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Fluorescence Studies of Selected 2-Alkylaminopyrimidines

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Abstract: The reactions of 2-chloropyrimidine with methylamine, ethylamine and piperidine gave the corresponding 2-*N*-methylamino-, 2-*N*-ethylamino- and 2*N*-piperidinopyrimidines, respectively. The fluorescence properties of these alkylamino derivatives in chloroform, ethyl acetate, carbon tetrachloride, acetone, ether, ethanol and methanol were studied. All the alkylamino derivatives showed the highest fluorescence intensity in polar protic solvents; thus 2-*N*-methylaminopyrimidine (highest fluorescence intensity at 377 nm when excited at 282 nm) and 2-*N*-ethylaminopyrimidine (highest fluorescence intensity at 375 nm, when excited at 286 nm) showed the highest fluorescence in methanol. In ethanol, 2-*N*-piperidinopyrimidine showed a fluorescence peak at 403 nm when excited at 360 nm and in chloroform it fluoresced at 392 nm when excited at 356 nm.

Keywords: Fluorescence spectroscopy, alkylaminopyrimidines, fluorometry, fluorogenic reagents

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Introduction

Science and technology are progressing very fast but advances in science, especially in the fields of biology, chemistry and medicine, are sometimes limited by the availability of suitable extremely sensitive analytical techniques. For example, analytical techniques are needed to examine mechanisms as well as for investigating the behaviour of certain analytical reagents at very low concentrations. In environmental science, a very sensitive technique is needed to detect chemical pollutants such as phenols, amines and aromatic hydrocarbons.

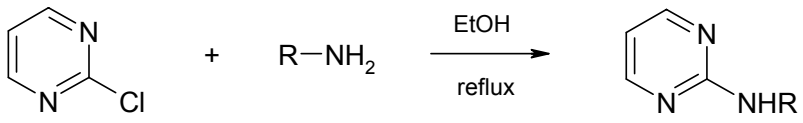
The fluorescence technique has been used extensively for the detection of amino acids [1-3]. For non-fluorescent amino acids, structural modifications can be carried out to give fluorescent products, which are then detected fluorometrically. Thus, for example, tryamine, found in animal urine and tissues, can be determined by first condensing it with formaldehyde, followed by oxidation to give a fluorescent product [4], which is then estimated by this technique. Obviously, fluorescent amines can also be detected fluorometrically. For example, serotine (hydroxyindole) is a very highly fluorescent compound and therefore can be easily detected using fluorescence spectroscopy [5].

Aliphatic amines are non-fluorescent compounds. Currently, the detection of individual aliphatic amines is done by first separating them using various chromatographic techniques, followed by identification, either by comparing the retention time on a chromatogram or using mass spectroscopy. When aliphatic amines are treated with a selected heterocyclic compounds, fluorescent products were obtained [6]. These results have been the starting point of a preliminary study towards detecting aliphatic amines using a fluorescence technique.

Results and Discussion

The secondary 2-alkylaminopyrimidines were obtained by reacting 2-chloropyrimidine with the respective primary alkylamines. Thus, 2-*N*-methylaminopyrimidine and 2-*N*-ethylaminopyrimidine were obtained when an ethanolic solution of 2-chloropyrimidine was treated with methylamine or ethylamine, respectively, as shown in Scheme 1.

Scheme 1



2-chloropyrimidine

R = CH_3 (2-*N*-methylpyrimidine)
R = CH_2-CH_3 (2-*N*-ethylpyrimidine)

The starting materials 2-chloropyrimidine, ethylamine and methylamine did not show any fluorescence properties in ethanol. Table 1 shows the fluorescence intensity of 2-*N*-methylpyrimidine and Table 2 shows the fluorescence intensity of 2-*N*-ethylaminopyrimidine in various solvents.

Table 1. Fluorescence peaks of 2-*N*-methylaminopyrimidine in various solvents

Solvents	Excitation wavelength (nm)	Fluorescence peak (nm)	Relative intensity*
Chloroform	278	361	0.0942
Ethyl acetate	280	366	0.0822
Carbon tetrachloride	286	351	0.0079
Acetone	335	370	0.0315
Ether	282	359	0.0356
Ethanol	282	377	0.4271
Methanol	283	351	0.4843

** Phenol was used as standard*

2-*N*-Methylaminopyrimidine showed the highest fluorescence peak in methanol followed by ethanol, as shown in Table 1. The same phenomenon was also observed with 2-*N*-ethylamino-pyrimidine, as indicated in Table 2. The fluorescence intensity of both compounds decreased markedly in non-polar solvents.

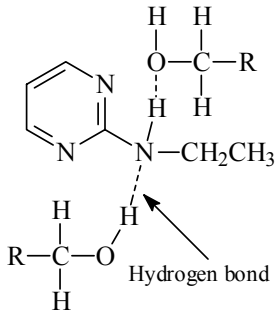
Table 2. Fluorescence peaks of 2-*N*-ethylaminopyrimidine in various solvents

Solvents	Excitation wavelength (nm)	Fluorescence peak (nm)	Relative intensity*
Chloroform	290	360	0.1540
Ethyl acetate	282	365	0.0712
Carbon tetrachloride	286	359	0.0720
Acetone	335	370	0.0400
Ether	289	358	0.0365
Ethanol	286	375	0.4401
Methanol	285	378	0.4672

**Phenol was used as standard*

The high fluorescence intensity observed in ethanol and methanol is probably due to hydrogen bonding by the solvents. The maximum intensity observed in these two solvents is due to the non-bonding electron of the solute, that is 2-*N*-methylaminopyrimidine and 2-*N*-ethylaminopyrimidine, which is bonded to the hydrogen atom of the solvent, forming a stable hydrogen-bonded complex as shown in Figure 1.

Figure 1

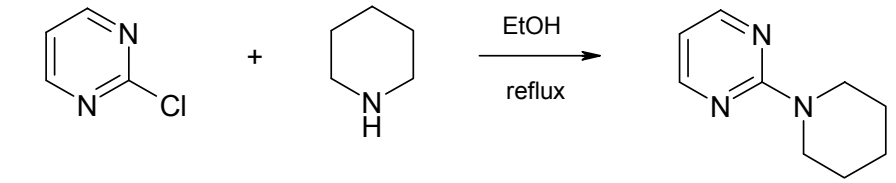


complex

This complex stabilizes the ground state as well as the excited state of the n → π* transition. However, the ground state of 2-*N*-methylaminopyrimidine and 2-*N*-ethylaminopyrimidine has two electrons in the non-bonding orbital whereas the excited state has only one, therefore the stabilisation of the ground state is greater. As the result, the energy of n → π* transition increases, thus favouring the low laying π → π* transition which is responsible for the higher fluorescence intensity.

2-*N*-Piperidinopyrimidine was obtained when 2-chloropyrimidine is treated with an alcoholic solution of piperidine as shown below (Scheme 2). Like the other amines, piperidine itself showed no fluorescence properties. Table 3 shows the fluorescence intensity of 2-*N*-piperidinopyrimidine in various solvents.

Scheme 2



2-chloropyrimidine piperidine 2-*N*-piperidinopyrimidine

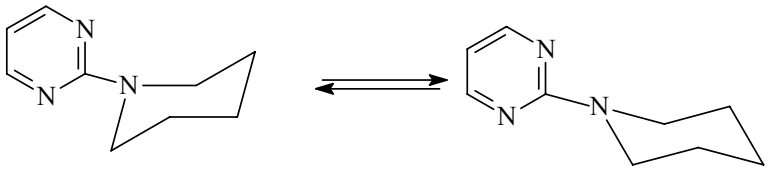
Table 3. Fluorescence peak of 2-*N*-piperidinopyrimidine in various solvents

Solvents	Excitation wavelength (nm)	Fluorescence peak (nm)	Relative intensity*
Chloroform	356	392	0.3657
Ethyl acetate	354	391	0.1710
Acetone	356	397	0.2041
Ether	350	380	0.1082
Ethanol	360	403	0.3514
Methanol	358	407	0.2852

**Phenol was used as standard*

The lower fluorescence intensity observed with 2-*N*-piperidinopyrimidine in ethanol compared to either 2-*N*-methylamino or 2-*N*-ethylaminopyrimidine is believed to be due to the piperidino ring flipping from one conformation to another, thus losing energy during the process (Figure 2). As the result, the energy emitted when the excited molecules fall to the ground state is less and consequently a low fluorescence intensity is observed. The highest fluorescence intensity observed in polar solvents such as ethanol and methanol is believed to be for the same reason as in the case of 2-*N*-methylamino- and 2-*N*-ethylaminopyrimidine.

Figure 2. Flipping of the piperidino ring in 2-*N*-piperidinopyrimidine



2-*N*-Piperidinopyrimidine also showed high fluorescence intensity in chloroform. There is no clear explanation for this observation because typically, when establishing a solvent-fluorescence relationship, hydrogen bonding, the dielectric constant of the solvent and its viscosity are frequently invoked to explain these relationships [7-8]. However, sometimes these effects may cancel each other out and as a result the solvent-fluorescence relationship is largely unpredictable. In the case of the 2-*N*-piperidino derivative, further work is in progress to understand the above phenomena.

Conclusions

2-Chloropyrimidine is a non-fluorescent compound, but when it reacts with selected aliphatic amines, fluorescent compounds are formed. Their fluorescence intensity was observed to be at maximum when the polar solvents are used.

Acknowledgements

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Experimental

General

The substituted pyrimidines were obtained as described below by treatment of 2-chloropyrimidine with piperidine, methylamine and ethylamine, respectively [9-10]. The fluorescence studies of the starting materials in ethanol and the studies of the 2-alkylaminopyrimidines in chloroform, carbon

tetrachloride, ethyl acetate, dichloromethane, acetone, ether, ethanol and methanol were carried out at room temperature in a quartz cell using a Hitachi Model 200 Fluorescence Spectrometer. Phenol was used as the standard. ^{1}H-NMR and ^{13}C-NMR spectra were recorded for $CDCl_3$ solutions using a JEOL JNM-GSX 270 FT NMR instrument. Mass spectra were recorded using a VG Prospec (Fisons Instruments) and infrared spectra were recorded using a Perkin-Elmer FTIR Model 1650 Spectrophotometer.

Alkylaminopyrimidine Syntheses

2-N-Methylaminopyrimidine. 2-Chloropyrimidine (0.16 g) was added to methylamine (5 mL) and the mixture heated under reflux in an oil bath at 120-140^{o}C for four hours, then cooled and extracted with ether (2 x 10 mL). The ethereal layer was washed with water and dried over anhydrous sodium sulphate. Filtration and evaporation of solvent gave crude product which was then recrystallised from hexane-acetone (10:1) to give colourless, transparent, plate-like crystals. Yield: 65%; m.p. 56-58^{o}C (lit. [12] 58-59^{o}C); IR (cm^{-1}): 3458.5; ^{1}H-NMR (δ): 8.27 (d, 2H, C_4 and C_6 of pyrimidine ring), 6.52 (t, 1H, pyrimidine ring C_5), 5.26 (b, 1H, N-H group), 2.98 (d, 3H, CH_3); ^{13}C-NMR (δ): 162.90 (pyrimidine ring C_2), 157.99 (pyrimidine ring C_4 and C_6), 110.36 (pyrimidine ring C_5), 28.33 (C methyl); MS: M^+= 109.1559 ($C_5H_7N_3$ requires M^+ = 109.0640).

2-N-Ethylaminopyrimidine. 2-Chloropyrimidine (0.15 g) was added to ethylamine (3 mL) and the mixture was heated under reflux in an oil bath at 120-140^{o}C for four hours and then cooled. The organic product was extracted with ether (2 x 15mL). The ethereal extracts were washed with water and dried over anhydrous sodium sulphate. Filtration and evaporation of solvent gave the crude product which was then recrystallised from hexane-chloroform to give white crystals. Yield: 60%; m.p. 49-53^{o}C. IR (cm^{-1}): 3443.8; ^{1}H-NMR (δ): 8.26 (d, 2H, pyrimidine ring C_4 and C_6), 6.51 (t, 1H, pyrimidine ring C_5), 5.11 (b, 1H, N-H group), 3.46 (q, 2H, ethyl group C_α), 1.24 (t, 3H, ethyl group C_β); ^{13}C-NMR (δ): 162.29 (pyrimidine ring C_2), 158.01 (pyrimidine ring C_4 and C_6), 110.37 (pyrimidine ring C_5), 36.23 (ethyl group C_α), 14.89 (ethyl group C_β); MS: M^+ = 123.2055 ($C_6H_9N_3$ requires M^+ = 123.0796).

2-N-Piperidinopyrimidine. 2-Chloropyrimidine (0.5 g), piperidine (5 mL) and ethanol (5 mL) were refluxed for two hours. The mixture was evaporated and the residue was extracted with ether (3 x 10 mL). The ether extracts were washed with water and dried over anhydrous sodium sulphate. Evaporation of the solvent gave a yellowish liquid. Yield: 48%; b.p. 120^{o}C/16mmHg (120 ^{o}C/ 16mmHg [11]); ^{1}H-NMR (δ): 8.27 (d, 2H, pyrimidine ring C_4 and C_6), 6.14 (t, 1H, pyrimidine ring C_5), 3.77 (m, 4H, piperidine ring $C_{2'}$ and $C_{6'}$), 1.65 (m, 6H, piperidine ring $C_{3'}$, $C_{4'}$ and $C_{5'}$); ^{13}C-NMR (δ): 161.62 (pyrimidine ring C_2), 157.63 (pyrimidine ring C_4 and C_6), 108.99 (pyrimidine ring C_5), 44.70 (piperidine ring $C_{2'}$ and $C_{6'}$), 25.68 (piperidine ring $C_{3'}$ and $C_{5'}$), 24.82 (piperidine ring $C_{4'}$); MS: M^+ = 163.2464 ($C_9H_{13}N_3$ requires M^+ = 163.1109).

General Procedure for Fluorescence Measurements: 2-N-Piperidinopyrimidine

2-*N*-Piperidinopyrimidine (5.1 mg) was dissolved in ethanol (25.0 mL) and the fluorescence measurements were taken at room temperature using an instrument sensitivity of 2.00. The same concentrations of 2-*N*-piperidinopyrimidine in chloroform, carbon tetrachloride, ethyl acetate, dichloromethane, acetone, ether and methanol were prepared and the measurements recorded under identical conditions. This procedure was repeated with 2-*N*-ethylamino and 2-*N*-methylamino-pyrimidine, using the same concentrations in the various solvents and the same instrument settings.

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Sample availability: Contact the authors