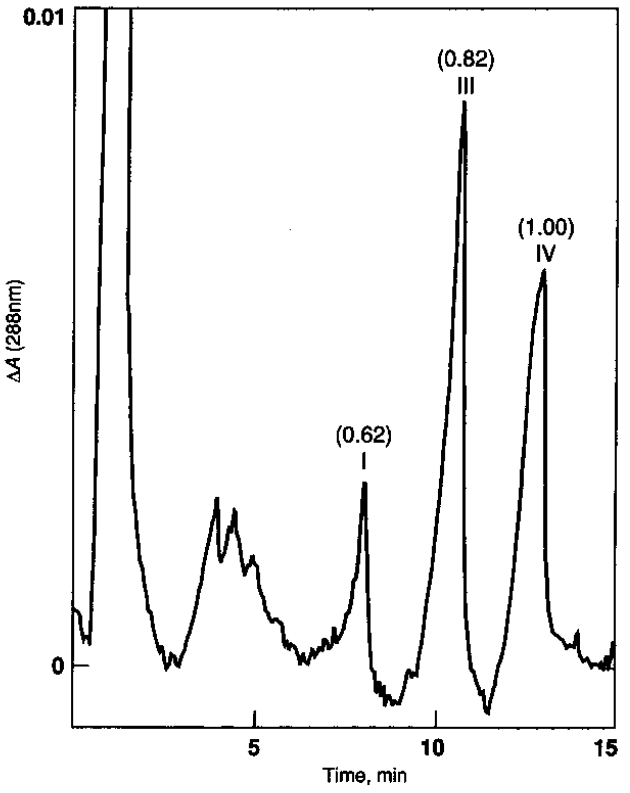

Figure 2 H.p.l.c. of a pyridine extract of cotton yarn, dyed with synthetic 6,6'-dibromoindigotin (IV), after u.v. irradiation in an alkaline reducing medium

of Leeds, UK), labelled 'Neel Indigo, Medak district, sample number A53' (supplied by Mrs S Grierson, Perth, Scotland). This was shown to contain similar amounts of I and indirubin (II) (Figure 3). Indirubin was identified according to spectral data published previously [8,12].

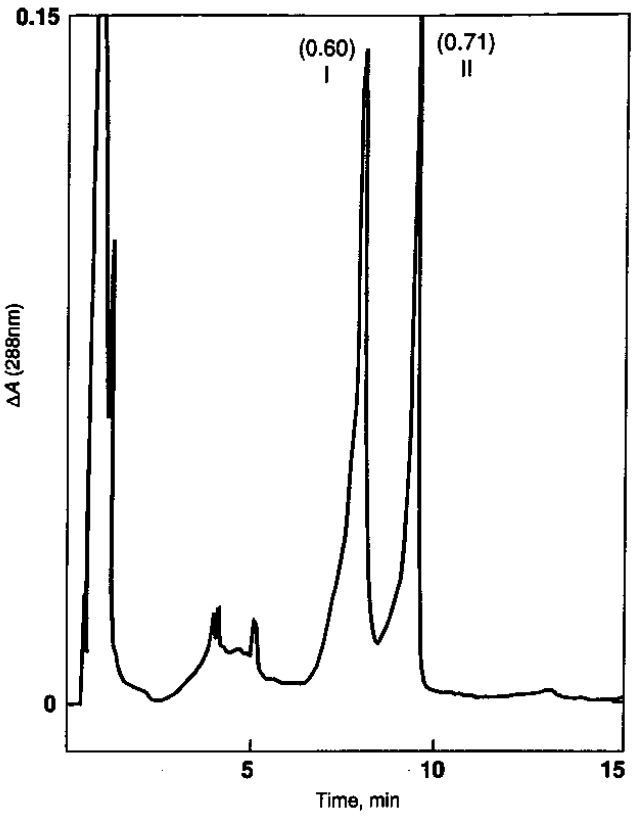

Figure 3 H.p.l.c. of a pyridine extract of indigo powder (for details see text)

The detection and quantification of these components, and maybe others, in vegetal blue dyes might be of interest for possible distinctions between indigo (*Indigofera* spp.) and woad (*Isatis tinctoria*) dyeings.

Sample 4

Combined from the preceding three in order that the separation of the four components of interest could be effectively displayed. As can be seen in Figure 4, a complete separation of all the components involved was achieved. Figure 5 is a contour plot, showing the relationship between retention time, absorption intensity and wavelength; this gives the investigator all the relevant analytical details at a glance. The rather red hue of II, contrasting with those of the other three compounds, which have their visible absorption maxima in the blue region is shown. The shift of absorption maximum from 613 nm (in I) to 606 nm (in III) to 600 nm (in IV) is also evident.

By means of the diode-array detection facility, not only could the detection wavelength be chosen to give an optimal response for each product, but also each peak in an unknown sample could be fully characterised by its u.v. and visible light absorption spectrum, taken on-line during chromatography. These spectra are given in Figures 6–9.

Sample 5

Silk fabric, stained with the contents of the hypobranchial gland of *Murex trunculus* as described previously [3]. The composition of a pyridine extract is given in Figure 10. According to the retention times and the spectral analysis

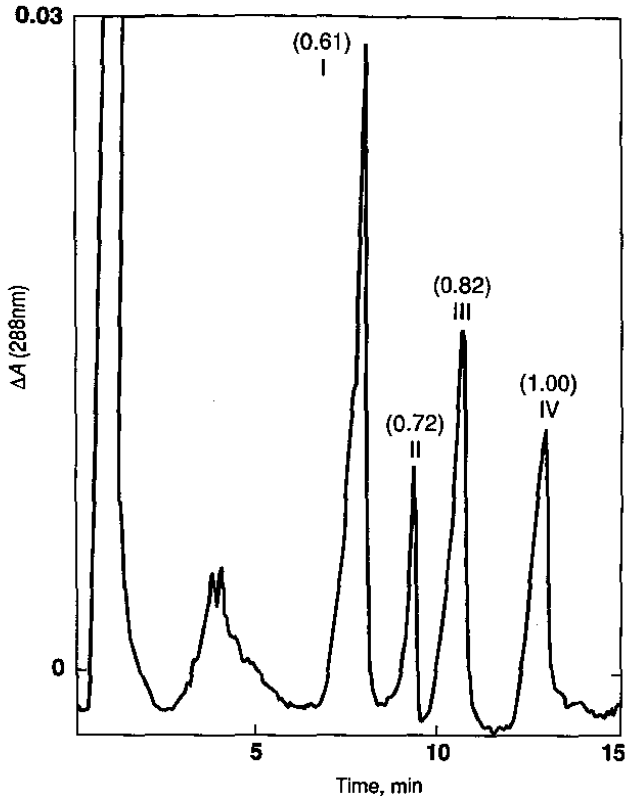

Figure 4 H.p.l.c. of indigoid dye components I–IV – normal chromatogram

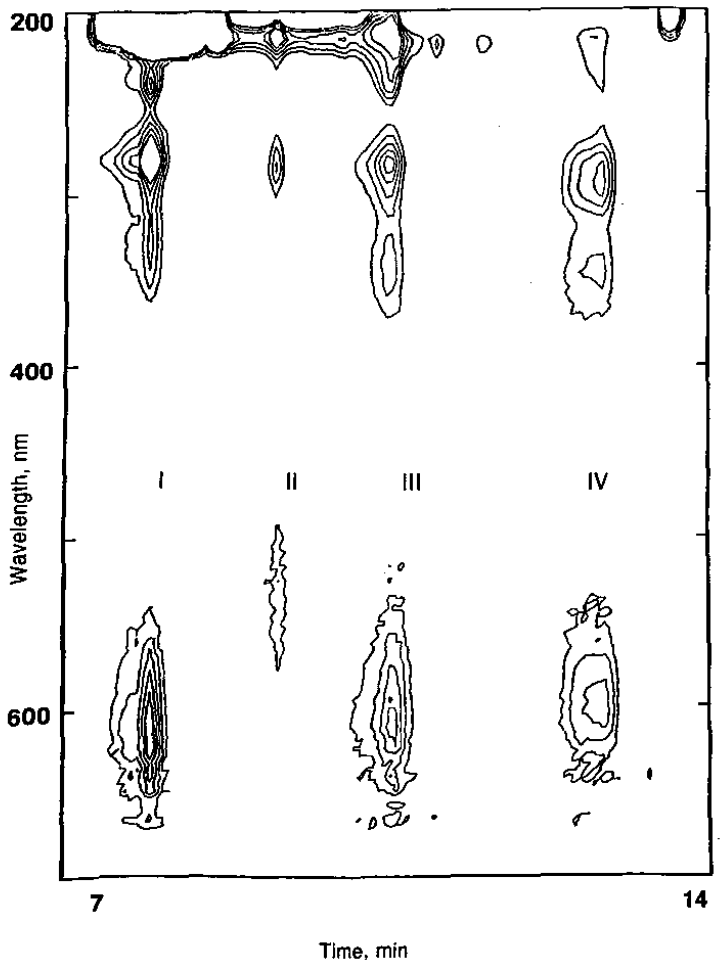

Figure 5 Contour plot of compounds I–IV in the time section 7 to 14 min from Figure 4

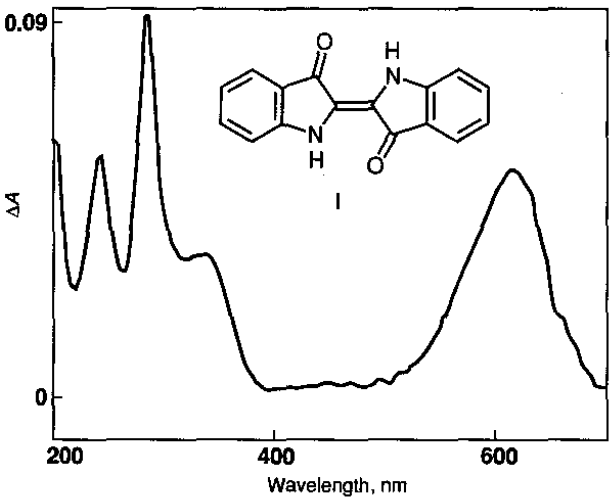

Figure 6 Absorption spectrum in the u.v. and visible light region of indigotin (I)

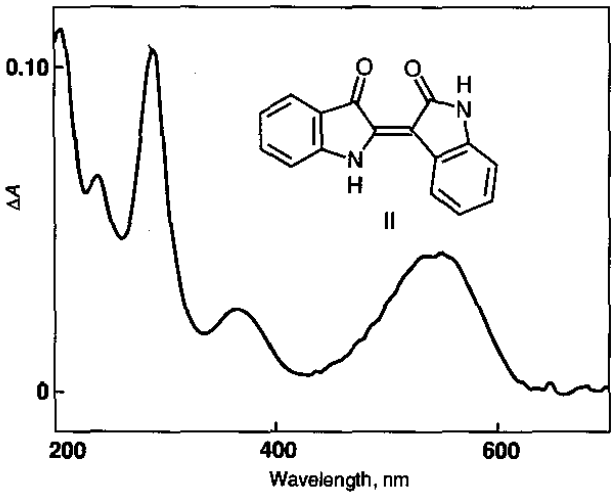

Figure 7 Absorption spectrum in the u.v. and visible light region of indirubin (II)

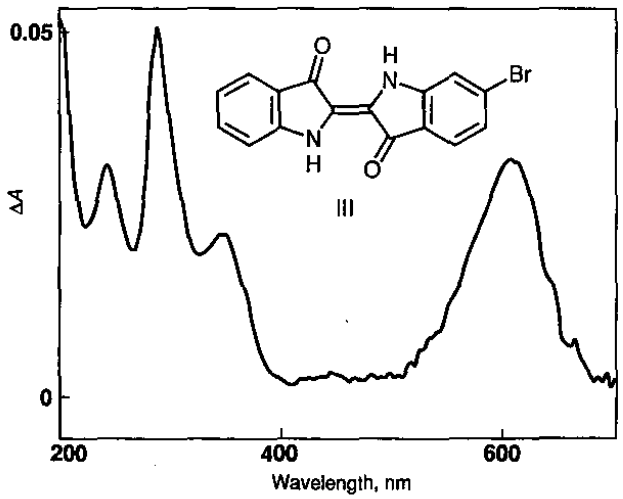

Figure 8 Absorption spectrum in the u.v. and visible light region of 6-bromoindigotin (III)

of each peak, I, III and IV were present. The presence of III in dyes prepared from *Murex trunculus* has never been shown before, although it has been suggested [5], possibly produced following treatment in an alkaline reducing medium [13]. This was, however, not the case with

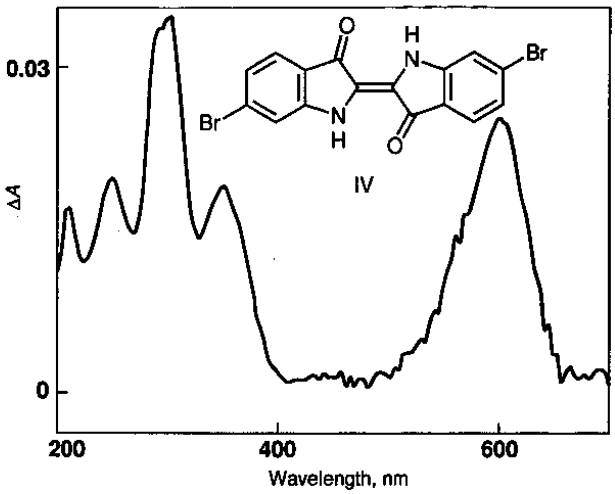

Figure 9 Absorption spectrum in the u.v. and visible light region of 6,6′-dibromoindigotin (IV)

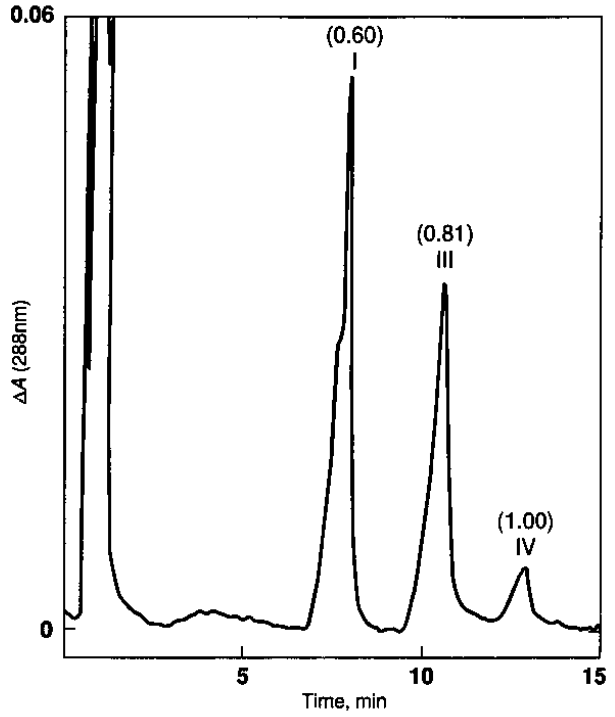

Figure 10 H.p.l.c. of a pyridine extract of silk fabric stained with the contents of the hypobranchial gland of *Murex trunculus* L

the present sample since the glandular contents were directly mixed and brushed onto the silk in the open air (no reduction) and in sunlight [3]. It may thus be stated that III is specifically formed from a brominated and a non-brominated precursor present in the hypobranchial gland of *Murex trunculus* L.

CONCLUSION

The analytical method described for indigoid dyes may greatly improve knowledge of the dyestuff composition of vegetable and animal blue and purple dyes. It does certainly broaden the applicability of the h.p.l.c. methodology for the separation of mordant dyes that was outlined in earlier papers [10,11]. It has been shown for the fist time that dye preparations from *Murex trunculus* contain three components: indigotin, 6-monobromoindigotin and 6,6′-dibromoindigotin. This particular composition may shed a new light upon the exact biological source that would have been used for the production of precious ancient blue and purple dyes. For instance, there has been much of speculation and discussion regarding the composition and origin of the 'tekhelet' dyes, because in no work presented (so far) has any separation, characterisation or quantification of all the dye components involved been given [14,15].

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