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(4*R*)-4-Hydroxy-1-nitroso-L-proline: synthesis, X-ray structure, ab initio and conformational calculations

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Abstract—4-Hydroxy-L-proline, an amino acid, an important component of collagen, was transformed into its *N*-nitroso-derivative, (4*R*)-4-hydroxy-1-nitroso-L-proline, **1** by butylnitrite in the acidic medium. The structure is a cyclic hydroxy-*N*-nitrosoacid with the carboxyl and hydroxyl groups trans to each other. The carboxyl group is in the *syn*-conformation. In the structure, the neutral molecules are connected via classical intermolecular O–H···O hydrogen bonds involving the hydroxyl and carboxyl groups [O···O=2.6251(14) Å], and form chains along the *a*-axis direction. The chains are linked into sheets via O–H···O hydrogen bond, [O···O=2.6813(15) Å] with participation of oxygen atom of nitroso group. Ab initio calculations based on density functional theory at the B3LYP/6-311++G(d, p) level of theory were performed to analyze the influence of 4-hydroxy-L-proline (Hyp) nitrosation on the conformation of the synthesized *N*-nitroso-compound. The geometry optimization of **1** and initial 4-hydroxy-L-proline was carried out in the gas phase and in solution using the polarizable continuum model. The single-point calculation was performed for the crystal structure of **1**. The most stable conformer of **1** is observed in an aqueous solution. In this state, the pyrrolidine ring adopts an envelope conformation, which is also maintained in the gas phase. The twisted conformation of the pyrrolidine ring is present in all states of Hyp and in the crystal structure of **1**. In **1** the interchange of five-membered ring conformation in solution and in the gas phase in comparison with the crystal is accompanied by an increase of the dipole moment of the molecule, which is maximal in solution.

1. Introduction

The functional specificity of proteins and polypeptides depends on the conformational behavior of the initial amino acids, which are extensively studied by theoretical and experimental methods.[1] L-Proline (Pro) and 4(*R*)-hydroxy-L-proline (Hyp) are abundant amino acids in collagen and exceptional among amino acids, as they are the only ones that have the amino group fixed within a pyrrolidine ring, making it rigid and directional in biological systems despite its conformational flexibility. Due to its crucial biological role (it is the most abundant protein in vertebrates) and very peculiar structure, collagen has been deeply investigated, together with several related polypeptides. Collagen is composed of approximately 300 repeats of the sequence $X_{aa}Y_{aa}Gly$, where X_{aa} and Y_{aa} are in most cases Pro and Hyp, respectively. The occurrence of Pro and Hyp in collagen restricts the orientational freedom of the chain in relation to the fiber axis and permits only left-handed helices.[2]

The importance of Pro and Hyp explains the extensive theoretical and structural studies of these amino acids and their derivatives including several di- and polypeptides. Aside from recent electron-density studies and quantum chemical investigations, the study of amino acid clusters in the gas phase has attracted considerable attention. The stereoelectronic effects crucial for the collagen stability have been studied, as well as quantum mechanical calculations for Pro, Hyp, and fluorinated dipeptide analogues in aqueous solution and for the Pro gaseous isomers have been fulfilled.[3] Moreover, in a recent paper, Conticello et al. discuss in detail the stereoelectronic effects in elastin-mimetic polypeptides due to Pro substitution.[4]

The structural studies of vitally important amino acids are fundamentally important. With respect to the metal free Pro and Hyp, so far the crystal structures of L-Pro and DL-Pro,[5,6]

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the monohydrates of L-Pro and DL-Pro[7,8] and numerous other solvates and salts of Pro have been determined. For Hyp and its derivatives, structural data are available for the zwitterion of 4-hydroxy-L-proline itself defined by X-ray analysis[9,10] and precision neutron diffraction,[11] 4-hydroxy-L-proline hydrate,[12] 4-hydroxy-*N*-methylproline,[13] and *N*,*N*′-dimethylated 4-hydroxy-D- and 4-hydroxy-L-proline chloride.[14] The acyclic peptides, ALB*-ALB-PRO* (three residues) and PRO*-PRO* were also described.[15,16]

As the only amino acids with the imino group, Pro and Hyp were extensively studied to elucidate the conditions responsible for the generation of carcinogenic *N*-nitroso-derivatives. A dose–response study on the endogenous formation of *N*-nitrosoproline was performed in rats, dogs, and in smokers and non-smokers. Solutions containing nitrite, proline, and in some cases ascorbic acid (ASC) and/or SCN^- were infused into the stomach and samples taken to determine gastric [nitrite], [NPro], [ASC], [SCN^-], and pH as functions of time. The ability of ASC to inhibit nitrosation (by reaction with nitrite) was shown to be highly dependent on initial [ASC] and on the rate of entry of O_2 into the stomach from blood. The rate of *N*-nitroso-Pro formation in the absence of ASC and SCN^-, the inhibitory effects on nitrosation of ASC, and the catalytic effects of SCN^- were all accurately predicted by the mathematical model.[17]

2. Results and discussion

In continuation of our previous studies devoted to the conditions of mutagenesis of the biologically important molecules to their carcinogenic *N*-nitroso-derivatives[18] and the stereochemistry of *N*-nitroso-compounds,[19] the nitrosation of 4-hydroxy-L-proline (**Hyp**) by butylnitrite[20] in the acidic medium has been fulfilled and the crystal structure of *N*-nitroso-derivative, 4-hydroxy-1-nitrosopyrrolidine-2-carboxylic acid **1**, was studied by X-ray crystallography (Fig. 1). The aim of this article is to study the product of **Hyp** nitrosation analyzing the structural peculiarities of *N*-nitrosooxyproline in the crystalline, liquid, and gaseous phases.

X-ray structural analysis was carried out at 100 K that permits the unambiguous localization of all the hydrogen atoms and to state that the molecule exists in the crystal in its neutral form. In the neutral molecule **1**, the hydroxyl and carboxyl groups are in the trans configuration relative to the ring, as found earlier for the zwitterions of L-Hyp,[9–11] 4-hydroxy-*N*-methylproline[13] and its hydrochloride salt[14] and differs from *cis*-zwitterion of Hyp in its dihydrate.[12] Molecular bond distances and bond angles as well as torsion angles, labeled according to the IUPAC conventions,[21] are given in Tables 1 and 2, respectively. The average C–C length of 1.530(2) Å is in agreement with 1.533(8) Å in Hyp dihydrate. The carboxylic group is in a *syn*-orientation and practically planar with the asymmetrical C(1)–O(2) 1.204(2) and C(1)–O(1) 1.328(2) Å distances which are consistent with the double and single C–O bonds. C(4)–O(3) length of 1.433(2) Å is consistent with 1.435(7) Å obtained by Shamala et al.[12] The *N*-nitroso-group has an ordinal geometry[19] with the equalized O(4)–N(2) 1.266(1) and N(1)–N(2) 1.289(2) Å distances and N–N–O bond angle O(4)–N(2)–N(1) 113.3(1)°. The dihedral angles formed by the carboxyl [O(1)/C(1)/O(2)] and nitroso [N(1)/N(2)/O(4)] groups with the pyrrolidine ring are equal 65.4(1)° and 11.4(1)°, respectively.

Two approaches could be applied to describe the puckering of the pyrrolidine ring. Generalization by Cremer and Pople[22] requires specification of an appropriate mean plane and uses the displacements of each atom from that plane to define puckering coordinates (q is a puckering amplitude and ψ is a phase angle). Geise et al.[23] use endocyclic torsion angles and thus avoid the need to define a mean plane. We use the advantages of both of these approaches and say that the pyrrolidine ring is in a twisted form with C^γ [C(4)] atom lying 0.608(2) Å [0.516 Å in Hyp[11]] below the plane through N, C^α, C^β, and C^δ, and with the latter four atoms coplanar to within 0.0367 Å [0.037(3) Å in Hyp]. The dihedral angle between the planes N–C^α–C^β–C^δ and C^α–C^β–C^γ–C^δ is 39.6(1)° [33.5° in Hyp]. The pyrrolidine ring conformation corresponds to the C^γ-*endo* or down puckering. The Cremer and Pople puckering ring parameters for **1** are gathered in Table 3 in comparison with those for Hyp and Pro derivatives. The closeness of the conformations for **1**, Hyp, and Pro monohydrates is evident.

To compare the puckering of the pyrrolidine ring in **1** and some derivatives of Hyp we use the data available in the Cambridge Structural Database.[24] Compound **1** and some structurally related molecules were fitted by the same atoms in the pyrrolidine ring skeleton. Figure 2 reveals the overlap

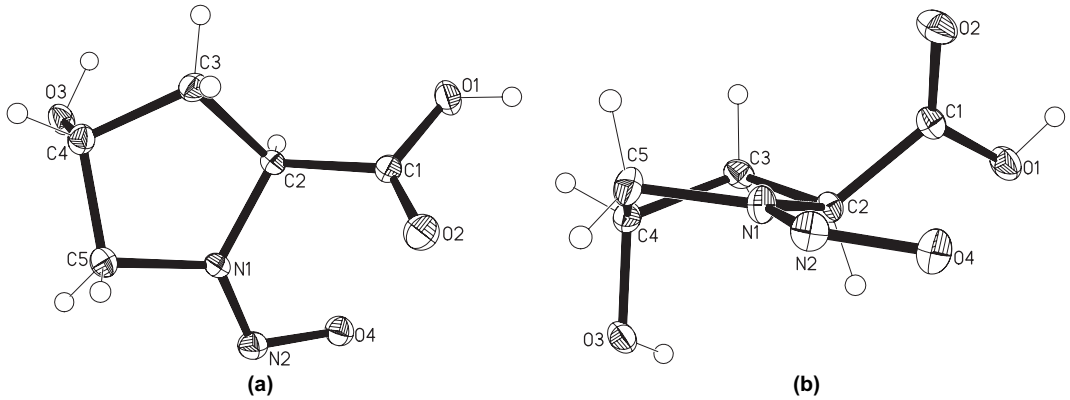

Figure 1. ORTEP (a) top and (b) side view of **1**. Thermal ellipsoids are drawn with the 50% probability level.

Table 1. Bond lengths (Å) and angles (degree) for **1** in crystal, in the gas phase (ε=1), and in solution (ε=78.4)

	Crystal	ε=1	ε=78.4
Bond distances			
O(1)–C(1)	1.328(2)	1.349	1.334
O(1)–H(1)	0.83(3)	0.97	0.99
O(2)–C(1)	1.204(2)	1.203	1.211
O(3)–C(4)	1.433(2)	1.426	1.427
O(3)–H(3O)	0.90(2)	0.96	0.98
O(4)–N(2)	1.266(1)	1.226	1.242
N(1)–N(2)	1.289(2)	1.319	1.299
N(1)–C(2)	1.473(2)	1.466	1.472
N(1)–C(5)	1.477(2)	1.465	1.472
C(1)–C(2)	1.529(2)	1.524	1.523
C(2)–C(3)	1.536(2)	1.547	1.548
C(2)–H(2)	0.97(2)	1.09	1.1
C(3)–C(4)	1.532(2)	1.541	1.541
C(3)–H(31)	0.98(2)	1.09	1.09
C(3)–H(32)	0.96(2)	1.09	1.09
C(4)–C(5)	1.522(2)	1.528	1.526
C(4)–H(4)	0.97(2)	1.1	1.1
C(5)–H(51)	0.91(2)	1.09	1.09
C(5)–H(52)	0.96(2)	1.09	1.09
Bond angles			
C(1)–O(1)–H(1)	109(2)	107	109
C(4)–O(3)–H(3O)	109(2)	109	109
N(2)–N(1)–C(2)	124.4(1)	123.4	124.2
N(2)–N(1)–C(5)	121.7(1)	121.6	121.6
C(2)–N(1)–C(5)	113.5(1)	114.2	113.6
O(4)–N(2)–N(1)	113.3(1)	114.4	115.2
O(2)–C(1)–O(1)	125.6(1)	123.9	124.5
O(2)–C(1)–C(2)	124.5(1)	124.8	124.5
O(1)–C(1)–C(2)	109.8(1)	111.2	111.0
N(1)–C(2)–C(1)	111.9(1)	111.6	111.9
N(1)–C(2)–C(3)	101.7(1)	102.2	102.4
C(1)–C(2)–C(3)	112.4(1)	112.0	112.4
N(1)–C(2)–H(2)	107(1)	109	109
C(1)–C(2)–H(2)	111(1)	109	109
C(3)–C(2)–H(2)	113(1)	113	112
C(4)–C(3)–C(2)	103.7(1)	104.5	104.4
C(4)–C(3)–H(31)	111(1)	109	109
C(2)–C(3)–H(31)	109(1)	113	110
C(4)–C(3)–H(32)	113(1)	110	112
C(2)–C(3)–H(32)	110(1)	113	112
H(31)–C(3)–H(32)	110(2)	108	109
O(3)–C(4)–C(5)	109.0(1)	107.1	107.5
O(3)–C(4)–C(3)	110.5(1)	112.4	112.1
C(5)–C(4)–C(3)	102.8(1)	103.1	102.9
O(3)–C(4)–H(4)	111(1)	111	111
C(5)–C(4)–H(4)	112(1)	112	112
C(3)–C(4)–H(4)	112(1)	113	111
N(1)–C(5)–C(4)	101.6(1)	102.5	102.4
N(1)–C(5)–H(51)	108(1)	111	110
C(4)–C(5)–H(51)	116(1)	110	113
N(1)–C(5)–H(52)	107(1)	112	110
C(4)–C(5)–H(52)	111(1)	111	111
H(51)–C(5)–H(52)	113(2)	110	110

of two molecules. It is evident that the best fitting was found for the zwitterion of Hyp, where the atoms of the pyrrolidine rings of the two molecules fit within 0.06 Å. Figure 2 also reveals an essential difference in an arrangement of the carboxylic and hydroxyl groups that is evident from the corresponding torsion angles. Torsion angles for Hyp in Table 2 are taken from Ref. 11.

A view of the molecular packing and hydrogen-bonding scheme is shown in Figure 3. The hydrogen-bonding geometry is summarized in Table 4. A classical hydrogen bond between the carboxyl hydrogen and the hydroxyl oxygen links the molecules into chains parallel to the [100] direction that can be described by the first level graph set, C(7).[26] Along the [010] direction the hydroxyl group acts as H-donor, being involved in an O–H···O hydrogen bond with the nitroso group oxygen via again C(7) graph set and the second level graph set, $R_4^4(23)$, thus generating the H-bonded sheet. Between the layers only one type of contact C(3)–H(31)···O(4) 3.152(2) Å deserves to be mentioned.

The rather close crystal packing is observed in Hyp itself,[9–11] where the molecules are also combined in the chains via the same C(7) O–H···$^-$OOC synthon, and the chains being further combined in the layer via NH_2^+(ammonium)···$^-$OOC(carboxyl) interactions analogous to those with nitroso group involvement in **1**.

In the analysis of the conformational and intermolecular characteristics of *N*-nitroso-compounds, it is of basic importance to consider environmental effects, so the ab initio calculations at the density functional theory (DFT) level have been thus fulfilled both in gas phase (ε=1) and in an aqueous solution (ε=78.4). To obtain a more definitive estimate of the conformational flexibility and relative stabilities of **1** in different states, geometry optimization was carried out in the solution using the polarizable continuum model (PCM) and in the gas phase. The single-point calculation was performed for the crystal structure. The computed SCF energies and energy differences for **1** in different states are summarized in Table 5.

The criteria of Cremer and Pople[22] have been used to analyze the non-planar character of the five-membered ring. Thus, a 'pure envelope' conformation with apex at 1 would be such that

$$z_2 = z_5,\ z_3 = z_4, \quad \psi = k \times 36, \tag{1}$$

and a 'pure twist' with axis through 1 would have

$$z_1 = z_2 + z_5 = z_3 + z_4, \quad \psi = k \times 36 + 18, \tag{2}$$

where the values of z_i are the displacements of *i*th atom perpendicular to the least-square plane.

However, it should be noted that for a ring with unequal lengths and angles, the conditions in Eq. 1 do not necessarily imply coplanarity of atoms 2–5. The list of z_i values for **1** in different states is summarized in Table 6.

The most stable conformer of **1** is observed in an aqueous solution. In this state according to the criteria of Cremer and Pople (Tables 3 and 6) the pyrrolidine ring adopts an envelope conformation, which is also maintained in the gas phase. The deviations of the carbon atom C(4) from the best-fit planes [N(1)/C(2)/C(3)/C(5)] in the solution and gas phase are equal to −0.57 and −0.56 Å, respectively. In the crystal structure of **1** the pyrrolidine ring adopts a conformation, which is twisted on C(3)–C(4) bond. Therefore for **1** we performed the conformational search (CS) implemented in Hyperchem 6.03 using the MM+ force field varying the dihedral angles inside the pyrrolidine ring. The envelope conformation of this ring is maintained in the lowest energy

Table 2. Torsion angles (degree) for **1** and L-Hyp[11]

IUPAC designation[21]	Atoms involved		**1**	L-Hyp[11]
ψ^1	O^1–C–C^α–N^1	O(1)–C(1)–C(2)–N(1)	151.2(1)	−3.2(2)
ψ^2	O^2–C–C^α–N	O(2)–C(1)–C(2)–N(1)	−31.6(2)	178.7(1)
χ^1	N^1–C^α–C^β–C^γ	N(1)–C(2)–C(3)–C(4)	−30.0(1)	−18.3(2)
$\chi^{2,1}$	C^α–C^β–C^γ–O^δ	C(2)–C(3)–C(4)–O(3)	−73.6(1)	−87.0(2)
$\chi^{2,2}$	C^α–C^β–C^γ–C^δ	C(2)–C(3)–C(4)–C(5)	41.4(1)	32.0(2)
$\chi^{3,1,1}$	$H^{\delta1}$–O^δ–C^γ–C^β	H(3O)–O(3)–C(4)–C(3)	−30.7(2)	−158.8(2)
$\chi^{3,1,2}$	$H^{\delta1}$–O^δ–C^γ–C^δ	H(3O)–O(3)–C(4)–C(5)	−142.4(2)	87.7(2)
$\chi^{3,2,1}$	O^δ–C^γ–C^δ–N	O(3)–C(4)–C(5)–N(1)	81.7(1)	84.0(1)
$\chi^{3,2,2}$	C^β–C^γ–C^δ–N^1	C(3)–C(4)–C(5)–N(1)	−35.2(1)	−33.5(2)
χ^4	C^γ–C^δ–N^1–C^α	C(4)–C(5)–N(1)–C(2)	17.3(1)	23.1(1)
χ^5	C^δ–N^1–C^α–C^β	C(5)–N(1)–C(2)–C(3)	8.0(1)	−3.1(1)
	O^4–N^2–N^1–C^α	O(4)–N(2)–N(1)–C(2)	4.6(2)	—
	O^4–N^2–N^1–C^δ	O(4)–N(2)–N(1)–C(5)	176.6(1)	—

Table 3. Computed ring puckering parameters for **1**, L-Hyp,[a] and Pro[b]

Compound	Crystal		Solution (ε=78.4)		Gas phase (ε=1)		Conformers **1a**, **1H**	
	q/Å	ψ/°	q/Å	ψ/°	q/Å	ψ/°	q/Å	ψ/°
1	0.403(1)	276.1(2)	0.373	0.363	279.3	281.9	0.330	280.2
L-Hyp	0.236	309.6	0.376	0.371	270.3	314.1	0.384	336.4
DL-Pro	0.403(2)	57.7(2)						
L-Pro	0.404	89.1						
DL-Pro monohydrate	0.4033(5)	308.63(7)						
L-Pro monohydrate	0.395(4)	309.9(6)						

[a] Atomic coordinates for the computing calculations are taken from Cambridge Structural Database (refcode HOPROL).
[b] All cited puckering parameters for Pro are taken from Ref. 6.

conformer **1a** according to the criteria of Cremer and Pople (Tables 3 and 6). In this molecule the deviation of the C^γ [C(4)] carbon atom from the best-fit plane [N(1)/C(2)/C(3)/C(5)] is equal to −0.51 Å and the values of O(2)–C(1)–C(2)–N(1) (−52.0°) and O(3)–N(2)–N(1)–C(2) (11.1°) torsion angles are maximal in comparison with those in other states. For the crystal, gas, and solvent states these values are equal to −31.6(2)°, 4.6(2)°; −41.18°, 6.55°; and −33.67°, 5.86°, respectively. The change of pyrrolidine ring conformation in solution and in gas phase in comparison with the crystal is accompanied by an increase in the dipole moment of the molecule, which is maximal in solution (Table 5).

In order to study the influence of nitrosation on the conformation of pyrrolidine ring, the DFT-based geometry optimizations as well as the CS implemented in Hyperchem 6.03 were carried out for hydroxy-L-proline (Hyp) in solution, gas, and crystal phases. The atomic numbering scheme for hydroxy-L-proline (Hyp) is the same as that of compound **1**. As in the case of **1**, the most stable conformer is observed in solution (Table 5). In the crystal and in solution, the pyrrolidine ring adopts the conformation twisted on the C(4)–C(5) bond. In the gas phase and in the conformer **1H** the pyrrolidine rings are twisted on C(3)–C(4) and N(1)–C(5) bonds, respectively. Thus, the twisted conformation of the pyrrolidine ring is present in all conformers of Hyp and in the crystal structure of **1**. However, due to nitrosation of Hyp, this ring in the crystalline state is twisted on the C(3)–C(4) bond, while in the same state of Hyp the ring is twisted along the C(4)–C(5) bond. In solution and in the gas phase after nitrosation the pyrrolidine ring in **1** adopts an envelope conformation. The optimized geometries of **1** and Hyp in solution and in gas phase as well as bond distances and bond angles in their crystal structures are listed in Table 1.

3. Conclusion

4-Hydroxy-L-proline, an amino acid, an important component of collagen, was transformed into its potentially carcinogenic *N*-nitroso-derivative, 4-hydroxy-1-nitroso-L-pyrrolidine-2-carboxylic acid by butylnitrite in the acidic medium. The X-ray structural analysis and ab initio calculations based on density functional theory at the B3LYP/6-311++G(d, p) level of theory were performed to analyze the influence of the *N*-nitroso-group on conformation of synthesized *N*-nitroso-compound. The most stable conformer of **1** was observed in an aqueous solution. In this state the pyrrolidine ring adopts an envelope conformation, which is maintained in the gas phase. In the crystal structure of **1** this ring adopts a twisted conformation. The change of the five-membered ring conformation in solution and gas phase in comparison with the crystal is accompanied by an increase of the dipole moment of the molecule, which is maximal in solution.

4. Experimental

4.1. General

The initial chemicals were used as received without further purification. IR spectra were recorded on a Specord-80 spectrophotometer as KBr disks. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker DPX-250 instrument (300 MHz ^{1}H; 75 MHz ^{13}C) in dimethylsulfoxide using TMS as an internal reference. Mass spectra were recorded on a MX-1321 device. Mass spectrometer operating at 70 eV. Perkin–Elmer 241 MC polarimeter was used for $[\alpha]_D^{20}$ measurements. Crystallographic measurements were carried out on a PX

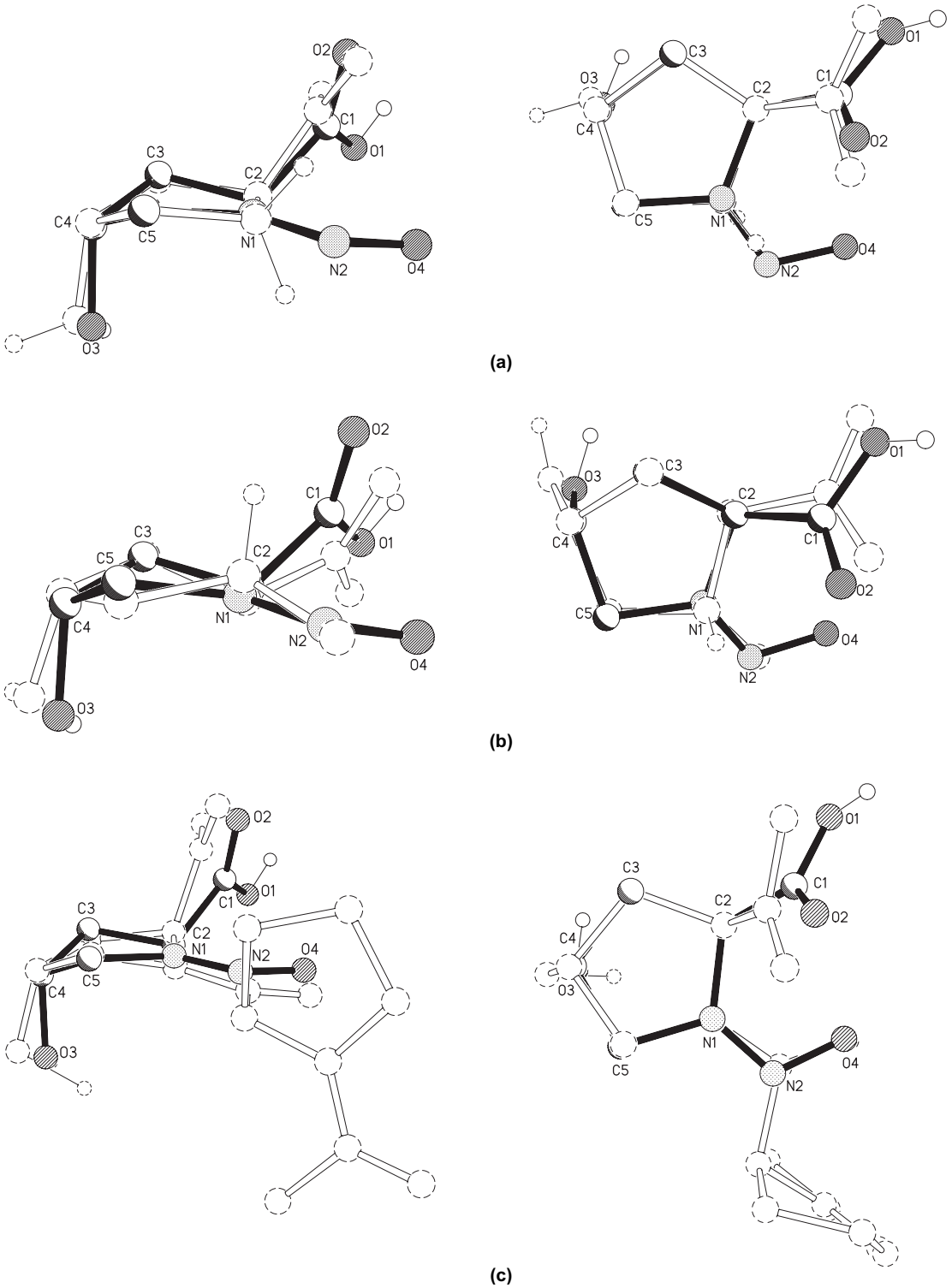

Figure 2. Fitting of the molecules **1** and Hyp derivatives (side and top views). Molecule of **1** is shown by solid lines. C-bound H-atoms are omitted for clarity. (a) **1** and 4-hydroxy-L-proline[11] (refcode HOPROL12 in CSD). Atoms of the pyrrolidine ring fit within 0.062 Å; (b) **1** and 4-hydroxy-*N*-methylproline[13] (refcode UGUHOT in CSD). Atoms of the pyrrolidine ring fit within 0.172 Å; and (c) **1** and *N*-acetyl-L-prolyl-l-4-hydroxyproline[25] (refcode GLHPRC in CSD). Atoms of the pyrrolidine ring fit within 0.168 Å.

kappa-geometry diffractometer with Onyx CCD camera at 100 K. The structure was solved by direct methods and refined by full-matrix least-squares on F^2 using SHELX-97 package.[27] All non-hydrogen atoms were refined anisotropically. Locations of all H-atoms were justified by difference Fourier synthesis and refined isotropically. The absolute configuration was not been defined.

Ab initio calculations were carried out using density functional theory with the Gaussian 03 package at the B3LYP/6-311++G(d, p) level of theory.[28] Polarizable continuum model (PCM) was included in the SCF procedure for the description of aqueous solution. The dielectric constant was set at 78.4. All calculations were carried out using the restricted spin formalism (closed-shell). Crystallographic

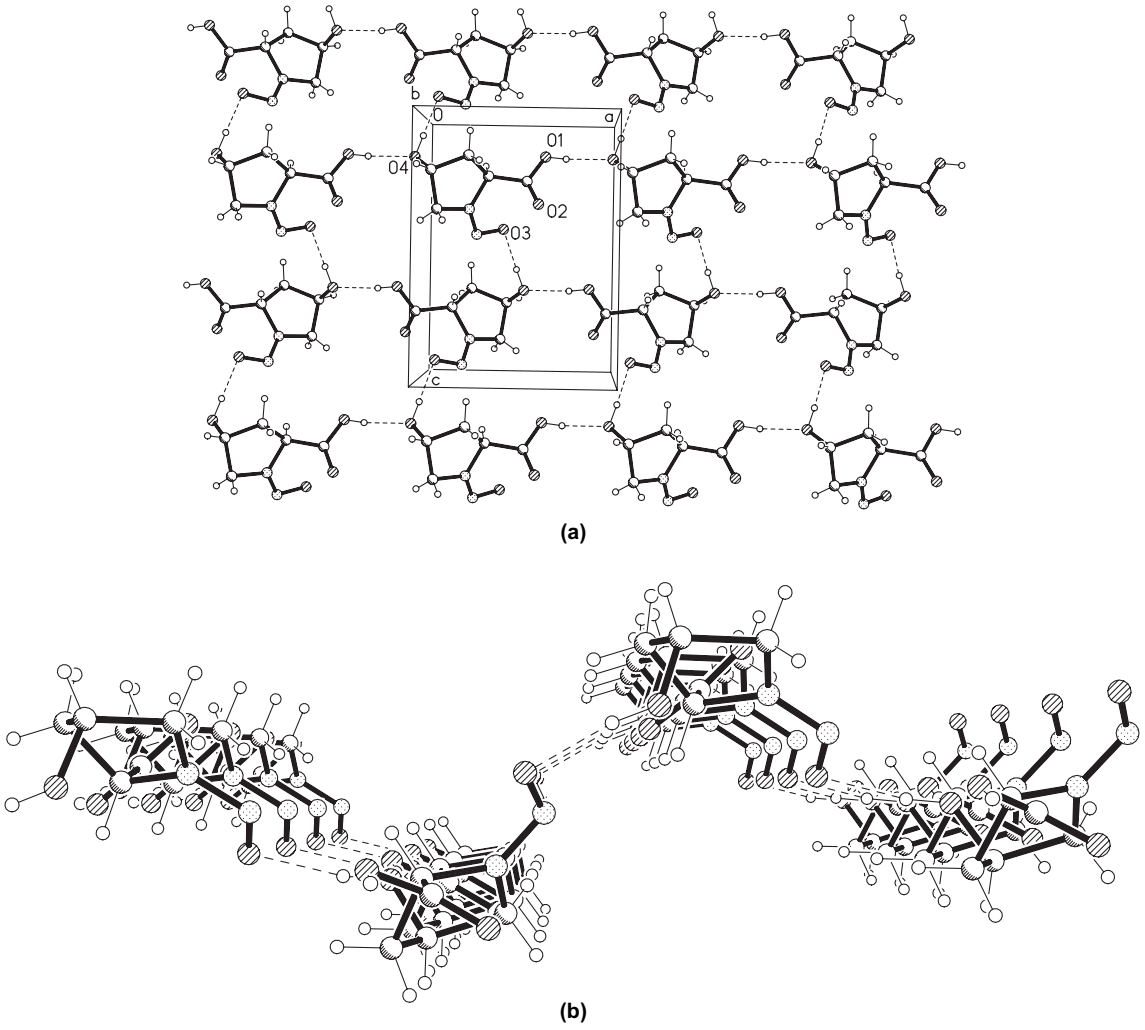

Figure 3. (a) Top and (b) side view of the layer of molecules **1**.

Table 4. Hydrogen-bonding geometry in **1**

D–H···A	*d*(H···A)/Å	*d*(D···A)/Å	∠DHA/°	Symmetry code for acceptor
O(1)–H(1)···O(3)	1.81(3)	2.625(1)	167(3)	*x*+1, *y*, *z*
O(3)–H(3O)···O(4)	1.79(2)	2.681(2)	171(2)	−*x*+1/2, −*y*+1, *z*−1/2
O(3)–H(3O)···N(2)	2.47(2)	3.254(2)	146(2)	−*x*+1/2, −*y*+1, *z*−1/2
C(3)–H(31)···O(4)	2.50(2)	3.152(2)	124(1)	−*x*+1, *y*+1/2, −*z*+1/2

data available for **1** and Hyp provided the initial geometries. The search of conformers was performed using the module conformational search implemented in Hyperchem 6.03[29] for finding low energy conformations of molecular systems by varying user-specified dihedral angles. The method involves random variation of dihedral angles to generate new structures and then energy minimizing of these angles using the MM+ force field. The dihedral angles in a ring are rotated by the 'torsion flexing' motion of Kolossvary and Guida,[30] which effectively leads to new ring conformations while avoiding large atomic displacements that can decrease the efficiency of optimization. The analysis of conformations has been carried out using the PLATON program package.[31]

Table 5. Computed SCF energies, energy differences, and dipole moments for **1** and Hyp compounds in the crystal, gas phase, and in solution and conformers **1a** and **1H**[a]

	1			Hyp		
	E/au	Δ*E*/au	*μ*/D	*E*/au	Δ*E*/au	*μ*/D
Crystal	−605.7354	0	6.3821	−476.3587	0	2.1041
Gas phase	−605.864	0.1286	6.0627	−476.5233	0.1646	1.87
Solution	−605.902	0.1666	9.1683	−476.5532	0.1945	2.4104
1a, **1H**	−605.7952	0.0598	6.0367	−476.4664	0.1077	5.7367

[a] Conformers found by the conformational search implemented in Hyperchem 6.03 for **1** (**1a**) and **Hyp** (**1H**).

Table 6. Displacements of pyrrolidine ring atoms from the least-square plane

	z_1	z_2	z_3	z_4	z_5
I					
Atoms of the pyrrolidine ring	N(1)	C(2)	C(3)	C(4)	C(5)
Crystal	0.027(1)	0.129(1)	−0.2324(14)	0.249(1)	−0.1707(14)
Gas phase	0.037	0.1032	−0.204	0.2269	−0.1631
Solution	0.0485	0.0963	−0.2044	0.2344	−0.1748
1a	0.0385	0.0894	−0.1831	0.2069	−0.1516
Hyp					
Crystal	0.0951	−0.0093	−0.0801	0.1389	−0.1446
Gas phase	0.0014	0.1369	−0.2229	0.2237	−0.1391
Solution	0.1657	−0.0337	−0.1112	0.2136	−0.2344
1H	0.223	−0.123	−0.0237	0.1613	−0.2374

4.1.1. 4-Hydroxy-1-nitroso-L-pyrrolidine-2-carboxylic acid, 1. 4-Hydroxy-L-proline (0.2 g) was stirred with the solution of butylnitrite (0.3 ml) and glacial acetic acid (0.2 ml) at 25 °C for 7 days. The colorless precipitate of 4-hydroxy-1-nitrosopyrrolidine-2-carboxylic acid was filtered off and recrystallized from a 1:1 mixture of *n*-butanol (5 ml) and methanol (5 ml). The yield of 4-hydroxy-1-nitrosopyrrolidine-2-carboxylic acid was 82%, mp=138–140 °C (dec). Anal. Calcd for $C_5H_8N_2O_4$: C, 37.50; H, 5.04; N, 17.49. Found: C, 37.47; H, 5.09; N, 17.53. IR (cm^{-1}, peaks of strong absorption only, Vaseline oil): 3500–3200, 3170, 3150, 3125, 3120, 3110, 3090, 3080, 3050, 3040, 3030, 3020, 3000–2800, 2780–2570, 1730, 1430, 1365, 1310, 1290, 1280, 1260, 1250, 1190, 1130, 1060. ^{1}H NMR δ (ppm) (DMSO-d_6, 300 MHz): 1.61 m (2H, CH_2), 2.78 m (2H, CH_2), 3.35 m (1H, CH). ^{13}C NMR δ (ppm) (DMSO-d_6, 300 MHz): 34.62 (C–CH_2–C), 54.15 (C–CH_2–N), 56.18 (C–CH–COOH), 67.54 (C–CH_2–OH), 172.94 (COOH). MS: *m/z* 160(22) (calcd for $C_5H_8N_2O_4$, 160.128) 130(5), 115(46), 88(20), 86(24), 85(16), 84(22), 68(15), 56(100); $[\alpha]_D^{20}$ −1.47.

Crystal data for (**1**), orthorhombic, $P2_12_12_1$, a=7.644(2), b=8.849(3), c=10.303(3) Å, V=696.9(4) Å^3, Z=4, D_x=1.526 g cm^{-3}, T=100 K, λ(Mo Kα)=0.71073 Å, μ=1.33 cm^{-1}, F(000)=336, GooF=1.097, R indices (all data) $R1$=0.0337, $wR2$=0.0836 for 1369 reflections and 132 parameters and R indices $R1$=0.0309, $wR2$=0.0821 for 1261 reflections obeying $I>2\sigma(I)$ criterion of observability.

Crystallographic data for the structural analysis of compound **1** have been deposited at the Cambridge Crystallographic Data Center, CCDC no. 602946. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1233 336 033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.06.097.

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