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Chemical extraction of indigo from *Indigofera tinctoria* while attaining biological integrity

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Abstract

Indigofera tinctoria was permeabilized with 20% methanol (v/v) at 25 °C and released 8 ± 2 μg indigo g^{-1} dry plant material (excluding roots). This is equivalent to 42 ± 11% of the total indican within the cells. The plants began to recover after 2 weeks.

Introduction

Indigo is currently being produced through chemical synthesis. We are interested in developing an environmentally benign and economically viable method to permeabilize the plant without destroying it, so it can be permeabilized again. Once indican is extracted from the plant, it can be filtered and oxidatively converted to indigo (Figure 1).

Materials and methods

Materials

Companion Plants (Athens, Ohio, USA) supplied whole plants of *Indigofera tinctoria*.

Assay of indigo

The extinction coefficient of indigo at 620 nm is 14.0 mm^{-1} cm^{-1}. Indican did not interfere with the indigo peak, so separation was not necessary.

Destructive extraction method

Fresh leaves were crushed in a mortar filled with liquid nitrogen. Half the leaves were placed in a drying oven at 40 °C for 24 h for a dry weight. The other half was mixed with 15 ml of 80% methanol (v/v) per gram fresh leaves. The solution was heated at 70 °C for 5 min. Evaporated methanol was replaced and then the solution was stirred for 20 min and filtered. This method was also performed on stems and roots (Maier *et al.* 1990).

Chemical permeabilization method of biologically active plants

To measure the fresh weight of the plant, the roots were removed and weighed separately from the rest of the plant. A one to one ratio of leaves plus stem to roots was established. A clamp apparatus was set-up to hold plants upside down. The roots were covered and the plant was placed into 20% (v/v) methanol (Komolpis *et al.* 1998). (Note: Water was used as the buffer system.) Permeabilization occurred for 4 h, and then the plants were removed and soaked in water for 6 h, changing the water every 2 h.

Oxidation of indican to indigo

The sample was dissolved in 1 ml of 2 M HCl per gram of fresh plant for 5 h at room temperature. The solution was filtered and evaporated under N_2 gas (Xia & Zenk 1992). The indigo was dissolved in dimethyl sulfoxide (DMSO) for spectral analysis. (Note: for

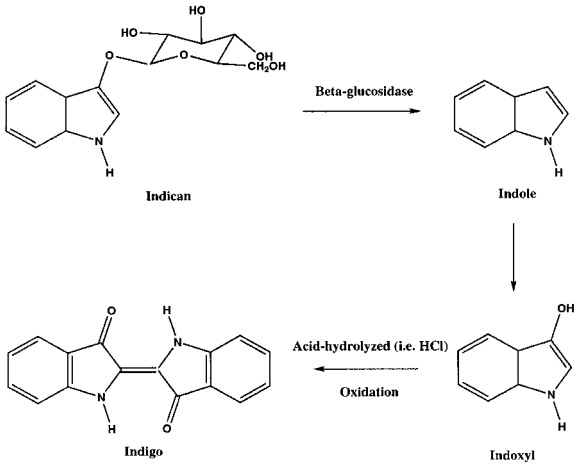

Fig. 1. The conversion of plant derived indican to indigo via oxidation (Strobel & Gröger 1989, Xia & Zenk 1992).

Table 1. Amount of indigo extracted by the destructive method.

	Indigo concentration (μg g^{-1} dry^{-1} w^{-1})	
	Present extraction	(Maier *et al.* 1990)[1]
Leaves	19	NA
Stems	11	32
Roots	9	24

[1]Extraction was performed on *Polygonum tinctorium*.

permeabilization the solution was filtered twice.) Synthetic indoxyl fl-D glucoside (97%) was converted into indigo and produced a 94.2% yield.

Results and discussion

Comparison of the destructive extraction and chemical permeabilization method

The amount of indigo destructively extracted was considerably less than the method used by Maier *et al.* (1990) (Table 1). Utilizing a different species of indigo-producing plant with different growth conditions may explain the difference.

Indigo was only permeabilized from the leaves and stems because roots require optimal conditions for growth. The amount of indigo extracted from chemical permeabilization had a mean yield of 8 μg g^{-1} dry plant (excluding roots) with a standard deviation of 2 (Table 2). The control run shows that only a minimal amount of indigo can be extracted using water as a buffer, proving that 20% (v/v) methanol is sufficient to release indican. Tables 1 and 2 show a lower amount of indigo released for the chemical permeabilization method. This is due to the fact that 20% (v/v) methanol was used for permeabilization instead of 80% (v/v) methanol for the destructive extraction method. 20% (v/v) methanol was used so the plant could recover after chemical permeabilization and be chemically permeabilized again. The varying amounts of indigo chemically extracted can be due to the various stages of plant life and the environmental conditions the plants were exposed to prior to chemical permeabilization. The percent release of indigo for chemical permeabilization as compared to destructive extraction has an average value of 42% with a standard deviation of 11%.

Table 2. Amount of indigo extracted and percentage released by the permeabilization method with control.

Plant	Indigo concentration (μg g^{-1} dry weight of plant)	% Release
Control	0.3	2
1	7.6	41
2	8.7	47
3	9.5	51
4	5.0	27

Viability assessment of plants before and after chemical permeabilization

The growth progression of the plants before and after the chemical permeabilization experiment was assessed. Plants 1 and 3 began to sprout new leaves 2 weeks after chemical permeabilization. This demonstrates that the plants can recover from chemical treatment and potentially be permeabilized again.

Purity of plant extract

The product extracted from the plants and converted into indigo was not pure. The solution can be purified using the TLC method in Maier *et al.* (1990). One possible explanation for the tainted solution is the presence of chlorophyll. To determine the influence of chlorophyll on the product, a known amount of indigo was added to the solution after indican had been extracted. The results show that the amount of indigo observed (original plus added indigo) corresponds to the amount added. Therefore, chlorophyll does not

affect the amount of indigo observed in the spectral analysis, but does produce a noticeable peak at about 665 nm.

Conclusion

One advantage of permeabilization is the potential for developing a continuous and intermittent permeabilization method while avoiding the environmental hazards of synthetic production. Other solvents or a mixture of solvents should be evaluated to find the optimal permeabilizing agents. There are also many other plant metabolites with medicinal properties that are difficult to produce synthetically. This chemical permeabilization concept should be investigated as an alternative for the production of synthetic products.

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