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Asymmetric Bioreductionof Ketones with a Pumpkin (*cucurbita*)

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Abstract

In this study, a general, efficient, and simple methodology for biocatalytic reduction of carbonyl compounds such as acetophenone 1a and 4-chloroacetophenone 2a using freshly cut ripen fruit of fresh pumpkin in the aqueous medium at room temperature was reported. The prochiral ketones can be reduced to chiral secondary alcohols in a generalized way. The obtained results indicated that the pumpkin fruits could be used as biochemical catalysts to contribute to the preparation of many pharmaceutical alcohols. This biochemical catalyst attracted much attention because of the low cost, high efficiency, and special selectivity for its environmental friendliness and its contribution to certain recommended green chemistry principles.

In this research, our aim was to contribute to this area by using biochemical catalysts with plant sources such as the pumpkin fruits in different states (fresh, juice). The prochiral ketones: acetophenone and 4-chloroacetophenone were chosen as typical ketones and the yield and optical purity were 77-88% and 50-96%, respectively. Mild reaction condition, simple operation, and easy availability of fresh pumpkin fruit revealed this protocol as an attractive and alternative eco-friendly option for a general reduction of all types of carbonyl compounds.

Keywords:pumpkin, cucurbita, biocatalyst, asymmetric reduction, chiral alcohols.

Introduction

Various types of biocatalysts have been used for beneficialbiotransformations. The biocatalytic transformations using plants can be applied in bioreduction of ketones (Machado et al.,2006; Rodríguez et al., 2007), enzymatic lactonization (Olejniczaket al., 2003), hydrolysis of esters (Maczkaet al., 2002), addition of hydrogen cyanide (Hamandezet al., 2004), and oxidation and hydroxylation reactions (Sakamakiet al., 2005).

In recent years, plant-based enzymes have generated a great deal of interest in their vast biotechnological potential (Bruniet al., 2002; Villa et al., 1998; Giriet al., 2001; Matsuo et al., 2008; Nakamura,et al., 2003). More recently, many of the plants and vegetables were used as biochemical catalysts in organic preparation instead of chemicals such as *Cynarascolymus L, Terfeziasp, Phoenix dactylifera L., and* figs (*Ficuscarica*) fruit *(Mespilusgermanica L)* (Khaled and Sekhri et al., 2019; Nedjimi, Sekhri et al., RJPBCS 2016; Nedjimi, Sekhri et al., Biomedical 2016; Bennamane et al., 2014; Bentayeb, et al.; 2018; Karthikeyan, et., al; 2018).

Many reports on the evaluation of bioreduction of prochiral ketones by using plants are available (Phukan& Devi; 2012; Matsuo et al., 2008; Sekhri et al., 2009; Yang et al., 2008; Andrade et al., 2006; Cordell et al., 2007).

Our aim in this research was to contribute to this area choosing the pumpkin. The merits of using vegetable pumpkin as biocatalysts are as follows:

(i)-Vegetable pumpkin are obtained all over the world, (ii) The pumpkin itself is a vegetable with a high yield, easy to grow, and therefore cheap (iii) it can be stored for a long time without damage due to the thickness of the wall of fruit, (iii) low cost, (iv) high versatility and efficiency (v) highly desirable chemical aspects such as chemical fusion, what made the biochemical reactions very attractive for the industrial sector. However few studies have referred to the promotion of asymmetric reduction of the pumpkin. This has led us to focus more on the study of the pumpkin as a biocatalyst.Acetophenone and 4-chloroacetophenone were chosenas typical ketones.

Pumpkin is known in the Arab world as Elyaktine and locally as Kaboya or Karaa and its scientific name is*Cucurbita*. Pumpkin has been mentioned once in the Holy Qur'an. It is the wisdom of germination of the pumpkin on the Prophet Yunus (peace be upon him) that the pumpkin plant grows fast, blooms, and gibes fruits, and the fruit contains the necessary nutrients for the body, and its fruits are eaten when they are green soft, or mature and can be stored for a long time without damage due to the thickness of the

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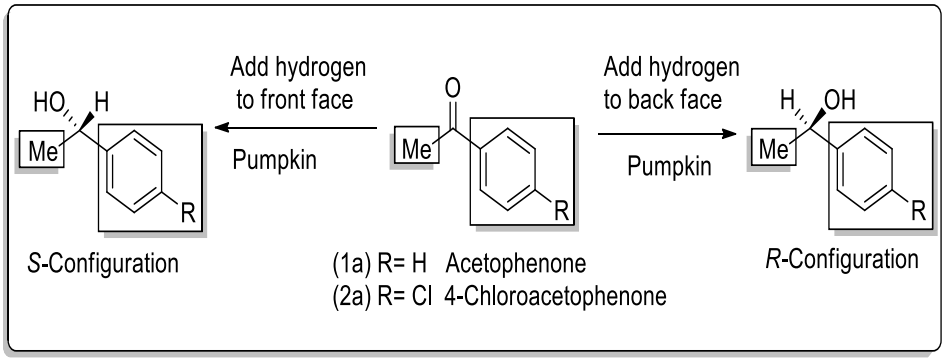
wall of fruit. The genus *cucurbita* includes five domesticated varieties from which three: *cucurbitapepo* L., *cucurbita maxima* D., and *cucurbita moo schata***(Figure 1)** are globally important both economically andnutrients useful for human (Whitaker and Davis, 1962; Qusti, et al., 2018).

Figure 1: Fresh pumpkins obtained from Lamniaa region, W. Ghardaia, Algeria

The pumpkin is considered to meet all the conditions for a healthy diet (Gajewski et al., 2008). This species is rich in phenols, flavonoids, vitamins (including β–carotene, vitamin A α-tocopherol, and vitamin C), amino acids and carbohydrates (Zhang, et al., 2000, 2002). This species has medicinal properties (Malik, et al., 2010, which has been useful in traditional Chinese medicine (Zhang and Sheng, 2003).

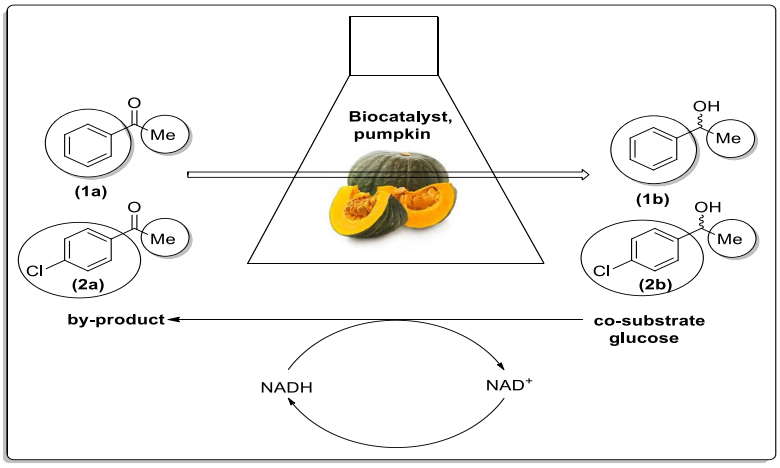
Results and Discussion

Asymmetric transformation always involves the conversion of 2D substrate into a 3D product. For prochiral ketones reduction such as acetophenone shown in **Scheme 1**, addition to the back face gives alcohol with *R* configuration, while adding to the back face gives alcohol with *S* configuration. The problem, of course, is that most common reducing agents, such as sodium borohydride or lithium aluminum hydride, react equally readily with either face. The most obvious solution to this problem is to use a hydride source, which itself is enantiomerically pure in principal such as a reagent that transfers the hydride to each face of the ketone through diastereoisomerically distinct transition state, which gives at least a fighting chance of an energy difference, and preferences for addition to one face over the other.



Scheme 1: Asymmetric conversion of two-dimensional substrate into a three-dimensional product.

Besides, plants are potential biocatalysts, which can be used as an alternative solution to this problem, since they are easily manipulated and easily obtainable from markets (Ahmad, et al., 2018). Asymmetric reduction reactions of acetophenone**1a** and 4-chloroacetophenone **2a**, using the pumpkin were investigated **(Scheme-2).**



Scheme 2: Asymmetric reduction reactions of acetophenone/4-chloroacetophenone using pumpkin

Experimental

General methods

Acetophenone**1a** and4-chloroacetophenone **2a** were bought from Aldrich and then, they were used without any purification. Thin-layer chromatography (TLC) was conducted using pre-coated plates (Aluminum foil, silica gel 60 F254 Merck, 0.25 mm). Merck 60 silica gel (230-400 mesh) was used for flash chromatography. Optical rotations were determined on EuromexPolarimeter PM. 5400 (Mitscherlich type polarimeter).

All 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR spectra were run on a Bruker AC 300 NMR spectrometer. Both ^{1}H NMR and ^{13}C NMR spectra were recorded using $CDCl_3$ as internal standard; Infrared spectra were recorded using a Perkin-Elmer 783 spectrometer equipped with a PE 600 data station.

Biocatalysts

Fresh pumpkin was obtained from Lamniaa region, WilayaGhardaia, Algeria and washed with water, then disinfected with ethanol. It was carefully cut into small thin pieces (approximately 1 cm long slice). The suspension of pumpkin (20 g) in water (80 ml) was stirred in an Erlenmeyer flask at 30 °C for 30 min.

Standard Procedure

The typical reaction mixture of 0.02 mol ketone, 3% (W/V) of glucose or i-PrOH (in the case of solid ketones), and 20 ml of phosphate buffer (pH=6.5) was added to 20 g of cultured plants, fresh pumpkin suspension in 80 mL deionized water. The mixture was stirredin orbital incubator shaker (150 rpm) at 30°C for 2 days. The progress of the reaction was monitored by TLC. Then the plant pieces were removed by filtration, washed with deionized water and the filtrate was extracted with petroleum ether (3×100ml). The petroleum ether fraction was dried over anhydrous ($MgSO_4$) and the solvent was evaporated to get the final product. Then enantioselectivity and chemical yield were determined. Each experiment was parallelly repeated at least 3 times. Then the average value and standard deviations were given.

The products were identified by comparing their data with those of authentic samples on TLC, by IR, ^{1}HNMR, and ^{13}CNMR spectra (Sekhri; 1998; Drew, et al., 1997). The presence of the alcoholic group in the final product was chemically confirmed by acetyl chloride test.

Determination of the optical activity of chiral products:

Optical properties of the products obtained from the prochiral were evaluated by using polarimeterEuromexPolarimeter PM. 5400 (Mitscherlich type polarimeter) using the method described in our paper reported recently (Nedjimi, Sekhri, et al.,2016).

Identification of chiral alcohols 1b by optical properties and spectroscopic data

Phenylethanol 1b:

Using Fresh figs: *(R)*-**(1b)** was obtained in (78% yield), $[\alpha]_D^{20}$ = +38 (*c* 5, MeOH); enantiomeric excess (ee=76%). The absolute configuration was estimated by analogy with {Lit., (Aldrich,1995/1996)$[\alpha]_D^{20}$ =+84 (*c* 5, MeOH) for *R*-isomer}.

Using dried figs:

(R)-**(1b)** was obtained in (85% yield), $[\alpha]_D^{20}$ = +36 (*c* 5, MeOH); enantiomeric excess (ee=80%). The absolute configuration was estimated by analogy with {Lit., (Aldrich,1995/1996)$[\alpha]_D^{20}$ =+45 (*c* 5, MeOH) for *R*-isomer}.

The IR, ^{1}H, and ^{13}C NMR spectra of **(1b)** were identical to those of authentic samples [(Sekhri et al., 1998); (Drew, et al., 1997)].

^{1}H ($CDCl_3$; 300 MHz): δ (ppm) 1.48 (3H, d, CH_3), 4.80 (1H, q,-CHOH) 3.99 (1H, br.s, OH), 7.25-7.36 (5H, m, Ar-H) **(Figure 2)**; ^{13}C ($CDCl_3$; 75 MHz): δ (ppm) 22.81(CH_3), 69.9 (-CHOH), 127.1 (-CH, Ar), 127.6 (-CH, Ar), 128.9 (-CH, Ar), 146.1 (C, Ar) **(Figure 3a** and **Figure 3b)**; νmax (KBr Disk, cm^{-1}): 3340-3060 (OH) **(Figure 4)**.

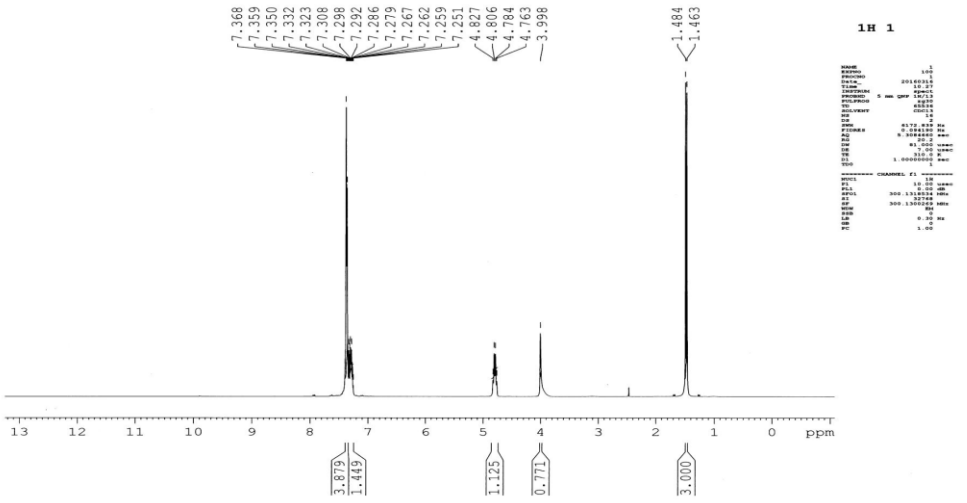


Figure 2: ^{1}H NMR spectrum of Phenylethanol **1b**

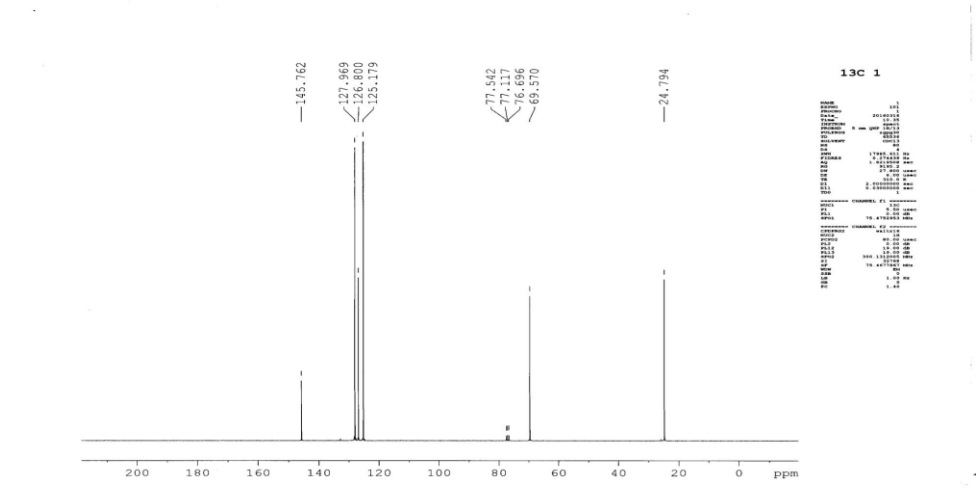


Figure 3a: ^{13}C NMR spectrum of Phenylethanol **1b**

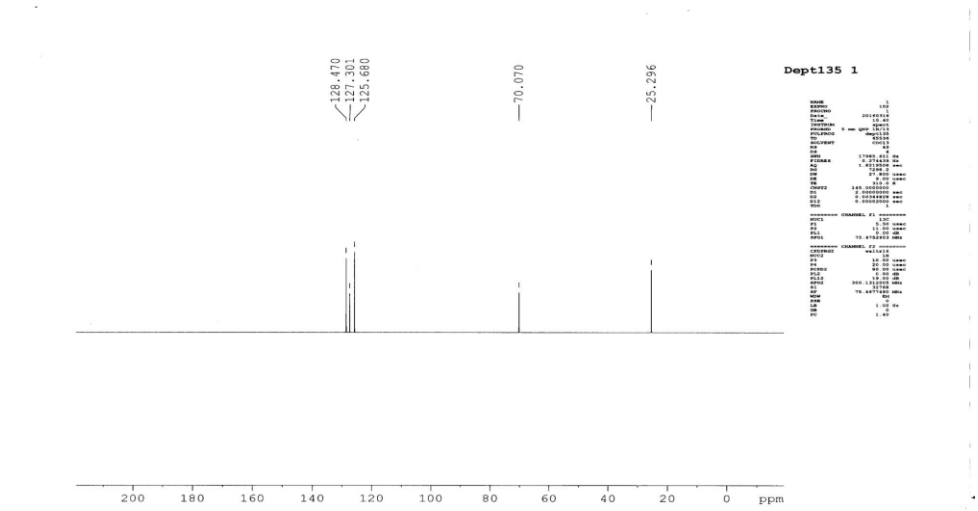


Figure 3b: ^{13}C NMR (DEPT-135) spectrum of Phenylethanol **1b**

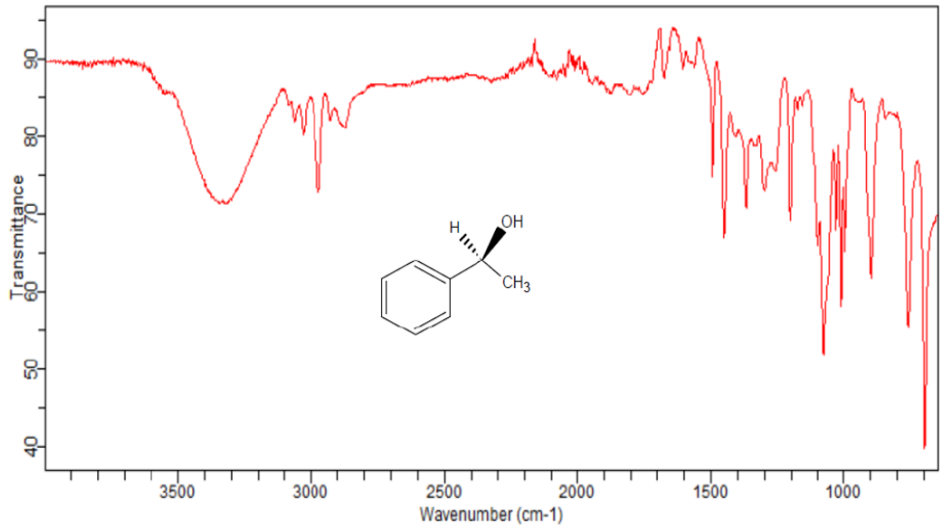


Figure 4: IR spectrum of Phenylethanol **1b**

4'-Chlorophenylethanol 2b:

(R)-**(2b)** was obtained in (70% yield), $[\alpha]_D^{20}$ =+22,8 (*c* 0,7 EtOH). The absolute configuration was estimated by analogy with {Lit., [33] $[\alpha]_D^{20}$ =+37 (*c* 0,7, EtOH) for *R*-isomer}.

The IR, and ^{1}H and ^{13}C NMR spectra of **(2b)** were identical to those of authentic samples [30, 31].

^{1}H (ppm) (300 MHz; $CDCl_3$): δ (ppm) 1.3 (3H, d, CH_3), 3.5 (1H, br.s, OH), 4.7 (1H, q, -CHOH), 7.0-7.3 (4H, m, Ar-H);^{13}C ($CDCl_3$; 100,62 MHz): δ (ppm) 28.08 (CH_3), 69.54 (-CHOH), 126.93 (-CH, Ar), 128.25 (-CH, Ar), 132.94 (C, Ar), 144.44 (C, Ar); νmax (KBr Disk, Cm^{-1}) : 3060-3340 (OH).

Conclusion

The bioreduction of acetophenone mixed with fresh pumpkincan be effectively reduced to the corresponding chiral alcohols by the applied plant tissue because the aromatic ketones such as acetophenone**1b** and 4-chloroacetophenone **2b** are more acceptable to plant cells. Moreover, only *R*- form configuration could be obtained through these asymmetric reduction reactions. This provides a new approach for the production of chiral alcohols, as the platform chemicals for enantiomerically pure pharmaceuticals, through asymmetric reduction of the corresponding prochiral ketones.

Among various co-substrates, glucose found to be the best for the regeneration of co-factors and the fresh pumpkinwas chosen as the biocatalysts. The acetophenone derivatives could be reduced to the corresponding chiral alcohols with attractive enantioselectivity by fresh fruits of this plant.

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