Synthesis and Biosynthesis of Isocordoin

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Received: July 25, 2000; Accepted: November 5, 2000

Abstract: **In the search of a convenient synthesis for isocordoin (1), a potential anticancer natural product, 2′,4′-dihydroxychalcone was inoculated in cell suspension cultures of *Morus nigra*, which were expected to contain an active prenyltransferase. After 24 hours the target compound was easily isolated from the metabolite extract. Optimization of the biotransformation resulted in a 85% yield of the prenyl derivative.**

A number of prenylated 2′,4′-dihydroxychalcones have been isolated from *Lonchocarpus neuroscapha* (1) or *Derris* species (2) and have been synthesized by condensation of a prenylated resacetophenone (3) and the appropriate benzaldehyde (4), (5). Preliminary studies on the natural chalcones and synthetic analogues revealed that isocordoin (**1**) was a potential new anticancer drug (6). The need for a larger availability stimulated our interest in the preparation of the compound. The synthesis of **1** from 3′-prenyl-resacetophenone (**2**) and benzaldehyde was considered unsatisfactory. Compound **2** is obtained by treatment of resacetophenone with 2-methyl-but-3-en-2-ol in the presence of $BF_3{\cdot}Et_2O$ (3) in only 25% yield and it is not easily separated from the other similar products (5′-prenyl and 3′,5′-diprenyl derivatives). Moreover, the condensation with benzaldehyde in alkali (4), (5) results in a poor yield (ca. 5%). Since the condensation of resacetophenone and benzaldehyde gives almost quantitatively the 2′,4′-dihydroxychalcone (**3**) and the reaction mixture does not require a chromatographic separation, we preferred the alternative pathway of the prenylation of the chalcone **3**, according to the general procedure of Bohlmann and Kleine (7). Even though isocordoin was obtained with a faster method and improved overall yield (8%), we turned our attention to the possibility of producing **1** biosynthetically. The enzymatic prenylation of a variety of phenolic compounds has previously been reported by a number of investigators (8).

On the other hand calli, derived from *Morus nigra* explants, have been shown in our laboratory to produce flavonoids and Diels-Alder type adducts similar to those in the parent plant (9).

Considering that the biosynthetic production of Diels-Alder adducts involves two prenylated chalcones and thus requires the presence of a prenyltransferase (10), we developed from

Planta Med 67 (2001) 475 – 477

ISSN: 0032-0943

the calli of *M. nigra* a cell suspension culture to obtain an exploitable active enzyme.

A stable cell line, which produced a pattern of metabolites comparable with those of calli and parent plant, was used for the biotransformation experiments. In a preliminary experiment 2′,4′-dihydroxychalcone (in EtOH/H_2O) was added to the culture medium; after 5 days cells and medium were separately extracted with EtOAc and the residues were examined by TLC ($CHCl_3$, two runs). Two yellow compounds, less polar than all the other components of the mixture, were detected in both extracts and easily separated on a small silica gel column by elution with $CHCl_3$.

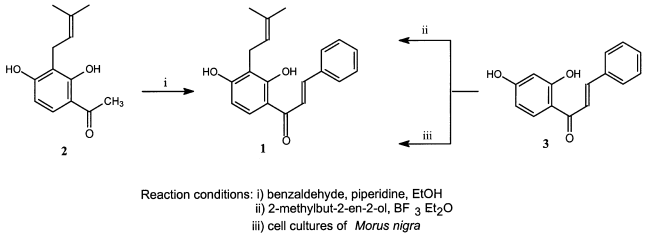
The first (major Rf) compound was identified as isocordoin by comparison with an authentic sample, while the second was the synthetic precursor **3**.

In further experiments the amounts of chalcones **1** and **3** were determined by integration of the relative peaks in HPLC chromatogram and extrapolation from a calibration curve.

In Figure **1** are summarized the results obtained when whole cells of *Morus nigra* were fed with different amounts (2.5, 5, and 10 mg per 10 g fresh wt.) of 2′,4′-dihydroxychalcone (**3**).

Isocordoin (**1**) was detected after 12 h in every case (ca. 1 mg per 10 g fresh wt.), but its amount increased significantly only for the 10 mg inoculum; in this case the target compound was isolated in a cumulative 85% yield (7 mg and 4 mg per 10 g fresh wt. in medium and cells, respectively) after 12 days.

The high content of the target metabolite in the culture medium makes biotechnologically promising the production in bioreactor. Studies on this possibility as well as on the characterization of the prenyltransferase enzyme are in progress.



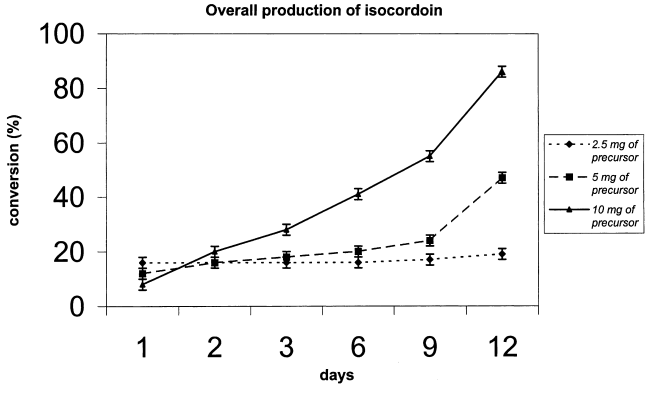


Fig. 1 Overall production of isocordoin per 10 g fr. wt. of cells.

Materials and Methods

Calli of *Morus nigra* (8) were inoculated (1 g fresh wt. per Erlenmeyer flask) in a MS62 (10) basal medium (65 mL), enriched with 2,4-D (0.01 ppm), NAA (0.1 ppm), kinetin (0.75 ppm) and casein (1 g L^{-1}). The suspended cells were maintained at 25 °C in the dark in a rotatory shaker and subcultured every 20th day (stationary phase) in fresh medium.

2′,4′-Dihydroxyacetophenone (1 g, 6.6 mM) and benzaldehyde (2 mL, 19 mM) in MeOH (20 mL) and KOH/H_2O (10 g/10 mL) were held at reflux for 45 min. The reaction mixture was poured into ice/6N HCl and the precipitate (2′,4′-dihydroxychalcone, **3**) was washed with abundant water and used as such. BF_3·Et_2O (1 mL, 8.2 mM) and 2-methylbut-3-en-2-ol (0.7 mL, 6.7 mM) in anhydrous THF (4 mL) were added to crude **3** (1.6 g) in anhydrous THF (28 mL); the resulting mixture was kept under stirring for 90 min at room temperature, poured into water and left under stirring overnight. After evaporation of THF the aqueous phase was extracted (×3) with Et_2O. The organic-phase residue on a silica gel column with CH_2Cl_2/hexane, 3 : 1, gave isocordoin (**1**, 163 mg, overall yield 8%), identical with the natural sample (1).

2′,4′-Dihydroxychalcone, dissolved in the minimum quantity of EtOH/H_2O, was inoculated in the medium of 4-day-old cell subculture of *Morus nigra* (2.5, 5, and 10 mg per 10 g fresh wt.). Cells and medium were separately extracted with EtOAc at intervals (6 h, 12 h, 24 h, 2 d, 3 d, 6 d, 9 d, 12 d) and the extract residues were analyzed by HPLC: column, RP-18 (100 × 4.6 i.d.); eluent system, MeOH/H_2O, 8 : 2; isocratic flow rate, 1 mL/min; UV detector, 320 nm.

Acknowledgements

The authors gratefully acknowledge the financial support of Progetto Finalizzato “Biotecnologia e Biostrumentazioni”.

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