UNIVERSIDADE FEDERAL DE SÃO CARLOS
CENTRO DE CIÊNCIAS EXATAS E DE TECNOLOGIA
DEPARTAMENTO DE QUÍMICA
PROGRAMA DE PÓS-GRADUAÇÃO EM QUÍMICA

"SYNTHESIS AND BIOTRANSFORMATION OF DIARYLPENTADIENONES"

ZACCHEAUS OLUWATAYO ALABI*

Dissertation presented as part of the requirements to obtain the title of MASTER IN CHEMISTRY, concentration area: ORGANIC CHEMISTRY.

Adviser: Prof. Dr. Edson Rodrigues Filho

***CAPES Scholar**

São Carlos – SP
2019

UNIVERSIDADE FEDERAL DE SÃO CARLOS

Centro de Ciências Exatas e de Tecnologia
Programa de Pós-Graduação em Química

Folha de Aprovação

Assinaturas dos membros da comissão examinadora que avaliou e aprovou a Defesa de Dissertação de Mestrado do candidato Zaccheaus Oluwatayo Alabi, realizada em 20/12/2019:

Prof. Dr. Edson Rodrigues Filho
UFSCar

Prof. Dr. Felipe Christoff Wouters
UFSCar

Profa. Dra. Taicia Pacheco Fill
UNICAMP

Dedication

To my parents, Mr. and Mrs. Alabi. They taught me to be strong.

To my siblings, Julius, Roseline and Samuel. They inspire me.

To the Almighty God, He gives grace and shows mercy.

Acknowledgment

My greatest praise goes to God Almighty, the giver of all life and knowledge. He brought me here and continues to steer me on.

Similarly, my thanks goes to my research advisor, Professor Edson Rodrigues-Filho of the Departamento de Quimica, Universidade Federal de Sao Carlos for his guidance, advice and patience in the course of the development of the project. He continued to steer me in the right direction and motivated me when the chips were down. Thank you very much.

I am grateful to Professor (Dr.) Felipe Christoff Wouters, Professor (Dr.) Moacir Rossi Forim and Professora (Dra.) Taicia Pacheco Fill for accepting to be part of my thesis committee and providing me with technical supports and encouragement in the course of my research.

Coming from an entirely different culture, settling in the laboratory would have been difficult if not for the immense help I received from my laboratory colleagues. I am grateful to Tatiana Salata, Diane Mirante, Alef Santos, Luciana Amaral, Rebecca Previate, Amanda Lima and several others who have contributed to my stay and work in the laboratory. I am also thankful for the companionship of Yoisel and Herika Vidal.

My stay here was more worthwhile because of the presence of my Nigerian and African friends. I am grateful to Bolaji Charles Dayo-Owoyemi, Oyebayo Oloyede, Olaoba Olamide, Amos Akinyemi, Elijah and several others. Thank you all.

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Similarly, I will forever relish the memory I have in the Department of Chemistry, Universidade Federal de Sao Carlos, Brazil. Thank you to all the members of faculty in the department and my professors in different courses, short courses, seminars and symposia for making learning worthwhile. I am grateful to both the technical, administrative and other staffs for their continuous help in the course of my research.

To conclude, my deepest appreciation goes to my parents and family, without whose love, support, prayers and encouragement I will not be here today.

Zaccheaus Oluwatayo Alabi

List of Abbreviations

^{1}H NMR	Proton Nuclear magnetic resonance
ADH	alcohol dehydrogenase
BV	Baeyer-Villiger
BVMO	Baeyer-Villiger monooxygenase
DIMCARB	Dimethyl ammonium dimethyle carbamate
DMSO	Dimethylsulfoxide
Ee	enantiomeric excess
ER	Ene/Enoate reductase
EWG	Electron-withdrawing group
FAD	flavin adenine dinucleotide
FMN	flavin mononucleotide
FMO	flavin-containing monooxygenase
G-6-PDH	glucose-6-phosphate dehydrogenase
HPLC	High performance liquid chromatography
LC-MS	Liquid Chromatography-Mass Spectrometry
Min	Minute
MO	Monooxygenase
NAD^{+}	β-nicotinamide adenine dinucleotide
NADH	reduced β-nicotinamide adenine dinucleotide

$NADP^+$	β-nicotinamide adenine dinucleotide phosphate
NADPH	Reduced β-nicotinamide adenine dinucleotide phosphate
NMR	Nuclear magnetic resonance
NTR	Nitroreductase
OYE	Old Yellow Enzyme
Pd/C	Palladium-on-carbon

List of Figures

List of Tables

List of Schemes

Abstract

SYNTHESIS AND BIOTRANSFORMATION OF DIARYLPENTADIENONES. Naturally-occurring compounds containing bis-aryl moiety are important components of fine chemicals required by pharmaceutical, agrochemical and flavor industries. These compounds have inspired the synthesis of several of their analogs such as the diarylpentadienones. These synthetic analogs possess equal or better potential compared to their lead compounds. As part of the ongoing efforts in our laboratory to enlarge the diarylpentadienones library, we synthesized some monoarylidenes and diarylpentadienones (compounds **2, 4, 5, 6**) and biotransformed them and some others obtained from the previous works in our laboratory (compounds **7, 8** and **9**) using *Penicillium brasilianum* and *Saccharomyces cerevisiae* to modify them and enlarge their library. In our finding, the resting cells of *P. brasilianum* reduced monoarylidene **2** to **10** and further oxidized it to **11** in a Baeyer-Villiger-like manner. However, no biotransformation product was detected when diarylpentadinones **4, 5, 7** and **8** were fed into the fungi. Also, baker's yeast *Saccharomyces cerevisiae* reduced **9** to **10** and **11** in a regioselective manner. Further investigation into why *P. brasilianum* was not able to reduce and/or oxidized diarylpentadienones used must be carried out.

Resumo

SÍNTESE E BIOTRANSFORMAÇÃO DE DIARYLPENTADIENONES. Os compostos de ocorrência natural contendo a porção bis-aril são componentes importantes de produtos químicos finos exigidos pelas indústrias farmacêutica, agroquímica e de aromas. Esses compostos inspiraram a síntese de vários de seus análogos, como as diarilpentadienonas. Esses análogos sintéticos possuem potencial igual ou melhor em comparação com seus compostos de chumbo. Como parte dos esforços em andamento em nosso laboratório para ampliar a biblioteca de diarilpentadienonas, sintetizamos algumas monoarilidenos e diarilpentadienonas (compostos **2**, **4**, **5**, **6**) e as biotransformamos e alguns outros obtidos nos trabalhos anteriores em nosso laboratório (compostos **7**, **8** e **9**) usando *Penicillium brasilianum* e *Saccharomyces cerevisiae* para modificá-las e ampliar sua biblioteca. Em nossa descoberta, as células em repouso de *P. brasilianum* reduziram o monoarilideno **2** para **10** e o oxidaram ainda mais para **11** de maneira semelhante a Baeyer-Villiger. No entanto, nenhum produto de biotransformação foi detectado quando as diarilpentadinonas **4**, **5**, **7** e **8** foram introduzidas nos fungos. Além disso, o fermento de padeiro *Saccharomyces cerevisiae* reduziu **9** para **10** e **11** de maneira regioseletiva. Uma investigação mais aprofundada sobre por que *P. brasilianum* não foi

capaz de reduzir e / ou diarilpentadienonas oxidadas usadas deve ser realizada.

Table of Contents

1 INTRODUCTION

Natural substances are as diverse in chemical composition as they are in biological and pharmacological activities. Literature is replete with the reports of biological activities of compounds of natural origin as well as the groups conferring those activities on them. Compounds containing benzene ring in their molecular structures are arguably one of the most extensively studied and the bisarylalkanoids have gained more prominence among these benzene-containing compounds. The "bisaryl" scaffold is important as the skeletal framework in compounds such as curcumin, chalcones and stilbenes, all of which are important in human health and diseases (DIN, n.d.). Based on the number of carbon atom in the inter-ring spacers (C_n), a series of bisaryl compounds with the molecular formula ArC_nAr' can be formed, where Ar and Ar' are the aryl groups, n is the number of carbon atom in the spacer and R_1, R_2, R_3 and R_4 are the ring substituents (FIGURE 1.1).

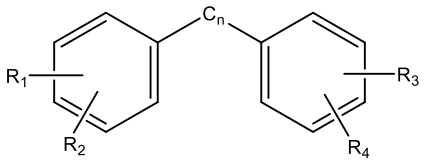
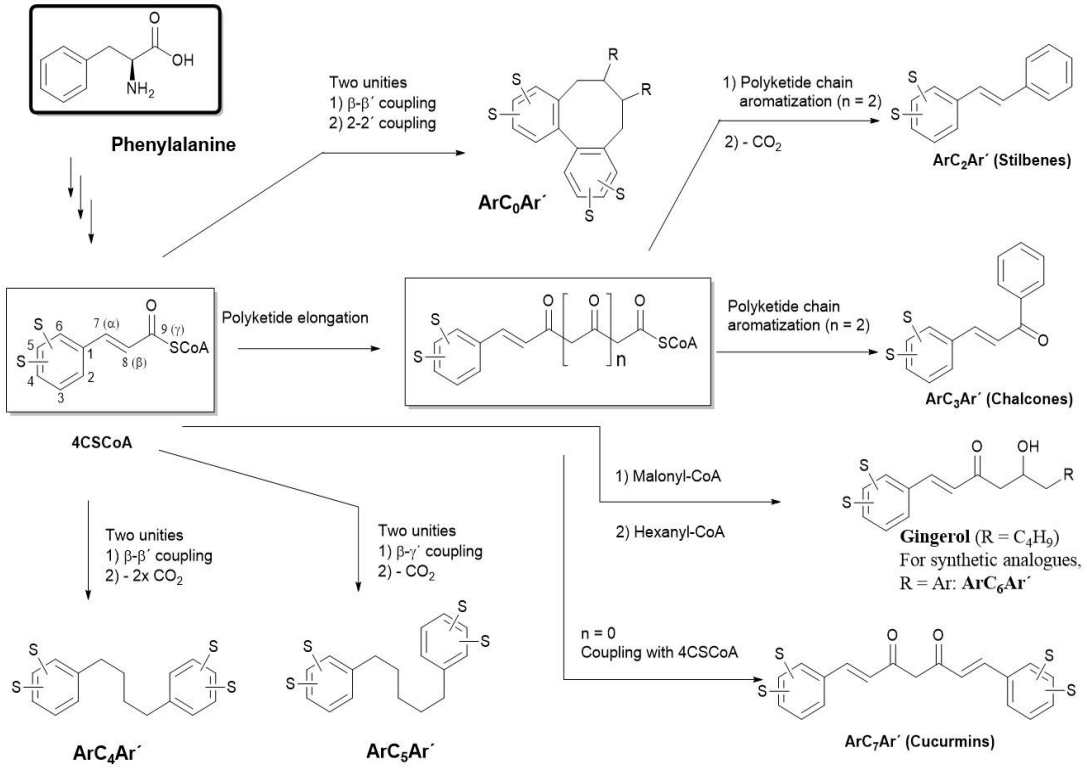


FIGURE 1.1 - The general formula for bisaryl compounds.

Similarly, the bisaryl compounds have been reported to possess similar biosynthetic origin from the amino acid phenylalanine as shown in SCHEME 1.1.



SCHEME 1.1 - Biosynthetic pathways for bisaryl compounds.

1.1 Diarylpentanoids

These are the bisarylalkanoids in which the two phenyl moieties are joined by the 5-carbon system (pentane) and having various substituents as shown in FIGURE 1.2 where R_1, R_2, R_3 and R_4 are the ring substituents (DIN, n.d.).

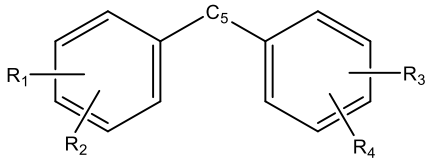


FIGURE 1.2 - The general structure of diarylpentanoids.

1.1.1 Diarylpentadienones

Diarylpentadienones are diarylpentanoids where the phenyl moieties are separated by a 5-carbon spacer to form an extended α, β-unsaturated carbonylic system. The representative member of diarylpentadienone is dibenzylideneacetone as shown in FIGURE 1.3. Diarylpentadienones derived their arrays of reported pharmacological activities majorly due to the presence of the conjugated system and the substituent groups.

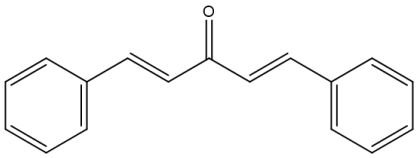
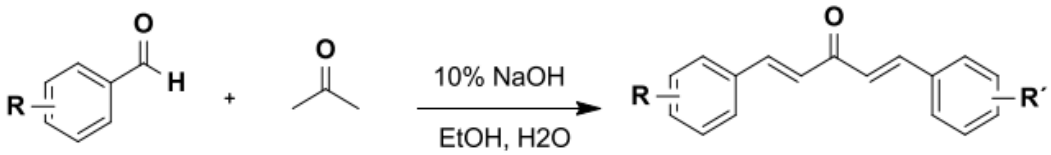


FIGURE 1.3 - Dibenzylideneacetone is a member of diarylpentadienones.

1.1.2 Synthesis of Diarylpentadienones

Literature is replete with methods of synthesis of diarylpentadienones in the laboratory (SHETTY ET AL., 2015). Diarylpentadienones are formed by

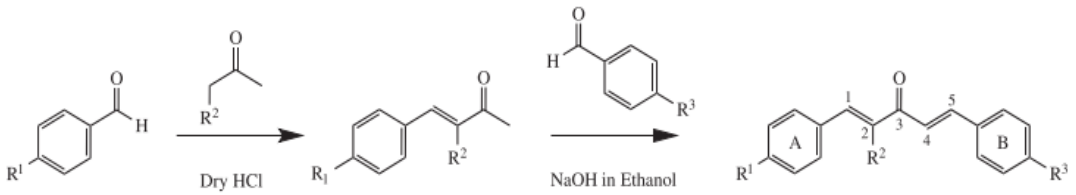
the vigorous mixing of benzaldehyde (or substituted benzaldehyde) with acetone under a basic or acidic catalytic condition in a conventional aldol condensation process. Asymmetrical diarylpentadienones are promising building blocks for arrays of compounds of interesting pharmacological activities (UD DIN ET AL., 2014). However, the direct reaction of aldehydes with ketones in a conventional acid/base-catalyzed aldol condensation yields symmetrical diarylpentadienones (SCHEME 1.2).



SCHEME 1.2 - Synthesis of diarylpentadienones.

To circumvent this challenge, chemists now synthesize the mono-2-arylidene derivatives of the ketones with a subsequent synthesis of the corresponding diarylpentadienones from the synthons (SCHEME 1.3). However, it is usually difficult to synthesize the monoarylidenes, especially those from cyclohexanone and cyclopentanone by aldol and Claisen-Schmidt condensations because the reaction usually proceed beyond mono-condensation forming the diadduct E,E-2,6-dibenzylidenecyclohexanone or

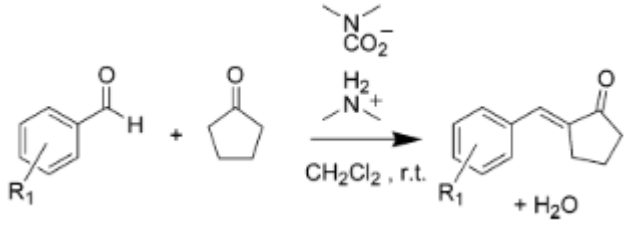
E,E-2,5-dibenzylidenecyclopentanone even when the molar ratio of starting aldehyde to ketone is substantially below 1:1 (BRAGA ET AL., 2014; RASTON & SCOTT, 2000; ZHENG ET AL., 2006). To overcome this challenges, chemists have resorted to the use of amine-based catalysts for the synthesis of monoarylidenes, though there are only view reports on this usage (KREHER ET AL., 2003; ROSAMILIA ET AL., 2007).



SCHEME 1.3 - Synthesis of asymmetrical diarylpentadienones.

Preparation of the monoarylidene adducts usually requires two steps, aldol condensation followed by a separate elimination (LIGHT & HAUSER, 1961). Kreher and co. reported the direct preparation of the monoarylidene derivatives of ketones and enolizable aldehydes in the ionic solvent dimethylammonium dimethyl carbamate (DIMCARB) as the catalyst (SCHEME 1.4). The product was produced with high selectivity in yields of about 74 to 91% (KREHER ET AL., 2003). DIMCARB, adduct of CO_2 and Me_2NH, is a relatively stable ionic liquid up to 50 °C. Owing to its ionic

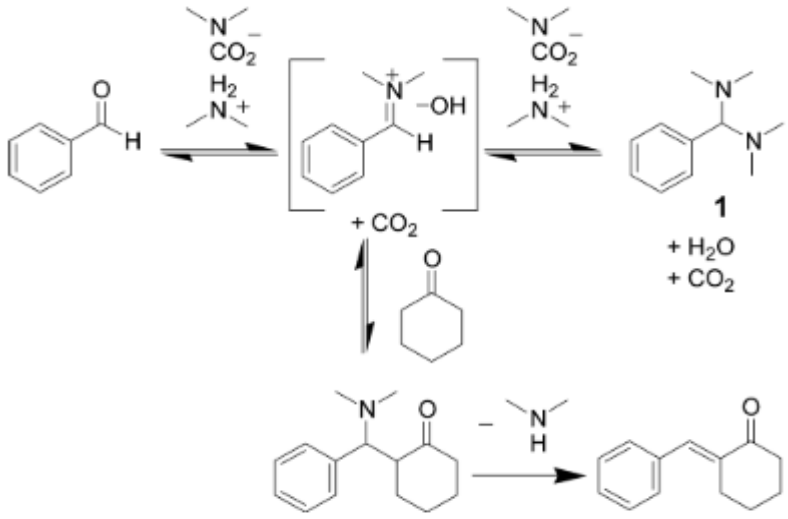
character, it can dissolve salts such as LiCl, NaCl, NaBr, KCl, and KI at levels between 2 and 5% w/v. Further, at 60 °C, DIMCARB dissociates to its constituent gasses CO_2 and Me_2NH which can be condensed, reassociated and reused (EARLE ET AL., 2004; WASSERSCHEID & KEIM, 2000).



SCHEME 1.4 - Synthesis of mono-2-arylidenes.

Kreher attempted effort to increase the yields were unsuccessful and this may be accounted for by the competing pathways in the reaction as shown in SCHEME 1.5. When 2 equivalents of dimethylamine react with 1 equivalent of the arylaldehyde, an aminal can be formed as a competiting species. However, when the dimethylamino moiety of the aminal is eliminated, the transient iminium species formed can undergo Mannich reaction with the ketone and the resulting adduct can undergo dimethylamine elimination to form the mono-2-arylidene derivative. They suggested that a vast excess of DIMCARB (up to 12 mol equiv used for experiments) may have helped to stabilize the aminal and could have accounted, at least in part, for the moderate yields of arylidene. The challenge associated with the one-

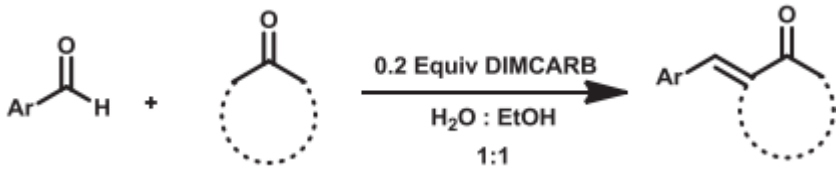
pot synthesis of monoarylidene by Kreher and co. is the use of dichloromethane (CH_2Cl_2), a chlorinated solvent as co-solvent. Apart from the implication of halogen-containing compounds as not being eco-friendly, when hydroxy- and carboxyarylaldehydes were employed in the reaction, they were usually insoluble in the co-solvent alone (KREHER ET AL., 2003).



SCHEME 1.5 - Reaction pathway of benzaldehyde and cylohexanone in DIMCARB.

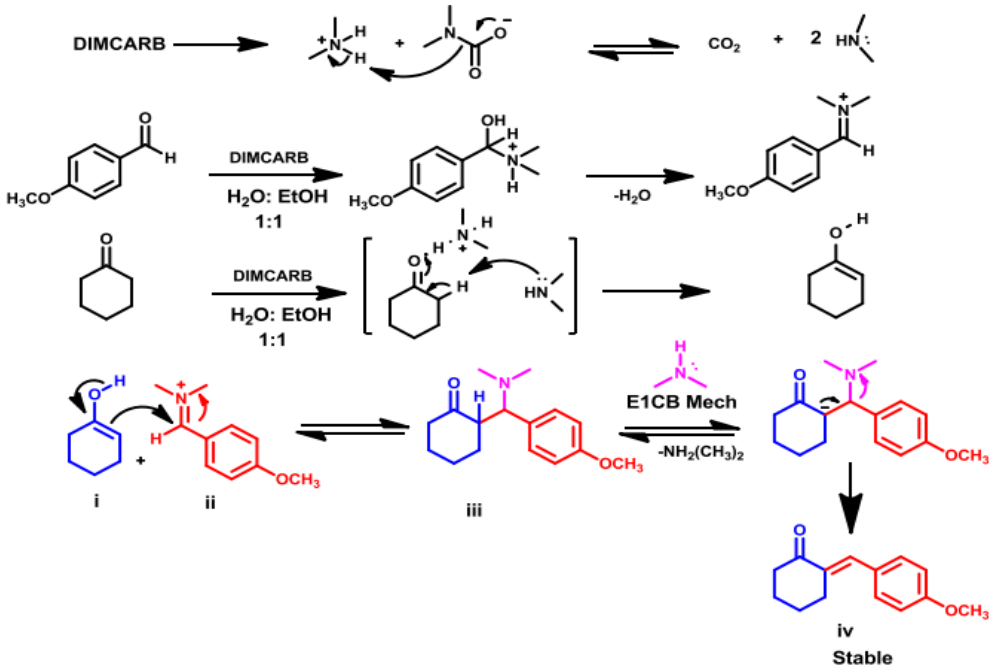
In our laboratory, efforts have been made to optimize the one-pot synthesis of monoarylidenes (and hence asymmetrical diarylpentadienones) using DIMCARB as a recyclable reaction medium and catalyst in green solvent of ethanol/water in ratio 1:1 (SCHEME 1.6). The advantages this confers on the general process are the reduction in the quantity of DIMCARB

used, the avoidance of the use of chlorinated solvent and a decrease in the formation of side products. In the attempt, Zia and Rodrigues-Filho reported that the reaction is more efficient in green solvent than in dichloromethane with HPLC analysis showing a 100 % conversion in ethanol water mixture compared to the 92 % conversion reported in dichloromethane. Further, when 1,8-diazabicycloundec-7-ene (DBU) was used instead of DIMCARB, excellent conversions were also observed (DIN & RODRIGUES-FILHO, 2016).



SCHEME 1.6 - Synthesis of monoarylidene ketones from different aldehydes and ketones.

The liquid chromatography-mass spectrometer (LC-MS) data showed that the reaction followed almost similar reaction mechanisms to the one described by Kreher *et al*., (SCHEME 1.7). The DIMCARB ionized into N,N-dimethyl amine, which reacts with the aldehyde yielding iminium species ii, which reacts with the enolic form of cyclohexanone I to yield the intermediate iii. On dehydration of iii, compound iv is formed.



SCHEME 1.7 - Mechanism of synthesis of monoarylidene by DIMCARB catalyzed reaction.

1.1.3 Biological activities of Diarylpentadienones

Owning to the presence of the two aromatic regions, the enone functionality and the substituents on the rings, which might be critical for protein-ligand binding, diarylpentadienones possess excellent structural scaffold with interesting arrays of biological activities. Literature has reported several findings where synthetic analogs of curcumin have been used as lead molecules for the design and development of therapeutic agents including anti-inflammatory, anticancer, antioxidant, antiviral, and antimicrobials (MAYDT ET AL., 2013).

1.1.3.1 Anti-inflammatory activity

Lee and co-workers synthesized a series of diarylpentanoids and evaluated them for their anti-inflammatory activities (LEE ET AL., 2009). Among the compounds tested, diarylpentanoid analogs in FIGURE 1.4 showed inhibitory action against nitric oxide (NO) production in macrophage cells (RAW 264.7) by either the inhibition of inducible nitric oxide synthase (iNOS) or its upstream regulatory protein rather than radical scavengers or antioxidant agents. They suggested that the potent anti-inflammatory property of the compounds might be conferred by the presence of the 2,5-dimethoxylated and 2-hydroxylated phenyl rings. This corroborated the findings of Weber and co-workers, who tested the anti-inflammatory potentials of analogs of curcumin in which the two aryl rings are separated by 7-carbon, 5-carbon or 3-carbon spacers, and showed that these compounds were able to inhibit tumor necrosis factor-alpha (TNF-α)-induced activation of nuclear factor-kappa-β (NF-κβ). Further, they showed that the most active analogues retain the enone functionality, although it is not essential for activity and that the analogues with the 5-carbon spacer are the most active. (WEBER ET AL., 2006). As such, bisaryl compounds with 5-carbon spacer would serve as excellent lead compounds in the rational design of anti-inflammatory agents with improved pharmacological properties.

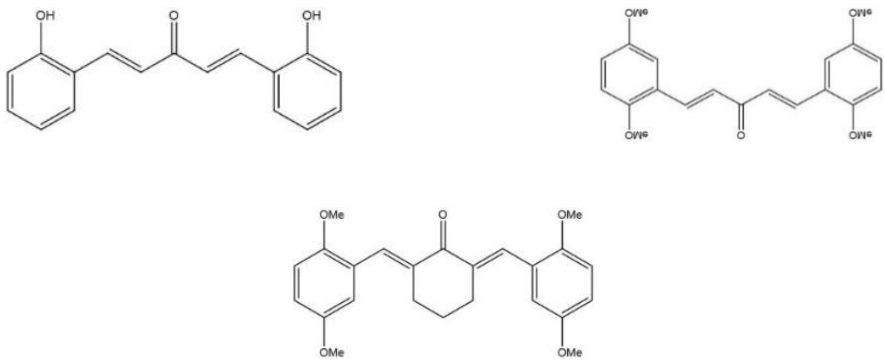


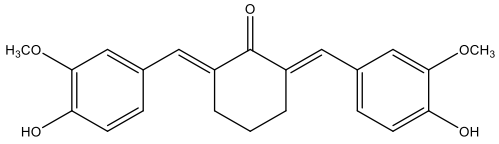
FIGURE 1.4 - Synthetic diarylpentanoids reported for their inhibitory effect against nitric oxide (NO) production in macrophage cells.

1.1.3.2 Anti-tumor activity

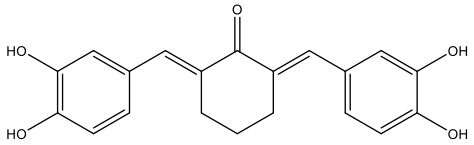
Like curcumin, structural analogs of curcumin have been reported to suppress tumor initiation and tumor progression. The anticancer activity of curcuminoids has been associated with inhibition of oncogenes expression, through the inhibition of c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase activation (ERK) pathways (CHEN & TAN, 1998; MOHAN ET AL., 2000). Similarly, curcuminoids may act as anti-inflammatory and antioxidant agents in the treatment of tumors, arthritis, and wound healing disorder, by inhibiting Fibroblast growth factor-2 (FGF-2)-induced angiogenesis (ABONIA ET AL., 2012; MOHAN ET AL., 2000). Two 2,6-bisbenzylidenecyclohexanones (FIGURE 1.5) namely 2,6-bis((3,4-dihydroxyphenyl)methylene)-cyclohexanone (BDHPC) and 2,6-bis((3-methoxy-4-hydroxyphenyl)-methylene)cyclohexanone (BMHPC) have

been synthesized and assessed for their activities as nuclear type II site and malignant cell growth antagonists (KITTRELL ET AL., 1992). The study demonstrated that both BMHPC and BDHPC bind to nuclear type II sites with similar affinities and inhibited Michigan Cancer Foundation-7 (MCF-7) human breast cancer cell proliferation in a dose-dependent manner.

Recently, Ahmed and co-workers evaluated the *in vitro* and *in vivo* cytotoxicity of 17 analogs of curcumin against Ehrlich ascites carcinoma (EAC). Results from the *in vivo* analysis show three (3) analogs of curcumin show high potency against EAC. The in vivo evaluation, however, only one compound was able to normalize hematological parameters compared with 5-fluorouracil, a well-known anticancer drug (FADDA ET AL., 2010).



BDHPC



BMHPC

FIGURE 1.5 - Structures of 2,6-bisbenzylidenecyclohexanones

1.1.3.3 Anti-Oxidant activity

Although there are mixed reports on the antioxidant activities of synthetic analogs of curcumin, some curcumin-analogs have shown improved anti-oxidant potentials when compared with the clinically-approved drugs. Li et al., synthesized and evaluated the antioxidant activities of 12 asymmetric monocarbonyl curcumin analogs with different substituent groups and degree of saturation of the aliphatic chain (LI ET AL., 2015). In an *in vitro* study using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and O^{2-} (NET) radical scavenging assays, some of these compounds were reported to show better anti-oxidant activities than ascorbic acid while others exhibited equal or more pronounced free radical scavenging activity than curcumin (FIGURE 1.6).

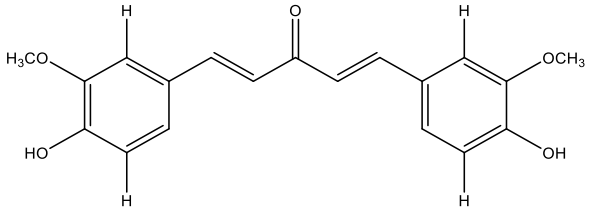
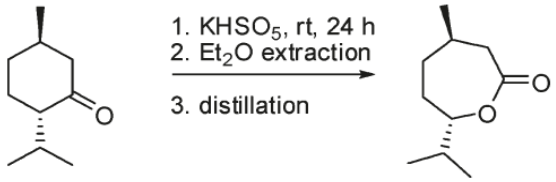


FIGURE 1.6 - This analog showed improved anti-oxidant activities.

1.2 Baeyer-Villiger oxidation of carbonyl compounds

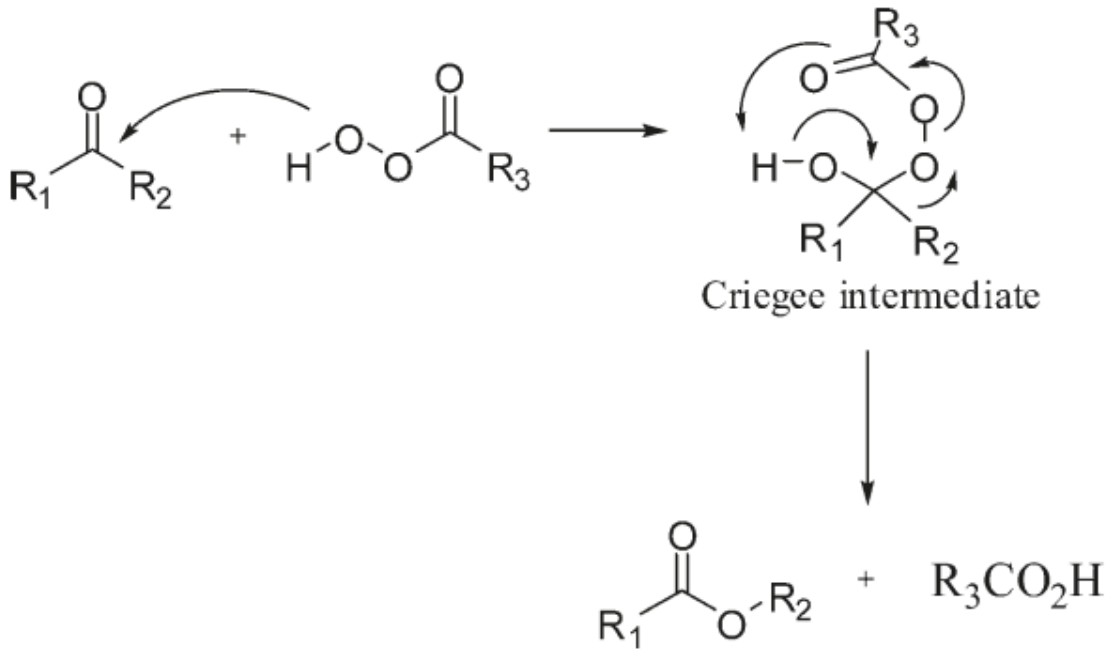
In 1899, Adolf von Baeyer and Victor Villiger reported on the use of potassium monopersulfate ($KHSO_5$) as an oxidant to convert cyclic ketones such as menthone, carvomenthone, and camphor to their lactone equivalents (BAEYER & VILLIGER, 1899). They reported a yield of up to 50% in a reaction carried out at room temperature for 24 hours (SCHEME 1.8). With time, organic chemists began to employ organic peroxy acids like 3-chloroperoxybenzoic acid and peroxotrifluoroacetic acid as stoichiometric oxidants to transform ketones into esters and cyclic ketones into lactones. This chemical conversion is known as Baeyer-Villiger (BV) oxidation. BV oxidation has been employed in the synthesis of a wide range of organic compounds, including steroids, antibiotics, and pheromones (KROW, 1993; LEISCH ET AL., 2011). Recently, there are several attempts to synthesize enantiomerically pure BVO products using chemoselective oxidants, transition metal catalysts and ionic solvents in a bid to make the process more environmentally-friendly (CORMA ET AL., 2001; MICHELIN ET AL., 2010).



SCHEME 1.8 - Baeyer-Villiger oxidation of menthone.

Over a half-century ago, Criegee studied the mechanism of the BVO (CRIEGEE, 1948). He proposed a mechanism that highlighted two key steps, the nucleophilic attack of the carbonyl carbon by the peracid to form a transient tetrahedral adduct known as Criegee intermediate, followed by a rate-limiting step involving the migration of one of the adjacent carbons to oxygen leading to the release of the carboxylate anion (SCHEME 1.9). Most time, the migrating carbon is chiral, conferring stereoselectivity on the reaction. This selectivity has made the BV oxidation most appealing to researchers. Besides, BV oxidation has a very wide range of carbonyl substrates and the regioselectivity is easily predictable. Furthermore, there are several oxidants to choose from. Despite the arrays of the aforementioned advantages, the chemical BV oxidation does not occur with high chemo-, regio-, and enantioselectivity and broad substrate specificity needed for efficient organic synthesis. Also, due to the shock-sensitivity and explosive character of peracids, large-scale reactions increase the potential risk for accidents. Furthermore, peracids are powerful oxidative agents and can

oxidized groups susceptible groups, thereby requiring protection and deprotection efforts. Lastly, the use of halogenated reagents and solvents is environmentally unfriendly (KAMERBEEK ET AL., 2003; LEISCH ET AL., 2011). As such, the use of the microbial enzymatic toolbox to catalyse the BV oxidation and other chemical reactions have gained momentum in recent years.



SCHEME 1.9 - Mechanism of the BV oxidation of ketones with peracids.

In 1948, G. E. Turfitt reported the first example of biological BV reaction in the biotransformation of cholesterol by the cholesterol-decomposing fungi *Proactinomyces erythropoli* (TURFITT, 1948). Since then, several organisms have been screened for the BV oxidation steps in

their biosynthetic pathways. Townsend *et al.* reported the BV-like oxidation on the methyl ketone derived from the opening of the averufin side chain in the biosynthesis of aflatoxin-B1 in the fungi *Aspergillus parasiticus* (TOWNSEND ET AL., 1982). In plants, Damtoft and co. also investigated the BV-like oxidation steps in the biosynthesis of iridoids (DAMTOFT ET AL., 1995) while Winter and co-workers reported the BV oxidation of 24-epicastasterone to give 24-epibrassinolide in the synthesis of brassinosteroids (WINTER ET AL., 1999). Similarly, the BV monooxygenases (BVMOs) provides an alternative source of fuel for most microorganisms as they can easily metabolize and grow on atypical carbon sources such as aliphatic methyl ketones, alicyclic hydrocarbons, aromatic compounds and terpenes (BRITTON & MARKOVETZ, 1977; DONOGHUE & TRUDGILL, 1975; FORNEY & MARKOVETZ, 1969; GRIFFIN & TRUDGILL, 1976; OUGHAM ET AL., 1983; SCHUMACHER & FAKOUSSA, 1999; VAN DER WERF & BOOT, 2000).

1.3 Microbial biotransformation of synthetic compounds

Microorganisms have a cornucopia of enzymatic machinery whose activities have been exploited to obtain new substances, including important chemicals. The earliest recorded use of microorganisms in the production of new substances was probably the use of yeast by the ancient Egyptians in the

production of alcoholic beverages and bread as well as the application of lactic acid bacteria in yoghurt production. Biotransformation process involves the use of microorganisms as biocatalysts to convert chemical compounds (substrates) to other compounds (KUMAR ET AL., 2009).

In recent years, there is a surge in the exploitation of microorganisms to produce industrially important compounds such as pharmaceuticals, agrochemicals, pheromones, flavors, fragrances and advanced materials. This is primarily due to increasing demand for chiral compounds by the pharmaceutical industries due to the recognition that enantiomers of a chiral compound could have different pharmacological effects and toxicity in the cell (PATEL, 2018). The use of microorganisms as biocatalyst has enormous advantages over the classical synthetic process and is sometimes the preferred route in the synthesis of fine chemicals and optically active compounds. They are excellent for the production of enantiomerically pure compounds, owing to their properties with respect to chemoselectivity, regioselectivity and, stereoselectivity (PANKE ET AL., 2004; SCHULZE & WUBBOLTS, 1999), and do not need protection and deprotection steps during synthesis, thereby minimizing the problems of isomerization, racemization, epimerization and rearrangement that are common in classical synthetic processes (KUMAR ET AL., 2009). Similarly, microorganisms are capable of

introducing desired functional groups at the sites of the molecule which are not easily accessible by chemical methods (SEDLACZEK & SMITH, 1988).

In addition, most microorganisms require moderate environmental conditions for their survival and therefore ensure that the biotransformation process can be carried out under environmentally friendly conditions thereby conforming to the principle and idea of green chemistry. Also, microbial cells and enzymes can be recycled and reused, and enzymes can be genetically engineered in large quantity thereby making the process efficient at low cost (PATEL, 2016, 2018).

Biotransformation process can be carried out with either the isolated enzymes or the whole cells of the microorganism. When isolated enzymes are used, the syntheses usually proceed with higher enantiomeric excess and the substrate are being fed to only a single enzyme, thereby avoiding the generation of unwanted products that might have been formed by competing enzymes (SILVA ET AL., 2012). However, most biocatalyst requires a cofactor, which must be generated *in situ* in another reaction step(s) (thereby complicating the reaction) or must be continually supplied exogenously in stoichiometric amounts if the reaction must proceed. Also, isolated enzymes can be easily denatured and deactivated if the reaction conditions are not met (ZHANG ET AL., 2008). This has made the use of whole-cell more attractive

since they can generate these cofactors by themselves and channel them for use when and where necessary (WANG ET AL., 2009).

1.3.1 Biotransformation by *Penicilium brasilianum*

Penicillium (FIGURE 1.7) is one of the most well-known fungi genera with over 354 acceptable species occurring in a diverse range of habitats, including soil, air, food products and vegetation (CHO ET AL., 2005; VISAGIE ET AL., 2014). Economically, they are of interest to man because of their roles in the decomposition of organic matter, food spoilage and food poisoning (FRISVAD & SAMSON, 2004; FRISVAD ET AL., 2004). Still, few have been reported for the beneficial use. Some species have been used for the production of food products such as speciality cheese and fermented sausage (GIRAUD ET AL., 2010; LUDEMANN ET AL., 2010). Others have been reported to synthesize both previously known and new physiologically active compounds with diverse structures (KOZLOVSKII ET AL., 2013). Perhaps, its most beneficial use is in the production of penicillin, an important antibiotic against bacterial infections (FLEMING, 1929). Owing to the array of beneficial use of Penicillium, most species have been screened for the production of enzymes capable of biotransforming compounds in a bid to improve their activities or produce entirely novel compounds (Y. LI ET AL., 2007). Many species have been shown to be prodigious regarding enzyme

production and biosynthesis of a great diversity of bioactive secondary metabolites with important pharmacological activities including antibacterial, antifungal, immunosuppressants and cholesterol-lowering (KWON ET AL., 2002; LUCAS ET AL., 2007; NICOLETTI ET AL., 2007; RANČIĆ ET AL., 2006).

FIGURE 1.7 - Some species of *P. brasilianum*.

One of the species that are attracting the attention of researchers is the fungus *Penicillium brasilianum* (synonyms: *Penicillium paraherquei, Penicillium ochrochloron var. paraherquei*). *P. brasilianum* has been reported as an interesting fungus with a great metabolic and underexplored enzymatic potential (BAZIOLI ET AL., 2017a). Different strains of *P. brasilianum* have been isolated from different sources including as

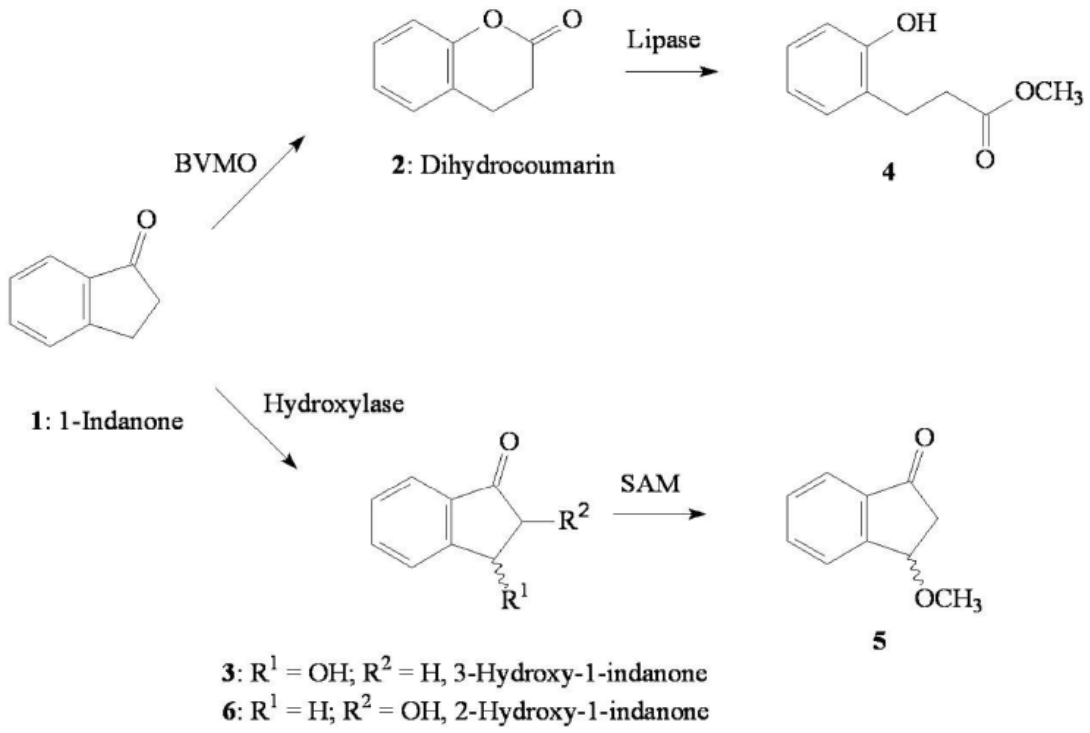
endophytes of plant *Melia azedarach* (TAICIA P. FILL ET AL., 2009; GERIS DOS SANTOS & RODRIGUES-FO, 2002), pathogens in onions (ZHELIFONOVA ET AL., 2010) and in soil samples in different parts of the world (FUJITA ET AL., 2002; JOURNAL, 2009; SCHÜRMANN ET AL., 2010).

P. brasilianum is a factory of important enzymes of biotechnological value. Thygesen and co-workers studied the production of cellulose and hemicellulose-degrading enzymes by *Penicillium brasilianum* IBT 20888 cultivated on wet-oxidised wheat straw and show that the highest enzyme activities were found in the culture broth originating from *P. brasilianum* (THYGESEN ET AL., 2003).

1.3.2 Baeyer-Villiger Monooxygenases in *P. brasilianum*

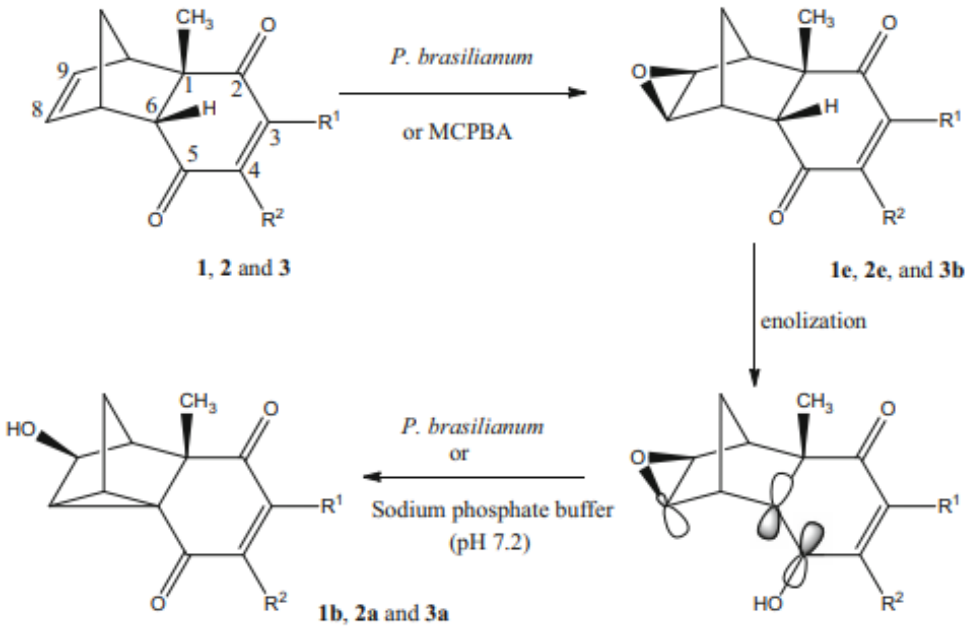
Though there are few reports on this, *P. brasilianum* has been studied for the BVMO activities of its enzymes. Previous works in our laboratory have tracked the activities of monooxygenases of certain strains of the fungus isolated as an endophyte from *Melia azedarach* (a Meliaceae plant) using 1-indanone as a substrate (TAICIA PACHECO FILL ET AL., 2012). It was observed that *P. brasilianum* transformed 1-indanone to dihydrocoumarin with the classical Baeyer-Villiger reaction regiochemistry, and (-)-(R)-3-hydroxy-1-indanone with high selectivity (78% ee) among other minor

products (SCHEME 1.10). Besides, they observed that all the fungi isolated from *M. azedarach* showed improved Baeyer-Villiger bioconversion of the ketone substrate to their lactone equivalents, the ability that might have been conferred on the fungi as a result of their endophytic association with the plant, since *M. azedarach* is a good producer of terpenoids containing esters and lactone groups.



SCHEME 1.10 - Biotransformation of 1-indanone by *P. brasilianum*.

In a continued effort by our group to exploit the BVMO activities in the cell of *P. brasilianum*, Zia *et al.,* biotransformed racemic Diels–Alder endo-cycloadducts in the cell of *P. brasilianum* (DIN ET AL., 2016). They obtained the Diels–Alder products from reacting cyclopentadiene or 2,3-dimethylbutadiene with alkylated para-benzoquinones, and biotransformed them with the resting cells of *P. brasilianum* to new functionalized polycyclic compounds. These biotransformations yielded novel products of oxidation and ring closure, reduction of the C=C or C=O in α, β-unsaturated system, and allylic hydroxylations. Further, evaluation of the enantioselectivity of the biotransformations revealed that the bioconversions were enantioselective (SCHEME 1.11).



SCHEME 1.11 – Mechanism of biotransformation of the Diels–Alder cycloadducts by *P. brasilianum*.

1.3.3 Biotransformation by *Saccharomyces cerevisiae*

Saccharomyces cerevisiae (Baker's yeast) is perhaps the most important microorganism in research and industry. They have proven to be a useful biocatalyst due to their rigid cell walls that ensure that their structures are retained when fed with various organic substrates and solvents. Besides, the reactivity of yeast as a biocatalyst has been improved upon by the development of methods aimed at making their cells more permeable to organic substrates and solvents (MATSUMOTO ET AL., 2001). Baker's Yeast (BY) is probably the most popular in research and industry. Apart from it being readily available and cheap, it is an excellent biocatalyst for

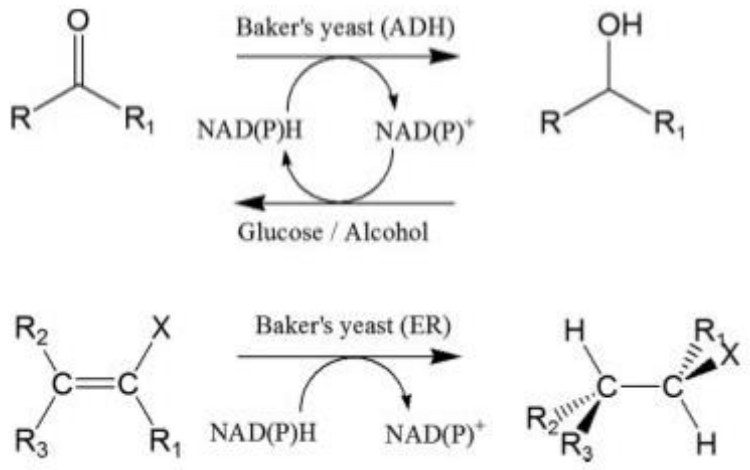
enantioselective biotransformation reactions involving carbon-carbon bond formation and oxidation-reduction reactions. (R)-phenylacetyl carbinol (PAC), the precursor of ephedrine, was synthesized from the carbon-carbon condensation reaction of benzaldehyde and acetaldehyde catalyzed by yeast (NIKOLOVA & WARD, 1992).

Most commonly, yeast has been used for the enantioselective reduction of ketones, β-ketoesters, imines, and α,β-unsaturated system with cabon-carbon double bond activated by strongly polarizing groups like nitro, carbonyl, or hydroxyl groups. The reduction of an α,β-unsaturated carbonyl compounds is usually accompanied by the formation of a mixture of saturated ketones or aldehyde, and/or saturated alcohol or allylic alcohol, and this clearly shows that there are many competing enzymes that reduce the carbon-carbon double bond and the carbonyl group (KHOR & UZIR, 2011; MATSUDA ET AL., 2009).

The two key enzymes in yeast reported to be responsible for the reduction of carbonyl compounds (pro-chiral) into hydroxyl groups-containing compound (chiral) are the cytosolic dehydrogenases and reductases. Yeast alcohol dehydrogenases (YADHs) reduce the carbonyl group while ene reductases (ERs) were found to reduce 'activated' carbon–carbon double bonds (FERREIRA ET AL., 2015; KHOR & UZIR, 2011). Like

most dehydrogenases and reductases, YADHs and ERs depends on the reduced nicotinamide adenine dinucleotide phosphate (NADPH) to supply the reducing equivalents. NADPH transferred hydride (H^-) to the carbonyl carbon of the substrate, becoming oxidized to $NADP^+$ in the process. For the reduction reaction to continue, there must be a continuous supply of NADPH and that is generated in a separate metabolic pathway. In the cytosol of the *S. cerevisiae*, the major sources of NADPH are the cytosolic $NADP^+$-dependent dehydrogenases, like glucose-6-phosphate dehydrogenase, acetaldehyde dehydrogenase, and isocitrate dehydrogenase. The pentose phosphate pathway (PPP), makes use of glucose, in a glucose-6-phosphate dehydrogenase-catalyzed reduction of $NADP^+$ to NADPH and is the primary source of cytosolic NADPH. NADPH can also be generated from ethanol in a reaction catalyzed by cyctosolic acetaldehyde dehydrogenase. Also, isocitrate dehydrogenase catalyzed the generation of NADPH from the secondary alcohol isocitrate (HENNINGSEN ET AL., 2015; MIYAGI ET AL., 2009). As a result, glucose and alcohols are excellent co-substrates to serve as hydrogen donors. SCHEME 1.12 illustrates how NADPH is being regenerated by yeast alcohol dehydrogenases and ethanoate reductases. Since the co-substrates are important for the bioreduction process, they must

be supplied in optimum amount for the biotransformation to continue (KHOR & UZIR, 2011).

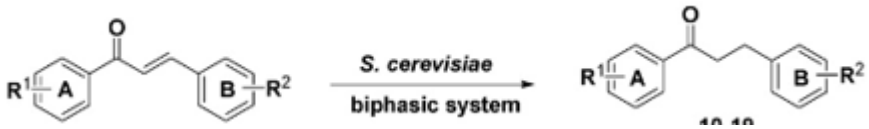


SCHEME 1.12 - Co-factor regeneration in baker's yeast reduction process.

Baker's yeast has a wide range of carbonyl-containing substrates whose enantioselective reductions have been reported. Such compounds include α- and β-diketones, keto esters and cycloalkanedione, oxocarboxylic acids, alkyl acetates and aromatic ketones (CHIN-JOE ET AL., 2002; ENGELKING ET AL., 2006; KHOR & UZIR, 2011; LIU ET AL., 2001). All these bioconversions have been done with products obtained at excellent yield and high enantiomeric ratio. Despite its well documented usage in asymmetric bioreduction of aromatic ketones, there are few reports on its use in the biotransformation of diarylpentadienones.

1.3.4 Bioreduction of α,β-unsaturated enones by *S. cerevisiae*

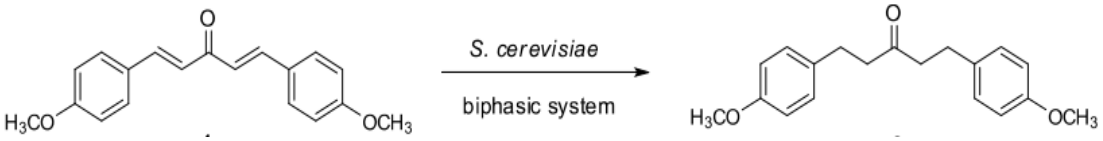
Recently, Silva and co-workers reported the application of *S. cerevisiae* (baker's yeast) for the biohydrogenation of the activated double bonds of 9 different chalcones (1,3-diaryl-2-propen-1-ones) to their corresponding dihydrochalcones in water/organic solvent biphasic systems (SCHEME 1.13). After optimizing the reaction conditions, they obtained ketone 10 in excellent yield (>99% conversions) though no correlation was observed between the effect of the donor or withdrawing electron groups (SILVA ET AL., 2010a).



SCHEME 1.13 - Biohydrogenation of chalcones by *S. cerevisiae.*

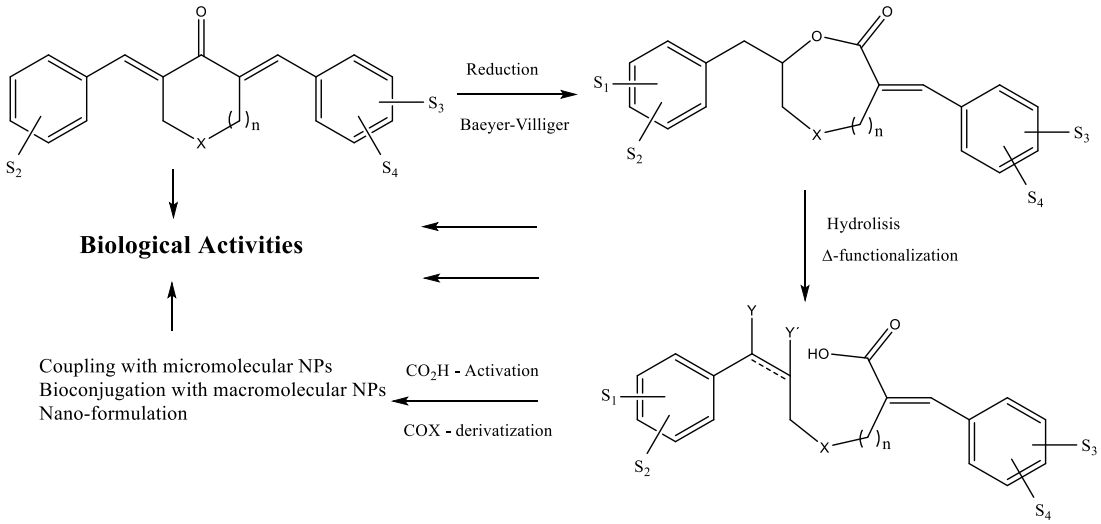
In continuation of their study, Silva and coworkers investigated the bioreduction of (1*E*,4*E*)-1,5-bis(4-methoxyphenyl)-1,4-pentadien-3-one in a buffer/n-hexane biphasic system. They also investigated the effect of various co-solvents on the biotransformation process to find the most appropriate organic solvent to dissolve the substrate. Also, optimum conditions such as yeast concentrations, temperature, pH, and volume of aqueous and organic phases were studied (SILVA ET AL., 2012). After optimizing the experimental

conditions, they obtained 1,5-bis-(4-methoxyphenyl)-3-pentanone in good conversion rate (82%) in a buffer/*n*-hexane biphasic system (SCHEME 1.14).



SCHEME 1.14 - Bioreduction of (1*E*,4*E*)-1,5-bis(4-methoxyphenyl)-1,4-pentadien-3-one by *S. cerevisiae* in a biphasic system.

The catalytic concept of these biotransformation processes is shown in SCHEME 1.15. The diarylpentadienones were subjected to reduction using both the conventional Palladium-on-carbon and *S. cerevisiae* followed by Baeyer-Villiger oxidation using *P. brasilianum*. The resulting lactones, labile and easily hydrolysable, can form polar intermediates that can interact with biological molecules and enzymes, thereby exerting their pharmacological activities (BERA ET AL., 2008; FANG ET AL., 2005; GUPTA ET AL., 2011).



SCHEME 1.15 – Predicted mechanism of biotransformation of diarylpentadienones.

As important as the above steps are for the biological activities of the compounds, the formation of polar/labile intermediates make these compounds unstable and either susceptible to attacks from detoxifying enzymes in microorganisms or render them soluble in water, thereby making them escape from the organic medium they naturally belong (DOS SANTOS & DE OLIVEIRA SILVA, 2019).

2 Objectives

The main objectives of the work include:

- ✓ To synthesize monoarylidenes and corresponding asymmetrical diarylpentadienones.
- ✓ To biotransform monoarylidenes and asymmetrical diarylpentadienones using endophytic fungus *Penicillium brasilianum*.
- ✓ To reduce the α,β-unsaturated double bonds of asymmetrical diarylpentadienones using *Saccharomyces cerevisiae* (Baker's yeast).

3 Experimental

3.1 Materials and Reagents used

Stationary phase for column chromatography:

- Silica gel 70-230 Mesh
- Silica gel 230-400 Mesh

Thin layer chromatography plate:

- TLC plate (MACHEREY-NAGEL) ALUGRAM® SIL G/UV_{254}

Column for analytical HPLC:

- PHENOMENEX Luna Phenyl-Hexyl Analytical Column 250 x 4.6mm (5μm);

Solvents

- Distilled solvents in the chemistry department;
- HPLC grade solvents from Tedia, JT Baker and Panreac®;
- Analytical purpose solvent from Panreac®, Synth, Vetex;
- Deuterated solvents Merck® and Aldrich® (98.0 – 99.9 %) for NMR spectra acquisition;
- Double distilled water with Milli-Q;

Growth media

- Potato Dextrose Agar;
- Czapek containing d-glucose; $NaNO_3$; $MgSO_4 \cdot 7H_2O$; KCl; $FeSO_4 \cdot 7H_2O$; K_2HPO_4; agar and yeast extract;
- Phosphate buffer at pH 7.2, 10 mM (containing monobasic sodium phosphate and dibasic sodium phosphate);
- Phosphate-Citrate buffer at pH 6.5 10mM

Reagents

- 4-methoxybenzaldehyde (98%) from Sigma Aldrich
- acetone (99.5%) from Merck;
- potassium phosphate buffer purchased from Vetec;
- citric acid from Acros;
- ammonium hydroxide;

Baker's yeast

- *Saccharomycetes cerevisiae* in dehydrated form (Dona Benta)

3.2 Equipments Used

NMR spectrometer

- BRUKER DRX 400.

High Performance Liquid Chromatography

- Analytical HPLC - Shimadzu LC-10AD: LC10AD gradient pumps, SHIMADZU; LC-8A, SHIMADZU; UV detector (PDA) for SHIMADZU SPD-10A and SPD-6A UV-Vis HPLC; communication module detector/computer in HPLC SHIMADZU CBM-10A; Communication BUS Module; Injector for HPLC SHIMADZU Auto Injector SIL-10ADVp, *software*: CLASS-VP.
- Preparative HPLC - Shimadzu LC-20AD composed of: SHIMADZU SIL-20AP Vp HPLC Injector, HPLC SHIMADZU CBM-20A Communication BUS Module, SHIMADZU LC20AP gradient pumps, HPLC UV Detector SHIMADZU SPD-20AV UV-Vis, DGU-20A degasser; software: LC SOLUTION;

Flash chromatography

- TELEDYNE-ISCO® *Combiflash* RF 200 UV/VIS

Rotary evaporator

- Rotaevaporador Tecnal (TE 120)
- Rotaevaporador Buchi 461-water bath (EL 131)

Laminar flow chamber

– Veco VL FS-12M;

Vertical Autoclave

– Phoenix AV 70;

Orbital shakers

– Tecnal TE 140;

Ultrassound bath

– Tecnal TE 140;

Analytical balance

– Tecnal;

– Metler Toledo

Furnace

– Quimis

Centrífuge

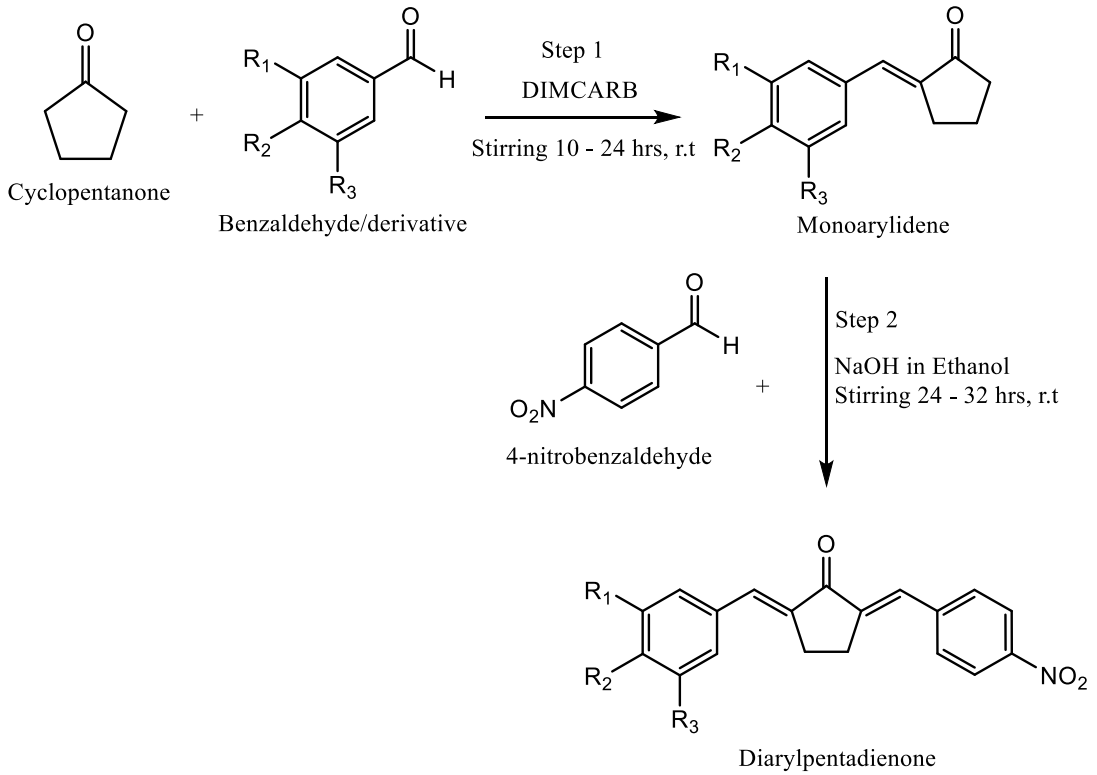
– Eppendorf 5810 R

Millipore filter paper 0.20 μm

3.3 Methods

3.3.1 Synthesis of Diarylpentadienones

The synthesis of diarylpentadienones was done after the method of Din and Rodrigues-Filho with some modifications (DIN & RODRIGUES-FILHO, 2016). In step 1 of SCHEME 3.1, monoarylidenes were synthesized from benzaldehyde and cyclopentanone. A mixture of cyclopentanone (10.95 mmol), aldehyde (7.3 mmol) and DIMCARB (0.2 equivalent) was taken in 100 mL round-bottom flask with 10mL of 1:1 mixture of ethanol and water as co-solvent. The reaction mixture was stirred vigorously for 10 - 24 hours after which the DIMCARB was removed from the reaction mixture by rotary evaporator at 60 °C. The crude product was extracted with ethyl acetate and 1 M HCl solution, dried and purified by column chromatography. The monoarylidenes obtained were recrystallized and used in step 2.



SCHEME 3.1 - Reaction conditions for the synthesis of compounds 1 - 6.

To synthesize diarylpentadienones (step 2 in SCHEME 3.1), a solution of pure monoarylidene and substituted benzaldehydes in ethanol (5 mL) was stirred for 5 min at room temperature after which added sodium hydroxide solution in ethanol (4 mL, 50 mmol) and stirring was continued for 24 – 32 hours. At the completion of the reaction, the solvent was evaporated on a rotary evaporator and the extract was worked up as described above. The yellow precipitate obtained was purified and characterized by ^{1}H NMR.

The monoarylidenes and diarylpentadienones synthesized in this work are shown in TABLE 3.1. Cyclopentanone and benzaldehyde were reacted to

form compound **1** which was further reacted with 4-nitrobenzaldehydes to form compound **4**. Compound **2** was obtained from the stirring of cyclopentanone and 4-methoxylbenzaldehyde while the reaction of compound **2** with 4-nitrobenzaldehydes yielded compound **5**. Monoarylidene **3** was obtained from the reaction of cyclopentanone and 3,4,5-methoxylbenzaldehyde. Further reaction of compound **3** with 4-nitrobenzaldehydes yielded compound **6**.

Compounds **7**, **8** and **9** and some part of compound **5** were obtained from the previous work in our laboratory through the combination of various ketones and aldehyde as shown in

TABLE 3.2. In **9,** the ketone is pentan-2-one.

TABLE 3.1 - The monoarylidenes and diarypentadienones synthesized in the present work.

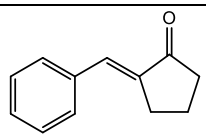
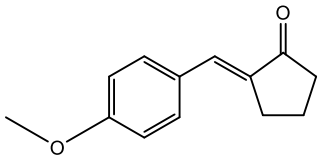
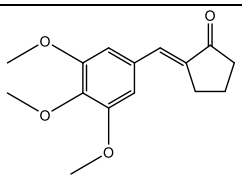
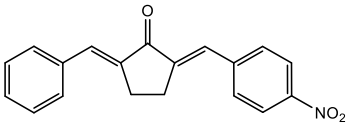
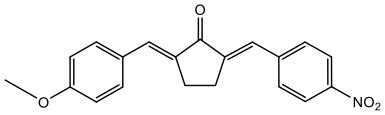
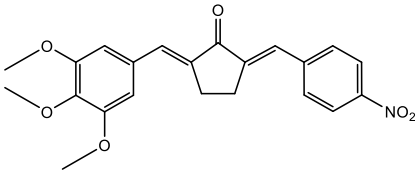
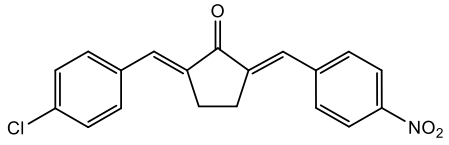
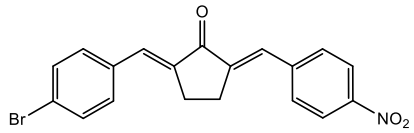
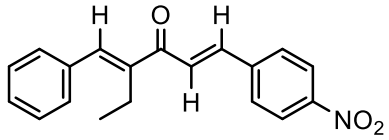
Compound	R_1	R_2	R_3
 **1**	H	H	H
 **2**	H	OCH_3	H
 **3**	OCH_3	OCH_3	OCH_3
 **4**	H	H	H
 **5**	H	OCH_3	H
 **6**	OCH_3	OCH_3	OCH_3

TABLE 3.2 - The diarypentadienones obtained from the previous works in our laboratory.

Compound	R_1	R_2	R_3
 **7**	H	Cl	H
 **8**	H	Br	H
 **9**	H	H	H

3.3.2 Biotransformation using *Penicillium brasilianum*

3.3.2.1 Cultivation of *Penicillium brasilianum*

4 strains of *Penicillium brasilianum*, an endophytic fungi isolated from the plant *Melia azedarach* and deposited as LaBioMMi 236, Δ630, PbΔNRPS 07 and PbΔNRPS 23 in the library of the Laboratório de Bioquímica Micromolecular de Micro-organismos (LaBioMMi) at the

Department of Chemistry, Universidade Federal de São Carlos, São Carlos, Brazil, were used in this experiment (GERIS DOS SANTOS ET AL., 2003). The inoculum of each strain was prepared by suspending spores from a 7-day old culture in sterilized water. A few drops of the inoculum was pipetted onto a potato-dextrose agar (PDA) medium in Petri-dishes and the fungi were allowed to grow for approximately 3 days before they were used for the biotransformation experiments (FIGURE 3.1).

FIGURE 3.1 - *Penicilium brasilianum*

3.3.2.2 Preparation of the growth media

1 liter of the Czapek media enriched with yeast extract was prepared by dissolving 3.0 g of $NaNO_3$, 1.0 g of K_2HPO_4, 0.5 g of $MgSO_4$, 0.5 g of KCl, 0.01 g of $FeSO_4 \cdot 7H_2O$ in 600 ml of distilled water. At the complete dissolution of the salts, 30.0 g of glucose was added to the solution and was

allowed to dissolve in the solution by shaking. Later, 20.0 g yeast extract was added to the solution, the solution was homogenized and made up to 1 liter (1000ml) with distilled water. 100 mL of the resulting solution was transferred into each of a 250 mL Erlenmeyer flask and the flasks were sterilized in an autoclave at 121°C for 15 minutes. The media were allowed to stand for 3 days to monitor any unwanted growth before being used for the various biotransformation experiments. In total, 12 replicates were prepared for each substrate. 3 flasks containing the fungi but no substrates were kept as control.

3.3.2.3 Inoculation of *P. brasilianum*

3 pieces of each of the *P. brasilianum* strains were transferred from the Petri-dishes into each flask and were allowed to grow for seven 7 days under static condition.

3.3.2.4 Biotransformation of compounds 4 and 5 in growing cells of *P. brasilianum*

Between 20 mg of **4** and **5** dissolved in 1.0 mL acetone was added to each flask and the fungi were allowed to grow under static condition. The control fungi were also allowed to grow albeit with no test compound added. After 15 days, the fungi was filtered and the filtrate (growth medium) was extracted with ethyl acetate (3 × 100 mL). The organic phase was dried with

Na_2SO_4 and evaporated. 20.0 g of solid NaCl was added to the mycelium suspended in 100 mL methanol for 24 hrs and the solution was homogenized, filtered, and the filtrate dried and concentrated. Both the ethyl acetate and methanol extracts were pooled together and transferred into a previously weighted, clean flask. The dried extracts were separated using column chromatography with hexane/ethyl acetate solvent mixture in gradient elution and each fraction was analyzed with TLC and possible biotransformation products were detected with ^{1}H NMR.

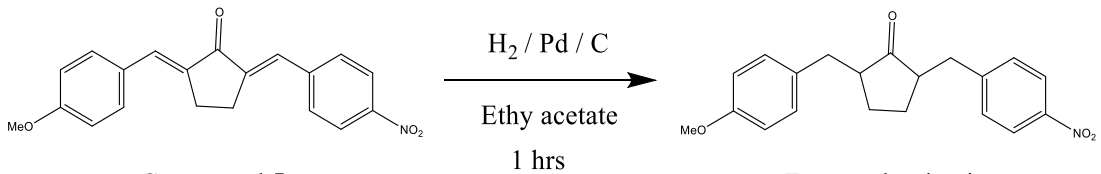
3.3.2.5 Biotransformation of compounds 2, 7 and 8 using the resting cells of *P. brasilianum*

The fungus was initially cultivated in Czapek liquid media enriched with yeast extract as described in item 3.3.2.4 above but with no diarylpentadienones added. The fungus was incubated in an orbital shaker at 120 rpm for 3 days and the mycelium was separated from the liquid medium by filtration. 100 ml of phosphate buffer (pH 7.0) was transferred to a new 250 ml Erlenmeyer's flask and autoclaved for 15 minutes at 121°C and allowed to cool to room temperature. The mycelium was transferred into the buffer solution under the septic condition and 20 mg each of **2, 7** and **8** dissolved in 1.0ml of acetone was carefully added. The resting cells were

stirred constantly at 120 rpm for 12 days. The extraction was worked up and analyzed as described in item 3.3.2.4

3.3.3 Catalytic hydrogenation of compound 5 using Pd/C

Palladium-on-carbon (Pd/C) was used for the selective catalytic hydrogenation of the olefinic double bonds in compound **5** according to SCHEME 3.2. 20 mg of compound **5** was added to a 100 mL round-bottom flask containing Pd/C in the presence of hydrogen and the reaction mixture was stirred for 1 hour at room condition.



Compound **5**

Expected reduction products of **5**

SCHEME 3.2 – Pd/C-catalysed hydrogenation of Compound 5.

3.3.4 Reduction of compound 9 by *S. cerevisiae*

S. cerevisiae meyen yeast strain in their active dry yeast form was obtained from Dona Benta, Angel Yeast, Co. Ltd, China (FIGURE 3.2). The pellets of dehydrated yeast cells were used directly in the reduction reactions. 4 g of Baker's yeast was transferred into a 100 mL round-bottom flask

containing 30 ml of phosphate buffer (pH 5.5) and 20 ml of n-hexane and the mixture was stirred in an orbital shaker for 30 minutes. 10 mg of **9** dissolved in 1ml acetone was transferred into the yeast aliquot and the reaction mixture was stirred vigorously for 14 days with a magnetic stirrer. Aliquots were periodically withdrawn from the aqueous and organic phases and extracted with ethyl acetate (3 × 50 mL) and the reactions were monitored by thin-layer chromatography (TLC) using n-hexane: ethyl acetate (9:1, v/v) as the eluent to see if a product is formed. After bioreduction was observed, the whole reaction was extracted with ethyl acetate (3 × 100 mL) and the organic phase was dried with anhydrous Na_2SO_4 and concentrated under vacuum pressure. The extract was purified by preparative TLC in an isocratic system containing n-hexane: ethyl acetate and the pure fractions isolated were characterized by ^1H NMR.

FIGURE 3.2 - *Saccharomyces cerevisiae* meyen yeast strain.

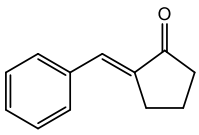
4 Results and Discussion

4.1 Synthesis of compounds 1-6

The reaction of benzaldehyde, 4-methoxy benzaldehyde and 3,4,5-trimethoxy benzaldehyde with cyclopentanone, with DIMCARB as a catalyst yielded the monoarylidenes **1-3**, which were purified and characterized by spectroscopic methods. Diarylpentadienones **4-6** were synthesized from the reaction of **1-3** with 4-nitrobenzaldehyde respectively. The compounds were purified, characterized by spectroscopy and the data were compared to literature. The yields of **1-6** are between 60-70 %.

4.1.1 Compound 1

The TLC plate for compound **1** is given in FIGURE 4.1.



1

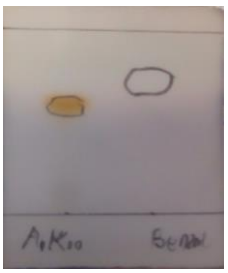
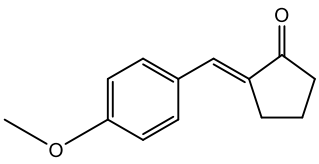


FIGURE 4.1 - TLC for compound 1.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 – 7.52 (m, 2H), 7.47 – 7.37 (m, 3H), 7.25 (s, 1H), 3.06 – 2.89 (m, 2H), 2.55 – 2.35 (m, 2H), 2.15 – 1.80 (m, 2H).

4.1.2 Compound 2

The TLC plate for compound **2** is given in FIGURE 4.2.



2

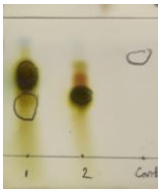
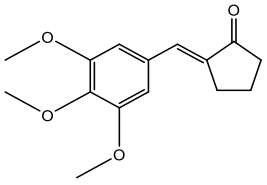


FIGURE 4.2 - TLC for compound 2.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, *J* = 8.6 Hz, 2H) 7.36 – 7.17 (m, 1H), 6.87 (d, *J* = 15.3 Hz, 2H), 3.77 (s, 3H), 2.95 – 2.80 (m, 2H), 2.32 (dd, *J* = 10.4, 5.2 Hz, 2H), 1.96 (dt, *J* = 15.1, 5.9 Hz, 2H).

4.1.3 Compound 3

The TLC plate for compound **3** is given in FIGURE 4.3.



3

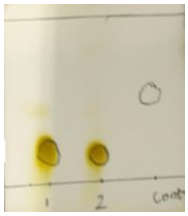
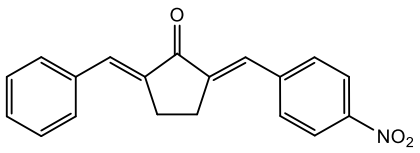


FIGURE 4.3 - TLC for compound 3.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (t, *J* = 2.7 Hz, 1H), 6.91 (s, 2H), 4.02 (s, 9H), 3.12 (td, *J* = 7.2 Hz, 2.7 Hz, 2H), 2.53 (t, *J* = 7.9 Hz, 2H), 2.18 (p, *J* = 7.6 Hz, 2H).

4.1.4 Compound 4

The structure of **4** is given below:

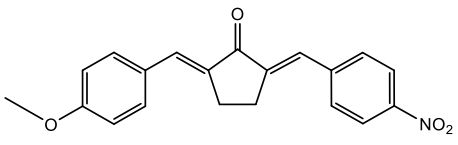


4

Percentage yield: 62.1%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, J = 12 Hz, 2H, ArH), 7.75 (d, J = 12 Hz, 2H, ArH), 7.67 (s, 1H, =CH), 7.63 (s, 1H, =CH), 7.39-7.51 (m, 5H ArH), 3.25 (s, 4H, CH2CH2).

4.1.5 Compound 5

The structure of **5** is given below:

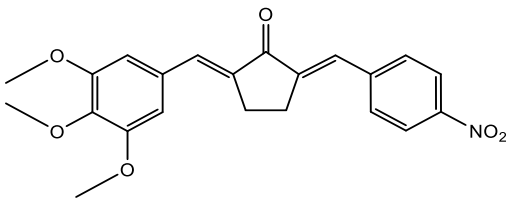


5

Percent yield: 65.7 %, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, *J* = 12 Hz, 2H, ArH), 7.75 (d, *J* = 12 Hz, 2H, ArH), 7.51 (s, 1H, =CH), 7.63 (s, 1H, =CH), 6.91 (d, *J* = 8 Hz, 2H, ArH), 3.85 (s, 3H, OCH_3), 3.17 (s, 4H, CH_2).

4.1.6 Compound 6

The structure of **6** is given below:



6

Percent yield: 69%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 - 8.27 (m, *J* = 8.9 Hz, 4H, ArH), 7.75 (s, 1H, =CH), 7.25 (s, 1H, =CH), 6.77 (s, 2H ArH), 3.81 (s, 6H, CH_3), 3.49 (s, 3H, CH_3), 3.12 (s, 4H, CH_2).

4.2 Biotransformation of compounds 5 and 4 using the growing cells *P. brasilianum*

In this experiment, compound **5** was chosen as a substrate because of the present of two groups with different electronic effects on the ring – an electron-donating methoxyl group and an electron-withdrawing nitro group. The chromatograms below (FIGURE 4.4 to FIGURE 4.7) show the fractions obtained from the purification of the extract from the biotransformation of compound **5** by 4 strains of *P. brasilianum*. In FIGURE 4.4, **a – h** correspond

to the 8 fractions obtained in the flash chromatography separation. ^{1}H NMR data of each fraction were acquired and fractions **b** and **d** were easily ruled out as fungi metabolites ergosterol and veruculogen respectively, other fractions except **c** are either impurities or fractions with no significance. The same treatment was given to the fractions in FIGURE 4.5, FIGURE 4.6 and FIGURE 4.7.

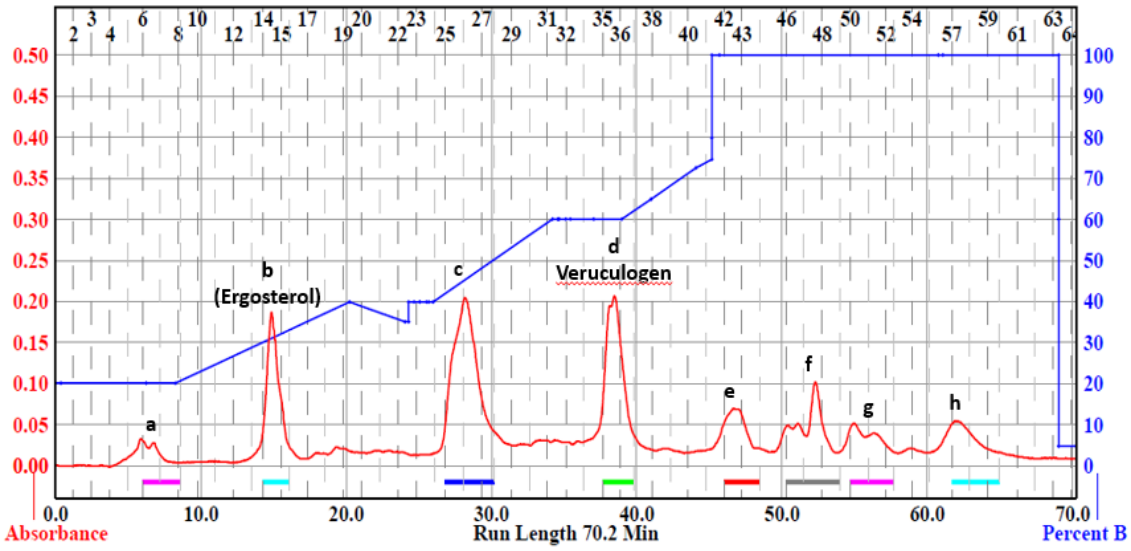


FIGURE 4.4 - The chromatogram for the biotransformation of compound 5 by *Pb*236. Separation conditions: RediSep Column: Silica 40g Gold, Flow Rate: 16 ml/min, Solvent A: n-hexane, Solvent B: ethylacetate, Wavelength 1 (red): 254nm, Wavelength 2 (purple): 280nm.

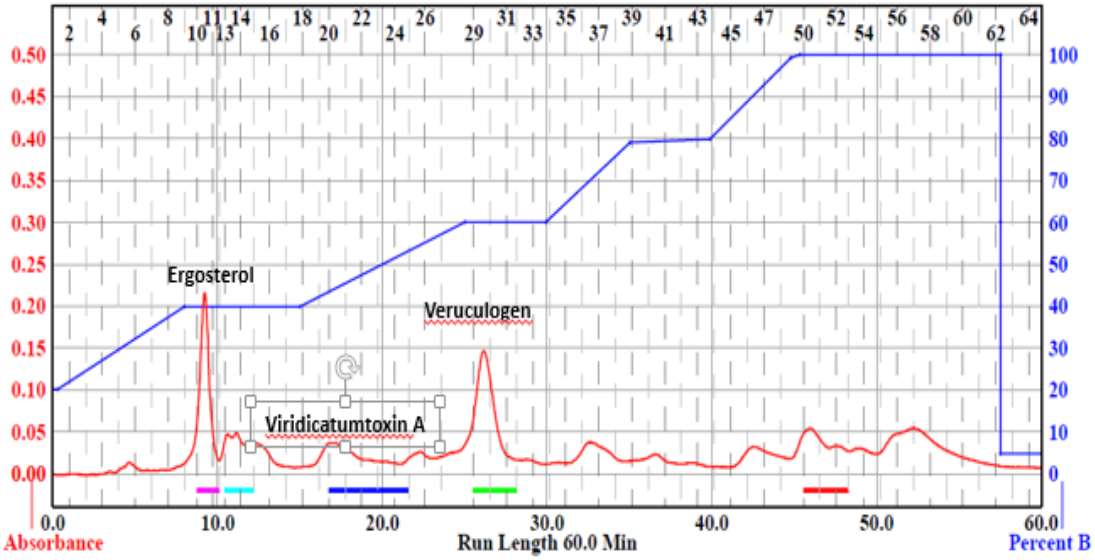


FIGURE 4.5 - The chromatogram for the biotransformation of compound 5 by *Pb*Δ630. Separation conditions: RediSep Column: Silica 40g Gold, Flow Rate: 16 ml/min, Solvent A: n-hexane, Solvent B: ethylacetate, Wavelength 1 (red): 254nm, Wavelength 2 (purple): 280nm.

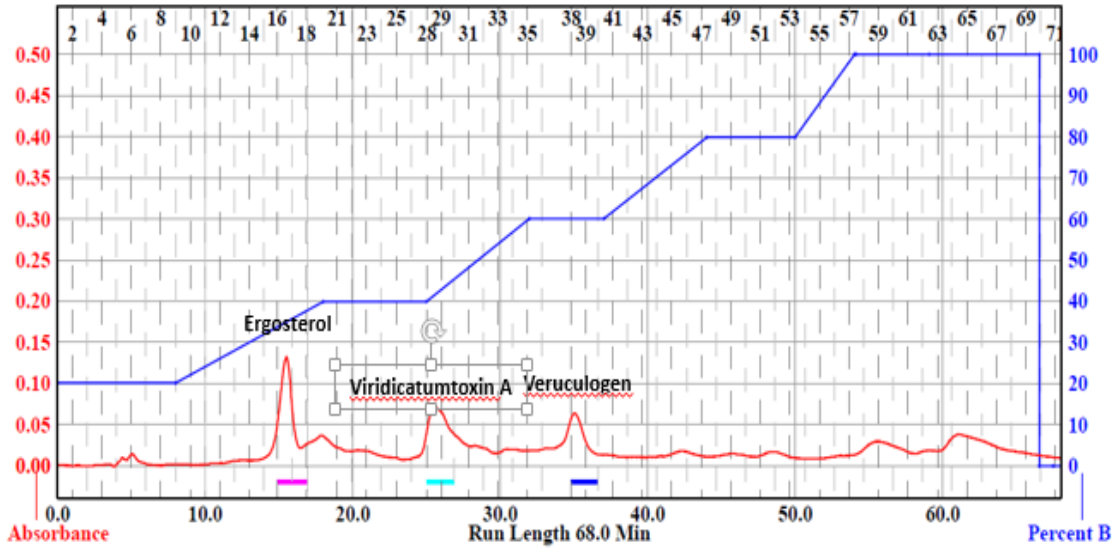


FIGURE 4.6 - The chromatogram for the biotransformation of compound 5 by *Pb*ΔNRPS07. Separation conditions: RediSep Column: Silica 40g Gold, Flow Rate: 16 ml/min, Solvent A: n-hexane, Solvent B: ethylacetate, Wavelength 1 (red): 254nm, Wavelength 2 (purple): 280nm.

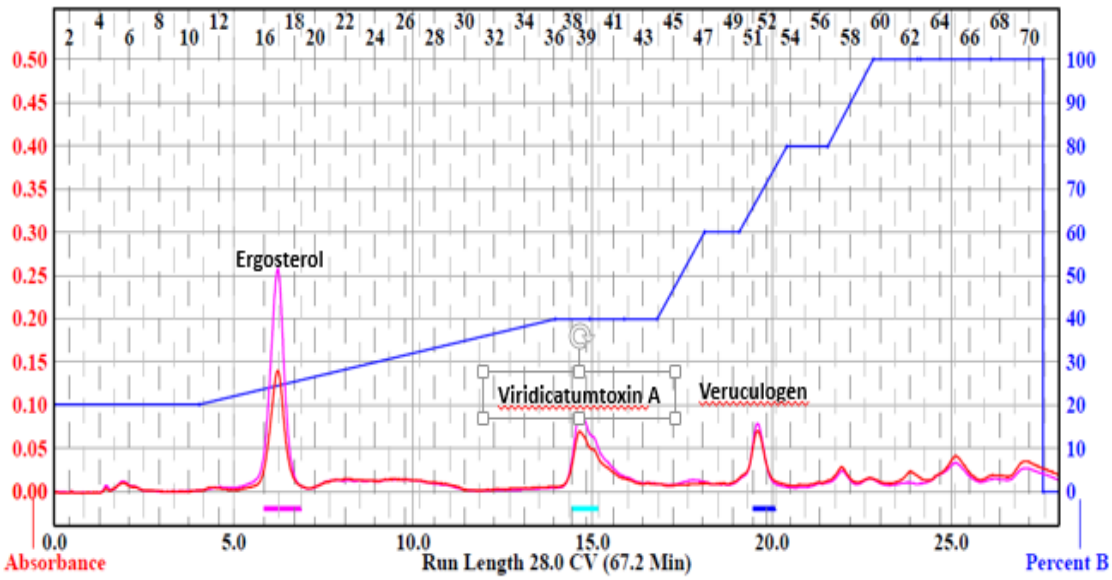


FIGURE 4.7 - The chromatogram for the biotransformation of compound 5 by *Pb*ΔNRPS23. Separation conditions: RediSep Column: Silica 40g Gold, Flow Rate: 16 ml/min, Solvent A: n-hexane, Solvent B: ethylacetate, Wavelength 1 (red): 254nm, Wavelength 2 (purple): 280nm.

In an attempt to identify the fraction labelled **c** in FIGURE 4.4 and search for biotransformation products, fraction **c** and the corresponding fractions in FIGURE 4.6 and FIGURE 4.7 were compared with compound **5** using analytical HPLC (FIGURE 4.8). From the chromatogram, it is evident that the 3 fractions are the same considering the similar retention time.

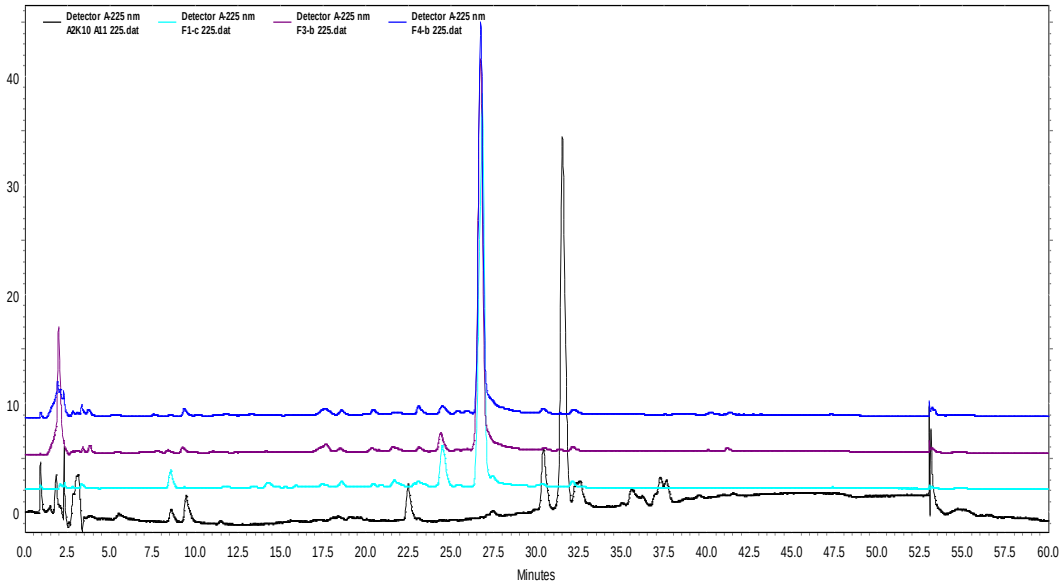


FIGURE 4.8 - Comparison of fraction c in FIGURE 4.4 and the corresponding fractions in FIGURE 4.6 and FIGURE 4.7 with compound 5. The black peak (bottommost baseline) represents compound 5, the green peak (the second bottommost baseline) represents fraction c where the red and blue lines (the third bottommost and the topmost baselines) represent the corresponding fractions from FIGURE 4.6 and FIGURE 4.7 respectively.

Pooling the 3 fractions together, the resulting fraction was compared with an already identified fungi toxin viridicatumtoxin A by analytical TLC. The fraction shown similar retention time with viridicatumtoxin A and fraction **c** in FIGURE 4.4 and the corresponding fractions in FIGURE 4.6 and FIGURE 4.7 were identified as viridicatumtoxin A. This observation was in agreement with several reports in the literature that *P. brasilianum* biosynthesize several metabolites including ergosterol, veruculogen and viridicatumtoxin A (BAZIOLI ET AL., 2017b).

A similar observation was recorded when compound **4** was used as a substrate of the growing cells of the 4 strains of *P. brasilianum* used in the experiment (FIGURE 4.9 to FIGURE 4.12).

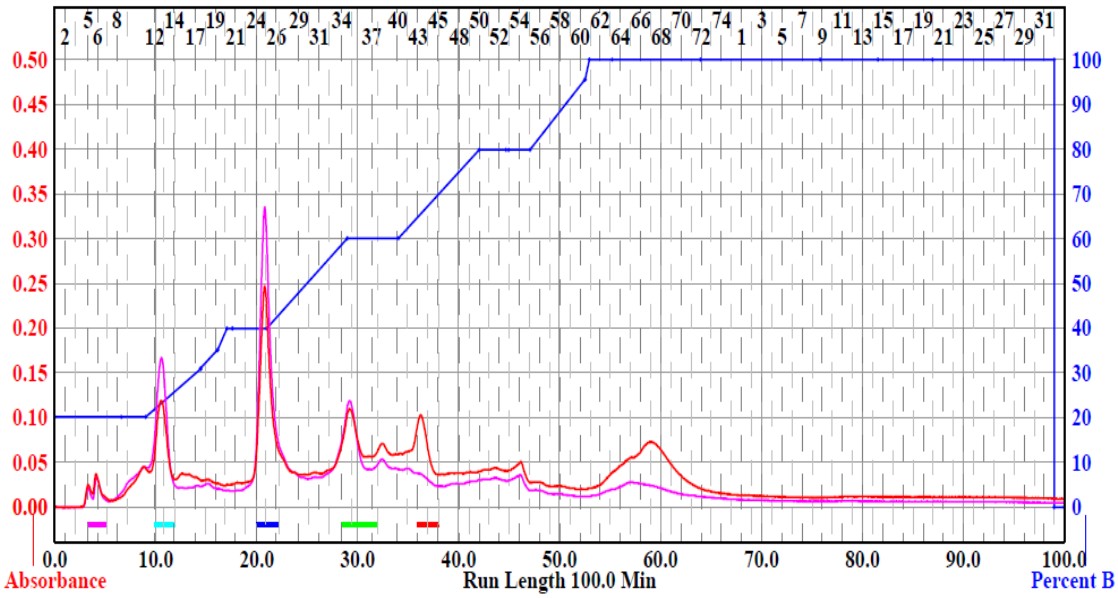


FIGURE 4.9 - The chromatogram for the biotransformation of compound 4 by *Pb*236. Separation conditions: RediSep Column: Silica 40g Gold, Flow Rate: 16 ml/min, Solvent A: n-hexane, Solvent B: ethylacetate, Wavelength 1 (red): 254nm, Wavelength 2 (purple): 280nm.

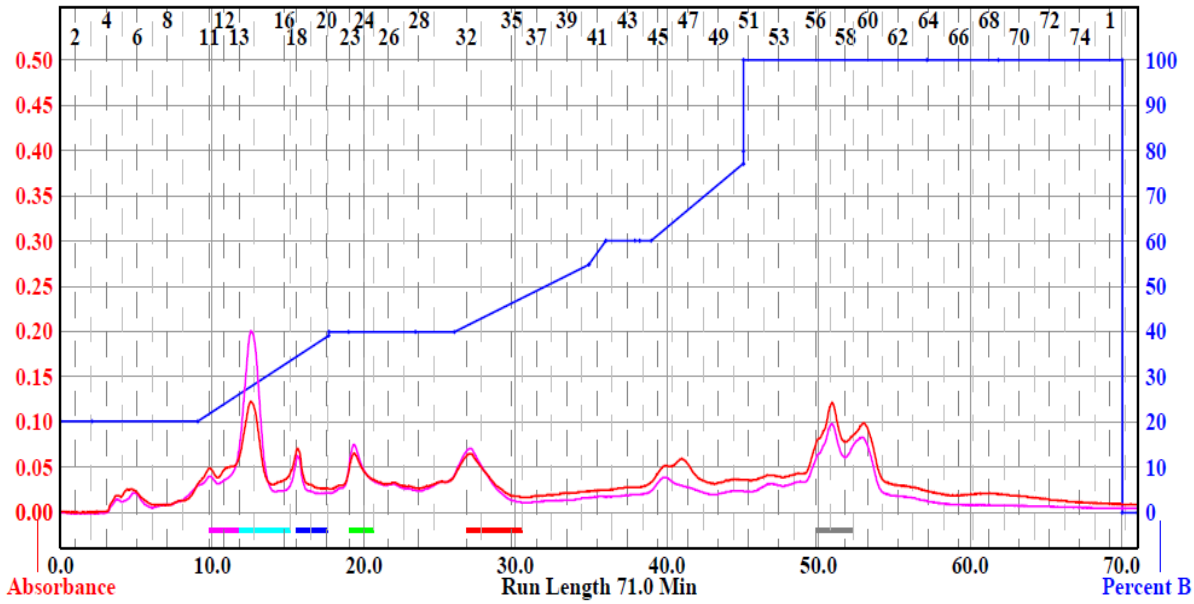


FIGURE 4.10 - The chromatogram for the biotransformation of compound 4 by *Pb*Δ630. Separation conditions: RediSep Column: Silica 40g Gold, Flow Rate: 16 ml/min, Solvent A: n-hexane, Solvent B: ethylacetate, Wavelength 1 (red): 254nm, Wavelength 2 (purple): 280nm.

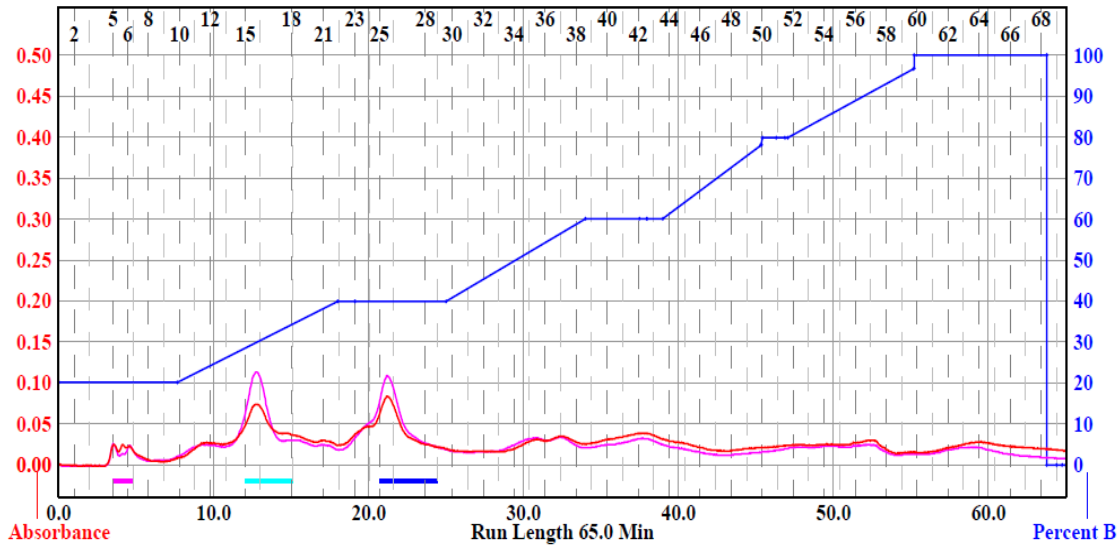


FIGURE 4.11 - The chromatogram for the biotransformation of compound 4 by *Pb*ΔNRPS07. Separation conditions: RediSep Column: Silica 40g Gold, Flow Rate: 16 ml/min, Solvent A: n-hexane, Solvent B: ethylacetate, Wavelength 1 (red): 254nm, Wavelength 2 (purple): 280nm.

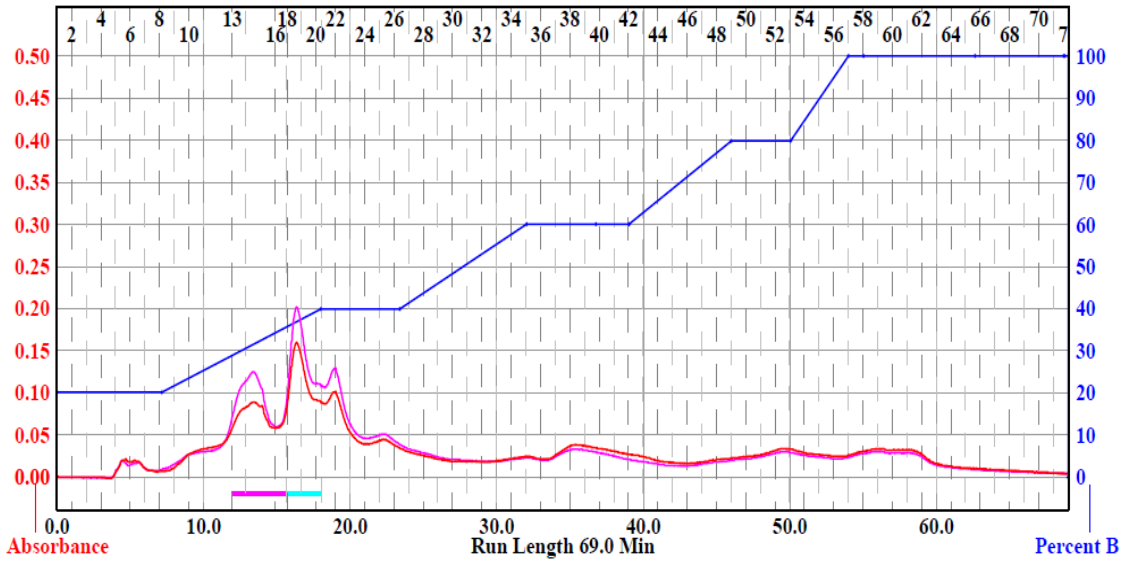


FIGURE 4.12 - The chromatogram for the biotransformation of compound 4 by *Pb*ΔNRPS23. Separation conditions: RediSep Column: Silica 40g Gold, Flow Rate: 16 ml/min, Solvent A: n-hexane, Solvent B: ethylacetate, Wavelength 1 (red): 254nm, Wavelength 2 (purple): 280nm.

Though there are no convincing explanations for the disappearance of compound **5** and **4** and their biotransformation products in the extract, there is a tendency that these compounds and their products of biotransformation interact with fungal proteins and other macromolecules. Compounds **5** and **4** are labile molecules with several groups, including the carbonyl, methoxyl, nitro as well as the two hydrophobic aromatic rings, capable of interacting with several proteins and biological molecules found in *P. brasilianum*. In the past, substituents and groups on curcuminoid analogs have been reported to bind strongly to biological molecules such as proteins, enzymes, metal ions, DNA and RNA and organelles like proteasome through covalent, non-

covalent hydrophobic, and hydrogen-bonding interactions with the side chains of amino acid residues such as serine, threonine, aspartate, histidine, and lysine and other polar, charged or aromatic groups (GUPTA ET AL., 2011). The carbonyl moiety on compounds **5** and **4** and other curcuminoid analogs is capable of undergoing keto-enol tautomerism, with the enol form being predominant and capable of accepting and donating hydrogen bonds, chelating charged metals that are present on the surface of proteins and acting as Michael acceptor to nucleophilic sulfhydryls of cysteine and Se^- moiety of selenocysteine (FANG ET AL., 2005; NECKERS ET AL., 2006; PEDERSEN ET AL., 1985; TØNNESEN, 1992). Similarly, the aryl moieties of these compounds are capable of bonding with the side chains of aromatic amino acids such as phenylalanine, tyrosine and tryptophan through a π-π van der Waals interactions (GUPTA ET AL., 2011). Finally, their polar substituents such as the nitro and methoxyl groups can interact with polar amino acids and the AT and AU-rich regions in DNA and RNA respectively by hydrogen-bonding to these molecules (BERA ET AL., 2008; NAFISI ET AL., 2009). Interaction of compounds **5** and **4** with any of the aforementioned molecules would result in the formation of polar complexes that escape with the aqueous medium during fungal extraction processes. Similarly, it is possible that *P. brasilianum* degraded these compounds to polar molecules

that also escaped with the aqueous medium (DOS SANTOS & DE OLIVEIRA SILVA, 2019). These factors may be the reasons why no biotransformation product of **5** and **4** were detected.

4.3 Biotransformation of compounds 2, 7 and 5 using the resting cells of *P. brasilianum*

One of the challenges encountered in the experiments involving the growing cell of *P. brasilianum* was the presence of a large number of fungal metabolites that interfered with the purification processes. To overcome this, we decided to biotransform compounds **2, 7** and **5** with the resting cells of *P. brasilianum* since the rate of metabolism in a resting cell is lower and only a few metabolites will be produced. At the end of the experiment, the extracts were analysed as described in section 3.3.2.5.

For compound **2**, both the mycelium and broth fractions were analyzed separately. The chromatogram comparing compound **2** to the extracts of the triplicates of mycelium used in the experiment is given in FIGURE 4.13. The triplicates were pooled together and separated by flash chromatography as shown in the chromatogram in FIGURE 4.14. Though the peaks were not conspicuous, all the fractions were collected and the NMR spectra of interesting fractions were acquired.

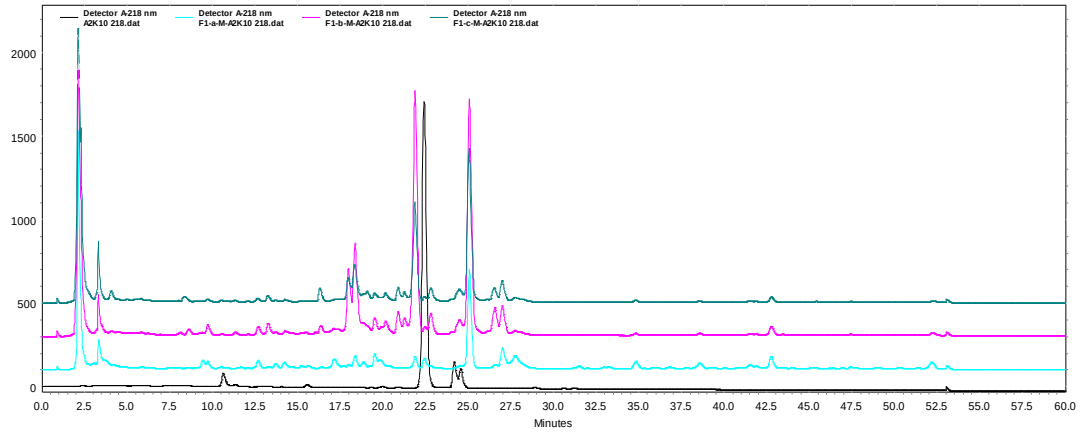


FIGURE 4.13 - Chromatogram for the biotransformation of compound 2 by the resting mycelium of *P. brasilianum Pb*236. The black line (bottommost) represents compound 2 while the others are the triplicate experiment. Column: Phenylhexyl, Solvent A: Water. Solvent B: Acetonitrile. Wavelength : 218 nm, flow : 0.8ml/min

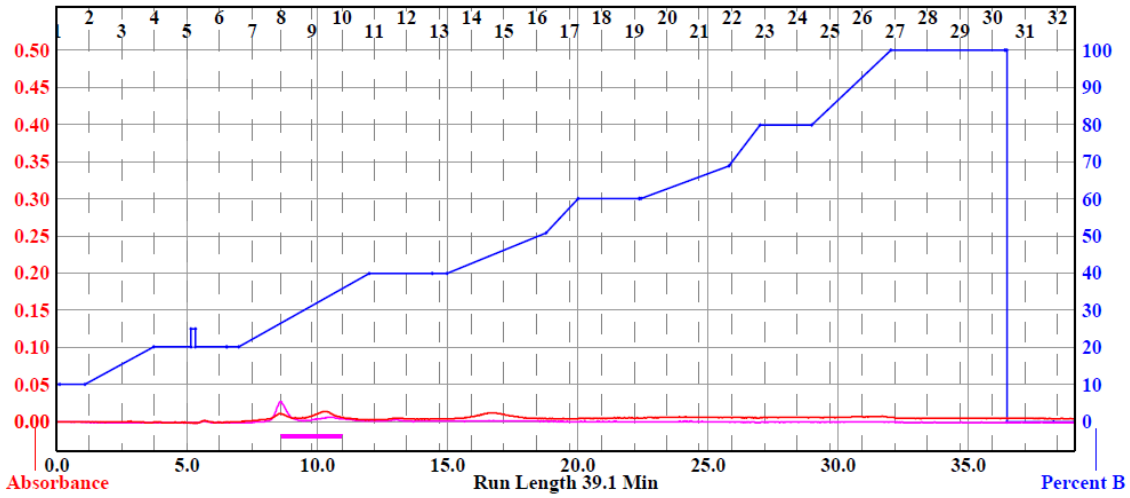


FIGURE 4.14 - The chromatogram for the biotransformation of compound 2 by *Pb*236. Separation conditions: RediSep Column: Silica 40g Gold, Flow Rate: 16 ml/min, Solvent A: n-hexane, Solvent B: ethyl acetate, Wavelength 1 (red): 254nm, Wavelength 2 (purple): 280nm.

FIGURE 4.15 is the ^{1}H NMR spectra of the first fraction in FIGURE 4.14. The aromatic region, there is a disappearance of the allylic carbon-carbon double bond (C=C) signal at δ_H 7.32 while there is an appearance of new signals between δ_H 2.0 and 3.5, confirming the reduction of the olefinic system to form compound **10** as shown in SCHEME 4.1.

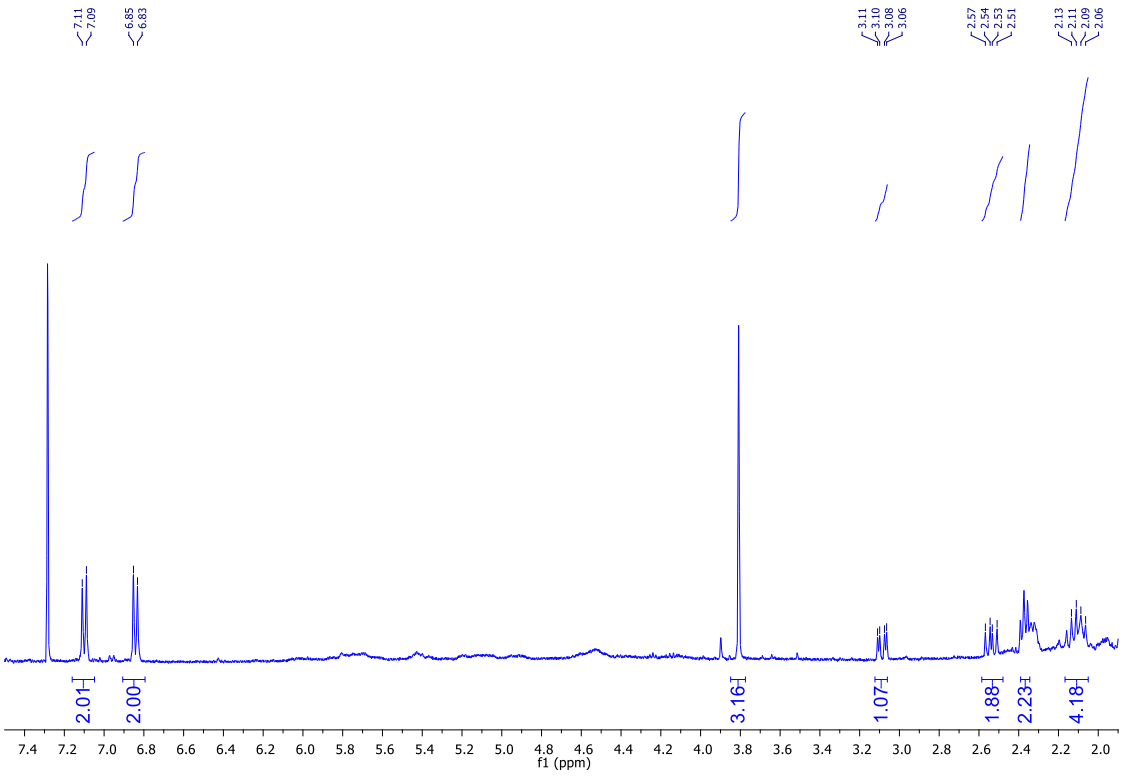
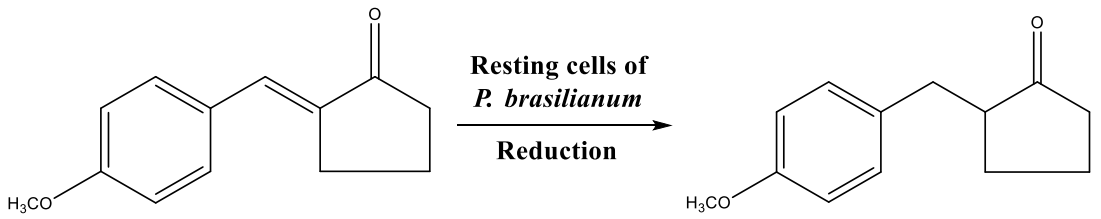


FIGURE 4.15 - ^{1}H NMR spectra of compound **10**.



Compound **2** Compound **10**

SCHEME 4.1 - Reduction of compound **2** to compound **10** by the resting mycelium of *P. brasilianum.*

Similarly, the extract from the biotransformation of compound **2** in the broth was also analysed. The idea was that in the initial growth process before the mycelia were transferred into the buffer, some of the enzymes of *P. brasilianum* would have been released into the medium. The experiment was analysed as described in section 3.3.2.5. The replicates were pooled, purified by flash chromatography (FIGURE 4.16) and analysed by NMR.

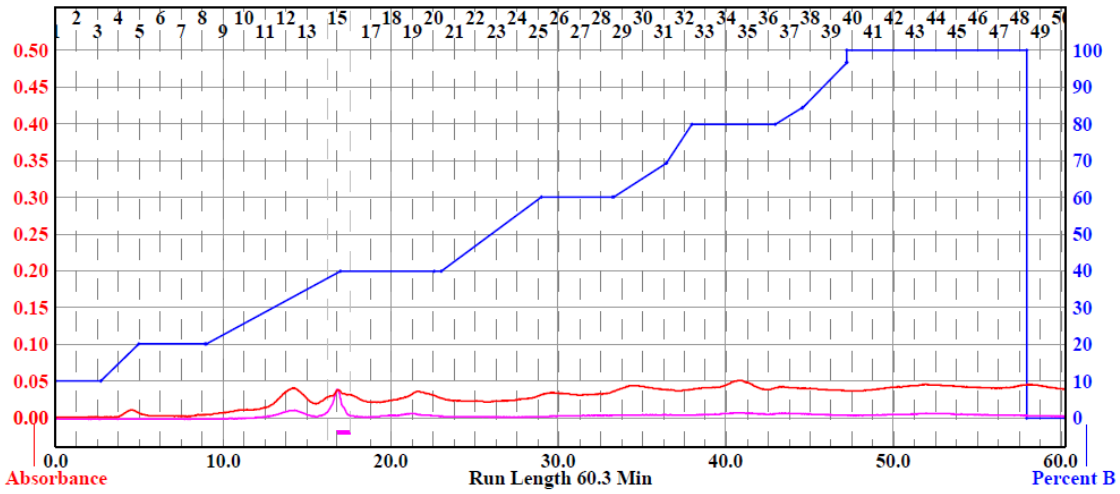


FIGURE 4.16 - The chromatogram for the biotransformation of compound 2 by broth of *Pb*236. Separation conditions: RediSep Column: Silica 40g Gold, Flow Rate: 16 ml/min, Solvent A: n-hexane, Solvent B: ethylacetate, Wavelength 1 (red): 254nm, Wavelength 2 (purple): 280nm.

FIGURE 4.17 is the ^{1}H NMR spectra of the major (first) fraction in the chromatogram. The signals around δ_H 7.0 and 6.7 correspond to the four aromatic hydrogens. However, there is a disappearance of the allylic C=C signal at δ_H 7.32 and an appearance of new signals between δ_H 3.0 and 4.0, confirming the reduction of the olefin bond to a methylene group. Further, there is an appearance of a downfield triplet at δ_H 4.15, which suggest that the carbon bearing these hydrogen is a neighbor to an electronegative atom. Based on further comparison with literature data, the data suggest that **2** was oxidized to **11** in a BV-like manner (SCHEME 4.2).

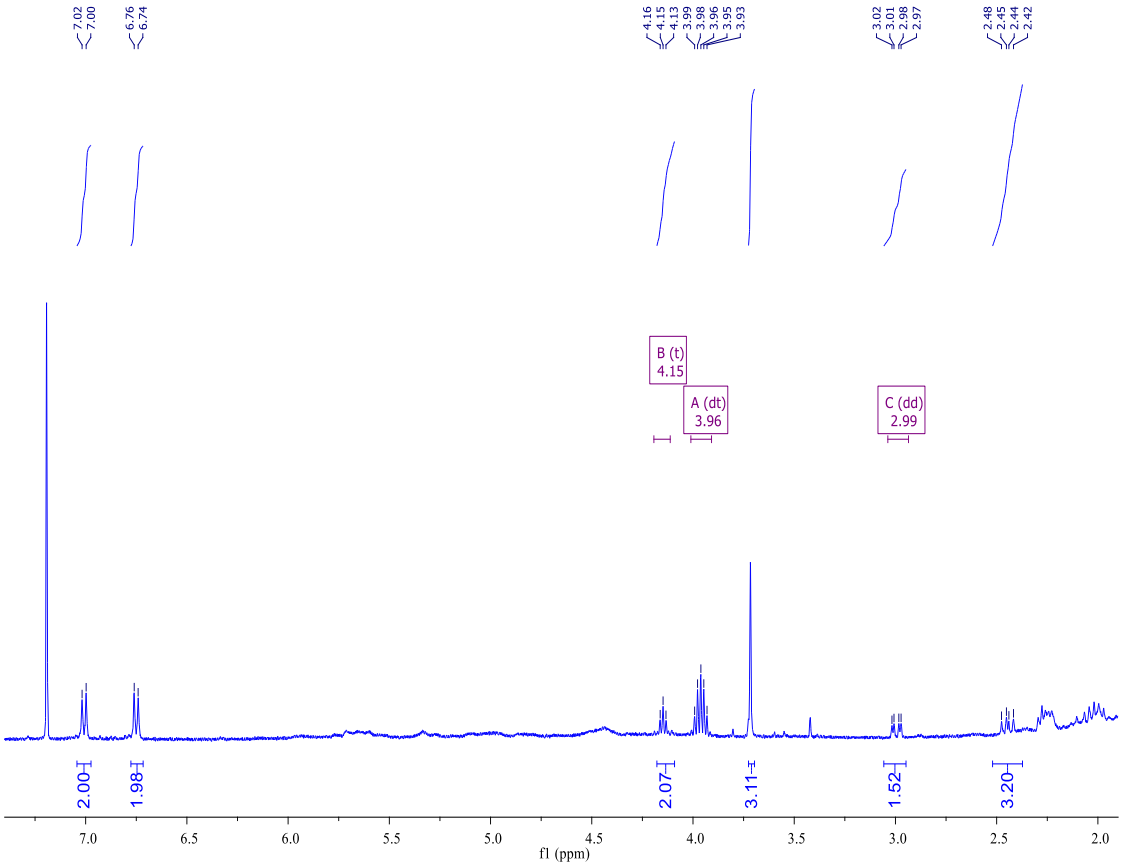
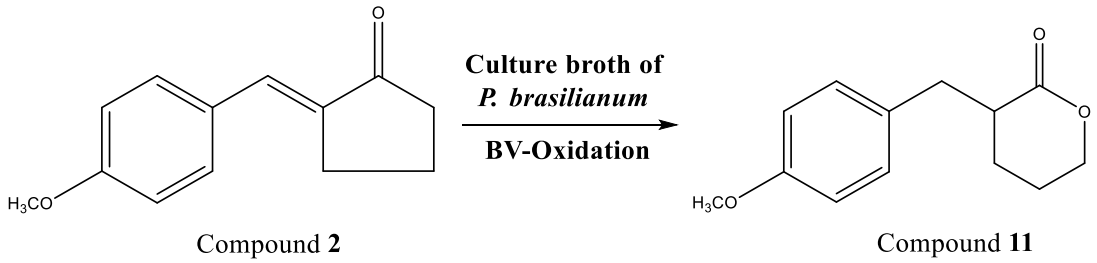


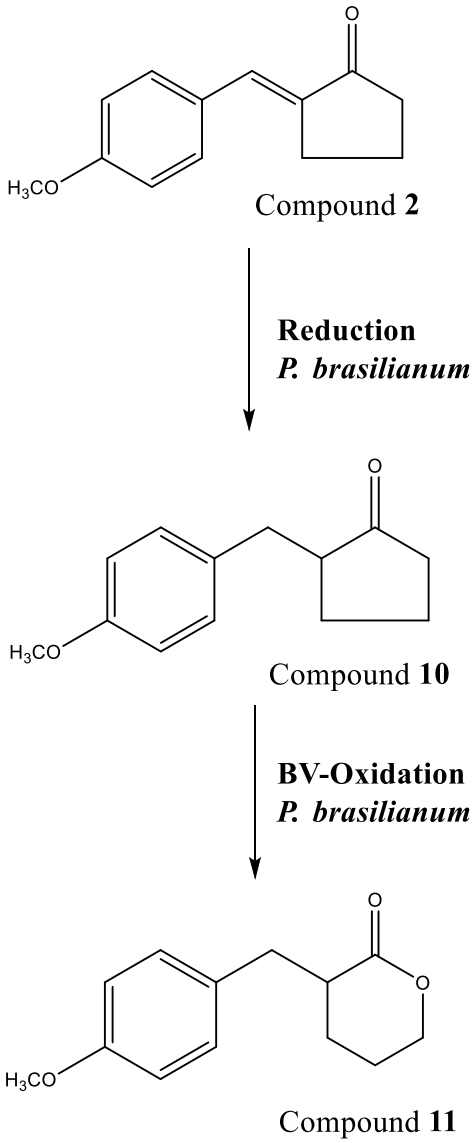
FIGURE 4.17 - ^{1}H NMR spectra of compound **11**.



Compound **2** Compound **11**

SCHEME 4.2 – BV-like oxidation of **2** to **11**.

Our findings showed that *P. brasilianum* was able to catalyze various form of reaction including reduction and Baeyer-Villiger-like oxidation when monoarylidene was fed as a substrate (SCHEME 4.3). However, there was no detection of any biotransformation product when diarylpentadienones are fed into the fungi, even though the reaction lasted for 26 days, with constant stirring in an orbital shaker. The observation that the microbial whole cells of *P. brasilianum* catalyzed the reduction and BV-oxidation of α,β-unsaturated enones agrees with previous observations in our laboratory. In our continuous effort to investigate the oxidative potential of the enzymes of *P. brasilianum* in our laboratory, we have shown that the resting cell of *P. brasilianum* oxidized 1-indanone, an α,β-unsaturated ketones, to dihydrocoumarin in a classical BV reaction as shown in SCHEME 1.10 (PACHECO ET AL., 2012). In another work, we showed that the whole cell of *P. brasilianum* was able to reduce the C=C double bond in Diels-Alder cycloadducts forming (DIN ET AL., 2016).



SCHEME 4.3 - Biotransformation of **2** to **10** and **11** by *P. brasilianum*.

The ability of *P. brasilianum* to produce enzymes of important biotechnological values have been documented. In recent work, the genome of a strain of *P. brasilianum* (LaBioMMi 136) was sequenced and screened for the genes responsible for the oxidoreductase and monooxygenase enzymes. LaBioMMi 136 strain of *P. brasilianum* genome was shown to

contain two conserved sequences similar to the sequences in the old yellow enzyme (OYE) in yeast *S. cerevisiae* (GONZAGA ET AL., 2018). Old yellow enzymes are group of flavin mononucleotide (FMN)-containing, NAD(P)H-dependent oxidoreductases capable of reducing activated C=C double bond of α,β-unsaturated ketones, aldehydes, carboxylic acids, and nitroalkenes through its ene-reductases activity (STEINKELLNER ET AL., 2014; TOOGOOD ET AL., 2010; TOOGOOD & SCRUTTON, 2018). The bioreduction of monoarylidene **2** to **10** and **11** is catalyzed by the ene-reductases. Similarly, over 18 monooxygenases (MOs) sequences were found in the genome of *P. brasilianum* and this clearly indicates that the enzymes of *P. brasilianum* are capable of catalyzing several oxidation reactions, including BV-oxidation. In this experiment, compound **2** was oxidized to compound **11** through the BV-monooxygenase activity of the enzymes in *P. brasilianum*.

For the extracts from the biotransformation of compounds **7** and **5**, classical column chromatography was used for the purification. The TLC photos in FIGURE 4.18 show the fractions obtained from the column chromatography separation of the extract from the biotransformation of compound **7** using the resting cells of *P. brasilianum*. The spot labeled 1 corresponds to the fraction from the gradient separation using Hexane/ethyl acetate solvent mixture in ratio 9:1, spot 2 with Hexane/ethyl acetate solvent

mixture in ratio 8:2, spot 3 corresponds to fraction with Hexane/ethyl acetate solvent mixture in ratio 3:7, while spot 4 comes from ethyl acetate/methanol mixture in ratio 9:1.

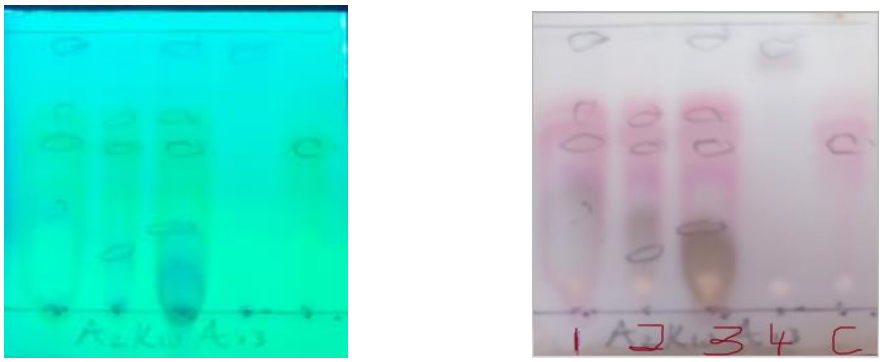


FIGURE 4.18 - The TLC for the extract of biotransformation of **7** by *P. brasilianum.* Photos under UV-Vis at 254nm (left) and immediately after the application of vanillin and heat (right). The spot labeled 1 corresponds to the fraction from the gradient separation using hexane/ethylacetate mixture in ratio 9:1, spot 2 with hexane/ethylacetate mixture in ratio 8:2, spot 3 with hexane/ethylacetate mixture in ratio 3:7 and spot 4 with ethylacetetate/methanol mixture in ratio 9:1.

Similar treatments were given to the extracts from the biotransformation of compound **5**. The TLC in FIGURE 4.19 show the fractions obtained from the separation of the extract from the biotransformation of compound **5** by *P. brasilianum*.

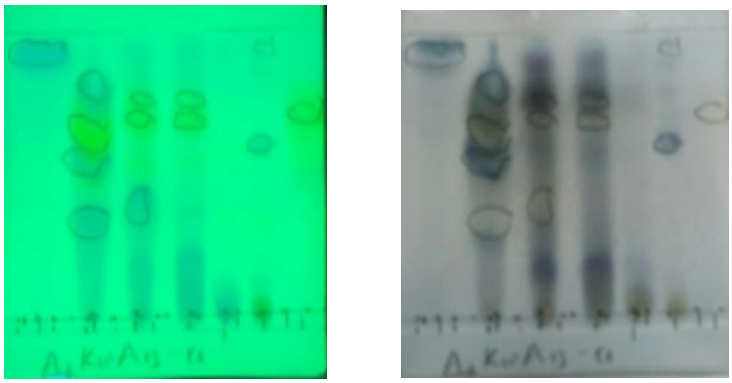
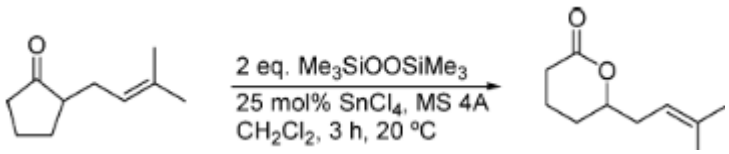


FIGURE 4.19 - The TLC spots correspond to the fractions obtained from the extract of the biotransformation of compound 5 using *P. brasilianum*. Photos under UV-Vis at 254nm (left) and immediately after the application of vanillin and heat (right).

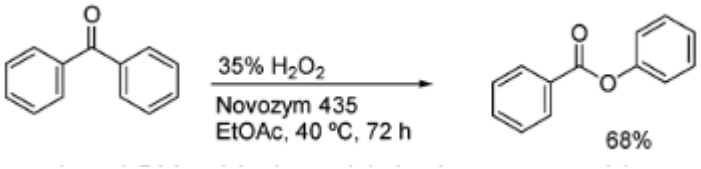
Currently, we do not know why there was no reaction between the fungi and diarylpentadienones. Diarylpentadienones are bis-α,β-unsaturated enones, forming an extended conjugated system. While literature search shows that Baeyer-Villiger oxidation takes place in an array of substrates including cyclic and substituted cyclic, aromatic and linear ketones, aldehydes, carboxylic acids and α-diketones, bicyclic ketones and various steroids (TEN BRINK ET AL., 2004; TOLMIE ET AL., 2019), there is almost no report of such oxidation in conjugated bis-α,β-unsaturated enones like the diarylpentadienones. In the instances of oxidation of unsaturated enones, chemical catalysts such as organic acids, hydrogen peroxide, dioxygen and organometallic catalysts were used. 2-(3-methyl- 2-butenyl)cyclopentanone, an isolated γ,δ-unsaturated enones, was oxidized under anhydrous conditions

with the Lewis acid tin(IV) chloride activating the carbonyl functionality for nucleophilic attack and bis-(trimethylsilyl)peroxide acting as the oxidant as shown in **Erro! Fonte de referência não encontrada.** (OLIVO ET AL., 2000; TEN BRINK ET AL., 2004).



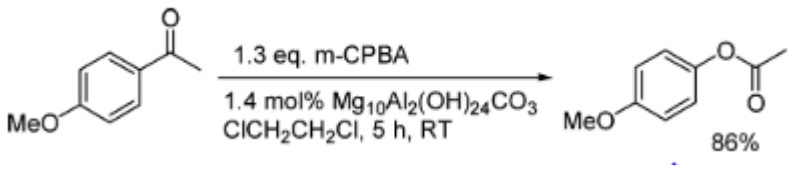
SCHEME 4.4 - BV-Oxidation of 2-(3-methyl- 2-butenyl)cyclopentanone.

In a report, lactases were shown to indirectly catalyse BV-oxidations of conjugated bis-α,β-unsaturated enones by mediating the *in situ* formation of peroxycarboxylic acids from a carboxylic acid or ester and hydrogen peroxide (SCHEME 4.5). As the lactase is not involved in the oxidation of the enones but only responsible for the generation of the oxidant, these reactions are not enantioselective (PCHELKA ET AL., 1998; TEN BRINK ET AL., 2004).



SCHEME 4.5 - Lipase-catalyzed BV oxidation with hydrogen peroxide.

In an extensive study, Kaneda and co-workers developed hydrotalcite catalysts that are active in BV oxidations with m-CPBA and O_2/aldehyde (SCHEME 4.6). Hydrotalcites are made of basic Brucite layers containing Lewis acidic metal cations and anionic interlayers (KANEDA ET AL., 1995, 1994; KANEDA & YAMASHITA, 1996).



SCHEME 4.6 - Hydrotalcite-catalyzed BV oxidation.

It is important to know that most of these reports used either a chemical catalyst or a combination of chemical and biological catalysts. There are few cases, however, in which microbial whole cells are used as a biocatalyst for BV-oxidation of α,β-unsaturated enones. In previous work in our laboratory, we showed that the growing cell of *P. brasilianum* oxidized

1-indanone, an α,β-unsaturated ketones, to dihydrocoumarin in a classical BV reaction as shown in SCHEME 1.10 (PACHECO ET AL., 2012).

Based on this observation, we decided to selectively reduce one olefin bond of the diarylpentadienones without reducing other groups such as the carbonyl functionality or the aromatic substituents. Initially, we employed palladium-on-carbon as a catalyst in the hydrogenation process before using baker's yeast as a biocatalyst.

4.3.1 Catalytic hydrogenation of compound 5 by palladium-on-carbon

Because we were unable to detect a biotransformation product of compound **5**, we decided to develop a marker to use as a calibrator in LC-MS analysis of the fractions by selective reduction of the carbon-carbon double bonds of compound **5**. Previous efforts to selectively reduce the carbon-carbon double bond in α,β-unsaturated enones has been reported to be very difficult (CAMPS ET AL., 1986; KEINAN & PEREZ, 1987; VON HOLLEBEN ET AL., 1994). In those attempts, provided the flow of hydrogen gas was controlled, the catalytic hydrogenation of enones usually gives saturated ketones and the catalysts employed include platinum, platinum oxide, platinum on carbon, rhodium on carbon, palladium on carbon among other metallic catalysts (UTLEY, 1985). The use of 10% palladium-on-

charcoal as the catalyst to hydrogenate the carbon-carbon double bond in enones under one atmosphere of hydrogen at room temperature has been reported to give roughly equal amounts of both the cis and the trans isomers of the corresponding saturated ketone when ethanol or methanol is the solvent. However, the addition of a small amount of dilute hydrochloric acid to the hydrogenation mixture gave only the pure cis isomer of the enones (AUGUSTINE, 1958). Based on this, we, therefore, selected palladium-on-carbon as a catalyst for the catalytic hydrogenation of the olefinic double bonds in compound **5**. After the completion of the reaction, the extract was separated by flash chromatography and the chromatogram for the separation is shown in FIGURE 4.20. The fractions obtained were further compared with compound **5** as shown in the chromatogram in FIGURE 4.21. From the chromatogram, the catalytic hydrogenation process gave a mixture of products in which the olefinic bond and the nitro group were reduced in a non-selective manner. The ^{1}H NMR data show a disappearance of the vinylic signals around δ_H 7.37 and 7.53 and the appearance of new signals around 3-4 ppm confirming the reduction of the vinylic spins. Also, the up-field shift in the ^{1}H NMR signals confirms the reduction of the nitro group.

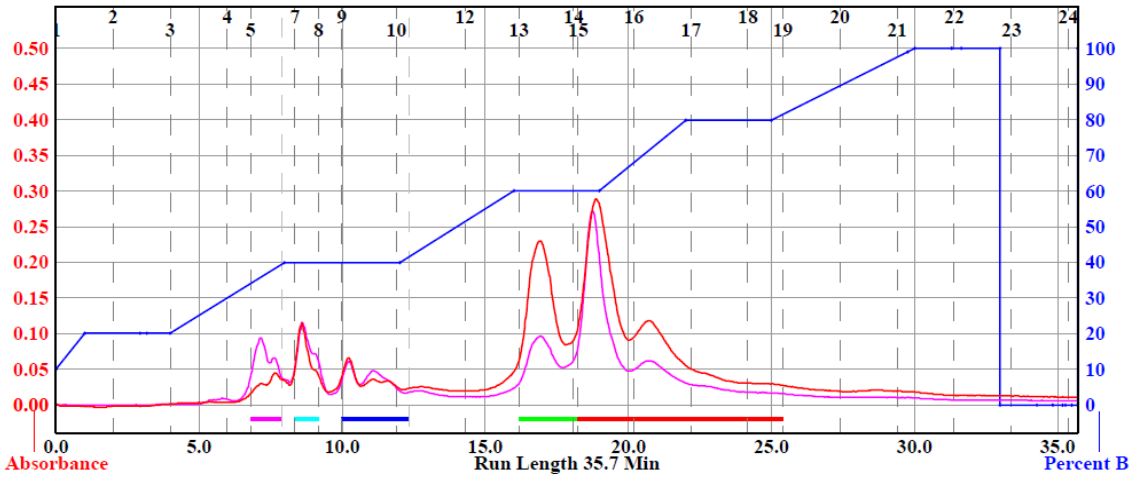


FIGURE 4.20 - The chromatogram for the extract of the catalytic hydrogenation of **5**.

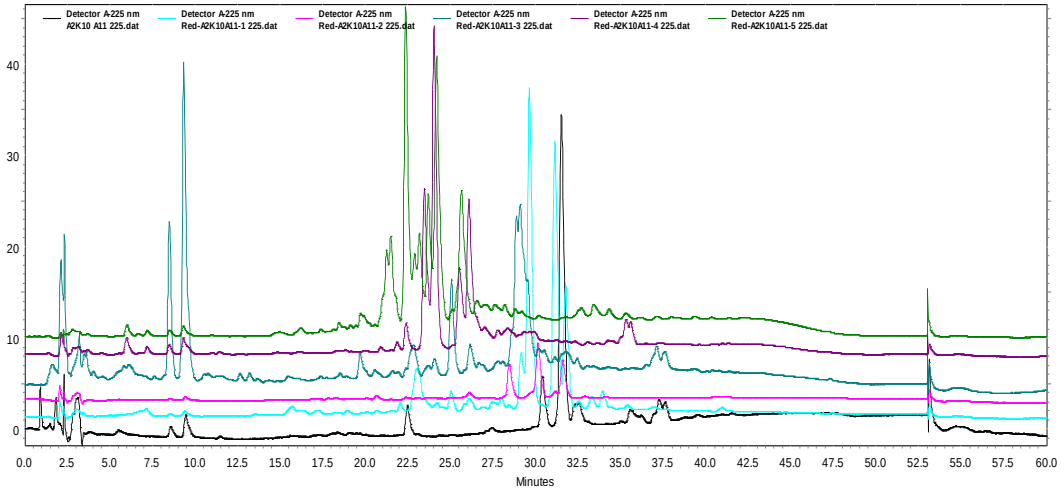


FIGURE 4.21 - Comparison of the fractions obtained in FIGURE 4.20 with **5** using analytical HPLC.

The catalytic hydrogenation of enones using palladium-on-carbon as catalyst reduces the double bonds as well as the nitro group in a non-selective manner. This observation was not completely surprising as previous attempts in our laboratory to hydrogenate bis- α,β-unsaturated enones gave similar results (DIN, n.d.).

4.3.2 Bioreduction of compound 9 by *S. cerevisiae*

To selectively reduce the double bonds in diarylpentadienones, we decided to use baker's yeast *Saccharomyces cerevisiae* as biocatalyst and chose compound **9** as the test compound. The TLC photo in FIGURE 4.22 shows the 10 fractions obtained from the separation of the extract from the bioreduction of compound **9** by baker's yeast. The elution solvent was a mixture of hexane/ethyl acetate in ratio 8:2. The spots labelled 1 to 10 on the plates correspond to each of the fraction in their order of elution while the spots labelled C corresponds to compound **9** (control).

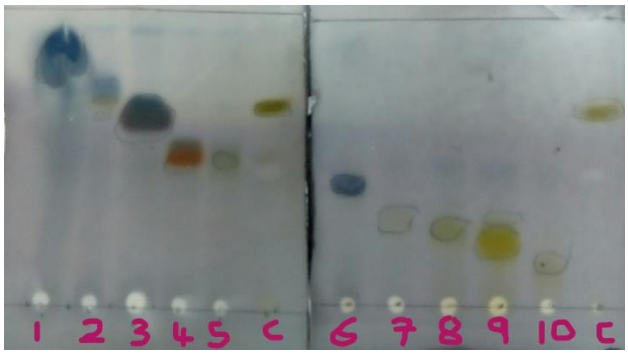


FIGURE 4.22 - The TLC analysis of the 10 fractions obtained from the bioreduction of **9** by baker's yeast. The solvent mixture was hexane/ethyl acetate (8:2). The spots labelled from 1 to 10 on the plates correspond to each of the fraction in their order of elution while the spots labelled C corresponds to the control (**9**).

Of immediate interest among the spots on the TLC is the spot labelled "3", not only because of its retention time that is almost similar to that of the original substrate but also because of its color that is different from that of the substrate. FIGURE 4.23 compares the ^{1}H NMR spectra of compound **9** and spot 3 in the TLC in FIGURE 4.22 above. Conspicuous are the aliphatic hydrogen signals between 1.0 ppm and 3.2 ppm, the aromatic hydrogen signals between 7.35 ppm and 8.2 ppm, the signal corresponding to water at 1.586 ppm and the deuterated chloroform at 7.26 ppm.

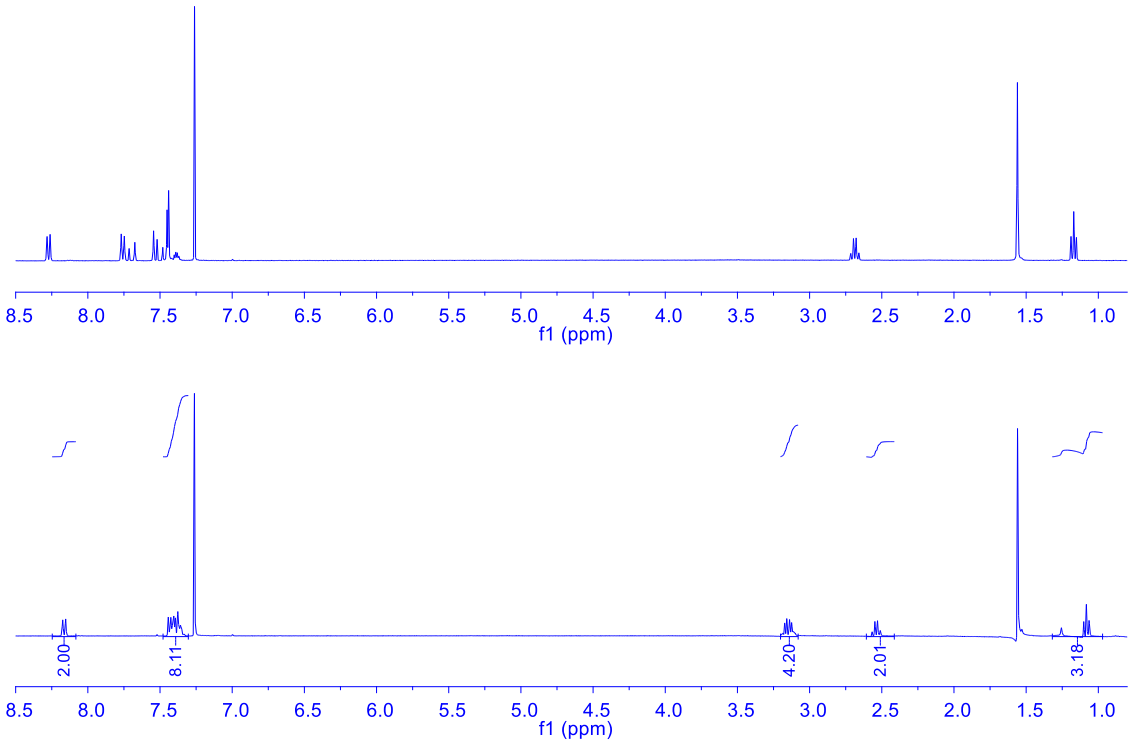


FIGURE 4.23 - Comparison of the ^{1}H NMR spectra of **9** (top) and spot 3 in the TLC (bottom).

The aliphatic region of the spectra is shown in FIGURE 4.24. The triplet at 1.08 ppm with an integral of 3H corresponds to a methyl (-CH_3) group adjacent to a methylene (-CH_2-) group. The quartet at 2.54 ppm with an integral of 2H corresponds to a methylene (-CH_2-) group either adjacent to a methyl group or flanked by a methylene and methine (-CH-) groups on either side. Since no signal corresponds to a methine in the aliphatic region, the methylene group is adjacent to a methyl group to form a –CH_2CH_3– system as found in the original substrate. The multiplet between 3.12 - 3.17 ppm

with an integral of 4H are two overlapping triplets that correspond to two adjacent methylene groups forming a $–CH_2CH_2–$ system, a result of the reduction of the non-aromatic double bond close to the nitro-substituted ring.

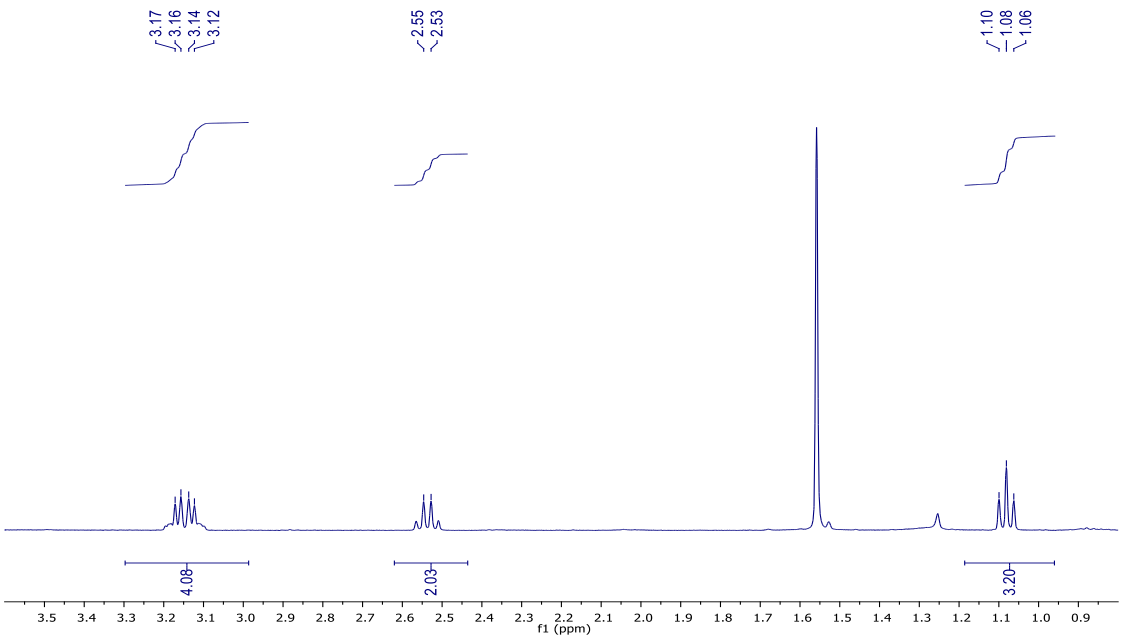


FIGURE 4.24 - The aliphatic region of the spectra of spot 3.

FIGURE 4.25 shows the signals of the aromatic hydrogen of the spectra. The downfield doublet at 8.16 ppm with an integral of 2H corresponds to protons that are close to an electron-withdrawing (an electronegative) group, the para-substituted nitro (NO_2) in this case. The multiplet between 7.30 and 7.45 corresponds to the signals of the aromatic and vinylic hydrogens.

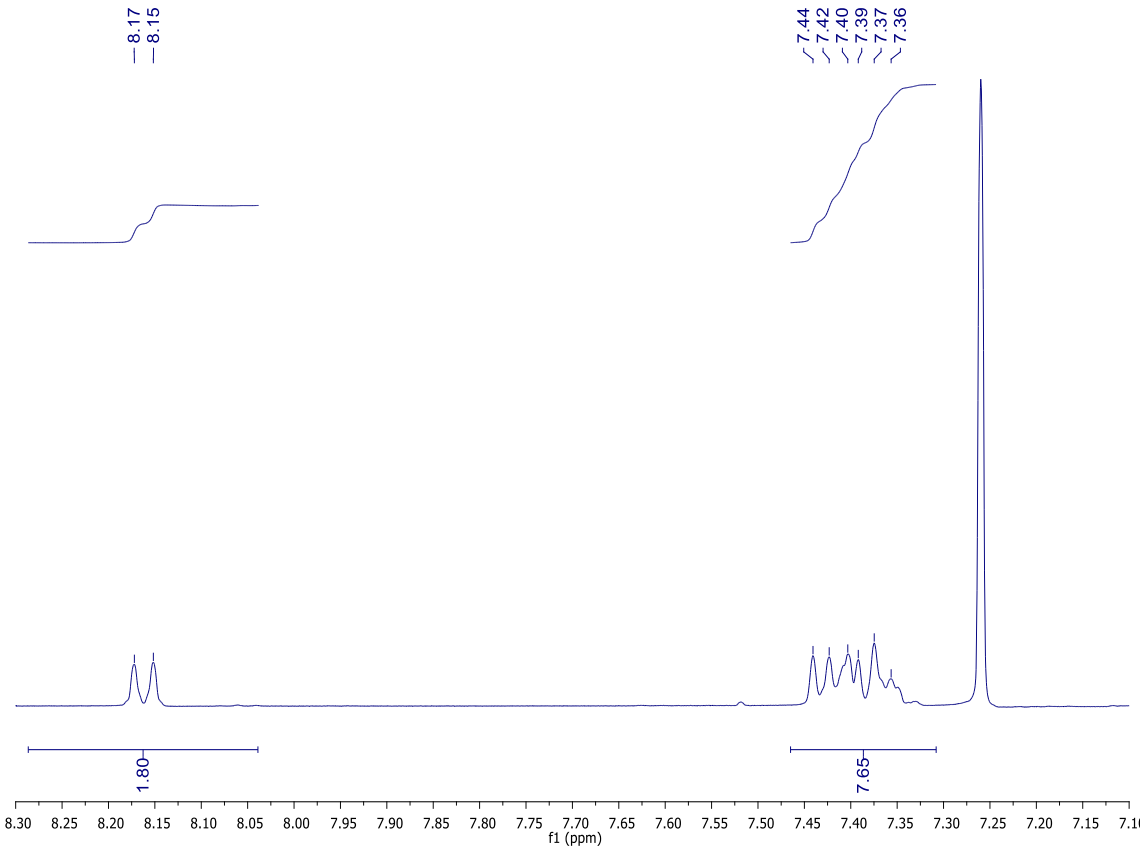
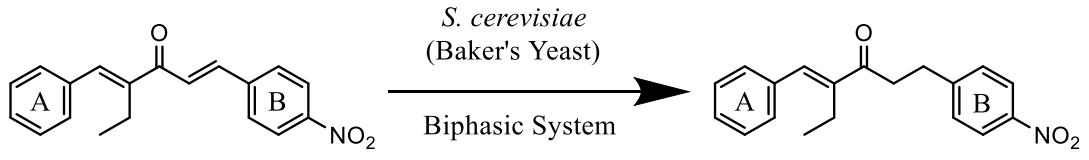


FIGURE 4.25 - The aromatic region of the spectra of spot 3.

Comparison of the spectra (FIGURE 4.23) shows that there is a reduction of the double bond close to the aromatic ring B to form compound **12** as shown in SCHEME 4.7.



Compound **9** Compound **12**

SCHEME 4.7 - The reduction of compound 9 to compound 12 by baker's yeast.

Similarly, the ^{1}H NMR spectra of compound **9** and spot 8 of the TLC are compared in FIGURE 4.26. The first observation was the disappearance of the most downfield signals (signals at around 8.3 ppm) present in compound **9** (FIGURE 4.26). A focus on the aromatic region of the NMR spectra of **9** reveals the presence of the most downfield signals at 8.4 ppm. These signals correspond to the signals of the two aromatic hydrogens on the two carbons flanking the nitro-substituted carbon in **9**. However, there is a displacement of those signals to around 6.60 ppm in fraction 8. Similarly, there was also a displacement of the signals of the other protons in the nitro-substituted ring to around 7.04 ppm. This observation clearly indicates that the nitro group has been reduced to an amine ($–NH_2$) group, thereby making the flanking carbons and their hydrogens less shielded and more upfield.

Furthermore, there was an appearance of two triplets at 3.05 and 2.88, signals that correspond to two adjacent methylene groups forming a $–CH_2CH_2–$ system, which is a result of the reduction of the non-aromatic double bond close to the nitro-substituted ring. The spectra shows signals that indicate that compound **9** was reduced at multiple positions by baker's yeast to form compound **13** as shown in SCHEME 4.8.

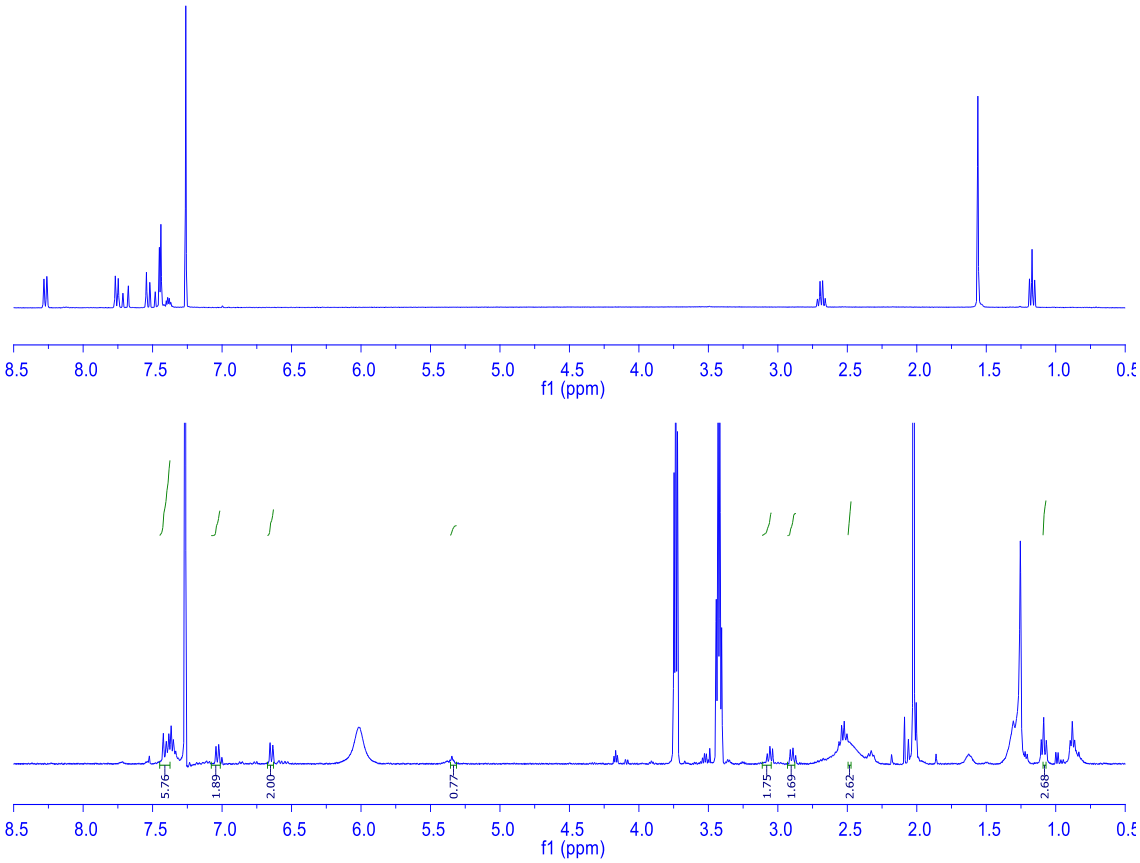
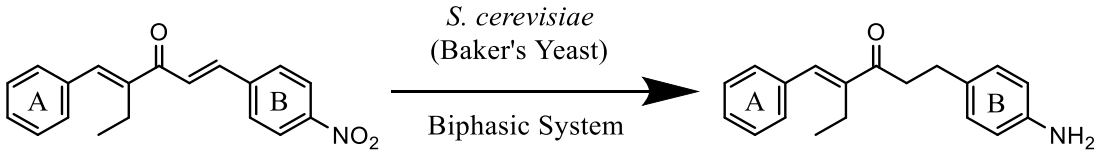


FIGURE 4.26 - Comparison of the ^{1}H NMR spectra of compound 5 (top) and spot 8 in the TLC in FIGURE 4.22 (bottom).

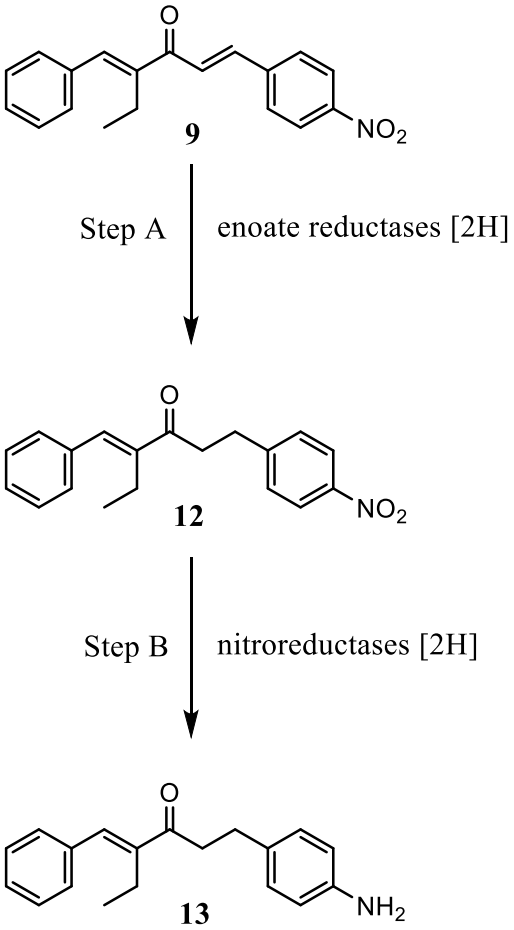


Compound **9** Compound **13**

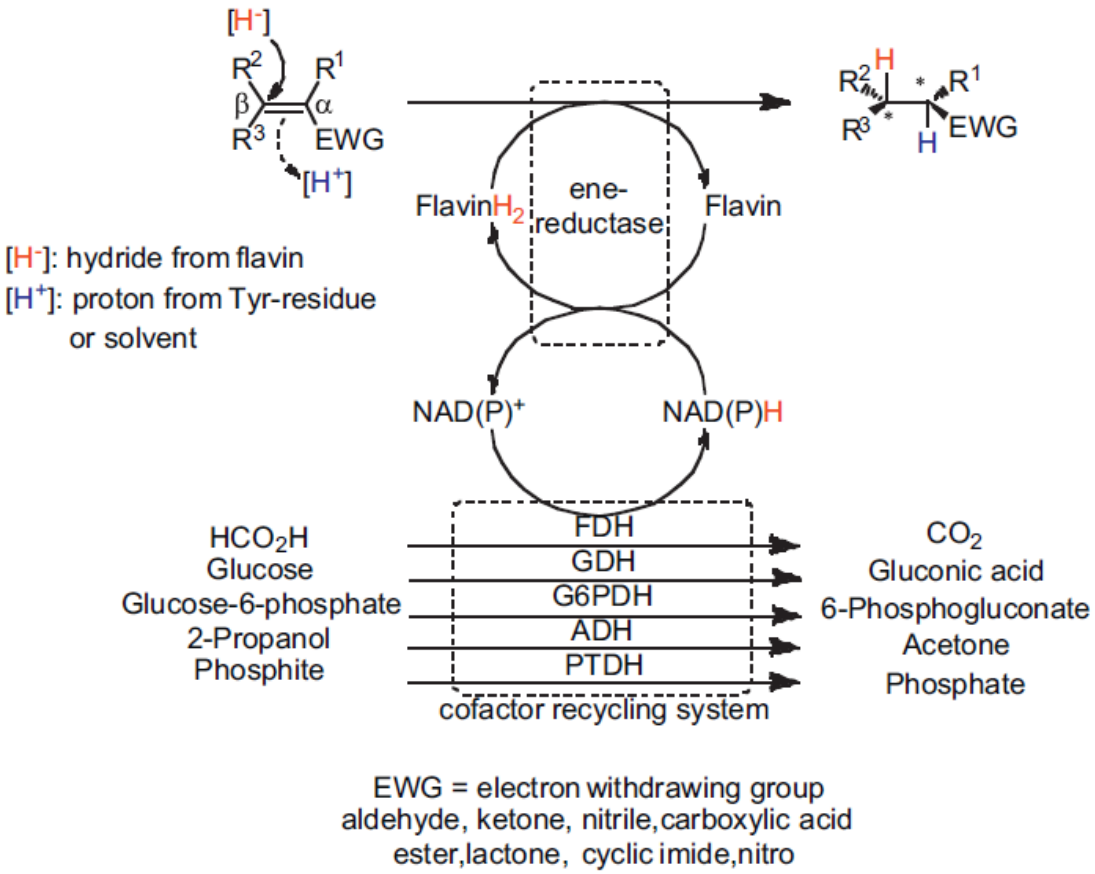
SCHEME 4.8 - The reduction of **9** to **13** by the baker's yeast.

Though there are several reports on the chemoselective bioreduction of the activated carbon-carbon double bonds in carbonyl group compounds, only a handful of data is available on the reduction of such bonds in bis-α,β-unsaturated enones like **9**. In those reports, microbial cells were able to reduce carbon-carbon double bonds and carbon-oxygen bonds forming ketones, alcohols and/or combination of both (ALBUQUERQUE ET AL., 2007; FERREIRA ET AL., 2015; KRAUßER ET AL., 2011; MACIEL FERREIRA ET AL., 2014; SCHAEFER ET AL., 2013; SILVA ET AL., 2012, 2010b). In yeast and other microbes, flavin-dependent ene reductases (ERs), family of the enzymes famously dubbed the Old Yellow Enzymes (OYEs) are responsible for catalyzing the reduction of activated C=C bonds in α,β-unsaturated compounds, giving products with high enantio- and stereo-selectivity in excellent yields (FERREIRA ET AL., 2015; WINKLER ET AL., 2012). The biohydrogenation of **9** in a water/n-hexane biphasic system by the baker's yeast was completely regioselective, resulting in the desymmetrization reduction of C=C bonds, and forming a mixture of mono-reduced enone **12** (SCHEME 4.7) and 4-amino-substituted enone **13** (SCHEME 4.8) after 14 days of constant stirring. Compound **12** results from the selective reduction of the C=C bond between the carbonyl group and ring B of the aromatic system. This suggests the activity of ene-reductase in baker's yeast (Step A

in SCHEME 4.9). The reduction observed was consistent with extant data in the literature that baker's yeast is capable of reducing the vinylic double bond in chalcones and other substituted carbonyl compounds in an aqueous system or biphasic system of aqueous/organic solvents (IRIMIE ET AL., 1997; SILVA ET AL., 2010a). α,β-unsaturated carbonyl enones are capable of promoting NADPH oxidation by OYE and can be reduced at the olefinic bonds. Mechanistically, the desymmetrization reduction involves hydride transfer from the R-face of the NADPH of ene-reductase to a β-carbon of the substrate. This is followed by a proton transfer from the solvent by a tyrosine residue of the enzyme to the α-carbon atom on the opposite face of the olefin as shown in SCHEME 4.10. The net addition of [2H] onto an olefin bond is a Michael-type addition of a complex hydride and results with relative trans-stereospecificity (DURCHSCHEIN ET AL., 2013; FERREIRA ET AL., 2015; HULT & BERGLUND, 2007; VAZ ET AL., 1995; WINKLER ET AL., 2012). In a somewhat complementary manner, the importance of carbonyl groups in the Markovnikov addition of nucleophiles to the vinyl double bonds of vinyl esters have been well documented. Several authors reported that the carbonyl oxygen is bound in an oxyanion hole or, coordinated to the metal ion at the enzyme active site, polarizing the double bond. The partial C=C bond is then attacked by the nucleophiles (WU ET AL., 2006).



SCHEME 4.9 - Biotransformation of **9** by enereductases and nitroreductases.



SCHEME 4.10 - Asymmetric reduction of activated alkenes by ene-reductases.

In chalcones and other α,β-unsaturated enals and enones, there is only one C=C bond available for reduction. However, there are two of such in bis-α,β-unsaturated compounds like enone **9**. The reduction of one C=C bond over the other by the ene reductases, leading to a desymmetrization reaction as seen in SCHEME 4.9 above is an interesting observation. Previous efforts involving the bioreduction of bis-α,β-unsaturated enones followed Markovnikov addition, yielding a mixture of products, including saturated

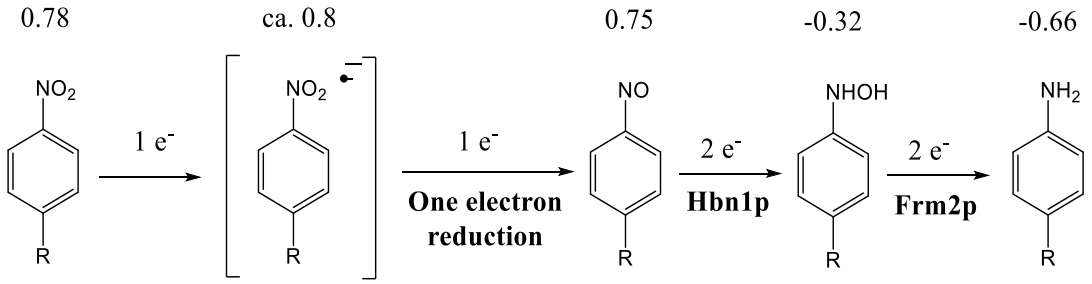
ketone, mono reduced ketone, saturated alcohol and mono reduced alcohol (FERREIRA ET AL., 2015). However, there have been reports of a chemoselective bioreduction of (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one mediated by baker's yeast in an aqueous/organic solvent biphasic system to form only the corresponding saturated ketone, and one would expect that a saturated ketone is also formed in this experiment (SCHAEFER ET AL., 2013). One possible explanation for the desymmetrization reaction is structural constraints. The ethyl ($-CH_2CH_3$) substituents at the β-carbon of the olefin bond between the carbonyl group and ring A of **9** may affect the rate of hydride transfer to the olefinic bond, consistent with a steric hindrance to hydride transfer from the N_5 position of the enzyme-bound reduced flavin (VAZ ET AL., 1995). Another possible reason is the electronic effect of the nitro group on the ring B of **9** by means of the conjugation of π-bonds on this side of the molecule. Nitro-containing compounds possess strong antibiotics properties and this had made it difficult for researchers to employ them as substrates for microorganisms in a biocatalytic process. However, the presence of an electron-withdrawing group on ring B increased the reactivity of the β-carbonyl carbon, making it possible for the Baker's Yeast to reduce the electron-deficient olefinic bond (OHTA ET AL., 1989, 1987; SILVA ET AL., 2010a).

Despite reports that the flavin-containing OYE family of enzymes is capable of acting as both an ene-reductase and a nitroreductase, only a little attention has been given to aromatic ring-substituted nitro group reduction in bis-α,β-unsaturated enones by nitroreductases (TOOGOOD & SCRUTTON, 2019). In this experiment, after the observation of compound **12** in the initial monitoring process, the reaction was left to progress due to a significant amount of unreacted substrate left in the reaction system. With the additional reaction time, the reduction of the nitro group on ring B was observed, forming compound **13**. This observation agrees with the reports that BY is capable of reducing aromatic nitro compounds containing electron-withdrawing groups in ortho, meta or para positions to their corresponding amino derivatives (BAIK ET AL., 1994; IRIMIE ET AL., 1997). The reduction of the nitro substituent, a strong electron-withdrawing group attached in the para position to ring B of compound **9**, is catalyzed by nitroreductases (Step B of SCHEME 4.9) and confirms the ability of the nitro group to increase the reactivity of the compound. The conversion of compound **9** to **13** is a two-step process (SCHEME 4.9) involving the reduction of the vinyl double bond by ene reductase followed by the reduction of the nitro group by nitroreductase (MACIEL FERREIRA ET AL., 2014).

Nitroreductases are widely distributed in bacteria and to a lesser extent, eukaryotes and are capable of reducing aromatic nitro groups to hydroxylamines and amines using FMN or FAD as prosthetic groups and NADH or NADPH as reducing agents (MÜLLER, 2018). There are two types of bacterial nitroreductases. The type I (oxygen-insensitive) catalyses the reduction of nitro groups by sequential two-electron reductions to nitroso, hydroxylamine intermediates and ultimately primary amines (KOBORI ET AL., 2001; ŠARLAUSKAS ET AL., 2004). They play important roles in the reduction of organic nitroaromatic and nitroheterocyclic compounds, such as nitrobenzene, 2,4,6-trinitrotoluene (TNT), nitrofurazone, metronidazole and nitrofurantoin (CABALLERO ET AL., 2005; HAN ET AL., 2007; MCCALLA ET AL., 1978). The type II (oxygen-sensitive) catalyses one-electron reduction of the nitro group, producing a nitro anion radicals that subsequently reacts with oxygen, forming a superoxide radical and regenerating the original nitroaromatic compound, thereby jeoparding the cells to oxidative damage as a result of the accumulation of radical (PETERSON & MASON, 1979; WASSERSCHEID & KEIM, 2000).

In the yeast *Saccharomyces cerevisiae*, two genes, *FRM2* (YCL026c-A) and *HBN1* (YCL026c-B) have been identified and characterized by *in silico* analysis as the genes that encode putative nitroreductase-like proteins

(DE OLIVEIRA ET AL., 2007). Though little is known about the roles of this reductases, experimental data suggest that *Frm2p* may be involved in lipid signaling pathway and cellular homeostasis (MCHALE ET AL., 1996). Further, de Oliveira and co-workers have shown that the *Frm2p* and *Hbn1p* nitroreductases influence the response to oxidative stress in *S. cerevisiae* yeast by modulating the reduced glutathione (GSH) contents and antioxidant enzymatic activities, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (DE OLIVEIRA ET AL., 2010). Based on the foregoing, BY may have earmarked compound **9** as a xenobiotic, prompting it to deploy its nitroreductases against its threat. In the metabolism of nitroaromatic compounds, the nitro reduction is an activating step because of the large electronic change that is generated by the metabolism of a nitro group. The Hammett substituent constant for a para-substituted nitro group is +0.78 while it is -0.34 for the corresponding hydroxylamine (4 electron reduction product) and -0.66 for amine (6 electron reduction product) as shown in SCHEME 4.11 below. This massive increase in electronic effect in this metabolic step result in the formation of electrophilic metabolites which are the basis of cytotoxicity in nitroaromatics (DENNY, 2005).



SCHEME 4.11 - Steps in the reduction of nitroaromatic compounds.

In the oxygen-sensitive reaction, transient nitroaromatic radical anion ($Ar–NO_2^-$) generated in the first step of the reduction process (SCHEME 4.11) can interact with oxygen in the medium and in turn reoxidized to ArNO2 with the formation of superoxide radical (O_2^-) which is acted upon by superoxide dismutase (SOD) to release hydrogen peroxide (H_2O_2), an equally dangerous reactive species. These species are capable of disrupting the cells of the organism and is the source of cytotoxicity in the nitroaromatic compound. The metabolism of **9**, however, appeared to have proceeded through the activity of the oxygen-insensitive nitroreductase in three consecutive two-electron transfers (MACIEL FERREIRA ET AL., 2014).

Several reports recorded the conversion of ketones to alcohols. It is noteworthy that no allylic and/or saturated alcohols were detected in this experiment. Interestingly, this observation was also recorded by Takeshita and co-workers, who noticed that whenever a carbonyl group existed in the

system, a selective reduction took place to give a combination of aminoketones and other compounds without the amino alcohol (TAKESHITA ET AL., 1989).

4.3.3 The biological yeast-catalyzed) and chemical (Pd/C-catalysed) reduction of diarylpentadienones

The biological (*Saccharomyces cerevisiae*, pH 6.5) and chemical (H_2/Palladium-on-carbon) reduction of diarylpentadienones were carried out to synthesized a saturated ketone. In our findings, both the yeast and catalytic hydrogenation processes using Pd/C reduced diarylpentadienones to a varying degree. Both the biological and chemical processes produced reduction products in which one/or both of the α,β-unsaturated double bonds in the diarylpentadienones were reduced. In the case of yeast, a mono-reduced ketone is detected whereas a mixture of products was detected in the Pd/C-catalyzed process. Further, both processes led to the reduction of the nitro groups, giving an amine. In the biological reaction, the reduction of the nitro group took place following the increased reaction time as nitro reduction was not detected in the first 14 days of the reaction.

Whereas the chemical process took between 3-4 hours, not until 14 days of constant stirring was a product detected in the yeast-catalysed reduction. What the biological process lacks in speed, it more than makes up for it in

efficiency and safety. The use of yeast as biocatalysts in the synthetic process offers a more environmentally-friendly alternative to chemical reduction, which uses explosive hydrogen and metal catalysts (TOLMIE ET AL., 2019). The biological reduction took place under mild conditions, such as room temperature and atmospheric pressure. Also, eco-friendly aqueous/organic biphasic solvent was used, minimizing the use of ethyl acetate and other dangerous solvents that are generally associated with chemical processes (PANKE ET AL., 2004). Further, the baker's yeast is cheap and commercially available in everyday store and the set-up of the reduction process is simple and efficient. For the chemical reduction process, specialized apparatus is needed. Besides, it can be difficult to regulate the flux of hydrogen gas used, making the monitoring of the reduction difficult and resulting in a lot of side reduction reaction, including reducing reactive substituent like nitro group (MACIEL FERREIRA ET AL., 2014).

Most biological processes give products with superior enantio- and regioselectivity. The bioreduction of α,β-unsaturated double bonds in enones by yeast gave products with high regioselectivity. This is perhaps the most important advantage of the biological reduction as enantiopure compounds are continually being demanded by the industry for the production of important chemicals including drugs, fragrance, agrochemical among others.

Though more than a single product was formed in the yeast-catalysed process, the other products were formed as a result of the extended reaction time. The reduction with palladium-on-charcoal was not chemoselective, giving a mixture of products that are reduced at various position (FERREIRA ET AL., 2015; MACIEL FERREIRA ET AL., 2014).

In general, baker's yeast is a reservoir of enzymes like ene reductase, nitroreductase and alcohol dehydrogenase, enzymes capable of catalyzing stereospecific reduction. Therefore, the use of microorganism as biocatalyst offers a lot of advantages compared to its chemical counterpart.

5 Conclusions

In the present work, we synthesized some monoarylidenes and diarylpentadienones (compounds **2, 4, 5, 6**) and biotransformed them and some others obtained from the previous works in our laboratory (compounds **7, 8** and **9**) using *Penicillium brasilianum* and *Saccharomyces cerevisiae* to modify them and enlarge the diarylpentadienones library. Our findings show that the resting cells of *P. brasilianum* reduced monoarylidene **2** to **10.** The broth of the fungi catalyzed Baeyer-Villiger oxidation of compound **2** to **11** indicating that *P. brasilianum* is a promising source of Baeyer-Villiger monooxygenases and reductases. However, no biotransformation product was detected when diarylpentadinones **4, 5, 7** and **8** were fed into the fungi. Baker's yeast *Saccharomyces cerevisiae* was able to reduce **9** to **10** and **11** in a regioselective manner. Further investigation into why *P. brasilianum* was not able to reduce and/or oxidized diarylpentadienones used must be carried out.

References

ABONIA, R., INSUASTY, D., CASTILLO, J., INSUASTY, B., QUIROGA, J., NOGUERAS, M., & COBO, J. (2012). Synthesis of novel quinoline-2-one based chalcones of potential anti-tumor activity. *European Journal of Medicinal Chemistry*, *57*, 29–40. https://doi.org/10.1016/j.ejmech.2012.08.039

ALBUQUERQUE, P. M., WITT, M. A., STAMBUK, B. U., & NASCIMENTO, M. DA G. (2007). Influence of sugars on enantioselective reduction using Saccharomyces cerevisiae in organic solvent. *Process Biochemistry*, *42*(2), 141–147. https://doi.org/10.1016/j.procbio.2006.07.034

ALEXANDER FLEMING. (1929). On the antibacterial action of cultures of a Penicillium, with special reference to their use in the isolation of B. injluenzae. *British]Ournal of Experimental Pathology*,.

AUGUSTINE, R. L. (1958). Stereochemistry of the Catalytic Hydrogenation of Some Bicyclic α, β- Unsaturated Ketones. *Journal of Organic Chemistry*, *23*(12), 1853–1856. https://doi.org/10.1021/jo01106a008

BAEYER, A., & VILLIGER, V. (1899). Einwirkung des Caro'schen Reagens auf Ketone. *Berichte Der Deutschen Chemischen Gesellschaft*. https://doi.org/10.1002/cber.189903203151

BAIK, W., LIM HAN, J., CHANG LEE, K., HO LEE, N., HYO KIM, B., & HAHN, J. T. (1994). Selective reduction of aromatic nitro compounds to aromatic amines by Baker's Yeast in basic solution. *Tetrahedron Letters*, *35*(23), 3965–3966. https://doi.org/10.1016/S0040-4039(00)76714-1

BAZIOLI, J. M., AMARAL, L. D. S., FILL, T. P., & RODRIGUES-FILHO, E. (2017a). Insights into penicillium brasilianum secondary metabolism and its biotechnological potential. *Molecules*. https://doi.org/10.3390/molecules22060858

BAZIOLI, J. M., AMARAL, L. D. S., FILL, T. P., & RODRIGUES-FILHO, E. (2017b, June 1). Insights into penicillium brasilianum secondary metabolism and its biotechnological potential. *Molecules*, Vol. 22. https://doi.org/10.3390/molecules22060858

BERA, R., SAHOO, B. K., GHOSH, K. S., & DASGUPTA, S. (2008). Studies on the interaction of isoxazolcurcumin with calf thymus DNA. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2007.08.010

BRAGA, S. F. P., ALVES, É. V. P., FERREIRA, R. S., FRADICO, J. R. B., LAGE, P. S., DUARTE, M. C., … DE OLIVEIRA, R. B. (2014). Synthesis and

evaluation of the antiparasitic activity of bis-(arylmethylidene) cycloalkanones. *European Journal of Medicinal Chemistry*. https://doi.org/10.1016/j.ejmech.2013.11.011

BRITTON, L. N., & MARKOVETZ, A. J. (1977). A novel ketone monooxygenase from Pseudomonas cepacia. Purification and properties. *Journal of Biological Chemistry*.

CABALLERO, A., LÁZARO, J. J., RAMOS, J. L., & ESTEVE-NÚÑEZ, A. (2005). PnrA, a new nitroreductase-family enzyme in the TNT-degrading strain Pseudomonas putida JLR11. *Environmental Microbiology*. https://doi.org/10.1111/j.1462-2920.2005.00801.x

CAMPS, F., COLL, J., & GUITART, J. (1986). Regiospecific reduction of unsaturated conjugated ketones with sodium dithionite under phase transfer catalysis. *Tetrahedron*, *42*(16), 4603–4609. https://doi.org/10.1016/S0040-4020(01)87304-X

CHEN, Y. R., & TAN, T. H. (1998). Inhibition of the c-Jun N-terminal kinase (JNK) signaling pathway by curcumin. *Oncogene*, *17*(2), 173–178. https://doi.org/10.1038/sj.onc.1201941

CHIN-JOE, I., HABERLAND, J., STRAATHOF, A. J. J., JONGEJAN, J. A., LIESE, A., & HEIJNEN, J. J. (2002). Reduction of ethyl 3-oxobutanoate using non-

growing baker's yeast in a continuously operated reactor with cell retention. *Enzyme and Microbial Technology*. https://doi.org/10.1016/S0141-0229(02)00165-5

CHO, H. S., HONG, S. B., & GO, S. J. (2005). First Report of Penicillium brasilianum and P. daleae Isolated from Soil in Korea . *Mycobiology*. https://doi.org/10.4489/myco.2005.33.2.113

CORMA, A., NEMETH, L. T., RENZ, M., & VALENCIA, S. (2001). Sn-zeolite beta as a heterogeneous chemoselective catalyst for Baeyer-Villiger oxidations. *Nature*. https://doi.org/10.1038/35086546

CRIEGEE, R. (1948). Die Umlagerung der Dekalin-peroxydester als Folge von kationischem Sauerstoff. *Justus Liebigs Annalen Der Chemie*. https://doi.org/10.1002/jlac.19485600106

DAMTOFT, S., FRANZYK, H., & JENSEN, S. R. (1995). Biosynthesis of iridoids in Syringa and Fraxinus: Secoiridoid precursors. *Phytochemistry*. https://doi.org/10.1016/0031-9422(95)00211-O

DE OLIVEIRA, I. M., HENRIQUES, J. A. P., & BONATTO, D. (2007). In silico identification of a new group of specific bacterial and fungal nitroreductases-like proteins. *Biochemical and Biophysical Research Communications*, *355*(4), 919–925.

https://doi.org/10.1016/j.bbrc.2007.02.049

DE OLIVEIRA, I. M., ZANOTTO-FILHO, A., MOREIRA, J. C. F., BONATTO, D., & HENRIQUES, J. A. P. (2010). The role of two putative nitroreductases, Frm2p and Hbn1p, in the oxidative stress response in Saccharomyces cerevisiae. *Yeast*. https://doi.org/10.1002/yea.1734

DENNY, W. (2005). Nitroreductase-based GDEPT. *Current Pharmaceutical Design*. https://doi.org/10.2174/1381612023394584

DIN, Z. U. (n.d.). *UNIVERSIDADE FEDERAL DE SÃO CARLOS "Synthesis, structural diversification, biological activities and biotransformation of dibenzylacetones: simple chemistry furnishing potent compounds."*

DIN, Z. U., FILL, T. P., DONATONI, M. C., DOS SANTOS, C. A. A., BROCKSOM, T. J., & RODRIGUES-FILHO, E. (2016). Microbial diversification of Diels–Alder cycloadducts by whole cells of Penicillium brasilianum. *Molecular Diversity*, *20*(4), 877–885. https://doi.org/10.1007/s11030-016-9680-0

DIN, Z. U., & RODRIGUES-FILHO, E. (2016). Optimized one-pot synthesis of monoarylidene and unsymmetrical diarylidene cycloalkanones. *Arabian Journal of Chemistry*. https://doi.org/10.1016/j.arabjc.2016.09.014

DONOGHUE, N. A., & TRUDGILL, P. W. (1975). The Metabolism of Cyclohexanol by Acinetobacter NCIB 9871. *European Journal of Biochemistry*. https://doi.org/10.1111/j.1432-1033.1975.tb20968.x

DOS SANTOS, V. H. P., & DE OLIVEIRA SILVA, E. (2019). Endophytic fungi from the Brazilian flora and their employment in biotransformation reactions. *Quimica Nova*. https://doi.org/10.21577/0100-4042.20170380

DURCHSCHEIN, K., HALL, M., & FABER, K. (2013). Unusual reactions mediated by FMN-dependent ene- and nitro-reductases. *Green Chemistry*, *15*(7), 1764–1772. https://doi.org/10.1039/c3gc40588e

EARLE, M. J., KATDARE, S. P., & SEDDON, K. R. (2004). Paradigm confirmed: The first use of ionic liquids to dramatically influence the outcome of chemical reactions. *Organic Letters*, *6*(5), 707–710. https://doi.org/10.1021/ol036310e

ENGELKING, H., PFALLER, R., WICH, G., & WEUSTER-BOTZ, D. (2006). Reaction engineering studies on β-ketoester reductions with whole cells of recombinant Saccharomyces cerevisiae. *Enzyme and Microbial Technology*. https://doi.org/10.1016/j.enzmictec.2005.07.007

FADDA, A. A., BADRIA, F. A., & EL-ATTAR, K. M. (2010). Synthesis and

evaluation of curcumin analogues as cytotoxic agents. *Medicinal Chemistry Research*, *19*(5), 413–430. https://doi.org/10.1007/s00044-009-9199-3

FANG, J., LU, J., & HOLMGREN, A. (2005). Thioredoxin reductase is irreversibly modified by curcumin: A novel molecular mechanism for its anticancer activity. *Journal of Biological Chemistry*, *280*(26), 25284–25290. https://doi.org/10.1074/jbc.M414645200

FERREIRA, I. M., DE VASCONCELLOS, S. P., DA CRUZ, J. B., COMASSETO, J. V., PORTO, A. L. M., & ROCHA, L. C. (2015). Hydrogenation of bis-α,β-unsaturated enones mediated by filamentous fungi. *Biocatalysis and Agricultural Biotechnology*, *4*(2), 144–149. https://doi.org/10.1016/j.bcab.2015.03.001

FILL, TAICIA P., DOS SANTOS, R. M. G., BARISSON, A., RODRIGUES-FILHO, E., & SOUZA, A. Q. L. (2009). Co-Production of bisphenylpropanoid amides and meroterpenes by an endophytic Penicillium brasilianum found in the root bark of Melia azedarach. *Zeitschrift Fur Naturforschung - Section C Journal of Biosciences*. https://doi.org/10.1515/znc-2009-5-609

FILL, TAICIA PACHECO, DA SILVA, J. V., DE OLIVEIRA, K. T., DA SILVA, B. F., & RODRIGUES-FO, E. (2012). Oxidative potential of some endophytic

fungi using 1-indanone as a substrate. *Journal of Microbiology and Biotechnology*, *22*(6), 832–837. https://doi.org/10.4014/jmb.1112.12014

FORNEY, F. W., & MARKOVETZ, A. J. (1969). An enzyme system for aliphatic methyl ketone oxidation. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1016/0006-291X(69)90876-6

FRISVAD, J. C., & SAMSON, R. A. (2004). Polyphasic taxonomy of Penicillium subgenus Penicillium: A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. *Studies in Mycology*.

FRISVAD, J. C., SMEDSGAARD, J., LARSEN, T. O., & SAMSON, R. A. (2004). Mycotoxins, drugs and other extrolites produced by species in Penicillium subgenus Penicillium. *Studies in Mycology*.

FUJITA, T., MAKISHIMA, D., AKIYAMA, K., & HAYASHI, H. (2002). New convulsive compounds, brasiliamides a and b, from penicillium brasilianum batista JV-379. *Bioscience, Biotechnology and Biochemistry*. https://doi.org/10.1271/bbb.66.1697

GERIS DOS SANTOS, R. M., & RODRIGUES-FO, E. (2002). Meroterpenes from Penicillium sp found in association with Melia azedarach.

Phytochemistry. https://doi.org/10.1016/S0031-9422(02)00379-5

GERIS DOS SANTOS, R. M., RODRIGUES-FO, E., CALDAS ROCHA, W., & SIMAS TEIXEIRA, M. F. (2003). Endophytic fungi from Melia azedarach. *World Journal of Microbiology and Biotechnology*. https://doi.org/10.1023/A:1026000731189

GIRAUD, F., GIRAUD, T., AGUILETA, G., FOURNIER, E., SAMSON, R., CRUAUD, C., … DUPONT, J. (2010). Microsatellite loci to recognize species for the cheese starter and contaminating strains associated with cheese manufacturing. *International Journal of Food Microbiology*. https://doi.org/10.1016/j.ijfoodmicro.2009.11.014

GONZAGA, L., ALMEIDA, P. DE, CERDEIRA, L. T., SAMBORSKYY, M., TEREZA, A., VASCONCELOS, R. DE, … RODRIGUES-FILHO, E. (2018). *crossm Draft Genome Sequence of the Fungus Penicillium brasilianum*. 1–2.

GRIFFIN, M., & TRUDGILL, P. W. (1976). Purification and Properties of Cyclopentanone Oxygenase of Pseudomonas NCIB 9872. *European Journal of Biochemistry*. https://doi.org/10.1111/j.1432-1033.1976.tb10222.x

GUPTA, S. C., PRASAD, S., KIM, J. H., PATCHVA, S., WEBB, L. J., PRIYADARSINI, I. K., & AGGARWAL, B. B. (2011). Multitargeting by

curcumin as revealed by molecular interaction studies. *Natural Product Reports*, *28*(12), 1937–1955. https://doi.org/10.1039/c1np00051a

HAN, F., LIU, S., HO, B., YAN, Z., & YAN, X. (2007). Alterations in rdxA and frxA genes and their upstream regions in metronidazole-resistant Helicobacter pylori isolates. *Research in Microbiology*, *158*(1), 38–44. https://doi.org/10.1016/j.resmic.2006.10.001

HENNINGSEN, B. M., HON, S., COVALLA, S. F., SONU, C., ARGYROS, D. A., BARRETT, T. F., … ZELLE, R. M. (2015). *Saccharomyces cerevisiae with NADPH-Specific Alcohol Dehydrogenase*. *81*(23), 8108–8117. https://doi.org/10.1128/AEM.01689-15.Editor

HULT, K., & BERGLUND, P. (2007). Enzyme promiscuity: mechanism and applications. *Trends in Biotechnology*, *25*(5), 231–238. https://doi.org/10.1016/j.tibtech.2007.03.002

IRIMIE, F. D., PAIZS, C., TOŞA, M. I., AFLOROAEI, C., & MICLĂUŞ, V. (1997). Bakers' yeast-mediated reductions of some nitro-dibenzofurans. *Heterocyclic Communications*, *3*(6), 549–553. https://doi.org/10.1515/HC.1997.3.6.549

JOURNAL, T. M. (2009). *Isolation and Identification of Antibiotics Produced By Penicillium Brasilianum Batista Isolated From Salahaddin Materials*

& Methods. *2009*(1).

KAMERBEEK, N. M., JANSSEN, D. B., VAN BERKEL, W. J. H., & FRAAIJE, M. W. (2003). Baeyer–Villiger Monooxygenases, an Emerging Family of Flavin-Dependent Biocatalysts. *Advanced Synthesis & Catalysis*, *345*(67), 667–678. https://doi.org/10.1002/adsc.200303014

KANEDA, K., UENO, S., & IMANAKA, T. (1995). Catalysis of transition metal-functionalized hydrotalcites for the Baeyer-Villiger oxidation of ketones in the presence of molecular oxygen and benzaldehyde. *Journal of Molecular Catalysis. A, Chemical*, Vol. 102, pp. 135–138. https://doi.org/10.1016/1381-1169(95)00055-0

KANEDA, K., UENO, S., IMANAKA, T., SHIMOTSUMA, E., NISHIYAMA, Y., & ISHII, Y. (1994). Baeyer-Villiger Oxidation of Ketones Using Molecular Oxygen and Benzaldehyde in the Absence of Metal Catalysts. *Journal of Organic Chemistry*, *59*(11), 2915–2917. https://doi.org/10.1021/jo00090a001

KANEDA, K., & YAMASHITA, T. (1996). Heterogeneous Baeyer-Villiger oxidation of ketones using m- chloroperbenzoic acid catalyzed by hydrotalcites. *Tetrahedron Letters*, *37*(26), 4555–4558. https://doi.org/10.1016/0040-4039(96)00902-1

KEINAN, E., & PEREZ, D. (1987). Silicon Hydrides and Molybdenum(0) Catalyst: A Novel Approach for Conjugate Reduction of α,β-Unsaturated Carbonyl Compounds. *Journal of Organic Chemistry*, *52*(12), 2576–2580. https://doi.org/10.1021/jo00388a042

KHOR, G. K., & UZIR, M. H. (2011). Saccharomyces cerevisiae: A potential stereospecific reduction tool for biotransformation of mono- and sesquiterpenoids. *Yeast*. https://doi.org/10.1002/yea.1827

KITTRELL, F. S., MEDINA, D., GREGORY, R. R., VARMA, M., & VARMA, R. S. (1992). Nuclear Type II Sites and Malignant Cell Proliferation: Inhibition by 2,6-Bis-benzylidenecyclohexanones. *Cancer Research*, *52*(9), 2482–2488.

KOBORI, T., SASAKI, H., LEE, W. C., ZENNO, S., SAIGO, K., MURPHY, M. E. P., & TANOKURA, M. (2001). Structure and site-directed mutagenesis of a flavoprotein from Escherichia coli that reduces nitrocompounds. Alteration of pyridine nucleotide binding by a single amino acid substitution. *Journal of Biological Chemistry*, *276*(4), 2816–2823. https://doi.org/10.1074/jbc.M002617200

KOZLOVSKII, A. G., ZHELIFONOVA, V. P., & ANTIPOVA, T. V. (2013). Fungi of the genus Penicillium as producers of physiologically active

compounds (Review). *Applied Biochemistry and Microbiology*. https://doi.org/10.1134/S0003683813010092

KRAUßER, M., WINKLER, T., RICHTER, N., DOMMER, S., FINGERHUT, A., HUMMEL, W., & GRÖGER, H. (2011). Combination of C=C bond formation by Wittig reaction and enzymatic C=C bond reduction in a one-pot process in water. *ChemCatChem*, *3*(2), 293–296. https://doi.org/10.1002/cctc.201000391

KREHER, U. P., ROSAMILIA, A. E., RASTON, C. L., SCOTT, J. L., & STRAUSS, C. R. (2003). Direct preparation of monoarylidene derivatives of aldehydes and enolizable ketones with DIMCARB. *Organic Letters*, *5*(17), 3107–3110.

KROW, G. R. (1993). The Baeyer-VilligerOxidation of Ketones and Aldehydes. In *Organic Reactions*. https://doi.org/10.1002/0471264180.or043.03

KUMAR, A. K., CHALAMAIAH, M., KUMAR, R. R., & BABU, K. N. (2009). Preliminary Studies on Biotransformation of Drumstick (Moringa oleifera) and Watermelon (Citrullus lanatus) Seed Oils using Baker's Yeast. *Asian Journal of Biological Sciences*, *2*(4), 118–123. https://doi.org/10.3923/ajbs.2009.118.123

KWON, O. E., RHO, M. C., SONG, H. Y., LEE, S. W., CHUNG, M. Y., LEE, J. H., … KIM, Y. K. (2002). Phenylpyropene A and B, new inhibitors of acyl-CoA: Cholesterol acyltransferase produced by Penicillin griseofulvum F1959. *Journal of Antibiotics*. https://doi.org/10.7164/antibiotics.55.1004

LEE, K. H., FARIDA, F. H., SYAHIDA, A., ABAS, F., SHAARI, K., ISRAF, D. A., & LAJIS, N. H. (2009). Synthesis and biological evaluation of curcumin-like diarylpentanoid analogues for anti-inflammatory, antioxidant and anti-tyrosinase activities. *European Journal of Medicinal Chemistry*. https://doi.org/10.1016/j.ejmech.2009.03.020

LEISCH, H., MORLEY, K., & LAU, P. C. K. (2011). Baeyer-villiger monooxygenases: More than just green chemistry. *Chemical Reviews*, *111*(7), 4165–4222. https://doi.org/10.1021/cr1003437

LI, Q., CHEN, J., LUO, S., XU, J., HUANG, Q., & LIU, T. (2015). Synthesis and assessment of the antioxidant and antitumor properties of asymmetric curcumin analogues. *European Journal of Medicinal Chemistry*, Vol. 93, pp. 461–469. https://doi.org/10.1016/j.ejmech.2015.02.005

LI, Y., CUI, F., LIU, Z., XU, Y., & ZHAO, H. (2007). Improvement of xylanase production by Penicillium oxalicum ZH-30 using response surface

methodology. *Enzyme and Microbial Technology*. https://doi.org/10.1016/j.enzmictec.2006.10.015

LIGHT, R. J., & HAUSER, C. R. (1961). Condensations of Dialkali β-Diketones with Ketones or Aldehydes to Form Hydroxy β-Diketones. Dehydration Products. Equilibrium Factors. *Journal of Organic Chemistry*. https://doi.org/10.1021/jo01065a007

LIU, X., ZHU, T. S., SUN, P. D., & XU, J. H. (2001). Asymmetric reduction of aromatic ketones by the baker's yeast in organic solvent systems. *Synthetic Communications*, *31*(10), 1521–1526. https://doi.org/10.1081/SCC-100104064

LUCAS, E. M. F., DE CASTRO, M. C. M., & TAKAHASHI, J. A. (2007). Antimicrobial properties of sclerotiorin, isochromophilone VI and pencolide, metabolites from a Brazilian cerrado isolate of Penicillium sclerotiorum Van Beyma. *Brazilian Journal of Microbiology*. https://doi.org/10.1590/S1517-83822007000400036

LUDEMANN, V., GRECO, M., RODRÍGUEZ, M. P., BASÍLICO, J. C., & PARDO, A. G. (2010). Conidial production by Penicillium nalgiovense for use as starter cultures in dry fermented sausages by solid state fermentation. *LWT - Food Science and Technology*.

https://doi.org/10.1016/j.lwt.2009.07.011

MACIEL FERREIRA, I., COUTINHO ROCHA, L., AKINOBO YOSHIOKA, S., NITSCHKE, M., HAROLDO JELLER, A., PIZZUTI, L., … PORTO, A. L. M. (2014). Chemoselective reduction of chalcones by whole hyphae of marine fungus Penicillium citrinum CBMAI 1186, free and immobilized on biopolymers. *Biocatalysis and Agricultural Biotechnology*, *3*(4), 358–364. https://doi.org/10.1016/j.bcab.2014.04.001

MATSUDA, T., YAMANAKA, R., & NAKAMURA, K. (2009). Recent progress in biocatalysis for asymmetric oxidation and reduction. *Tetrahedron Asymmetry*. https://doi.org/10.1016/j.tetasy.2008.12.035

MATSUMOTO, T., TAKAHASHI, S., KAIEDA, M., UEDA, M., TANAKA, A., FUKUDA, H., & KONDO, A. (2001). Yeast whole-cell biocatalyst constructed by intracellular overproduction of Rhizopus oryzae lipase is applicable to biodiesel fuel production. *Applied Microbiology and Biotechnology*. https://doi.org/10.1007/s002530100733

MAYDT, D., DE SPIRT, S., MUSCHELKNAUTZ, C., STAHL, W., & MÜLLER, T. J. J. (2013). Chemical reactivity and biological activity of chalcones and other α,β-unsaturated carbonyl compounds. *Xenobiotica*. https://doi.org/10.3109/00498254.2012.754112

MCCALLA, D. R., KAISER, C., & GREEN, M. H. L. (1978). Genetics of nitrofurazone resistance in Escherichia coli. *Journal of Bacteriology*, *133*(1), 10–16.

MCHALE, M. W., KROENING, K. D., & BERNLOHR, D. A. (1996). Identification of a class of Saccharomyces cerevisiae mutants defective in fatty acid repression of gene transcription and analysis of the frm2 gene. *Yeast*. https://doi.org/10.1002/(SICI)1097-0061(19960330)12:4<319::AID-YEA914>3.0.CO;2-#

MICHELIN, R. A., SGARBOSSA, P., SCARSO, A., & STRUKUL, G. (2010). The Baeyer-Villiger oxidation of ketones: A paradigm for the role of soft Lewis acidity in homogeneous catalysis. *Coordination Chemistry Reviews*. https://doi.org/10.1016/j.ccr.2009.09.014

MIYAGI, H., KAWAI, S., & MURATA, K. (2009). Two sources of mitochondrial NADPH in the yeast Saccharomyces cerevisiae. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M804100200

MOHAN, R., SIVAK, J., ASHTON, P., RUSSO, L. A., PHAM, B. Q., KASAHARA, N., … FINI, M. E. (2000). Curcuminoids inhibit the angiogenic response stimulated by fibroblast growth factor-2, including expression of matrix metalloproteinase gelatinase B. *Journal of Biological Chemistry*,

275(14), 10405–10412. https://doi.org/10.1074/jbc.275.14.10405

MÜLLER, F. (2018). Chemistry and biochemistry of flavoenzymes. In *Chemistry and Biochemistry of Flavoenzymes* (Vol. 2). https://doi.org/10.1201/9781351070584

NAFISI, S., ADELZADEH, M., NOROUZI, Z., & SARBOLOUKI, M. N. (2009). Curcumin binding to DNA and RNA. *DNA and Cell Biology*. https://doi.org/10.1089/dna.2008.0840

NECKERS, L., TREPEL, J., LEE, S., CHUNG, E.-J., LEE, M.-J., JUNG, Y.-J., & MARCU, M. (2006). Curcumin is an Inhibitor of p300 Histone Acetylatransferase. *Medicinal Chemistry*, *2*(2), 169–174. https://doi.org/10.2174/157340606776056133

NICOLETTI, R., LOPEZ-GRESA, M. P., MANZO, E., CARELLA, A., & CIAVATTA, M. L. (2007). Production and fungitoxic activity of Sch 642305, a secondary metabolite of Penicillium canescens. *Mycopathologia*. https://doi.org/10.1007/s11046-007-9015-x

NIKOLOVA, P., & WARD, O. P. (1992). *Whole cell yeast biotransformations in two-phase systems : effect of solvent on product formation and cell structure*. *10*, 169–177.

OHTA, H., KOBAYASHI, N., & OZAKI, K. (1989). Asymmetric Reduction of

Nitro Olefins by Fermenting Bakers Yeast. *Journal of Organic Chemistry*, *54*(8), 1802–1804. https://doi.org/10.1021/jo00269a011

OHTA, H., OZAKI, K., & TSUCHIHASHI, G. (1987). Asymmetric Hydrogenation of 2-Aryl-1-nitropropenes by Fermenting Bakers' Yeast. *Chemistry Letters*. https://doi.org/10.1246/cl.1987.191

OLIVO, H. F., VELÁZQUEZ, F., & TREVISAN, H. C. (2000). Synthetic studies on the trans-chlorocyclopropane dienyne side chain of callipeltoside A. *Organic Letters*, *2*(25), 4055–4057. https://doi.org/10.1021/ol006696i

OUGHAM, H. J., TAYLOR, D. G., & TRUDGILL, P. W. (1983). Camphor revisited: Involvement of a unique monooxygenase in metabolism of 2-oxo-Δ3-4,5,5-trimethylcyclopentenylacetic acid by Pseudomonas putida. *Journal of Bacteriology*, *153*(1), 140–152.

OZTURK, K., SAYIN, S., & CELEBI OLCUM, N. (2016). Computational identification of promiscous enzyme activity for the Morita-Baylis-Hillman reaction. *FEBS Journal*.

PACHECO, T., VINICIUS, J., OLIVEIRA, K. T. DE, FERREIRA, B., & RODRIGUES-FO, E. (2012). *Oxidative Potential of Some Endophytic Fungi Using 1-Indanone as a Substrate*. *22*, 832–837.

PANKE, S., HELD, M., & WUBBOLTS, M. (2004). Trends and innovations in

industrial biocatalysis for the production of fine chemicals. *Current Opinion in Biotechnology*. https://doi.org/10.1016/j.copbio.2004.06.011

PATEL, R. N. (2016). Green Processes for the Synthesis of Chiral Intermediates for the Development of Drugs. In *Green Biocatalysis* (pp. 71–114). https://doi.org/10.1002/9781118828083.ch4

PATEL, R. N. (2018). CHAPTER 5: Bristol-Myers Squibb: Preparation of Chiral Intermediates for the Development of Drugs and APIs. In *RSC Catalysis Series*. https://doi.org/10.1039/9781782629993-00125

PCHELKA, B. K., GELO-PUJIC, M., & GUIBÉ-JAMPEL, E. (1998). Chemoenzymatic autocatalytic Baeyer-Villiger oxidation. *Journal of the Chemical Society - Perkin Transactions 1*, (17), 2625–2627. https://doi.org/10.1039/a804426k

PEDERSEN, U., RASMUSSEN, P. B., & LAWESSON, S. -O. (1985). Synthesis of Naturally Occurring Curcuminoids and Related Compounds. *Liebigs Annalen Der Chemie*, *1985*(8), 1557–1569. https://doi.org/10.1002/jlac.198519850805

PETERSON, J., & MASON, P. (1979). and Jordan. *The Journal of Biological Chemistry*, *254*(151), 4009–4014.

RANČIĆ, A., SOKOVIĆ, M., KARIOTI, A., VUKOJEVIĆ, J., & SKALTSA, H.

(2006). Isolation and structural elucidation of two secondary metabolites from the filamentous fungus Penicillium ochrochloron with antimicrobial activity. *Environmental Toxicology and Pharmacology*. https://doi.org/10.1016/j.etap.2005.12.003

RASTON, C. L., & SCOTT, J. L. (2000). Chemoselective, solvent-free aldol condensation reaction. *Green Chemistry*, *2*(2), 49–52. https://doi.org/10.1039/a907688c

ROSAMILIA, A. E., STRAUSS, C. R., & SCOTT, J. L. (2007). Distillable ionic liquids for a new multicomponent reaction. *Pure and Applied Chemistry*, *79*(11), 1869–1877. https://doi.org/10.1351/pac200779111869

ŠARLAUSKAS, J., NEMEIKAITE-ČENIENE, A., ANUSEVIČIUS, Ž., MISEVIČIENE, L., JULVEZ, M. M., MEDINA, M., … ČENAS, N. (2004). Flavoenzyme-catalyzed redox cycling of hydroxylamino- and amino metabolites of 2,4,6-trinitrotoluene: Implications for their cytotoxicity. *Archives of Biochemistry and Biophysics*, *425*(2), 184–192. https://doi.org/10.1016/j.abb.2004.02.043

SCHAEFER, C. A., SILVA, V. D., STAMBUK, B. U., & DA NASCIMENTO, M. G. (2013). Use of Saccharomyces cerevisiae yeasts in the chemoselective bioreduction of (1E,4E)-1,5-bis(4-methoxyphenyl)-1,4-pentadien-3-one

in biphasic system. *Journal of the Brazilian Chemical Society*. https://doi.org/10.5935/0103-5053.20130140

SCHULZE, B., & WUBBOLTS, M. G. (1999). Biocatalysis for industrial production of fine chemicals. *Current Opinion in Biotechnology*. https://doi.org/10.1016/S0958-1669(99)00042-7

SCHUMACHER, J. D., & FAKOUSSA, R. M. (1999). Degradation of alicyclic molecules by Rhodococcus ruber CD4. *Applied Microbiology and Biotechnology*, *52*(1), 85–90. https://doi.org/10.1007/s002530051491

SCHÜRMANN, B. T. M., SALLUM, W. S. T., & TAKAHASHI, J. A. (2010). Austin, dehydroaustin and other metabolites from penicillium brasilianum. *Quimica Nova*. https://doi.org/10.1590/S0100-40422010000500007

SEDLACZEK, L., & SMITH, L. L. (1988). Biotransformations of steroids. *Critical Reviews in Biotechnology*, *7*(3), 187–236. https://doi.org/10.3109/07388558809146602

SHETTY, D., KIM, Y. J., SHIM, H., & SNYDER, J. P. (2015). Eliminating the heart from the curcumin molecule: Monocarbonyl curcumin mimics (MACs). *Molecules*. https://doi.org/10.3390/molecules20010249

SILVA, V. D., STAMBUK, B. U., & NASCIMENTO, M. D. G. (2012). Asymmetric

reduction of (4R)-(-)-carvone catalyzed by Baker's yeast in aqueous mono- and biphasic systems. *Journal of Molecular Catalysis B: Enzymatic*. https://doi.org/10.1016/j.molcatb.2012.01.012

SILVA, V. D., STAMBUK, B. U., & NASCIMENTO, M. DA G. (2010a). Efficient chemoselective biohydrogenation of 1,3-diaryl-2-propen-1-ones catalyzed by Saccharomyces cerevisiae yeasts in biphasic system. *Journal of Molecular Catalysis B: Enzymatic*. https://doi.org/10.1016/j.molcatb.2010.01.010

SILVA, V. D., STAMBUK, B. U., & NASCIMENTO, M. DA G. (2010b). Efficient chemoselective biohydrogenation of 1,3-diaryl-2-propen-1-ones catalyzed by Saccharomyces cerevisiae yeasts in biphasic system. *Journal of Molecular Catalysis B: Enzymatic*, *63*(3–4), 157–163. https://doi.org/10.1016/j.molcatb.2010.01.010

STEINKELLNER, G., GRUBER, C. C., PAVKOV-KELLER, T., BINTER, A., STEINER, K., WINKLER, C., … GRUBER, K. (2014). Identification of promiscuous ene-reductase activity by mining structural databases using active site constellations. *Nature Communications*. https://doi.org/10.1038/ncomms5150

TAKESHITA, M., YOSHIDA, S., KIYA, R., HIGUCHI, N., & KOBAYASHI, Y.

(1989). Reduction of Aromatic Nitro Compounds With Baker's Yeast. *Chemical and Pharmaceutical Bulletin*. https://doi.org/10.1248/cpb.37.615

TEN BRINK, G. J., ARENDS, I. W. C. E., & SHELDON, R. A. (2004). The Baeyer-Villiger reaction: New developments toward greener procedures. *Chemical Reviews*, *104*(9), 4105–4123. https://doi.org/10.1021/cr030011l

THYGESEN, A., THOMSEN, A. B., SCHMIDT, A. S., JØRGENSEN, H., AHRING, B. K., & OLSSON, L. (2003). Production of cellulose and hemicellulose-degrading enzymes by filamentous fungi cultivated on wet-oxidised wheat straw. *Enzyme and Microbial Technology*, *32*(5), 606–615. https://doi.org/10.1016/S0141-0229(03)00018-8

TOLMIE, C., SMIT, M. S., & OPPERMAN, D. J. (2019). Native roles of Baeyer-Villiger monooxygenases in the microbial metabolism of natural compounds. *Natural Product Reports*, *36*(2), 326–353. https://doi.org/10.1039/c8np00054a

TØNNESEN, H. H. (1992). *Chemistry of Curcumin and Curcuminoids*. https://doi.org/10.1021/bk-1992-0506.ch011

TOOGOOD, H. S., GARDINER, J. M., & SCRUTTON, N. S. (2010). Biocatalytic

Reductions and Chemical Versatility of the Old Yellow Enzyme Family of Flavoprotein Oxidoreductases. *ChemCatChem*. https://doi.org/10.1002/cctc.201000094

TOOGOOD, H. S., & SCRUTTON, N. S. (2018). Discovery, Characterization, Engineering, and Applications of Ene-Reductases for Industrial Biocatalysis. *ACS Catalysis*. https://doi.org/10.1021/acscatal.8b00624

TOOGOOD, H. S., & SCRUTTON, N. S. (2019). *Ene reductases* (Vol. 8). https://doi.org/10.1021/acscatal.8b00624.Discovery

TOWNSEND, C. A., CHRISTENSEN, S. B., & DAVIS, S. G. (1982). Bisfuran Formation in Aflatoxin Biosynthesis: The Role of Versiconal Acetate. *Journal of the American Chemical Society*, *104*(22), 6154–6155. https://doi.org/10.1021/ja00386a070

TURFITT, G. E. (1948). The microbiological degradation of steroids; fission of the steroid molecule. *The Biochemical Journal*, *42*(3), 376–383. https://doi.org/10.1042/bj0420376

UD DIN, Z., FILL, T. P., DE ASSIS, F. F., LAZARIN-BIDÓIA, D., KAPLUM, V., GARCIA, F. P., … RODRIGUES-FILHO, E. (2014). Unsymmetrical 1,5-diaryl-3-oxo-1,4-pentadienyls and their evaluation as antiparasitic agents. *Bioorganic and Medicinal Chemistry*.

https://doi.org/10.1016/j.bmc.2013.12.020

UTLEY, J. H. . (1985). Reductions in organic chemistry. *Endeavour*, Vol. 9, p. 108. https://doi.org/10.1016/0160-9327(85)90056-0

VAN DER WERF, M. J., & BOOT, A. M. (2000). Metabolism of carveol and dihydrocarveol in Rhodococcus erythropolis DCL14. *Microbiology*, *146*(5), 1129–1141. https://doi.org/10.1099/00221287-146-5-1129

VAZ, A. D. N., CHAKRABORTY, S., & MASSEY, V. (1995). Old Yellow Enzyme: Aromatization of Cyclic Enones and the Mechanism of A Novel Dismutation Reaction. *Biochemistry*, *34*(13), 4246–4256. https://doi.org/10.1021/bi00013a014

VISAGIE, C. M., HOUBRAKEN, J., FRISVAD, J. C., HONG, S. B., KLAASSEN, C. H. W., PERRONE, G., … SAMSON, R. A. (2014). Identification and nomenclature of the genus Penicillium. *Studies in Mycology*. https://doi.org/10.1016/j.simyco.2014.09.001

VON HOLLEBEN, M. L. A., ZUCOLOTTO, M., ZINI, C. A., & OLIVEIRA, E. R. (1994). A selective reduction of α,β-unsaturated ketones. *Tetrahedron*, *50*(4), 973–978. https://doi.org/10.1016/S0040-4020(01)80811-5

WANG, W., ZONG, M. H., & LOU, W. Y. (2009). Use of an ionic liquid to improve asymmetric reduction of 4′-methoxyacetophenone catalyzed by

immobilized Rhodotorula sp. AS2.2241 cells. *Journal of Molecular Catalysis B: Enzymatic*. https://doi.org/10.1016/j.molcatb.2008.05.010

WASSERSCHEID, P., & KEIM, W. (2000). Ionic Liquids—New "Solutions" for Transition Metal Catalysis. *Angewandte Chemie*, *39*(21), 3772–3789. https://doi.org/10.1002/1521-3773(20001103)39:21<3772::aid-anie3772>3.0.co;2-5

WEBER, W. M., HUNSAKER, L. A., ROYBAL, C. N., BOBROVNIKOVA-MARJON, E. V., ABCOUWER, S. F., ROYER, R. E., … VANDER JAGT, D. L. (2006). Activation of NFκB is inhibited by curcumin and related enones. *Bioorganic and Medicinal Chemistry*, *14*(7), 2450–2461. https://doi.org/10.1016/j.bmc.2005.11.035

WINKLER, C. K., TASNÁDI, G., CLAY, D., HALL, M., & FABER, K. (2012). Asymmetric bioreduction of activated alkenes to industrially relevant optically active compounds. *Journal of Biotechnology*, *162*(4), 381–389. https://doi.org/10.1016/j.jbiotec.2012.03.023

WINTER, J., SCHNEIDER, B., MEYENBURG, S., STRACK, D., & ADAM, G. (1999). Monitoring brassinosteroid biosynthetic enzymes by fluorescent tagging and HPLC analysis of their substrates and products. *Phytochemistry*. https://doi.org/10.1016/S0031-9422(98)00760-2

WU, W. B., XU, J. M., WU, Q., LV, D. S., & LIN, X. F. (2006). Promiscuous acylases-catalyzed Markovnikov addition of N-heterocycles to vinyl esters in organic media. *Advanced Synthesis and Catalysis*. https://doi.org/10.1002/adsc.200505342

ZHANG, Y., LI, J., HAN, D., ZHANG, H., LIU, P., & LI, C. (2008). An efficient resolution of racemic secondary alcohols on magnetically separable biocatalyst. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1016/j.bbrc.2007.10.205

ZHELIFONOVA, V. P., ANTIPOVA, T. V., & KOZLOVSKY, A. G. (2010). Secondary metabolites in taxonomy of the Penicillium fungi. *Microbiology*. https://doi.org/10.1134/S002626171003001X

ZHENG, M., WANG, L., SHAO, J., & ZHONG, Q. (2006). *A Facile Synthesis of α , α ' -bis (Substituted Benzylidene) cycloalkanones Catalyzed by bis (p -ethoxyphenyl) telluroxide (bmpto) Under Microwave Irradiation*. *7911*. https://doi.org/10.1080/00397919708005039