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PRECURSOR FEEDING OF AILANTHUS ALTISSIMA CELL SUSPENSION CULTURES WITH L-TRYPTOPHAN

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High alkaloid yielding cultures of Ailanthus altissima (Mill.) Swingle have been maintained in our laboratories for several years and are being used to study alkaloid production (Anderson et al 1983,1985). The cultures produce 1-methoxycanthin-6-one as the major alkaloid together with canthin-6-one and 1-hydroxycanthin-6-one as minor alkaloids. Our studies have shown that a number of factors, for example, the seed source and the composition of the basal medium, influence alkaloid production in these cultures (Anderson et al 1986).

Initial feeding experiments with the biosynthetic precursor L-[methylene-^{14}C]-tryptophan have demonstrated that almost 50% of the labelled precursor is taken up by freshly subcultured cells within 15 minutes (Figure 1). Preparative TLC and HPLC of the extracted alkaloids followed by scintillation counting techniques have shown incorporation of the radio-label into the canthinone alkaloids. Labelled alkaloids, however, were not detected until 48 hours after feeding.

Having demonstrated that labelled L-tryptophan added to the culture medium could be taken up by the suspension cells and the radio-label incorporated into the alkaloids further experiments were undertaken to ascertain whether the alkaloid yields could be improved by feeding unlabelled L-tryptophan. Freshly subcultured cells were fed aseptically with L-tryptophan, dissolved in an aliquot of culture medium, at levels of 250 and 500 mgl^{-1}. The supplemented flasks and controls were harvested after 30 days, the cells filtered from the medium and the alkaloids extracted. The medium was analysed directly by HPLC in order to determine the levels of L-tryptophan remaining.

Figure 1 UPTAKE OF L-[METHYLENE-^{14}C]-TRYPTOPHAN BY AILANTHUS ALTISSIMA CELLS IN SUSPENSION CULTURES

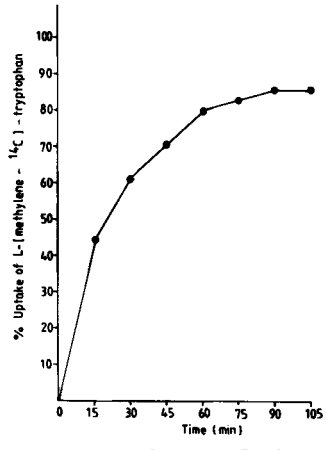
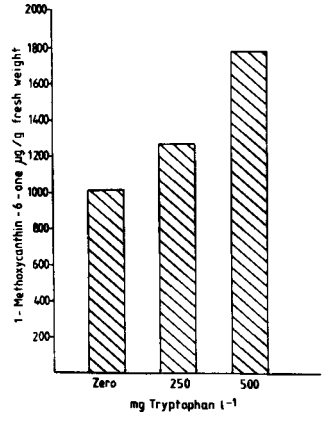


Figure 2 EFFECT OF TRYPTOPHAN ON 1-METHOXYCANTHIN-6-ONE YIELD FROM AILANTHUS ALTISSIMA (RBG line)



Quantitative HPLC analysis of the alkaloidal extracts showed improved alkaloid yields with the L-tryptophan fed cultures (Figure 2). Supplementation of the cultures with 500 mgl^{-1} L-tryptophan, in particular, resulted in a 76% increase in the yield of 1-methoxycanthin-6-one.

These experiments illustrate that, in addition to other parameters (Anderson et al 1986), the levels of available precursors also need to be optimised to ensure maximum alkaloid yield from A. altissima cell cultures.

Acknowledgement. We gratefully acknowledge financial support from SERC.

Anderson L.A. et al (1983) J. Nat. Prod. 46: 374-378
Anderson L.A. et al (1985) J. Pharm. Pharmac. 37: 45P
Anderson L.A. et al (1986) Alkaloid production by plant cells: Proceedings of 'Process possibilities for plant and animal cell cultures', UMIST, Manchester