FLUORESCENCE CHARACTERISTICS OF PHENOXY DERIVATIVES OF PYRIDINE

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Abstract
2-Chloropyridine was used as the starting material for the preparation of 2-phenoxypyridine and 2-(4-methyl)phenoxypyridine. Fluorescence studies were carried out in various solvents, capped and uncapped condition at room temperature. The fluorescence study of the above compounds with respect to time was also carried out. 2-Phenoxypyridine showed the highest fluorescence peak in methanol. The fluorescence peak was observed at 321 nm, when excited at 290 nm for 2-phenoxypyridine. 2-(4-methyl)phenoxypyridine on the other hand showed the highest fluorescence peak in ethyl acetate, whereby fluorescence peak was observed at 613 nm when excited at 310 nm. The fluorescence intensity of 2-Phenoxypyridine and 2-(4-methyl)phenoxypyridine decreased with time for capped condition.

Keywords: Phenoxypyridine, fluorescence, solvents

Introduction
Fluorescence is one of the most promising techniques in biology and medicine for the beginning of the 21st century. Fluorescence spectrophotometers are forecast to be the fastest growing market of all the molecular spectroscopy instruments through 1995, with biological research as the most important application. Advance in science especially biology and medicine are limited by the available analytical techniques. Scientists require not only diverse analytical procedures but extremely sensitive one as well, since they are continually examining mechanisms and investigating the functions of analytical reagents which are active in extremely low concentrations, for example in tissue. One of the methods available now is fluorescence spectroscopy.

The sensitivity of fluorescence detection is so great that it has served many of the functions for which radioactive isotopes are now used. In pharmacology and related fields of biology it has become almost a requirement to synthesis a new metabolite, drug or insecticide in radioactive from in order to determine its in vivo disposition. This procedure is used with great success to determine blood levels and rates of absorption and excretion. Where the compound is fluorescent, it may often be detected at a very low concentration by fluorescence assay.

In the course of fluorescence measurement, the following observables can be determined; intensity of fluorescence, emission spectrum, excitation spectrum, absorption spectrum, quantum yield, polarization and lifetime. The measurements can be performed in two principle ways. The classic measurement is simpler and provides time-averaged, so called steady-state values. Time-resolve-measurements are more demanding and require more expensive equipment, but they provide information on how the fluorescence parameters change on a nanosecond scale following excitation.

Fluorescence is one of the most active research fields in science today, as evidenced by the increasing number of paper, reviews and monographs published each year. It provides some of the most sensitive and selective methods of chemical analysis.

The pyridine ring systems are very widely distributed in nature, especially in the plant kingdom. Many important alkaloids are pyridine derivatives[11]. Synthetic pyridine derivatives are important as therapeutic agents, for examples isoniazide is a major antituberculosis agent, prialdoxime is an antidote for poisoning by organophosphates, sulphapyridine is one of sulfonamide antibacterial and lacidipine is one of several antihypertensive 1,4-dihydropyridines. The first pyridine was obtained from the isolation of bone oil and from coal tar in 1846 by Anderson[2]. The pyridine ring plays a key role in several biological processes such as the oxidation/reduction coenzyme nicotine adenine dinucleotide (NAPP), whereby the vitamin niacin is required for
its biosynthesis[3]. Korner and Dewar, in 1869, suggested the cyclic nature of pyridine as a hexagonal structure having alternate double and single bonds[4].

Today, the pyridine ring can be synthesized using two main synthetic routes, namely Hantzsch synthesis and Guareshi synthesis. The most important and widely used pyridine synthesis is by using Hantzsch method, which involved the condensation of two molecules of $\beta$-keto ester with an aldehyde and ammonia. Pyridine and its derivatives undergo electrophilic and nucleophilic substitutions. The fluorescence characteristic of pyridine or other heterocycles are not extensively studied, eventhough a wide variety of heterocyclic compounds are known to be fluorescent[5-7]. This work involves synthesizing various pyridine derivatives followed by study their fluorescence characteristic in various solvents. In this paper, the fluorescence characteristic of 2-phenoxy pyridine and 2-(p-methyl)phenoxy pyridine will be reported.

### Experimental

#### Synthesis

**2-Phenoxy pyridine**

Phenol (1.8900 g) was mixed with potassium hydroxide pellet (1.1515 g) in minimum volume of water (2 ml). The mixture was heated to remove the water and yielded a dry brownish crystal. 2-Chloropyridine (2.2963 g) was then added and refluxed for 10 hours. The mixture was extracted with ether and washed with water for 3 times and dried over anhydrous magnesium sulphate. Evaporation of solvent gave the product a dark brownish liquid (1.6963 g, 50%). The yield was purified and separated with T.L.C in 1:20 (EA : Hexane) solvent system.

50%, IR (cm$^{-1}$): 3064.23-3037.97, 1588.12, 1466-1423.88, 1261.68-1155.98; $^1$H NMR (CDCl$^3$) $\delta$: 8.08, dd, 1H (H-6), 7.55, dd, 1H (H-3), 7.30, dd, 2H (H-3', H-5'), 7.06, dd, 3H (H-5, H-4, H-4'), 6.88, dd, 2H (H-2', H-5'); M$^+$: 170.00; C$\text{_{11}}$H$\text{_{9}}$NO requires M$^+$: 170.21

**2-(4-methyl)phenoxy pyridine**

$p$-Cresol (2.1500 g) was mixed with potassium hydroxide pellet (1.1585 g) in minimum volume of water (2 ml). The mixture was heated to remove the water and yielded a dry brownish crystal. 2-chloropyridine (2.2987 g) was then added and refluxed for 7 hours. The mixture is extracted with extracted with sodium hydroxide, chloroform and washed with water for 3 times and dried over anhydrous magnesium sulphate. Evaporation of solvent gave the product a dark brownish liquid (1.1676 g, 32%).

32%, IR (cm$^{-1}$): 3064.23-3037.97, 1588.12, 1466-1423.88, 1261.68-1155.98, 778.31; $^1$H NMR (CDCl$^3$) $\delta$: 8.24, dd, 1H (H-6), 7.68, dd, 1H (H-3), 7.26, dd, 2H (H-3', H-5'), 7.06, dd, 3H (H-5, H-4, H-4'), 6.92, dd, 2H (H-2', H-5'); 7.00, ddd, 2H (H-5), 6.92, ddd, 1H (H-4), 2.40, s, (CH$_3$); M$^+$:184.00; C$\text{_{12}}$H$_{11}$NO requires M$^+$: 184.25

#### Spectroscopic analysis

All solvents were redistilled before use. Melting points were determined with Electrothermal Melting Point Apparatus and were not corrected. Infrared spectra were recorded using Perkin Elmer 298 Infrared Spectrometer and FTIR Perkin Elmer 1600 Series. $^1$H-NMR spectra were recorded on JEOL JNM-LA400FT NMR System. Mass spectrum was recorded using GCMS Hewlett-Packard HP 6890 Series with mass selective indicator.

#### Fluorescence Studies

2-Phenoxy pyridine and 2-(4-methyl)phenoxy pyridine with the same concentration were prepared in methanol, ethanol, tetrahydrofuran, ether, acetonitrile and ethyl acetate. The fluorescence measurement was carried out in a quartz cell, using Fluorescence Spectrometer Model F-2000 Hitachi at room temperature with the same instrument setting.

#### Results and Discussion

2-Phenoxy pyridine (1) and 2-(4-methyl)phenoxy pyridine (2) were obtained when commercially available 2-chloropyridine (3) was reacted with phenol and $p$-cresol respectively, as shown in Scheme 1. The structures of both compounds were confirmed by infrared, $^1$H - NMR, $^{13}$C -NMR and mass spectra.
The structure of 2-phenoxy pyridine (1) was confirmed by $^1$H-NMR, $^{13}$C-NMR, IR and GC-MS spectra. The $^1$H-NMR spectra showed a doublet of doublet at $\delta$ 8.08 ppm which was due to H-6 proton resonance. At $\delta$ 7.55 ppm, the spectrum showed a doublet of doublet of doublet peak which was due to H-3 proton. A doublet of doublet of doublet peak was observed at $\delta$ 7.30 ppm, which was due to H-3' and H-5' protons of the phenolic ring. A multiplet peak was observed at $\delta$ 7.06 ppm, which is due to the protons of H-5, H-4 of pyridine ring, and H-4' of phenolic ring. At $\delta$ 6.88 ppm, a multiplet recorded which was due to the protons of H-2' and H-5' of phenol ring respectively.

The $^{13}$C NMR spectrum showed absorptions of relatively low intensity at $\delta$ 163.747 ppm which was due to C$_2$ of pyridine ring and peak at $\delta$ 153.965 ppm was due to C$_1$ of phenolic ring. Higher intensity absorption were observed at $\delta$ 147.770 ppm for C$_6$ of pyridine ring, $\delta$ 139.394 ppm for C$_4$ of phenolic ring and $\delta$ 129.670 ppm for C$_3$ and C$_5$ of phenolic ring. Absorption at $\delta$ 124,651 ppm was due to C$_1$ of pyridine ring and at peak $\delta$ 121,147 was due to C$_2$ and C$_6$ of phenolic ring. The absorption which recorded at $\delta$ 118.432 ppm, was assigned to C$_3$ of pyridine ring and peak at $\delta$ 111.496 ppm was due to C$_2$ of pyridine ring. The numbering of carbon is as shown in Figure 1.

![Scheme 1](image)

The gas chromatography mass spectrum (GCMS) led to the molecular formula of C$_{11}$H$_9$NO and M$^+$ was recorded at 170.2100.
The structure of 2-(4-methyl)phenoxy pyridine (2) was also confirmed by $^1$H-NMR, $^{13}$C-NMR, IR and GC-MS spectra. The $^1$H-NMR spectra showed a doublet of doublet at $\delta$ 8.24 ppm which was due to H-6 of pyridine ring. At $\delta$ 7.68 ppm, a doublet of doublet peak was recorded which was due to H-3 proton. A doublet peak was recorded at $\delta$ 7.26 ppm, which was due to H-3’ and H-5’ protons of phenolic ring. While, a doublet peak was recorded at $\delta$ 7.10 ppm, which due to the H-2’ and H-5’ protons of phenolic ring. A triplet peak was observed at $\delta$ 7.00 ppm which was due to H-4 proton of pyridine ring. A singlet peak was observed at $\delta$ 2.40 ppm, was due to the protons of methyl group.

The $^{13}$C-NMR spectrum showed absorptions of relatively low intensity at $\delta$ 164.1690 ppm which were due to C$_2$ of pyridine ring and peak at $\delta$ 151.9349 ppm was due to C$_1$ of phenolic ring. Higher intensity absorption peaks were recorded at $\delta$ 147.8249 ppm for C$_6$ of pyridine ring, $\delta$ 139.4613 ppm for C$_3$ of pyridine ring and $\delta$ 134.3263 ppm was due to C$_8$ of the phenolic ring. Peak at $\delta$ 129.670 ppm was assigned to C$_6$ and C$_8$ of phenolic ring while absorption at $\delta$ 121.2970 was assigned to C$_2$ and C$_8$ of the phenolic ring. Absorption which was recorded at $\delta$ 118.3559 ppm and $\delta$ 111.4102 ppm were assigned to C$_3$ and C$_4$ of pyridine ring. A peak was observed at $\delta$ 21.0103 ppm, which was assigned to a peak of a methyl group. The assignment of carbon absorption based on $^{13}$C-NMR is as shown in Figure 2.

The gas chromatography mass spectrum (GCMS) led to the molecular formula C$_{12}$H$_{11}$NO and M$^+$ was recorded at 184.2500.

The fluorescence studies of these two compounds were carried out in methanol, tetrahydrofuran, ethanol, ether, ethyl acetate and acetonitrile in capped condition. The concentrations of these compounds were the same and the measurements were taken at room temperature. Table 1 and 2 show the fluorescence characteristics of 2-phenoxy pyridine and 2-(4-methyl)phenoxy pyridine in various solvents. Figure 3 shows the fluorescence spectra for 2-phenoxy pyridine in various solvents.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Solvent</th>
<th>Excitation wavelength/nm</th>
<th>Fluorescence Wavelength/nm</th>
<th>Intensity/I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capped</td>
<td>Methanol</td>
<td>290</td>
<td>321</td>
<td>3.951</td>
</tr>
<tr>
<td></td>
<td>Tetrahydrofuran</td>
<td>289</td>
<td>306</td>
<td>3.178</td>
</tr>
<tr>
<td></td>
<td>Ether</td>
<td>258</td>
<td>340</td>
<td>2.157</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>268</td>
<td>288</td>
<td>1.732</td>
</tr>
<tr>
<td></td>
<td>Acetonitrile</td>
<td>251</td>
<td>499</td>
<td>0.396</td>
</tr>
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</table>
It can be seen from the Table 1 that, 2-phenoxypyridine showed the highest fluorescence intensity in methanol, followed by in tetrahydrofuran. The highest fluorescence intensity which was recorded in methanol is may be due to the formation of the complex with the solvent through hydrogen bonding as suggested in Figure 4.

2-Phenoxypyridine is believed to be less polar in excited state, so solvent relaxation stabilization is greater in the ground state than excited state. The high fluorescence intensity in methanol is may also due to the strong interaction of atom on the aromatics ring with the methanol, especially when charge-transfer between the two ring system occurs in excited state, removes the forbidden of these bands by distorting the π-electron systems and this results in an increasing absorptivity of 2-phenoxypyridine. It may also be due to the lone pair electrons of oxygen are involved in formation of the H-bonding. Thus are not available for n → π' transition. As the result, high fluorescence intensity was observed.
Table 2: Fluorescence characteristic of 2-(4-methyl)phenoxyypyridine in various solvents (6.126 X 10^{-6}M)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Solvent</th>
<th>Excitation wavelength/nm</th>
<th>Fluorescence Wavelength/nm</th>
<th>Intensity/I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capped</td>
<td>Ethyl acetate</td>
<td>310</td>
<td>613</td>
<td>2.236</td>
</tr>
<tr>
<td></td>
<td>Ether</td>
<td>311</td>
<td>617</td>
<td>1.992</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>312</td>
<td>620</td>
<td>1.225</td>
</tr>
<tr>
<td></td>
<td>Acetonitril</td>
<td>369</td>
<td>737</td>
<td>0.859</td>
</tr>
<tr>
<td></td>
<td>Tetrahydrofuran</td>
<td>370</td>
<td>738</td>
<td>0.692</td>
</tr>
</tbody>
</table>

Table 2 showed the fluorescence characteristic of 2-(4-methyl)phenoxyypyridine in various solvents in capped condition. From Table 2, it can be seen that the highest fluorescence intensity was observed in ethyl acetate, followed by ether, ethanol, acetonitrile and tetrahydrofuran, for capped condition. The highest intensity in ethyl acetate maybe due to the formation of complex between 2-(4-methyl)phenoxyypyridine with this solvent via hydrogen bonding as in the case of 2-phenoxyypyridine. Figure 5 showed the fluorescence spectra of 2-(4-methyl)phenoxyypyridine in various solvents.

![Fluorescence spectra for 2-(4-methyl)phenoxyypyridine in various solvents](image)

Fig. 5: Fluorescence spectrum for 2-(4-methyl)phenoxyypyridine in various solvents

It can be seen from Table 1 and 2 that 2-phenoxyypyridine is the more fluorescent compared to 2-(4-methyl)phenoxyypyridine. Low fluorescence intensity of 2-(p-methyl)phenoxyypyridine is probably due to the structure as shown in Figure 6.
The methyl group –CH₃ is an electron donating substituent. The presence of electron donating substituent does not increase the fluorescence intensity. The low fluorescence intensity observed is probably due to the non-rigidity of the structure. The non-rigidity of the structure arises from the -O- linkage between the pyridine and phenolic ring. As the result, some of energy absorb was dissipated as heat. The fluorescence wavelength peak for 2-(4-methyl)phenoxydpyridine higher than 2-phenoxydpyridine. It probably due to the presence of -CH₃, supply the electrons to the ring thus high mobility of electron in the system occurs and as the result a shift in fluorescence wavelength peak observed.

Table 3: Fluorescence characteristic of 2-phenoxydpyridine versus time in methanol

<table>
<thead>
<tr>
<th>Condition</th>
<th>Duration/min</th>
<th>Excitation wavelength/nm</th>
<th>Fluorescence Wavelength/nm</th>
<th>Intensity/I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capped</td>
<td>0</td>
<td>290</td>
<td>296</td>
<td>3.409</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>290</td>
<td>303</td>
<td>3.274</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>290</td>
<td>304</td>
<td>3.253</td>
</tr>
</tbody>
</table>

Fig. 7: Fluorescence spectra for 2-phenoxydpyridine versus time in methanol for capped sample.
Table 4: Fluorescence characteristic of 2-(4-methyl)phenoxy pyridine versus time in ether.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Duration/min</th>
<th>Excitation wavelength/nm</th>
<th>Fluorescence Wavelength/nm</th>
<th>Intensity/I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capped</td>
<td>0</td>
<td>311</td>
<td>618</td>
<td>1.944</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>618</td>
<td>1.943</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td></td>
<td>618</td>
<td>1.902</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td></td>
<td>617</td>
<td>1.892</td>
</tr>
</tbody>
</table>

Fig. 8: Fluorescence spectra for 2-(4-methyl)phenoxy pyridine in ether versus time for capped sample

Table 3 and Table 4 show the fluorescence characteristic of 2-phenoxy pyridine and 2-(4-methyl)phenoxy pyridine with time respectively. Figure 7 and 8 show the fluorescence spectra of 2-phenoxy pyridine and 2-(4-methyl)phenoxy pyridine with time. 2-Phenoxy pyridine and 2-(4-methyl)phenoxy pyridine showed a decreased in fluorescence intensity with time. The decrease in fluorescence intensity with time probably due to on prolonged standing, the energy loss trough mode increased. As the result, low fluorescence intensity was recorded.

Conclusion

2-Phenoxy pyridine showed the highest fluorescence peak in methanol, while 2-(4-methyl)phenoxy pyridine showed the highest fluorescence peak in ethyl acetate. The fluorescence intensity of capped sample is higher than uncapped sample. Fluorescence peak was also reduced with time for both compounds.

Acknowledgement

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References