IN VITRO ANTI-OXIDANT ACTIVITY OF HINDERED PIPERIDONE DERIVATIVES

S. NITHIYA1*, N. KARTHIK2, J. JAYABHARATHI3

1Department of Chemistry, Periyar University, Salem, India, 2Department of Chemistry, Rajalakshmi Engineering College, Chennai, India.
3Department of Chemistry, Annamalai University, Annamalai Nagar, India. Email: nith.subu@gmail.com

ABSTRACT

The present study was designed to synthesize isomeric alcohols from corresponding piperidone 1 by sodium ethoxide reduction and the alcohols were separated by column chromatography. The synthesized compounds were characterized by IR, 1H, 13C NMR studies. In addition to that in vitro potential antioxidant activity of the compounds was analyzed via DPPH assay and compared with standard antioxidant such as Ascorbic acid and proposed the suitable mechanism of antiradical action. It has been found that piperidone 1 demonstrated excellent radical scavenging activity, much better than its isomeric alcohols 2 and 3.

Keywords: Piperidone, Isomeric alcohols, NMR, DPPH.

INTRODUCTION

Several thousand piperidine compounds have been mentioned in clinical and preclinical studies1. Besides the interesting structural features, these compounds are also of pharmaceutical interest as they exhibit a wide range of biological activities2. Nevertheless, the variety of functionality and substitution patterns found in piperidine targets and the widely accepted concept that the biological properties of piperidines are highly dependent on the type and location of substituents on the heterocyclic ring3. Piperidine derivatives are found to possess pharmacological activity and form an essential part of the molecular structures of important drugs such as raloxifene and minoxidil4. Selective inhibition of a number of enzymes involved in the binding and processing of glycoproteins has rendered piperidone aldehyds as important tools in the study of biochemical pathways5. Piperidones are somewhat less prominent, but often they serve a role as advanced intermediates prior to their conversion to piperidines. Hydroxylated piperidine aldehyds are frequently found in living systems and display a wide range of biological activities due to their ability to imitate carbohydrates in a variety of enzymatic processes6. In many different disciplines antioxidants become more interested in new compounds, either synthesized or obtained from natural sources that could provide active components to prevent or reduce the impact of oxidative stress on cell7. Antioxidants are extensively studied for their capacity to protect organism and cell from damage that are induced by oxidative stress. Oxidative damages play a significantly pathological role in human diseases. Cancer, emphysema, cirrhosis, atherosclerosis and arthritis have all been correlated with oxidative damage. Also, excessive generation of reactive oxygen species (ROS) induced by various stimuli and which exceeds the antioxidant capacity of the organism leads to variety of pathophysiological processes such as inflammation, diabetes, genotoxicity and cancer8. In the present study wide range applications of radical scavenging and antioxidant activities of newly synthesized compounds were investigated using DPPH method.

Chemistry

Synthesis and isomerization of piperidine-4-ols have been described by Noller and Balasubramanian9,10.

Biology

The in vitro antioxidant activity of the synthesized compounds was evaluated by DPPH assay method11.

Statistical analysis

Statistical significance of difference between the mean activities of the subgroups of compounds was determined by utilizing ANOVA for variables with normal distributions.

MATERIALS AND METHODS

IR spectra

Infrared spectra were recorded on a NICOLET AVATAR 360 FT-IR spectrometer. The sample was mixed with KBr and the pellet technique was adopted to record the spectra.

1H NMR spectra

Proton spectra were recorded on a AMX 400 NMR instrument operating at 400 MHz. Samples were prepared by dissolving 10 mg of the substance in 0.5 mL of CDCl3 containing 1% TMS. The spectral parameters used are, number of transients, 32; number of data points, 32 K; spectral sweep width 4000 Hz.

13C NMR spectra

Proton decoupled 13C NMR spectra were recorded on a AMX 400 NMR instrument operating at 100 MHz. Solutions for the measurement of spectra were prepared by dissolving 0.5 g of the sample in 2.5 mL of CDCl3 containing a few drops TMS as internal reference. The solvent chloroform-d also provided the internal field frequency lock signal. The spectral parameters used are, number of transients 5000; number of data points 32K; pulse width 6 s (45°), spectral sweep width, 2200 Hz.

Synthesis of compound 1

Dry ammonium acetate (0.5 mole) was dissolved in 50 mL of distilled ethanol was mixed with 4-chlorobenzaldehyde (1 mole) and ethylisopropylketone (0.5mol) in distilled ethanol12 was heated and cooled. After cooling the viscous liquid was dissolved in ether (50mL) and shaken with concentrated HCl (5mL) resulting the piperdin-4-one was precipitated as its hydrochloride then the precipitate was filtered and washed with 50mL of Ether: Ethanol mixture (4:1) to remove colored impurities. By adding aqueous ammonia and acetone the base was liberated from ethanolic solution. It was recrystallized from ethanol.

1H-NMR: 7.35[m,H(ortho)], 7.27[m,H(meta)], 4.03[s, H(6)], 3.88[s,H(2)], 2.85[t, J(5H)-2(5E)], 1.86[s,NH], 1.17[s,EqCH3], 0.94[s,Ax CH3].

13C-NMR: 211.86 C(4), 141.34(ipso), 137.32(Ph), 68.72 C(2), 60.84 C(6), 49.66 C(3), 47.04 C(5), 20.29 [EqCH3], 19.82 [Ax CH3].

Synthesis of compounds 2 and 3

The 4-hydroxy piperidines 2 and 3 were prepared from the corresponding piperidin-4-one 1 by adopting the general procedure11. 12. About (0.5mol) of Piperidin-4-one 1 in distilled ethanol was refluxed with (1mol) of liquid Na at 60°C for 7hrs. Reaction mixture was subjected to Co-TLC to check the formation of isomeric alcohols.
After the formation reaction mixture was poured into acidified ice water. The crude was separated by ether separation and these isomeric alcohols were separated by column chromatography using neutral alumina as adsorbent and benzene: ethyl acetate (90:10) as eluent.

2. \( ^1\)H NMR: 7.43 m, H(ortho), 7.26 m, H(meta), 4.27 (dd, H(4)), 4.13 (s, H(6)), 3.58 (s, H(2)), 1.01 (s, Eq CH3), 0.86 (s, Ax CH3).

\( ^13\)C NMR: 143.39 (ipso), 142.58 (Ph), 75.29 (C(4)), 68.36 (C(2)), 62.64 (C(6), 55.32 (C(3), 37.45 (C(5), 24.43 (Eq CH3), 1.95 (Ax CH3).

3. \( ^1\)H NMR: 7.34 m, H(ortho), 7.27 m, H(meta), 3.87 (dd, H(4)), 3.86 (s, H(6)), 3.48 (H(2)), 1.48 (s, H(10)), 0.90 (s, Eq CH3), 0.98 (s, Ax CH3).

\( ^13\)C NMR: 142.60 (ipso), 132.92 (Ph), 76.70 (C(4)), 68.80 (C(2)), 59.79 (C(6), 39.71 (C(3), 37.89 (C(5), 23.89 (Eq CH3), 1.27 (Ax CH3).

### Table 1: IR stretching frequencies of compounds 1-3

<table>
<thead>
<tr>
<th>Piperidone 1</th>
<th>Axial alcohol 2</th>
<th>Equatorial alcohol 3</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3311</td>
<td>1632</td>
<td>1628</td>
<td>( \nu_{\text{C-H}} )</td>
</tr>
<tr>
<td>-</td>
<td>3427</td>
<td>3442</td>
<td>( \nu_{\text{OH}} )</td>
</tr>
<tr>
<td>2974, 2931</td>
<td>2964</td>
<td>2923, 2853</td>
<td>( \nu_{\text{C-H}} )</td>
</tr>
<tr>
<td>1698</td>
<td>-</td>
<td>-</td>
<td>( \nu_{\text{C=O}} ) and ( \nu_{\text{C=O}} ) in plane bending vibration</td>
</tr>
<tr>
<td>1489, 1462</td>
<td>1488, 1441</td>
<td>1542, 1490</td>
<td>( \nu_{\text{C=C}} ) stretching vibration of aromatic ring</td>
</tr>
<tr>
<td>1360</td>
<td>1317</td>
<td>1325</td>
<td>Symmetric bending vibration of methyl group</td>
</tr>
<tr>
<td>1089, 1012</td>
<td>1088, 1046</td>
<td>1017</td>
<td>( \nu_{\text{C-N}} )</td>
</tr>
<tr>
<td>940, 822</td>
<td>824, 753</td>
<td>826</td>
<td>Aromatic C-H out of plane bending vibrations</td>
</tr>
<tr>
<td>685, 513</td>
<td>690, 558</td>
<td>670, 461</td>
<td>Aromatic C-C out of plane bending vibrations</td>
</tr>
</tbody>
</table>

### Antioxidant activity

Free radical scavenging activity of compounds was measured by DPPH, using the method of Blois. Briefly, 0.1 mM solution of DPPH in ethanol was prepared, and this solution (1 mL) was added to sample solutions in CHCl₃ (3 mL) at different concentrations (1-10 mg/mL). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH Concentration (mM) in the reaction medium was calculated from the following calibration curve and determined by linear regression (R²: 0.997): Absorbance = 0.00034 DPPH. – 0.0174. The capability to scavenge the DPPH radical was calculated using the following equation:

\[
\text{DPPH Scavenging effect (\%)} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

Where, \( A_0 \) is the absorbance of the control reaction and \( A_1 \) is the absorbance in the presence of the samples or standards.

### RESULTS AND DISCUSSION

#### IR spectra

The prominent peaks around 3427, 3442 cm⁻¹ in the IR spectra are attributed to \( \nu_{\text{OH}} \) mode in 2 and 3 respectively shows that C=O in piperidone 1 was reduced to secondary alcohol –CH–OH the values are reproduced in (Table 1).

#### \(^1\)H NMR

The signals in the \(^1\)H NMR spectra were assigned based on their positions, multiplicities and integrals. Due to syn,1,3-diaxial interaction with protons at C(2) and C(6) equatorial proton at C(4) in axial alcohol 2 resonated at 4.27 ppm whereas axial proton at C(4) in equatorial alcohol 3 resonated at 3.87 ppm. It confirms that He (4) in axial alcohol 2 gets deshielded by axial hydroxy group at C(4) than in equatorial alcohol 3. In compound 2 the hydroxyl group causes polarization of the CH bond so that the hydrogen gets positive charge and the carbon gets negative charge. Thus, hydrogen was deshielded and the carbon gets shielded.

#### DPPH Radical scavenging activity

The model of scavenging the stable DPPH radical model is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their electron/hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants, because of reaction between antioxidant molecules and radical, progresses, which result in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants such as ascorbic acid were determine by using DPPH method. The inhibitory effect of synthesized compounds 1-3 on DPPH radical was showed in (Fig 1). With the increase of concentration, the scavenging effect also increased. It was reported that reducing power serves as a significant indicator of antioxidant activity for a compound. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radicals chain by donating a hydrogen atom or electrons.
CONCLUSION

In vitro antioxidant evaluation by DPPH assay concludes that piperidone exhibit strong antioxidant activity by electron donating mechanism (i.e.) it donate unshared pair of electrons on exocyclic carbonyl oxygen atom. Reduction of C=O into –OH resulted in a mechanism (i.e.) it donate unshared pair of electrons on exocyclic carbonyl double bond it absorbs at higher wavelength it was evidenced from (Table 2). Among the isomeric alcohols, equatorial alcohol 3 shows much better antiradical activity than axial alcohol 2. As a result of 1,3-diaxial interaction axial alcohol 2 shows lower antiradical scavenging effect to DPPH radical. The order of antioxidant activity: 1>3>2.

ACKNOWLEDGEMENT

One of the author Mr. N. Karthik convey his sincere thanks to Dr. P. Rajasekar, Senior Lecturer and Dr. Johanna Rajkumar, Professor & Head, Department of Biotechnology, RajalakshmiEngineering College, Chennai, India for providing necessary chemicals, instruments and ANOVA software to evaluate Antioxidant Activity.

REFERENCES