

Structural studies of some thiotriazole compounds and their effect on algae and bacteria

Hendrik C. A. van Beek A. B. Tadros*

Laboratory of Chemical Technolgy, delft University of Technology, Julianalaan 136, Delft, The Netherlands.

* National Institute of Oceanography & Fisheries, Kayet Bey, Alexandria, Egypt.

ABSTRACT

Three new derivatives of 4-amino-3-hydrazino-5-thio-1,2,4-triazole: 4-(2,4-dichlorobenzylideneamino)-3-hydrazino-5-thio-1,2,4-triazole (H_2Cl , BATrztz), 4-(Octylamino)-3-(Octylhydrazin)-5-thio-1,2,4-triazole (H_2 OAOHTrztz) and 4-(methylisothiocyanateamino)-3-hydrazino-5-thio-1,2,4-triazole (H_2 MTAHTrztz) and their copper complexes have been prepared. H_2 OAOHTrztz, Although it was prepared in 1:1 molar ratio between 4-amino-3-hydrazino-5-thio-1,2,4-triazole and octyl aldehyde, the dioctyl product is formed. The molecular weight of the prepared ligands were confirmed from elemental analysis and mass spectral measurements.

Ethanol or hydrochloride salt and water molecules are suggested to be present in the crystal structure of H_2Cl_2 BAHTrztz ligand which depends on whether the ligand is prepared in ethanolic or aqueous medium.

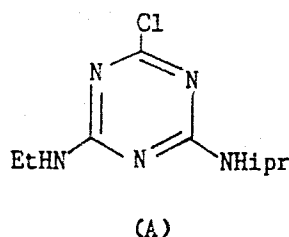
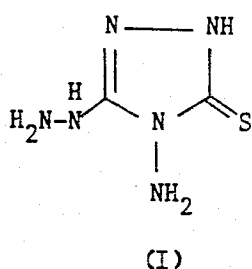
All copper complexes were prepared in 1:1 copper ligand ratio, and the only one formed with ML_2 ratio is $Cu(H_2OAOHTrztz)_2Cl_2 \cdot 2H_2O$ complex. Both (H_2Cl_2 BAHTrztz) and (H_2 MTAHTrztz) ligands behave as monovalent bidentat and their copper complexes formed with the general formula CuL_x (where x is stand for chloride).

The antibacterial and antialgal effects of some of the prepared compounds have been tested. Their EC_{50} values have been calculated and compared with the standard test substance TBTO.

INTRODUCTION

The coordination behaviour of 4-amino -3-hydrazino -5-thio -1, 2, 4-triazole (I) and its mono and dibenzylidene derivatives with copper ion has been examined (1, 2).

Now the authors report the synthesis and structural studies of three new derivatives of compound (I) derived from its reaction with 2, 4-dichlorobenzaldehyde, octylaldehyde and methylisothiocyanate. The choice of these three different compounds was based on studying the effect of different functional activity of compound (I) and its benzylidene derivative. For example, methyl group has an influence on the biological action, because it changes the electronic effect and alters the biological properties. Its effect is found in the triazine herbicides compound A.



One of the striking cases were an inert substituent exerts a profound change in biological action in the homologous series each member is usually found to be more biologically than the previous one until suddenly of the last one more-CH₂ group severely diminishes(3). Also, the presence of an electron attracting group such as chloro-and nitr-groups in the phenyl ring enhances the activity as have been observed with chloroproquinol and halogenated 8-quinolinols which display broad spectrum antibacterial and antifungal activity (3).

A combination of heavy metal atom with organic molecule sometimes

increases the antifungal action and more important the selective toxicity of the metal (4). So, the copper complexes of the last ligands have been prepared and their effective lethal concentration EL_{50} has been estimated through the standard test using bacterium phosphoreum species and chlamydomonas unicellular algae.

EXPERIMENTAL

1. preparation of the ligands. 4- (Dichloro- benzylideneamino)-3-hydrazino-5-thio-1, 2, 4-triazole, 4-(octyl-amino)- 3-(octylhydrazino)-5-thio- 1, 2, 4-triazole were prepared using the same procedure described by Ronald G. Dickinson (5) :(1.45 g) 4- amino-3-hydrazino-5- thio-1, 2, 4-triazole (I) was dissolved in hot (1 M HCl) 75 ml, then 2,4-dichlorobenzol- dehyd (1.75 g) in ethanol or water, octyaldehyde (1.28 g) in ethanol was added with stirring over 20 min with ligand (I), the product was collected after cooling, washed and recrystallized from ethanol.

4- (Methylisothiocyanate amino)-3-hydrazino-5-thio-1, 2, 4- triazole was prepared by adding equimolar amounts of (I) and methylisothiocyanate in dry DMF. The mixture was refluxed for 24 hrs, separated by drying against air and recrystallized from ethanol.

2. Preparation of copper complex. An aqueous solution of $CuCl_2$ (0.1 mole) was added with stirring to a hot suspended ethanolic solution of the ligand (0.1 mole). All the complexes appear immediatly. The precipitates were washed with water, ethanol and dried under vacuum at $50^{\circ}C$.

3. Analyses. The chemical analysis of carbon, hydrogen, sulphur, chlorine and nitrogen were done by analytical laboratory, physical treatment, chemical Technology Department, Technical University, Delft, Holland. The estimation of

Structural studies of some thiotriazole.....

copper was carried out by standard complexometric titrations.

Infra-red spectra of the ligands and their complexes were done on perkin Elmer 389 infra-spectrophotometer in the range $4000-400\text{ cm}^{-1}$ using potassium bromide pellets of the sample.

^1H NMR Spectra of the ligands and diamagnetic complexes in deuterated DMSO were recorded at 35°C on 60 MHz varian EM-360 spectrophotometer. The mass spectra of the ligands were done on mass spectrometer MAT 311 A.

4. Biological laboratory test. 4.1. Test organisms. The fresh water green flagellate *Chlamydomonas reinhardtii* (CCAP 11/32C) was produced from the culture collection of algae and protozoa (CCAP), 36 story 's way, Cambridge, CB 3DDT, England 6.

Freeze-dried cultures of the bacterium *photobacterium phosphoreum* were supplied by Beckman Inc.

4.2. Preparation of test solutions. The concentrations to be tested were chosen on basis of the solubility of the compounds in sea- water. DMSO was used as solvent for the stock solution.

4.3. Growth inhibition test with *C. reinhardtii*. The test was essentially the same as that described in NEN 6506 (7). An algal suspension containing 10^4 cells per ml was prepared from a preculture. The test vessels were 180 ml culture bottles containing 100 ml of algal suspension, to which was added 100 μl of the DMSO solution (or suspension) of test substance.

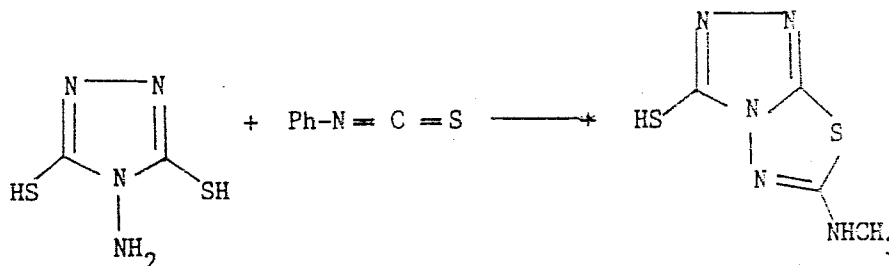
4.4. Microtox. test. The microtox. Test was that described in the

microtox. manual (8). A suspension of bacteria was prepared in saline poured into cuvettes. Solutions of test substances were prepared in saline by adding 200 μ l of the DMSO solution or suspensions to 100 ml of saline in volumetric flask.

RESULTS AND DISCUSSION

The elemental analysis along with some physical properties as reported in Table I. The elemental analysis of H₂ OAOHTtrz ligand indicates its dioctyl structure. Its molecular weight is deduced from mass spectral measurement (Fig. 1).

The reaction between 2,5-dimercapto-4- amino-1, 2, 4-tri- azole and phenylisothiocyanate was reported to give the following product. The behaviour which is not observed between the reaction of 4-amino-3-hydrazino-5-thio-1, 2, 4-triazole and methylisothiocyanate and the product is suggested to have open side chain-structure(VI) which is probably due to the delocalization of mercapto to form zwitter ion (9). ¹HNMR spectra of the ligands and their diamagnetic complexes were measured in DMSO and their chemical shift data are given in Table II.



The presence of one-NH₂ group proton signal at 5.3±0.2 PPM with H₂Cl₂ BAHTtrz and H₂ MTTAHTtrz ligands indicate that, only one amino group of the parent compound H₂ATtrz is blocked through its condensation with 2, 4-dichlorobenzaldehyde and methylisothiocyanate. The situation differs with H₂ OAOHTtrz ligand in which, no signal due to NH₂ group appears indicating that, the

Structural studies of some thiotriazole.....

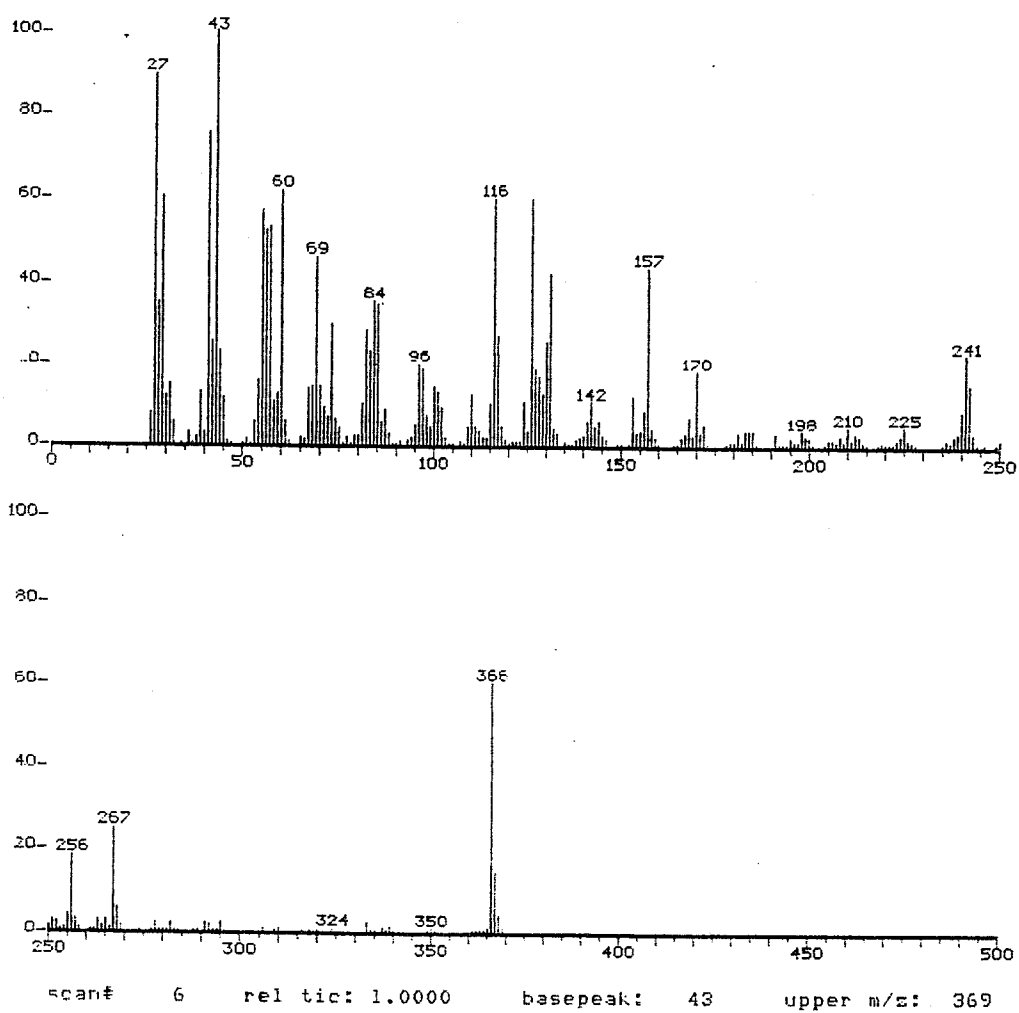


Fig. 1: Mass spectrum of 4-(octylamino)-3-(octylhydrazino)-5-thio-1,2,4-triazole

TABLE I
List of investigated compounds

No.	Compound	Structure	Color, m.p. (°C) Yield, % Analytical data Calc., (Found)
I	4-(2,4-Dichlorobenzylidene-amino)-3-hydrazino-5-thio-1,2,4-triazole. (H ₂ Cl ₂ BAHTtrz).EtOH		Yellow, 243 98 C, 37.82, (37.09) H, 3.75, (3.72) N, 24.06, (24.68) S, 9.18, (9.25) Cl, 20.29, (20.46)
II	Cu(HCl ₂ BAHTtrz).Cl.4H ₂ O.EtOH		Green, > 300 55 C, 25.44, (25.90) H, 4.08, (4.00) N, 16.18, (16.04) S, 8.17, (7.92) Cl, 20.48, (20.24) Cu, 12.22, (12.20)
III	4-(2,4-Dichlorobenzylidene-amino)-3-hydrazino-5-thio-1,2,4-triazole (H ₂ Cl ₂ BARTtrz).HCl.2H ₂ O		Yellow, 252 98 C, 28.85, (28.25) H, 2.69, (2.66) N, 22.42, (21.98) S, 6.56, (8.25) Cl, 28.38, (28.10)
IV	4-(Octylbenzylideneamino)-3-(octylbenzylidenehydrazino)-5-thio-1,2,4-triazole. (H ₂ OAOHTtrz)		Yellow, 120 36 C, 58.98, (58.33) H, 8.80, (9.30) N, 22.92, (22.40) S, 18.75, (18.38)
V	Cu(H ₂ OAOHTtrz) ₂ .Cl ₂		Black, 133 68 C, 47.85, (47.91) H, 8.03, (7.79) N, 18.60, (18.40) S, 7.10, (7.00) Cl, 7.85, (6.99) Cu, 7.03, (7.00)
VI	4-(Methylisothiocyanateamino)-3-hydrazino-5-thio-1,2,4-triazole (H ₂ MTAHTtrz)		Violet, 241 40 C, 21.91, (21.00) H, 4.13, (4.07) N, 44.71, (45.55) S, 29.24, (28.91)
VII	Cu(HMTAHTtrz).Cl		Grey, 255 55 C, 25.35, (25.00) H, 2.54, (2.55) N, 30.91, (30.57) S, 20.15, (20.74) Cu, 19.96, (20.10)

Table (II) : Characteristic ^1H NMR signals of the ligands and their diamagnetic complexes in DMSO δ (ppm).

Compound	-NH ₂	-NH	H - \emptyset	H	-SH	-CH ₃	CH ₂	-OH
		-NH		-C=H	H ₂ O			
(H ₂ Cl ₂ BAHT ^{tr}) ₂ . EtOH	5.5	11.7	7.49 - 7.9	8.69	3.6	1.1	3.4	4.4
		13.06						
(H ₂ Cl ₂ BAHT ^{tr}) ₂ . HCl. 2H ₂ O	5.53	11.14	7.67 - 7.88	8.69	3.3	-	-	-
		13.03						
H ₂ OAOHT ^{tr}		8.27		7.2	3.4	0.86	1.25	
		13.00		7.6				
Cu (H ₂ OAOHT ^{tr}) ₂ . 2H ₂ O. Cl ₂					0.86	1.23		
H ₂ MTAHT ^{tr}	5.25	8.0			3.36	2.7 - 2.9		
		8.2						
		8.9						
		12.5						
Cu (HMTAHT ^{tr}) ₂ . Cl	5.25	8.0			3.36	2.7 - 2.95		
		8.6						
		9.26						
		12.8						

condensation reaction in 1:1 molar ratio between H_2 AHTtrz and octylaldehyde gave the dioctyl derivative instead of the expected mono derivative. Also, the mass spectrum of the ligand supports the proposed structure, Fig. 1. Thione-thiol tautomerism is suggested to be present with all ligands due to the presence of both 1- NH and -SH proton signals at 12.8 ± 1.5 and 3.4 ± 0.1 PPM, respectively (10), while the signal of the other NH group appears round 9.8 ± 1.5 PPM, for the three ligands.

The signals of ethanol or water molecules for the H_2Cl_2 BAHTtrz ligand appear at (1.1, 3.4 and 4.4) and 3.3 PPM, respectively.

1H NMR spectrum of cu (HMTAHTtrz). Cl complex does not give direct indication about the bonding site, due to the presence of more than one imino proton and the possibility of the tautomeric isomerism with the triazole ring and also in the side chain. The position of -NH₂ signal does not change by complexation, which excludes its coordination to the cooper ion. On the other hand, all the imino proton signals are deshielded by complexation, Table II.

The I.R spectra of the ligands exhibit a band at 1050 ± 50 cm^{-1} . The absence of any absorption bands in the region 2400-2600 cm^{-1} with all ligands indicates their existance in the thione form. The position of the ν_{NH} and ν_{NH_2} groups in the ligands H_2Cl_2 BAHTtrz and H_2 MTTAHTtrz are distinguished by comparison with the 4-(benzylideneamino)-3-(benzylidenehydra-zino)-5-thio-1, 2, 4-triazole spectrum which does not contain -NH₂ group (11). The absnce of any absorption band due to -NH₂ group in the spectrum of H_2 OAOHTtrz ligand supports its dioctyl structure.

Structural studies of some thiotriazole.....

Generally, the I.R. Spectra of the three ligands are fairly complex due to the presence of many functional groups. They contain outstanding bands which can be assigned with reasonable confidence. The stretching frequency of ν OH of the absorbed ethanol or water molecules to the H_2CL_2 BAHTtrz ligand appears at 3400 and 3360 cm^{-1} respectively. In case of its copper complex (CuHCl_2 BATrz). ($4\text{H}_2\text{O}$.CL). EtOH, one of ν NH bands disappeared which indicates either Cu-N Cu-S bond is formed, in which the later band is possible through the thiol form of the ligand. On the other hand, the shift of thioamide bands support sulfur chelation, since thioamide bands in III and IV locate at 970 and 830 cm^{-1} in the free ligand, mainly due to (ν C=N major + ν C=S minor) and (ν C=S major + ν C=N minor), respectively, nearly disappeared while thioamide band in II present at 1315 cm^{-1} is positively shifted to 1340 cm^{-1} due to increase of ν C=N character resulting from (δ (NH) major + ν C=N major). Also, the lower shift of thioamide band in I due to (δ (NH) major + ν (C=N) major) from 1490 to 1460 cm^{-1} after complexation in keep sulfur chelation (12, 13), Table III.

The electronic spectra (Table IV) rule out an octahedral geometry for ($\text{Cu}(\text{HCl}, \text{BAHTtrz}) \cdot 4\text{H}_2\text{O}$). EtOH. Cl complex. Its electronic spectra show a broad band centered at 11,760 cm^{-1} due to ${}^2\text{E}_g - {}^2\text{T}_{2g}$ transition. The broadness and position of the band indicate a probable configuration of the complex (14,15). The low magnetic moment of this complex (1.56) B.M. can be explained on the basis of spin-spin interaction between Cu(II) ions present in polymeric structure. Both Cu ($\text{H}_2\text{OAOHTtrz}$) $_2 \cdot 2\text{H}_2\text{O} \cdot \text{Cl}_2$ and Cu (HMTAHTtrz). Cl complexes are diamagnetic and their e.s. Show a band $\sim 15,870 \text{ cm}^{-1}$ In addition to weak bands or shoulders at 13,330 and 11,111 cm^{-1} . These spectral data are similar to those reported to square planar chelates (16).

Table (III) : major Characteristic IR bands of the ligands and complexes (Cm^{-1})

Compound	Assignment				Thioamide bands				
	νNH_2	νNH	δNH_2	$\nu\text{C}=\text{N}$	νOH	I	II	III	IV
$(\text{H}_2\text{Cl}_2\text{BAHTtz}) \cdot \text{EtOH}$	3300w	3020	1590s	1640	3400	1490	1315	970	830
$\text{Cu}(\text{HCl}_2\text{BAHTtz}) \cdot 4\text{H}_2\text{O} \cdot \text{Cl}_2 \cdot \text{EtOH}$	3260w	3040	1590m	1630s	3400b	1460m	1340m	960m	820
	3280w	3140w							
$(\text{H}_2\text{Cl}_2\text{BAHTtz}) \cdot 2\text{H}_2\text{O} \cdot \text{HCl}$	3300	3080	1580	1640s	3360	1490m	1305	950w	870sh
		3140		1610m					
$\text{H}_2\text{OAOHTtz}$		3150w		1625s		1540m	1310m	1095	845
		3220b							
$\text{Cu}(\text{H}_2\text{OAOHTtz})_2 \cdot \text{Cl}_2 \cdot 2\text{H}_2\text{O}$		3180		1620s	3400b	1530	1305	1090	840
		3200							
$\text{H}_2\text{MTAHTtz}$	3300	3040w	1600m	1635m		1490	1325	960s	860m
		3100b				1500	1290		
$\text{Cu}(\text{HMTAHTtz}) \cdot \text{Cl}$	3380	3160m	1610	1630w		1490s	1320w	960w	
		3285				1290w			

Structural studies of some thiotriazole.....

Table (IV) : Electronic spectral data of the copper complexes in nujol mull and their diamagnetic moment

Compound	μ_{eff} B.M.	LMCT	d - d assignment cm^{-1} .			
			2E_g ${}^2T_{2g}$	${}^2B_{1g}$ ${}^2B_{2g}$	${}^2B_{1g}$ ${}^2B_{2g}$	${}^2B_{1g}$ ${}^2A_{1g}$
Cu (HCl) ₂ BAHTTtz). Cl. 4H ₂ O. ETOH (CuL.Cl.4H ₂ O.EtOH)	1.56	19,230	13,333 - 10,000			
Cu (H ₂ OAOBTtz) ₂ .Cl ₂ .2H ₂ O (Cu(L) ₂ .Cl ₂ .2H ₂ O)	Diam	18,181	15,870	13,333	11,111	
Cu(HMTAHTtz). Cl (CuL.Cl)	Diam	20,000	15,870	13,699	11,904	

Where L stands for the ligand.

Table (V) : Results of the Tests of the compounds with *Chlamydomonas reinhardtii*, and *Bacterium photobacterium* phosphorium

Compound*	Solubility (mg.L ⁻¹)		Algal test EC ₅₀ mg.L ⁻¹	NO EC mg.L ⁻¹	Microtox test EC ₅₀ mg.L ⁻¹	pH
	sea	Algal medium				
(H ₂ Cl ₂ BAHT ^{tr} z).	5.0	Insol.	>>56	≥5.6	1.8<EC ₅₀ <10	8.5
Cu (HCl ₂ BAHT ^{tr} z).cl.4H ₂ O	1.0	Insol.	> 0.1	0.00032	>0.1	8.5
H ₂ MTTAHT ^{tr} z	3.5	Insol.	7.86	3.2	12	8.3-8.46
Cu (H ₂ OAOHT ^{tr} z). Cl ₂ .2H ₂ O	0.65	0.22	18.8	3.2	9	8.7
TBTO	0.018		0.018	0.001	>0.01	8.2-8.5

* See Table 1.

Structural studies of some thiotriazole.....

Algal and microtox test. Table V lists the results of both algal and microtox tests. The calculated EC₅₀ values exhibit relatively wide 95% confidence limits, because the cell were counted only after three days. The data revealed that, Cu(HCl, BAHTtrz). Cl. 4H₂O complex is more toxic than its organic part, 4-(dichlorobenzylideneamino) -3- hydrazino-5-thio-1, 2, 4-triazole, and in general, copper complexes have antialgal and antibacterial action than ligands. On the other hand, the standard test substance TBTO, the well knowm algicides, is more toxic than the prepared compounds.

AKNOWLEDGEMENT

The authors wish to thank prof. Dr. A.O. Hanstviet, Institute voor Kunststoffen, Rubber, Verfen Verpakkingen, TMO, Delft, the Netherlands, for doing the algal and microtox test of the compounds, and the calculation of the EC₅₀ values.

REFERENCES

1. A. B. Tadros, J. Chem. Tech. Biotechnol., 45 (1989) : P. 213 - 221.
2. A. B. Tadros, J. Chem. Tech. Biotechnol., under publication.
3. Adrien Albert, Selective Toxicity, Fifth Edition, London Chapman and Hall, 1973.
4. Buger, Medical Chemistry, Fourth Edition , Mnfred E. Walff P. 533.
5. R. G. Dickinson, N.W. Jacobsen, Org. Prep. Proceed. Enst., 6(3), 1974. 56 - 57.
6. NEN 6506, Water determination of algal toxicity in Dutch, Netherlands, Normalisatie - Institut, Delft, 1979.

7. Phyllis D. Uja, 3, 183, 241. Appl. Feb. 11, 1963. 4PP. U.S.
8. D. de Zwark, W. Sluoff, Aquatic Toxicol., 4, (1983) : 129 - 138.
9. Micheal E. Senko, H. David, Acta Cryst., 11, 1958, 808.
10. Jhon R. Dyer, An introduction to spectroscopic methods for the identification of organic compounds, Vol. 2, pergamon press 1970.
11. A. B. Tadros, Paint & Resin, Dec. 1987, Vol. 57, NO. 6, 25 - 27.
12. B. Singh, M.M.P. Rukhalyor, R.J. Sinha, J. Inorg. Nucl. Chem., 39, 1977, 29-32.
13. Campbell, R. Grzeskowiak, J. Chem. Soc., 1967, 396 - 404.
14. B. N. Figgis, Lewis, J. Modern Coordination Chem. Inter-Science Publishers Inc., New York, 1960, P. 403.
15. C. K. Jorgensen, Acta Chem. Scand., 9, 1955, P. 1362.
16. A. B. P. Lever, "Inorganic Electronic spectroscopy". Second Ed. Elsevier, New York, 1984.