New Antimicrobial Complex of Copper (II) with Benzothiazole Derivative: Synthesis and Application in Leather Industry*

by

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Abstract

Leather goods, especially leather shoes, are often damaged by microorganism and their antimicrobial performances are increasing demanded. However, the normal leather fungicides can not meet the requirement to the antimicrobial agents used in the leather goods; it is significant to develop an effective, harmless and broad-spectrum antimicrobial compound for leather goods. In this study, a novel ligand, 4-[(2-benzothiazolylimino) methyl] phenoxyacetic acid (HL), was prepared by 2-amino benzothiazole and 4-formyl phenoxyacetic acid in absolute ethyl alcohol and the copper complex was synthesized by HL and copper acetate in ethanol at 65°C. The chemical structures of the ligand and copper complex were characterized by elementary analysis, UV, IR, and ^{1}H NMR. Results show that the copper ion coordinate with two oxygen atoms of phenoxyl and carboxyl in the ligand and two stable pentagonal rings are formed. The antimicrobial activity of the complex against representative species of bacteria and fungi isolated from leather shoes was assessed by the determination of minimal inhibitory concentrations (MICs). Results show that MICs of the copper complex were 100-200 mg L^{-1} to bacteria, 1-50 mg L^{-1} to moulds and 1 mg L^{-1} to yeasts, respectively. The copper complex with the concentration of 0.5-7.5 g L^{-1} in acetone was applied to shoe lining, and the inhibitory effects of the treated shoe lining were evaluated by inhibition zone method, plate culture medium method and inhibition ratio method. Results indicate that the shoe lining fabric containing more than 27.03 g kg^{-1} of the antimicrobial compound can form clear inhibition zones with diameters of 25-55 mm, inhibit the growth of microorganism after inoculated on plate culture mediums and achieve more than 90% inhibition ratio. So, the new complex is a potential compound as a leather antimicrobial agent.

Resumen

Las manufacturas de cuero, especialmente calzado de cuero, son estropeadas a menudo por microorganismos y por lo tanto el comportamiento antimicrobiano de las manufacturas tiene una exigencia creciente. Sin embargo, los fungicidas de cuero habituales no pueden resolver los requisitos como agentes antimicrobianos usados en las manufacturas de cuero; será significante desarrollar un compuesto antimicrobiano eficaz, inofensivo y de amplio espectro para las manufacturas de cuero. En este estudio, un nuevo agente quelante, 4-[(benzotiazol-2-imino) –metil-fenoxiacético (HL), fue preparado a partir de 2-aminobenzotiazol y el ácido 4-formylfenoxiacético en alcohol etílico absoluto y el correspondiente complejo de cobre se obtuvo disolviendo HL en una solución de acetato de cobre (II) en etanol a 65°. Las estructuras químicas del ligante y del complejo de cobre fueron caracterizadas por análisis elemental, UV, IR, y ^{1}H NMR. Los resultados demuestran que el enlace por coordinación del ión de cobre con los dos átomos de oxígeno del oxil fenol y el carboxilo en el ligante, forman dos anillos pentagonales estables. La actividad antimicrobiana del complejo contra especies representativas de bacterias y de hongos aislados en los calzados de cuero fue realizada por la determinación de las concentraciones inhibitorias mínimas (MICs). Los resultados demuestran que MICs del complejo de cobre era 100-200 mg L^{-1} a las bacterias, 1-50 mg L^{-1} a los hongos y 1 mg L^{-1} a las levaduras, respectivamente. El complejo del cobre con la concentración de 0.5-7.5 g L^{-1} en acetona fue aplicado al forro del calzado, y los efectos inhibitorios del forro tratado del calzado fueron evaluados por método de la zona de la inhibición, método de cultivo en placa y método de la tasa de inhibición. Los resultados indican que la tela del forro del calzado conteniendo más de 27.03 g kg^{-1} del compuesto in vitro antimicrobial activities of the copper complex were evaluated with serial dilution method by using by beef extract and peptone medium for bacteria, Czapek-Dox medium for moulds and Potato medium (PDA) for yeasts. The test concentrations of the compound, which was dissolved in dimethyl sulfoxide, were ranged in 1-1000 mg L^{-1}. The MICs were defined as the

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conteniendo más de 27.03 g kg^{-1} del compuesto antimicrobiano puede formar zonas definidas de inhibición con diámetros de 25-55 milímetros, para inhibir el crecimiento de los microorganismos después de que estén inoculados por medio de cultivo de placa y alcanzan una tasa de inhibición mayor al 90%. De esta manera, el nuevo complejo es un compuesto potencial como agente antimicrobiano del cuero.

Introduction

Hides and leathers can be damaged by bacteria, which are mainly responsible for the decomposition of untanned proteins (in raw hides and during soaking), and fungi, which thrive on leather containing carbohydrates, fats and proteins.[1] Generally, there are two types of antimicrobial agents - preservative and fungicide - needed to prevent microbial biodeterioration of hides and leathers. The former is used to inhibit the growth of bacteria in raw hides while the latter is often added in tanning or fat-liquoring process to protect wet blue or crust leather. However, little attention was paid to the microbial growth on leather goods in the past few decades.[2-4] Bacterial and fungal growth is not only a serious problem for leather goods such as shoes, garments and gloves, but also for human organisms due to their close contact with human bodies. Statistical data indicates that 20-50% of population in the Western Europe suffers from foot mycelial (mycotic) diseases which are caused by fungal microorganisms arising as a result of abnormal foot sweating, uncared hygiene, human immune system disorders, airtight footwear and so on.[5] So, it is necessary to develop an antimicrobial agent which can inhibit the microorganism on the leather goods, especially leather shoes. Because current fungicides used in the leather industry can not meet the requirement to the antimicrobial agents used in the leather goods[6-8], the main subject of our interest is the development of new antimicrobial compounds and especially compounds which have the dual behavior of being a bactericide and fungicide.

In general, benzothiazole and its derivatives have good antifungal activity while phenoxyacetic acid compounds are conventional bactericides.[9,10] Considering this, a novel antimicrobial compound with these two chemical backbones was synthesized by a condensation reaction of 2-amino benzothiazole and 4-formyl phenoxyacetic acid. And to improve its antimicrobial activity, the compound as a ligand reacted with copper ion to produce a new antimicrobial complex. Then, the antimicrobial activity of the complex against representative species of bacteria and fungi isolated from leather shoes was assessed by the determination of minimal inhibitory concentrations (MICs) and its application characteristic in the leather shoe lining material was also studied.

Experimental

Synthesis

Materials and Instruments

4-formyl phenoxyacetic acid was synthesized by this laboratory.[11] Other reagents were research grade. Elemental analyses (C, H, N) were performed on a Carlo-Erba 1106 analyser and copper ion content was determined at a ICP-AES. Infrared spectra were obtained on a FT-IR spectrophotometer (MAGNA.IR506, Nicolet Ltd., USA) by using KBr disk in the range 4000-400cm^{-1}. ^{1}H NMR spectra were recorded on a Bruker Avance 600 instrument in deuterated dimethyl sulfoxide solutions. Chemical shifts are reported as δ (ppm) relative to TMS as internal standard. The electronic spectra in the 200-600 nm range were obtained in absolute ethyl alcohol solutions on a Perkin Elmer Lambda 25 UV-vis spectrophotometer.

Preparations

Preparation of 4-[(2-benzothiazolylimino) methyl] phenoxyacetic acid

A solution of 2-amino benzothiazole (3.0 g, 20 mmol) and 4-formyl phenoxyacetic acid (3.6 g, 20 mmol) in anhydrous ethanol (50 mL) was refluxed at 85°C for 5 h. The mixture kept overnight at room temperature gave a precipitate which was removed by filtration and purified by recrystallization from anhydrous ethanol to give 4-[(2-benzothiazolylimino) methyl] phenoxyacetic acid as yellowish green solid (4.12 g, 66.0%). *Anal.* Calc. for $C_{16}H_{12}N_2SO_3$: C, 61.53; H, 3.87; N, 8.97%. Found: C, 61.25; H, 3.82; N, 8.34%. IR (KBr, cm^{-1}): υ (OH), 3299.46; υ (CH_2), 2920.33, 2869.78; υ (C=O, -COOH), 1715.74; υ (C=N), 1639.08, 1607.05; υ (C=C, Ar), 1568.57, 1513.93, 1483.77, 1457.75; υ (Ar-O-C), 1163.78; δ (Ar-H, ortho), 750.27; δ (Ar-H, para), 846.74. ^{1}H NMR (DMSO-d_6, ppm) δ: 9.8969 (s, 1H, N=CH-), 7.8477, 7.8624 (d, 2H, Ar-H), 7.5721, 7.5589 (d, 1H, Ar-H), 7.4988, 7.4854 (d, 1H, Ar-H), 7.3646, 7.3509, 7.3389 (t, 1H, Ar-H), 7.2040, 7.1907, 7.1784 (t, 1H, Ar-H), 7.0821, 7.0675 (d, 2H, Ar-H), 4.7357 (s, 2H, -OCH_2-).

Preparation of copper complex

To a stirred solution of the ligand, 4-[(2-benzothiazolylimino)methyl] phenoxyacetic acetic acid (0.624 g, 2 mmoL) in anhydrous ethanol (40 mL) was slowly added copper acetate solution (0.1997 g, 1 mmoL in 20 mL anhydrous ethanol) within 30 min at 65°C. The solution was stirred at 65°C for 2 h and a green powered solid was obtained after cooling. Then the solid was filtrated, washed with ethanol and dried under vacuum. *Anal.* Calc. for $CuC_{32}H_{28}N_4S_2O_8$: C, 53.21; H, 3.63; N, 7.76; Cu, 8.80%. Found: C, 52.97; H, 3.79; N, 7.60; Cu, 8.85%. IR (KBr, cm^{-1}): υ (OH), 3315.09; υ (CH_2), 2929.83; υ (C=N), 1650.69, 1598.39; υ (C=C, Ar), 1568.71, 1512.78, 1482.74, 1456.84; υ (Ar-O-C), 1170.98; δ (Ar-H, ortho), 755.75; δ (Ar-H, para), 833.97. ^{1}H NMR (DMSO-d_6, ppm) δ: 9.8372 (s, 1H, N=CH-), 7.8156, 7.8010 (d, 2H, Ar-H), 7.6490, 7.6362 (d, 1H, Ar-H), 7.4817 (br, 2H, H_2O), 7.3447, 7.3315 (d, 1H, Ar-H), 7.2135, 7.2015, 7.1894 (t, 1H, Ar-H), 7.0389, 7.0243 (d, 2H, Ar-H), 7.0196, 7.0057, 6.9929 (t, 1H, Ar-H), 4.5935 (s, 2H, -OCH_2-).

Antimicrobial Activity

The tested bacterial stains *Escherichia coli, Staphylococcus aureus and fungal stains Aspergillus niger, Aspergillus flavus, Penicillium citrinum, Mucor racemosus, Candida albicans, Candida parapsilosis,* were isolated from leather shoes. The

lowest concentrations that produce no visible microbial growth after the incubation time.

Application

Antimicrobial treatment of shoe lining material

The copper complex was dissolved in 100mL acetone to obtain solutions for treatment of shoe lining material. The concentrations were 7.5, 5.0, 2.5, 1.0 and 0.5 g L^{-1}, respectively. The common polyester fiber was used as the shoe lining material, which was cut into discs of 24.00mm in diameter. 25 sample discs were soaked in acetone for 1h, air dried and weighed. Then, these discs were soaked in the copper complex solution for 2h, removed to dry and subsequently weighed. According to the weight and area of discs, the distribution characteristic of the copper complex in the shoe lining was obtained.

Antimicrobial test of the treated sample

Inhibition zone method, plate culture medium method and inhibition ratio method were used to test the inhibitory effects of the treated sample discs against bacteria and fungi as 2.2. The inhibition zone method and plate culture medium were previously described. [7] In the inhibition ratio method, 2g the treated shoe lining material (10x10mm) and 0.2mL mixed seeded solution were added to a triangular flask containing 20mL sterilized physiological saline solution. Then, the triangular flask was shaken for 2h at 200r/min in a water-bath oscillator. The colony forming units (cfu) of the remained solution before and after oscillation were determined by plating technique. The inhibition ratio (*IR*) of the treated shoe lining was calculated using the following formula:

$$IR = \frac{C_0 - C_t}{C_0} \times 100\% \qquad (1)$$

where C_0 and C_t are the colony forming units of the solution before and after oscillation, respectively.

RESULTS AND DISCUSSION

Hides and leathers can be damaged by bacteria, which are mainly responsible for the decomposition of untanned proteins (in raw hides and during soaking)

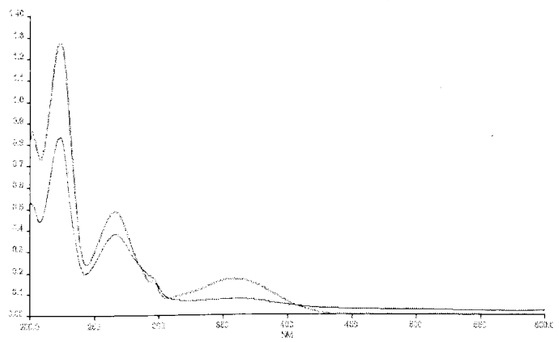

Figure 1: UV-vis absorption spectra of ligand and its copper complex (a – ligand; b – copper complex)

Structure

The amino group of 2-amino benzothiazole reacted with the aldehyde group of 4-formyl phenoxyacetic acid to produce 4-[(2-benzothiazolylimino) methyl] phenoxyacetic acid, which can be indicated by IR and ^{1}H NMR experiments.

For the copper complex, there is no coordination between the copper ion and the nitrogen atom of -N=CH group, which was suggested by the similar ^{1}H NMR and UV-vis absorption spectrum (Fig.1) of 4-[(2-benzothiazolylimino) methyl] phenoxyacetic acid, the ligand, and the copper complex. The molecular structure of the copper complex can be attained as Fig. 2, where two stable pentagonal rings are formed as a consequence of the coordination of copper ion to two oxygen atoms of phenoxyl and carboxyl.

Antimicrobial activity

It can be seen from Table 1 that different antimicrobial effects were observed against different microorganism stains, but the copper complex still showed good antibacterial and antifungal activity. The MICs of the copper complex were 100-200 mg L^{-1} to bacteria, 1-50 mg L^{-1} to moulds and 1 mg L^{-1} to yeasts, respectively.

Application

The absorption characteristic of copper complex for shoe lining fabric was listed in Table 2. The results of inhibitory effects of the treated sample discs against bacteria and fungi are given in Table 3, 4 and 5.

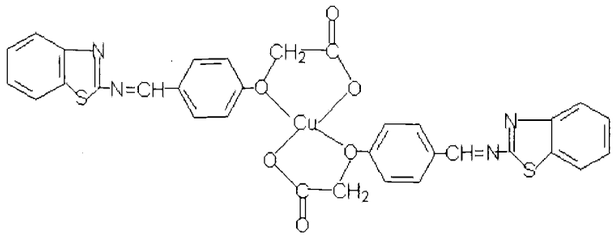

Figure 2: Proposed structure of the copper complex

Table 1 MICs of the copper complex

Species	MIC (mg L^{-1})	Species	MIC (mg L^{-1})
Escherichia coli	200	*Penicillium citrinum*	10
Staphylococcus aureus	100	*Mucor racemosus*	1
Aspergillus niger	50	*Candida albicans*	1
Aspergillus flaves	25	*Candida parapsilosis*	1

Results of three evaluation methods show that the treated shoe lining has better inhibitory effects against fungi, especially yeasts, than bacteria. As shown in Table 3, the minimum concentrations of the copper complex to form clear inhibition zones are 2.5 g L^{-1} for bacteria, 0.5-2.5 g L^{-1} for moulds and 0.5 g L^{-1} for yeasts, respectively. In the severe plate culture medium method, when the concentration of the copper complex is up to 5.0 g L^{-1}, namely, its content in the lining fabric is 27.03 g kg^{-1} or 2.79 g m^{-2}, the treated lining material can inhibit entirely the growth of bacteria and moulds in the 7 days period of incubation. Also, 2.5 g L^{-1} of the copper complex solution is required for the inhibition of yeasts.

When inhibition ratio is more than 99.0%, it can be considered that the sample have excellent antimicrobial activity. At the concentration of 5.0 g L^{-1}, the inhibition ratios of the shoe lining against mixed moulds and yeasts reach 99.7% and 99.9%, respectively. For mixed bacteria, the inhibition ratio is 99.9% when the concentration of the copper complex reaches 7.5 g L^{-1}.

Table 2 Absorption of copper complex for shoe lining

Concentrations (g L^{-1})	7.5	5.0	2.5	1.0	0.5
Absorption per disc (mg)	1.54	1.26	0.56	0.23	0.09
Distribution in weight (g kg^{-1})	33.41	27.03	12.14	5.03	2.02
Distribution in area (g m^{-2})	3.40	2.79	1.23	0.51	0.20

Table 5 Inhibition ratio of the shoe lining (%)

Species	Concentrations (g L^{-1})		
	7.5	5.0	2.5
Bacteria	99.9	91.6	84.4
Moulds	99.9	99.7	92.5
Yeasts	99.9	99.9	95.7

CONCLUSIONS

The novel ligand, 4-[(2-benzothiazolylimino) methyl] phenoxyacetic acid and its copper complex have not been previously reported. In the structure of the copper complex = shown in Fig.2, two stable pentagonal rings are formed as a consequence of the coordination of copper ion to two oxygen atoms of phenoxyl and carboxyl. The copper complex as a potential antimicrobial compound for shoe lining fabric exhibits strong antibacterial and antifungal activity. The shoe lining fabric containing more than 27.03 g kg^{-1} of the antimicrobial compound can form clear inhibition zones with diameters of 25- 55 mm, inhibit the growth of microorganism after inoculated on plate culture mediums and achieve more than 90% inhibition ratio.

Table 3 Diameters of inhibition zones for the shoe lining discs (mm)

Concentrations (g L^{-1})	*Escherichia coli*	*Staphylococcus aureus*	*Aspergillus niger*	*Aspergillus flavus*	*Penicillium citrinum*	*Mucor racemosus*	*Candida albicans*	*Candida parapsilosis*
7.5	27.62	35.14	32.98	34.13	42.02	52.20	55.43	54.26
5.0	26.17	29.14	24.45	30.71	31.17	33.83	46.33	48.17
2.5	24.00	25.34	24.00	26.09	27.61	29.50	41.04	42.82
1.0	×	×	×	24.00	24.65	28.35	37.52	38.14
0.5	×	×	×	×	24.00	26.08	30.12	30.14
0	×	×	×	×	×	×	×	×

Note: (1) All the values are given as the mean values of two measured values. (2) × - indicates serious microbial growth on sample discs.

Table 4 Antimicrobial activity of the treated shoe lining fabric by plate culture medium method

days	Bacteria						Moulds						Yeasts					
	7.5	5.0	2.5	1.0	0.5	0	7.5	5.0	2.5	1.0	0.5	0	7.5	5.0	2.5	1.0	0.5	0
1	–	–	–	–	×	×	–	–	–	–	–	–	–	–	–	–	–	×
2	–	–	–	×			–	–	–	–	–	×	–	–	–	–	–	
3	–	–	–				–	–	–	–	×		–	–	–	–	×	
4	–	–	×				–	–	–	×			–	–	–	–		
5	–	–					–	–	–				–	–	–	×		
6	–	–					–	–	×				–	–	–			
7	–	–					–	–					–	–	–			

Note: (1) — - indicates no growth of microorganism on sample discs. (2) × - indicates microbial growth on sample discs.

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