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Synthesis, Characterization and Crystal Structure of Optically Active L- and D-Histidine Norcantharimide

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L- and D-histidine norcantharimide was showed significant PP2A inhibitors effect. They were synthesized through a highly efficient combinatorial approach , to a solution of norcantharidin and L-histidine in 95 % EtOH under temperature affords L-histidine norcantharimide in 97 % yield. L-histidine norcantharimide was characterized by X-ray. This single crystal was orthorhombic with the space group P2(1)2(1)2(1). One unit cell dimensions were a = 10.1553(4) Å, b = 10.4840(5) Å and c = 12.8749(6) Å, respectively. $\alpha = \beta = \gamma = 90°$, Mr = 305.29, V = 1370.77(11) Å^3, D = 1.479 Mg/m^3, F(000) = 640 and Z = 4. The crystal structure is stabilized by a network of intermolecular hydrogen bonds involving N-H and COO^- group.

Keywords: L-norcantharimide, D-histidine norcantharimide, Crystal Structure, Synthesis.

INTRODUCTION

Cantharidin, a well-known natural toxin of the traditional Chinese medicine, has been used as a natural remedy for over 2000 years. However, its toxicity on the renal system and suppression effect on bone marrow limit its clinical usage. Norcantharidin, the synthetic demethylated analogue of cantharidin, also possesses anticancer activity but with less toxicity and is now clinically available in China[1]. From the last decade, studies showed that both cantharidin and norcantharidin were potent inhibitors of PP2A, a trimeric holoenzyme which mediated most cellular processes and was recognized as a potential target for medical research, especially for cancer treatment[2-4]. Thus, as a promising lead compound, the structure modification of cantharidin and norcantharidin aiming at PP2A inhibition aroused great attention in recent years[5-8]. In recent years McCluskey and co-workers have reported the synthesis and PP2A inhibition activities of a series of amino acid derivatives of norcantharidin, either in ring closed imide form or in ring-opened acid-amide form[3,5,6,8]. One of the most interesting compound is L-histidine norcantharimide, which proved to be a more potent inhibitor of PP1 and PP2A (PP1 IC_{50} = 2.82 ± 0.6 μM; PP2A IC_{50} = 1.35 ± 0.3 μM) than norcantharidin[6] (PP1 IC_{50} = 5.31 ± 0.76 μM; PP2A IC_{50} = 2.9 ± 1.04 μM). In light of these results, we were interested in the synthesis and structural elucidation of L- and D-histidine norcantharimide.

EXPERIMENTAL

Melting points were determined on a thiele melting point apparatus and were uncorrected. Optical rotations were taken on Autopol-IV automatic polarimeter (Rudolph Research Analytical). The IR spectrum was measured in KBr on an Analect RFX-65A FT-IR spectrometer. NMR spectra were recorded on a Bruker DRX-400 spectrometer in DMSO-d_6. Spectra were referenced internally by using the residyal solvent resonance (δ = 7.26 for $CDCl_3$) relative to $SiMe_4$. The positive ion FAB-MS spectra was obtained on a VG ZAB-HS instrument by bombarding 3-nitrobenzyl alcohol matrixes of the samples with 8 keV (about 1.28×10^5 J) Xe atoms. Elemental analyses were performed on an Elementar Vario EL elemental analyzer. L-histidine was provided by Shanghai Bio Life Science & Technology Co., Ltd. in China and was used as received. Norcantharidin was synthesized in accord with the published procedure[9,10]. All other chemicals used in this study were of analytical grade.

Synthesis

L-Histidine norcantharimide: In a typical procedure, recrystallized norcantharidin (0.37 g, 2.2 mmol), along with L-histidine (0.31 g, 2 mmol) was mixed in 30 mL 95 % ethanol. The heterogeneous mixture was kept refluxing for 2 days until it turned to be a colorless solution, which indicated the end of the reaction. Then the solution was evaporated and the residue

mass was recrystallized from acetone-distilled water mixture. Finally, L-histidine norcantharimide was collected as colorless crystal by filtration and washed with anhydrous ethanol (0.59 g, 97 %); m.p. 264 °C. ^{1}H NMR(D_2O, δ/ppm, J/Hz): 1.53-1.63 (4H, m, 4-H and 5-H), 2.96 (2H, d, *J* = 7.2 Hz, 2-H), 3.02 (2H, d, *J* = 7.2 Hz, 7-H), 3.28-3.30 (2H, m, 11-H), 4.47-4.71 (3H, m, 3-H, 6-H and 10-H), 7.05 (1H, s, 13-H), 8.38 (1H, s, 14-H). ^{1}H NMR (DMSO, δ/ppm, *J*/Hz): 1.56-1.58 (4H, m, 4-H and 5-H), 2.96 (2H, d, *J* = 7.2 Hz, 2-H), 3.02 (2H, d, *J* = 7.2 Hz, 7-H), 3.09-3.22 (2H, m, 11-H), 4.58 (1H, s, 3-H), 4.64 (1H, dd, *J* = 10.4 and *J* = 4.8, 10-H), 4.67 (1H, s, 6-H), 5.52 (1H, s, C=N-H), 6.74 (1H, s, 13-H), 7.64 (1H, s, 14-H). FAB-MS *m/z* = 306, $[M + H]^+$. IR (KBr, ν_{max}, cm^{-1}): 3469 (N-H), 3160, 3120, 3006 (C=C-H), 2965, 2883 (CH_2), 1781 (ν_{asym} imide), 1708 (ν_{sym} imide), 1637 (ν_{asym} COO^-), 1400 (ν_{sym} COO^-), 1189 (C-O-C). $[\alpha]_D^{25}$ = -90.9° (c = 0.01019, H_2O). Anal. Calcd. for $C_{14}H_{15}N_3O_5$: C, 55.08; H, 4.95; N, 13.76; found C, 54.47; H, 5.06; N, 13.93.

D-Histidine norcantharimide: Following the similar produres of L-histidine norcantharimide, but with D-histidine instead of L-histidine. D-histidine norcantharimide was collected as colorless crystal; m.p. 264 °C. $[\alpha]_D^{25}$ = + 90.8°(c = 0.01021, H_2O). ^{1}H NMR, (D_2O, δ/ppm, *J*/Hz): 1.56-1.66 (4H, m, 4-H and 5-H), 2.98 (1H, d, *J* = 7.2 Hz, 2-H), 3.04 (1H, d, *J* = 7.2 Hz, 7-H), 3.30-3.32 (2H, m, 11-H), 4.49-4.73 (3H, m, 3-H, 6-H and 10-H), 7.07 (1H, s, 13-H), 8.42 (1H, s, 14-H)

X-ray crystallography: A suitable single crystal of the compound was formed by slow evaporation of the acetone-distilled water mixture (3:1) at room temperature. Single-crystal data of the compound was collected at 173 (2) K on a Bruker Smart 1000 CCD diffractometer with MoK_α radiation (λ = 0.71073 Å). All empirical absorption corrections were applied by using the SADABS program[11]. The structure was solved using direct method, which yielded the positions of all non-hydrogenatoms. These were refined first isotropically and then anisotropically. All the hydrogen atoms of the ligands were placed in calculated positions with fixed isotropic thermal parameters and included in structure factor calculations in the final stage of full-matrix least-squares refinement. All calculations were performed using the SHELXTL system of computer programs[12]. The experimental details together with crystal data are given in Table-1.

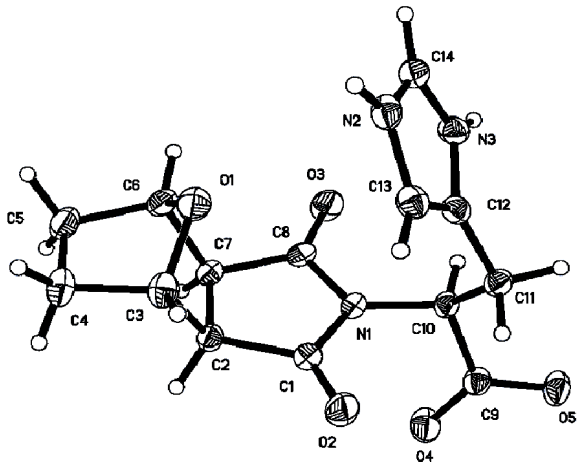


Fig. 1. Molecular structure of L-histidine norcantharimide showing the atom numbering scheme

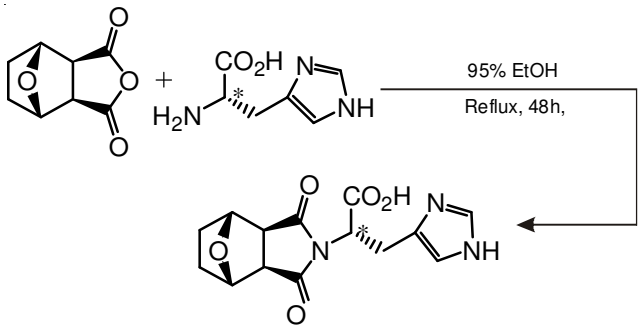
TABLE-1
CRYSTAL DATA AND STRUCTURE REFINEMENT FOR L-HISTIDINE NORCANTHARIMIDE

Empirical formula	$C_{14}H_{15}N_3O_5$
Formula weight	305.29
Temperature	173(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	P2(1)2(1)2(1)
Unit cell dimensions	a = 10.1553(4) Å, α = 90°
	b = 10.4840(5) Å, β = 90°
	c = 12.8749(6) Å, γ = 90°.
Volume	1370.77(11) $Å^3$
Z	4
Density (calculated)	1.479 mg/m^3
Absorption coefficient	0.114 mm^{-1}
F(000)	640
Crystal size	0.47 × 0.46 × 0.27 mm^3
Theta range for data collection	2.51 to 27.02°.
Index ranges	-12≤h≤12, -13≤k≤10, -16≤l≤16
Reflections collected	9198
Independent reflections	1723 [$R_{(int)}$ = 0.0229]
Completeness to theta = 27.02°	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9698 and 0.9482
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	1723/0/200
Goodness-of-fit onF^2	1.057
Final R indices [I > 2σ (I)]	R1 = 0.0337, wR2 = 0.0928
R indices (all data)	R1 = 0.0354, wR2 = 0.0952
Extinction coefficient	0.021(3)
Largest diff. peak and hole	0.544 and -0.205 e.$Å^{-3}$

RESULTS AND DISCUSSION

Synthesis and characterization: We carried out the condensation reaction between norcanthridin and L-histidine in a number of solvents at reflux temperature, including toluene, acetone, acetonitrile, *etc*., but no product could be obtained efficiently unless 95 % EtOH. The reaction proceeded smoothly at reflux temperature for 2 days leading to the target compound in 97 % yield (**Scheme-I**), which was about 7-fold enhencement compared with that reported by McCluskey *et al.*[6]. As former literature did not mention the optical rotation data of L-histidine norcantharimide[6], so we set out to elucidate this important constant with accuracy. A control experiment was carried out and confirmed that L-histidine in 95 % EtOH under the same reaction conditions maintained the specific optical rotation ($[\alpha]_D^{20}$ = -39.4, c = 1.13, H_2O). In addition, D-histidine reacted with norcantharimide under the same conditions to afford D-histidine norcantharimide in comparable yield of 95 % and give a substantially equal but reversed $[\alpha]_D$ value ($[\alpha]_D^{25}$ = -90.9° (c = 0.01019, H_2O) for L-histidine norcantharimide; whereas $[\alpha]_D^{25}$ = +90.8° (c = 0.01021, H_2O) for D-histidine norcantharimide). These results demonstrated that the configuration of the histidine-derived norcantharimide was maintained during the coupling reaction, furnishing the L- or D-histidine norcantharimide, respectively, with the same stereochemical orientation as the starting material.

X-ray crystal structure: Analysis in order to get a deeper insight into the crystalline and molecular structure of the compound, in particular, the features of the imide group, the single X-ray crystal diffraction study was undertaken.



Scheme-I: Synthesis of both enantiomers of histidine norcantharimide

The crystallographic data of the compound is summarized in Table-1. As a result, this single crystal was orthorhombic with the space group P2(1)2(1)2(1). One unit cell dimensions were a = 10.1553(4) Å, b = 10.4840(5) Å and c = 12.8749(6) Å, respectively. The molecular conformation of L-histidine norcantharimide is shown in Fig. 1. The molecular of the compound contains the norcantharidin moiety and the L-histidine moiety in a ratio of 1:1 through imide bond, the geometry of L-histidine moiety is similar to the geometry of pure L-histidine reported earlier[13]. It exists as a zwitter ion in which the carboxyl hydrogen atom transferred to the imidazole nitrogen atom.

Selected bond lengths and angles are listed in Table-2. Interestingly, X-ray structural determinations reveal that aromatic π-electrons are delocalized in the protonated imidazole ring. This is evident from the observed bond lengths C(12)-N(3) (1.382(3)Å), C(13)-N(2) (1.381(3)Å), C(14)-N(2) (1.323(3)Å), C(14)-N(3) (1.325(3)Å) and the torsion angles N(3)-C(14)-N(2)-C(13) (0.00°), C(12)-C(13)-N(2)-C(14) (1.0(0)°), which indicating a planar imidazole ring. Also, in the crystal, the carboxylic group is disordered. The observed tendency of equalisation of the C-O and C=O bond lengths and the angles of the carboxylic group is a result of this disorder. Due to the H-bonds the C=O bond is somewhat elongated and the C-O bond slightly shortened and the difference between the C-O and C=O bond lengths amounts only 0.041 Å. Intermolecular interactions between L-histidine norcanth-arimide molecules to form the crystal structure were also investigated. The crystal structure is stabilized by a network of intermolecular hydrogen bonds involving N-H and COO^- group. In the crystal structure, an four-membered ring is formed by a N(3)-H(3A)···O(5) hydrogen bond and N(3)-H(3A)···O(4) hydrogen bond. They are further bonded to each other by N(2)-H(2A)···O(5) hydrogen bond. As a result, a network of hydrogen bonds formed in the crystal structure of L-histidine norcantharimide (Table-3, Fig. 2).

TABLE-3
HYDROGEN BONDS (Å) AND ANGLES (°) FOR L-HISTIDINE NORCANTHARIMIDE

D-H···A	Distance (D-H)	Distance (H-A)	Distance (D-A)	Angle
N(3)-H(3A)···O(5)#1	0.88	1.89	2.747(2)	165.3
N(3)-H(3A)···O(4)#1	0.88	2.53	3.193(2)	133.0
N(2)-H(2A)···O(5)#2	0.88	1.91	2.756(2)	161.5

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

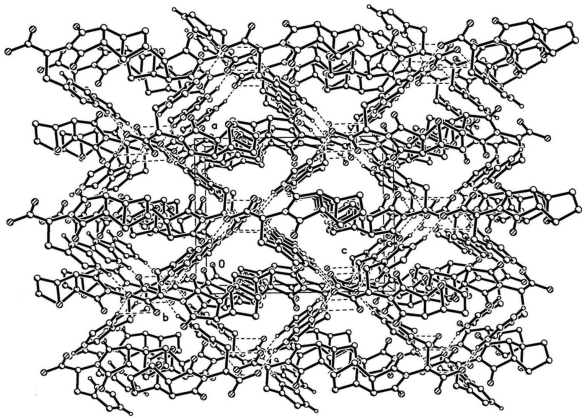


Fig. 2. Packing diagram of L-histidine norcantharimide showing the hydrogen-bond interactions

Conclusion

In conclusion, the procedure represented a simple and highly efficient way for the preparation of both enantiomers of histidine norcantharimide. Moreover, the physical and spectral

TABLE-2
SELECTED BOND LENGTHS (Å) AND ANGLES (°) FOR L-HISTIDINE NORCANTHARIMIDE

Bond lengths					
C(1)-O(2)	1.208(2)	C(9)-O(4)	1.230(3)	C(12)-N(3)	1.382(3)
C(1)-N(1)	1.384(3)	C(9)-O(5)	1.271(3)	C(13)-N(2)	1.381(3)
C(2)-C(7)	1.544(3)	C(9)-C(10)	1.547(3)	C(13)-H(13)	0.9500
C(3)-O(1)	1.449(3)	C(10)-N(1)	1.459(2)	C(14)-N(2)	1.323(3)
C(6)-O(1)	1.444(2)	C(10)-C(11)	1.542(3)	C(14)-N(3)	1.325(3)
C(7)-C(8)	1.514(3)	C(10)-C(11)	1.542(3)	C(14)-H(14)	0.9500
C(8)-O(3)	1.203(2)	C(11)-C(12)	1.489(3)	N(2)-H(2A)	0.8800
C(8)-N(1)	1.384(2)	C(12)-C(13)	1.361(3)	N(3)-H(3A)	0.8800
Bond angles					
O(2)-C(1)-N(1)	124.72(18)	N(3)-C(12)-C(11)	122.55(18)	C(1)-N(1)-C(10)	124.67(16)
O(2)-C(1)-C(2)	126.64(18)	C(12)-C(13)-N(2)	107.21(17)	C(8)-N(1)-C(10)	122.33(16)
N(1)-C(1)-C(2)	108.64(16)	C(12)-C(13)-N(2)	107.21(17)	C(14)-N(2)-C(13)	108.53(17)
O(3)-C(8)-N(1)	124.01(18)	C(12)-C(13)-H(13)	126.4	C(14)-N(2)-H(2A)	125.7
O(3)-C(8)-C(7)	126.97(18)	N(2)-C(13)-H(13)	126.4	C(13)-N(2)-H(2A)	125.7
N(1)-C(8)-C(7)	109.01(16)	N(2)-C(14)-H(14)	125.5	C(14)-N(3)-C(12)	109.04(17)
C(13)-C(12)-N(3)	106.18(17)	N(3)-C(14)-H(14)	125.5	C(14)-N(3)-H(3A)	125.7
C(13)-C(12)-C(11)	131.26(19)	C(1)-N(1)-C(8)	112.87(16)	C(12)-N(3)-H(3A)	125.7

data of the compounds were fully illuminated, the optical rotation data is reported for the first time and the crystal structure of L-histidine norcantharimide was determined by X-ray crystallographic analysis. Further synthesis and evaluation of norcantharimide analogues are currently underway in our laboratories and will be reported in due course.

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