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# Ether formation on the tridentate Schiff base ligands of copper(II) complexes

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## Abstract

A series of copper(II) complexes, CuL·imidazole, where L2− are tridentate Schiff base ligands formed by condensation of salicylaldehyde with a series of amino acids, have been synthesized. Visible spectral data indicate that copper(II) in these complexes are five coordinate in the solid state and in solution. Electrospray mass spectrometry has been used to show how these complexes react in alcohol/NaOH solutions with and without the presence of d-galactose. In the absence of d-galactose where the amino acid in the ligand is serine, the alcohol group on the ligand is converted to its alkyl ether after sonication of the solution for up to 4 h. In the presence of d-galactose, an alkoxy group is added to the ligands except for the ligand containing serine after sonication of the solutions for up to 4 h. At the same time, d-galactose is oxidized to its aldehyde. Where the ligand contains methionine, oxygen is also added to the ligand, most likely to the thioether sulfur.



## Keywords

Copper(II) Schiff base complexes, Electrospray mass spectrometry, Ether formation, d-galactose oxidation


## Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences


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## Authors

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# Ether Formation on the Tridentate Schiff Base Ligands of Copper(II) Complexes

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## Abstract

A series of copper(II) complexes, CuL.imidazole, where $L^{2-}$ are the tridentate Schiff Base ligands formed by the condensation of salicylaldehyde with a series of amino acids have been synthesised. Visible spectral data indicates that the copper(II) atoms in these complexes are five coordinate in the solid state and in solution. Electrospray mass spectrometry has been used to show how these complexes react in alcohol/NaOH solutions with and without the presence of D-galactose. In the absence of D-galactose where the amino acid in the ligand is serine, the alcohol group on the ligand is converted to its alkyl ether after sonication of the solution for up to four hours. In the presence of D-galactose an alkoxy group is added to the ligands except for the ligand containing serine after sonication of the solutions for up to four hours. At the same time D-galactose is oxidised to its aldehyde. Where the ligand contains the amino acid methionine an oxygen atom is also added to the ligand most likely to the thioether sulfur atom.
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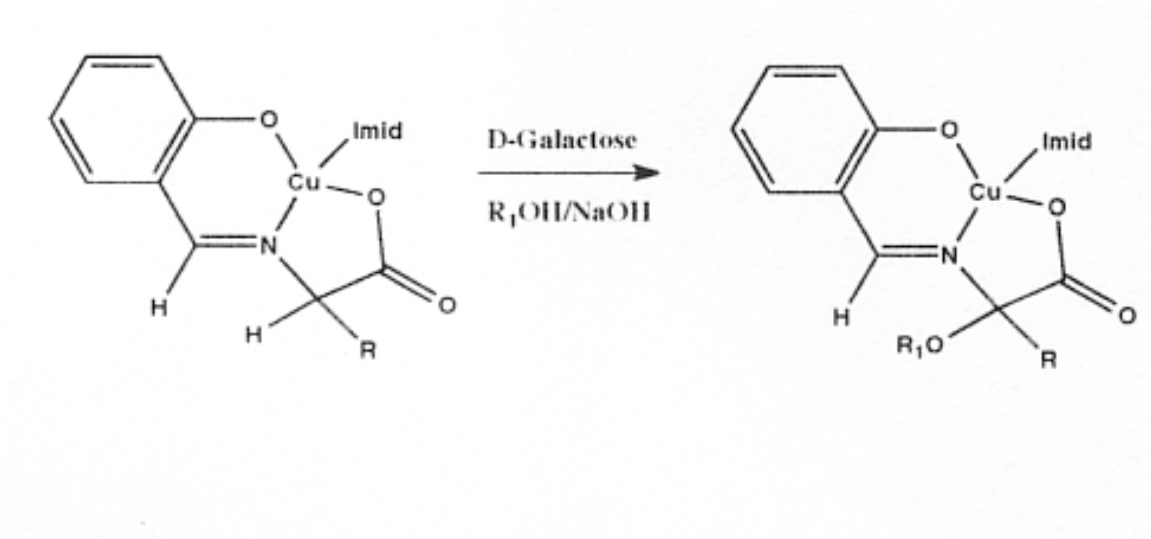
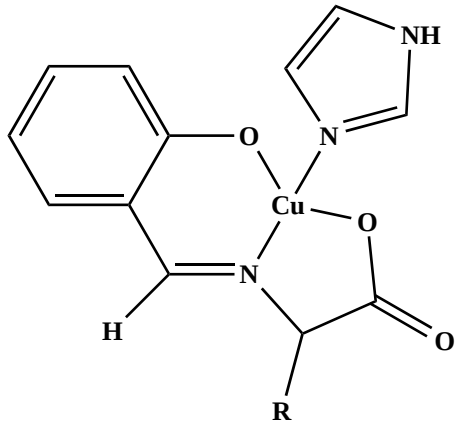
*Keywords*: Copper(II) Schiff Base complexes; electrospray mass spectrometry; ether formation; D-galactose oxidation.



## 1. Introduction

Copper is used by nature in many different enzymes that catalyse a variety of biochemical reactions. One such enzyme is the fungal enzyme galactose oxidase which catalyses the oxidation of primary alcohols to their corresponding aldehydes along with the reduction of dioxygen to hydrogen peroxide [1,2].
The crystal structure of the inactive Cu(II) form of galactose oxidase [3,4] reveals a mononuclear copper site with two histidyl nitrogen atoms, two tyrosyl oxygen atoms and a water molecule or acetate oxygen atom bound to the copper atom in a distorted square pyramidal environment around the copper atom.
We have synthesised a series of copper(II) complexes of tridentate Schiff Base ligands formed by the reaction of salicylaldehyde with some amino acids.
The structure of these complexes, CuSalaa.imid, is shown in figure 1.

Figure 1. CuSalaa.imid




We have used two physico-chemical techniques to study the reaction of these complexes in solution with and without the presence of D-galactose. The main technique used was electrospray mass spectrometry.

## 2. Experimental

### *2.1 Materials and Measurements*

All reagents were purchased from commercial sources and used as supplied.
Visible spectra in the range 400-900 nm were measured using a Shimadzu UV-2401 spectrophotometer. Solid state spectra were measured as nujol mulls on filter paper and solution spectra were run using approximately 0.0025 M copper complexes.
ESI (electrospray ionisation) mass spectra were obtained using a Micromass VG quattro-2$^{tm}$ triple quadrupole mass spectrometer (Altringham, United Kingdom) utilising a Z-spray ion source. Data was gathered using Masslynx$^{tm}$ v45 (Micromass Ltd) mass spectrometer software. Samples were introduced into the mass spectrometer via an Isco

SFC-500$^{tm}$ syringe pump and a Rheodyne U6K ten microlitre sample loop injector. HPLC grade solvents and ultra pure water were used. The following operational parameters were typical.
Solvent flow: 20 microlitres per minute, capillary voltage: 2.5, voltage lens: 0.40, cone voltage: 20-30 volts, high and low mass resolution:15, desolvation gas temperature: 80$^{o}$C.
Sonication of the solutions for up to four hours was carried out using a Unisonic ultrasonic cleaner.
Sonication is a technique often used to clean glassware and to help dissolve compounds in solvents but it is also a useful technique to decrease the time taken for a reaction to take place.
eg. A reaction that might take place in ten hours occurs in one or two hours if the solution is sonicated.
The solutions (10 mL) were sonicated in a 10 mL volumetric flask (limited supply of oxygen) or a 35 mL cylindrical glass sample container.
Carbon, hydrogen and nitrogen microanalyses were carried out by the University of Queensland Microanalytical Service.

## 2.2 Syntheses

***Cu(aminoacid anion)$_2$.H$_2$O***
Copper sulphate pentahydrate (0.1 mole), the amino acid (0.2 mole) and sodium hydroxide (0.2 mole) were dissolved in hot water (300 mL) and the solution heated until a blue precipitate started to appear. The solution was then cooled to room temperature and the blue solid collected by suction filtration, washed with acetone and air dried. When the amino acid used was serine the volume of water used was reduced to 100 mL because of the increased solubility of the product in water.

**CuSalaa.imidazole.**
Cu(amino acid anion)$_2$.H$_2$O (3 g) was suspended in methanol (100 mL). Salicylaldehyde (2 mole equivalent) and triethylamine (7 mL) were added to the methanol solution which was refluxed for one hour. The solution was filtered by gravity and imidazole (1.2 mole equivalent) dissolved in methanol (50 mL) was added to the filtered solution. The solution was allowed to slowly evaporate at room temperature until the blue green product precipitated from solution. The product was washed with 40-60 petroleum ether and air dried. The C,H,N analytical data for CuSalaa.imid are shown in table 1.

**Table 1.** Microanalytical Data for CuSalaa.imid[a]

| Compound | Formula | | C | H | N |
|---|---|---|---|---|---|
| CuSalala.imid | $C_{13}H_{13}N_3O_3Cu$ | calcd. | 48.08 | 4.03 | 12.94 |
| | | found | 48.14 | 4.03 | 12.72 |
| CuSalgly.imid | $C_{12}H_{11}N_3O_3Cu$ | calcd. | 46.67 | 3.59 | 13.61 |
| | | found | 46.38 | 3.57 | 13.41 |
| CuSalleu.imid | $C_{16}H_{19}N_3O_3Cu$ | calcd. | 52.67 | 5.25 | 11.52 |
| | | found | 52.45 | 5.19 | 11.41 |
| CuSalmet.imid.0.5H$_2$O | $C_{15}H_{18}N_3O_{3.5}SCu$ | calcd. | 45.97 | 4.63 | 10.72 |
| | | found | 46.21 | 4.44 | 10.54 |
| CuSalnleu.imid | $C_{16}H_{19}N_3O_3Cu$ | calcd. | 52.67 | 5.25 | 11.52 |

| | | | | | |
|---|---|---|---|---|---|
| | | found | 52.37 | 5.99 | 11.58 |
| CuSalnval.imid | $C_{15}H_{17}N_3O_3Cu$ | calcd. | 51.35 | 4.88 | 11.98 |
| | | found | 51.01 | 4.97 | 11.82 |
| CuSalphenala.imid | $C_{19}H_{17}N_3O_3Cu$ | calc. | 57.21 | 4.30 | 10.53 |
| | | found | 57.05 | 4.23 | 10.56 |
| CuSalphengly.imid.0.5$H_2O$ | $C_{18}H_{16}N_3O_{3.5}Cu$ | calcd. | 54.89 | 4.09 | 10.67 |
| | | found | 55.24 | 3.72 | 10.62 |
| CuSalser.imid | $C_{13}H_{13}N_3O_4Cu$ | calcd. | 46.09 | 3.87 | 12.40 |
| | | found | 46.14 | 3.82 | 12.38 |
| CuSaltyr.imid.$H_2O$ | $C_{19}H_{17}N_3O_4Cu$ | calcd. | 52.47 | 4.32 | 9.66 |
| | | found | 52.71 | 4.42 | 9.71 |
| CuSalval.imid | $C_{15}H_{17}N_3O_3Cu$ | calcd. | 51.35 | 4.88 | 11.98 |
| | | found | 51.00 | 4.85 | 11.95 |

[a]ala (alanine), gly(glycine), leu(leucine), met(methionine), nleu(norleucine), nval(norvaline), phenala(phenylalanine), phengly (phenylglycine), ser(serine), tyr(tyrosine), val(valine), imid(imidazole).

## 3. Results

The structures of thirty five complexes of the type CuSalaa.base have previously been determined by x-ray crystallography [5-41]. In each of these complexes the copper atom is in an approximately square pyramidal environment ($CuN_2O_3$) with five coordination occurring through:
(i) addition of a molecule of water [5-16].
(ii) formation of a dimer bridged by ligand phenolic oxygen atoms [17-27, 41].
(iii) formation of a polymer with an adjacent carboxyl oxygen atoms occupying the fifth coordination site [28-40].
In the case of CuSalala.imid [22,41] five coordination is achieved via dimer formation. Each complex has a broad solid state spectral band peak in the range 590-630 nm (Cusalala.imid: 610 nm) and it seems reasonable to conclude that this band represents five coordinate $CuN_2O_3$. In methanol solution the spectral band peak occurs in the range 615-622 nm suggesting a $CuN_2O_3$ environment with a methanol ligand occupying the fifth coordination site. In $CH_3CN/H_2O$ solution the spectral band peak occurs in the range 605-616 nm again suggesting a $CuN_2O_3$ environment with a water molecule occupying the fifth coordination site.

### 3.1 Electrospray Mass Spectroscopy of CuSalaa.imid + D-Galactose(1:2) in methanol.

The major peaks and their assignments are shown in table 2. The copper peaks appear as a double peak (Cu-63, Cu-65) and the peak values shown in table 2 are those containing the dominant isotope (Cu-63).

(i) The electrospray mass spectra of all the compounds contain $[CuL.imid - H]^-$ and $[CuL.imid + H]^+$ peaks. There are also in some cases $[CuL.imid + Na]^+$ peaks with the sodium ions coming from sea spray in the atmosphere. There are also dimer peaks, $[Cu_2L_2.imid - H]^-$, $[Cu_2L_2 + H]^+$ and $[Cu_2L_2 + Na]^+$, which is consistent with the compounds being dimers although dimerization or depolymerization could occur within the mass spectrometer.

(ii) Galactose peaks, $[Gal - H]^-$, $[Gal + Cl]^-$, $[Gal + HCO_2]^-$, $[2Gal - H]^-$ and $[Gal + Na]^+$ also occur. The chloride ion comes from sea spray while the formate ion persists in the mass spectrometer after formic acid was used as an ionizing medium in previous mass spectral measurements.

(iii) $[CuL + Gal - H]^-$ and $[Cu_2L_2 + Gal - H]^-$ peaks occur showing that D-galactose binds to the copper compounds replacing the imidazole. This is consistent with D-galactose binding to the copper compounds before being oxidized.

(iv) When the electrospray mass spectra of CuL.imid are measured in methanol in the absence of D-galactose, the spectra obtained are similar to the spectra in table 2 without the peaks containing D-Galactose.

**Table 2.** Electrospray Mass Spectra of CuSalaa.imid + D-Galactose (1:2) in Methanol.

| Assigned Peak | Gly | Ala | Val/ Nval | Leu/ Nleu | Ser | Met | Tyr | Phen gly | Phen ala |
|---|---|---|---|---|---|---|---|---|---|
| $[Cu_2L_2 + Gal - H]^-$ | 659 | 687 | | 771 | 719 | 807 | 871 | 839 | |
| $[Cu_2L_2.imid - H]^-$ | 547 | 575 | 631 | 659 | | 695 | 759 | 699 | 727 |
| $[CuL + Gal - H]^-$ | 419 | 433 | 461 | 475 | 449 | 493 | 525 | 495 | 509 |
| $[2Gal - H]^-$ | 359 | | | 359 | 359 | 359 | 359 | | |
| $[CuL.imid - H]^-$ | 307 | 321 | 349 | 363 | 337 | 381 | 413 | 383 | 397 |
| $[Cu(L\text{-}CO_2).imid - H]^-$ | 263 | 277 | 305 | 319 | | 337 | 369 | 339 | 353 |
| $[Gal + HCO_2]^-$ | 225 | 225 | | 225 | | 225 | | | 225 |
| $[Gal + Cl]^-$ | 215 | | 215 | 215 | 215 | 215 | | 215 | 215 |
| $[Gal - H]^-$ | 179 | 179 | 179 | 179 | 179 | 179 | 179 | 179 | 179 |
| $[Cu_2L_2.imid + H]^+$ | | 577 | 633 | 661 | | 697 | 761 | 701 | 729 |
| $[Cu_2L_2 + Na]^+$ | | 531 | 587 | 615 | 563 | 651 | 715 | 655 | 683 |
| $[Cu_2L_2 + H]^+$ | | 509 | 565 | 593 | 541 | 629 | 693 | 633 | |
| $[CuL.imid_2 + H]^+$ | | 391 | 419 | 433 | | | 483 | | 467 |
| $[2Gal + Na]^+$ | 383 | | 383 | 383 | 383 | | 383 | | |
| $[CuL.imid + Na]^+$ | 331 | | 373 | | 361 | 405 | | 453 | 421 |
| $[CuL.imid + H]^+$ | 309 | 323 | 351 | 365 | 339 | 383 | 415 | 385 | 399 |
| $[CuL.MeOH + H]^+$ | | 287 | 315 | 329 | | 347 | 379 | 349 | 363 |
| $[CuL + Na]^+$ | | | | | | 315 | 347 | | |
| $[Gal + Na]^+$ | 203 | 203 | 203 | 203 | 203 | 203 | 203 | 203 | 203 |

### 3.2 Reactions of CuSalaa.imid in $R_1OH$/NaOH Solutions

#### 3.2.1 Oxidation of D-Galactose

The ratio of CuSalaa.imid:D-galactose of 1:2 was chosen because it was found that the D-galactose peaks in the mass spectra are easier to observe at the higher D-galactose concentrations.

When 10 mL solutions of CuSalaa.imid (0.0025 M) + D-galactose (0.0050 M) in NaOH/MeOH (0.05 M) are sonicated in the presence of a limited supply of oxygen

(10 mL volumetric flasks) the blue/green solutions turn yellow and a white solid is precipitated from solution. The Cu(II) d-d spectral bands at 605-612 nm disappear and electrospray mass spectral peaks containing copper either disappear completely or have their intensities substantially reduced. Mass spectral peaks occur at m/z 179 [D-galactose – H]$^-$ and m/z 195 [oxidised galactose + OH]$^-$ in the negative ion electrospray mass spectra of the solutions. The relative intensities of the two D-galactose peaks depends on the complex used, the concentration of hydroxide ion and the time of sonication with an increase in the concentration of hydroxide and time of sonication increasing the intensity of the 195 peak which represents oxidation of the $CH_2OH$ group on the D-galactose to its aldehyde. This indicates that the D-galactose is oxidised while the Cu(II) complex is reduced to the Cu(I) complex which precipitates from solution as the white solid as shown in reaction (1).

$$2\text{Cu(II)L.imid} + \text{GalCH}_2\text{OH} + 2\text{NaOH} \rightarrow 2\text{NaCu(I)L.imid} + \text{GalCHO} + 2\text{H}_2\text{O} \quad (1)$$

On exposure to air these yellow solutions slowly turn blue-green as the white precipitate dissolves and the copper peaks reappear in the mass spectra of the compounds. If excess D-galactose is present in the solutions the cycle can be repeated on further sonication of the solutions.
When the solutions are sonicated in 35 mL sample containers only the complexes where the amino acid in the ligand is tyrosine or methionine turn yellow. The visible spectra of the remaining complexes contain a d-d band in the 607-11 nm region indicating little change in the immediate environment around the copper atom.
The negative ion mass spectra (table 4) of the complexes contain both peaks for galactose (179) and oxidised galactose (195) indicating that the redox reaction involving the copper complexes and D-galactose still occur even when the colour change to yellow is not observed.

### 3.2.2 CuSalser.imid in $R_1OH$/NaOH Solution with Sonification

When CuSalser.imid (0.0025 M) is dissolved in a 0.05 M solution of sodium hydroxide dissolved in methanol, a visible spectral band peak at 607nm occurs indicating the environment around the copper is square pyramidal. The negative ion electrospray mass spectral peaks are similar to those in methanol solution with [CuL.imid –H]$^-$ at m/z 337, and with the addition of a small peak at m/z 351.
After sonication for two hours the visible spectral band shifts to 604 mn indicating minimal change to the copper environment.
In the negative ion electrospray mass spectrum of the complex, the [CuL.imid – H]$^-$ peak at m/z 337 is replaced by a peak at m/z 351 (figure 2, table 3) and the [CuL.imid + Na]$^+$ peak in the positive ion spectrum moves from m/z 361 to 375 (table 3)

Figure 2. The negative ion electrospray mass spectrum (intensity vs m/z) of CuSalser.imid in MeOH/NaOH solution after sonication for two hours.

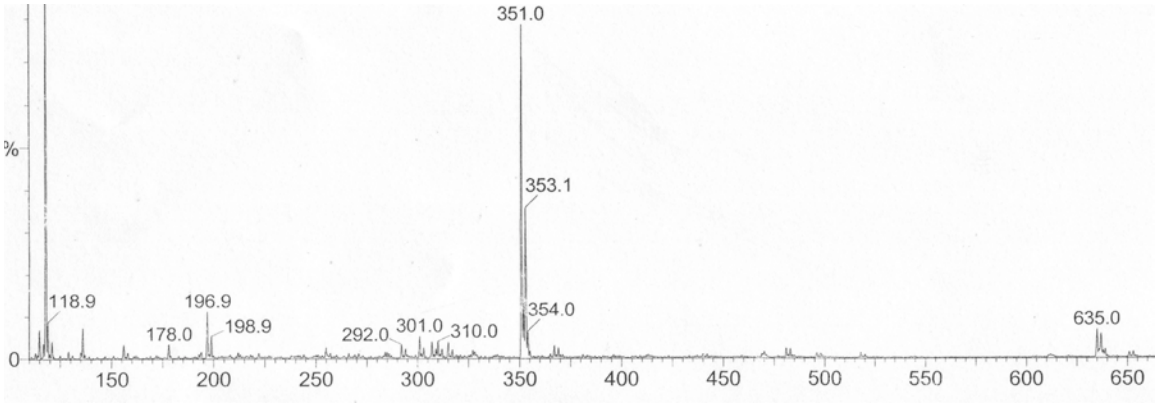



Table 3. Electrospray Mass Spectra of CuSalser.imid in $R_1OH$/NaOH after sonication for up to four hours.

| | $CH_3OH$/ NaOH Sonicate | $C_2H_5OH$/ NaOH Sonicate | n-$C_3H_7OH$/ NaOH Sonicate |
|---|---|---|---|
| Assigned Peak | | | |
| $[Cu_2(LR_1)_2.imid - H]^-$ | 635 | | |
| $[CuLR_1.imid - H]^-$ | 351 | 365 | 379 |
| $[CuLR_1.imid + Na]^+$ | 375 | 389 | 403 |
| $[Cu(LR_1\text{-}CH_2).imid + H]^+$ | 339 | 353 | 367 |

This indicates the addition of a methyl group to the complex and loss of a hydrogen atom from the complex giving an addition of 14 to the molecular ions. When ethanol is used instead of methanol the $[CuLR.imid - H]^-$ peak occurs at m/z 365 and the $[CuLR.imid + Na]^+$ peak occurs at m/z 389 adding another 14 to the molecular ions (table 3). When n-propanol is used instead of methanol 42 is added to the original molecular ions (table 3). If a 50:50 mixture of methanol and ethanol is used peaks representing the addition of both methyl and ethyl groups to the complex are obtained.

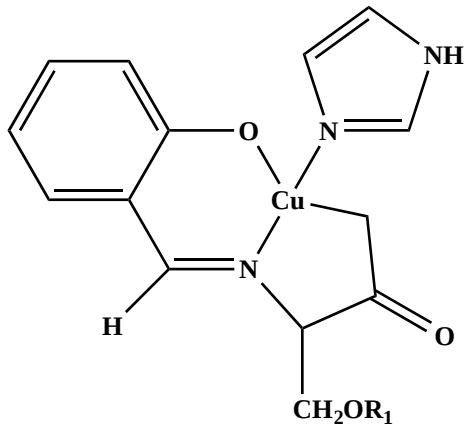
When the MS/MS spectrum of the $[CuL.imid - H]^-$ peak at m/z 337 is measured a daughter ion peak at m/z 293 representing the loss of $CO_2$ from the ligand is obtained. When the MS/MS spectrum of the $[CuLMe.imid - H]^-$ peak at m/z 351 is measured daughter ions at m/z 307 (loss of $CO_2$) and m/z 292 (loss of $CH_3 + CO_2$) are obtained. MS/MS data on the daughter peak at m/z 307 showed the further loss of $CH_3$ to produce the peak at m/z 292. This suggests that the alkyl group is not part of ester formation on the ligand which would require simultaneous loss of $CO_2$ and the methyl group rather than initial loss of $CO_2$.

This suggests that the hydrogen atom on the “OH” group on the serine part of the ligand has been replaced by an alkyl group to form an ether as shown in reaction (2).

$CuLCH_2OH.imid + R_1OH \rightarrow CuLCH_2OR_1.imid + H_2O$ (2)

The proposed structure of the ether product is shown in figure 3.

Figure 3. The suggested structure of the product of the reaction of CuSalser.imid after sonication in $R_1OH$/NaOH for two hours.



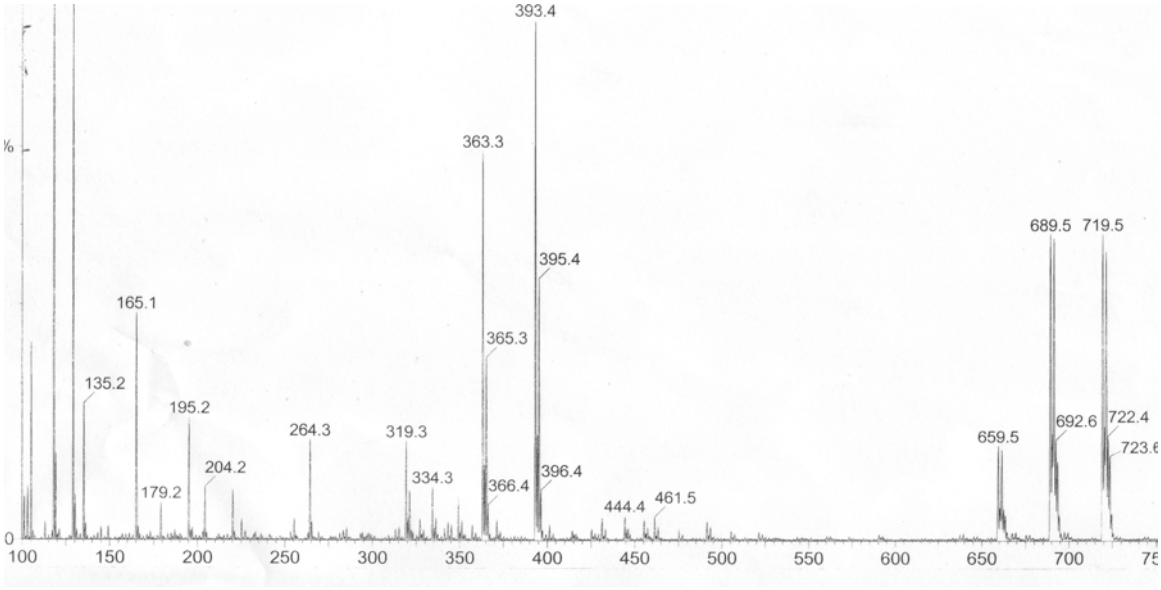

### 3.2.3 CuSalaa.imid + D-Galactose(1:2) in $R_1OH$/NaOH/Air Solution with Sonification.

When CuSalaa.imid (0.0025 M) is dissolved in 0.05 M solutions of NaOH in methanol visible spectral bands occur at 605-616 nm which shift by 0-4 nm when D-galactose is added to the solutions. When the solutions of these complexes in MeOH/NaOH/Air are sonicated for two hours, the mass spectra of these solutions resemble those of the complexes in MeOH/NaOH before sonication indicating that no additional reactions have taken place except in the case where the amino acid in the ligand is leucine, norleucine or methionine where a small additional peak $[CuL.imid - H]^-$ + 30 occurs.
When D-Galactose is added to the solutions before sonication and the solutions are then sonicated for up to two hours mass spectral data indicates that a reaction takes place. Where the solvent is MeOH/NaOH, the mass spectral peaks of the parent compounds are partially replaced by peaks increasing by 30 in monomer peaks and 60 in dimer peaks indicating the replacement of a proton on the ligand by a methoxy group (figure 4, tables 4,5). When EtOH/NaOH is used the monomer peaks increase by 44 (table 5) indicating the replacement of a proton by an ethoxy group. This indicates that the following reaction takes place as shown in reaction (3).

$CuL.imid + R_1OH \rightarrow CuLOR_1.imid + H_2O$ (3)

When the MS/MS spectra of CuSalala.imid in MeOH is measured the $[CuSalala.imid - H]^-$peak at m/z 321 produces a daughter ion peak at m/z 277 representing the loss of $CO_2$.

When the MS/MS spectra of the sonicated solution [CuLOMe.imid – H]$^-$ peak at m/z 351 is measured daughter ions at m/z 307 (loss of $CO_2$) and m/z 292 (further loss of $CH_3$) are obtained in a similar pattern to the serine based ligand system.
Figure 4. The negative ion electrospray mass spectrum (intensity vs m/z) of CuSalleu.imid + D-galactose(1:2) in MeOH/NaOH/Air after sonication for two hours.




Where the ligand amino acid is glycine no copper containing peaks with an addition of an OR group to the ligand are obtained although a weak peak representing [LOMe + H]$^-$ at m/z 208 is obtained suggesting that a small amount of reaction may occur. The presence of this [LOMe + H]$^-$ peak in the negative ion mass spectra of most of the compounds (table 4) shows that the methoxy group is added to the ligand and not to the imidazole ring.

**Table 4.** Electrospray Mass Spectra of CuSalaa.imid + D-Galactose (1:2) in Methanol/NaOH/Air after sonication for 2 hours.

| | Gly | Ala | Val/ Nval | Leu/ Nleu | Ser | Met | Tyr | Phen gly | Phen ala |
|---|---|---|---|---|---|---|---|---|---|
| [$Cu_2$(LOMe)$_2$.imid – H]$^-$ | | 635 | 691 | 719 | | | | 759 | 787 |
| [$Cu_2$LMe.(LMe.O).imid – H]$^-$ | | | | | 651 | | | | |
| [$Cu_2$(LMe)$_2$.imid – H]$^-$ | | | | | 635 | | | | |
| [$Cu_2$L.LOMe.imid – H]$^-$ | | 605 | 661 | 689 | | | | 729 | 757 |
| [$Cu_2L_2$.imid – H]$^-$ | | 547 | 575 | 631 | | 659 | | | 727 |
| [CuLO.OMe.imid – H]$^-$ | | | | | | 427 | | | |
| [CuLOMe.imid – H]$^-$ | | 351 | 379 | 393 | | 411 | 443 | 413 | 427 |
| [CuLMe.O.imid – H]$^-$ | | | | | 367 | | | | |
| [CuLO.imid – H]$^-$ | | | | | | 397 | | | |
| [CuLMe.imid – H]$^-$ | | | | | 351 | | | | |
| [CuL.imid – H]$^-$ | 307 | 321 | 349 | 363 | | 381 | 413 | 383 | 397 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| $[Cu(LMe\text{-}CO_2).imid - H]^-$ | | | | | 307 | | | | |
| $[Cu(L\text{-}CO_2).imid - H]^-$ | 263 | 277 | 305 | 319 | | | | 339 | 353 |
| $[LOMe + H]^-$ | 208 | 222 | 250 | 264 | | | | 284 | 298 |
| $[Gal + HCO_2]^-$ | | 225 | 225 | 225 | | | 225 | | |
| $[Oxid.\ Gal + OH]^-$ | 195 | 195 | 195 | 195 | 195 | 195 | 195 | 195 | 195 |
| $[Gal - H]^-$ | 179 | 179 | 179 | 179 | | 179 | 179 | 179 | 179 |
| $[Cu_2(LMe.O)LMe + Na]^+$ | | | | | 607 | | | | |
| $[Cu_2(LOMe)_2 + Na]^+$ | | | 647 | 675 | | | | | |
| $[Cu_2(LMe)_2 + Na]^+$ | | | | | 591 | | | | |
| $[Cu_2(LO)_2 + Na]^+$ | | | | | | 683 | | | |
| $[Cu_2L.LOMe + Na]^+$ | | 561 | 617 | 645 | | | | | |
| $[Cu_2L.LO + Na]^+$ | | | | | | 667 | | | |
| $[Cu_2L_2 + Na]^+$ | 503 | 531 | 587 | 615 | | 651 | | | |
| $[CuLO.OMe.imid + Na]^+$ | | | | | | 451 | | | |
| $[CuLMe.O.imid + Na]^+$ | | | | | 391 | | | | |
| $[CuLOMe.imid + Na]^+$ | | 375 | | 417 | | | | 437 | 451 |
| $[2Gal + Na]^+$ | | 383 | 383 | 383 | | 383 | 383 | | 383 |
| $CuL.Me.imid + Na]^+$ | | | | | 375 | | | | |
| $[CuL.imid + Na]^+$ | 331 | 345 | 373 | 387 | | 405 | 437 | | 421 |
| $[CuLMe.imid + H]^+$ | | | | | 353 | | | | |
| $[CuL.imid + H]^+$ | | | | | 339 | | | | |
| $[Gal + Na]^+$ | 203 | 203 | 203 | 203 | 203 | 203 | 203 | 203 | 203 |

**Table 5.** Electrospray Mass Spectra of CuSalaa.imid + D-Galactose (1:2) in Methanol(Ethanol)/NaOH/Air after sonication for 2 hours.

| Assigned Peak | Ala | Norval | Norleu | Phengly | Phenala |
|---|---|---|---|---|---|
| $[Cu_2(LOMe)_2.imid - H]^-$ | | | 719 | | 787 |
| $[Cu_2(LOEt)_2.imid - H]^-$ | | 719 | 747 | | 815 |
| $[Cu_2L(LOMe).imid - H]^-$ | | 661 | 689 | | 757 |
| $[Cu_2L(LOEt).imid - H]^-$ | | 675 | 703 | | 771 |
| $[CuLOMe.imid - H]^-$ | 351 | 379 | 393 | 413 | 427 |
| $[CuLOEt.imid - H]^-$ | 365 | 393 | 407 | 427 | 441 |
| $[CuLOMe.imid + Na]^+$ | | 403 | 417 | | 451 |
| $[CuLOEt.imid + Na]^+$ | | | 431 | 451 | 465 |

Where the amino acid in the ligand is serine the $[CuLMe.imid - H]^-$ peak at m/z 351 and other peaks representing the methyl ether ligand (table 4) appear in the negative and positive ion electrospray mass spectra of the sonicated solutions as happens when the solutions are sonicated without the presence of D-galactose. In addition there are small extra +16 peaks (table 4) which appear to represent the loss of a proton and the addition of an “OH” group to the ligand. These additional peaks only occur in the presence of D-galactose.

### 3.2.4 Reaction of CuSalaa.imid + D-Galactose (2:1) in MeOH/NaOH/Air after Sonication for Different Periods of Time

In order to eliminate the possibility that the reactions of the ligands are taking place inside the mass spectrometer and are not related to the time of reactions in solution, the mass spectra of the solutions were measured after no sonication and after sonication of the solutions for 1.5 and 4 hours. The ratio of CuSalaa.imid:D-galactose of 2:1 was chosen because one mole of D-galactose is required to reduce two moles of the copper compound. The ratio of the intensities of $[CuLOMe.imid - H]^-$:$[CuL.imid - H]^-$ can be used as a qualitative measure of the relative reactivity of the different ligands as the amino acid is changed in the ligands (table 6).

Before the commencement of sonication visible spectral bands occur at 606-616 nm and there is no mass spectral evidence for the formation of the ether products. After a reaction time of 1.5 hours visible spectral bands occur in the range 605-613 nm and mass spectral peaks representing formation of the ethers have started to appear with some of the compounds especially where the amino acid in the ligand is phenylglycine. After sonication for four hours visible spectral bands appear in the range 612-622 nm and mass spectral peaks indicate that the reaction with phenylglycine has gone to completion and there has been a considerable amount of formation of the ether products in the other compounds except where the amino acid in the ligand is glycine. The small change in the visible spectral peaks suggests that the environment of the copper atom in the complexes does not substantially change after reaction takes place (16 nm for the phenylglycine compound vs 5 nm for the glycine compound).

The reaction rate depends on the “R” group on the ligand suggesting a likely site of reaction as shown in figure 5.

Figure 5. The suggested structure of the ether formed by the reaction of CuSalaa.imid + D-galactose(1.2) after sonication in $R_1OH$/NaOH/Air for two hours.

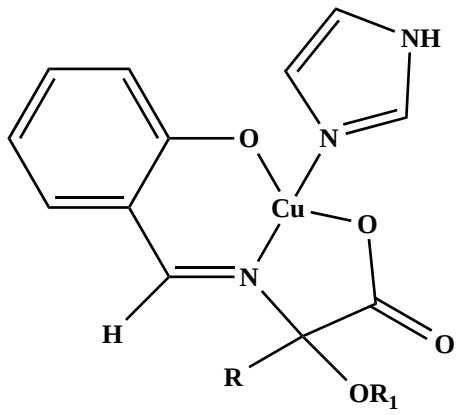



**Table 6.** Visible Spectra(nm) and the Ratio of $[CuLOMe.mid - H]^-$: $[CuL.imid - H]^-$ in the Negative Ion Electrospray Mass Spectra of CuSalaa.imid + D-Galactose (2:1) in Methanol/NaOH/Air Solutions after Sonication for 0, 1.5 and 4 Hours.

| Gly | Ala | Val | Nval | Leu | Nleu | Met | Tyr | Phen gly | Phen ala |
|---|---|---|---|---|---|---|---|---|---|

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Time: 0 Hours | | | | | | | | | |
| 610 | 607 | 608 | 608 | 607 | 607 | 616 | 605 | 606 | 609 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Time: 1.5 Hours | | | | | | | | | |
| 611 | 608 | 609 | 607 | 610 | 607 | 607 | 605 | 613 | 609 |
| 0 | 0.3 | 0.1 | 0.5 | 0.1 | 0.4 | 0.2 | 0 | 2.1 | 0.8 |
| Time: 4 Hours | | | | | | | | | |
| 615 | 617 | 614 | 612 | 612 | 612 | 619 | 614 | 622 | 612 |
| 0 | 2.0 | 0.5 | 1.3 | 1.0 | 1.0 | 1.2 | 0.5 | 100 | 1.1 |

### 3.2.5 CuSalmeth.imid + D-Galactose (1:2) in Methanol/NaOH /Air Solution after Sonication.

When a solution of CuSalmeth.imid + D-galactose (1:2) is sonicated for two hours, a peak for $[CuLOMe.imid - H]^-$ at m/z 411 appears in the negative ion electrospray mass spectra (figure 9, table 4) as expected. There are, however, additional peaks at m/z 397 in the negative ion mass spectra and m/z 667 and 683 in the positive ion mass spectra (table 4) which represent the addition of 16 to the monomer peak and 32 to the dimer peaks which can be assigned to $[CuLO.imid - H]^-$ and $[Cu_2L.LO + Na]^+$ and $[Cu_2(LO)_2 + Na]^+$.

## 4. Discussion

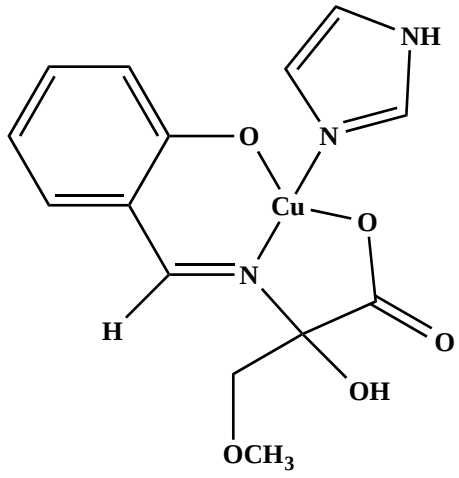
Mass spectral data on the reactions of these copper(II) complexes in $R_1OH$/MeOH solutions indicate the formation of ether groups on the ligands after sonication of the solutions.

(a) Where the amino acid in the ligand is serine the reaction takes place on the alcohol part of the ligand as shown in reaction (2), figure 3. This reaction does not require the presence of a reducing agent such as D-galactose. In the presence of D-galactose the same reaction occurs with the presence of a minor product containing an additional “OH”group.

(b) Where the amino acid in the ligand is not serine or possibly glycine, the presence of the reducing agent D-galactose is required for the addition of an ether group to the ligands in more than trace amounts. The most likely way for this to occur is via a redox reaction and a hydroxylated intermediate species

Karlin et al [43-48] have pioneered the study of aromatic hydroxylation ligand reactions in binuclear copper(I) compounds. Less common are hydroxylation reactions that occur on the non aromatic parts instead of aromatic parts of the ligands of copper complexes. This type of reaction [49-53] has been suggested to be a model system of the reactions of copper monooxygenase enzymes such as dopamine β-monooxygenase.
A possible structure of the “hydroxylated” minor product that occurs when solutions of CuSalser.imid are sonicated in the presence of D-galactose is shown in figure 6.

Figure 6. The suggested structure of the minor product of the reaction of CuSalser.imid + D-galactose(1:2) after sonication in MeOH/NaOH for two hours.




A possible reaction scheme for the formation of ether groups on the ligands is shown in figure 8.

Figure 7. The suggested reaction scheme for the aliphatic ligand hydroxylation of the copper(II) complex of the ligand in references [51, 52].

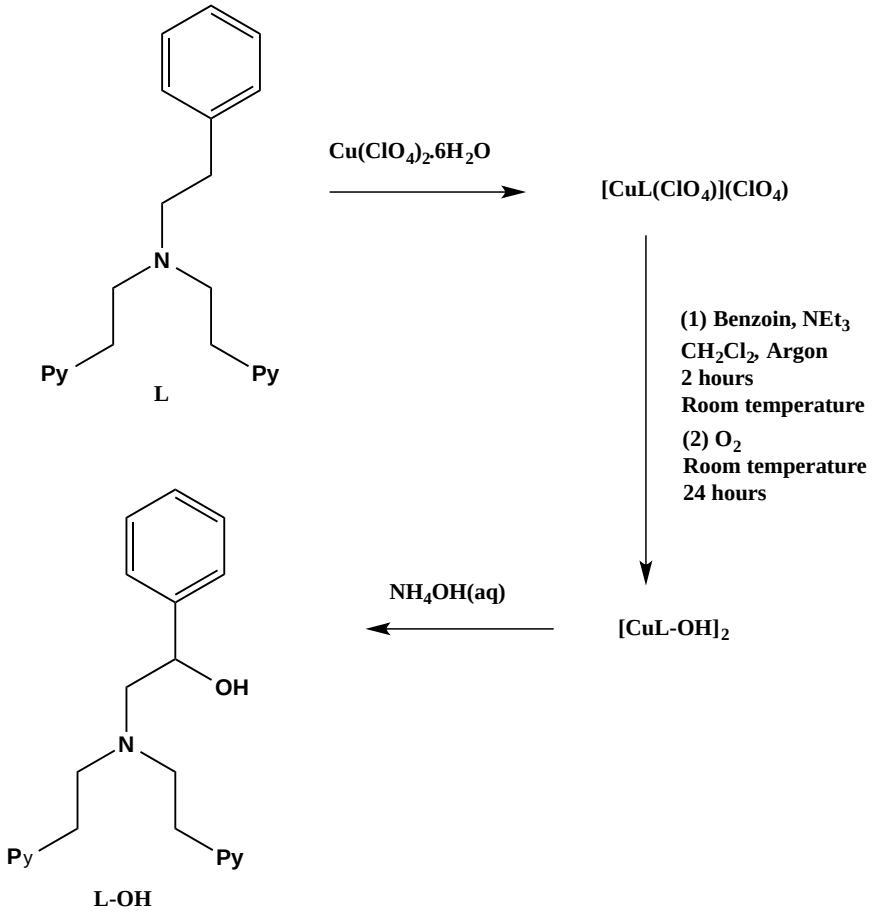
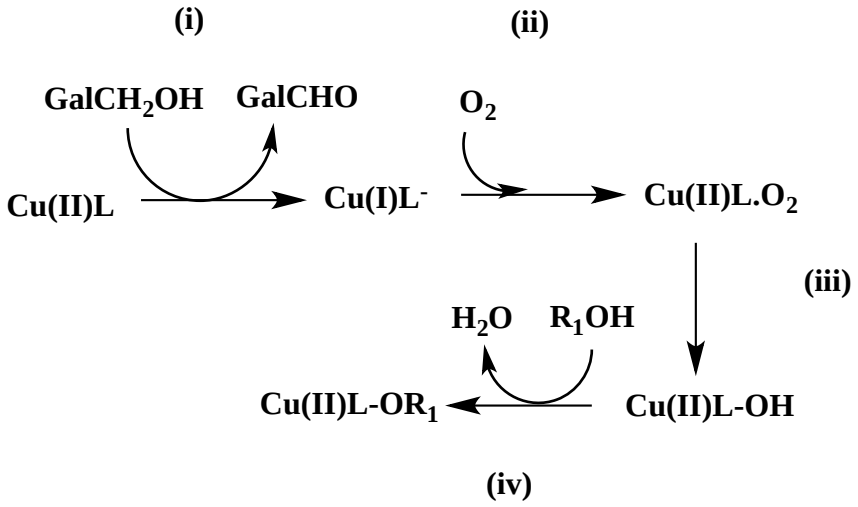


Figure 8. The proposed scheme for the reaction of CuL.imid + D-Galactose in $R_1OH$/NaOH/Air after sonication.




The reaction scheme shown in figure 8 is similar to that reported by Itoh et al [49, 50] for the aliphatic ligand hydroxylation of the copper(II) complex of the tridentate ligand [N, N-bis[2-(2-pyridyl)ethyl-2-phenylethylamine)] by dioxygen in the presence of triethylamine and the reducing agents benzoin or hydroquinone (figure 7). In this work an additional step involves the conversion of the alcohol to the ether.

(i) The copper(II) complex is reduced to the copper(I) complex and D-Galactose is oxidised to its aldehyde. The reduction of Cu(II) to Cu(I) can be seen by the change in colour of the solutions from blue-green to yellow with the formation of white precipitates along with the disappearance of the Cu(II) bands in the visible spectra together with the substantial reduction of intensity of the copper peaks in the mass spectra where the reactions take place in the presence of limited amounts of dioxygen. The oxidation of D-galactose is confirmed by the appearance of the $[OxidGal + OH]^-$ peak in the negative ion electrospray mass spectra.

(ii) The exposure to air of the sonicated solutions results in the colour of the solutions changing from yellow to blue green indicating re-oxidation of the solutions. There are many reported examples of the formation of dioxygen adducts after the addition of dioxygen to solutions of Cu(I) complexes but in most cases these adducts are not stable at room temperature. There is no mass spectral evidence to show the presence of stable dioxygen adducts at room temperature in solutions of these complexes.

(iii) Itoh et al [49, 50] with their Cu(II) ligand system predicted that after the formation of a superoxo monomeric adduct dimerization occurs to form a peroxo bridged dimer which is then followed by breaking of the O – O bond to form a bis-μ–oxobridged Cu(III) dimer. They then predicted that an intramolecular hydrogen abstraction reaction and a direct oxygen insertion mechanism might take place with the bis-μ-oxo Cu(III) compound or with a Cu(II)-oxo radical monomeric species. Maiti et al [51] and Peterson et al [52]

have suggested the possibility of Cu(II)–OOH and Cu(II)–O· (cupryl) intermediate species being involved in the hydroxylation reaction.
The presence of the $[CuLMe.O.imid - H]^-$ peak at m/z 367 in the negative ion electrospray mass spectrum of CuSalser.imid + D-galactose and other CuLMe.O peaks after sonication for two hours are consistent with the presence of the ligand hydroxylated intermediates.

(iv) Ether formation then takes place with the reaction of the hydroxylated products in basic methanol or ethanol solutions as has been shown to occur in the serine based ligand system. Tano et al. [53] reported that the reaction of phenols on the end on superoxide adduct of the copper(II) complex used in reference 49 produced $[LCu(II)OAr]^+$ species in acetone solution at -85ºC but did not report any addition of the OAr groups to their ligand.

Where the amino acid in the ligand is methionine it was initially thought that the +16 peaks might represent the intermediate hydroxylated species as may occur with the serine based ligand. There are, however, additional peaks at m/z 443 in the negative ion mass spectrum representing $[CuLO.OMe.imid - H]^-$ and at m/z 451 in the positive ion mass spectrum representing $[CuLO.OMe.imid + Na]^+$. While this might represent addition of both OH and OMe to the ligand it is more likely that this +16 represents addition of oxygen to the sulfur thioether atom in the methionine thioether part of the ligand to form a sulfoxide sulphur atom as shown in figure 10.
Lee et al. [54] have determined the structure of a sulfoxide product of the reaction of the Cu(I) compound of a $N_2S$ tridentate thioether ligand with dioxygen and suggested a similar reaction scheme to that of the hydroxylation reaction reported by Maiti et al. [51]. In the presence of excess $H_2O_2$ a sulfone ($SO_2$) product was also formed with their ligand system but there is no evidence of the formation of a sulfone in this work.

Figure 9. The negative ion electrospray mass spectrum (intensity vs m/z) of CuSalmeth.imid + D-galactose(1:2) in MeOH/NaOH/Air after sonication for two hours..

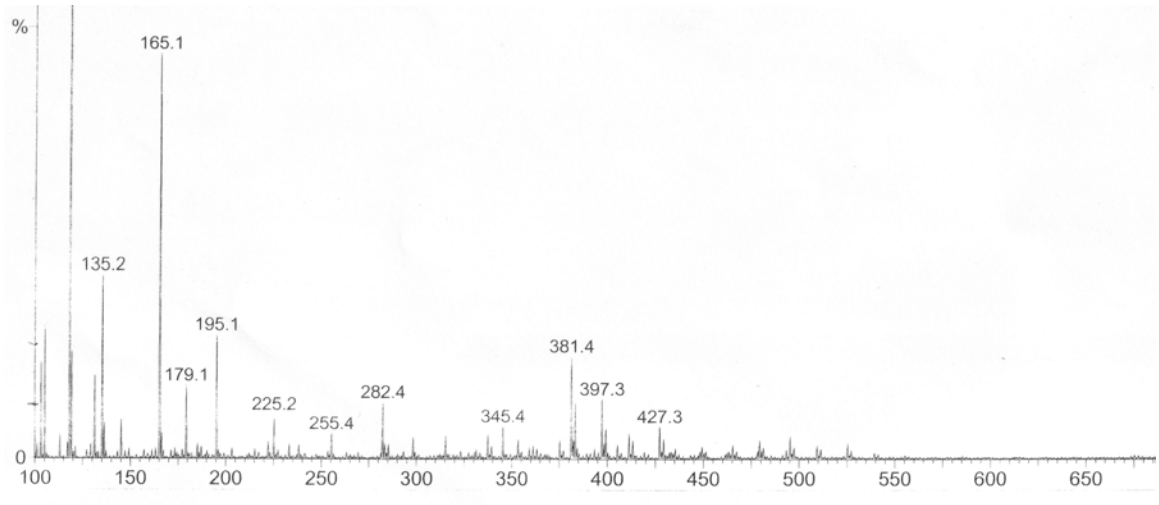
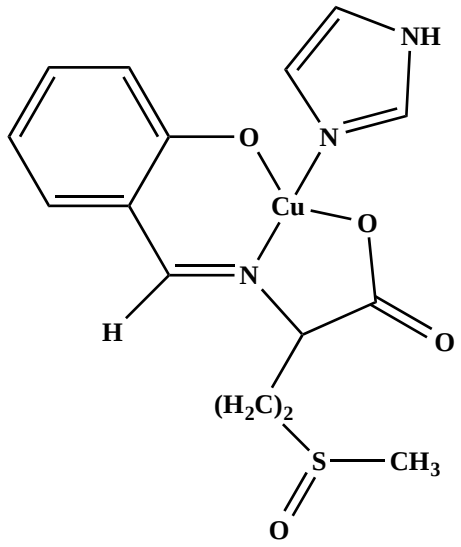


Figure 10. The suggested structure of the minor product formed by the reaction of CuSalmeth.imid + D-Galactose in MeOH/NaOH/Air after sonication for two hours.




## 5. Conclusion.

We have found that electrospray mass spectrometry is a useful technique to study the reactions of a series of copper(II) compounds of tridentate NNO Schiff Base ligands and D-Galactose in basic methanol solution. In addition to the expected D-galactose redox reactions we have identified the formation of ether and sulfoxide groups on the copper(II) compound ligands with the nature of the product depending on the amino acid part of the ligand.

Sonication has been found to be a useful technique to reduce the reaction times for the reactions studied.

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