

ENHANCED ALKALOID YIELDS IN *CINCHONA LEDGERIANA* ROOT ORGAN  
SUSPENSION CULTURES SUPPLEMENTED WITH L-TRYPTOPHAN

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Root organ suspension cultures of *Cinchona ledgeriana* MOENS which produce low levels of quinine and quinidine, have been maintained in culture for 3 years in our laboratories (Anderson *et al* 1982). It has been reported that alkaloid levels are affected by feeding tryptophan, one of the biosynthetic precursors of the alkaloids, to suspension cultures of *Cinchona* species (Koblitz *et al* 1983; Hunter *et al* 1982).

In an attempt to improve the alkaloid yields in our cultures, experiments have been undertaken to study the effect of the addition of L-tryptophan to the culture medium. Initial experiments using cultures fed with L-[methylene-<sup>14</sup>C]-tryptophan have demonstrated that the labelled precursor is taken up rapidly from the medium into the root organs. Preparative TLC of the extracted alkaloids followed by scintillation counting techniques has shown incorporation of the radioactive label into quinine and quinidine, at levels of 0.1 and 0.3%, respectively.

The results demonstrating the incorporation of the labelled precursor into the alkaloids prompted further investigations into the effect of feeding high levels of unlabelled tryptophan to the cultures. Root organ suspensions were filtered aseptically and the root organs distributed into flasks containing 40 ml medium giving an initial inoculum of 4g fresh weight per flask. Freshly subcultured flasks were fed aseptically with L-tryptophan, dissolved in an aliquot of culture medium, at levels of 250 and 500 mg l<sup>-1</sup>. The supplemented flasks and controls were harvested after 15 days, the root organs were filtered from the medium and the alkaloids extracted using standard techniques and analysed by HPLC with fluorescence detection. The medium was analysed directly by HPLC in order to determine the levels of L-tryptophan remaining.

The results of the experiment (Table 1) show that significant levels of L-tryptophan are taken up by the cells. Furthermore, no evidence was found from the HPLC analyses of the presence of tryptophan metabolites such as 5-hydroxytryptophan, tryptamine, 5-hydroxytryptamine or indole acetic acid in the medium.

Table 1. Effect of L-tryptophan on alkaloid yields in *Cinchona ledgeriana* root organ suspension cultures

L-Tryptophan in Medium (mg l <sup>-1</sup> )		Alkaloids in Root Organs (μg l <sup>-1</sup> ) at 15 days	
Zero Time	At 15 Days	Quinine	Quinidine
0	0	117.5	103.8
250	24	187.5	150.0
500	120	625.0	525.0

Analysis of the alkaloidal extracts clearly shows that supplementation of the medium with L-tryptophan at levels of 500 mg l<sup>-1</sup>, in particular, results in a remarkable five-fold increase in the levels of quinine and quinidine. The results from the labelled and unlabelled feeding experiments suggest that the increased alkaloid production observed with the unlabelled tryptophan is, in part, due to the improved availability of the biosynthetic precursor.

Acknowledgement. One of us (CAH) gratefully acknowledges receipt of a SERC Quota award.

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