SOME NEW ASYMMETRICAL PYRIDYL-SUBSTITUTED PYRAZINE AND QUINOXALINE LIGANDS FOR COPPER AND IRON

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The work reports the preparation of seven new pyridyl- substituted quinoxaline, dihydropyrazine and pyrazine ligands containing cuproin and ferroin functionalities. The absorptiometric properties of their reactions towards copper and iron have been studied. The reagents 2-(2'-pyridyl)-3-[2"-6"- methylpyridyl)]quinoxaline(V) and 2-(2'-pyridyl)-3-[2"-6- methylpyridyl)]-6-methylpyrazine (XI) for copper and 2-(2'-pyridyl)3-[2"-(6"-methylpyridyl)]-5,6-dihydropyrazine (VI) and 2- (2'-pyridyl)-3-(2"-(6"-methylpyridyl)]-5-methyl-pyrazine (X) are investigated for their possible use for the simultaneous determination of copper and iron in a single aliquot.

Key words: Pyrazine, Quinoxaline, Chromogenic reagents.

Introduction

Few organic reagents have been so thoroughly investigated as those of the ferroin and cuproin type chromogens [1]. Less work has been reported on pyridyl substituted pyrazines and quinoxalines, the related compounds of the same group. Goodwin and Lions [2], Buu-Hoi and Sain-Ruff [3], Jensen and Pflam [4], Peiffer and Case [5], Schilt and Hoyle [6], Stephen *et. al.* [7-10] have prepared a number of reagents to study these as chromogen reagents and their carcinogenic properties.

Goodwin and Lions [2] have observed that, when one of the nitrogen atom of the pyrazine ring has become involved in the metal ligand bond, the other nitrogen atoms lacks completely any tendency to do so, on this basis, they argued that 2,3-bis(2'- pyridyl)-5,6-dihydropyrazine and 2,3-bis(2'-pyridyl)pyrazine act only as bidentate, but not as bis-bidentate chelating agent. These findings are now confirmed by the work of other investigators [8].

With the above facts in mind, it was thought, if pyridyl substituted pyrazine compounds are designed in such a way that one half of the molecule contains, the "ferroin" atomic grouping and the other "cuproin" one, such compounds would form more sensitive and stable copper (I) complexes and maintain their reactions toward iron (II), and could be applied as a complexing reagent for copper and iron in a mixture. The molecules which formed metal ligand bonds with iron (II) would not react with copper (I), but more reagent would be required to form copper (I) complex and form the basis for simultaneous determination of copper and iron in a single aliquot.

Experimental

Preparation of the Reagents.

6-Methyl-2,2'-pyridoin(1-hydroxy-1-(2'-pyridyl)-2-[2"-(6"-methylpyridyl)]ethan-2-one). A mixture of 2-

pyridine-carboxalidehyde (10.7 g) and 6- methyl-2-pyridine-carboxaldehyde (12.1 g) dissolved in pyridine (30 ml) and water (200 ml), was warmed on a water barth. Potassium cyanide (0.7 g) dissolved in water (10 ml) was then added slowly with constant shaking. The reaction mixture was heated for 30 min. and cooled in ice bath to 3°. The solidified mass was filtered and washed well with distilled water.

Pyridine (50 ml) was added to the dry, crude material with continuous stirring. Most of the product dissolved in pyridine and the solution was filtered and cooled at -5° for 3-4 hr. The mixture was mixed well and precipitated material was filtered off immediately. Water (200 ml) was added to the filtrate and the precipitate was filtered and dried in air. Yield (35%), m.p.=82°, $C_{13}H_{12}N_2O_2$ requires % C=68.4, H=5.2, N=12.3; found % C=68.6, H=5.1, N=12.4.

6-Methyl-2,2'-pyridyl (1,2-Dioxo-1-(2'-pyridyl)-2-[2"-(6"-methyl- pyridyl)]ethane. Dry 6-methyl-2,2'-pyriodoin (10 g) was dissolved in ethanol (200-250 ml) with stirring and mixture was slightly warmed to complete the dissolution. The solution was filtered, and oxygen was bubbled through the orange solution for 6-8 hr. The solution changed to lemon yellow and was concentrated to 50-60 ml. Precipitated material was recrystallised from ethanol. Yield 50%, m.p. 122°, $C_{13}H_{10}N_2O_2$ requires % C=69.92, H=4.42, N=12.39, found % C=69.3, H=4.45, N=12.4.

2-(2'Pyridyl)-3-[2"-(6"methylpyridyl)]quinoxaline(V). 1,2-Diaminobenzene (2.2 g) dissolved in ethanol (10 ml) was slowly added to the refluxing solution of 6-methyl-2,2'-pyridyl (4.4 g) in ethanol (25 ml). The mixture was heated for 1 hr and half of the ethanol was distilled off, and remaining solution was diluted with water until permanent turbidity was apparent. The mixture was cooled to 3°and filtered. Recrystallised from petroleum spirit (60-80°), m.p. 102; $C_{15}H_{14}N_4$ requires % C=76.51, H=4.69, N=18.79; found % C=76.5, H=4.8, N=18.7.

2-(2'-Pyridyl)-3-[2"-(6"-methylpyridyl)]-5,6-dihydropyrazine (VI). 1,2-Diaminoethane (1.2 g) dissolved in ethanol (4 ml) was slowly added to the solution of 6-methyl-2,2'-pyridyl (IV) (4.2 g) in ethanol (25 ml). The mixture was heated for 30 min. and cooled to -5°. Precipitate filtered and recrystallised from ethanol or benzene - petroleum spirit (60-80°), m.p. 167°; $C_{15}H_{14}N_4$ requires % C=72.0, H=5.6, N=22.4; found % C=72.3, H=5.8, N=22.6.

2-(2'-Pyridyl)-3-[2"-(6"-methylpyridyl)]-5-methyl-5,6-dihydropyrazine (VII) and 2-(2'-pyridyl)-3-[2"-(6"-methylpyridyl)]-6-methyl- 5,6-dihydropyrazine (VIII). 1,2-Diaminopropane (1.3 g) in ethanol (4 ml) was slowly added to the hot solution of 6-methyl-2.2'-pyridyl (4.2 g) in ethanol (25 ml). The mixture was refluxed for 30 min. and most of the ethanol was distilled off. The dark residue was dissolved in benzene and benzene was removed on rotary evaporator under reduced pressure. This process was repeated first with benzene- petroleum spirit (60-80°), then with petroleum spirit (60-80°) only. The gummy mass solidified slowly to a yellow-brown solid m.p. = 85, $C_{16}H_{16}N_4$ requires % C=72.7, H=6.1, N=12.2; found % C=72.5, H=6.0, N=20.9. The mixture of brown and yellow solid was separated on the basis of fractional crystallisation as under:

The mixture was dissolved in petroleum spirit (60-80°) and the solution was left until most of the brown precipitate has crystallized out first and the yellow precipitate had started to deposit over the brown one. The solution was transferred to other flask and the brown precipitate was recrystallised twice from petroleum spirit (60-80°), m.p. 125°, found % C=72.9, H=6.1, N=21.3. The product is assumed to be 2-(2'-pyridyl)-3-[2"-(6"- methylpyridyl)]-6-methyl-5,6-dihydropyrazine (VIII).

The petroleum spirit in the solution transferred to the flask was evaporated out, and the yellow product so obtained was dissolved in a minimum amount of petroleum spirit (60-80°). The solution was again separated from the mixture of the brown and yellow precipitate which was deposited initially, and the product was then allowed to crystallise out. The yellow solid thus obtained was recrystallized from n-hexane, m.p. 77-9°; found % C=72.5, H=6.1, N=20.9. The product is thought to be 2-(2'- pyridyl)-3-[2"-(6"-methylpyridyl)]-5-methyl-5,6-dihydropyrazine (VII).

2-(2'-Pyridyl)-3-[2"-(6"-methylpyridyl)]pyrazine(IX), 2-(2'-pyridyl)-3-[2"-(6"-methylpyridyl)]-5-methylpyrazine(X) and 2-(2'-pyridyl)-3-[2"-(6'-methylpyridyl)]-6-methylpyrazine (XI). The dihydropyrazine compounds VI, VII or VIII (5.0 g) were dissolved in mesitylene (30 ml) containing palladium charcoal (10%, 0.5g). The mixture was refluxed for 30 hr and then filtered while hot. The clear filtrate was concentrated to 10 ml and 10

ml of n-hexane was added to the residue. The solution was cooled overnight at -5°. Precipitates recrystallised from n-hexane or petroleum spirit (60-80°). IX melted at 121-23, $C_{15}H_{12}N_4$ requirres % C=72.56, H=4.86, N=22.56; found % C=72.5, H=5.0, N=22.4; X melted at 125-27°; $C_{16}H_{14}N_4$ requires % C=73.3, H=5.3, N=21.4; found % C=73.3, H=5.2, N=21.4. XI melted at 102°, $C_{16}H_{14}N_4$ requires % C=73.3, H=5.3, N=21.4; found % C=73.4, H=5.3, N=21.2.

Solutions. Solutions containing 1 mg/ml of a cation were prepared from AR grade $(NH_4)_2SO_4$. $FeSO_4.6H_2O$, $CuCl_2.2H_2O$, $ZnSO_4.H_2O$, $Co(NO_3)_2$. $6H_2O$, $Cr(NO_3)_3$, $9H_2O$, $CdSO_4$. $8H_2O$ and $RuCl_3$. $5H_2O$. A range of conventional buffers at unit pH interval in the range of 1-13 was prepared using where appropriate one or more of the following: Hydrochloric acid, potassium chloride (2 M). acetic acid (2 M) sodium acetate (2 M), ammonium chloride (2 M), aqueous ammonia and sodium hydroxide.

Analytical procedure was followed as reported earlier [16]. The absorption spectra in visible region were recorede on a Unicam SP 800 recording Spectrophotometer and Cecil CE 505 Spectrophotometer in conjection with recorder CE 500.

Analysis of tap water. Three samples (200 ml) of tap water from hot water supply were collected from the laboratory after running the water for 5 min. The samples were allowed to cool at room temperature and 5 ml of 1% W/V ascorbic acid, 5 ml of acetate buffer pH 4.6, 10 ml of the reagent solution VI, followed by 4 g of sodium perchlorate and 7-8 ml of chloroform was then added. The contents were mixed well. The organic layer was collected in a 25 ml volumetric flask and extraction was repeated twice with 5 ml of chloroform and 1 ml of ethanol was added before dilution to volume with chloroform. The absorbances of the solutions were measured at 600 nm and 539 nm against reagent blank prepared from deionized water.

Results and Discussion

When 2-pyridinecarboxaldehyde and 6-methyl-2-pyridine-carboxaldehyde were condensed in the presence of potassium cyanide three possible products 2,2'-pyridoin (I) 6-methyl-2,2'- pyridoin (II) and 6,6'-dimethyl-2,2'-pyridoin (III) were actually obtained during the course of reaction and asymmetric 6-methyl- 2,2'-pyriodoin (II) was separated from the remaining symmetrical products on the basis of solubility in pyridine, where 6-methyl- 2,2-pyridoin is more soluble than the other two products (Fig. 1). However it changed to a semi gummy mass on keeping. It was always used in the next step on the same day. 6-methyl-2-pyridoin was oxidized to diketone (IV) by passing oxygen through its solution in ethanol but an attempt to oxidize 6-methyl-2,2'- pyridoin (II),
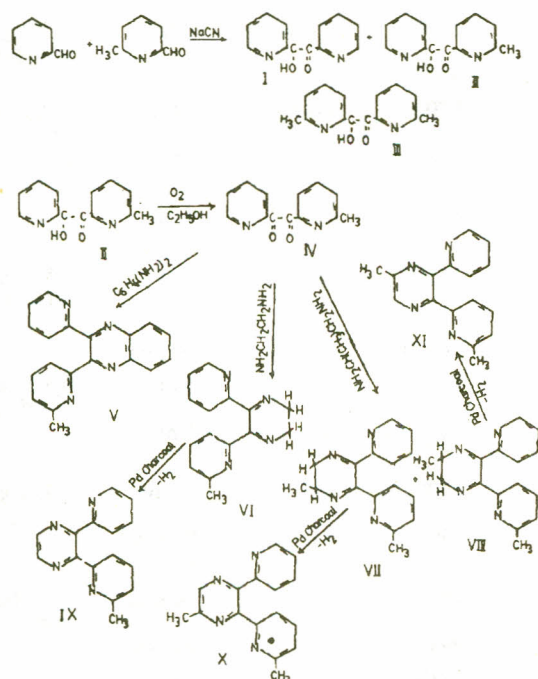
Fig. 1 The pictorial Scheme for the preparation of the ligands.

with dilute nitric acid yielded only some gummay mass. The colour of diketone (IV) changed to green on the surface due to exposure to light and atmosphere, but it retains normal reaction.

It is interesting to note that 6-methyl-2-pyridyl condenses with 1,2-diaminopropane to form VII and VIII which were identified on basis of their reactions towards iron (II) (Fig. 1). Where VIII reacts with iron (II) in aqueous ethanolic media to form greenish blue complex of low intensity, but VII show fairly good chromophoric sensitivity in same solvent system (Table 1). This is further supported by the dehydrogenation of VII to VIII, where the latter does not show any reaction toward iron (II) as expected, but corresponding compound X formed by the dehydrogena-tion of VII maintained colour reactions towards iron (II).

The cuproin reagents V and XI form maximum colour reactions with copper (I) in pH range 4-7 and obey the Beer's law within 0.5-$12 \mu g/ml$ of copper, with relative error $\pm 2\%$ for analysis of copper solutions. Co(II), Ni(II), Fe(II), Ru(II), Cr(III), Bi(II), citrate, tartrate and phosphate did not interfere

TABLE 1. QUANTITATIVE ABSORPTIOMETRIC DATA OF COPPER (I) AND IRON (II) COMPLEXES.

Reagent	Metal ion	Solvent	Colour of complex	λ-max nm	ε-mole^{-1} cm^{-1}	pH of max colour formate	Solution stability	Linear calibration ranges
2-(2'-pyridyl)-3-2"-(6"-methyl pyridyl)] quinoxaline	Cu (I)	Ethanol	Purple	524	6660	–	24 hr	0.5-12μg/ml of copper.
	" "	1,2-Dich-loroethane	" "	522	6700	3.5-7	24 hr	
2-(2'-pyridyl)-	Cu (I)	Water	Purple red	549	11000	–	30 min	
3[2"-(6"-methyl-pyridyl)]-5,6-di-hydropyrazine	"	Chloro-form	" "	539	10800	4-7	2 hr	0.5-7.0μgml of copper.
	Fe (II)	Water	Blue	605	15000	–	30 min	–
	" "	Chloroform	" "	600	14700	4-7	2 hr	0.5-5μg/ml iron.
							The absorbance them decreased with some bathochromic shift.	
		Nitrobenzene	Blue	600	15900	–	24 hr	
2-(2'-pyridyl)-3-[2"-(6"-methyl-pyridyl)]-6-methyl-	Cu (I)	Water	Red purple	550	11800	–	3 hr	–
-5, 6-dihydro pyrazine	" "	1,2-Di-chloro-ethane	" "	535	10900	4-7	4 hr	0.5-7.0μg/ml. of copper.
	Fe (II)	Water	Greenish blue	61d2	3700	–	Absorbance slowly increased.	
		1,2-Dichlo-roethane	Blue	600	13600	4.2-5.0	2 hr	0.5-5.0μg/ml for iron.
2-(2'-pyridyl)-3-2"-(6"-methyl-	Cu (I)	Water	Orange	466	6200	–	2 hr	
pyridyl)-6-methyl-pyrazine	." "	1,2-Dichlo-roethane	" "	458	7100	4-7	16 hr	0.5-12 μg/ml. of copper.

Contd. Table 1.

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2-(2'-pyridyl)-3-2"-(6"-methyl-pyridyl) pyrazine	Cu (I)	1,2-Dichloroethane	Orange	460	6390	4-6	3 hr	1-12μg/ml. of copper.
	" "	Nitroben-	" "	460	6400	" "	1 h	–
	Fe (II)	1,2-Dichloroethane	Reddish pink	532	9700	44-6	24 hr	1-8μg/ml of iron.
	" "	Nitrobenzene	" "	534	108001	–	24 hr	–
2-(2'-pyridyl)-3-[2"-(6"-methyl-pyridyl)]-5-methyl-5,6-dihydropyrazine	Cu (I)	Water	Red purple	522	12300	–	2 hr	–
	" "	1,2-Dichloroethane	" "	536	11300	4-7	8 hr	0.5-7.0μg/ml of copper
	Fe (II)	Water	Blue	605	14000	–	30 min	–
	" "	1,2-Dicloroethane	" "	600	14200	4-6.5	4 hr	0.5-5.0μg/ml of iron (II).
2-(2'-pyridyl)-3-[2"(6"-methyl-pyridyl)]-5-methyl-pyrazine	Cu (I)	Water	Orange	471	6400	–	1 hr	–
	" "	1,2-Dichloroethane	" "	460	7100	4-7	2 days	1-12 μg/ml
	" "	Nitrobenzene	" "	461	6700	–	2 days	–
	Fe (II)	Water	Reddish pink	524	2850	–	30 min	0.5-7.0μg/ml ofiron
	" "	1,2-Dichloroethane	" "	522	9980		2 days	
	" "	Nitrobenzene	" "	524	10100	–	–	–

TABLE 2. VALUES OF MOLAR ABSORPTIVITY AT THE -MAX OF COPPER AND IRON COMPLEXES.

Reagent solvent		Copper (I)		Iron (II)					
		λ-max nm	$\varepsilon l.\,1.mole^{-1}\,cm^{-1}$	λ-max nm	$\varepsilon l.\,mole^{-1}\,cm^{-1}$	λ-max nm	$\varepsilon l.mole^{-1}\,cm^{-1}$	λ-max nm	$\varepsilon l.mole^{-1}\,cm^{-1}$
VI	Chloroforma	539	10801	600	3177	600	14799	539	8795
	Nitrobenzene	539	10801	600	3177	600	15916	539	9095
VII	1,2-Dichloroethane	536	11357	600	2209	600	14240	536	7958
X	" "	460	6398	532	3359	532	9773	460	4886

at ten times the concentration of copper.

The reagents VI, VII, VIII, IX and X react with both copper(I) and iron (II) and their complexes are extractable in 1,2-dichloroethane and nitrobenzene as perchlorate ion association complex, with maximum colour formation within pH 4-7 (Fig. 2) except iron (II) complex of VIII which is extractable within a narrow pH range of 4.2-5. The ion association complex of IX slowly breaks down with loss of sensitivity, but the complex in 1,2-dichloroethane, when collected over anhydrous sodium sulphate shows excellent chromophoric stability. Linear calibration curves were obtained for both copper and iron with relative percentage error within ± 3% for analysis of test solutions of copper and iron.

For simultaneous determination of copper and iron in a single aliquot, various procedures with different reagents have been reported Banerjea *et. al.* [11], Schill *et al.* [15], Yee *et al.* [14], Stephen *et. al.* [13], Bhaskara *et. al.* [12]. The reagent VI, VII, IX and X could be used for the simultaneous determination of copper and iron in a single aliquot, after
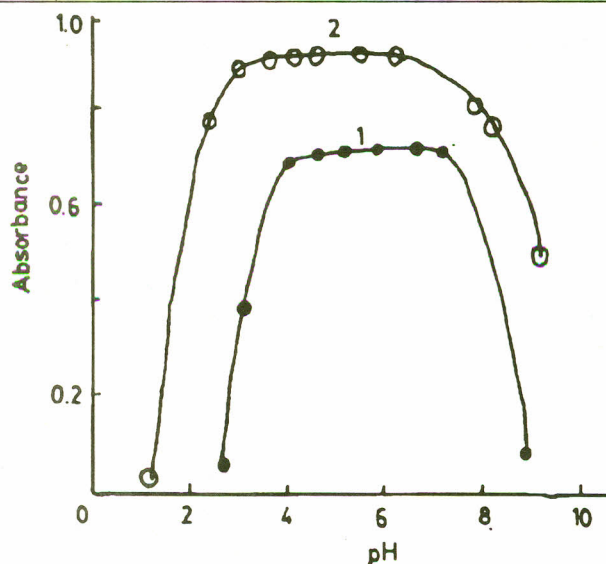

Fig. 2. The effect of variation in pH on the extraction of (1) iron (II) and (2) copper(I) complexes with 2-(2'-pyridyl)-[2"-(6'-methylpyridyl)]-5-methylpyrazine in 1,2-dichloroethane as perchlorate ion association complex at a final conc. of 4.0 mg/ml and 8.0 mg/ml of iron (II) and copper (I) respectively.
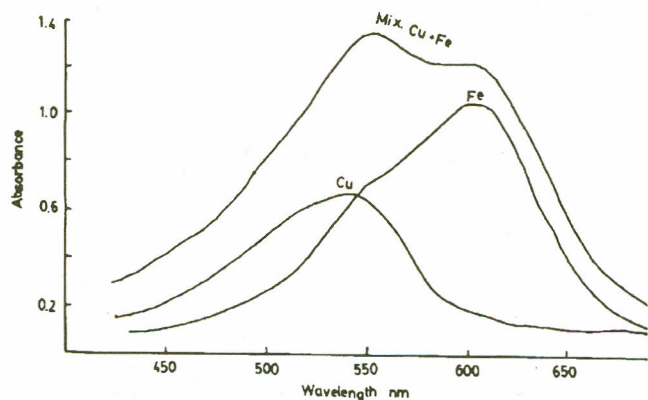
Fig 3. Absorption spectra of copper (I)(4.0μg/ml), iron (II) (4.0 μg/ml) and a mixture of copper (I) and iron (II) (4.0 μg/ml copper + 4.0 μg/ml iron) chelates of 2-(2'-pyridyl)-3-[2"-(6-methylpyridyl)]-5,6- dihydropyrazine in chloroform as perchlorate ion association complex

extraction of the complexes in 1,2-dichloroethane or nitrobenzene as a perchlorate ion association complex and measuring the absorbances at their respective wavelengths of maximum absorbances of both copper(I) and iron(II) complexes. (Fig. 3). A separate simultaneous equation is required to obtain the concentration of metal ion. The reagent VI and X proved to be better reagents for the simultaneous determination of copper and iron with an error within ± 6% Ni(II) and Co(II) only interferred in the determination of copper and iron. Their presence in moderate amounts enhanced the absorbance of the test solutions, due to the formation of coloured complexes, but with amounts as large as 40   μg/ml, the absorbance decreased on account of consumption of the reagent.

The cations and anions Cd(II), Mn(II), Cr(III), Ru(II), V(IV), Pb(II), Bi(II), Zn(II), Citrate, tartrate and phosphate, when present ten times the concentration of copper and iron did not interfere.

Finally the tap water was analysed for the copper and iron contains using reagent VI and were found to be 0.395 and 0.025 μg/ml with coefficient of variation of 3.2% and 2.9%

respectively.

The amount of copper in tap water was also analysed by an independent spectrophotometric method using diethyl dithiocarbamate. The amount of copper found was 0.41ug/ml. Both the results agree fairly well with each other.

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