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REACTION OF ISONIAZID (INH) WITH RHENIUM (Re) USING DIFFERENT REDUCING AGENTS, CHARACTERIZATION OF COMPLEXES AND X-RAY CRYSTAL STRUCTURE OF INH DIMER

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Reactivity of INH with NH_4ReO_4 was studied using $SnCl_2•2H_2O$ and $NaBH_4$ as reducing agents. ^{99m}Tc labelled INH was also prepared according to the reported procedure for comparison. A complex (1) similar to ^{99m}Tc-INH (2) was formed with NH_4ReO_4 in the presence of $SnCl_2•2H_2O$. On the other hand, $NaBH_4$ gave a dimer of INH (3) which was confirmed by x-ray crystallography. The products, 1, 2 and 3 were characterized by HPLC, IR spectra and x-ray crystallography.

Keywords: Reactivity, Reduction, Isoniazid, ^{99m}Tc-INH, Dimerization, Crystallography.

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1. Introduction

INH is one of the most effective agents in tuberculosis therapy. It inhibits the synthesis of long chain fatty acids (mycolic acid) in the cell wall of mycobacterium tuberculosis [1]. Studies on introduction of ^{11}C and ^{123}I on a substituent of pyridine ring of isoniazid have been published [2, 3]. INH derivative with 2-iminothiolane has been labelled with ^{99m}Tc by conventional method and has shown promising results as tubercular lesion imaging agent [4]. Direct labelling of INH with ^{99m}Tc and the factors affecting labelling efficiency have been studied earlier. The results suggested that ^{99m}Tc-INH is a specific and cost effective radiopharmaceutical for diagnosis and localization of tubercular lesions [5]. Isoniazid has amino and carbonyl groups to form bonds with ^{99m}Tc, although details of the chemistry of formation and molecular structure are unknown.

A very sensitive catalytic spectrophotometeric method for the determination of copper (II), in concentration as low as 0.01 ng/ml has been reported. This method is based on the oxidation of INH by hydrogen peroxide in ammoniacal medium, catalyzed by copper(II). Various metal interferences have been reported. Structure of the oxidation product is not known [6]. Isonicotinyl hydrazones obtained by the primary antituberculosis agent isoniazid, have been used as monoanionic ligands (L) to prepare copper(II) and nickel(II) octahedral complexes of stoichiometry $[MeL_2(H_2O)_2]$. Their antimycobacterial in-vitro activity was evaluated against *Mycobacterium tuberculosis H37Rv* in comparison with the ligands [7].

A number of organomercury (II) complexes involving isoniazid (I), of the type RHgCl(L) (II) R = phenyl(C_6H_5), o-hydroxyphenyl (o-HOC_6H_4), p-hydroxyphenyl (p-HOC_6H_4), p-acetoxyphenyl (p-$AcOC_6H_4$), 2-furyl (2-C_4H_3O); L = isoniazid] have been synthesized and characterized. Conductance measurements indicate that the complexes are nonelectrolytes. From IR and UV studies, it is concluded that isoniazid acts as a bidentate ligand, coordinating through hydrazinic nitrogen and carbonyl oxygen. ^{1}H and ^{13}C NMR support the stoichiometry of the complexes. The $C_6H_5HgCl(L)$ and p-$HOC_6H_4HgCl(L)$ complexes have been screened for tuberculosis activity [8]. Isoniazid, an anti-tuberculosis (TB) drug has been coordinated with chromium, molybdenum, and tungsten metal carbonyls and three new zero-valent complexes *fac*-$[M(CO)_3(isoniazid)_3]$ (M = Cr, Mo, and W) (isoniazid = 4-$H_2NHNOCC_5H_4N$) have been synthesized. Reaction of the complex precursors *fac*-$[M(CO)_3(CH_3CN)_3]$ (M = Cr, Mo, and W) prepared 'in situ' with three equivalents of isoniazid

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in methanol at room temperature afforded high yields of isoniazid substituted metal carbonyl complexes. The complexes have been characterized by elemental analyses, mass analysis, thermal analysis (TGA, EGA), FT-IR, UV/visible and ^1H NMR spectroscopic techniques and powder X-ray diffraction (XRD) [9].

A series of 12 complexes of cobalt(II) and nickel(II) with N-isonicotinamido-2',4'-dicholoro-benzalaldimine (INH-DCB) with the general composition MX_2.n(INH-DCB) [M = Co(II), or Ni(II), X = Cl^-, Br^-, NO_3^-, CH_3COO^-, n = 2; X = ClO_4^-, n = 3] have been synthesized. The nature of bonding and the stereochemistry have been deduced from elemental analysis, electronic spectra, and magnetic susceptibility and conductivity measurements. An octahedral geometry has been suggested for all complexes. The metal complexes were screened for antifungal and antibacterial properties on different species of pathogenic fungi and bacteria and their biopotency has been discussed [10].

In this work reactivity of INH towards NH_4ReO_4 is studied using different reducing agents. Corresponding complex of INH with ^{99m}Tc is also synthesized for comparison purposes. The products obtained are characterized by HPLC, IR spectra and x-ray crystallography.

2. Experimental

2.1. *Materials and methods*

All the chemicals used were of analytical grade and purchased from Merck., Germany. Radiochemical yield of ^{99m}Tc labelled INH was assessed by thin layer chromatographic method using ITLC-SG and Whatman paper No.3. Free $^{99m}TcO_4^-$ in the preparation was determined by using Whatman paper No.3 as the stationary phase and acetone as the mobile phase. Reduced and hydrolyzed activity was determined by using ITLC-SG as the stationary phase and 0.5 M NaOH as mobile phase.

The samples were passed through a millipore filter carefully and injected into the HPLC column (peptide system). The Hitachi L-6200 Intelligent pump and L-4200 UV-Vis detector systems were used for HPLC analysis. Analytical reverse phase HPLC analysis was performed with an analytical C18 column (RP-18 Lichrosorb, 25, 0.45 cm) using a continuous gradient of methanol/water (20:80) to (80:20) in 30 min. Elutions were performed at a flow rate of 1.0 ml /min after an injection volume of 20 µL tracer. The eluted radioactivity was monitored on line using a NaI probe (Raytest-Steffi) collected fractions were also measured by well type gamma counter. Typical elution profiles of complexes from an analytical HPLC were obtained.

IR spectra were determined by FT-IR spectrophotometer of Thermo Electron Corporation, NICOLET 6700, spectral resolution 0.125, MID + IR (7800-350). This facility was provided by the Department of Advanced Polymer Materials (DAPM), Pakistan Institute of Engineering and Applied Sciences (PIEAS), Nilore, Islamabad, Pakistan. Elemental analysis (C, H, N) of (3) was performed by the Chemical Analysis Center of the University of Tsukuba, Japan.

2.2. *Preparation of 1*

NH_4ReO_4 (4.0 mg, 0.016 mmol) dissolved in 10 ml of H_2O. INH (2.0 mg, 0.014 mmol) was added to this clear solution. $SnCl_2 \cdot 2H_2O$ (3.0 mg, 0.012 mmol) was then added. The solution was stirred at room temperature for 30 min. Dark reddish brown precipitate was obtained which was filtered and dried under vacuum.

2.3. *Preparation of 2*

By a little modification of the reported method [5], INH (2.0 mg, 0.08 mmol) was dissolved in 1.0 ml of H_2O. $SnCl_2 \cdot 2H_2O$ (100 µg, 0.00004 mmol) was added, the pH of solution was ~ 7. ^{99m}Tc (1.0 ml, 16mCi) was added and the solution was incubated at room temperature for 10 minutes and heated in boiling water for 5 minutes. This complex being radioactive could not be characterized by elemental analysis, IR etc, while HPLC was performed.

2.4. *Preparation of 3*

INH (140 mg, 1.0 mmol) was dissolved in 2-3 ml of H_2O. NH_4ReO_4 (268 mg, 1.0 mmol) was then added followed by the addition of $NaBH_4$ (40 mg, 1.0 mmol). Deep brownish red solution was stirred at room temperature for 1.0 hour. The solution was filtered and filtrate kept in refrigerator for 3-4 days resulting in brown chip crystals (0.012 g) suitable for X-ray crystallography. [Analytically calculated for $C_{12}H_{14}N_4O_4$ (%): C, 51.74; H, 5.03; N, 20.12. Found: C, 50.85; H, 4.99; N, 19.83.

Table 1. Summary of crystallographic data for $C_{12}H_{14}N_4O_4$ (3).

Empirical Formula	$C_{12}H_{14}N_4O_4$
Formula Weight	278.27
Crystal Dimensions	0.20 × 0.15 × 0.05 mm
Crystal System	orthorhombic
Lattice Parameters	a = 28.166(3) Å
	b = 24.329(2) Å
	c = 3.8134(4) Å
	V = 2613.0(4) $Å^3$
Space Group	Fdd2 (#43)
Z value	8
D_{calc}	1.415 g/cm^3
μ(MoKα)	1.086 cm^{-1}
*F*000	1168.00
Temp. (°C)	23 ± 1 °C
2θ range (deg)	54.9°
Reflections collected	4497
R_{int}	0.036
Unique reflections	1414
No. of observations (I>2.00σ(I))	1227
No. of variables	120
Reflect./Para ratio	10.23
Residuals: R1 (I>2.00σ(I))	0.0504
Residuals: wR2 (I>2.00σ(I))	0.1260
Goodness of Fit Indicator	1.010
Max.Min peak ($eÅ^{-3}$)	0.37, -0.31

2.5. *Crystal structure determination*

X-ray crystallography was performed by Dr. Yoshitaro Miyashita of Department of Chemistry, University of Tsukuba, Japan. A brown chip crystal of $C_{12}H_{14}N_4O_4$ having approximate dimensions of 0.20 × 0.15 × 0.05 mm was mounted on a glass fiber. All measurements were made on a Rigaku Mercury Charged-Coupled Devices (CCD) area detector with graphite monochromated Mo-Kα radiation. The crystallographic data and experimental parameters are summarized in Table 1. The data were corrected for Lorentz and polarization effects [11].

The structure was solved by direct methods and expanded using Fourier techniques [12, 13]. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined isotropically. The final cycle of full-matrix least-squares refinement on F^2 was based on 1227 observed reflections and 120 variable parameters and converged (largest parameter shift was 0.00 times its esd) with unweighted and weighted agreement factors.

Least Squares function minimized:
$\Sigma w(F_o^2-F_c^2)^2$ where w = Least Squares weights.

The standard deviation of an observation of unit weight was 1.01.

Standard deviation of an observation of unit weight:
$[\Sigma w(F_o^2-F_c^2)^2/(N_o-N_v)]^{1/2}$
Where: N_o = number of observations
N_v = number of variables

A Sheldrick weighting scheme was used. Plots of Σ w $(|Fo| - |Fc|)^2$ versus |Fo|, reflection order in data collection, sin θ/λ and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.37 and -0.31 $e^-/Å^3$, respectively. The absolute structure was deduced based on Flack parameter, -7(9), refined using 561 Friedel pairs [14]. Neutral atom scattering factors were taken from Cromer and Waber [15]. Anomalous dispersion effects were included in Fcalc the values for Δf' and Δf" were those of Creagh and McAuley [16, 17]. The values for the mass attenuation coefficients are those of Creagh and Hubbell [18]. All calculations were performed using the Crystal Structure crystallographic software package [19, 20].

3. Results and Discussion

In HPLC the UV chromatogram showed UV peak at 254 nm in 30 minutes at 5.80 min (1) (70%), 5.90 min (2) (99.7%) and 10.86 min (3) (98.7%). This result supports that similar type of complexes are formed (1 and 2) when INH reacts with ^{99m}Tc or NH_4ReO_4 in the presence of $SnCl_2$•$2H_2O$ under similar conditions. The complex obtained by the reaction of INH with NH_4ReO_4 and $SnCl_2$•$2H_2O$ seems to be neutral because of its poor solubility in different solvents. This is also similar to ^{99m}Tc-isoniazid which showed neutral complex in electrophoresis [5]. The complex obtained by the reaction of INH with NH_4ReO_4 and $SnCl_2$•$2H_2O$ was not easily soluble when prepared on milligram scale. Its poor solubility was a

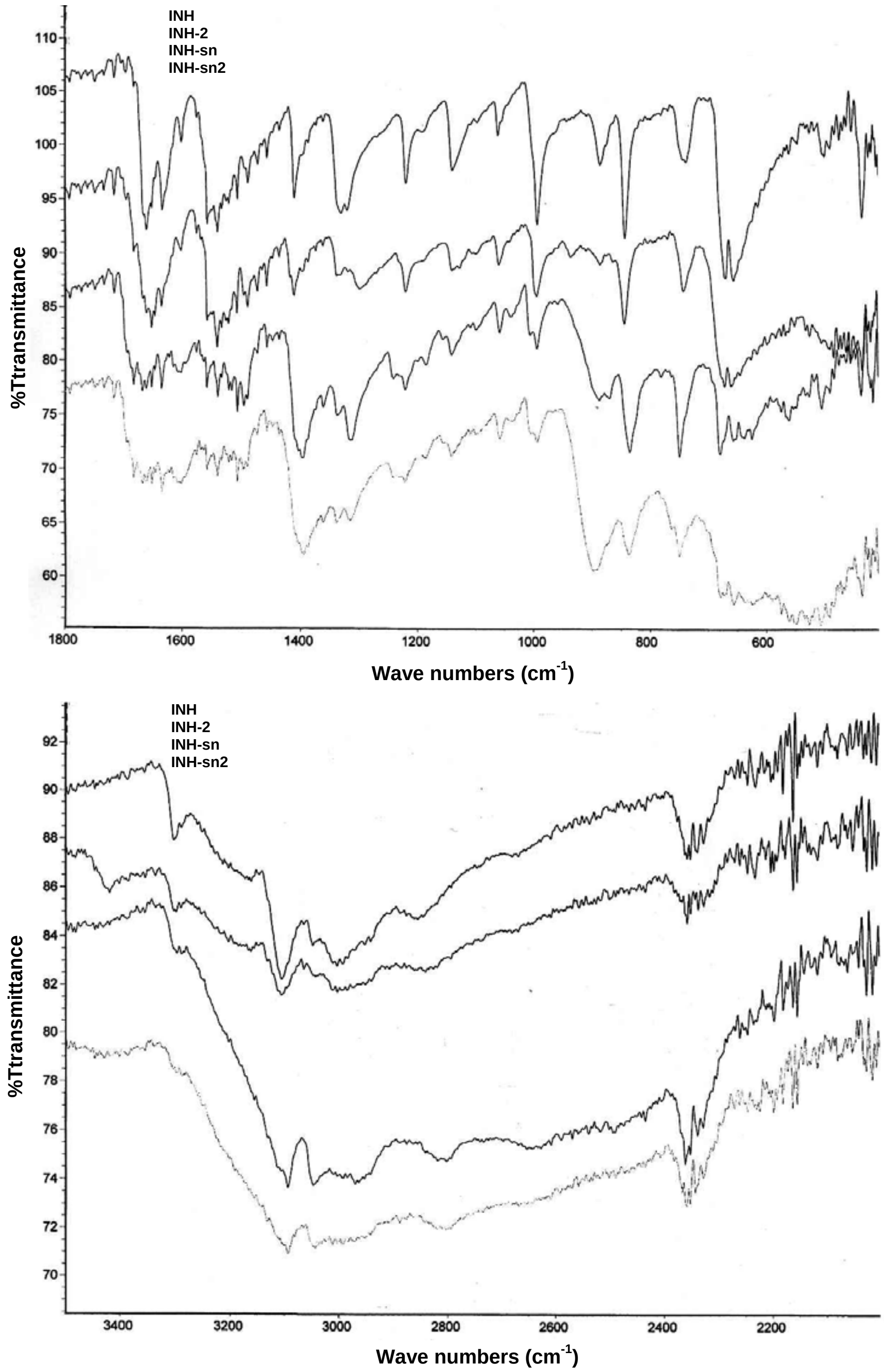

Figure 1. IR spectra top-bottom; INH, INH-2 (3), INH-Sn (milligram scale) (1) and INH-Sn2 (microgram scale) (1).

hindrance in its further classification. HPLC result of 3 showed that complex similar to 1 or 2 was not formed which was further confirmed by IR spectra and x-ray crystallography.

The IR spectrum of 1 is quite different from that of 3 (Figure 1) [IR (cm^{-1}) for INH: 3902.5, 3868.7, 3862.9, 3851.9, 3838.0, 3819.4, 3814.5, 3800.1, 3748.8, 3742.6, 3734.1, 3709.7, 3687.9, 3674.2, 3668.5, 3648.2, 3627.2, 3627.3, 3586.2, 3302.6,

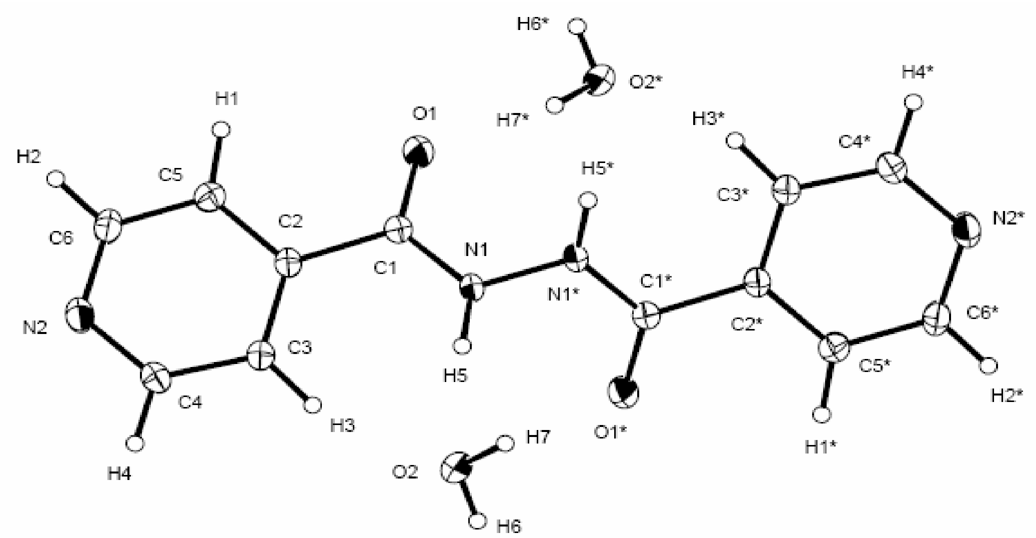

Figure 2. ORTEP diagram of $C_{12}H_{14}N_4O_4$ (3). Non-H atoms are represented with 50% probability ellipsoids.

3104.7, 3002.7, 2352.3, 2339.8, 2328.2, 1661.7, 1634.7, 1557.7, 1540.1, 1521.1, 1506.9, 1489.1, 1472.4, 1456.7, 1410.6, 1331.3, 1220.2, 1140.3, 994.2, 886.3, 843.6, 739.1, 671.3, 657.0, 562.4, 501.8, 492.3, 437.0, 426.5, 421.8, 412.9, 408.4. IR (cm^{-1}) for 1: 3093.4, 3043.5, 2359.2, 2342.7, 2246.7, 2224.4, 2198.6, 2181.7, 2163.2, 2155.4, 1683.1, 1668.3, 1652.3, 1635.2, 1602.0, 1557.8, 1539.8, 1520.7, 1506.5, 1495.9, 1394.4, 1338.6, 1316.3, 1221.2, 1141.5, 994.3, 897.6, 836.3, 749.8, 678.7, 656.5, 624.7, 574.3, 547.9, 526.5, 506.9, 491.9, 470.1, 436.3, 427.1, 422.0, 413.8, 408.4. IR (cm^{-1}) for 3: 3852.2, 3749.3, 3734.3, 3710.0, 3648.4, 3105.2, 3002.4, 2358.2, 2017.9, 1652.6, 1635.5, 1557.6, 1540.3, 1521.3, 1507.1, 1489.2, 1472.7, 1456.9, 1436.1, 1411.0, 1338.4, 1229.4, 1220.3, 1140.8, 1060.1, 995.1, 937.3, 886.4, 844.2, 743.3, 672.0, 661.2, 599.3, 574.8, 567.5, 552.0, 526.5, 491.4, 485.0, 476.5, 470.3, 456.0, 436.3, 426.6, 421.7, 408.1]. This indicates that different complexes are formed in the presence of $SnCl_2{\cdot}2H_2O$ and $NaBH_4$. N-H stretch in the region 3000-3600 cm^{-1} is seen in all spectra. The spectral pattern in 1 was somewhat different than 3, from this it might be deduced that NH_2 is involved in coordinate bond formation with rhenium. The C=O stretch is shown between 1200-1600 cm^{-1}. The spectral pattern in this region changed in 1 as compared to 3. This shows that C=O might also be involved in the formation of coordinate bond with rhenium. N-H and C-H bending is shown in the region of 1500-1700 cm^{-1} and 1200-1800 cm^{-1}, C-C stretch in the region of 800-1300 cm^{-1}, C=C, C=O and C-N stretch is observed in the region of 1500-1900 cm^{-1}, C-H stretch was observed between 2700-3800 cm^{-1} and C-N ring stretching between1430-1600 cm^{-1}.

Table 2. Selected bond distances (Å) and angles (^{o}) for **3**

Bond lengths			
O(1)–C(1)	1.225(3)	N(1)–N(1)$^{1)}$	1.397(2)
N(1)–C(1)	1.338(3)	N(2)–C(4)	1.323(3)
N(2)–C(6)	1.325(3)	C(1)–C(2)	1.497(3)
C(2)–C(3)	1.374(3)	C(2)–C(5)	1.377(3)
C(3)–C(4)	1.389(3)	C(5)–C(6)	1.375(3)
N(1)$^{1)}$–N(1)	1.397(2)		
Bond angles			
N(1)$^{1)}$–N(1)–C(1)	118.8(2)	C(4)–N(2)–C(6)	116.6(2)
O(1)–C(1)–N(1)	122.6(2)	O(1)–C(1)–C(2)	121.0(2)
N(1)–C(1)–C(2)	116.4(2)	C(1)–C(2)–C(3)	123.9(2)
C(1)–C(2)–C(5)	118.2(2)	C(3)–C(2)–C(5)	118.0(2)
C(2)–C(3)–C(4)	118.6(2)	N(2)–C(4)–C(3)	123.8(2)
C(2)–C(5)–C(6)	119.2(2)	N(2)–C(6)–C(5)	123.8(2)
C(1)–N(1)–N(1)$^{1)}$	118.8(2)		
(1) –X + ½	-Y + ½	Z (symmetry)	Operators

The reaction of INH with NH_4ReO_4 in the presence of $NaBH_4$ gave brown crystals. It has been revealed by the X-ray structural analysis that the brown chip crystal consists of dimers of INH formed by the removal of N_2H_4 and the crystal contains two H_2O molecules. The absence of anions implies that the compound is neutral. Elemental analysis result supported the formula $C_{12}H_{14}N_4O_4$. Summary of crystallographic data for $C_{12}H_{14}N_4O_4$ (3) is given in Table 1. Perspective view of crystal is given in Figure 2 and the selected bond distances and angles are given in Table 2. The two INH molecules are linked with each other by dinitrogen bond with a bond length of 1.397(2) Å.

The N(1)–C(1) distance (1.338(3) Å) is slightly longer than N(2)–C(6) (1.325(3) Å) and N(2)-C(4) (1.323(3) Å). The distances between carbon atoms C(1)–C(2) (1.497(3) Å) is larger than C(2)–C(3) (1.374(3) Å) and C(2)–C(5) (1.377(3) Å). The C(1)–O(1) (1.225(3) Å) distance shows double bond of carbonyl. The angle N(1)$^{1)}$–N(1)–C(1) (118.8(2)°) is smaller than O(1)_C(1)_N(1) (122.6(2)°) and O(1)_C(1)_C(2) (121.0(2)°) but nearly equal to N(1)_C(1)_C(2) (116.4(2)°) and C(4)_N(2)_C(6) (116.6(2)°). The angle N(1)_C(1)_C(2) (116.4(2)°) is smaller than N(2)_C(4)_C(3) (123.8(2)°) and N(2)_C(6)_C(5) (123.8(2)°). The angles between carbon atoms of the ring are nearly equal (118(2)-119(2)°) while that of C(1)_C(2)_C(3) is 123.9(2)°.

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