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Enantioselective Reduction by Crude Plant Parts: Reduction of Benzofuran-2-yl Methyl Ketone with Carrot (*Daucus carota*) Bits

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For several years the ACS as well as other scientific societies have promoted the incorporation of green chemistry aspects in the chemistry curricula. These efforts resulted in several chemistry articles, published in this *Journal* and presented at scientific meetings *(1–6).* In Latin America green chemistry techniques are playing a major role in development owing to the important concerns about environmental degradation caused by traditional chemistry procedures.

Biotransformations are a worthy alternative when trying to substitute a conventional chemical process by a greener method in organic chemistry *(7).* They are carried out in environmentally benign conditions, are economically favorable, and are a very reliable source of chirality when the generation of chiral centers is desired.

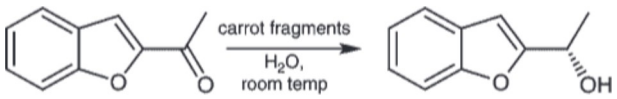
One of the oldest biotransformations reported by organic chemists is the reduction of ketones and ketoesters to the corresponding alcohols by baker's yeast *(8, 9).* For years this reaction has attracted attention because of the simplicity of operation and the often high yields and enantiomeric excesses (ee) obtained in the products. Many other microorganisms aside from baker's yeast are capable of performing the same reduction, but their use requires particular expertise and lab facilities that are not always available to the traditional chemist. Later, it was reported that not only yeast but also other eukaryotes can perform the reaction. In that sense, there are reports of reductions carried out by cultured cells of several plants such as camellia *(10),* gardenia, carrot, and tobacco *(11),* among others *(12).* Unfortunately, the work with cultured cells is frequently more complicated than growing prokaryote microorganisms (bacteria), thus the use of cultured plant cells is limited to a few organic chemistry laboratories around the world. Therefore, these kind of techniques are not suitable to be carried out in an introductory course of organic chemistry and, aside from the well known baker's yeast reduction of ketones *(13–16),* there are few examples of biotransformation experiments designed for laboratory courses *(17, 18).*

Since 1999, several groups from India *(19–21),* Italy *(22, 23),* Poland *(24, 25),* and Brazil *(26)* have reported the reduction of prochiral ketones by fragmented parts of different plants including carrot, onion, cucumber, eggplant, and so forth. These findings provided an environmentally friendly procedure, carried out in water without the addition of cosolvents even for slightly soluble substrates. Prochiral compounds are reduced in diverse degrees of enantioselectivity ranging from 70% to 100% ee. In general the results obtained are comparable or often better than the corresponding reductions by baker's yeast. Although several plants have been tested, *Daucus carota* seems to systematically yield the best results. We have not found any study on the biochemical pathway of the reaction, but the exogenous substrate seems to be reduced by enzymes excreted to the extracellular medium *(12).* Although nonenzymatic reducing pathways are possible in vegetal tissues, the involvement of enzymes is presumed, since high enantiomeric excesses are obtained and because the reaction seems to be extremely sensitive to temperature changes.

Our goals in designing this experiment for an organic chemistry class are:

- To teach the advantages of biotransformations applied to organic synthesis.
- To introduce biotransformations as a green alternative to conventional chemical procedures.
- To illustrate some green chemistry concepts, such as solvent substitution and alternatives to hazardous procedures.
- To stimulate the use of inexpensive and nonhazardous reagents.
- To show the stereoselectivity of enzymatic pathways.
- To teach separation techniques such as extraction and chromatography.

In carrying out the reduction, peeled carrots were added to a suspension of the liquid or solid substrate in water. The use of a food processor or blender does not improve the yield of the reaction; conversely it caused problems during the separation stage. Among the ketones tested in our teaching lab were acetophenone, *p*-nitroacetophenone, indanone, and benzofuran-2-yl methyl ketone. The reaction mixture was allowed to stir at 20 °C for times ranging from 3 to 48 hours and the solid plant pieces were separated mechanically. The plant-free suspension was extracted with ethyl acetate and the product isolated from the organic solvent. The reaction did work, although some of the ketones tested were reduced only after



Scheme I. Reduction of benzofuran-2-yl-methyl ketone.

several hours of reaction. One particular substrate, benzofuran-2-yl-methyl ketone, was reduced rapidly enough for a laboratory experiment (Scheme I).

Procedure

One small carrot was washed with 100 mL of distilled water. Utilizing an ordinary kitchen peeler or grater, the carrot was carefully peeled in fine slices. Nearly 24 g of carrot fragments were added to an Erlenmeyer flask containing 75 mL of distilled water. Finally, 20 mg of benzofuran-2-yl-methyl ketone were added to the Erlenmeyer flask, and the reaction was magnetically stirred very slowly at room temperature (below 28 °C). The Erlenmeyer must not be in contact with the stirrer plate, since increased temperature is detrimental to the reaction progress. The reaction was monitored by TLC (R_f = 0.2 (alcohol); R_f = 0.4 (ketone); hexanes/ethyl acetate, 85/15) using 254 nm UV light or anisaldehyde reagent (a solution of anisaldehyde and sulfuric acid in ethanol) as the detection agent. Samples (1-mL) were taken from the reaction mixture, and micro-extractions with ethyl acetate (1-mL) inside a test tube were performed to monitor the progress by TLC. This procedure was performed at 10, 30, 60, 100, and 120 min. After 10 min a weak spot corresponding to the alcohol was observed and at 120 min the reduction was nearly complete. The carrot bits were separated utilizing a common strainer. They were washed with 25 mL of water and separated again. The aqueous solution was extracted four times with 25 mL of ethyl acetate; the organic layers were combined, dried over anhydrous $MgSO_4$, and concentrated at reduced pressure to an orange oil. About 20 mg of extract was purified by column chromatography. The separation was performed in a Pasteur pipet half filled with silica gel and eluted with a mixture of 15 mL of hexanes and 2.5 mL of ethyl acetate to provide approximately 8 mg of optically pure 1-benzofuran-2-yl-ethanol: $[\alpha]_D^{20}$ -16° (c = 1.0, $CHCl_3$), Lit. value: $[\alpha]_D^{27}$ -16.6° (c = 1.0, $CHCl_3$); yield = 39%.

Hazards

Precaution should be observed when handling the compounds used in this experiment: benzofuran-2-yl-methyl ketone, 1-benzofuran-2-yl-ethanol, sodium borohydride, ethyl acetate, hexanes, ethanol, chloroform, and deuterated chloroform. Sodium borohydride can produce hydrogen, which is a flammable gas. Chloroform and deuterated chloroform are halogenated organic compounds and should be disposed in a separate and properly labeled container. The visualization of TLC plates using anisaldehyde and the preparation of the optical rotation and NMR samples must be performed in a fume hood to avoid the irritant or hazardous vapors. Although the extraction with ethyl acetate is better performed in a fume hood, it can be carried out in a well-ventilated laboratory facility. Proper care of our environment dictates that we should minimize waste in our teaching labs; therefore, we recommend that all chromatographic eluents and extraction solvents be distilled and reused.

Conclusions

This experiment can be carried out by students who have already been trained in organic chemistry laboratory manipulations, to teach green chemistry, enantioselective reductions, and spectroscopic analyses. This experiment can also be used to teach TLC and column chromatography. The technique is suitable for a teaching laboratory that can be carried out in a regular laboratory session (3 hours and 30 minutes). This period is very useful to practice TLC monitoring of the reaction progress. Any extra time can be used to discuss the spectral data of the product and the stereochemistry concepts involved in the procedure (stereospecificity, optical rotation, enantiomeric excess, etc.).

In addition, it is possible to run a parallel reduction with $NaBH_4$ in order to compare stereoselectivity and environmental impact of the enzymatic process related to the conventional one. The comparison of the enzymatic process versus a nonchiral alternative is valuable for the students to appreciate the scope and limitations of both techniques. Moreover, the latter experiment provides an opportunity to critically discuss the chromatographic method chosen for reaction monitoring.

In summary, the experiment furnishes a good chance to demonstrate the power and environmental friendliness of biotransformations. It renders pure material and provides opportunities for further modifications and development such as trying different enzymatic sources (plants) and substrates (ketones) as reported in the provided literature.

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Supplemental Material

Instructions for the students and notes for the instructor are available in this issue of *JCE Online*.

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