Asian Journal of Chemistry Vol. 22, No. 8 (2010), 5993-5999

Synthesis, Crystal Structure and Antiproliferative Activity of Mn(II) Complexes of Demethylcantharate

YUN-YUN WANG†, RUI-DING HU†, QIU-YUE LIN*†, YU-LING ZHAO† and NA WANG†
Zhejiang Key Laboratory for Reactive Chemistry on Solid Surfaces, Institute of Physical Chemistry, Zhejiang Normal University, Jinhua, Zhejiang 321004, P.R. China
Fax: (86)(579)82282269; Tel: (86)(579)82283353; E-mail: sky51@zjnu.cn

A complex with the molecular formula $[Mn(C_8H_8O_5)(H_2O)_2]$ formed by Mn(II) with demethylcantharate (DCA = 7-oxa-bicyclo[2.2.1]heptane-2,3-bicarboxylate, $C_8H_8O_5$) was characterized by elemental analysis, IR and single crystal X-ray diffraction. The complex was crystallized in the orthorhombic crystal system and Iba2 space group with a = 1.89914(5) nm, b = 1.03558(2) nm, c = 1.04856(2) nm; $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$, V = 2.06221(8) nm^3, Dc = 1.772 g/cm^3, Z = 8, F(000) = 1128, R = 0.0361, wR = 0.0981 [$I > 2\sigma(I)$]. The antiproliferative activity test indicates that the complex has a higher inhibitive effect on human hepatoma cells SMMC7721 ($IC_{50} = 38.0 \pm 0.9$ μmol L^{-1}). The inhibition rates of the complex are much higher than those of demethylcantharidin (NCTD).

Key Words: Demethylcantharidin, Manganese, Crystal, Antiproliferative activity.

INTRODUCTION

Cantharidin is an effective ingredient of blister beetles, which are often used as folk medicine to treat malignant tumor. Therefore, it has a great medical functional value but irritates the urine system and digestive tube[1-3]. Demethylcantharidin (NCTD) and disodium demethylcantharate (Na_2(DCA), DCA = 7-oxa-bicyclo-[2.2.1]heptane-2,3-bicarboxylate), two derivatives of cantharidin, could also inhibit the growths of cancer cells and raise the amount of leucocytes. They can remove the side effect on urinary system substantially compared to cantharidin[4]. Manganese is an essential microelement in human body. It plays an important role in the synthesis and activation of many enzymes, the metabolism of sugar and fat in human body, the acceleration of the synthesis of protein, vitamin B and C[5]. Several complexes of demethylcantharate have been synthesized which have strong anticancer, antibacterium and antiproliferative activity[6]. There is no complex of manganese of demethylcantharate reported so far. So, it is worthwhile to synthesize a new manganese complex of demethylcantharate and its single crystals were obtained. The antiproliferative testing result indicates that the complex has inhibitive activity against human hepatoma cells and lung cancer cells.

†College of Chemical and Life Science, Zhejiang Normal University, Jinhua, Zhejiang 321004, P.R. China.

EXPERIMENTAL

All the chemicals were obtained commercially and used without further purification. NCTD was purchased in Nanjing Zelang Medical Technological Co. Ltd, manganese chloride (AR, $MnCl_2 \cdot 4H_2O$) was obtained from Beijing Double Circles Chemical Reagent Co. and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma corporation. The other reagents were all of analytical grade. Double distilled water was used in the experiments. Human hepatoma cells SMMC7721 and human lung cancer cells A549 were obtained from Shanghai Cell Bank of Chinese Academy of Sciences.

Infrared spectra were recorded as KBr pellet by using a NEXUS-670 FT-IR spectrometer. Diffraction intensities for the complex were collected at 296K on a Bruker SMART APEX II CCD diffractometer. Elemental analyses of C and H were carried out in a Vario EL III elemental analyzer. DG3022A ELISA instrument was used to perform antiproliferative activity.

Synthesis of the complex: A mixture of NCTD (1 mmol), $MnCl_2 \cdot 4H_2O$ (1 mmol) and distilled water (15 mL) was sealed in a Teflon-lined stainless vessel (25 mL) and heated at 443 K for 3 d, then cooled slowly to room temperature. The solution was filtered and colourless crystals were obtained. Anal. calcd. for $C_8H_{12}MnO_7$ (%): C, 34.80; H, 4.33. Found (%): C, 34.93; H, 4.40.

X-ray crystallography of the complex: The single crystal of the complex with appropriate dimension of 0.327 mm × 0.173 mm × 0.091 mm was placed on Bruker SMART APEXII X-ray diffractometer. Intensity data were collected with a graphite monochromated MoK_α radiation ($\lambda = 0.071073$ nm) using the ω scan technique in the range of $2.14° \leq \theta \leq 27.46°$ at 296(2) K. A total of 11532 reflections were collected, of which 2355 reflections ($R_{int} = 0.0241$) were independent and 2255 were observed with $I > 2\sigma(I)$. The structure was solved by direct methods and the position of the rest non-hydrogen atoms was determined by successive Fourier syntheses. Hydrogen atoms of water were located by Fourier methods and the rest hydrogen atoms were positioned hydrogenation geometrically. The position and anisotropic parameters of all non-hydrogen atoms were refined on F^2 by full-matrix least-squares method by using the SHELXL-97 program package. Crystal data and structure refinement for the complex $[Mn(C_8H_8O_5)(H_2O)_2]$ are listed in Table-1.

Antiproliferative activity[7]: The antiproliferative activity of the complex and NCTD was evaluated by using human hepatoma cells SMMC7721 and human lung cancer cells A549. The antiproliferative activity was measured by the MTT assay. Growth cells in the exponential phase were assayed by adding 100 μL stock solution directly to culture wells. After the cells were seeded for 24 h, the complexes and NCTD were added. Then the cells were incubated for 72 h, followed by adding 100 μL MTT (1 mg/mL, dissolved in DMEM nutrient solution) into each well. Later, the liquid in each well was abandoned and then 150 μL acidifying isopropanol (containing 0.04 mol/L HCl) was added. The mixture was placed in a dark area for 0.5 h. The inhibition rate and IC_{50} were calculated.

TABLE-1
CRYSTAL DATA OF THE Mn COMPLEX OF DCA

Empirical formula	$Mn(C_8H_8O_5)(H_2O)_2]$	Formula weight	275.12
Temperature (K)	296(2)	Crystal system	Orthorhombic
Space group	Iba2		
a (Å)	18.9914(5)	α (º)	90
b (Å)	10.3558(2)	β (º)	90
c (Å)	10.4856(2)	γ (º)	90
Volume $(Å)^3$	2062.21(8)	Z	8
Dcalc (g/cm^3)	1.772	Absorption coefficient (mm^{-1})	1.298
F(000)	1128	θ Range (º)	2.14 to 27.46
h, k, l ranges	-24 22, -11 13, -13 13	Reflections collected/unique	11532 / 2355 [R(int) = 0.0241]
Data/restraints/parameters	2355 / 7 / 157	Goodness-of-fit on F^2	1.037
R indices [I>2σ(I)]	R1 = 0.0361, wR2 = 0.0981	R indices (all data)	R1 = 0.0377, wR2 = 0.0994
Largest ΔF peak and hole $(e/Å^3)$	0.324 and -0.339		

$R = \Sigma|F_0|$, $wR = [\Sigma[w(F_O^2-F_C^2)^2]/\Sigma w(F_O^2)^2]]^{1/2}$.

RESULTS AND DISCUSSION

IR spectra of the complex: For the complex, the broad band of 3400 cm^{-1} is assigned to $\nu(H_2O)$. The band of $\nu_{as}(-COO^-)$ shifts to 1679, 1564 cm^{-1} and $\nu_s(-COO^-)$ to 1440, 1375 cm^{-1}. One value of $\Delta[\nu_{as}(COO-)-\nu_s(COO^-)]$ is 239 cm^{-1} and the other is 189 cm^{-1}, indicating that there are two coordinated carboxylic groups in the complex. One is coordinated to manganese ion by single oxygen ($\Delta\nu > 200$ cm^{-1}), the other by bridging double oxygens ($\Delta\nu$ =140-200 cm^{-1})[8,9]. The shifts of ν(C-O-C) and the cyclic skeleton structure vibration bands appear at 1258, 987, 1057 cm^{-1} in the complex, indicating that the cyclic ether oxygen atom is also coordinated to manganese ion.

Structure description of the complex: Mn(II) complex with demethylcantharate $[Mn(DCA)(H_2O)_2]$ is isostructural with the Cu(II)[10] and Ni(II)[11] analogues. Fig. 1 is the molecule structure of the complex and Fig. 2 is the crystal packing diagram. It is clear from Fig. 1 that each Mn(II) ion is six-coordinated by two oxygen atoms (O1W,O2W) from water, one bridge oxygen (O5), two different carboxy-late oxygen atoms (O2,O4) in two different carboxylate groups and one carboxylate oxygen atom (O1A) in another asymmetric unit.

The main bond lengths and bond angles are listed in Table-2 and hydrogen bonds are listed in Table-3. O2, O5, O1W and O2W lie in the equatorial plane with the torsion angle -4.243(83)°. Carboxylate oxygen atom O1 and O4 are in the axial positions. The bond angle of O1-Mn1-O4 is 176.890(86)°, so it forms a distorted octahedral. Owing to the binding of the bridge oxygen atom with Mn, two six-membered rings (Mn1-O5-C5-C6-C7-O2 and Mn1-O5-C2-C1-C8-O4) are created.

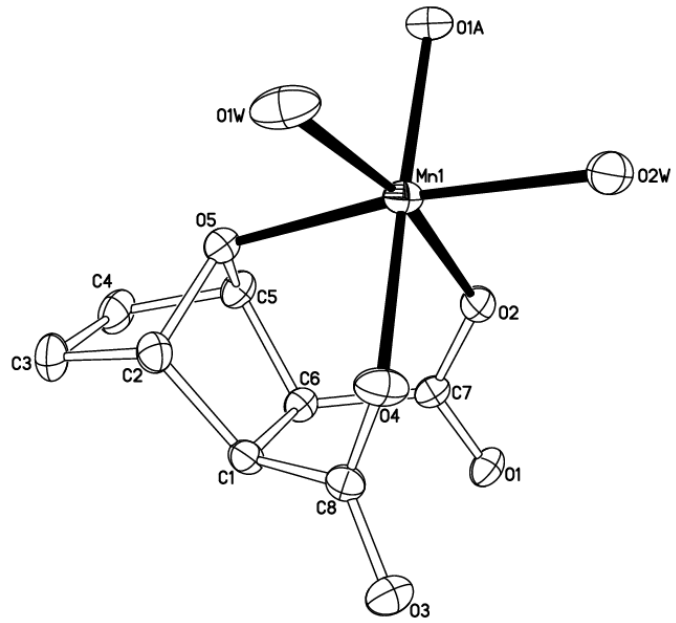

Fig. 1. Molecular structure of the complex showing 30 % atomic displacement ellipsoids

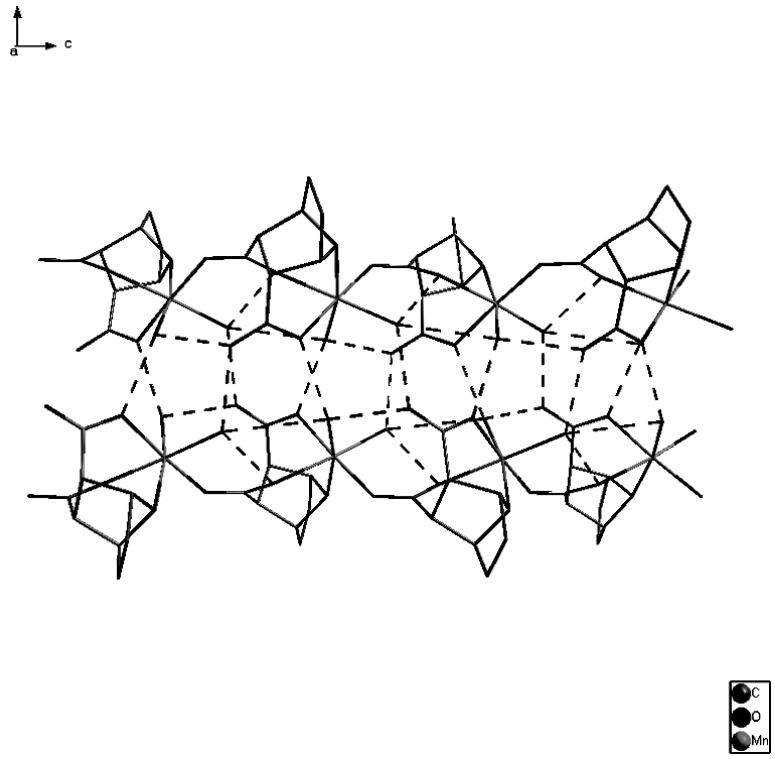

Fig. 2. Packing diagram of the complex, hydrogen bonds as dash lines

TABLE-2
SELECTED BOND LENGTHS (Å) AND ANGLES (º) FOR THE Mn COMPLEX OF DCA

O(1W)-Mn(1)	2.174(3)	O(1)-Mn(1)#1	2.091(2)
O(2W)-Mn(1)	2.191(2)	O(2)-Mn(1)	2.192(2)
O(4)-Mn(1)	2.152(2)	O(5)-Mn(1)	2.2679(19)
Mn(1)-O(1)#2	2.091(2)		
O(1)#2-Mn(1)-O(4)	176.89(9)	O(1)#2-Mn(1)-O(1W)	91.33(10)
O(4)-Mn(1)-O(1W)	87.68(11)	O(1)#2-Mn(1)-O(2W)	83.41(9)
O(4)-Mn(1)-O(2W)	93.98(9)	O(1W)-Mn(1)-O(2W)	104.49(12)
O(1)#2-Mn(1)-O(2)	97.51(9)	O(4)-Mn(1)-O(2)	83.87(9)
O(1W)-Mn(1)-O(2)	168.33(10)	O(2W)-Mn(1)-O(2)	84.17(9)
O(1)#2-Mn(1)-O(5)	98.68(8)	O(4)-Mn(1)-O(5)	84.22(8)
O(1W)-Mn(1)-O(5)	87.19(11)	O(2W)-Mn(1)-O(5)	168.12(10)
O(2)-Mn(1)-O(5)	83.97(8)		

Symmetry transformations used to generate equivalent atoms: #1 -x+1, y+0, z-1/2 #2 -x+1, y+0, z+1/2.

TABLE-3
HYDROGEN BONDS FOR THE Mn COMPLEX OF DCA

D-H...A	d(D-H)	d(H...A)	d(D...A)	∠(DHA)
O(1W)-H(1WA)···O(2)#2	0.831(19)	2.10(3)	2.822(3)	145(5)
O(1W)-H(1WA)···O(2W)#2	0.831(19)	2.57(4)	3.238(4)	138(4)
O(1W)-H(1WB)···O(3)#3	0.849(19)	1.97(2)	2.798(3)	164(5)
O(2W)-H(2WA)···O(3)#2	0.839(19)	2.00(3)	2.809(3)	162(5)
O(2W)-H(2WB)···O(4)#4	0.835(19)	1.87(2)	2.696(3)	173(5)

Symmetry transformations used to generate equivalent atoms: #1 -x+1, y+0, z-1/2 #2 -x+1, y+0, z+1/2 #3 x+0, -y+0, z+1/2 #4 -x+1, -y, z.

In addition, a seven-membered ring (Mn1-O2-C7-C6-C1-C8-O4) is formed because of the coordination of carboxylate oxygen atoms O2 and O4. It is noted that intermolecular hydrogen bonds of the complex make the compound more stable. Two different Mn(II) are connected by a bridging carboxyl group to form a one-dimension chain, which forms a one-dimension double-strand structure along c axis by intermolecular hydrogen bonds, which are formed by the coordinated water and the carboxyl groups of the monodentate ligands of two adjacent complexes.

Antiproliferative evaluation: The antiproliferative effects of the complex and NCTD on human hepatoma cells SMMC7721 and human lung cancer cells A549 are reported in Figs. 3 and 4. As shown in Fig. 3, at the tested doses (0-200 μmol L^{-1}), the inhibition effect of the complex is more than that of NCTD against SMMC7721 cells. At lower doses (50 μM), inhibition rates of the complex (70.9 ± 1.9 %) is much higher than that of NCTD (8.2 ± 1.1 %) against SMMC7721 cell lines. And in Fig. 4, for A549 cells, the complex has an inhibition effect the same as NCTD.

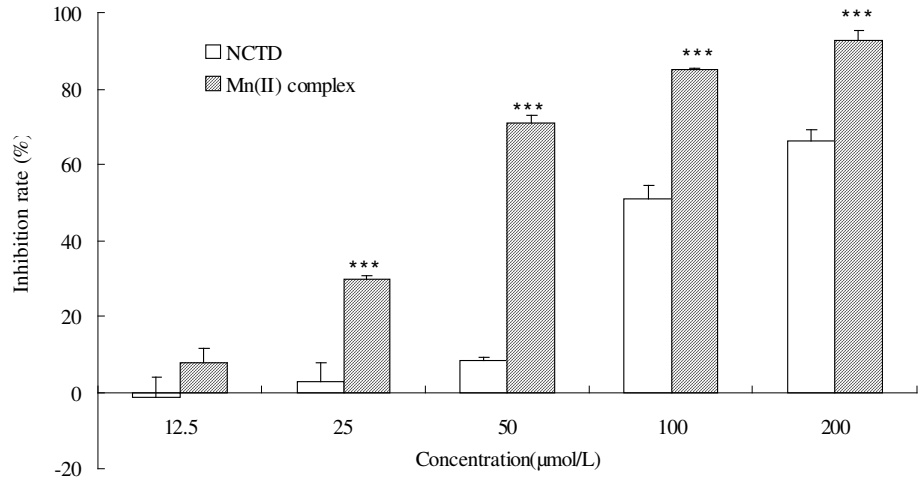

Fig. 3. Inhibition effects of NCTD and the complex on SMMC7721 cell growth. Data represent mean ± SD and all assays were performed in triplicate for three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001 *vs.* NCTD in the same concentration, t test

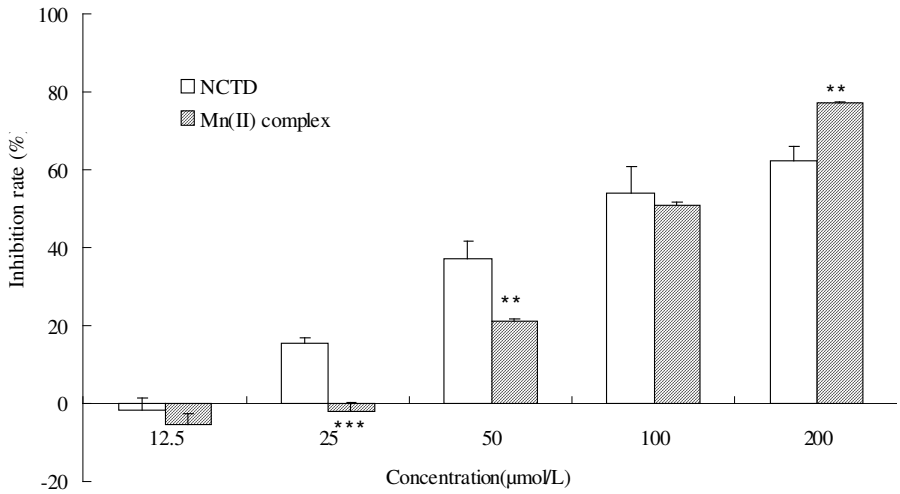

Fig. 4. Inhibition effects of NCTD and the complex on A549 cell growth. Data represent mean ± SD and all assays were performed in triplicate for three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001 *vs.* NCTD in the same concentration, t test

The concentrations of the compounds for 50 % inhibition (IC_{50}) on the SMMC7721 and A549 cell lines were determined and the results were summarized in Table-4. The test indicates that the complex has a higher inhibitive effect on human hepatoma cells SMMC7721 (IC_{50} = 38.0 ± 0.9 μmol L^{-1}) and human lung cancer cells A549 (IC50 = 105.9 ± 2.4 μmol L^{-1}). The inhibition rates of the complex on human hepatoma cells SMMC7721 are much higher than those of NCTD. Therefore, it may be concluded that the complex has better antiproliferative

activity against cancer cells than NCTD. These observations suggest that chelation of Mn(II) ion is a very important process, useful to afford new chemical features to the complex.

TABLE-4
IC_{50} VALUES (72 h) OF TEST MATERIALS ON SMMC7721 AND A549 CELLS. DATA REPRESENT MEAN ± SD. ALL ASSAYS WERE PERFORMED IN TRIPLICATE FOR THREE INDEPENDENT EXPERIMENTS

IC_{50}	SMMC7721	A549
NCTD	115.5 ± 9.5 μmol L^{-1}	88.7 ± 12.8 μmol L^{-1}
Mn(II) complex	38.0 ± 0.9 μmol L^{-1}	105.9 ± 2.4 μmol L^{-1}

Conclusion

Mn(II) complex $[Mn(C_8H_8O_5)(H_2O)_2]$ with demethylcantharate was synthesized and characterized by elemental analysis, IR and single crystal X-ray diffraction. The antiproliferative activity test showed that the complex has a higher inhibitive effect on human hepatoma cells SMMC7721 (IC_{50} = 38.0 ± 0.9 μmol L^{-1}). The inhibition rates of the complex are much higher than those of NCTD. All the above could provide that chelation of Mn(II) ion is a very important process. Therefore, further studies of novel demethylcantharate with various metal cations are underway.

ACKNOWLEDGEMENTS

The authors thank Institute of Material Medica of Zhejiang Academy of Medical Sciences for help with anti-proliferative activity test. Financial support from Natural Science Foundation of Zhejiang Province (Grant no. Y407301) is gratefully acknowledged.

REFERENCES

1. F.L. Liu, T. Jiang and X. Qi, *Chin. J. Org. Chem.*, **22**, 761 (2002).
2. Z.H. Zhou and R.Y. Chen, *Chem. J. Chin. Univ.*, **21**, 721 (2000).
3. L.H. Jiang and M.Y. Wang, *Herald Med.*, **23**, 385 (2004).
4. S.N. Yi, J. Wass, P. Vincent and H. Iland, *Leuk. Res.*, **15**, 883 (1991).
5. Z.C. Zhang, Z.X. Zhang and R.G. Zhang, *Stud. Trace Elem. Health*, **20**, 59 (2003).
6. F.L. Yin, J. Shen, J.J. Zou and R.C. Li, *Acta Chim. Sinica*, **61**, 556 (2003).
7. X.L. Zheng, H.X. Sun, X.L. Liu, Y.X. Chen and B.C. Qian, *Acta Pharmacol. Sin.*, **25**, 1090 (2004).
8. F. Zhang, Q.Q. Zhang, W.G. Wang and X.L. Wang, *J. Photochem. Photobiol. A*, **184**, 241 (2006).
9. K. Nakamot, Infrared and Raman Spectra of Inorgnic and Coodination Compounds, D.R. Huang and R.Q. Wang, Translator, Chemical Industry Press, Beijing, p. 257 (1991).
10. Y.Y. Wang, R.D. Hu and Y.J. Wang, *Acta Cryst.*, **E65**, m169 (2009).
11. Y.Y. Wang, R.D. Hu, W.Z. Zhu and Q.Y. Lin, *Acta Cryst.*, **E65**, m787 (2009).

(*Received*: 19 September 2009; *Accepted*: 30 April 2010) AJC-8652