Scientific paper

N’-(2-Hydroxybenzylidene)-3-Methylbenzohydrazide and its Copper(II) Complex: Syntheses, Characterization, Crystal Structures and Biological Activity

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Abstract

The hydrazone compound N’-(2-hydroxybenzylidene)-3-methylbenzohydrazide (H₂L) was prepared. With H₂L and copper acetate a new copper complex [Cu(HL)(NCS)]·CH₃OH was synthesized. Both the hydrazone and the copper complex were characterized by physico-chemical methods and single crystal X-ray diffraction techniques. The complex is a thiocyanato-coordinated copper(II) species. The Cu atom in the complex is in square planar geometry. The complex is a promising urease inhibitor.

Keywords: Hydrazone; copper complex; crystal structure; biological activity

1. Introduction

In recent years, much efforts have been focused on Schiff bases because they have a wide range of biological activities such as antibacterial,¹ antitumor,² anti-inflammatory³ and cytotoxic⁴ etc. Some chloro, fluoro, iodo, and bromo-substituted compounds have remarkable antimicrobial activities.⁵ Some hydrazones have strong urease inhibitory activities.⁶ In addition, hydrazones are a kind of versatile ligands during the coordination with metal ions.⁷ Vanadium complexes derived from hydrazides show interesting urease inhibitory activities.⁸ You and coworkers have found that some Schiff base complexes are effective urease inhibitors,⁹ and some hydrazones have various biological properties.¹⁰ In pursuit of new urease inhibitors, in this work, a new copper(II) complex, [Cu(HL)(NCS)]·CH₃OH, derived from N’-(2-hydroxybenzylidene)-3-methylbenzohydrazide (H₂L, Scheme 1), was presented.

2. Experimental

2.1. Materials and Methods

Salicylaldehyde and 3-methylbenzohydrazide were purchased from Sigma-Aldrich Co. Ltd, and were used as received. Other reagents were obtained from commercial suppliers with AR grade. Elemental analyses for C, H and N were performed on a Perkin-Elmer 240C elemental analyzer. IR spectra were recorded on a Jasco FT/IR-4000 spectrometer as KBr pellets in the 4000–400 cm⁻¹ region. UV-Vis spectra were recorded on a Lambda 35 spectrometer. ¹H NMR spectrum for the hydrazone was recorded...
on a Bruker 300 MHz spectrometer. Single crystal X-ray diffraction was carried out on a Bruker SMART 1000 CCD diffractometer.

2. 2. Synthesis of H$_2$L

Salicylaldehyde (1.22 g, 0.01 mol) and 3-methylbenzohydrazide (1.50 g, 0.01 mol) were dissolved in methanol (70 mL). The mixture was heated under reflux for 30 min, and the solvent removed under reduced pressure. The solid was re-crystallized from methanol to give colorless single crystals. Yield 2.12 g (83%). Anal. Calc. for C$_{15}$H$_{14}$N$_2$O$_2$: C, 70.8; H, 5.5; N, 11.0. Found: C, 71.0; H, 5.6; N, 10.9%. IR data (cm$^{-1}$): 3370 ν(NH), 2027 ν(NCS), 1648 ν(C=O), 1610 ν(C=N), 1234, 1208, 1139, 1040, 901, 786, 752, 523, 507. UV–Vis data (methanol, λ/nm (ε/L cm$^{-1}$)): 205 (18,320), 235 (22,100), 286 (15,516), 300 (15,670), 323 (8,737), 387 (2,210). 1H NMR (300 MHz, δ (ppm)): 2.31 (s, 3H, C–H$_1$), 7.65–7.35 (m, 4H, Ar–H$_4$), 7.10 (t, 1H, Ar–H$_3$), 6.97–6.7 (1H, Ar–H$_2$), 7.82 (d, 1H, Ar–H$_5$), 7.76 (s, 1H, Ar–H$_6$), 8.72 (s, 1H, C–H$_7$). The collected data were reduced with SAINT,$^{11}$ and multiple reflections were collected at 298(2) K with MoK$\alpha$ radiation (λ = 0.71073 Å).

H$_2$L (1.0 mmol, 0.25 g), Cu(CH$_3$COO)$_2$·H$_2$O (1.0 mmol, 0.20 g) and NH$_4$NCS (1.0 mmol, 0.20 g) were dissolved in methanol. The mixture was stirred for 30 min at room temperature and filtered. The filtrate was kept in air for a few days, to form deep blue single crystals. Yield: 157 mg (39%). Anal. Calc. for C$_{17}$H$_{17}$CuN$_3$O$_3$S: C, 50.2; H, 4.2; N, 10.5. Found: C, 71.0; H, 5.6; N, 10.9%. IR data (KBr, cm$^{-1}$): 3413 ν(OH), 3257 ν(NH), 3055, 2961, 2925, 2852, 1690 ν(C=O), 1612 ν(C=N), 1534, 1457, 1437, 1282, 1324, 1208, 1139, 1040, 901, 786, 752, 523, 507. UV–Vis data (methanol, λ/nm (ε/L cm$^{-1}$)): 205 (18,320), 235 (22,100), 286 (15,516), 300 (15,670), 323 (8,737), 387 (2,210). 1H NMR (300 MHz, δ (ppm)): 12.95 (s, 1H, OH$_2$), 8.72 (s, 1H, CH=N), 7.76 (d, 1H, Ar–H$_3$), 7.65–7.35 (m, 4H, Ar–H$_4$), 7.10 (t, 1H, Ar–H$_2$), 6.97 (d, 1H, Ar–H$_1$), 2.31 (s, 3H, CH$_3$).

2. 4. X-ray Crystallography

Diffraction intensities for the compounds were collected at 298(2) K with MoKα radiation (λ = 0.71073 Å). The collected data were reduced with SAINT,$^{11}$ and multi-scan absorption correction was performed with SADABS.$^{12}$ Structures of the hydrazone and the copper complex were solved by direct methods and refined against $F^2$ by full-matrix least-squares method with SHELXTL.$^{13}$ All of the non-hydrogen atoms were refined anisotropically. The amino H atoms in H$_2$L and the complex were located from difference Fourier maps and refined isotropically. The N–H distances are restrained to 0.90(1) Å, and the remaining hydrogens were placed in calculated positions and constrained to ride on their parent atoms. Crystallographic data for the hydrazone and the copper complex are summarized in Table 1.

| Table 1. Crystal data for H$_2$L and the copper complex |
|----------------------------------------------------------|
| **H$_2$L** | **the copper complex** |
| Formula | C$_{15}$H$_{14}$N$_2$O$_2$ | C$_{17}$H$_{17}$CuN$_3$O$_3$S |
| FW | 254.28 | 406.94 |
| Space group | Pn$\bar{a}$2$_1$ | P$2_1$2$_1$2$_1$ |
| a (Å) | 22.1876(18) | 5.7269(15) |
| b (Å) | 5.0760(13) | 13.087(2) |
| c (Å) | 11.0325(19) | 23.720(2) |
| V (Å$^3$) | 1242.5(4) | 1777.8(6) |
| Z | 4 | 4 |
| I (MoKa) (Å$^{-1}$) | 0.71073 | 0.71073 |
| m (MoKa) (cm$^{-1}$) | 0.092 | 1.367 |
| Reflections/parameters | 6791/177 | 9105/232 |
| Unique reflections | 2119 | 3211 |
| Observed reflections | 1789 | 2940 |
| $R_1$ ($I > 2\sigma (I)$) | 0.0462, 0.1054 | 0.0274, 0.0649 |
| $R_1$ (all data) | 0.0578, 0.1148 | 0.0321, 0.0667 |

3. Results and Discussion

3. 1. Chemistry

The hydrazone compound N′-(2-hydroxybenzylidene)-3-methylbenzohydrazide was obtained by the reaction of 1:1 molar ratio of salicylaldehyde and 3-methylbenzohydrazide in methanol solution. The copper complex was obtained by the reaction of 1:1:1 molar ratio of H$_2$L, copper acetate and ammonium thiocyanate in methanol solution. The complex in methanol is of non-electrolytic nature, as evidenced by low molar conductivity value.$^{14}$

3. 2. Structure Description of the Hydrazone H$_2$L

The molecular structure of the hydrazone H$_2$L is shown in Fig. 1. Selected bond lengths and angles are given in Table 2. The molecule is in an E configuration about the methylidene group. The methylidene bond, with the distance of 1.278(4) Å, indicates a definitely double bond. In the –C(O)–NH– group, the C–N bond is shorter and the C=O bond is longer than usual, which is caused by the conjugation character in the hydrazone molecule. All the bond distances of the compound are within normal ranges.$^{10c}$ The two benzene rings form a dihedral angle of 28.7(5)°. In the crystal structure, the hydrazone molecules are linked via C–H···O and N–H···O hydrogen bonds (Table 3), to form two-dimensional layers along the bc plane (Fig. 2).

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Table 2. Selected bond lengths (Å) and angles (°) for H$_2$L and the copper complex

|               | H$_2$L       | Complex          |
|---------------|--------------|------------------|
| C7–N1         | 1.278(4)     | Cu1–O1 1.0996(16) |
| N2–O2         | 1.364(4)     | Cu1–O2 1.9955(17) |
| N1–N2         | 1.376(3)     | Cu1–N1 1.921(2)  |
| N2–C8         | 1.364(4)     | O1–Cu1–N3 93.00(9) |
| C8–O2         | 1.2219(3)    | O1–Cu1–O2 166.67(10) |
| N3–Cu1–O2     | 93.47(9)     | N3–Cu1–O2 80.58(8) |
| N3–Cu1–N1     | 168.67(10)   | N3–Cu1–N3 91.07(8) |

3. 3. Structure Description of the Copper Complex

The molecular structure of the copper complex is shown in Fig. 3. The asymmetric unit contains a [Cu(HL)(NCS)] complex molecule and a methanol molecule. The complex is linked to the methanol solvate molecule through N2–H2–O4 hydrogen bond (Table 3). The Cu atom is in a square planar geometry. The four donor atoms come from the phenolate O, imino N and carbonyl O atoms of the hydrazone ligand, and the thiocyanate N atom. The Cu atom deviates by 0.175(2) Å from the least squares plane defined by the donor atoms. The Cu–O bond lengths of 1.90–2.00 Å and Cu–N bond lengths of 1.92 Å are similar to the copper(II) complexes with square planar geometry. The cis and trans bond angles of the Cu atom in the basal plane are 80.58(8)–93.47(9)° and 167.02(8)–168.67(10)°, respectively. The two benzene rings of the hydrazone ligand form a dihedral angle of 6.2(5)°.

In the crystal structure, the complex molecules are linked by methanol molecules through O–H···O and N–H···O hydrogen bonds (Table 3), to form one-dimensional chains along the b axis (Fig. 4).
salts are known urease inhibitors. Copper perchlorate was treated with IC50 value of 28.1 μmol L–1. The urease inhibitory activity of the hydrazone and the copper complex is similar to the bromo- and thiocyanato-coordinated copper complexes with pyridine based hydrazone ligands, and stronger than the other copper complex with the above mentioned hydrazone ligand. In general, the copper complexes have much better activity than the complexes with other metals. Thus, the present copper complex is a promising urease inhibitor.

### Table 3. Hydrogen bond distances (Å) and bond angles (°) for H2L and the complex

| D–H⋯A | d(D–H) | d(H⋯A) | d(D–A) | Angle (D–H⋯A) |
|-------|--------|--------|--------|----------------|
| H2L   |        |        |        |                |
| O1−H1−N1 | 0.82   | 1.88   | 2.597(3) | 146(4)         |
| N2−H2−O1 | 0.90(1) | 2.16(2) | 3.032(3) | 164(4)         |
| C6−H6−O2 | 0.93   | 2.53   | 3.327(3) | 143(4)         |
| C7−H7−O2 | 0.93   | 2.38   | 3.216(3) | 150(4)         |

Complex

| O4−H4−O1 | 0.82   | 1.90   | 2.719(3) | 173(3)         |
| N2−H2−O4 | 0.90(1) | 1.80(1) | 2.690(3) | 173(3)         |

Symmetry codes: i: ½ − x, −½ + y, −½ + z; ii: ½ − x, ½ + y, −½ + z; iii: −x, −½ + y, 3/2 − z.

### Table 4. Inhibition of urease by the tested materials

| Tested materials              | Inhibition rate (%) | IC50 (μmol L–1) |
|-------------------------------|---------------------|-----------------|
| H2L                           | 8.3 ± 1.6           | > 100           |
| the copper complex             | 89.8 ± 2.7          | 2.8 ± 1.3       |
| Copper perchlorate             | 70.2 ± 3.3          | 8.5 ± 1.7       |
| Acetohydroxamic acid           | 85.5 ± 3.9          | 28.1 ± 3.6      |

* The concentration of the tested material is 100 μmol L–1.

### 3. 4. IR and UV-Vis Spectra

The weak absorption centered at 3413 cm–1 in the IR spectrum of the hydrazone is attributed to the phenol group. The sharp bands observed at 3257 and 3370 cm–1 for H2L and the complex, respectively, are due to the N–H vibrations. The intense absorptions at 1690 cm–1 for H2L and 1648 cm–1 for the copper complex are due to the carbonyl groups. The typical absorption for the azomethine groups, C=N, are located at 1612–1610 cm–1. The strong band at 2027 cm–1 for the copper complex is assigned to the NCS ligand. In the electronic spectra of H2L and the copper complex, the bands centered at 260–290 nm are assigned to the intra-ligand π–π* transition of the aromatic groups. The charge transfer LMCT band of the copper complex is located at 390 nm. The weak absorption centered at 3413 cm–1 in the IR spectrum of the hydrazone is attributed to the phenol group. The sharp bands observed at 3257 and 3370 cm–1 for H2L and the complex, respectively, are due to the N–H vibrations. The intense absorptions at 1690 cm–1 for H2L and 1648 cm–1 for the copper complex are due to the carbonyl groups. The typical absorption for the azomethine groups, C=N, are located at 1612–1610 cm–1. The strong band at 2027 cm–1 for the copper complex is assigned to the NCS ligand.

In the electronic spectra of H2L and the copper complex, the bands centered at 260–290 nm are assigned to the intra-ligand π–π* transition of the aromatic groups. The charge transfer LMCT band of the copper complex is located at 390 nm. The complex has weak d–d electronic transition centered at 640 nm, which is assigned to $^2E_g(D) \rightarrow ^2T_{2g(D)}$. The typical absorption for the azomethine groups, C=N, are located at 1612–1610 cm–1.

### 3. 5. Biological Activity

The assay of the urease inhibitory activity was carried out according to the literature method. The urease inhibitory activity of the hydrazone and the copper complex is given in Table 4. The hydrazone has obvious weak activity on the urease. While the copper complex has remarkable activity (IC50 = 2.8 μmol L–1). The assay of the urease inhibitory activity was carried out according to the literature method. The urease inhibitory activity of the hydrazone and the copper complex is given in Table 4. The hydrazone has obvious weak activity on the urease. While the copper complex has remarkable activity (IC50 = 2.8 μmol L–1). Inorganic copper perchlorate was treated with IC50 value of 28.1 μmol L–1. The urease inhibitory activity of the hydrazone and the copper complex is given in Table 4. The hydrazone has obvious weak activity on the urease. While the copper complex has remarkable activity (IC50 = 2.8 μmol L–1).

### 4. Conclusion

A new hydrazone $N'$-(2-hydroxybenzylidene)-3-methylbenzohydrazide was prepared and structurally characterized. With the hydrazone compound, a new copper(II) complex was synthesized and characterized. Single crystal structures of the hydrazone compound and the oxido-vanadium(V) complex were determined. The hydrazone compound coordinate to the Cu atom through the NOO donor set. The complex is a thiocyanato-coordinated copper(II) species. The Cu atom in the complex is in square planar geometry.

### 5. Supplementary Data

CCDC 1887945 for H2L, and 1445986 for the copper complex contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

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Povzetek
Sintetizirali smo hidrazon \(N^\prime\)-(2-hidroksibenziliden)-3-metilbenzohidrazid (H\(_2\)L). Z H\(_2\)L in bakrovim acetatom smo sintetizirali nov bakrov kompleks [Cu(HL)(NCS)]-CH\(_3\)OH. Hidrazon in bakrov kompleks smo okarakterizirali s fiziko-kemijskimi metodami in monokristalno rentgensko difrakcijo. Kompleks je bakrova(II) zvrst koordinirana s ti-ocianato ligandom. Cu atom ima kvadratno planarno geometrijo. Kompleks ima obetavne lastnosti kot inhibitor ureaze.