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Synthesis, characterization and the antioxidative activity of copper(II), zinc(II) and nickel(II) complexes with naringenin

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Abstract

New complexes of $Cu^{(II)}$, $Zn^{(II)}$ and $Ni^{(II)}$ with naringenin have been synthesized and characterized on the basis of elemental analyses, molar conductivities, ^{1}H-n.m.r., i.r. spectra, u.v. spectra, thermal analyses, and fluorescence spectra. In addition, the suppression ratio for $O_2^{-}\cdot$ (a) and $OH\cdot$ (b) of the complexes were studied by spectrophotometric methods. The results show that the effect of the $Cu^{(II)}$-complex IC_{50} (a) = 0.003 μM, IC_{50} (b) = 0.06 μM is the most remarkable, and the average scavenger ability of the complexes (IC_{50} = 0.06–2.67 μM) against $OH\cdot$ is higher than that of the ligand (IC_{50} = 28.5 μM). Taken together, these results indicate that the scavenger effect can be enhanced by the formation of metal-ligand coordination complexes, and the transition-metal ions may have differential and selective roles.

Introduction

Because activated oxygen and free radicals often play a role in exacerbating cellular injury and the ageing process, the study on the relief factor has attracted much uninterrupted attention over the past 20 years [1]. Particularly, an excess of activated oxygen species in the forms of superoxide anion ($O_2^{-}\cdot$) and hydroxyl radical ($OH\cdot$), generated by normal metabolic processes, may cause various diseases such as carcinogenesis, drug-associated toxicity, inflammation, atherogenesis, and aging in aerobic organisms [2–4]. Although the naturally occurring antioxidants can scavenge free radicals in the body, many naturally occurring antioxidants have been confined by their low effectiveness even though they are considered to be active in eliminating reactive oxygen and controlling toxic effects. These economic, health, and environmental concerns have highlighted the need to develop new types of antioxidants. The potential value of antioxidants has prompted investigators to search for the cooperative effect of metal complexes with natural compounds for improving antioxidant activity and cytotoxicity [5].

Flavonoids are a group of low cytotoxicity polyphenols existing widely in fruits and vegetables. The natural compounds are potential antibacterial, anticancer, antioxidant, antiinflammatory, and antiallergic agents since they stimulate or inhibit a wide variety of enzyme systems as pharmacological agents [6]. The best-known flavonoids, such as quercetin, morin and rutin, have been thoroughly studied in references [7–8], but naringenin is one of the lesser-known flavonoids except for being used as antioxidant, antibacterial and anticancer studied as agents [9]. Until now the biological activity of its complexes has not been reported. This aroused our interest in synthesis of its complexes in view of evaluating the biological activities (scavenger effects on $OH\cdot$ and $O_2^{-}\cdot$).

Experimental

Materials

Nitroblue tetrazolium (NBT), methionine (MET), and vitamin B_2 ($VitB_2$) were purchased from Sigma Chemical Co. Trichloroacetic acid (TCA), safranin, EDTA, naringenin, $M(OAc)_2 \cdot nH_2O$ [M = Cu(II), $n = 1$; M = Zn(II), $n = 2$; M = Ni(II), $n = 4$] were produced in China. All chemicals used were of analytical grade. EDTA–Fe(II) and KH_2PO_4–K_2HPO_4 buffers were prepared with deionized water.

Physical measurements

Carbon and hydrogen were analyzed on an Elemental Vario EL analyzer. The metal contents of the complexes were determined by titration with EDTA. The i.r. spectra were obtained in KBr discs on a Thermo Mattson FTIR spectrometer in the 4000–400 cm^{-1} region. ^{1}H-n.m.r. spectra were recorded on a Varian VR 300-MHz spectrometer in DMSO-d_6 with TMS as an internal standard. The u.v. spectra of the compounds (DMSO solution) were recorded on a Varian Cary 100

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Conc spectrophotometer. The fluorescence spectra were measured on a Hitachi RF-4500 spectrofluorophotometer. The molar conductance values were performed in DMSO with a DDS-11A conductometer at 25 °C. The thermal behavior was monitored on a PCT-2 differential thermal analyzer. The antioxidative activity was performed in DMSO with a 72 spectrophotometer.

Preparation of the complexes

Cu(II) complex

Naringenin (1.0 mmol, 0.272 g) was dissolved in MeOH (4 cm^3). Then, a solution of $Cu(OAc)_2 \cdot H_2O$ (1.0 mmol, 0.200 g) in MeOH (25 cm^3) was added dropwise to the ligand solution with stirring. Immediately, a large amount of light green precipitate appeared. After stirring for 4 h at room temperature, the light green precipitate was separated by suction filtration, purified by washing six times with MeOH, and dried for 48 h in vacuum.

Zn(II) and Ni(II) complexes

Naringenin (1.0 mmol, 0.272 g) was dissolved in absolute EtOH (6 cm^3). Then, a solution of $Zn(OAc)_2 \cdot 2H_2O$ (1.0 mmol, 0.220 g) in absolute EtOH (10 cm^3) was added dropwise and the mixture solution was refluxed on a oil-bath for 24 h at 60 °C with stirring. After cooling to room temperature, a light yellow precipitate separated. It was purified by washing six times with EtOH, and dried for 48 h in vacuum. The Ni(II) complex was precipitated by adding ethyl acetate to the reaction system.

^{1}H-n.m.r. spectra

The ^{1}H-n.m.r. spectra of the ligand (naringenin) and its Zn(II) complex are assigned as follows: H_3L ($(CD_3)_2CO$), δ(ppm):2.72 (1H, dd, $J=12.3$, 17.0 Hz, 3(a)-H), 3.06 (1H, dd, $J=3.0$, 17.0 Hz, 3(e)-H), 5.28 (1H, dd, $J=3.0$, 12.3 Hz, 2(a)-H), 5.98 (1H, d, $J=4.8$ Hz, 6-H), 5.99 (1H, d, J = 4.8 Hz, 8-H), 6.87 (2H, d, $J=8.4$ Hz, 3′, 5′-H), 7.23 (2H, d, $J=8.4$ Hz, 2′, 6′-H), 9.04 (1H, s, 4′-OH), 9.50 (1H, s, 7-OH), 10.52 (1H, s, 5-OH), $[Zn(H_2L)_2 \cdot 2H_2O] \cdot H_2O$ (DMSO-d_6), 2.62 (2H, d, $J=9.0$ Hz, 3(a)-H), 3.34 (4H, Broad peak, H_2O), 3.60 (2H, d, $J=9.0$ Hz, 3(e)-H), 5.33 (2H, Broad peak, 2(a)-H), 5.47 (2H, $J=9.6$ Hz, 6-H), 5.54 (2H, d, $J=9.6$ Hz, 8-H), 6.77 (4H, d, $J=8$ Hz, 3′, 5′-H), 7.29 (4H, d, $J=8$ Hz, 2′, 6′-H), 9.56 (2H, s, 4-OH), 9.86 (2H, s, 7-OH). In the complex, hydrogen of the –OH–5 (A) group is replaced by metal. All of these observations prove that the complex has formed.

Scavenger measurements of $O_2^{-}\cdot$

The superoxide radicals ($O_2^{-}\cdot$) were produced by the MET/VitB$_2$/NBT system [10].The amount of $O_2^{-}\cdot$ and suppression ratio for $O_2^{-}\cdot$ can be calculated by measuring the absorbance at 560 nm, because NBT can be reduced quantitatively to blue formazan by $O_2^{-}\cdot$. The solution of MET, VitB$_2$, and NBT were prepared with 0.067 M phosphate buffer (pH = 7.8) at avoiding light. The tested compounds were dissolved in DMSO. The 10 cm^3 reaction mixture contained MET (0.01 M), NBT (4.6×10^{-5} M), VitB$_2$ (3.3×10^{-6} M), and the tested compound (the final concentration: $C_{i(i=1\text{–}4)} = 0.16$, 1.6, 16, 160 μM). After illuminating with a fluorescent lamp at 30 °C for 10 min, the absorbances (A_i) of the samples were measured at 560 nm. The sample without the tested compound was used as the control and its absorbance was A_0. The suppression ratio for $O_2^{-}\cdot$ was calculated from the following expression:

$$\text{Suppression ratio} = 100\frac{A_0 - A_i}{A_0} \qquad (1)$$

where A_i = the absorbance in the presence of the ligand or its complexes, A_0 = the absorbance in the absence of the ligand or its complexes.

Hydroxy radical scavenging activity

The hydroxyl radical in aqueous media was generated through the Fenton reaction [11]. The solution of the tested compound was prepared with DMSO. The sample contained 1 cm^3 of 0.15 M phosphate buffer (pH = 7.4), 0.5 cm^3 of 114 μM safranin, 1 cm^3 of 945 μM EDTA–Fe(II), 1 cm^3 of 3% H_2O_2, and 1 cm^3 of the solution of the tested compound (the final concentration: $C_{i(i=1\text{–}4)} =$ 0.22, 2.2, 22, 220 μM). The sample without the tested compound was used as the control. The reaction mixtures were incubated at 37°C for 30 min in a water-bath. Absorbances (A_i, A_0) at 520 nm were measured. The suppression ratio for OH· was calculated from the following expression:

$$\text{Suppression ratio} = 100\frac{A_i}{A_0} \qquad (2)$$

where A_i = the absorbance in the presence of the ligand or its complexes, A_0 = the absorbance in the absence of the compound tested and EDTA–Fe(II).

Result and discussion

The complexes are air stable for extended periods and soluble in DMF and DMSO; slightly soluble in MeOH; insoluble in water and ether. The molar conductance values of the complexes are in the range 3.1–9.5 S cm^2 mol^{-1} in DMSO (Table 1), showing that all complexes are non-electrolytes in DMSO [12]. The elemental analyses (Table 1) show that the formula of the complexes are $[M(H_2L)_2 \cdot 2H_2O] \cdot H_2O$ (M(II) = Cu, Zn and Ni).

Table 1. Elemental analyses and molar conductivity

Compound	Yield (%)	Color	C (Calcd.) %	H (Calcd.) %	Me (Calcd.) %	Λ (S cm^2)
$[Cu(H_2L)_2 \cdot 2H_2O] \cdot H_2O$ $C_{30}H_{28}O_{13}Cu$	90.0	Green	54.7 (54.5)	4.25 (4.2)	9.7 (9.7)	3.1
$[Zn(H_2L)_2 \cdot 2H_2O] \cdot H_2O$ $C_{30}H_{28}$ O_{13} Zn	60.4	Yellow	54.4 (54.5)	4.2 (4.2)	9.8 (9.8)	5.8
$[Ni(H_2L)_2 \cdot 2H_2O] \cdot H_2O$ $C_{30}H_{28}$ $O_{13}Ni$	50.9	Green	54.8 (55.0)	4.1 (4.3)	9.1 (9.01)	9.5

Table 2. Some main i.r. data of the ligand and its complexes

Compound	νO–H	(νO–H, δOH)		νC=O	νC=C	δAr–H	νM–O
H_3L	3285m	–	–	1633s	1601s	834s	—
$[Cu(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	3382m	3223m	594w	1614s	1589s	829s	484w
$[Zn(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	3339m	3197m	592w	1609s	1588s	832s	480w
$[Ni(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	3317m	3201m	585w	1605s	1583s	835s	478w

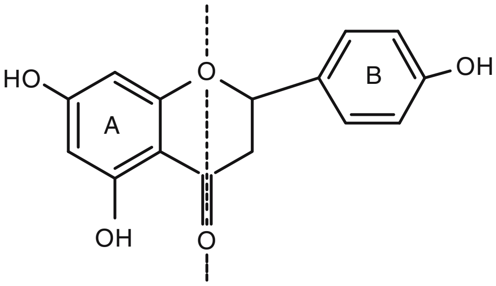


Fig. 1. The structure of naringenin.

I.r. spectra

The main stretching frequencies of the i.r. spectra of the ligand and its complexes are tabulated in Table 2. The ν(C=O) vibration of the free ligand is at 1633 cm^{-1}; for the complexes, the peaks shift to 1605–1614 cm^{-1}, $\Delta\nu_{(\text{ligand-complexes})}$ is equal to 23–28 cm^{-1}. This shift confirms that the group loses its original characteristics and forms coordinative bonds with metal [13]. The weak band at 480 cm^{-1} is assigned to ν(M–O) [14–16]. These further demonstrate that the oxygen of the carboxyl has formed coordinative bonds with the transition metal ions [16]. The aqueous ν(O–H) and δ(OH) bands of the complexes appear at 3223 and 594 cm^{-1}, respectively, showing that there is some crystal water in the complexes [17].

U.v. spectra

The u.v. spectra data of the ligand and its complexes in DMSO are listed in Table 3. The ligand has an intensive band at $\lambda_{max} = 321$ nm and a less intensive band at $\lambda_{max} = 269$ nm. Figure 1 shows the structure of the ligand. It follows from the literature that the band at 321 nm is related to the absorbance of the B ring (cinnamoyl system), whereas the band at 269 nm is related to the $\pi \rightarrow \pi^*$ transition absorbance (benzoyl system) in the A ring [18]. But in the complexes, the bands at 269 nm are shift to 218, 242, and 244 nm, respectively. These results suggest that the complexes have formed.

Table 3. U.v. data of the ligand and its complexes

Compound	(I) λ_{max} (nm)	ε×10^{-4} (L/mol cm)	(II) λ_{max} (nm)	ε×10^{-4} (L/mol cm)
H_3L	321	4.53	269	2.86
$[Cu(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	298	5.81	218	1.53
$[Zn(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	293	9.37	244	2.65
$[Ni(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	290	7.21	242	3.09

Table 4. Thermal analyses data of the compounds

Compound	D t_1 (°C)	H_2O loss Calcd. (%)	D t_2 (°C)	H_2O loss Calcd. (%)	Decomp. (°C)		Residue (%)
					t_3	t_4	
$[Cu(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	67	2.9 (2.7)	158	5.5 (5.4)	351	434	12.0 (12.1)
$[Zn(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	84	2.5 (2.7)	146	5.2 (5.4)	343	491	12.1 (12.3)
$[Ni(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	85	2.6 (2.7)	131	5.3 (5.5)	335	425	11.2 (11.5)

Table 5. Fluorescence data of the ligand and the complexes

Compound	Excitation wavelength (nm)	Emission wavelength (nm)	Relative fluorescence intensity
H_3 L	369	443	295
$[Cu(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	369	453	69
$[Zn(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	369	464	844
$[Ni(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	369	456	679

Thermal analysis

Some data of thermal analyses are listed in Table 4. The complexes have their first peaks between 67–85 °C, corresponding to 2.54–2.93%, coinciding with one crystal water molecule. In addition, the DTA curves of the $Cu^{(II)}$, $Zn^{(II)}$ and $Ni^{(II)}$ complexes have endothermic peaks at 158, 146 and 131 °C, respectively. The corresponding TG curves show that the weight losses are equal to two coordinative water molecules. The results are in accordance with the composition of the complexes as determined by elemental analyses. While being heated to 800 °C, the complexes changed into oxides. The residue is in accordance with the calculation.

Fluorescence analysis of the ligand and complexes

The fluorescence analysis shows that the ligand itself exhibits a strong fluorescence. The emission wavelength of the complexes all shifted to higher wavelength by 9.6–21 nm under the same excitation wavelength, and the fluorescence intensity of the Cu(II) complex is weaker than that of the ligand.This result indicated that Cu(II) complex has the function of decrease in the fluorescence. However, the fluorescence intensity of the $Zn^{(II)}$ and $Ni^{(II)}$ complexes are stronger than that of the ligand. These results indicated that the two complexes have the function of increase in the fluorescence. The fluorescence data are listed in Table 5.

On the basis of above evidence and analyses, the possible structures of the complexes are shown in Figure 2.

Average suppression ratio (%) for $O_2^{-}\cdot$ and $OH\cdot$

The data of the suppression ratio for $O_2^{-}\cdot$ are listed in Table 6. We find that the average suppression ratio for $O_2^{-}\cdot$ increases with the increase of the compound concentration. The $Cu^{(II)}$ complex ($IC_{50} = 0.003$ μM) is the most effective inhibitor, which is the only one more effective than the ligand ($IC_{50} = 0.262$ μM). The $Zn^{(II)}$ complex ($IC_{50} = 40.1$ μM) is the poorest one. It indicates that the transition-metal ions such as $Cu^{(II)}$, $Zn^{(II)}$ and $Ni^{(II)}$ may have differential and selective nature for scavenging $O_2^{-}\cdot$.

The comparison of the inhibitory effect on $OH\cdot$ is shown in Table 7. We can find that the average suppression ratio of the ligand ($IC_{50} = 28.5$ μM) for $OH\cdot$ is the least in all compounds, and the average suppression ratio for $OH\cdot$ increases with the increase of the compound concentration. The $Cu^{(II)}$ complex ($IC_{50} = 0.06$ μM) is the most effective in three complexes for scavenging $OH\cdot$, whereas the $Ni^{(II)}$ complex ($IC_{50} = 2.67$ μM) has the poorest inhibitory. It is clear that the scavenger effect on $OH\cdot$ can be enhanced by the formation of metal-ligand coordination complexes and the metal ions also affect the ability.

Conclusions

Taken together, complexes of $Cu^{(II)}$, $Zn^{(II)}$ and $Ni^{(II)}$ with naringenin have been prepared and characterized. The suppression ratio for $O_2^{-}\cdot$ and $OH\cdot$ of ligand and complexes were investigated by spectrophotometric methods for the first time. The results show that the effect of the $Cu^{(II)}$ complex is the most remarkable, and the average scavenger ability of the complexes

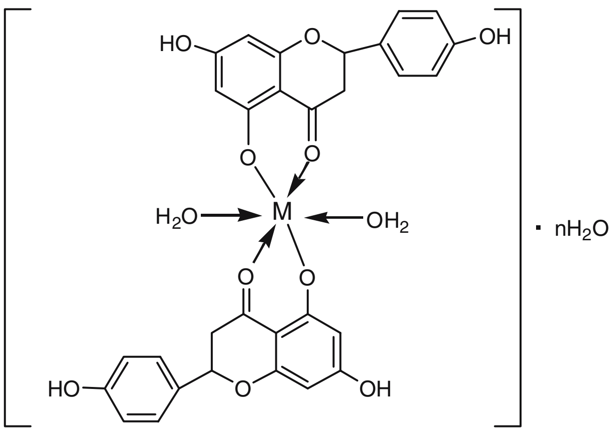
Table 6. The influence of investigated compounds for $O_2^{-}\cdot$

Compound	η_a(%)	η_a(%)	η_a(%)	η_a(%)	Equation	IC_{50}^{a} μM	R^2
H_3 L	40.4	42.8	61.9	66.7	$y = 9.80x + 114.49$	0.262	0.907
$[Cu(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	58.5	69.8	84.0	92.8	$y = 11.71x + 149.81$	0.003	0.993
$[Zn(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	25.3	38.3	44.2	56.6	$y = 9.93x + 93.66$	40.1	0.977
$[Ni(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	26.3	42.1	52.4	66.7	$y = 9.87x + 101.43$	6.16	0.979

[a] IC_{50} values were calculated from regression lines where: x was log of the tested compound concentration and y was percent inhibition of the tested compounds. When the percent inhibition of the tested compounds was 50%, the tested compound concentration was IC_{50}. R^2 = correlation coefficient.

Table 7. The influence of investigated compounds for $OH\cdot$

Compound	η_b(%)	η_b(%)	η_b(%)	η_b(%)	Equation	IC_{50}^{a} μM	R^2
H_3 L	72.6	76.8	78.0	82.6	$y = 9.15x + 91.58$	28.5	0.960
$[Cu(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	73.6	75.9	76.5	78.6	$y = 4.57x + 83.19$	0.06	0.959
$[Zn(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	72.9	74.6	76.5	78.8	$y = 5.75x + 84.54$	0.97	0.996
$[Ni(H_2L)_2 \cdot 2H_2O] \cdot H_2O$	75.3	76.5	78.8	82.5	$y = 7.01x + 89.06$	2.67	0.949



(M = Cu^{2+}, Ni^{2+}, Zn^{2+}, n = 1)

Fig. 2. The possible structure of complexes.

against OH· are higher than that of the ligand. $Cu^{(II)}$ complexes are well known to have superoxide dismutase activity and some relevant safety to life. So, information obtained from the present is helpful to the development of new potent antioxidant and new therapeutic reagent for some diseases.

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References

1. D. Harman, *J. Gerontol.*, **11**, 298 (1956).
2. B.N. Ames, M.K. Shigenaga and T.M. Hagen, *Proc. Natl. Acad. Sci. USA*, **90**, 7915 (1993).
3. B. Halliwell, J.M.C. Gutteridge and C.E. Cross, *J. Lab. Clin. Med.*, **119**, 598 (1992).
4. A.A. Horton and S. Fairhurst, *Crit. Rev. Toxicol.*, **18**, 27 (1987).
5. S.F. Lo, V. Mulabagal, C.L. Chen, C.L. Kuo and H.S. Tsay, *J. Agric. Food Chem.*, **52**, 6916 (2004).
6. F. Cheng and Breen Kevin, *Bio Metals*, **13**, 77 (2000).
7. Y.M. Song, J.W. Kang, Z.H. Wang, X.O. Lu, J.Z. Guo and L.F. Wang, *J. Inorg. Biochem.*, **91**, 470 (2002).
8. J. Zhou, L.F. Wang and N. Tang, *J. Inorg. Biochem.*, **83**, 41 (2001).
9. A.S. Ramaswamy, S. Jayaraman, M. Sirsi and K.H. Rao, *Indian J. Exp. Biol.*, **1**, 72 (1972).
10. C.C. Winterbourn, *Biochem. J.*, **182**, 625 (1979).
11. C.C. Winterbourn, *Biochem. J.*, **198**, 125 (1981).
12. W.J. Geary, *Coord. Chem. Rev.*, **7**, 81 (1971).
13. Y.B. Zeng, N. Yang, W.S. Liu and N. Tang, *J. Inorg. Biochem.*, **97**, 262 (2003).
14. Z.Y. Yang, R.D. Yang, Q. Li and F.S. Li, *Synth. React. Inorg. Met-Org. Chem.*, **29**, 205 (1999).
15. Z.Y. Yang, *Synth. React. Inorg. Met-Org. Chem.*, **32**, 903 (2002).
16. F. Marchetti, C. Pettinari, R. Pettinari, D. Leonesi and A. Lorenzotti, *Polyhydron*, **18**, 3041 (1999).
17. Z.Y. Yang, R.D. Yang, F.S. Li and K.B. Yu, *Polyhydron*, **19**, 2599 (2000).
18. Q.X. Zhen, B.H. Ye, Q.L. Zhang, J.G. Liu, H. Li, L.N. Ji and L. Wang, *J. Inorg. Biochem.*, **76**, 47 (1999).