



Accidental synthesis of a trimer of pyrazolone and comparison of its antioxidant activity: an investigatory report

ARTI JAIN^{a,*} , SUSHMA YADAV^{a,b} and PRITI MALHOTRA^a

^aDepartment of Chemistry, Daulat Ram College, University of Delhi, Delhi 110007, India

^bDepartment of Chemistry, University of Delhi, Delhi 110007, India

E-mail: jainarti21@gmail.com

MS received 25 February 2021; revised 17 May 2021; accepted 19 May 2021

Abstract. Untargeted synthesis leading to the formation of a significant product is a common practice and has been successfully achieved after holistic characterization of the accidentally formed molecule of the trimer of pyrazolone. Its significance was further explored in the pharmaceutical field emphasizing the need for the synthesis and validating the newly established pathways for its synthesis. It was known that pyrazolone exhibit a plethora of applications ranging from catalysis, decolourisation of dyes and metallurgical extractions. Paramount importance has been attributed to pyrazolones in recent years for their broad-spectrum biological activities manifested in their anti-inflammatory, analgesic, anticancer and anti-tubercular functions. In the ongoing research 4,4-Bis-(3-methyl-phenyl-pyrazolin-5-on-4-yl)-3-methyl-1-phenyl-pyrazolin-5-one was synthesized economically and efficiently *via* a novel one-step bio-catalytic pathway using laccase as a catalyst. To validate the utility of our accidental discovery, we have also calculated its antioxidant activity against ascorbic acid as a standard compound. DPPH and ABTS have been used to study the scavenging of free radicals *in-vitro*. This is the first report of the enzyme driven synthesis of trimeric form of pyrazolone. These results will emphasise the utilization of pyrazolone trimers as eco-friendly compounds which exhibit a promising natural antioxidant property in physiological environments.

Keywords. Laccase; Trimer; Pyrazolone; One step novel synthesis; Antioxidant activity.

1. Introduction

Biotechnology can contribute to the development of 'green tools' for the transformation of organic moieties by providing tailor-made biocatalysts. Laccase is amongst the most sought enzymes and is widely acknowledged by scientists for its limited requirement for air and restriction of by-products as only water is the common by-product expected during any reaction. It spears heads its category of 'most green' enzymes and has humungous catalytic applications pioneering in the 21st century. Laccase uses molecular oxygen for oxidizing the substance and is capable of removing hydrogen from the hydroxyl group thereby oxidizing it.¹⁻¹⁰ Pyrazolone derivatives form a significant class of organic compounds and feature among major scientific researchers focused on biological, analytical

applications, catalysis, dyes and extraction metallurgy.¹¹⁻¹⁴ Therefore, inordinate efforts have been diverted towards the synthetic manipulation of pyrazolone derivatives to find more valuable compounds.¹⁵ Although various routes have been promoted for various targeted organic synthesis and among them, innumerable methods are available for the conversion of functional groups, yet unanticipated conversions have gained enough significance. Experts continue to be amazed by the appearance of unexpected transformations. These fortuitous discoveries enrich and expand the collection of principle designs for the planning of synthetic schemes involving intentional pathways and targeted products.

In this paper, we report a new, efficient and serendipitously discovered 4,4-Bis-(3-methyl-phenyl-pyrazolin-5-on-4-yl)-3-methyl-1-phenyl-pyrazolin-5-one.

*For correspondence

Supplementary Information: The online version contains supplementary material available at <https://doi.org/10.1007/s12039-021-01943-0>.

As shown in our previous research paper¹⁶ coupling reaction of hydroquinone (1) with *N*-phenyl-3-methylpyrazolin-5-one (2) in the presence of enzyme laccase gave 60% coupled compound (3) in major amount, but surprisingly an unexpected compound (4) was also formed along with the desirable expected compounds as shown in Scheme 1. This unexpected compound prepared accidentally was characterized with the help of various spectral techniques (¹H NMR, ¹³C NMR, IR, and Mass Spectroscopy) and was found to be the trimeric form of *N*-phenyl-3-methylpyrazolin-5-one. The same compound was earlier prepared in 1953 via a two-step procedure by Westoo and the group.¹⁷ This group used various hazardous chemicals such as chloroform along with two different reactants which make this reaction somehow tedious to proceed. With these peculiar results in our hands, we tried this reaction with *N*-phenyl-3-methylpyrazolin-5-one as a sole reactant with laccase as a catalyst. Self-condensation product was achieved in good yield (Scheme 2).

2. Material and methods

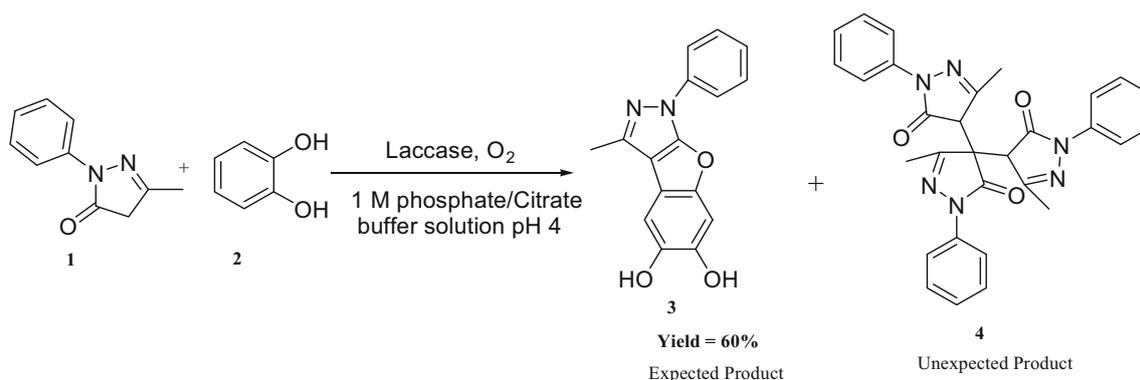
2.1 Chemicals

3-methyl-*N*-phenyl-pyrazolin-5-one, ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), Ascorbic acid were purchased from Aldrich. All media components and chemicals used were of analytical grade.

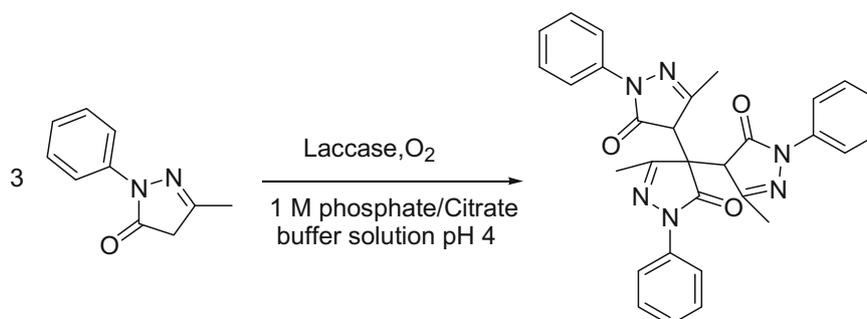
Microorganisms The laccase from *Trametes Versicolor*; was purchased from Sigma Aldrich.

2.2 General procedure for the synthesis of trimer

N-phenyl-3-methylpyrazolin-5-one and laccase (100U) in buffer solution of 1 M phosphate/Citrate pH 4 were stirred at room temperature for 5 h and 30 min. After completion of the reaction, solid compound was precipitated in the reaction media. The solid precipitate was filtered and dried. The dried white compound was characterized by ¹H-NMR, ¹³C-NMR, IR and mass spectroscopy.



Scheme 1. Reaction between catechol and 3-methyl-1-phenyl-pyrazolin-5-one using laccase in the phosphate-citrate buffer of pH = 4 at room temperature.



Scheme 2. Synthesis of trimer of pyrazolone using laccase enzyme.

2.3 Antioxidant activity

Various methods exploring the antioxidant activity methods have been used to monitor and compare the antioxidant potential of various such compounds. The main characteristic of an antioxidant is its ability to trap free radicals which is generally measured by using free radicals such as ABTS, DPPH (2,2-diphenyl-1-picrylhydrazyl), TBARS (Thiobarbituric acid reactive substance), MDA (Malondialdehyde), *etc.* or abstraction of a proton from antioxidants. Herein, DPPH and ABTS have been used to study of scavenging of free radical *in vitro*.¹⁸

2.3a DPPH antioxidant assay: 2 mg DPPH was dissolved in 50 mL methanol. This solution is now of 0.1 mM DPPH concentration. 0.5 mL sample compound and 1 mL DPPH was taken and kept in dark for 30 min. Optical density (A) was taken at 515 nm. Control is taken with 0.5 mL methanol and 1 mL DPPH. Methanol is considered for blank measurement.¹⁹ The DPPH radical scavenging activity of synthesized trimer was compared with ascorbic acid.

2.3b ABTS radical activity: ABTS was dissolved in water to obtain 7 mM concentration. ABTS radical cation ($\text{ABTS}^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use.²⁰ The $\text{ABTS}^{\bullet+}$ solution was diluted with phosphate buffer saline (PBS), pH 7.4, to an absorbance of 0.70 (60.02) at 734 nm. After the addition of 1.0 mL of diluted $\text{ABTS}^{\bullet+}$ solution ($A_{734\text{nm}} = 0.7006020$) to 10 μL of antioxidant compounds or ascorbic acid standards (final concentration 0.01–0.2 mg/mL), the absorbance reading was taken 1 min after initial mixing and up to 6 min. Appropriate solvent blanks were run in each assay. All determinations were carried out in triplicate.

3. Results and Discussion

In the present work, we designed an efficient, green protocol for the synthesis of trimer of pyrazolone *i.e.*, 4,4-Bis-(3-methyl-phenyl-pyrazolin-5-on-4-yl)-3-methyl-1-phenyl-pyrazolin-5-one. The reaction conditions were optimized at different concentration of enzymes, temperature, pH and different solvents. The synthesized trimer of pyrazolone exhibits a promising

natural antioxidant property in physiological environments.

3.1 Optimization of reaction condition

3.1a Optimization of solvent Motivated by encouraging preliminary results a conscientious effort was made towards optimising the reaction conditions for enhanced yields. Attempts were made to run the reaction in various reaction conditions so that good yields of the product with minimised inputs were obtained. Variations in the amount of catalyst and choice of suitable solvents in various combinations were carried out to optimise more productive reaction parameters. An array of reactions was performed in various solvents and it was observed that in DMSO (Dimethylsulfoxide), DMF (Dimethylformamide) and MeCN like solvents no reaction took place for which it can be inferred that probably denaturation of laccase was caused by them which resulted in its deactivation. An important observation led to the conclusion that the highest yield could be obtained at pH = 4 in phosphate-citrate buffer solution whereas the reaction ceased in a buffer solution having pH > 5, further indicating that at higher pH the activity of laccase is stunted. It was also seen that in non-inter miscible organic and aqueous phases diminished yields were obtained which also incurred from the lowered activity of laccase in poorly miscible solvents. Moreover, it emphasised the generalisation that the oxidative coupling reaction progressed with higher reactivity and selectivity in an aqueous medium as compared to the commonly used organic solvents (Table 1).

3.1b Optimization of temperature Furthermore, the effect of reaction temperature was studied by varying temperature from 5 °C to 60 °C (Table 2). The optimal reaction temperature was found to be the room temperature (25 °C) because the stability of laccase enzyme used in the study was found to be maximum at 25–30 °C temperature range.

3.1c Effect of enzyme concentration on trimer formation Enzyme catalysis reactions are widely impacted by the concentration of enzyme and each reaction requires an optimum concentration of the enzyme. The formation of the trimer, in this case, is also governed by minute variation in enzyme concentration and so the optimal effect of enzyme concentration on trimer formation was studied. In order to determine the threshold amount of enzyme essentially required for maximised yield, the optimisation of enzyme

Table 1. Solvent optimization for the synthesis of trimer using N-phenyl-3-methylpyrazolin-5-one^a.

Sl. No.	Solvent	Yield ^b
1	1 M Acetate buffer pH 4	23%
2	Water	52%
3	THF	40%
4	Ethanol	34%
5	1 M phosphate/Citrate buffer pH 5.5	–
6	1 M phosphate/Citrate buffer solution pH 4	78%
7	DMSO	–
8	MeCN	–
9	DMF	–
10	Methanol	35%
11	1,4 Dioxane	38%
12 ^c	1 M phosphate/Citrate buffer pH 5.5	–
13 ^c	1 M phosphate/Citrate buffer solution pH 4	–
14 ^c	1 M Acetate buffer pH 4	–

^aReaction conditions: 1 mmol of reactant was taken in 10 mL of solvent. 100 U of laccase (*T. versicolor*) was added and the resultant solution was stirred well for 4 h. ^bisolated yield.

^cReaction was carried out in the absence of enzyme laccase.

Table 2. Synthesis of trimer using N-phenyl-3-methylpyrazolin-5-one at different temperatures using different 100 U laccase (*T. versicolor*) in phosphate-citrate buffer of pH=4^a.

Sl. No.	Temperature (°C)	Yield ^b (%)
1	0–5 °C	0
2	25 °C	75
3	50 °C	0
4	100 °C	0

^aReaction conditions 1 mmol of reactant was taken in 10 mL of buffer solution. Different temperatures were employed, and the resultant solution was stirred well for 4 h. ^bisolated yield.

Table 3. Synthesis of trimer using N-phenyl-3-methylpyrazolin-5-one at room temperature using different concentration of laccase (*T. versicolor*) in phosphate-citrate buffer of pH=4^a.

Sl. No.	Concentration of enzyme	Yield ^b (%)
1	30 U	25
2	50 U	45
3	100 U	75
4	150 U	80

^aReaction conditions 1 mmol of reactant was taken in 10 mL of buffer solution. Different Units of laccase was added and the resultant solution was stirred well for 4 h. ^bisolated yield.

concentration is minutely followed in the reaction mixture. Both the rate of the reaction and the amount of product formed are closely monitored for the optimisation of the concentration of the enzyme in the reaction mixture because they play a crucial role in stimulating the rate of the reaction and in the amount of product formed. The ongoing observations gave a clear indication that the formation of the product relies directly on laccase concentration and increases with an increase in concentration and after approaching the optimum concentration of enzyme, it starts decreasing. The optimal enzyme concentration for trimer formation was found to be 100 units and concentration beyond this did not show any significant change in the amount of trimer formation (Table 3). Hence 100 U of

laccase was observed to be the optimum concentration for the enzyme for subsequent reactions.

3.1d Optimization of incubation time The optimal effect of incubation time on trimer formation was also studied to determine the minimum time required for its maximum formation. It is essential to optimize the incubation time of the reaction because kinetic studies reveal that the maximum formation occurs in the beginning and then the rate of the reaction decreases subsequently and there is a point in a reaction where the reaction doesn't produce any more product hence this is the maximum time given to the reaction to form maximum amount of product. The optimal time for trimer formation was found to be 4 h and above which

Table 4. Synthesis of trimer using *N*-phenyl-3-methylpyrazolin-5-one at room temperature using different incubation time with laccase (*T. versicolor*) in phosphate-citrate buffer of pH=4^a.

Sl. No.	Incubation time (min)	Yield ^b (%)
1	60	40
2	120	55
3	140	57
4	160	62
5	180	71
6	240	75
7	300	76
8	330	76

^aReaction conditions 1 mmol of reactant was taken in 10 mL of buffer solution with 100 U laccase. Different incubation time was incorporated, and the resultant solution was stirred well. ^bisolated yield.

there was no appreciable change in the yield of trimer formation (Table 4). Hence 4 h incubation period was used as a standard time for subsequent reactions.

3.1e Spectral data of the synthesized compounds 4,4-Bis-(3-methyl-phenyl-pyrazolin-5-on-4-yl)-3-methyl-1-phenyl-pyrazolin-5-one

Isolated yield: 75%; M.p. 198-199 °C. IR (KBr) ($\nu_{\max}/\text{cm}^{-1}$): 3338 (NH stretch), 3067 (aromatic C-H), 2920 (Alkyl C-H), 1708 (amide group).

¹H NMR (DMSO-*d*₆, 400 MHz): δ (ppm) 2.03(s, 3H, CH₃), 2.14 (s, 3H, CH₃), 2.38 (s, 3H, CH₃) 7.14-7.7.84 (m, 15H, 15 Aromatic proton), 11.32 (s, br, 2NH). ¹³C NMR (DMSO-*d*₆, 100 MHz): δ (ppm) 12.77, 13.18, 100.09, 117.92119.12, 120.06, 124.42, 125.03, 128.92, 136.88, 138.15, 148.95, 161.32, 172.38; Anal. Calcd for C₁₀H₁₄N₂O₂: C, 69.48; H, 5.05; N,16.21. Found: C, 69.40; H, 5.14; N, 16.50. ESI-MS 517.1942.

3.1f Tentative mechanism of trimer formation From a mechanistic point of view this biotransformation could result from the initial formation radical (A)²¹ followed by oxidation to intermediate (B),^{10,22} then (B) reacted again with intermediate (A) to form (C), (C) reacted with another (A) to form the final trimerized product shown in Scheme 3.

3.1g Antioxidant activity It is well-established that disparity and disturbances of the balance between the production of free radicals in the body and the ability of the body to counteract by producing antioxidants are key factor in various prevalent diseases of the human body. It generally leads to oxidative stress and

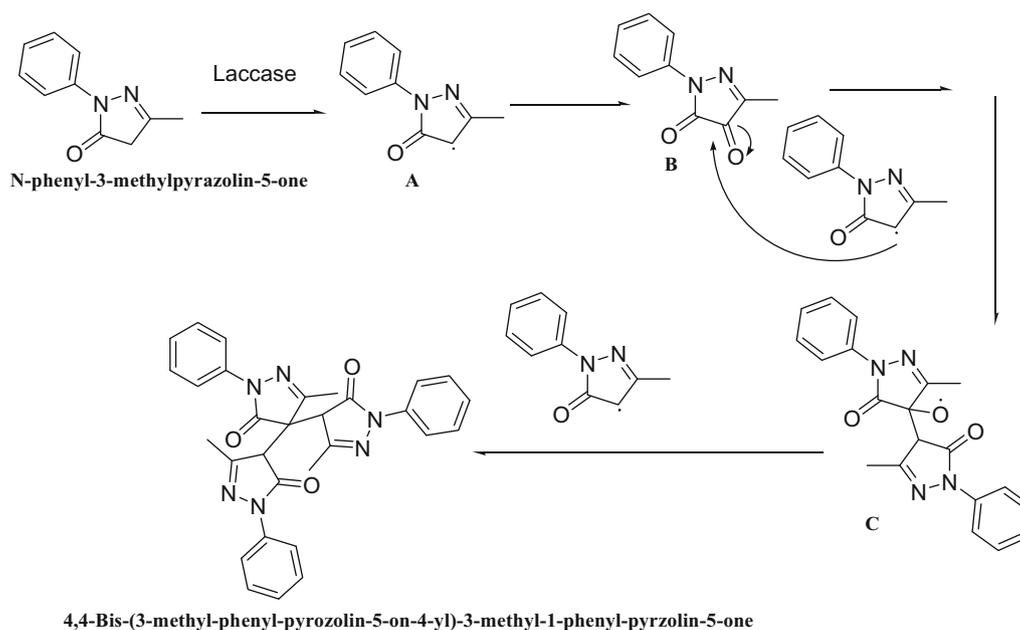
the weakens the natural mechanism of the body to prevent various chronic diseases, hypertension, cardiovascular disorders, cancer and diabetes mellitus.²³ Free radicals are the most reactive entities and mainly consist of reactive oxygen species (ROS) which essentially need antioxidants and prooxidants to suppress their damaging activity caused to the cells and tissues of the body.²⁵⁻²⁶ The underlying mechanism of antioxidant lies in either donating an electron or hydrogen to the free radicals without losing their own stability and thereby neutralising the electrons of free radicals.²⁷ Among potential antioxidants are Methyl-1-phenyl-2-pyrazolin-5-one (MCI -186) which acts as an inhibitor for lipoxygenase metabolism by removing hydroxyl radicals²⁸ and have also proved to be efficient in treating patients suffering from cerebral ischemia.^{29,30} These findings encouraged us to run trials for finding antioxidant properties of the trimeric MCI-186 and a remarkably enhanced activity was observed in the trimer.

Furthermore, the antioxidant property of trimer of pyrazolone was tested by evaluating their free radical uptake potential using the ABTS and DPPH assays. The percentage ability inhibiting the free radical by ABTS/DPPH ratio was calculated as follows:

$$\text{Free Radical removing activity } \% = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100$$

The trimer of pyrazolone synthesised by enzyme catalysis was tested for its radical scavenging activity and was expressed as IC₅₀ (concentration of compounds in mM required for 50% reduction of ABTS/DPPH radicals).

The percentage inhibition of absorbance is calculated and plotted as a function of the concentration of the antioxidant compound and with reference to ascorbic acid for the standard reference data (Figure 1). The results obtained during the assessment of the potential of DPPH for removal of free radicals was quite promising as it was found that among DPPH radical scavenging activity of reactant, vitamin C and trimer of pyrazolone, the trimer of pyrazolone exhibited high efficacy as an antioxidant with IC₅₀ value 0.096 mM. Even when ABTS based methodology was employed for evaluating the efficacy of removal of radicals for gauging in vitro antioxidant ability a similar observation was witnessed. A decrease in the percentage of absorbance at 734 nm is plotted against the concentration of the antioxidant compound and with reference to ascorbic acid for the standard reference data (Figure 2). The calculated value of IC₅₀ showed that trimer of



Scheme 3. Probable mechanism for the formation of trimer of pyrazolone.

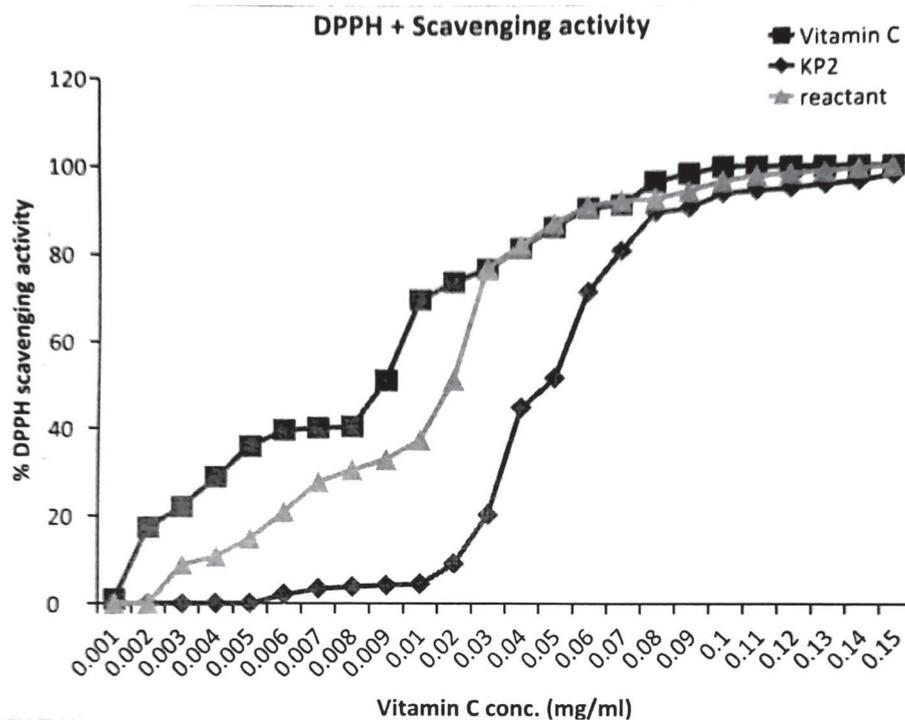


Figure 1. The DPPH scavenging effects of ascorbic acid, trimer of pyrazolone presented at different concentrations of these antioxidants.

pyrazolone ($IC_{50} = 0.482$ mM) demonstrated good potential in scavenging activity than reactant ($IC_{50} = 1.034$ mM) and ascorbic acid ($IC_{50} = 0.909$ mM). A comprehensive study of both DPPH and ABTS assay showed that the trimer of pyrazolone has

pronounced antioxidant properties as compared to vitamin C and other molecules and can be used as an excellent antioxidant also in the medicinal field. The in-depth investigations have revealed the main reasons for its high efficacy leading to the

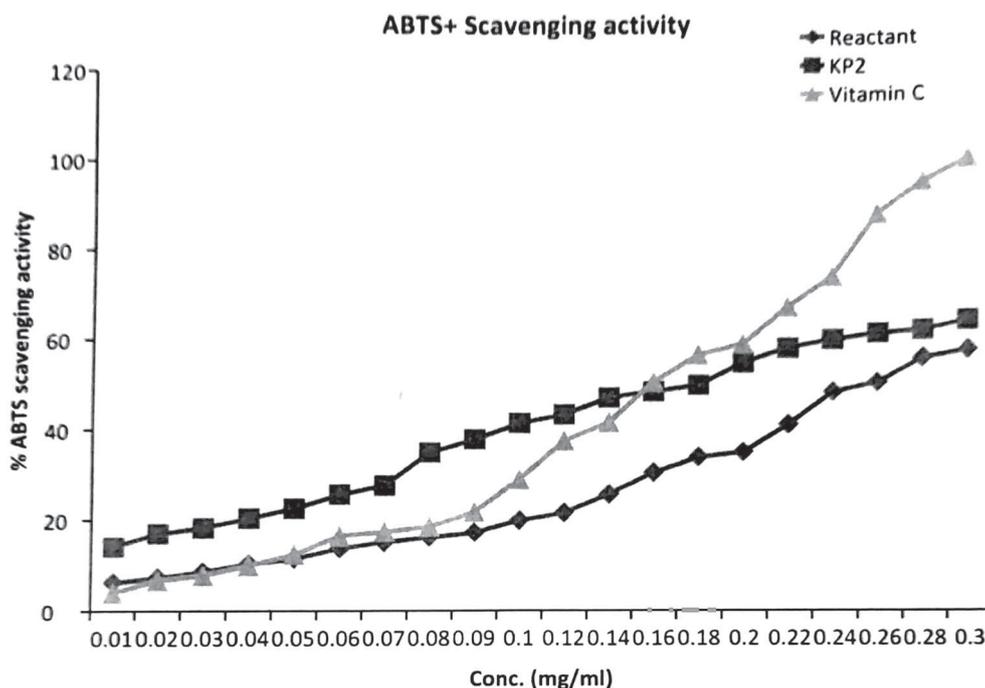


Figure 2. The ABTS scavenging effects of ascorbic acid, trimer of pyrazolone presented at different concentrations of these antioxidants. Note: The term KP2 in Figures 1 and 2 is referred to the product i.e., trimer of pyrazolone.

conclusion that H-atom at position 4 of MCI-186 is the main entity responsible for its high antioxidant potential. In the past several studies have elucidated the mechanism of edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) to scavenge DPPH radical which are also proved by density functional theory (DFT) calculations. It has been strongly observed that the more probable release of H-atom at position 4 of edaravone is the key factor in enabling the antioxidant property rather than the electron-transfer reaction. Owing to the fact there is no effect of substituent's on the release of H from C-H bond; 2-pyrazolin-5-one is recognized as the active centre for edaravone.³¹ This validates our conclusions that in our trimer we have two pyrazoles rings (each from MCI-186) having H-atom at position four.

This type of investigation encourages the chemists to modify MCI-186 to evaluate its activity other than that of antioxidant activity.

4. Conclusions

Conclusively, it is verified that biocatalytically driven synthesis of trimeric pyrazolone is a significant outcome leading to the formation of pharmaceutically important 4,4-Bis-(3-methyl-phenyl-pyrazolin-5-on-4-yl)-3-methyl-1-phenyl-pyrazolin-5-one. The trimeric form of the product was observed to play a crucial role

in enhancing its antioxidant activity. In future many more synthetic protocols may be developed in designing the trimeric pyrazolone and its antioxidant activity may be extended to different domains including physiological areas.

Acknowledgements

S. Yadav acknowledges UGC-India for providing a Senior Research Fellowship.

Declarations

Conflict of interest There is no conflict of interest to declare.

References

1. Maté D, García-Burgos C, García-Ruiz E, Ballesteros A O, Camarero S and Alcalde M 2010 Laboratory evolution of high-redox potential laccases *Chem. Biol.* **17** 1030
2. Sharma A, Jain K K, Jain A, Kidwai M and Kuhad R C 2018 Bifunctional in vivo role of laccase exploited in multiple biotechnological applications *Appl. Microbiol. Biotechnol.* **102** 10327
3. Witayakran S and Ragauskas A J 2009 Synthetic applications of laccase in green chemistry *Adv. Synth. Catal.* **351** 1187
4. Kunamneni A, Camarero S, García-Burgos C, Plou F J, Ballesteros A and Alcalde M 2008 Engineering and

- applications of fungal laccases for organic synthesis *Microb. Cell Fact.* **7** 1
- Rodríguez Couto S and Toca Herrera J L 2006 Industrial and biotechnological applications of laccases: a review *Biotechnol. Adv.* **24** 500
 - Riva S 2006 Laccases: blue enzymes for green chemistry *Trends Biotechnol.* **24** 219
 - Majumder A B, Ramesh N G and Gupta M N 2009 A lipase catalyzed condensation reaction with a tricyclic diketone: yet another example of biocatalytic promiscuity *Tetrahedron. Lett.* **50** 5190
 - Majumder A B and Gupta M N 2014 Lipase-catalyzed condensation reaction of 4-nitrobenzaldehyde with acetyl acetone in aqueous-organic cosolvent mixtures and in nearly anhydrous media *Synth. Commun.* **44** 818
 - Kidwai M, Jain A, Sharma A and Kuhad R C 2012 Ecofriendly approach for detection of phenols in water using laccase from different fungi *Water Sci. Technol.* **66** 385
 - Kidwai M, Jain A, Sharma A and Kuhad R C 2012 First time reported enzymatic synthesis of new series of quinoxalines: a green approach *J. Mol. Catal. B Enzym.* **74** 236
 - Raman N, Kulandaisamy A, Shunmugasundaram A and Jeyasubramanian K 2001 Synthesis, spectral, redox and antimicrobial activities of Schiff base complexes derived from 1-phenyl-2,3-dimethyl-4-aminopyrazol-5-one and acetoacetanilide *Transit. Met. Chem.* **26** 131
 - Uzoukwu B A, Adiukwu P U, Ai-juaid S S, Hitchcock P B and Smith J D 1996 Pyrazolonato complexes of lead. Crystal structures of bis(1-phenyl-3-methyl-4-acetyl pyrazolonato) lead(H) and bis (1-phenyl-3-methyl-4-butanoylpyrazolonato) lead (II) *Inorg. Chim. Acta* **250** 173
 - Yang Z Y, Yang R D, Li F S and Yu K B 2000 Crystal structure and antitumor activity of some rare earth metal complexes with Schiff base *Polyhedron* **19** 2599
 - Chiba P, Holzer W, Landau M, Bechmann G, Lorenz K, Plagens B, et al. 1998 Substituted 4-acylpyrazoles and 4-acylpyrazolones: Synthesis and multidrug resistance-modulating activity *J. Med. Chem.* **41** 4001
 - L'Abbé G, Emmers S, Dehaen W and Dyall L K 1994 5-Chloropyrazole-4-carbaldehydes as synthons for intramolecular 1,3-dipolar cycloadditions *J. Chem. Soc. Perkin Trans.* **1** 2553
 - Kidwai M, Jain A, Sharma A and Kuhad R C 2013 Laccase - A natural source for the synthesis of benzofuro[2,3-c]pyrazolin- 5-ones *Catal. Sci. Technol.* **3** 230
 - Westoo G 1953 Studies on pyrazolones III. Michael Condensation between pyrazole blue and the Addenda 1-phenyl-3-methyl-5-pyrazolone, 1-p-Bromophenyl-3-methyl-5-pyrazolone and 1-Phenyl-3-methyl-40bromo-5-pyrazolone *Acta Chem. Scand.* **7** 355
 - Kuş C, Ayhan-Kilcigil G, Özbey S, Kaynak F B, Kaya M, Çoban T and Can-Eke B 2008 Synthesis and antioxidant properties of novel N-methyl-1,3,4-thiadiazol-2-amine and 4-methyl-2H-1,2,4-triazole-3(4H)-thione derivatives of benzimidazole class *Bioorg. Med. Chem.* **16** 4294
 - Sharma O P and Bhat T K 2009 DPPH antioxidant assay revisited *Food Chem.* **113** 1202
 - Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C 1999 Antioxidant activity applying an improved ABTS radical cation decolorization assay *Free Radic. Biol. Med.* **26** 1231
 - Yamamoto Y, Kuwahara T, Watanabe K and Watanabe K 1996 Antioxidant activity of 3-methyl-1-phenyl-2-pyrazolin-5-one *Redox. Rep.* **2** 333
 - Wellington K W 2012 In: Green chemistry Application of laccases in organic synthesis: A review p. 1-43, ISBN 978-1-61324-877-5
 - Schewe T 2002 15-lipoxygenase-1: a prooxidant enzyme *Biol. Chem.* **383** 365
 - Halliwell B 2009 The wanderings of a free radical *Free Radic. Biol. Med.* **46** 531
 - Mena S, Ortega A and Estrela J M 2009 Oxidative stress in environmental-induced carcinogenesis *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **674** 36
 - Van Horsen J, Witte M E, Schreibelt G and de Vries H E 2011 Radical changes in multiple sclerosis pathogenesis *Biochim. Biophys. Acta Mol. Basis Dis.* **1812** 141
 - Litwinienko G and Ingold K U 2007 Solvent effects on the rates and mechanisms of reaction of phenols with free radicals *Acc. Chem. Res.* **40** 222
 - Murota S-I, Morita I and Suda N 1990 The control of vascular endothelial cell injury *Ann. N. Y. Acad. Sci.* **598** 182
 - Minhaz U, Tanaka M, Tsukamoto H, Watanabe K, Koide S, Shohtsu A and Nakazawa H 1996 Effect of MCI-186 on postischemic reperfusion injury in isolated rat heart *Free Radic. Res.* **24** 361
 - Houkin K, Nakayama N, Kamada K, Noujou T, Abe H and Kashiwaba T 1998 Neuroprotective effect of the free radical scavenger MCI-186 in patients with cerebral infarction: clinical evaluation using magnetic resonance imaging and spectroscopy *J. Stroke Cerebrovasc. Dis.* **7** 315
 - Wang L F and Zhang H Y 2003 A theoretical investigation on DPPH radical-scavenging mechanism of edaravone *Bioorg. Med. Chem. Lett.* **13** 3789