Malaysian Journal of Chemistry, 2007, Vol. 9, No. 1, 022 - 028

2-N-Anilinopurine, 2-N-Piperidinopurine, 2-N-Phenylalaninopurine and 2-N-Prolinopurine: Fluorescence Characteristics

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Abstract : 2-N-Anilinopurine, 2-N-Piperidinopurine, 2-N-Phenylalaninopurine and 2-N-Prolinopurine were obtained when 2-fluoropurine was reacted with aniline, piperidine, phenylalanine and proline respectively. The structures of all the compounds were confirmed by infrared, ^{1}H NMR and mass spectrometries. The fluorescence characteristics of these 2-substituted purines were studied in 75% ethanol. 2-N-Anilinopurine showed the highest fluorescence intensity followed by 2-N-Piperidinopurine, 2-N-Phenylalaninopurine and 2-N-Prolinopurine showed the least intensity. 2-N-Anilinopurine, 2-N-piperidinopurine and 2-N-phenylalaninopurine were excited at 340 nm and the fluorescence peaks were observed at 473, 490 and 490 nm respectively, while 2-prolinopurine was excited at 385 nm, and fluoresced at 470 nm.

Abstrak : 2-N-Anilinopurina, 2-N-Piperidinopurina, 2-N-Fenilalaninopurina and 2-N-Prolinopurina diperolehi apabila 2-fluoropurina ditindak balas dengan anilina, piperidina, fenilalanina dan prolina. Struktur sebatian ini disahkan menggunakan spektroskopi inframerah, spektroskopi ^{1}H NMR dan spektroskopi jisim. Ciri pendafluoran sebatian ini dikaji dalam pelarut 75% etanol. 2-N-Anilinopurina menunjukkan keamatan pendafluoran yang tertinggi, diikuti oleh 2-N-Piperidinopurina, 2-N-Fenilalaniopurina dan yang paling kurang adalah 2-N-Prolinopurina. 2-N-Anilinopurina, 2-N-Piperidinopurina dan 2-N-Fenilalaninopurina diuja pada 340 nm dan puncak pendafluoran direkodkan pada 473, 490 dan 490 nm, manakala 2-prolinopurina pula diuja pada 385 nm dan berpendafluor pada 470 nm.

Received : 26.10.05; accepted : 22.11.07

Introduction

The fact that a number of organic compounds emit visible light when they are exposed to radiation from the sun been known for several years, and the first monograph on phosphorescence was published in 1640 [1]. Several centuries later, Herschel [2] reported on a solution of quinine sulphate that it emitted a strong luminescence when it was held in the sunlight. The study on the luminescence characteristic of organic compounds was then extended to the frozen aromatic compounds [3-6].

The progress in the fluorescence study of heterocycles is very slow although a wide variety of heterocyclic compounds are known to be fluorescent. In general, heterocycles tend to be most fluorescent in polar solvents whilst others are fluorescent in acidic solution. In these conditions, the lone pair of electrons of the heterocycles are bound to the solvent, and the longest absorption wavelength corresponds to π to π^* instead of n to π^* [7]. The fluorescence characteristics of nitrogen containing heterocycles are poorly understood at present and it is therefore very difficult to generalize. However, it appears that those heterocycles in which the longest absorption wavelength corresponds to n to π^* are non-fluorescent whereas those correspond to π to π^* are likely to fluoresce [7]. The fluorescence characteristic of selected heterocycles, such as diazines [8 – 9] and other systems [10-11] were reported.

In this study, various 2-substituted purines were prepared and their fluorescence characteristics in various solvent were studied. Comparisons between 2-substituted purines with 6-substituted purines were also studied. This paper will report on the fluorescence characteristic of 2-substituted purines in 75% ethanol.

Experiments

Preparation of 2-Substituted Purines

2-N-Piperidinopurine

Piperidine (20 mg) in ethanol (4 mL) was added to a solution of 2-fluoropurine (40 mg) [12] in ethanol (7 mL) and the mixture was refluxed for 1 hour at 100 ^{o}C. The mixture was cooled and ethanol was evaporated off. The slurry was extracted twice with ether. The ethereal layer was washed with water and dried over anhydrous sodium sulphate. Evaporation of ether gave crude product, which was recrystallised from petroleum ether.

The yield is 50%, decomposed above 215 ˚C, IR (cm^{-1}): 3115, 1673, 1620; ^{1}H NMR ($CDCl_3$) δ: 8.70, s, 1H (H_6), 8.00, s, 1H (H_8), 3.35, m, 4H ($H_{2'}$, $H_{6'}$), 1.68, m, 6H ($H_{3'}$, $H_{4'}$, $H_{5'}$); M^+: 203.1163, calculated: 203.117

2-N-Anilinopurine

2-Fluoropurine (80 mg) was added to aniline (3 mL) and warmed at 60 ^{o}C for four hours. The mixture was cooled and refrigerated overnight. 2-N-Anilinopurine was crystallized out of the reaction mixture. The crystal was filtered, washed with ice-cold water and dried. Pure product was obtained after recrystallisation from dichloromethane.

The yield of 73.5%, decomposed above 200 ˚C, IR (cm^{-1}): 3330, 1670, 1630; ^{1}H NMR ($_{d6}$-DMSO) δ: 8.65, s, 1H (H_6), 8.00, s, 1H (H_8), 7.20, m, 3H ($H_{3'}$, $H_{4'}$, H_5'), 6.90, d, 2H ($H_{2'}$ and $H_{6'}$), 5.40, d, 2H, (N-H); M^+:211.0858, calculated: 211.086.

2-N-Phenylalaninopurine

Phenylalanine (45 mg) was dissolved in sodium hydroxide solution (0.1 mol L^{-1}, 3 mL) and the pH was adjusted to 9.5. The solution was then added to a solution of 2-fluoropurine (40 mg) in ethanol (6 mL) and the mixture was refluxed for 45 minutes. The mixture was cooled and ethanol was evaporated off. Water was added to the residue and the pH of the solution was adjusted to 7 and shaken in ether. The ethereal layer was discarded. The pH of the solution was further adjusted to 3.4 and extracted three times with ether (3 x 4 mL), washed with water and dried over anhydrous sodium sulphate. Evaporation of ether gave crude product. Pure product was obtained using preparative thin layer chromatography using ethyl acetate: dichloromethane (1:2).

The yield is 30%, decomposed > 230^{o}C; IR (cm^{-1}): 3120, 1752, 1680, 1615; ^{1}H NMR ($_{d6}$-DMSO) δ: 10.20, b, 1H (H of COOH), 8.70, s, 1H (H_6), 8.10, s, 1H (H_8), 7.00-7.300, b, 5H ($H_{2'}$, $H_{3'}$, $H_{4'}$, H_5', $H_{5'}$), 3.00, b, 2H (aliphatic 2H of phenylalanino ring), 5.75, b(w), 1H, (N-H); M^+: Found: 283.1069, calculated: 283.1070.

2-N-Prolinopurine

2-Fluoropurine (55.5 mg) was dissolved in absolute ethanol (2 mL) and added to a solution of proline (70 mg) in sodium hydroxide solution (0.1 mol L^{-1}, 6 mL). The pH of the solution was adjusted to 9.35. The mixture was refluxed for 10 minutes, followed by the addition of another equivalent amount of sodium hydroxide solution ((0.1 mol L^{-1}). The mixture was refluxed for further 30 minutes. The mixture was then cooled and the solvent was evaporated off. A minimum volume of water was added and the pH of the mixture was adjusted to 7.00 and shaken in ether. The ethereal layer was discarded. The pH of the aqueous layer was further adjusted to 3.6 and extracted three times with ether (3 x 10 mL). The etherial layer was washed with water, and dried over anhydrous sodium sulphate. Ether was distilled off. Pure product was obtained after TLC separation using a mixture of methanol:ethylacetate (5:1).

Yield of 15%; mpt: 201-203˚C, IR (cm^{-1}): 3460, 1725, 1670, 1630, 1625; ^{1}H NMR ($_{d6}$-DMSO) δ: 12.00, 1H, (H of COOH), 8.65, s, 1H (H_6), 8.05, s, 1H (H_8), 4.23, m, 1H ($H_{1'}$), 3.40, m, 2H ($H_{4'}$), 2.09, m, 4H ($H_{2'}$ and $H_{3'}$), 5.50, b, 1H, (N-H); M^+:found: 233.067, calculated: 233.077.

General Analysis

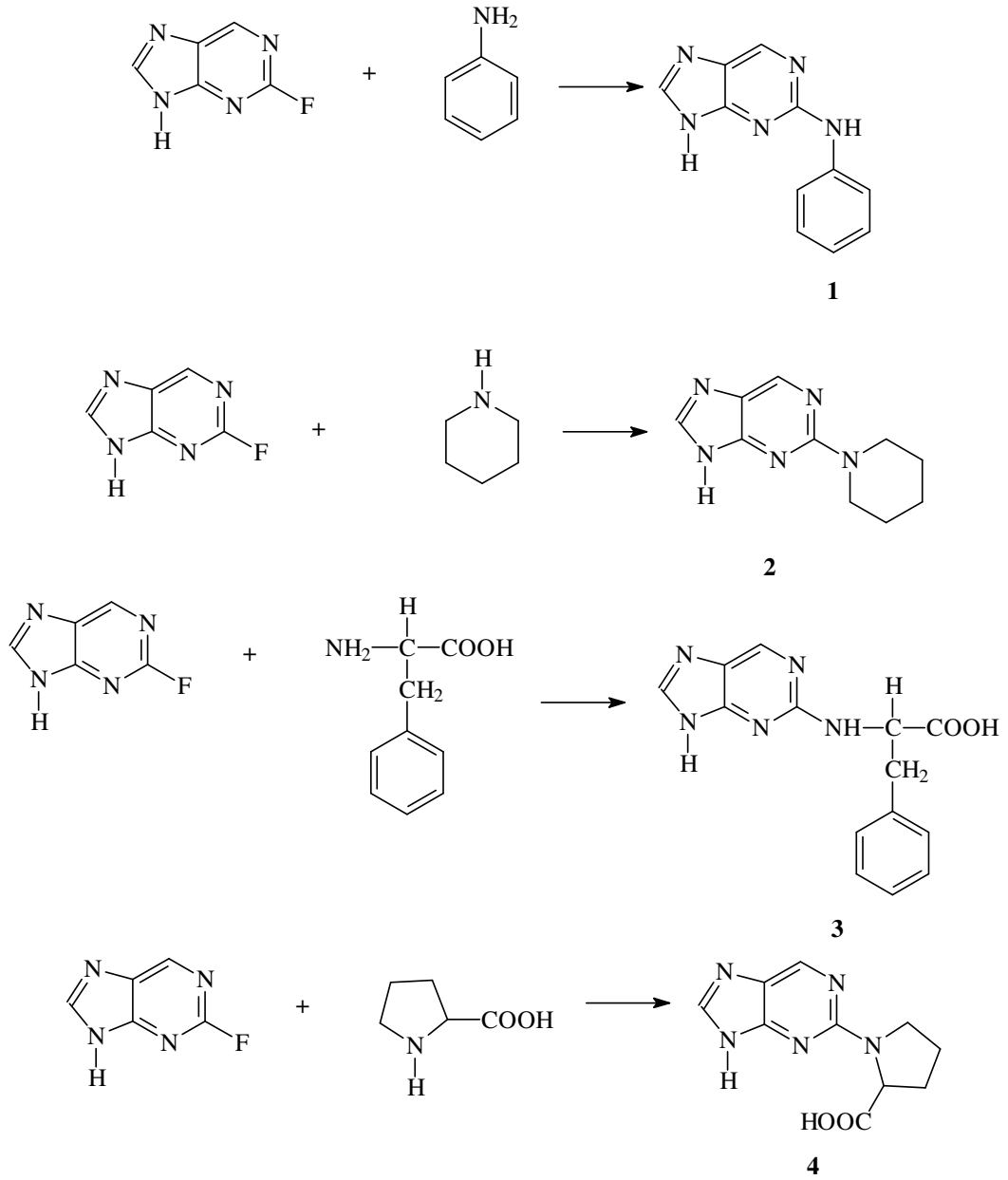
All solvents were redistilled before use. Melting points were determined with Electrothermal Melting Point Apparatus and were not corrected. The infrared spectra were recorded using Perkin Elmer 298 Infrared Spectrometer and FTIR Perkin Elmer 1600 Series. The ^{1}H NMR spectra were recorded on Bruker WP-80 and Bruker AM 250. The fluorescence measurement was carried out using Fluorescence Spectrophotometer Model F-2000 Hitachi.

Measurement of Fluorescence Intensity

2-Substituted purines of the same concentration were prepared in 75% ethanol. Quinine sulphate at the same concentration was used as the standard. The fluorescence intensity of quinine sulphate was taken to be 1.00. The measurements were recorded at room temperature in a quartz cell.

Results and Discussion

Treatment of 2-fluoropurine [12] with aniline, piperidine, phenylalanine and proline gave 2-N-anilinopurine (1), 2-N-piperidinopurine (2), 2-N-phenylalaninopurine (3) and 2-N-prolinopurine (4) respectively, as shown in Scheme 1.



Scheme 1

The structures of the above compounds were confirmed by spectroscopic methods, as recorded in the experimental section.

Figure 1-5 show the fluorescence emission spectra of 2-N-aminopurine, 2-N-anilinopurine, 2-N-piperidinopurine, 2-N-phenylalaninopurine and 2-N-prolinopurine in 75% ethanol respectively, and their relative fluorescence intensities are as shown in Table 1.

2-N-Aminopurine

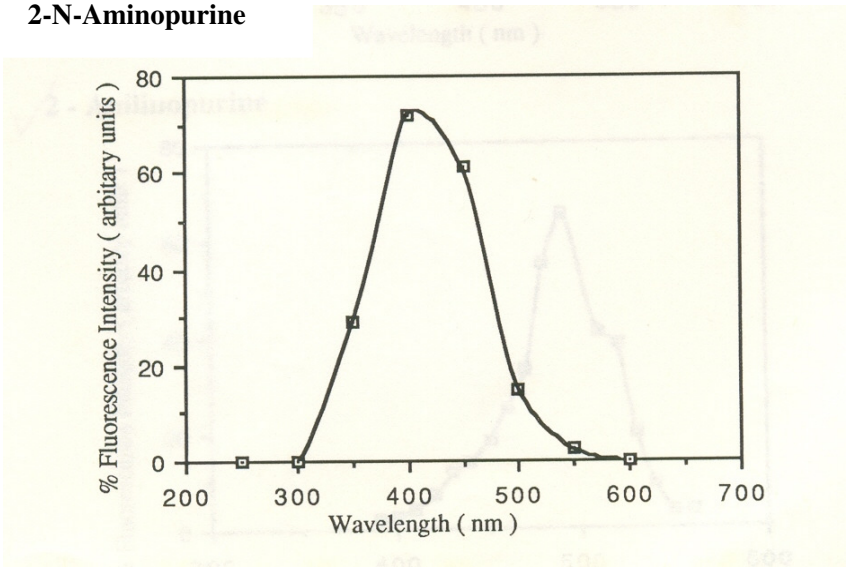


Figure 1: Fluorescence emission pectrum of 2-N-aminopurine (3.712 x 10^{-6} M)

Table 1: Fluorescence peaks of 2-X purine in 75% ethanol.

2-N-X Purine	Excitation wavelength/nm	Fluorescence peak/nm	Relative intensity*
Amino	340	380	0.964
Anilino	340	490	0.371
Piperidino	340	473	0.253
Phenylalanino	340	420, 490	0.481 (490 nm)
Prolino	385	470	0.037

*The fluorescence intensity of quinine sulphate is taken to be 1.00.

It can be seen from the table that the fluorescence intensities of all the substituted amino compounds are reduced relative to the unsubstituted aminopurine, and the emission maxima are shifted by about 100 nm towards the high wavelength. The shifting to a higher wavelength value is believed to be due the increased in the availability of electrons in the systems, which occurs either through sigma bonds in aliphatic substituents or increased mobility through the π system in the benzene ring [13].

2-N-Anilinopurine

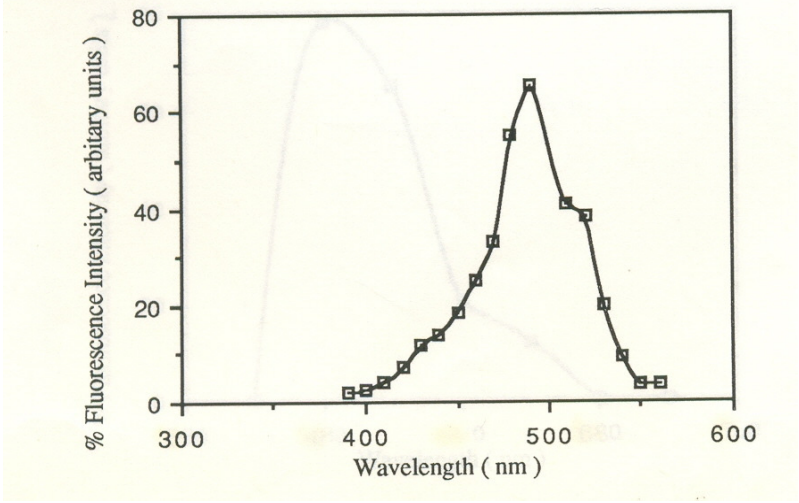


Figure 2 : Fluorescence spectrum of 2-N-anilinopurine (8.890 x 10^{-4} M)

2-N-Anilinopurine is more fluorescent than 2-N-piperidinopurine. This is probably partly due to the aniline part, whereby aniline itself fluoresced in water and ethanol at 345 nm [14], which is not far from that of 2-N-aminopurine. However, when the two are combined in the 2-N-anilinopurine, the fluorescence peak is shifted to 490 nm i.e. to a higher wavelength as shown in Figure 2, and the fact indicates an interaction between the two systems. The combined fluorescence characteristics from both rings i.e aniline and purine result in the relatively high fluorescence intensity, as recorded. Piperidine on the other hand, does not show any fluorescence characteristic either in water or organic solvents at room temperature, but 2-N-piperidinopurine shows nearly as much fluorescence as the 2-N-anilino derivative as shown in Figure 3. It is thought that the $-N(CH_2)_5$ group, like the $-NH_2$ group at C_2, changes the relationship of low lying n-π^* transition to π - π^*, as a result fluorescence becomes the predominant emission for this compound.

2-N-Piperidinopurine

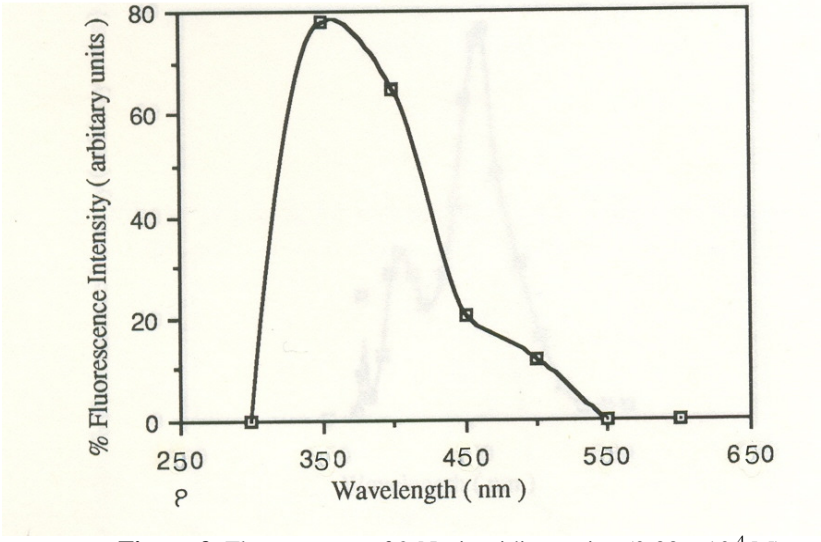


Figure 3: Fluorescence of 2-N-piperidinopurine (9.88 x 10^{-4} M)

2-N-Phenylalaninopurine

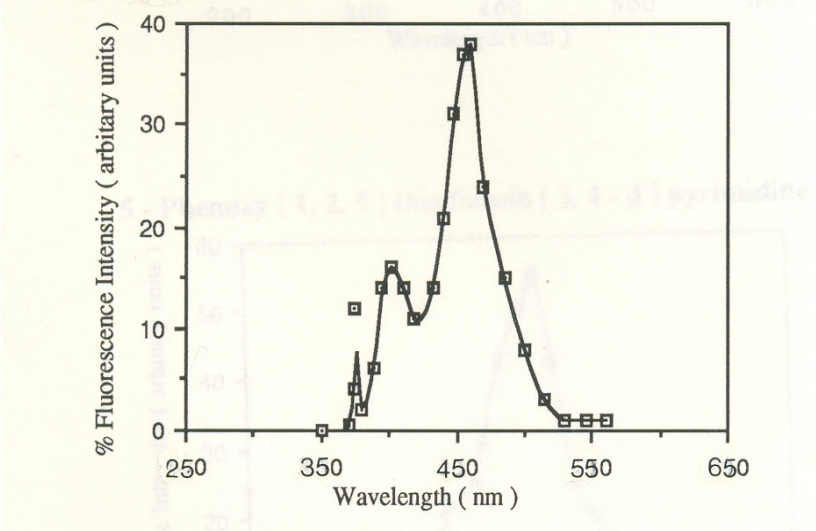


Figure 4: Fluorescence spectrum of 2-N-phenylalaninopurine (5.712 x 10^{-4} M)

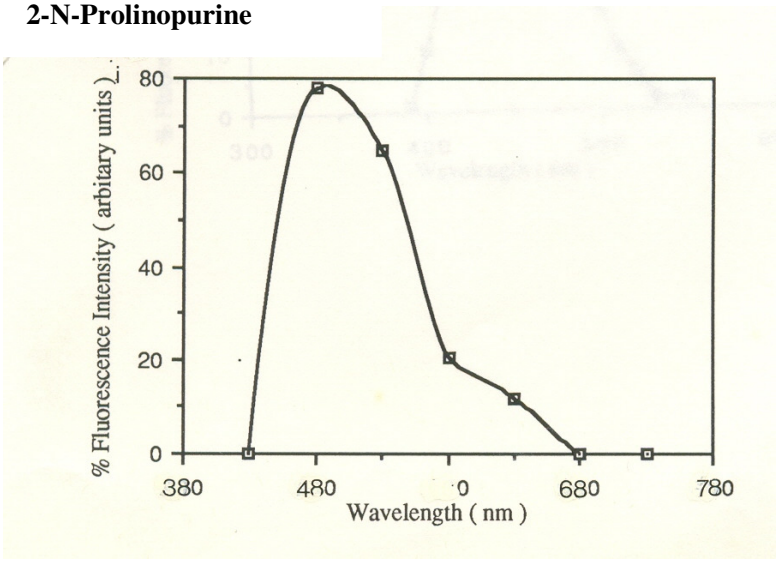


Figure 5: Fluorescence spectrum of 2-N-prolinopurine (3.712 x 10^{-4} M)

A fluorescence peak was observed when 2-N-phenylalaninopurine and 2-N-prolinopurine were dissolved in 75% ethanol, as shown in Figure 4 and Figure 5.

Both amino acids substituents are supplying the electrons to the purine ring even though the phenylalanino group is a better electron donator compared to the prolino substituent. In general, most of aromatic amino acids are fluorescent. Studies of phenylalanine [15] indicated that the fluorescence emission spectrum of phenylalanine in the solid state was shifted towards the visible region, as compared to that in the solutions. The native fluorescence of phenylalanine in solution was studied by Feitelson [16], and it was found that there was a decreased in fluorescence intensity when the carboxyl group was unionised or when it is esterified. The undissociated carboxyl group interacts with the excited aromatic ring causing quenching. This quenching effect results from the intermolecular charge transfer, and thus explains the decrease in the fluorescence intensity of phenylalanine in solutions. Therefore, the observed fluorescence intensity of 2-N-phenylalaninopurine which is less than that of 2-N-anilinopurine, is believed to be due to the above reason. 2-N-Prolinopurine is less fluorescent compared to 2-N-phenylalaninopurine, as expected. This is probably due to the proline itself, which is a non- fluorescent amino acid. The presence of a carboxyl group in proline ring does not increase the fluorescence characteristic of 2-N-prolinopurine, but it rather decreases the fluorescence intensity due to the same reason as in the phenylalanino system.

Conclusion

2-N-Aminopurine showed the highest fluorescence peak in 75% ethanol, followed by 2-N-anilinopurine, 2-N-piperidinopurine, 2-N-phenylalaninopurine and 2-N-prolinopurine. 2-N-Anilinopurine is the most fluorescent compounds amongst substituted aminopurine, which is may be due to the increase in mobility of electrons in the systems. Lower fluorescent intensity observed with amino acid derivatives is due to the quenching effect of the carboxyl group. The fluorescence characteristics of other amino and amino acid derivatives are under progress before a concrete conclusion can be made on the fluorescence characteristic of substituted purines.

Acknowledgements

The author wishes to thank University Malaya and Academy of Science for their financial support for this project.

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