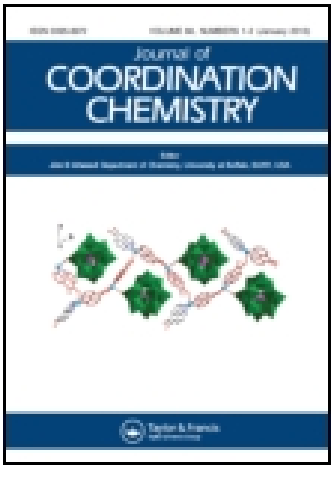

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Ether formation on the tridentate Schiff base ligands of copper(II) complexes

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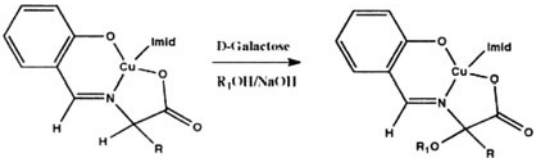

A series of copper(II) complexes, CuL·imidazole, where L^{2-} are tridentate Schiff base ligands formed by condensation of salicylaldehyde with a series of amino acids, have been synthesized. Visible spectral data indicate that copper(II) in these complexes are five coordinate in the solid state and 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

1. Introduction

Copper is used by nature in many different enzymes that catalyze a variety of biochemical reactions. One such enzyme is the fungal enzyme galactose oxidase which catalyzes the oxidation of primary alcohols to their corresponding aldehydes along with reduction of dioxygen to hydrogen peroxide [1, 2].

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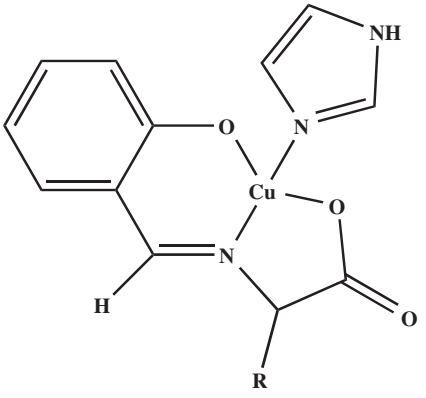

Figure 1. CuSalaa·imid.

The crystal structure of the inactive Cu(II) form of galactose oxidase [3, 4] reveals a mononuclear copper site with two histidyl nitrogens, two tyrosyl oxygens, and a water or acetate bound to copper in a distorted square pyramidal environment around copper.

We have synthesized a series of copper(II) complexes of tridentate Schiff base ligands formed by reaction of salicylaldehyde with amino acids.

The structure of CuSalaa·imid is shown in figure 1.

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 from 400 to 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 ionization) mass spectra were obtained using a Micromass VG quattro-2^{tm} triple quadrupole mass spectrometer (Altringham, United Kingdom) utilizing a Z-spray ion source. Data were 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 10 µL sample loop injector. HPLC grade solvents and ultra-pure water were used. The following operational parameters were typical: solvent flow: 20 µL min^{-1}, capillary voltage: 2.5, voltage lens: 0.40, cone voltage: 20–30 V, high and low mass resolution: 15, desolvation gas temperature: 80 °C.

Sonication of the solutions for up to 4 h was carried out using a Unisonic ultrasonic cleaner. Sonication is a technique often used to clean glassware and to help dissolve compounds, but it is also a useful technique to decrease the time taken for a reaction to take place. For example, a reaction that might take place in 10 h occurs in one or 2 h if the

solution is sonicated. 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*

2.2.1. Cu(aminoacid anion)$_2$·H$_2$O. Copper sulfate pentahydrate (0.1 M), amino acid (0.2 M), and sodium hydroxide (0.2 M) 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 was 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.

2.2.2. CuSalaa·imidazole. Cu(amino acid anion)$_2$·H$_2$O (3 g) was suspended in methanol (100 mL). Salicylaldehyde (2 M equivalent) and triethylamine (7 mL) were added to the methanol solution which was refluxed for 1 h. The solution was filtered by gravity and imidazole (1.2 M 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 mL petroleum ether and air dried. 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	$C_{19}H_{17}N_3O_3Cu$	Calcd	57.21	4.30	10.53
		Found	57.05	4.23	10.56
CuSalphengly·imid·0.5H$_2$O	$C_{18}H_{16}N_3O_{3.5}Cu$	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$_2$O	$C_{19}H_{17}N_3O_4Cu$	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 35 complexes of the type CuSalaa·base have previously been determined by X-ray crystallography [5–41]. In each of these complexes, the copper is in an approximately square pyramidal environment (CuN_2O_3) with five coordination occurring through: (1) addition of a molecule of water [5–16]; (2) formation of a dimer bridged by ligand phenolic oxygens [17–27, 41]; (3) formation of a polymer with an adjacent carboxyl oxygen occupying the fifth coordination site [28–40].

For CuSalala·imid [22, 41], five coordination is achieved via dimer formation. Each complex has a broad solid state spectral band at 590–630 nm (Cusalala·imid: 610 nm) and it seems reasonable to conclude that this band represents five-coordinate CuN_2O_3. In methanol, the spectral band occurs at 615–622 nm, suggesting a CuN_2O_3 environment with a methanol occupying the fifth coordination site. In CH_3CN/H_2O solution, the spectral band occurs at 605–616 nm, again suggesting a CuN_2O_3 environment with water 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. Copper causes a double peak (Cu-63, Cu-65) and the peak values are shown in table 2 are those containing the dominant isotope (Cu-63).

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

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	
$[Cu_2L_2{\cdot}imid - H]^-$	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		
$[CuL{\cdot}imid - H]^-$	307	321	349	363	337	381	413	383	397
$[Cu(L\text{-}CO_2){\cdot}imid - H]^-$	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
$[Cu_2L_2{\cdot}imid + H]^+$		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	
$[CuL{\cdot}imid_2 + H]^+$		391	419	433			483		467
$[2Gal + Na]^+$	383		383	383	383		383		
$[CuL{\cdot}imid + Na]^+$	331		373		361	405		453	421
$[CuL{\cdot}imid + H]^+$	309	323	351	365	339	383	415	385	399
$[CuL{\cdot}MeOH + H]^+$		287	315	329		347	379	349	363
$[CuL + Na]^+$						315	347		
$[Gal + Na]^+$	203	203	203	203	203	203	203	203	203

(2) Galactose peaks, $[Gal - H]^-$, $[Gal + Cl]^-$, $[Gal + HCO_2]^-$, $[2Gal - H]^-$, and $[Gal + Na]^+$, also occur. The chloride comes from sea spray while the formate persists in the mass spectrometer after formic acid was used as an ionizing medium in previous mass spectral measurements.
(3) $[CuL + Gal - H]^-$ and $[Cu_2L_2 + Gal - H]^-$ peaks occur showing that D-galactose binds to copper replacing the imidazole. This is consistent with D-galactose binding to copper compounds before being oxidized.
(4) When 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.

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 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 [oxidized galactose + $OH]^-$ in the negative ion electrospray mass spectra of the solutions. The relative intensities of the two D-galactose peaks depend on the complex used, the concentration of hydroxide 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 oxidized while the Cu(II) complex is reduced to Cu(I) complex which precipitates from solution as the white solid as shown in reaction (1).

$$2Cu(II)L \cdot imid + GalCH_2OH + 2NaOH \rightarrow 2NaCu(I)L \cdot imid + GalCHO + 2H_2O \quad (1)$$

On exposure to air, these yellow solutions slowly turn blue-green as the white precipitate dissolves and the copper peaks reappear in mass spectra of the compounds. If excess D-galactose is present in the solutions, the cycle can be repeated on further sonication.

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 at 607–11 nm, indicating little change in the immediate environment around copper.

The negative ion mass spectra (table 4) of the complexes contain both peaks for galactose (179) and oxidized galactose (195), indicating that the redox reaction involving the copper complexes and D-galactose still occur even when the color 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 at 607 nm occurs, indicating the environment around the copper is

square pyramidal. The negative ion electrospray mass spectral peaks are similar to those in methanol with $[CuL{\cdot}imid - H]^-$ at *m/z* 337, with the addition of a small peak at *m/z* 351.

After sonication for 2 h, 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 $[CuL{\cdot}imid - H]^-$ peak at *m/z* 337 is replaced by a peak at *m/z* 351 (figure 2, table 3) and the $[CuL{\cdot}imid + Na]^+$ peak in the positive ion spectrum moves from *m/z* 361 to 375 (table 3).

This indicates the addition of a methyl to the complex and loss of a hydrogen from the complex giving an addition of 14 to the molecular ions. When ethanol is used instead of methanol, the $[CuLR{\cdot}imid - H]^-$ peak occurs at *m/z* 365 and the $[CuLR{\cdot}imid + Na]^+$ 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 to the complex are obtained.

When the MS/MS spectrum of the $[CuL{\cdot}imid - H]^-$ 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 $[CuLMe{\cdot}imid - H]^-$ 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 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 rather than initial loss of CO_2.

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

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

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

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 605–16 nm which shift by 0–4 nm when D-galactose is added to

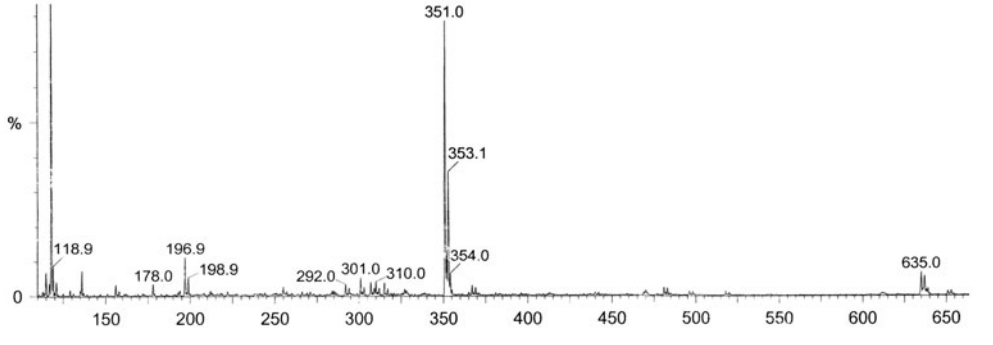

Figure 2. The negative ion electrospray mass spectrum (intensity *vs. m/z*) of CuSalser·imid in MeOH/NaOH solution after sonication for 2 h.

Table 3. Electrospray mass spectra of CuSalser·imid in R_1OH/NaOH after sonication for up to 4 h.

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

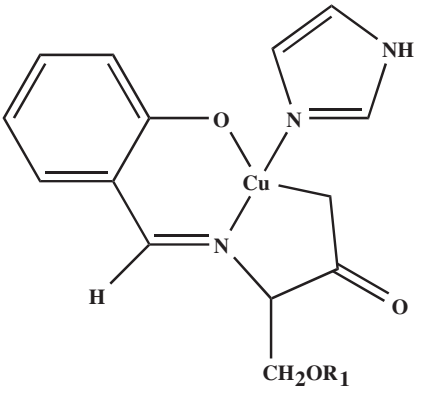

Figure 3. The suggested structure of the product of the reaction of CuSalser·imid after sonication for 2 h in R_1OH/NaOH/air solution.

the solutions. When the solutions of these complexes in MeOH/NaOH/air are sonicated for 2 h, the mass spectra 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 $[CuL\cdot imid - H]^- + 30$ occurs.

When D-galactose is added to the solutions before sonication and the solutions are then sonicated for up to 2 h, 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 replacement of a proton on the ligand by a methoxy (figure 4, tables 4 and 5). When EtOH/NaOH is used, the monomer peaks increase by 44 (table 5), indicating replacement of a proton by an ethoxy. This indicates that the reaction takes place as shown in reaction (3).

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

When the MS/MS spectrum of CuSalala·imid in MeOH is measured, the $[CuSalala\cdot imid - H]^-$ 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 $[CuLOMe\cdot imid - H]^-$ 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.

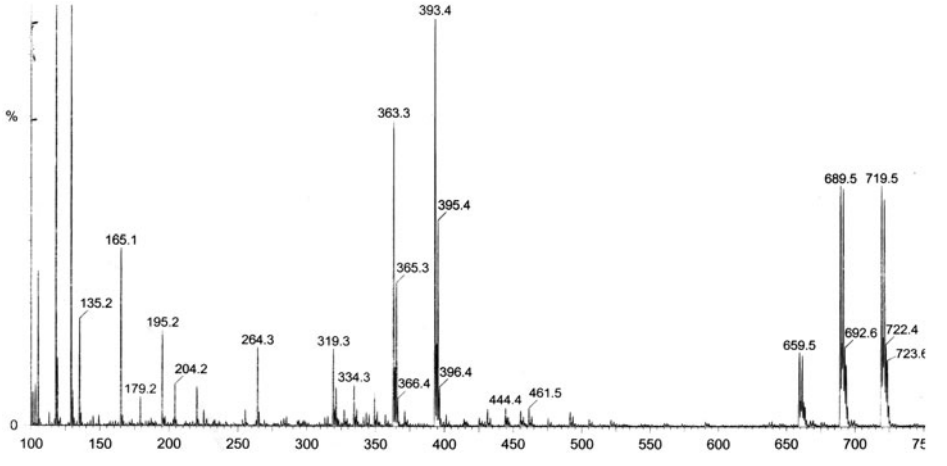

Figure 4. The negative ion electrospray mass spectrum (intensity *vs. m/z*) of CuSalleu·imid + D-galactose (1 : 2) in MeOH/NaOH/air solution after sonication for 2 h.

Table 4. Electrospray mass spectra of CuSalaa·imid + D-galactose (1 : 2) in methanol/NaOH/air after sonication for 2 h.

	Gly	Ala	Val/Nval	Leu/Nleu	Ser	Met	Tyr	Phen gly	Phen ala
$[Cu_2(LOMe)_2\cdot imid - H]^-$		635	691	719				759	787
$[Cu_2LMe\cdot(LMe.O)\cdot imid - H]^-$					651				
$[Cu_2(LMe)_2\cdot imid - H]^-$					635				
$[Cu_2L\cdot LOMe\cdot imid - H]^-$		605	661	689				729	757
$[Cu_2L_2\cdot imid - H]^-$		547	575	631		659			727
$[CuLO\cdot OMe\cdot imid - H]^-$						427			
$[CuLOMe\cdot imid - H]^-$		351	379	393		411	443	413	427
$[CuLMe\cdot O\cdot imid - H]^-$					367				
$[CuLO\cdot imid - H]^-$						397			
$[CuLMe\cdot imid - H]^-$					351				
$[CuL\cdot imid - H]^-$	307	321	349	363		381	413	383	397
$[Cu(LMe\text{-}CO_2)\cdot imid - H]^-$					307				
$[Cu(L\text{-}CO_2)\cdot imid - H]^-$	263	277	305	319				339	353
$[LOMe + H]^-$	208	222	250	264				284	298
$[Gal + HCO_2]^-$		225	225	225			225		
$[Oxid\cdot Gal + OH]^-$	195	195	195	195	195	195	195	195	195
$[Gal - H]^-$	179	179	179	179		179	179	179	179
$[Cu_2(LMe\cdot O)LMe + Na]^+$					607				
$[Cu_2(LOMe)_2 + Na]^+$			647	675					
$[Cu_2(LMe)_2 + Na]^+$					591				
$[Cu_2(LO)_2 + Na]^+$							683		
$[Cu_2L\cdot LOMe + Na]^+$		561	617	645					
$[Cu_2L\cdot LO + Na]^+$						667			
$[Cu_2L_2 + Na]^+$	503	531	587	615		651			
$[CuLO\cdot OMe\cdot imid + Na]^+$						451			
$[CuLMe\cdot O\cdot imid + Na]^+$					391				
$[CuLOMe\cdot imid + Na]^+$		375		417				437	451
$[2Gal + Na]^+$		383	383	383		383	383		383
$[CuL\cdot Me\cdot imid + Na]^+$					375				
$[CuL\cdot imid + Na]^+$	331	345	373	387		405	437		421
$[CuLMe\cdot imid + H]^+$					353				
$[CuL\cdot imid + H]^+$					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 h.

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

Where the amino acid is glycine, no copper-containing peaks with an addition of an OR 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 methoxy is added to the ligand and not to the imidazole ring.

Where the amino acid in the ligand is serine, the $[CuLMe \cdot imid - H]^-$ 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" 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 take 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 h. 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 $[CuLOMe \cdot imid - H]^-$: $[CuL \cdot imid - H]^-$ 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).

Table 6. Visible spectra (nm) and the ratio of $[CuLOMe \cdot mid - H]^-$: $[CuL \cdot imid - H]^-$ 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 h.

	Gly	Ala	Val	Nval	Leu	Nleu	Met	Tyr	Phen gly	Phen ala
Time: 0 h										
	610	607	608	608	607	607	616	605	606	609
	0	0	0	0	0	0	0	0	0	0
Time: 1.5 h										
	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 h										
	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

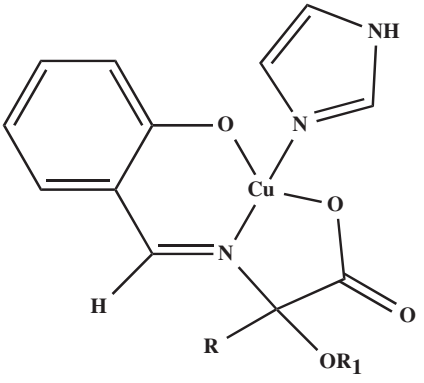

Figure 5. The suggested structure of the ether formed by the reaction of CuSalaa·imid + D-galactose (1 : 2) after sonication in R_1OH/NaOH/air for 2 h.

Before sonication, visible spectral bands occur at 606–616 nm and there is no mass spectral evidence for formation of the ether products. After a reaction time of 1.5 h, visible spectral bands occur at 605–613 nm and mass spectral peaks representing formation of the ethers start to appear with some of the compounds, especially where the amino acid in the ligand is phenylglycine. After sonication for 4 h, visible spectral bands appear at 612–622 nm and mass spectral peaks indicate that the reaction with phenylglycine has gone to completion and there has been considerable 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 in the complexes does not substantially change after reaction takes place (16 nm for the phenylglycine compound *versus* 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.

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 2 h, a peak for $[CuLOMe{\cdot}imid - H]^-$ at *m/z* 411 appears in the negative ion electrospray mass spectra (figure 6, 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 $[CuLO{\cdot}imid - H]^-$, $[Cu_2L{\cdot}LO + Na]^+$, 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 formation of ether groups on the ligands after sonication of the solutions.

(1) 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

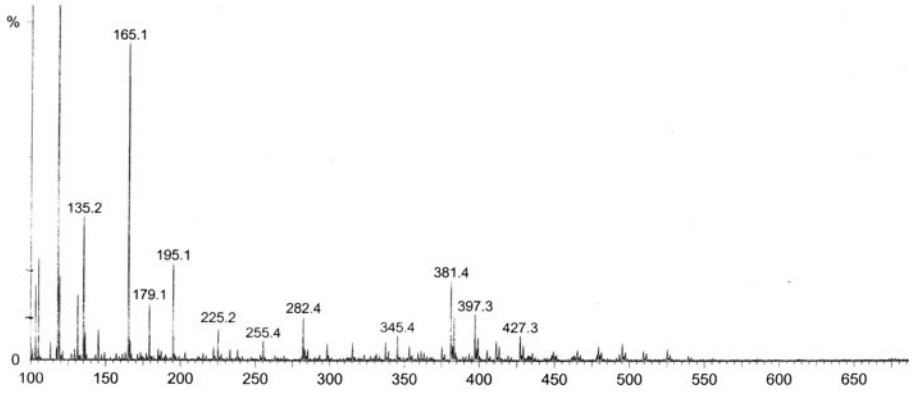

Figure 6. The negative ion electrospray mass spectrum (intensity vs. *m/z*) of CuSalmeth·imid + D-galactose (1 : 2) in MeOH/NaOH/air solution after sonication for 2 h.

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.

(2) Where the amino acid in the ligand is not serine or possibly glycine, the presence of the reducing agent D-galactose is required for 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.

Karlin *et al.* [42–47] have pioneered the study of aromatic hydroxylation 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 [48–52] 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 7.

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

The reaction scheme shown in figure 8 is similar to that reported by Itoh *et al.* [48,49] for aliphatic 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 9). In this work, an additional step involves conversion of the alcohol to the ether.

(1) The copper(II) complex is reduced to the copper(I) complex and D-galactose is oxidized to its aldehyde. The reduction of Cu(II) to Cu(I) can be seen by the change in color of the solutions from blue-green to yellow with 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 $[\text{OxidGal} + \text{OH}]^-$ peak in the negative ion electrospray mass spectra.

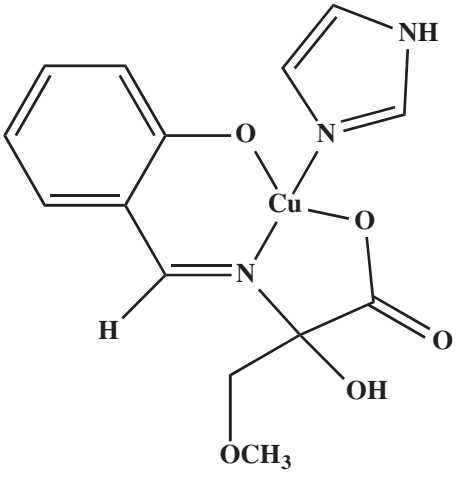

Figure 7. The suggested structure of the minor product of the reaction of CuSalser·imid + D-galactose (1 : 2) after sonication in MeO/NaOH/air for 2 h.

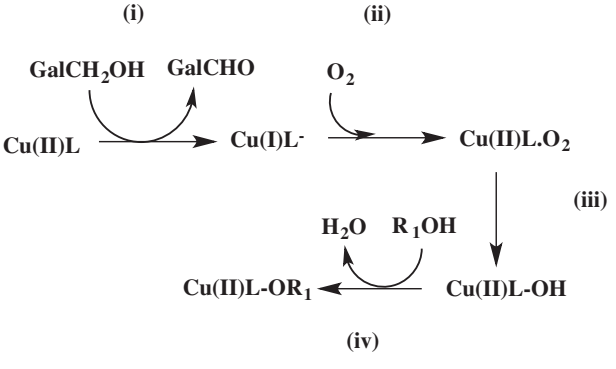

Figure 8. The proposed scheme for the reaction of CuL·imid + D-galactose in R_1OH/NaOH/air after sonication.

(2) The exposure to air of the sonicated solutions results in the color of the solutions changing from yellow to blue green, indicating re-oxidation of the solutions. There are many reported examples of formation of dioxygen adducts after 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.

(3) Itoh *et al.* [48, 49] with their Cu(II) ligand system predicted that after 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 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.* [50] and Peterson *et al.* [51] have suggested the possibility of Cu(II)–OOH and

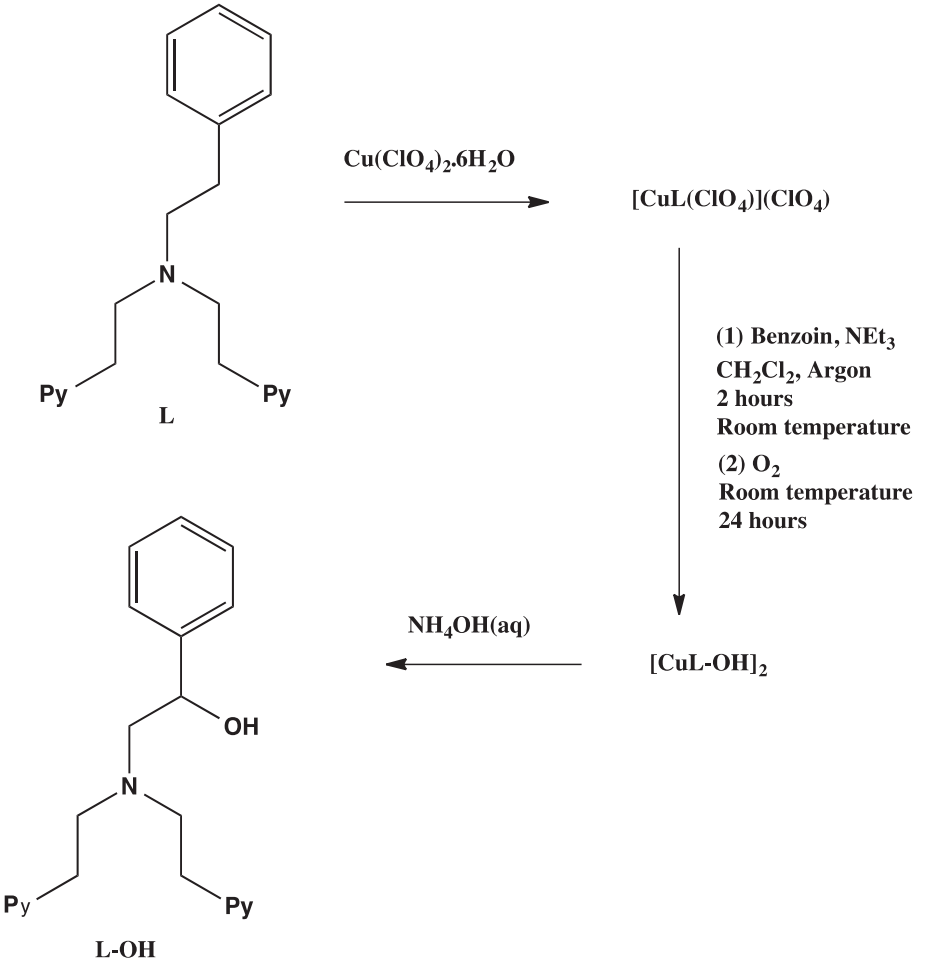

Figure 9. The suggested reaction scheme for the aliphatic ligand hydroxylation of the copper(II) complex of the ligand in references [48, 49].

Cu(II)–$O^{\cdot}$ (cupryl) intermediates 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 2 h are consistent with the presence of the ligand hydroxylated intermediates.

(4) Ether formation then takes place with reaction of the hydroxylated products in basic methanol or ethanol solutions as shown to occur in serine-based ligand system. Tano *et al.* [52] reported that reaction of phenols on the end of superoxide adduct of the copper(II) complex used in reference 49 produced [LCu(II)OAr]$^{+}$ species in acetone solution at −85 °C, but did not report any addition of the OAr groups to their ligand.

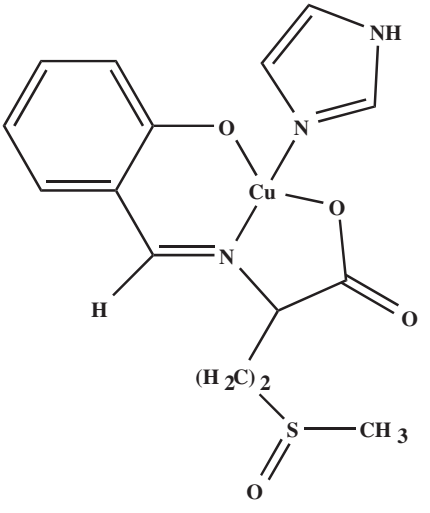

Figure 10. The suggested structure of the minor product formed by the reaction of CuSalmeth·imid + D-galactose (1 : 2) after sonication in MeOH/NaOH/air for 2 h.

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 $[CuLO{\cdot}OMe{\cdot}imid - H]^-$ and at *m/z* 451 in the positive ion mass spectrum representing $[CuLO{\cdot}OMe{\cdot}imid + Na]^+$. 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 in the methionine thioether part of the ligand to form a sulfoxide as shown in figure 10.

Lee *et al.* [53] 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.* [50]. 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.

5. Conclusion

We have found that electrospray mass spectrometry is a useful technique to study 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) ligands with the nature of the product depending on the amino acid part of the ligand. Sonication is a useful technique to reduce the reaction times for the reactions studied.

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