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Biocatalytic Preparative Methods of Asymmetric Alcohols using garlic (*Allium sativum*)

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Abstract

In this study,a mild, environmentally friendly method was reported for reduction of carbonyl compounds such as 4'-bromoacetophenone **1a** and 4'-iodoacetophenone **2a**by the biocatalyticcut ripe fresh garlic***(****Allium sativum)***at room temperature in an aqueous medium, to the corresponding chiral alcohols **1b** and **2b**.The results showed that garlic can 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. The prochiral ketones: 4'-bromoacetophenone **1a** and 4'-iodoacetophenone **2a**mixed with the biocatalyticcut of ripe fresh garlic***(****Allium sativum)***were chosen as typical ketones and the yield and optical purity were (65-75%) and (86-92%), respectively. Mild reaction condition, simple operation, and easy availability of freshvegetable revealed this protocol as an attractive and alternative eco-friendly option for a general reduction of all types of carbonyl compounds.

Keywords: Garlic, *Allium sativum*, biocatalyst, asymmetric reduction, chiral alcohols.

Introduction

The biochemical potential of plant cells to produce special secondary metabolites including agrochemicals, pigments, flavors, and drugs, is of considerable interest in connection with their biotechnological utilization (Bruni et al., 2002; Munir, et al., 2018). A wide range of chemical compounds such as terpenoids, coumarins, alkaloids, steroids, and aromatics can undergo biotransformation by using organ cultures, enzymes, and plant cells. Many plants and vegetables were used as biochemical catalysts in organic preparation instead of chemicals (Machado et al., 2006; Rodriguez et al., 2007), enzymatic lactonization (Olejniczaket al., 2003), hydrolysis of esters (Maczkaet al., 2002), addition of hydrogen cyanide (Hamandezet al., 2004), and hydroxylation and oxidation reaction (Sakamakiet al., 2005). A large number of reports about the assessment of bioreduction of prochiral ketones using plants is available (Phukan et al., 2012; Matsuo et al., 2008; Sekhri et al., 2009; Cordell et al., 2007).

In recent years, plant-based enzymes have attracted a lot of attention in their vast biotechnological potential (Bruni et al.,2002; Matsuo et al., 2008; Bennamane et al., 2014). More recently, it has been reported that the use of many vegetables and plants such as *Cynarascolymus L, Terfeziasp, Phoenix dactylifera L.* (Nedjimi, Sekhri et al., RJPBCS 2016**;** Nedjimi, Sekhri et al., Biomedical 2016; Karthikeyan, et., al; 2018; Bentayeb, et al.; 2018) *and* figs (*Ficuscarica*) fruit *(Mespilusgermanica L)* (Khaled, Sekhri et al., 2019) as biochemical catalysts.

This research was conducted to contribute to this area choosing **garlic** ***(****Allium sativum).* The merits of using vegetable garlic as biocatalysts are as follows:

(i)Garlic is easy to grow and therefore cheap; (ii) it has long been a common seasoning worldwide; (iii) low cost; (iv) high versatility and efficiency (v) can be domestically stored for a long time without damage, it is stored warm above 18 °C and dry to keep it dormant (to inhibit sprouting); (vi) Peeled cloves may be stored in vinegar in the refrigerator (Harris et al., 2016). Commercially, it is stored at 0 °C, in a dry environment. Garlic will keep longer if the tops remain attached (Orono et al., 2011); (vi) highly desirable chemical aspects such as chemical fusion, what made the biochemical reactions very attractive for the industrial sector. However, few studies refer to the promotion of asymmetric reduction of garlic.

This has led us to focus more on the study of garlic as a biocatalyst and 4-bromoacetophenone **1a** and 4-iodoacetophenone **2a** were chosenas typical ketones.

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Garlic is locally known in the Arab world as Toum and their scientific name is*Allium sativum.* Garlic has been mentioned once in the Holy Quran. Garlic is an essential component in the diet of various regions including southern Europe, northern Africa, Southeast Asia, South Asia, eastern Asia, the Middle East, and parts of Latin America.The fresh and dried garlic is illustrated in [**figure 1** and **figure 2].**

Figure 1: Dried garlic obtained from Algerian market

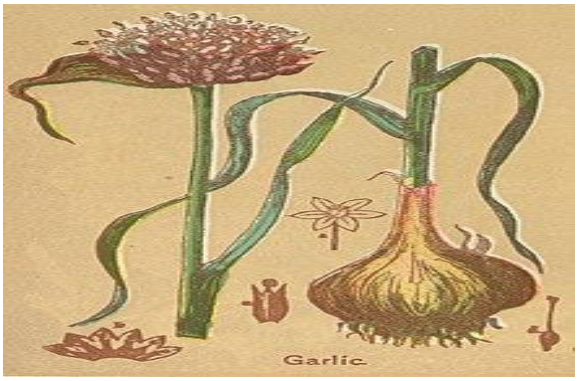


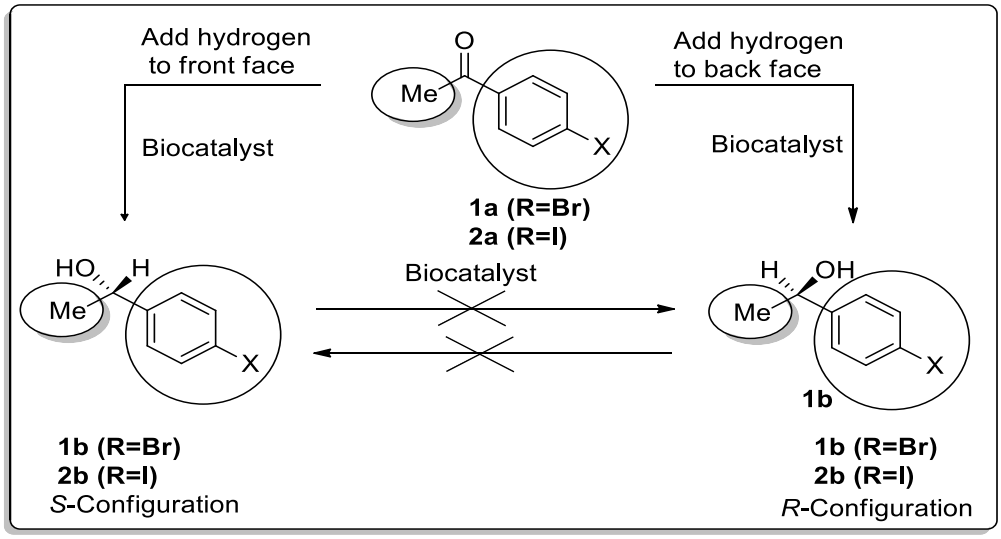
Figure 2: Fresh garlic obtained from Algerian market

Garlic meets all the conditions for treating many chronic diseases related to the heart and blood system, such as highblood pressure and highcholesterol. It isbelieved to reduce the risk of several types of cancers, such as colon cancer, stomach cancer (Borrelli et al., 2007; Kodali et al., 2014; Turati et al., 2014), rectal cancer, lung cancer, breast cancer, prostate cancer, and bladder cancer. Treatschronic fatigue syndrome, and reduces enhanced cholesterol level scaused by HIV medicines. The meta-analysis of observational epidemiological investigations showed that the consumption of garlic lowers the risk of stomach cancer (Woo et al., 2014; Saloua et al., 2018).

This species is rich in volatile oils, which are responsible for most of itsmedicinalproperties. Moreover, itcontains 17 aminoacids; glycosides; arginine; many types of mineralsaltslikeselenium; and a group of enzymes includingmyrosinase, allinase, peroxidase, etc.

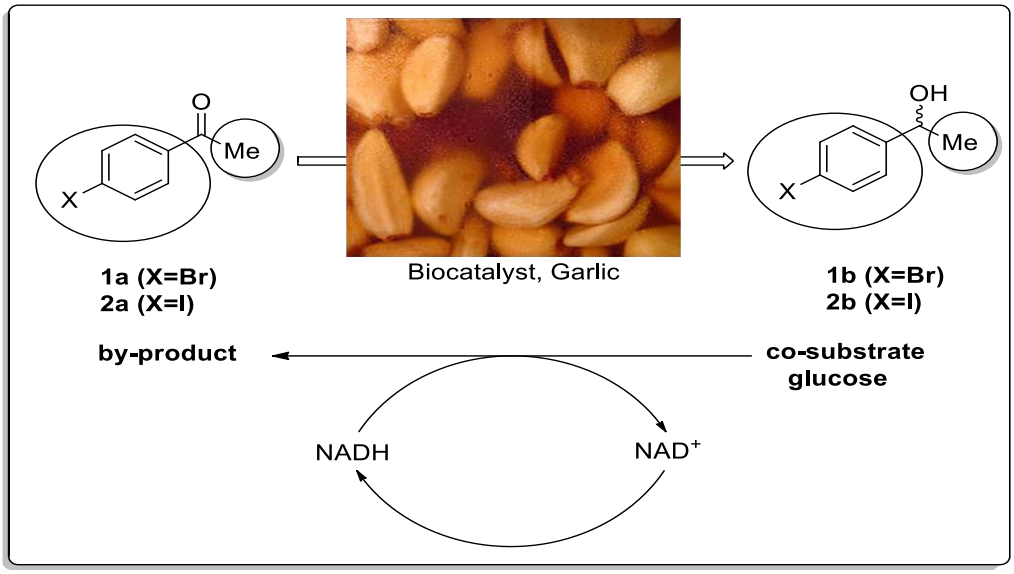
Results and Discussion

Asymmetric transformation involves the conversion of 2D substrate into a 3D product. In the reduction of prochiral ketones such as 4-bromoacetophenone **1a** and 4-iodoacetophenone **2a,** 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, $NaBH_4$, or lithium aluminum hydride ($LiAlH_4$) equally and readily react with either face. The most obvious solution to this problem is to use a hydride source, which itself is enantiomerically pure in principle and as a reagent, it will transfer the hydride to each face of the ketone through diastereoisomerically distinct transition state, that gives at least a fighting chance of an energy difference, and preference 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, because they are available and can easily be manipulated (Ahmad, et al., 2018). Asymmetric reduction reactions of 4-bromoacetophenone **1a** and 4-iodoacetophenone **2a**, using garlic were investigated (**Scheme-2).**



Scheme 2: Asymmetric reduction reactions of 4-Bromooacetophenone **1a** and 4-iodoacetophenone **2a** using garlic.

Experimental

General methods

4-Bromoacetophenone **1a** and 4-iodoacetophenone **2a** were prepared from Aldrich and then, they were used without any purification. Thin-layer chromatography (TLC) was run 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. The Optical rotations were determined on EuromexPolarimeter PM. 5400 (Mitscherlich type polarimeter).

All 75 MHz ^{13}C NMR and 300 MHz ^{1}H 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; IR spectra were recorded using a Perkin-Elmer 783 spectrometer equipped with a PE 600 data station.

Biocatalysts

Fresh garlic was obtained from Algerian market, WilayaOuargla, 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 garlic (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 ketone), and 20 ml of phosphate buffer (pH = 6.5) was added to 20 g of the cultured plants, fresh garlic suspension in 80 mL deionized water. The mixture was shaken in 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 (3x100ml). The petroleum ether fraction was dried over anhydrous ($MgSO_4$). The meta-analysis and the solvent were evaporated to get the final product. Then, enantioselectivity and chemical yield were determined. All the experiments were parallelly repeated at least 3 times. Then the average value and standard deviations were given.

The product was identified by comparing its spectroscopic data with those of an authentic sample on TLC by ^{13}CNMR I, ^{1}HNMR, and IRspectra (Sekhri et al., 1998; Drew, Sekhri, et al., 1987). 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 assessed by using polarimeter Euromex Polarimeter PM. 5400 (Mitscherlich type polarimeter) using the method described in our paper reported recently (Nedjimi, et al., 2016).

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

4'-Bromophenylethanol 1b:

(R)-**(1b)** was obtained in (65% yield), $[\alpha]_D^{20}$ =+35 (*c* 1 $CHCl_3$). The absolute configuration was estimated by analogy with {Lit., (Aldrich Catalogue, 1995/1996) $[\alpha]_D^{20}$ =+39 (*c* 1 $CHCl_3$) for *R*-isomer; ee= 90%, R=95%}. The IR, ^{1}H, and ^{13}C NMR spectra of **(1b)** were identical to those of an authentic samples (Sekhri, 1998; Drew et al., 1997).

^{1}H ($CDCl_3$; 300 MHz): δ (ppm): 1.5 (3H, d, -CH_3), 4.7 (1H, q, -CHOH), 5.2 (1H, br.s, OH), 7.3-7.9 (4H, m, Ar-H) **(Figure 3)**; ^{13}C ($CDCl_3$; 75 MHz): δ (ppm): 28.03 (CH CHOH), 169.54 (-CHOH),) 126.93 (-CH, Ar), 128.25 (-CH, Ar), 132.94 (C, Ar), 144.44 (C, Ar) (**Figure 4** and **Figure 5**); νmax (KBr Disk, cm^{-1})3050-3330 (OH).

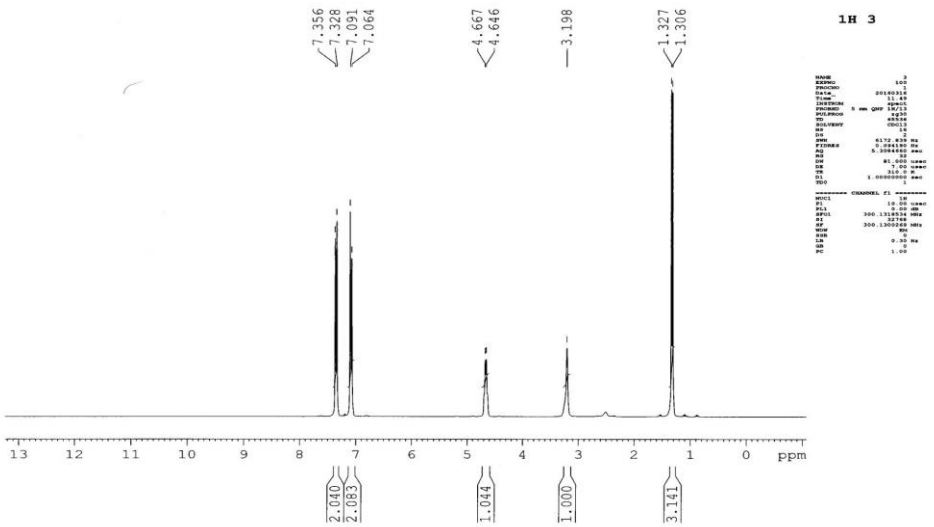


Figure 3: [1]H NMR spectrum of 4'-Bromophenylethanol **1b**

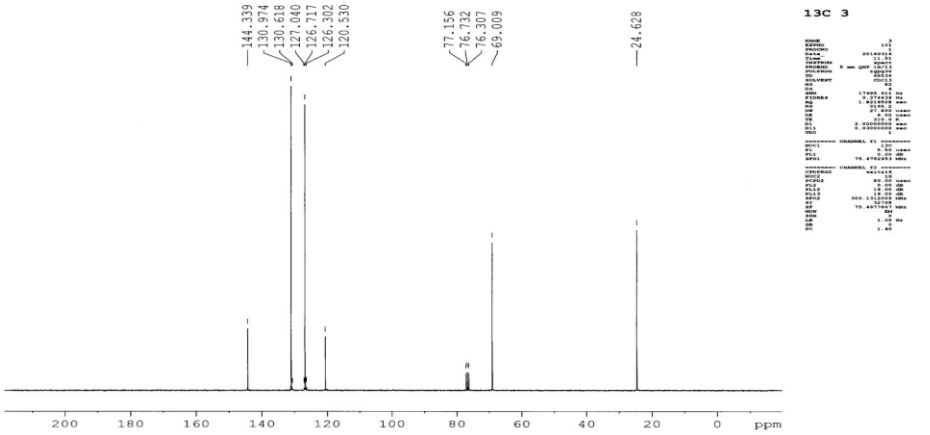


Figure 4: [13]C NMR spectrum of 4'-Bromophenylethanol **1b**

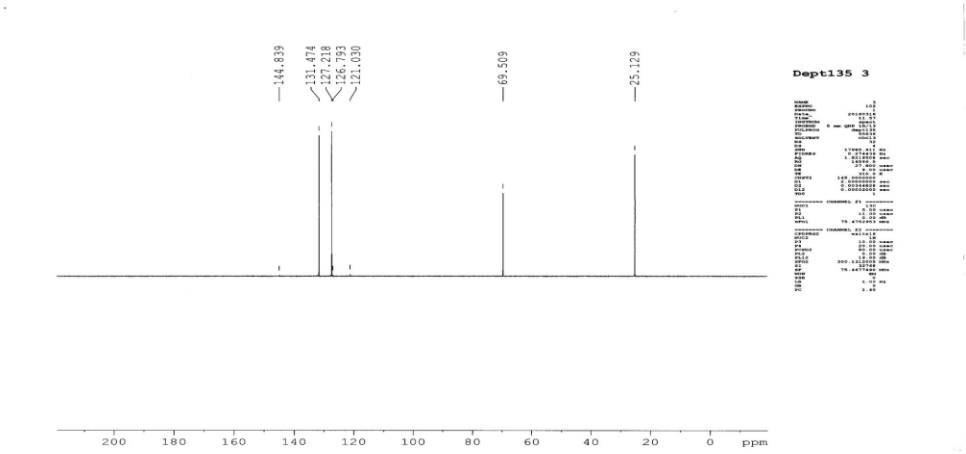


Figure 5: [13]C NMR (DEPT 135) spectrum of 4'-Bromophenylethanol **1b**

4'-iodophenylethanol 2b:

4'-iodophenylethanol **2b** was prepared from the reduction of 4-iododacetophenone **2a** in a similar way to that of previous 4'-Bromophenylethanol **1b** as oil.

(R)-**(2b)** was obtained in (70% yield), $[\alpha]_D^{20}$ =+43° (*c* 1 $CHCl_3$). The absolute configuration was estimated by analogy with {Lit., (Aldrich Catalogue, 1995/1996) $[\alpha]_D^{20}$ =+50° (*c* 1 $CHCl_3$) for *R*-isomer; ee= 86%, R=93%}. The IR, ^1H, and ^{13}C NMR spectra of **(2b)** were identical to those of an authentic sample (Sekhri, 1998; Drew et al., 1997).

^1H ($CDCl_3$; 300 MHz): δ (ppm): 1.3 (3H, d, CH_3), 2.0 (1H, br.s, OH), 4.7 (1H, q, -CHOH), 6.5-7.0 (2H, d, Ar-H), 7.8 (2H, d, Ar-H); νmax (KBr Disk, cm^{-1}): 3330-3040 (OH).

Conclusion

By using4'-bromoacetophenone **1a** and 4'-iodoacetophenone **2a**mixed withbiocatalyticfreshgarlic, an environmentally benign, efficient, simple, economically, and enantioselective method was reported for the reaction of acetoph enonederivatives to the corresponding alcohols. It is because the aromatic ketones like acetophenone**1a** and **2a** are more acceptable for plant cells. Moreover, only the *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 prochiral ketones.

Among various co-substrates, glucose found to be the best for the regeneration of co-factors and fresh garlicwas chosen as the biocatalyst.

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