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Searching for local biocatalysts: Bioreduction of aldehydes using plant roots of the Province of Córdoba (Argentina)

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ABSTRACT

A screening for the capacity of wild plants growing in the Province of Córdoba to bioreduce benzaldehyde was carried out. From this study, thirteen species showed quantitative reduction yields to benzyl alcohol, with *Conium maculatum* showing the best reduction efficiency. This plant was also tested against different substituted benzaldehydes, and quantitative yields of substituted benzylic alcohols were obtained, except for vanillin, where only 27% of vanillic alcohol was formed (main product: 2-methoxyphenol at a 73% yield). A scaling study of the reaction using *C. maculatum* and benzaldehyde was carried out, and it was observed that high substrate–catalyst relationships reduced the efficiency of the reaction due to side reactions of oxidation. The bioreduction method presented here permits substituted benzylic alcohols to be obtained using an environmentally friendly methodology, with excellent yields produced on a laboratory scale.

1. Introduction

The reduction of the carbonyl group is among the most important reactions in organic chemistry, with today's organic chemists having a wide range of appropriate reduction systems at their disposal. In general, most of these use heavy metals or their hydrides and organic solvents as the reaction medium, which are able to provide excellent yields of the desired alcohols [1]. However, comparatively few reduction methodologies have been developed taking into account the concept of green chemistry (environmentally friendly reaction systems) in order to avoid the formation of toxic waste that may pollute the environment [2].

In recent years, chemical reactions using plant parts and their cell cultures as biocatalysts have received great attention due to the large biotechnological potential of enzymatic reactions. Some important characteristics of these biocatalysts are their low cost, high versatility and efficiency, in addition to highly desirable chemical aspects such as chemoselectivity, regioselectivity, and enantioselectivity, with the combination of these factors having made biocatalytic reactions very attractive to the industrial sector [3].

Many transformations of different substrates, such as hydroxylation and oxidation reactions (*Gynostemma pentaphyllum*) [4], hydrolysis of esters (*Solanum tuberosum*, *Helianthus tuberosus*) [5], bioreduction of ketones and aldehydes (*Daucus carota*, *Foeniculum vulgare*, *Cucurbita pepo*, *Phaseolus aureus*, *Cocos nucifera*, *Saccharum officinarum*, *Manihot dulcis*, *Manihot esculenta*) [3,6–16], enzymatic lactonization (*Malus sylvestris*, *Helianthus tuberosus*) [17], glycosylation (*Ipomoea batatas*, *Eucalyptus perriniana*) [18], etc., have been performed, and have produced very good results using plants and their cultured cells.

The use of plants as biocatalysts has many advantages. First of all, a large array of taxonomically different plants is available at a very low cost. Another important aspect is that the separation of the product from the reaction mixture can be carried out very easily by filtration/centrifugation and the remaining material is easily disposed of. Moreover, these systems have the advantage of being environmentally friendly, due to the reaction being carried out in water as the solvent and the catalyst being biodegradable [19], as opposed to the classic reactions of organic chemistry where heavy metal disposal may be an issue.

In summary, it can be stated without equivocation that plants represent an alternative source of "new" enzymes for use in organic synthesis.

Recently, as a part of a major program on the study of the flora in the Province of Córdoba, a project was commenced with the

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aims of finding green alternatives and economically viable procedures to synthesize chemical products of commercial interest using biocatalytic processes.

In the particular case of the benzylic alcohols, several of these are considered to be key starting materials in the synthesis of scented substances for cosmetics, fragrances and the flavour industry [20], which in general are more expensive than the corresponding aldehydes from which they are obtained. However, the reduction of benzaldehydes may be potentially carried out through biocatalytic methodologies, if an efficient and affordable biocatalyst is available which is also capable of generating the desired product in adequate quantities. With this objective in mind, the screening of the native flora was initiated to find plants that could be used as biocatalysts in reduction reactions of aromatic aldehydes.

2. Experimental

2.1. General methods

Benzaldehyde, benzoic acid, 2-methoxyphenol and substituted benzaldehydes were purchased from the Sigma–Aldrich Chemical Company (Argentina). 4-(*N*,*N*-dimethylamino) benzaldehyde was obtained from Fluka. Benzyl alcohol and substituted benzyl alcohols were purchased from the Sigma–Aldrich Chemical Company. 4-(*N*,*N*-dimethylamino)benzyl alcohol, 2-methylthiobenzyl alcohol and vanillic alcohol were synthesized by a methodology described in the literature [21], and sterile deionized water was used as the solvent in all experiments. The crude reaction products were extracted with ethyl acetate, the organic solutions were evaporated, and the products were filtered on a short column with silica gel (70–230 mesh) using ethyl acetate as the eluent. GC analyses were made on a Shimadzu GC-14B instrument, with FID detector and GC–MS analyses were carried out on a Shimadzu GC-17A/QP-5000 instrument. ^{1}H NMR spectra were recorded on a Bruker AC 200 MHz using $CDCl_3$ as the solvent. All products were characterized by comparison of their GC retention time (GC Rt) with authentic samples, and by comparison of their MS and ^{1}H NMR spectra with literature data [21–26].

2.2. GC-FID and GC–MS analyses

The GC separations were performed on a Hewlett Packard HP-5 fused silica capillary column (Crosslinked 5% PhMe Siloxane, 30 m, 0.32 mm, 0.25 μm film thickness) with GC conditions of: split 1/50, injector 220 °C, detector FID: 220 °C, carrier gas: N_2 to 1 mL/min, temp: $T_1 = 50$ °C (5 min), $\Delta T = 5$ °C/min, $T_2 = 150$ °C (5 min). The yields of the reactions were determined by GC using the normalized peak areas without a correction factor. The GC–MS (70 eV) analyses were performed using the same conditions as those used in the GC analysis and the same capillary column.

2.3. Biocatalysts

Healthy and intact plants were collected in the Punilla Valley (Province of Córdoba, Argentina) and identified by a botanist. To carry out this study, plants were selected whose roots were similar in form and texture to that of carrot. The aerial parts were discarded, and the roots were washed with distilled water to remove traces of soil.

2.4. Bioreductions

The reactions were conducted immediately after acquisition of the plant to assure the integrity of the enzymatic system. A typical reaction was conducted as follows: fresh plant roots were washed with distilled water and maintained in a 5% sodium hypochlorite aqueous solution for 20 min. Then, they were washed with sterile deionized water and the external layer was removed, with the remaining roots being cut into small thin slices (1 cm) with a sterile cutter. Both the treated and cut plant roots (10 g) were added to a sterile Erlenmeyer flask (250 mL) with sterile deionized water (80 mL), and the corresponding aldehyde (50 mg) was added to this suspension and the reaction carried out by stirring on an orbital shaker at room temperature with the Erlenmeyer flask closed. The reaction's progress was monitored every 24 h for 7 days, and the samples (5 mL, saturated with sodium chloride) were extracted by shaking with ethyl acetate (2 mL). The organic layer was collected, sodium sulfate was added to remove the dissolved water, and the organic solution was filtered and analyzed (1 μL) by GC.

2.5. Scaling study

This study was carried out using treated and cut roots (10 g), sterile deionized water (80 mL) and an orbital shaker at room temperature. In this system, the concentration of the substrate and the reaction time were modified to optimize the conditions, with the evolution of the reactions being periodically monitored by GC-FID analysis. The crude reaction mixture described in Table 3 (entry 6) was filtered and the aqueous solution was extracted with ethyl acetate (3× 20 mL). Then, the combined organic layer was dried over calcium sulfate, and the solution was preconcentrated on a rotary evaporator. The crude solution was filtered on a short column with silica gel (70–230 mesh) using ethyl acetate as eluent, and benzyl alcohol was isolated (192 mg, 96% yield). The presence of benzoic acid in the reactions (Table 3; entries 9 and 10) was determined by GC, using a standard sample of benzoic acid and through GC–MS analysis by comparing the obtained spectra with library data.

2.6. Spectroscopic and GC data

2.6.1. Benzyl alcohol

GC Rt: 13.5 min (benzaldehyde GC Rt: 10.7 min), MS *m*/*z*: 109 (M^+ +1, 5%), 108 (M^+, 60%), 107 (41%), 91 (13%), 79 (100%), 78 (13%), 77 (62%), 65 (10%), 63 (10%), 53 (14%), 52 (14%), 51 (50%), 50 (27%). ^{1}H NMR δ (ppm): 2.30 (s, 1H), 4.61 (s, 2H), 7.20–7.40 (m, 5H).

2.6.2. Benzoic acid

GC Rt: 19.6 min, MS *m*/*z*: 277 (M^+ +1, 9%), 276 (M^+, 93%), 245 (100%), 217 (14%), 90 (24%), 89 (17%), 63 (8.5%).

2.6.3. 4-Chlorobenzyl alcohol

GC Rt: 18.9 min (4-chlorobenzaldehyde GC Rt: 14.9 min), MS *m*/*z*: 143 (M^+ +1, 17%), 142 (M^+, 84%), 125 (24%), 113 (24%), 107 (52%), 89 (11%), 79 (53%), 77 (100%), 51 (25%). ^{1}H NMR δ (ppm): 2.30 (s, 1H), 4.61 (s, 2H), 7.05–7.50 (m, 4H).

2.6.4. 4-Methoxybenzyl alcohol

GC conditions: $T_1 = 50$ °C (2 min), $\Delta T = 5$ °C/min, $T_2 = 200$ °C (2 min), GC Rt: 17.5 min (4-methoxybenzaldehyde GC Rt: 16.3 min), MS *m*/*z*: 139 (M^+ +1, 10%), 138 (M^+, 100%), 137 (67%), 121 (56%), 109 (71%), 107 (27%), 105 (22%), 94 (17%), 77 (36%), 65 (15%), 63 (16%), 51 (18%), 39 (16%). ^{1}H NMR δ (ppm): 2.30–2.42 (s, 1H), 3.77 (s. 3H), 4.52 (s, 2H), 6.87 (d, 2H), 7.21 (d, 2H).

2.6.5. 4-(N,N-Dimethylamino)benzyl alcohol

GC retention time: 19.6 min (4-(*N*,*N*-dimethylamino)benzaldehyde GC Rt: 21.6 min), MS *m*/*z*: 152 (M^+ +1, 5%), 151 (M^+, 49%), 135 (100%), 134 (67%), 120 (34%), 119 (40%), 118 (42%), 105 (11%), 91 (45%), 89 (19%), 77 (22%), 65 (18%),