Structure and Hydrogen Bonding Interactions in Methoxysalicylaldehyde Thiosemicarbazone Derivatives in Solution by NMR and DFT Methods*

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Abstract. Solution state structure and hydrogen bonding in 4- and 5-methoxysalicylaldehyde 4-phenylthiosemicarbazones, **1** and **2**, have been studied by a combined use of NMR spectroscopy and DFT calculations. It has been shown that both compounds existed in hydroxy-thione tautomeric form in solution as previously found in solid state. Two kinds of hydrogen bonds were found to exist in solution, *i.e.* *intra*- and *inter*-molecular. DFT calculations and chemical shift values support the conclusion that three center hydrogen bonds were formed between the studied compounds and the solvent used, involving OH and NH groups. The solution state structures of **1** and **2** were found to differ slightly from those observed in the solid state which was attributed to a low barrier of rotation around the N–N bond.

Keywords: salicylaldehyde 4-phenylthiosemicarbazones, NMR, DFT, hydrogen bonds

INTRODUCTION

Thiosemicarbazones and their corresponding metal complexes are widely known compounds possessing diverse pharmacological activities, such as antitumor, antivirial, antibacterial, antifungal, antimalarial, *etc.*[1–3] Bioactivity is closely related to molecular conformation which can significantly be affected by the presence of *intra*- and *inter*-molecular hydrogen bonds. Moreover, *inter*-molecular hydrogen bonding interactions play an important role in ligand-receptor recognition processes.

Salicylaldehyde thiosemicarbazones can exist in several tautomeric forms (Figure 1) with both *intra*- and *inter*-molecular hydrogen bonds. X-ray structural analysis of different salicylaldehyde thiosemicarbazone derivatives showed a presence of the *intra*-molecularly hydrogen-bonded hydroxy-thione tautomer in the solid state (resonance assisted hydrogen-bond OH···N between the hydroxyl group and the azomethine N atom).[4,5] *Intra*-molecular proton transfer, and therefore the hydroxy-imino-ketoamino and thione-thiol tautomeric equilibrium, are governed by the shape and energy barrier between the minima in the corresponding potential wells.

A combined use of NMR spectroscopy and quantum chemical calculations has proven useful to study hydrogen-bonding of organic compounds in solution.[6–9]

Our previous investigations of hydrogen-bonding interactions in salicylaldehyde 4-phenylthiosemicarbazone by using experimental and theoretical methods[10,11] showed that the latter predominantly existed in the localized hydroxy-thione tautomeric form in solution, adopting conformation somewhat different compared to that in the solid state.

The present paper deals with two methoxy derivatives of salicylaldehyde 4-phenylthiosemicarbazone, *i.e.* 4- and 5-methoxysalicylaldehyde 4-phenylthiosemicarbazones (**1** and **2,** Figure 2). We are particularly interested to see whether the methoxy substituents will perturb hydrogen bonding and the tautomeric equilibrium and shift it toward other possible tautomers. This information can be useful in designing derivatives possessing bioactivity. Hence, we employ one- and two-dimensional NMR spectroscopy and DFT calculations to study conformation and hydrogen bonds in solution of **1** and **2**.

EXPERIMENTAL

NMR Measurements

NMR spectra were recorded on Bruker Avance 300 and 600 spectrometers from samples in 5 mm NMR tubes at
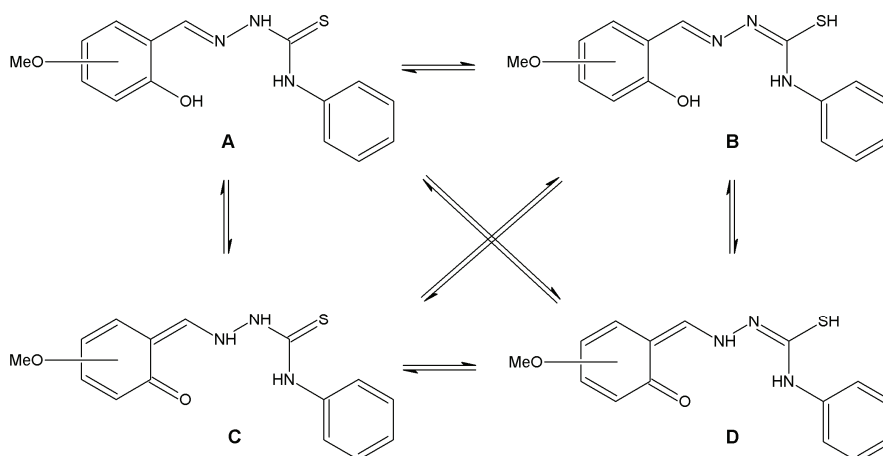

Figure 1. Tautomerism in methoxysalicylaldehyde thiosemicarbazones (**A** = hydroxy-thione; **B** = hydroxy-thiol; **C** = keto-thione; **D** = keto-thiol).

298 K. $CDCl_3$, CD_3COCD_3, CD_3OD, and DMSO-d_6 were used as solvents and TMS as internal standard. Proton spectra with spectral width of 6200 Hz and a digital resolution of 0.09 Hz per point were measured with 8–16 scans.

APT spectra with spectral widths of 7000 Hz and a digital resolution of 0.11 and 0.17 Hz per point, respectively, were collected with 1500−12000 scans.

Assignment of the ^{1}H and ^{13}C NMR signals in $CDCl_3$, CD_3COCD_3, CD_3OD, and DMSO-d_6 was performed using gradient-selected two-dimensional homo- and heteronuclear correlation experiments (gCOSY, gHSQC and gHMBC). In the gCOSY experiment 2046 points in the *f*2 dimension and 512 increments in the *f*1 dimension were used. For each increment 8 scans and the spectral width of 4007 Hz were applied. Digital resolution was 1.97 and 7.82 Hz per point in *f*2 and *f*1 dimensions, respectively. Typical spectral conditions for gHSQC and gHMBC spectra were as follows. Spectral width was 3906 Hz in *f*2 and

18870 Hz in *f*1 dimension for both experiments. 2K data points were applied in the time domains and for each data set 157 and 246 increments were collected for gHSQC and gHMBC spectra, respectively. The resulting digital resolution was 3.81 Hz per point in *f*2 dimension and 34.3 and 36.9 Hz per point in *f*1 dimension in gHSQC and gHMBC spectra, respectively.

Phase-sensitive TPPI NOESY spectra were recorded under the following conditions: spectral width was 6000 Hz in both dimensions, 2K data points were applied in the time domain and 512 increments were collected for each data set with linear prediction to 1K and zero filling to 2K. 4 scans were applied for each increment. A relaxation delay was 1.5 s. The spectra were obtained with the mixing time of 400 ms and processed with sine squared function shifted by π/2 in both domains.

Molecular Modeling

Quantum chemical calculations were performed at the B3LYP/6-311++G(d,p) level of the theory and the Gaussian 03 program package[12] was used. Geometry optimizations were performed separately for hydroxy-thione forms of **1** and **2**. For all these optimized structures harmonic frequencies were calculated to insure that the structures correspond to a local minimum on potential energy surface. Potential energy surfaces for internal rotation were computed at the same level and transition states were confirmed by calculating the harmonic frequencies. Calculated rotational barriers were estimated using the harmonic zero-point energy (ZPE) corrections of the minima and respective transition states.

The solvent effects of methanol, chloroform, acetone, DMSO and dichloromethane were introduced in the calculation using the reformulation of PCM (polarizable continuum method)[13,14] known as integral equa-
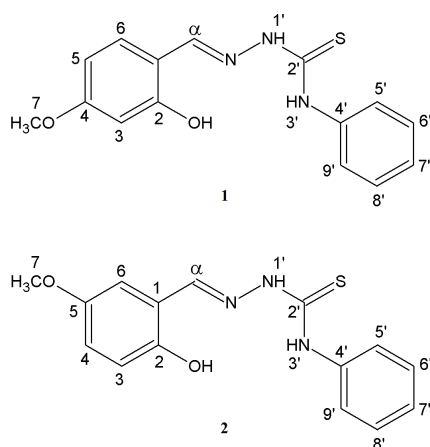

Figure 2. Structures and atom numbering of **1** and **2**.

tion formalism (IEFPCM; integral equation formulation of the polarizable continuum model) of Tomasiand co-workers.[15–18] Separately, for each molecule the complexes with one explicit molecule of solvent were formed and optimized. For determination of stabilization energy by *intra*-molecular hydrogen bond complexes without hydrogen bond were formed and optimized.

RESULTS AND DISCUSSION

NMR Assignments

Proton and carbon chemical shifts of **1** and **2** were deduced from a combined use of one- (^{1}H and APT) and two-dimensional NMR techniques (COSY, HSQC and HMBC). A comparison of chemical shifts of **1** and **2** in CD_3COCD_3, CD_3OD, and DMSO-d_6 with those in $CDCl_3$ are given in Table 1 and Table 2, respectively.

Chemical shift values point toward hydroxy-thione form as the predominant form of both **1** and **2** in all solvent used at ambient temperature, as was also found in the solid state.[19,20] The C-2' atom signals were found at ≈ 176 ppm in both **1** and **2**, which is similar to the value observed in non-substituted salicylaldehyde 4-phenylthiosemicarbazone.[10,11] Chemical shifts of C-2 atoms in **1** and **2** assigned at ≈ 158 ppm and ≈ 151 ppm, respectively, and C-α chemical shifts in the range

≈ 140–147 ppm (Tables 1–2) indicated the hydroxy form as the predominant one in **1** and **2** in solution. In the proton transferred NH-form, carbonyl C-2 atom is expected to be significantly deshielded (≈ 180 ppm),[10,11] and splitting of the H-α signal should be observed due to the scalar coupling to nitrogen. The shielding of C-2 atom in **2** with respect to those in **1** and salicylaldehyde 4-phenylthiosemicarbazone[6,7] was attributed to electron donating ability of the methoxy substituent in the *para* position. The same shielding effect was observed for the OH signal, which was found in the ranges ≈ 8.84–9.54 ppm and ≈ 9.45–10.05 in **1** and **2**, respectively. The broad OH signal and chemical shift values indicated the presence of the *intra*-molecular OH···N=C-α hydrogen bond in both **1** and **2** in solution, as was also detected in the solid state.[21,22] The OH proton in **2** exhibited an up-field shift with respect to **1** and the parent salicyl-aldehyde 4-phenylthiosemicarbazone which can be attributed to somewhat weaker *intra*-molecular hydrogen bonding in **2**. The thiourea H-1' and H-3' chemical shifts in both **1** and **2** were observed in the ranges ≈ 9.6–11.8 and ≈ 8.3–10.1, respectively. Compared to the corresponding proton shifts in the parent molecule, negligible differences were found, implying that the methoxy substituent did not have any significant effect on the structure of thiosemicarbazone part of the molecules **1** and **2**, and therefore on their interactions with solvent molecules. Correlation signals in HMBC spectra

Table 1. A comparison of ^{1}H and ^{13}C chemical shifts of **1** in CD_3COCD_3, CD_3OD and DMSO-d_6 with respect to $CDCl_3$

Atom	CDCl₃		CD₃COCD₃		CD₃CO		DMSO-d₆	
	$^1H\,\delta$ ppm	$^{13}C\,\delta$ ppm	Δ_H [(a)] ppm	Δ_C [(b)] ppm	Δ_H ppm	Δ_C ppm	Δ_H ppm	Δ_C ppm
α	7.99	147.49	0.46	−2.81	0.24	−4.59	0.41	−6.32
1		110.31		2.26		1.68		3.28
2		159.50		−0.45		−1.35		−0.99
3	6.52	101.58	−0.03	−0.12	−0.08	−1.46	−0.08	−0.36
4		163.61		−0.33		−1.06		−1.12
5	6.55	107.91	−0.02	−0.74	−0.18	−2.00	−0.09	−1.15
6	7.17	133.03	0.41	−1.83	0.41	−3.96	0.81	−4.03
7	3.84	55.58	−0.03	−0.35	−0.11	−1.66	−0.09	−0.02
1'	9.61		0.93		−		2.03	
2'		175.48		1.24		≈ -0.52		0.26
3'	8.31		1.32		−		1.66	
4'		137.57		2.07		0.86		2.06
5', 9'	7.58	124.88	0.11	0.23	−0.06	−0.07	0.00	0.95
6', 8'	7.42	128.99	−0.07	−0.53	0.13	−1.48	−0.07	−0.61
7'	7.31	126.68	−0.13	−1.22	−0.16	−2.18	−0.13	−1.29
OH	9.45		0.10		−		0.60	

[(a)] $\Delta_H = \delta_H$ (solvent) $- \delta_H$ ($CDCl_3$)

[(b)] $\Delta_C = \delta_C$ (solvent) $- \delta_C$ ($CDCl_3$)

Table 2. A comparison of ^{1}H and ^{13}C chemical shifts of **2** in CD$_3$COCD$_3$, CD$_3$OD and DMSO-d$_6$ with respect to CDCl$_3$

Atom	CDCl$_3$		CD$_3$COCD$_3$		CD$_3$CO		DMSO-d$_6$	
	^{1}H δ ppm	^{13}C δ ppm	Δ_H (a) ppm	Δ_C (b) ppm	Δ_H ppm	Δ_C ppm	Δ_H ppm	Δ_C ppm
α	8.01	146.58	0.53	−4.56	0.30	−5.11	0.47	−6.11
1		116.91		2.98		2.54		4.14
2		151.54		−0.54		−0.84		−0.17
3	6.93	117.89	−0.06	−0.77	−0.20	−1.65	−0.12	−0.45
4	6.96	119.61	−0.05	−1.39	−0.16	−1.60	−0.09	−1.06
5		153.26		−0.08		−0.66		−0.41
6	6.78	114.93	0.58	−3.13	0.55	−4.89	0.85	−3.91
7	3.79	55.99	−0.03	≈ −0.99	−0.08	−1.56	−0.06	0.14
1'	9.62		1.06		−		2.14	
2'		175.76		≈ 1.24		0.16		0.55
3'	8.40		1.37		−		1.67	
4'		137.42		1.79		0.99		2.26
5', 9'	7.58	124.91	0.13	−0.19	−0.06	−0.21	−0.02	1.45
6', 8'	7.42	129.02	−0.06	−0.92	−0.12	−1.51	−0.05	−0.53
7'	7.29	126.82	−0.09	−1.68	−0.14	−2.12	−0.09	−1.12
OH	8.85		−0.01		−		0.69	

(a) $\Delta_H = \delta_H$ (solvent) − δ_H (CDCl$_3$)

(b) $\Delta_C = \delta_C$ (solvent) − δ_C (CDCl$_3$)

between H-1' and C-α and between H-3' and C-5', 9', as well as long-range correlation between the H-α and H-1' protons in COSY spectra corroborated the existence of the thione form for both **1** and **2** in solution.

Conformation and Hydrogen Bonding in Solution

NOESY spectra provided further evidence on structural characteristics of **1** and **2** in solution. The NOE cross peaks are listed in Table 3. The NOE connectivities were mostly in accordance with the solid state structure and calculated molecular geometry. All NOE's that were found for the parent compound[11] were also found in the NOESY spectra of **1** and **2** indicating similar conformation of these compounds (Table 3). Furthermore,

Table 3. Non-trivial NOE cross peaks of **1** and **2** in DMSO-d$_6$ and proton–proton distances calculated by DFT

NOE cross peaks	Distance / pm	
	1	**2**
H-α, H-1'	2.238	2.242
H-α, H-6	2.352	2.354
H-α, OH	3.465	3.485
H-3', H-6	6.025	6.030
H-3', H-5',9'	3.739, 2.187	3.739, 2.190
H-3', OH	2.292	2.281
H-3, OH	3.520	3.508

previously observed NOE cross peak between protons H-6 and H-3' was also observed for **1** and **2** which strongly point towards the fact that solid state and solution state conformation slightly differ. The optimized geometries of hydroxy-thione form for **1** and **2** are presented in Figure 3. Obtained geometrical parameters are in a good agreement with experimental data.[19,20] In order to explain the close proximity of protons H-6 and
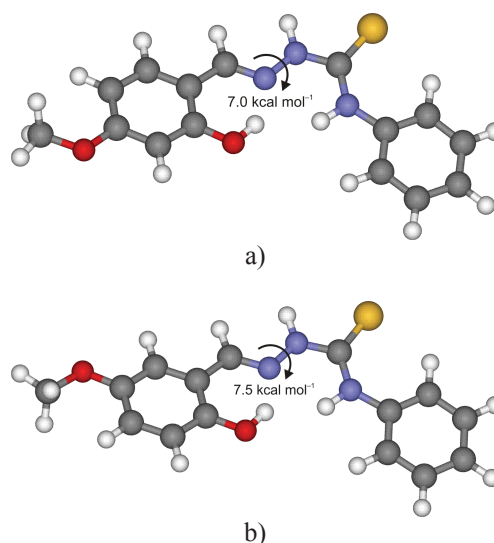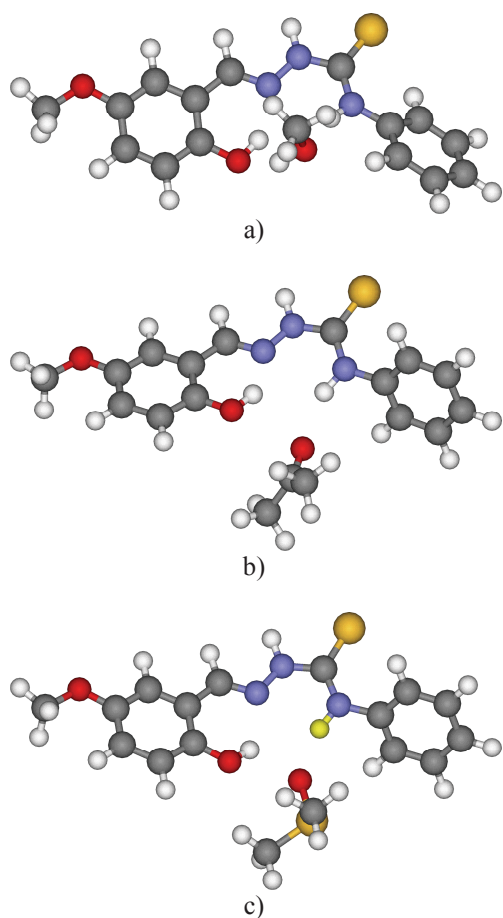

a)

b)

Figure 3. Geometry of the studied molecules: a) **1**, and b) **2** optimized at B3LYP/6-311++G(d,p) level of the theory.

Table 4. Relative energies of **1** and **2** calculated at the B3LYP/6-311++G(d,p) level of theory and using the IEFPCM solvent model

Relative energy kcal mol^{-1}	**1**	**2**
vacuum	0.0	0.0
CCl_4 ($\varepsilon_r = 2.23$)	−6.9	−6.9
$CHCl_3$ ($\varepsilon_r = 4.90$)	−12.4	−12.4
CH_2Cl_2 ($\varepsilon_r = 8.93$)	−15.5	−15.4
CH_3COCH_3 ($\varepsilon_r = 20.70$)	−17.9	−17.8
CH_3OH ($\varepsilon_r = 32.63$)	−19.2	−19.1
DMSO ($\varepsilon_r = 46.70$)	−19.1	−18.9

H-3' in solution we also calculated barriers of rotation around several bonds in the molecules. The lowest barriers were observed for the rotation around N–N bond and these were 7.0 kcal mol^{-1} and 7.5 kcal mol^{-1} in **1** and **2**, respectively (Figure 3). This evidence supports the conclusion that unlike in solid state the structure of salicylaldehydethiosemicarbazone part is not planar in solution and that the molecules exhibit conformational flexibility with portions of skewed conformations, as already found in the parent molecule.[11]

Since the NMR chemical shifts and correlation peaks in the two-dimensional spectra strongly points toward the hydroxy-thione form as the predominant form in solution we have next calculated relative energies of the hydroxy-thione forms for **1** and **2** using the IEFPCM approach. These are listed in Table 4. Furthermore, in order to explain in more details specific interactions in solution we calculated the complexes of **1** and **2** with explicit solvent molecules according to NMR data and stabilization energies by employing the PCM model. Several complexes of **1** and **2** with solvent molecules were investigated and the most interesting are shown in Figure 4 and Figure 5, respectively. In each complex the solvent molecule is creating the three center hydrogen bond with the oxygen and nitrogen atoms of **1** and **2**. Stabilization energies by *intra*-molecular hydrogen bond are getting lower with the dielectric constant of the solvent (Table 5). Therefore, in the more polar solvent the creation of *inter*-molecular hydrogen bond between the solvent and the parent molecule is facilitated. In the case of **1** this effect is more pro-

Figure 4. Complexes of **1** with one molecule of solvent: a) methanol, b) acetone, and c) DMSO, optimized at B3LYP/6-311++G(d,p) level of the theory.
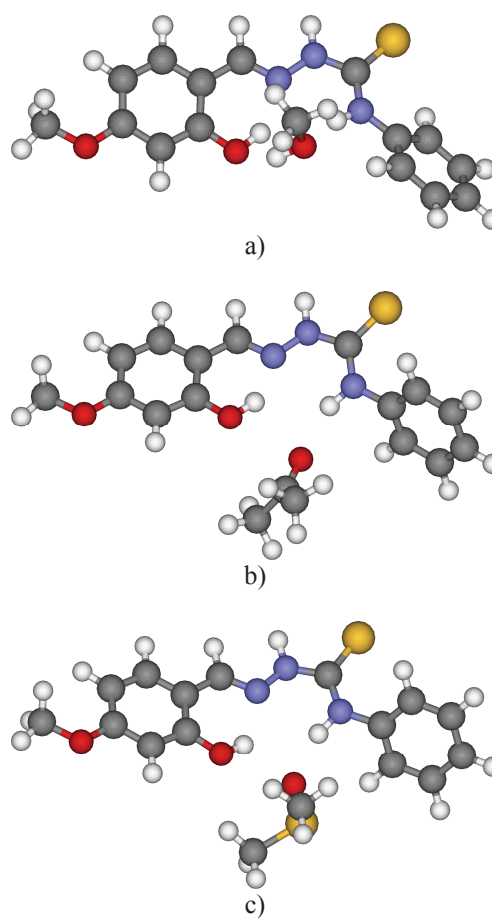

Figure 5. Complexes of **2** with one molecule of solvent: a) methanol, b) acetone, and c) DMSO, optimized at B3LYP/6-311++G(d,p) level of the theory.

Table 5. Comparison of stabilization energies by *intra*-molecular hydrogen bond for **1** and **2** calculated at the B3LYP/6-311++G(d,p) level of the theory *in vacuo* and using the PCM solvent model

Stabilization energy kcal mol^{-1}	**1**	**2**
vacuum	−6.6	−5.9
CCl$_4$ ($\varepsilon_r = 2.23$)	−5.6	−4.9
CHCl$_3$ ($\varepsilon_r = 4.90$)	−4.8	−4.2
CH$_2$Cl$_2$ ($\varepsilon_r = 8.93$)	−4.1	−4.0
CH$_3$COCH$_3$ ($\varepsilon_r = 20.70$)	−4.0	−2.7
CH$_3$OH ($\varepsilon_r = 32.63$)	−3.3	−2.4
DMSO ($\varepsilon_r = 46.70$)	−3.8	−2.6

nounced with respect to **2** and the parent salicylaldehyde 4-phenylthiosemicarbazone molecule.[10,11] This is in a good agreement with the observed OH chemical shift values. Namely, the largest deshielding effect was observed for OH group in compund **1** (Tables 1 and 2) reflecting the strongest *inter*-molecular H-bonding *inter*-actions.

Supplementary Materials. – Supporting informations to the paper are enclosed to the electronic version of the article. These data can be found on the website of *Croatica Chemica Acta* (http://public.carnet.hr/ccacaa).

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SAŽETAK

Istraživanje strukture i vodikovih veza derivata metoksisalicilaldehid tiosemikarbazona u otopini pomoću metoda NMR i DFT

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Istražene su struktura i vodikove veze u otopini 4- i 5-metoksisalicilaldehid 4-feniltiosemikarbazona, **1** i **2**, primjenom NMR i DFT metoda. Dokazano je da obje molekule postoje u hidroksi-tionskom tautomernom obliku u otopini, kao i u čvrstom stanju. Utvrđeno je postojanje dviju vrsta vodikovih veza u otopini, *intra-* i *inter*-molekulskih. Prema DFT izračunima i vrijednostima kemijskih pomaka može se zaključiti, da postoje trocentrične vodikove veze (koje uključuju OH i NH skupine) između istraživanih molekula tiosemikarbazona te molekula otapala. Opaženo je da se struktura molekula **1** and **2** u otopini nešto razlikuje od onih u čvrstom stanju, što je pripisano malom iznosu energije barijere rotacije oko N–N veze.