Arch. Pharm. Res. **15**(2), 130-133 (1992)

Copper Complexes of Some Amino Acid Derivatives of Substituted Coumarilic Acid

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(Received February 26, 1992)

Abstract ☐ A series of copper complexes of some amino acid derivatives of 6-methoxy-3-methyl-coumarilic acid were prepared. The infrared, visible spectra and magnetic susceptibility of these compounds were reported. All copper complexes were found to have antimicrobial activity against gram-positive bacteria only.

Keywords ☐ Coupling reactions, amino acid derivatives, copper complexes, biological activity.

Recently, copper complexes of some amino acid derivatives were reported in our laboratory[1,2]. In continuation of our study in the same field, now we prepare a series of copper complexes of amino acids and dipeptide of 6-methoxy-3-methyl-coumarilic acid **3a-d** and study their IR, visible spectra, magnetic susceptibility and biological properties.

EXPERIMENTAL

Melting points were determined using an electrothermal melting point apparatus and are uncorrected. Thin layer chromatography (TLC, R_f values) was performed on silica-gel-G using benzene-ethyl acetate (3:1) and iodine-KI (20%) as detection reagent. Benzidine, ninhydrin were used for the detection of amino acid derivatives (spot reactions). The microanalysis were performed by microanalytical center at Cairo University. Infrared spectra were recorded using Shimadzu IR spectrometer (IR 440). Samples were mixed with KBr and all spectra were scanned in the range 5050-300 cm^{-1}. Visible spectra were obtained in the range 900-500 nm. (11,111-20, 000 cm^{-1}) in the liquid phase using Perkin Elmer λ3b spectrometer. Magnetic susceptibility measurements were obtained at room temperature using Guoy method[3]. ^{1}H-NMR spectra were recorded on a Varian EM-360L spectrophotometer using DMSO-d_6 as a solvent and TMS as an internal standard.

6-Methoxy-3-methyl-coumariloylamino acids 1a-c.

6-Methoxy-3-methyl-coumariloyl chloride (0.02 mole) was dissolved in benzene (25 m*l*) and added dropwise to a solution of the appropriate amino acid (0.07 mole) in 1M NaOH (20 m*l*) at 7℃. The reaction mixture was stirred at 10℃ for 75 min., followed by three hours stirring at room temperature. The benzene layer was separated out and the aqueous layer was cooled to 0℃ and acidified with 2M HCl until become acidic to congo red (pH 4.5). The separated crude products **1a-c** were filtered off, washed with cold water and recrystallized from ethanol-water. The compounds **1a-c** were chromatographically homogeneous when developed with benzidine, iodine solution and gave ninhydrin negative test.

The ^{1}H-NMR spectra of compounds **1a-c** exhibited (δ) at: 10.7(s, 1H, COOH), 5.8(s, 1H, NH) and other protons assignable to each individual amino acid residues.

6-Methoxy-3-methyl-coumariloyl-DL-valyl-L-tyrosine methyl ester 2.

DL-Tyrosine methyl ester hydrochloride (0.01 mole) was suspended in THF (20 m*l*) and then triethylamine hydrochloride filtered off. The filtrate was added to a solution of 6-methoxy-3-methyl-coumariloyl-DL-valine (**1c**; 0.01) in THF (20 m*l*). The mixture was cooled to 0℃, and dicyclohexylcarbodimide (0.011 mole) was added and the mixture stirred for three hours at 0℃, then left for 24 hours at room temperature. The dicyclohexylurea was filtered off and the filtrate was evaporated *in vacuo*. The residual solid was recrystallized from ethanol-water. Compound 2 was chromatographically homogeneous when developed with benzidine, iodine solution and gave positive hydroxamate reaction and ninhydrin negative test.

The ^{1}H-NMR spectra of compounds 2 exhibited (δ) at: 2.75(s, 3H, $COOCH_3$), 5.8(s, 1H, NH): 7.8-8.4(s, aromatic protons) and other protons characteristic of amino acids.

Preparation of copper complexes of (1:1) molar ratio 3a-d

A 0.01M solution of 6-methoxy-3-methyl-coumariloyl-amino acids or dipeptide derivtive (ligands **1a-2**) in absolute ethanol was prepared, Hot ethanolic solution of $CuCl_2 \cdot 2H_2O$ was dropwise added to stirred hot ethanolic solution of ligands **1a-2**. On cooling, copper complex was separated out, and recrystallized from ethanol-water.

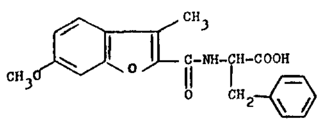
RESULTS AND DISCUSSION

Chemistry

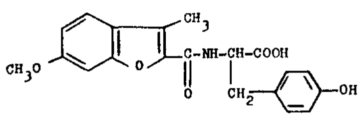
Coupling of 6-methoxy-3-methyl-coumariloyl chloride with the appropriate amino acid in benzene-sodium hydroxide medium at 7℃, gave the desired 6-methoxy-3-methyl-coumariloylamino acids **1a-c** in 61-80% yields (cf. Fig. 1 and Table I). The time required for the completion of the reaction was monitored by TLC. Synthesis of Tyr-derivative 2 did not require the prior protection of the side chain and no side reaction was observed. Complete acid hydrolysis (6M-HCl, 24 hrs, 100℃) of **1a** followed by subsequent paper chromatography, gave a ninhydrin positive spot of phenylalanine.

Also, coupling of the amino acid with 6-methoxy-3-methyl-coumariloyl chloride in water-THF-Et_3N medium using Theodoropolulos method[4], gave the compounds **1a-c** with the same melting points, R_f as those obtained by the first method. The products obtained by this method needed crystallization several times (20-32% yields), so the benzene-sodium hydroxide method was preferred.

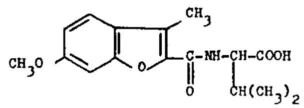
Coupling of compound **1c** and DL-tyrosine methyl ester hydrochloride in THF-triethylamine medium and using (DCC) method[5] afforded the dipeptide **2** in 65% yield (cf. Fig. 1 and Table I). Complete acid hydrolysis (6M-HCl, 24 hrs, 100℃) of **2** followed by subsequent paper chromatography yielded two positive spots of valine and tyrosine.



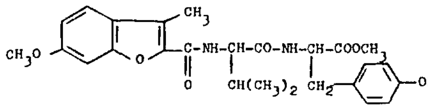
6-Methoxy-3-methyl-coumariloyl-DL-phenylalanine. **1a**



6-Methoxy-3-methyl-coumariloyl-DL-tyrosine **1b**.



6-Methoxy-3-methyl-coumariloyl-DL-valine **1c**.



6-Methoxy-3-methyl-coumariloyl-DL-valyl-DL-tyrosine **2**

Fig. 1. Structures of compounds 1a-2.

Copper complexes of 6-methoxy-3-methyl-coumariloylamino acids and dipeptide **3a-d** of (1:1) molar ratio were prepared by adding alcoholic solution of $CuCl_2 \cdot 2H_2O$ to alcoholic solution of the appropriate ligand **1a-2**. Copper was determined complexometrically as reported before[6].

The main IR absorption bands of the ligands **1a-2** and their Cu^{+2} complexes **3a-d** are listed in Table II. It was clear from the IR spectra of the ligands (compounds **1a-2**) that an absorption feature was observed in the range 3200-3400 cm^{-1} and this absorption band was assigned to ν(OH), also a strong absorption peak was clearly appeared in the range 1700-1730 cm^{-1} and this absorption frequency was attributed to ν(C=O) stretching mode. The band appeared in the range 1135-1150 cm^{-1} most probably attributed to ν(C-O) of the furan ring. ν(NH) was observed at ~3300 cm^{-1}, while δ(NH) appeared in the range 1525-1576 cm^{-1}. The strong absorption feature located in the region 1628-1600 cm^{-1} was assigned to ν(C=C) or the phenyl ring.

Comparison of the infrared spectra of ligands

Table I. Physical properties of 6-methoxy-3-methyl-coumariloylamino acid derivatives (ligands 1a-c).

Compd. No.	(*) Yield	M.P. °C	R_f	Molecular formula	Elemental analysis (%) Calculated/Found C	H	N	C	H	N
1a	73	133-4	0.89	$C_{20}H_{19}NO_5$	67.99	5.38	3.97	68.2	5.5	3.0
b	61	150	0.70	$C_{20}H_{19}NO_6$	65.04	5.15	3.79	65.2	5.3	3.7
c	80	121-2	0.93	$C_{16}H_{19}NO_5$	62.95	6.23	4.59	63.1	6.4	4.4
2	65	90-1	0.86	$C_{26}H_{30}N_2O_7$	64.73	6.22	5.81	64.8	6.4	5.7

(*)Crystallization solvent for compounds **1a-2** =ethanol-water.

Table II. Frequencies in cm^{-1} of IR absorption peaks.

Compound No.	ν(NH)	ν(NH)*	ν(C=O)	ν(C=C)	δ(NH)	ν(C−O)
1a	3390	3390	1730	1607	1555	1140
3a	3390	3390	1720	1605	1552	1135
1b	3400	3400	1720	1605	1573	1135
3b	3400	3400	1713	1603	1572	1118
1c	~3400	3400	1727	1605	1570	1115
3c	3400	3400	1720	1605	1570	1138
2	~3420	3350	1730	1628	1575	1142
3d	3420	3350	1722	1625	1572	1138

* ν(NH) and ν(OH) are overlapped

Table III. Magnetic susceptibility data and absorption peaks in cm^{-1}

Complex No.	Colour	μ_{eff} B.M.	Absorption peaks cm^{-1}	
3a	brown	1.95	12,077	12,165
b	brown	1.88	11,961	12,165
c	brown	1.92	11,961	12,165
d	yellow	1.87	11,961	12,165

and their Cu^{+2}-complexes revealed that ν(C=O) stretching frequency was clearly shifted to lower frequency upon complexation, also ν(C−O) was slightly shifted to lower frequency. Thus, we suggest that complexation takes place *via* the coordination sites carbonyl oxygen and furan ring oxygen.

It was reported[7] that coordinated water absorbs in the range 3500-3200 cm^{-1} (OH stretching) and at 1630-1600 cm^{-1} (HOH bending). Strong and extremely broad absorption feature was clearly observed in the range 3200-3400 cm^{-1} in the IR spectra of Cu^{+2}-complexes and this feature was attributed to ν(OH) of coordinated water molecules. Water bending mode occured at ~1630 cm^{-1} as a shoulder on the strong peak which recorded in the region 1628-1600 cm^{-1}.

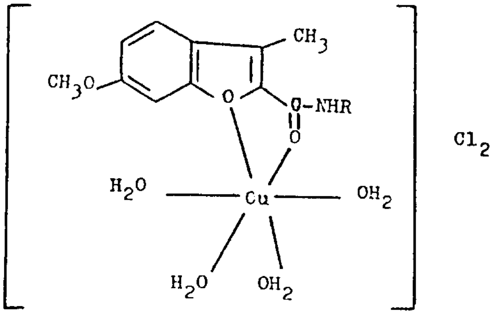
Magnetic susceptibility data were measured at room temperature using Guoy method[3]. The data indicated that these ligands have weak ligand field, μ_{eff} are listed in Table III.

Absorption peaks of Cu^{+2}-complexes in the visible region are listed in Table III. Broad absorption peaks observed at ~12,000 and 12,165 cm^{-1} indicate that Cu^{+2}-complexes are octahedrally coordinated[8]. The absorption bands can be assigned to $^2E_g \rightarrow {}^2T_{2g}$. The weak features observed in the absorption spectra may be attributed to Jahn-Teller effect in Cu^{+2}-complexes[9].

In conclusion to our study, the experimental data suggest an octahedral structure for Cu^{+2}-complexes. The proposed structure is shown in Fig. 2.

Biological screening:

The antimicrobial activities of copper complexes **3a-d** were tested using the hole plate and filter paper disc methods[10,11]. All compounds were tested against gram-positive and gram-negative bacteria: *Bacillus subtilis* (ICC-strain), *Bacillus mycoides*



R=amino acid residue

Fig. 2. Proposed structure for copper complexes.

(USSR), *Escherichia coli* (NRRL-B-210), *Salmonella typhosa* (NRRL-B-573) and selected fungi: *Candida utilis*. The results were compared with the activity of the parent compounds (Ligands **1a-2**) which were found to possess a various antimicrobial activity against gram-positive bacteria only (*B. subtilis* and *B. mycoides*) at MIC ranging from 100 to 250 μg/m*l*. The data for the minimal inhibitory concentrations (MIC in μg/m*l*) of active compounds are summarized in Table IV.

The copper complexes of 6-methoxy-3-methylcoumariloyl-DL-Phe **3a** and corresponding DL-Tyr **3b** and DL-Val **3c** were found to possess an interesting antimicrobial activity against gram-positive bacteria (MIC ranging 100 to 250 μg/m*l*) [cf. Table IV]. The copper complex of dipeptide derivative **3d** was found to possess a moderate activity against gram-positive bacteria only (MIC 100 μg/m*l*) [cf. Table IV].

All the ligands **1a-2** and copper complexes **3a-d** were found to possess a very low antimicrobial activity against gram-negative bacteria (*E. coli* and *S. typhosa*) and the testd fungi (*Cand. utilis*) [MIC 500 μg/m*l*].

The achieved results revealed that introducing copper atom to 6-methoxy-3-methyl-coumariloylamino acids or dipeptide derivative improve the biological properties of the prepared complexes **3a-d**.

Table IV. Minimal inhibitory concentration (MIC in μg/m*l*) of the biologically active compounds(*).

Compd. No.	*Bacillus subtilis*	*Bacillus mycoides*
1a	250	250
b	100	100
c	250	250
2	125	250
3a	50	50
b	10	10
c	25	10
d	100	100

(*)Compounds **1a-3d** were found to possess a very low antimicrobical activities against *E. coli*, *S. typhosa* and *Cand. utilis* with MIC values 500 μg/m*l*.

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