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Coconut water (*Cocos nucifera* L.)—A new biocatalyst system for organic synthesis

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ABSTRACT

A series of aliphatic and aromatic aldehydes and ketones was reduced using plant cell preparations from coconut juice, *Cocos nucifera*, also called ACC (água-de-coco do Ceará). The reduced products were typically obtained in excellent yields (%) and with very high enantiomeric excess. Esters, amides, and nitrobenzene, yielded acids, amines and an azoxyderivative with satisfactory results.

1. Introduction

Recently, plants have been considered as a potential source for catalytic biotransformation, and it is well established that crude preparations of certain common vegetables can serve as selective, high-yield reducing agents in organic synthesis [1,2]. Several samples of whole plants were reported as sources of reductase activity with alcohol dehydrogenase systems such as *Daucus carota* [3,4], *Manihot* species [5], *Saccharum officinarum* [6], and *Passiflora edulis* [7]. Previous work from this laboratory demonstrated the effectiveness of manihot (cassava) to serve as a powerful, enantioselective reagent for the reduction of a diverse array of aldehydes and ketones [5,8]. An investigation was therefore initiated to explore a range of roots, tubers, seeds, and fruits from the Brazilian northeast used for nutritional and medicinal purposes for their potential as biocatalytic reagents. In these continuing investigations to find sources of reductase from Brazilian plants to be used for biocatalysis, coconut juice from *Cocos nucifera* L.(Arecaceae) was investigated (Fig. 1).

Coconut juice is a pleasant and refreshing beverage derived from the fruit of *C. nucifera*. It is also called "coconut water", and is widely consumed as a nourishing soft drink in tropical countries. The juice is flavorful, sweet, slightly acid, and rich in phosphorus and potassium [9,10]. It also contains proteins, fats, minerals, and is very rich in carbohydrates [11]. This work reports the use of coconut juice as a biocatalyst for reduction and hydrolytic reaction processes.

2. Experimental

2.1. General

The aldehydes and ketones were purchased from Aldrich Chemical Co., Milwaukee, WI, USA. The pure starting materials and the reactions products were analyzed by GC–MS on a Hewlett-Packard (Model 5971) using a (5%-phenyl)-methylpolysiloxane DB-1 capillary column (30 m × 0.25 mm) with film thickness 0.1 μm; carrier gas helium, flow rate 1 mL/min with split mode. The injector temperature and detector temperature were 250 °C and 200 °C, respectively. The column temperature was programmed at 4 °C/min from 35 °C to 180 °C, and then at 10 °C/min from 180 °C to 250 °C. The enantiomeric excess was calculated using a Thermo Electron GC–FID model Trace GC Ultra instrument equipped with Varian Capillary chiral column Chirasil-Dex CB (β-cyclodextrin, 25m × 0.25 mm × 0.25 μm, #CP7502); carrier gas helium, flow rate 1 mL/min with split mode. The injector temperature and detector temperature were 220 °C. The column temperature was programmed at 2 °C/min from 130 °C to 160 °C. Column chromatog-

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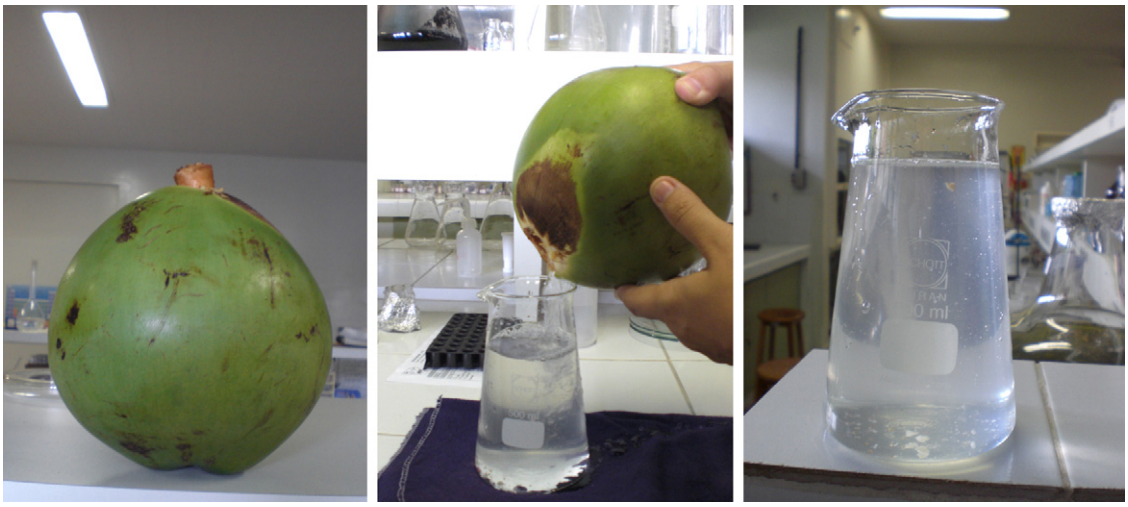


Fig. 1. Photography of fruit and coconut juice of *C. nucifera* ("coco"fruit).

raphy was run using silica gel 60 (70–230 mesh, Vetec). The optical rotations were measured on a PerkinElmer 341 digital polarimeter using chloroform as solvent. NMR spectra were recorded on a Bruker Avance DRX-500 (500 MHz) using $CDCl_3$ as solvent.

2.2. Plant material

The species *C. nucifera* was identified by botanist Prof. Edson P. Nunes. Voucher specimen (no. 35458) has been deposited at the Herbarium Prisco Bezerra of the Departamento de Biologia, Universidade Federal do Ceará, Fortaleza, Brazil.

2.3. General bioreduction of compounds *1*–*16*, dimerization of *18*, and hydrolysis of compounds *17* and *19*–*22*

2.3.1. Extraction and isolation of *1′*–*22′*

The coconut juice, identified as ACC (água-de-coco do Ceará) was obtained by perforating the fruit with a metallic sharp object (knife). The coconut juice was filtered using filter paper: WHATMAN, 47 mm for the elimination of residues.

In separate experiments, substrates **1**–**22** (200 mg) were added to the freshly prepared coconut juice of *C. nucifera* (300 mL). The mixtures were shaken (160 rpm) at room temperature for 72 h, and the reaction process was monitored by TLC. Each individual suspension was filtered, and the residue was washed with water. The aqueous solutions were then extracted with EtOAc (3 × 100 mL), and the organic phases were dried with Na_2SO_4 and evaporated under reduced pressure. The residues were filtered on a short silica gel column, using EtOAc:hexane (2:8) as eluent, to afford the reduced products, for *C. nucifera*, yielding: **1′** (133 mg), **2′** (120 mg), **3′** (171 mg), **4′** (101 mg), **5′** (99 mg), 6′**a** (87 mg), **6′b** (41 mg), **7′a** (107 mg), **7′b** (21 mg), **8′** (64 mg), **9′** (95 mg), **10′** (142 mg), **11′** (134 mg), **12′** (59 mg), **13′** (117 mg), **14′a** (58 mg), **14′b** (32.40 mg), **15′** (103 mg), **16′** (61 mg), **17′** (80 mg), **18′** (127 mg), **19′** (100 mg), **20′** (81 mg), and **22′** (152 mg). The residue of substrate **21** was submitted to column chromatography on Sephadex LH-20 using MeOH as eluent to give 15 fractions after TLC analysis, yielding **21′a** (79 mg) and **21′b** (43 mg).

2.4. Spectroscopic data

The products were analyzed by GC–MS and ^{1}H NMR spectral data in comparison with literature values, and were fully characterized according to their spectral data [12–14]. ^{1}H NMR spectral data of the isolated products **14′b** and **18′** are presented below, and are in agreement with literature data [15].

2.4.1. Cyclohexanone-2,3-butylene ketal *14′b*

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.06 (d; 6H, J = 5.52 Hz), 1.18 (m; 4H), 1.36 (m; 5H) 4.78 (q; 2H, J = 6.45 Hz), MS (EI$^+$, *m/z*): 170 (7%), 141 (11%), 127 (60%), 55 (100%), and 41 (25%).

2.4.2. Diazene diphenyl-1-oxide *18′*

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.32 (m; 2H), 7.53 (m; 2H), 7.58 (m; 1H), 8.18 (m; 2H), 7.50 (m; 2H), 7.41 (m; 1H), MS (EI$^+$, *m/z*): 198 (15%), 77 (100%), and 51 (32%).

2.5. Acylation of compound *11* and determination of enantiomeric excess

The enantiomeric excess of **11** was determined through the corresponding acylated derivative **11′acyl**. Commercially available racemate **11′** and the reaction product obtained by the reduction of **11** with ACC were separately acylated with Ac_2O/pyridine at room temperature using a literature procedure [16]. Both acylated products were analyzed by Chiral GC and the *ee* of the bioreduction products were determined as 95%. GC conditions: 40 °C (10 min), 2 °C/min, 160 °C (10 min); *tR*(*R*) 6.945 min, *tR*(*S*) 8.797 min.

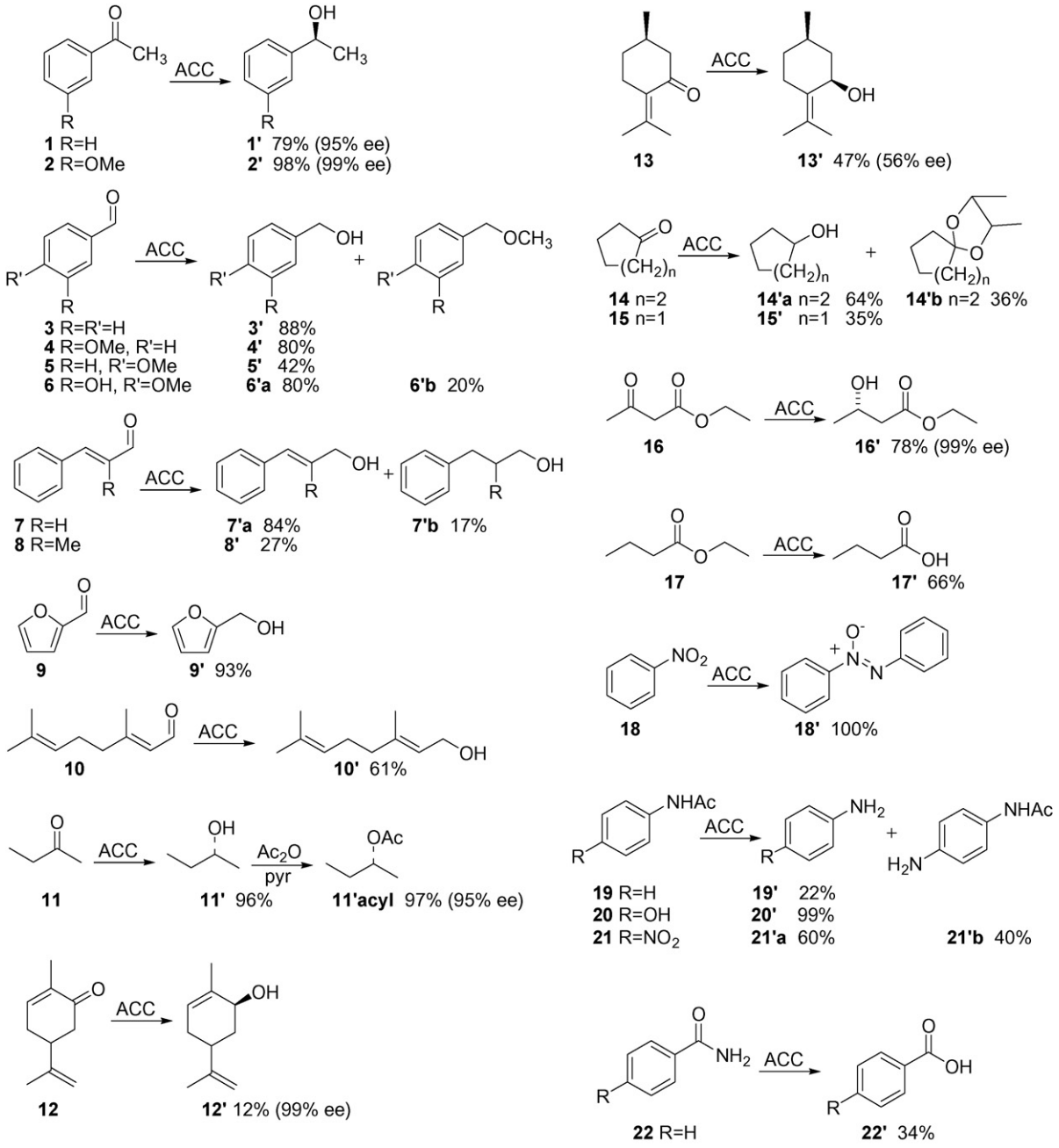
2.6. Determination of enantiomeric excess of compounds *1′*, *2′*, *12′*, *13′*, and *16′*

Commercially available racemic **1′**, **2′**, **12′**, **13′**, and **16′**, and the reaction products obtained by the reduction of compounds **1**, **2**, **12**, **13**, and **16** with coconut juice were separately analyzed by GC–FID and the enantiomeric excesses were determined as 95%, 99%, 99%, 56%, and 99%, respectively.

3. Results and discussion

The initial experiment was to determine the total protein in the enzymatic systems [17,18] using the Hartree method [19]. For coconut juice, the total protein was determined as 1.2%, in accordance with literature values of about 1% [20].

A series of simple aromatic and aliphatic: aldehydes, ketones, esters and amides (**1**–**22**, Scheme 1) was treated with the fresh coconut juice, referred to as ACC (água-de-coco do Ceará), obtained from the fruits of *C. nucifera* [21]. The process of the reaction was



Scheme 1. Reaction of coconut juice (ACC) with aromatic and aliphatic carbonyl compounds (aldehydes, ketones, and esters), amides, and nitrobenzene.

monitored by TLC and ^{1}H NMR spectroscopic analysis and all conversions were based on NMR and GC–MS measurements.

The aldehydes **3**–**5** yielded the corresponding primary alcohols: benzylic alcohol **3′** (88%), 3-methoxybenzyl alcohol **4′** (80%), and 4-methoxybenzyl alcohol **5′** (42%) in good yields. Vanillin **6** was modified by the crude enzyme system yielding two compounds: **6′a**/**6′b**, identified as vanillic alcohol **6′a**, and an unexpected vanillic ether **6′b** in the 8:2 ratio, respectively.

A regioselective reaction was observed with the aldehyde **7** and **8**, where reduction occurred selectively at the carbonyl group, yielded only the corresponding alcohol **8′** (27%), without modification of the double bond [22]. It is possible that the presence of the alkyl group attached to the double bond at the alpha position of carbonyl modulated the yield and products. For the aldehyde **7** the expected alcohol and the tetrahydro product with reduction of the carbonyl and the double bond, namely **7′b**, were obtained in the ratio 84:17, for **7′a** and **7′b**, respectively, in agreement with the literature [23].

Enantioselectivity, induced by *C. nucifera*, was observed in the reduction of the pro-chiral ketones **1**/**2**, where the alcohols 1-phenyl ethanol **1′** (79%) and 3-methoxy-1-phenyl ethanol **2′** (98%) were formed as the unique enantiomer with the "*S*" configuration, in accordance with Prelog's rule, and with the *ee* ranging from 95% to 99%, respectively.

Reduction of the indicated aldehydes and ketones with fresh coconut juice yielded the respective alcohols in excellent yield and with results superior to those reported for *D. carota* [3], *Zygosaccharomyces rouxii* [24], and manihot species [5].

The enzymatic reaction was then extended to other types of carbonyl-containing compounds including: aliphatic and alicyclic

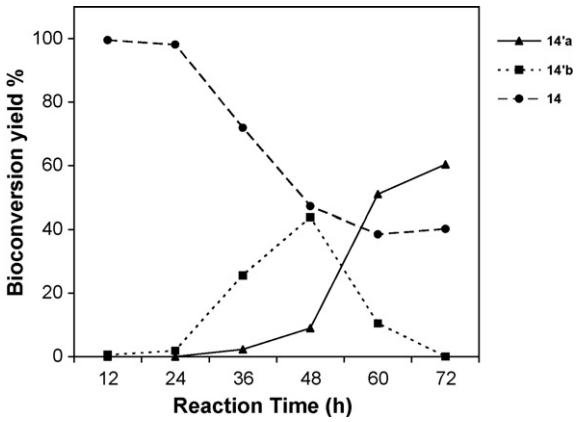


Fig. 2. Bioconversion of cyclohexanone (**14**) to cyclohexanol **14′a**, and a ketal (cyclohexanone-2,3-butylene ketal **14′b**) using coconut juice (ACC) at room temperature.

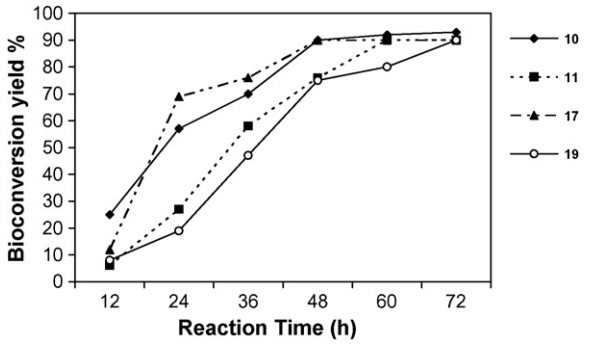


Fig. 3. Bioconversion of citral **10**, butan-2-one **11**, ethyl butyrate **17** and acetanilide **19** using coconut juice (ACC) at room temperature.

ketones, and simple carboxylic acid derivatives, including esters, an amide, and a nitro compound. An excellent yield was obtained with **11**, an aliphatic ketone to afford **11′**. The reduction was chemoselective to give the corresponding butan-2-ol (96%), and the *ee* was determined as 95% by chiral GC analysis of its acetylated derivative. The reduction of the α,β-unsaturated ketone **12** was chemo- and stereoselective in low yield (12%); however, with high *ee* (99%) and an "*S*" configuration. For compound **13**, the reduction to the alcohol **13′** was also regio- and enantioselective; however, it was only moderately effective, with 47% yield, 56% *ee*, and an "*R*" configuration. The compound **14** produced a mixture of two products, the expected alcohol **14′a** (64%), and an unexpected ketal **14′b** (36%). The formation of **14′b** can be attributed to the presence of butane-2,3-diol, identified by CG/MS in the chemical composition of coconut juice (ACC) [25]. These results were examined through a kinetic study, Fig. 2, where maximum conversion of **14′a** was observed after 72 h, whereas for **14′b** the maximum yield was obtained after 48 h, with a decrease to zero at 72 h.

The next step was to observe the enzymatic system with other carbonyl and functional groups. The selected compounds were an aliphatic β-keto-ester **16**, ethyl butyrate **17**, nitrobenzene **18**, acetanilide **19**, 4-hydroxyacetanilide **20**, and benzamide **22**. The products obtained for the esters were derived from hydrolysis corresponding to carboxylic acids, rather than reduction to the alcohols.

Complete chemo- and enantio-stereoselectivity was observed with β-keto-ethyl-butyrate **16**, through the exclusive reduction of the keto group at C-3 yielding 3*S*-(+)-hydroxy-ethyl-butyrate **16′** (78%) with an *ee* value of 99%.

4-Nitroacetanilide **21** yielded two products, **21′a/21′b**, identified as nitroaniline and 4-aminoacetanilide, respectively in the ratio 60:40. The enzymatic ester hydrolysis of **17** by coconut juice (ACC) is quite effective for an aliphatic ester yielding butyric acid **17′** (66%). However, hydrolysis proceeds in much lower yield with benzamide producing benzoic acid **22′** in only 34% yield, and no benzylamine.

The other tested compound was nitrobenzene **18**, which yielded the unexpected dimeric azoxy derivative **18′**. This product has not been reported previously from any biocatalytic process, and was identified through spectroscopic data 1D and 2D NMR, and EIMS.

Maximum bioconversion yield was observed after 3 days for the selected compounds: aldehyde **10**, ketone **11**, ester **17**, and the amide, acetanilide **19**. As expected, aldehydes were more reactive than ketones. In addition, the hydrolysis reaction of ethyl butyrate 17 showed a faster reaction rate when compared with the bioreduction rates of ketones and aldehydes. Aliquots were analyzed by GC–MS with reaction times varied from 12 h, 24 h, 36 h, 48 h, 60 h to 72 h. Experiments were performed in duplicate and the results are presented in Fig. 3.

4. Conclusion

The juice of the coconut species *C. nucifera* was very effective in selectively reducing a range of aromatic and aliphatic carbonyl groups showing substantial regio- and enantioselectivity in the products. Following this preliminary work, studies are aimed at expanding the kinetic experiments to other substrates, the purification, stabilization, and cloning and expression of the reductase(s) from the coconut species, as well as exploring other plant systems for their potential for regio- and stereoselective synthesis in organic chemistry. The encouraging results obtained here using the crude aqueous preparation of a common fruit (the coconut) for biocatalysis may offer new possibilities for the reduction of selected carbonyl compounds as a critical step in a synthetic organic pathway, specifically avoiding the use of non-sustainable, hydride reducing agents. As a result of these and previous studies with common vegetables, it is apparent that an opportunity for developing a new area of synthetic organic chemistry has been established. Utilizing the vast potential of abundant and sustainable natural sources, work in biocatalysis, through its selectivity, simplicity, employment of benign processing technology, and ultra-low cost, may become a significant alternative approach for large-scale synthetic chemical transformations.

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