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Solid-State Structure and Conformation of the Nootropic Agent 4-Hydroxy-2-oxo-1-pyrrolidineacetamide: X-Ray and Theoretical Self-Consistant Field Molecular Orbital (SCF-MO) Studies

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The crystal and molecular structure of 4-hydroxy-2-oxo-1-pyrrolidineacetamide was determined by X-ray analysis. The molecular conformation found in the solid was compared with results of theoretical quantum mechanical calculations carried out by using an *ab-initio* method at the STO-3G level.

Crystals (orthorhombic) of the studied nootropic agent were as follows: $a=7.162$ (6), $b=8.852$ (3), $c=11.340$ (8) Å; $Pbc2_1$. The five-membered ring deviates from planarity, while the planes of the amide group and of the four atoms N(1)–C(1)–C(2)–C(4) are almost perpendicular in *cis* arrangement, with the 4-hydroxy group in the axial position. In the crystals, molecules are linked by two intermolecular hydrogen-bonds, namely O(3)···O(2) and N(2)···O(1), which are 2.77 and 2.92 Å long, respectively.

Results of theoretical calculations indicate for the free molecule a twisted *cis* conformation, with the 4-hydroxy goup in the axial position, as the most preferred one. This appears to be determined by the intramolecular hydrogen-bond between the O(1) atom and amide group hydrogen atoms. The relative conformational energies indicate that several conformations about the minimum can be populated in the solution state.

The geometry and conformational profile of the pharmacologically active investigated molecule were found to reproduce, with the exception of the puckered conformation of the five-membered ring, those previously determined for the related analogous nootropic agent 2-oxo-1-pyrrolidineacetamide (piracetam), whose chemical structure differs only in the lack of the 4-hydroxy group in the β-position to the carbonyl group.

Keywords——nootropic agent; 4-hydroxy-2-oxo-pyrrolidineacetamide; X-ray analysis; solid-state structure; *ab-initio* theoretical calculation; molecular conformation

In the previous studies, the main conformational features of 2-oxo-1-pyrrolidineacetamide (2-PNA), which is the best-known representative of the nootropic drugs, were elucidated.[1-3] The 2-PNA molecule was found to exist in solution in the preferred twisted-*cis* conformation, the intramolecular hydrogen bond between the O(1) atom and the amide group being essentially the conformation-determining factor.

The present investigation on 4-hydroxy-2-oxo-1-pyrrolidineacetamide (4H-2-PNA) was undertaken as a part of a research program on the determination of the requisite conformational characteristics of drugs exerting nootropic activity. The drug 4H-2-PNA is a new γ-amino-β-hydroxybutyric acid (GABAOB)*-related nootropic[4,5] whose chemical structure differs from that of 2-PNA only in having the hydroxy group in the β-position to the carbonyl group. Recent clinical studies[6,7] on patients with organic brain syndrome showed that 4H-2-PNA gives a greater improvement in memory factor, while 2-PNA is more active in paranoid

ideation and agitation. In general, 4H-2-PNA was found to be two to three times more active than 2-PNA. These differences in activity of 4H-2-PNA with respect to that of the parent compound 2-PNA, in spite of the minor difference in the chemical structure, prompted us to examine in detail the molecular structure and the conformation of 4H-2-PNA, in order to investigate any possible structural and conformational changes accompanying the replacement of the hydrogen atom in the β-position with a hydroxy group. Here we present the result of a solid state structure analysis (an X-ray diffraction study on the crystal), together with additional information provided by self-consistant field molecular orbital (SCF-MO) *ab initio* calculations of conformational energies.

Experimental

The sample of 4H-2-PNA (a gift from ISF, Trezzano S/N, Milano Italy) was recrystallised from methanol (mp 168 °C).

Crystal Structure Determination——X-Ray data were collected with a Philips PW/100 diffractometer. The radiation was graphite-monochromated Mo$K\alpha$, $\lambda = 0.7107$ Å for which $\mu = 1.10\,cm^{-1}$. The measurement was made at 21 °C. The crystals ($C_6H_{10}N_2O_3$; M_r 158.16) were orthorhombic, $Pbc2_1$ (alternative setting of $Pca2_1$, No. 29), $a = 7.162(6)$, $b = 8.852(3)$, $c = 11.340(8)$ Å, $V = 718.9(8)$ Å^3, D_m (by flotation) = 1.47, $Z = 4$, $D_c = 1.46\,gcm^{-3}$. The intensities of 1173 unique reflections for which $I > 3\sigma(I)$ (total number of reflections is 1472) were measured by scanning for 1.4° in 2ϑ with a scan speed of $0.04\,s^{-1}$. Periodic measurements of three standard reflections were made and no significant changes in the intensities were observed. Corrections for Lorentz and polarization effects were applied, but no corrections were made for absorption or extinction. The distribution of the normalized structure factors confirmed the acentric choice for the space group. Application of direct methods yielded the atomic coordinates of all nonhydrogen atoms and the model gave a reliability index R of 0.11. Hydrogen atoms appeared on a difference map and the refinement of coordinates and anisotropic temperature factors (isotropic for the H atoms) was continued until no shift was greater than 0.5 times the estimated standard deviation in the corresponding parameter, using a weighting scheme of the type $w = K/[\sigma^2(F_o) + a(F_o)^2]$ ($K = 1.94$, $a = 1.08 \times 10^{-3}$). The final R factor was 0.046 and $R_g = [\sum w(|F_o| - |F_c|)^2 / \sum w|F_o|^2]^{1/2}$ was 0.060. Calculations were performed with SHELX-76.[8]

Positional coordinates for the nonhydrogen atoms are given in Table I, while pertinent distances and angles, with some other geometrical data, are listed in Tables II and III.

Anisotropic thermal parameters, and positional and thermal parameters for the H atoms are given in Tables IV and V, respectively.

Observed and calculated structure factors are available as Supplementary Material.

Theoretical Calculations——Conformational energy calculations were performed with the use of *ab-initio* molecular orbital theory (restricted Hartree–Fock with STO-3G basis set). The calculations were carried out using the standard Gaussian 70 program.[9] The crystal structure of 4H-2-PNA was used as the geometrical input data, while the standard values of 1.09 and 1.01 Å were assumed for all of the C–H and N–H bond lengths, respectively. Calculations were performed for each conformation denoted by the angles φ, ω that were assumed to change in clockwise direction, along the N(1)–C(5) and C(5)–C(6) axes, starting from the $\varphi = \omega = 0°$ conformation outlined in Fig. 1

TABLE I. Positional Parameters with e.s.d.'s ($\times 10^{-4}$) of Nonhydrogen Atoms

Atom	x/a	y/b	z/c
O(1)	2431 (3)	6115 (2)	4274 —[a]
O(2)	1375 (3)	4354 (2)	1338 (3)
O(3)	7430 (3)	7662 (2)	3252 (3)
N(1)	3728 (3)	6433 (2)	2453 (3)
N(2)	−70 (4)	6200 (3)	335 (3)
C(1)	3761 (3)	6043 (2)	3600 (3)
C(2)	5727 (4)	5529 (3)	3887 (3)
C(3)	6897 (3)	6188 (3)	2890 (3)
C(4)	5520 (4)	6229 (3)	1866 (3)
C(5)	2063 (4)	6942 (3)	1852 (3)
C(6)	1099 (3)	5712 (2)	1144 (3)

e.s.d., estimated standard deviation. *a*) Fixed to define the origin.

TABLE II. Molecular Geometry of 4H-2-PNA

(a) Bond lengths (Å) with e.s.d.'s in parentheses

N(1)–C(1)	1.346 (5)	C(2)–HC(2)′	0.97 (4)
C(1)–O(1)	1.223 (3)	C(2)–HC(2)″	0.86 (4)
C(1)–C(2)	1.515 (4)	O(3)–HO(3)	0.95 (6)
C(2)–C(3)	1.523 (4)	C(3)–HC(3)	1.05 (4)
C(3)–O(3)	1.420 (3)	C(4)–HC(4)′	1.02 (4)
C(3)–C(4)	1.524 (4)	C(4)–HC(4)″	0.85 (4)
C(4)–N(1)	1.457 (4)	C(5)–HC(5)′	0.91 (4)
N(1)–C(5)	1.446 (4)	C(5)–HC(5)″	0.97 (4)
C(5)–C(6)	1.519 (4)	N(2)–HN(2)′	0.89 (4)
C(6)–O(2)	1.238 (3)	N(2)–NH(2)″	0.98 (5)
C(6)–N(2)	1.315 (4)		

(b) Bond angles (°) with e.s.d.'s in parentheses

N(1)–C(1)–O(1)	125.2 (2)	C(1)–C(2)–HC(2)′	113 (3)
N(1)–C(1)–C(2)	107.5 (3)	C(1)–C(2)–HC(2)″	105 (3)
O(1)–C(1)–C(2)	127.2 (3)	HC(2)′–C(2)–HC(2)″	105 (3)
C(1)–C(2)–C(3)	103.7 (2)	C(3)–O(3)–HO(3)	114 (4)
C(2)–C(3)–O(3)	106.6 (2)	C(2)–C(3)–HC(3)	108 (3)
O(3)–C(3)–C(4)	111.9 (2)	C(3)–C(4)–HC(4)′	114 (3)
C(2)–C(3)–C(4)	102.6 (2)	C(3)–C(4)–HC(4)″	115 (3)
C(3)–C(4)–N(1)	103.0 (3)	HC(4)′–C(4)–HC(4)″	96 (4)
C(4)–N(1)–C(1)	113.2 (3)	N(1)–C(5)–HC(5)′	113 (3)
C(1)–N(1)–C(5)	123.4 (3)	N(1)–C(5)–HC(5)″	108 (3)
C(4)–N(1)–C(5)	123.4 (3)	HC(5)′–C(5)–HC(5)″	106 (3)
N(1)–C(5)–C(6)	113.6 (2)	C(6)–N(2)–HN(2)′	121 (3)
C(5)–C(6)–O(2)	122.0 (2)	C(6)–N(2)–NH(2)″	115 (3)
O(2)–C(6)–N(2)	123.0 (2)	HN(2)′–N(2)–HN(2)″	124 (4)
C(5)–C(6)–N(2)	115.0 (2)		

(c) Least-squares planes with the deviations (Å) for relevant atoms

Plane	Deviations
N(1), C(1), C(2), C(4)	N(1), C(1), C(2), C(4), O(1) 0.00; C(3) 0.49; O(3) 1.91; C(5) 0.04
C(5), C(6), O(2), N(2)	C(5), C(6), O(2), N(2) 0.00; N(1) 0.43; C(1) −.42; HN(2)′ −.11: HN(2)″ 0.02
Dihedral angle: 87.9°	

(d) Torsion angles (°)

N(1)–C(1)–C(2)–C(3)	18.8	C(1)–N(1)–C(5)–C(6)	−98.6
C(1)–C(2)–C(3)–C(4)	−29.6	C(4)–N(1)–C(5)–C(6)	78.7
C(2)–C(3)–C(4)–N(1)	29.8	N(1)–C(5)–C(6)–N(2)	−161.5
C(3)–C(4)–N(1)–C(1)	−19.8	N(1)–C(5)–C(6)–O(2)	20.0
C(4)–N(1)–C(1)–C(2)	0.7		

(e) Non-bonded contacts (Å)

N(1)---C(2)	2.31	O(2)---N(2)	2.24	O(3)---C(2)	2.36
N(1)---C(3)	2.33	O(2)---N(1)	2.80	O(3)---C(4)	2.44

TABLE III. Geometry of Hydrogen Bonds[a)]

O(3)---O(2)[b)]	2.77 Å;	O(3)–HO(3)	0.95 Å;	HO(3)---O(2)	1.85 Å;	O(3)–HO(3)---O(2)	165°
N(2)---O(1)[c)]	2.29 Å;	N(2)–HN(2)′	0.89 Å;	HN(2)′---O(1)	2.03 Å;	N(2)–HN(2)′---O(1)	180°

a) The codes for symmetry-related atoms are as follows. *b)* $1-x, 1/2+y, z$. *c)* $1-x, 1-y, 1/2+z$.

TABLE IV. Anisotropic Thermal Parameters ($\times 10^2$)[a]

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O(1)	5.02	4.13	4.78	−.41	2.30	−.69
O(2)	4.53	2.19	4.69	0.36	−.89	0.03
O(3)	4.70	3.58	4.31	−.10	−.41	−1.54
N(1)	2.38	3.43	2.98	−.05	−.32	−.22
N(2)	4.66	2.91	5.39	0.37	−2.42	−.28
C(1)	3.43	2.30	2.94	−.29	0.37	−.60
C(2)	3.84	2.83	3.18	0.65	−.50	−.30
C(3)	2.64	2.85	3.48	−.02	−.18	−.10
C(4)	3.17	4.26	2.43	−.06	0.35	−.25
C(5)	3.27	2.35	5.00	−.22	−1.26	−.05
C(6)	2.36	2.22	3.14	0.18	−.15	−.17

a) The anisotropic temperature factors expression is of the form $\exp[-2\pi^2(U_{11}a^{*2}h^2+\cdots+2U_{12}a^*b^*hk)]$.

TABLE V. Positional ($\times 10^3$) and Thermal ($\times 10^2$) Parameters for Hydrogen Atoms

Atom	x/a	y/b	z/c	U	Atom	x/a	y/b	z/c	U
HO(3)	804	823	266	8.5	HC(3)	804	546	274	3.8
HN(2)′	−81	556	−5	5.0	HC(4)′	552	527	137	4.9
HN(2)″	−15	730	26	5.6	HC(4)″	581	682	131	5.4
HC(2)′	617	589	465	3.0	HC(5)′	227	775	138	5.8
HC(2)″	565	456	396	5.0	HC(5)″	118	728	244	5.4

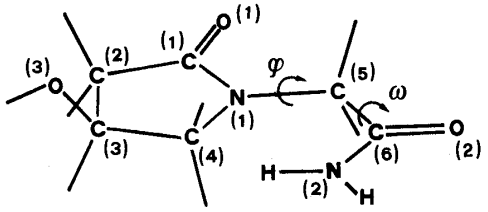

Fig. 1. Numbering Scheme of the 4H-2-PNA Molecule

The unstable "starting" conformation, assumed as $\varphi=\omega=0°$ is outlined. In this conformation the planes C(1)–N(1)–C(4) and C(5)–C(6)–N(2) are coplanar, with the C(6)–N(2) bond *syn* to N(1)–C(5) bond. Rotations are defined in the clockwise sense along the C(5)–N(1) (φ) and C(6)–C(5) (ω) bonds, starting from the above conformation. The possible conformers (φ, ω) are therefore obtained as combinations of the rotation angles φ and ω, which vary in the sense shown by the arrows.

TABLE VI. *Ab-initio* Calculated Energy Differences (kcal mol^{-1}) for Selected Conformations of 4H-2-PNA

(φ, ω)	(270, 300)	(90, 0)	(90, 60)	(270, 0)	(180, 90)	(180, 270)
(270, 300)	0	1.318	2.222	3.026	5.806	6.101
(90, 0)		0	0.904	1.707	4.488	4.783
(90, 60)			0	0.803	3.584	3.879
(270, 0)				0	2.982	3.076
(180, 90)					0	0.295
(180, 270)						0

The conformational energies are given as the difference (ΔE) relative to that for the most stable conformation (270, 300).

Results and Discussion

X-Ray Analysis

The molecule of 4H-2-PNA is essentially composed by two planes: the plane formed by the atoms C(5), C(6), N(2) and O(2) (acetamide group), and the plane of the pyrrolidone ring, as shown in Fig. 2. However, the five-membered ring has large deviations from a planar arrangement and it adopts an envelope conformation (C_s symmetry)[10] with C(3) at the flap of the envelope, lying 0.49 Å from the best plane through N(1), C(1), C(2), C(4) which are coplanar to within 0.003 Å. The conformational equations of De Tar and Luthra[11] [$\chi_i = a_0 \cos(t + 4\pi(i-2)/5)$] confirm the almost perfect envelope conformation. In the present case, the values are: $a_0 = 31°$ and $t = 197.5°$ (additional parameter $d_1 = 5.5°$). Consequently, the torsion angle C(4)–N(1)–C(1)–C(2) tends to be much closer to zero than any other torsion angle. A puckered conformation has previously been found in molecules containing the pyrrolidine ring (in particular, the proline residue[12]). However, the data available from 40 X-ray determinations[12] confirm that the ring is a rather flexible one and its conformation is affected by various factors including intramolecular and/or intermolecular interactions, such as hydrogen bonds. The plane of the amide group and that of the four coplanar atoms in the

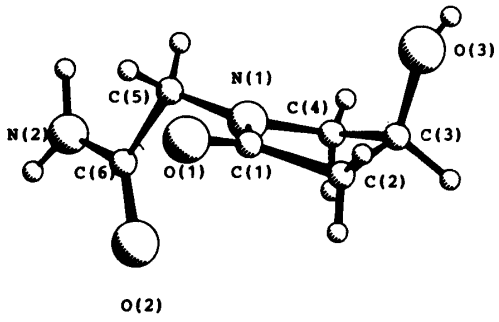

Fig. 2. Numbering System and Molecular Conformation as Found in the Crystal of 4H-2-PNA (Viewed along the *c* Axis)

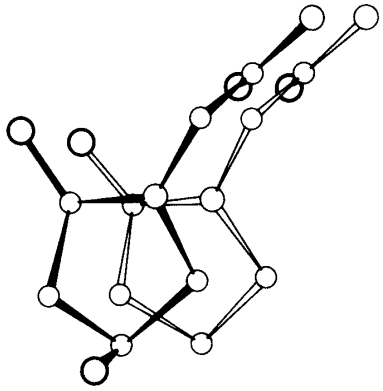

Fig. 3. Superimposition of 4H-2-PNA (Black) and 2-PNA (White) Molecules as Found in the Crystal

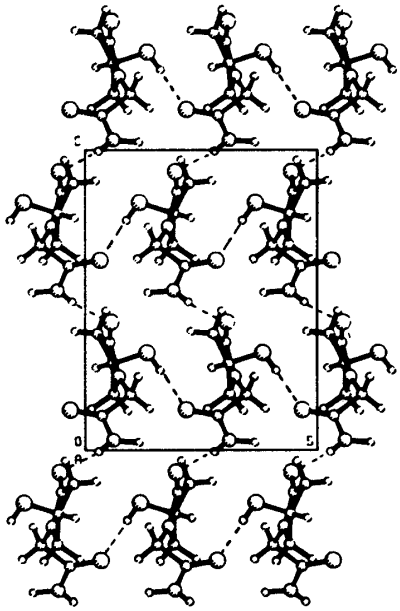

Fig. 4. Crystal Structure Projected along the *a* Axis, Showing the Intermolecular Hydrogen-Bonding System in 4H-2-PNA

ring are almost perpendicular to each other, the corresponding dihedral angle being 87.9°, a value similar to that found in 2-PNA (88.2°).[1] The conformation about the C(5)–C(6) bond is such that the N(1) atom is *syn*-periplanar to the carbonyl O(2) atom [torsion angle N(1)–C(5)–C(6)–O(2) being 20°] and *anti*-periplanar to the aminic N(2) atom [torsion angle N(1)–C(5)–C(6)–N(2) being −161.5°].

Superimposition[13] of 2-PNA and 4H-2-PNA, with its hydroxyl group bonded to C(3) in the axial position, shows a near perfect fit of their structures (Fig. 3), apart from the high degree of planarity for the five-membered ring in 2-PNA.

As found in 2-PNA, the distance N(1)–C(1) [1.346(5) Å] in the pentatomic ring is shorter than a single C–N bond, indicating a partial conjugation between the π-bonding of O(1)–C(1) and the lone pair on the nitrogen atom. In fact, in the N(1)–C(4) and N(1)–C(5) bonds, where a single bond character can be expected, the distances are 1.457(4) and 1.446(4) Å, respectively. The C(3)–O(3) bond length of 1.420(3) Å is close to the accepted value for the C(sp^3)–O single bond length (1.43 Å).

Other aspects of the molecular structure appear to be quite normal in terms of previously known structures containing the 2-pyrrolidone ring[1] and proline derivatives.[11]

The essential difference between 2-PNA and 4H-2-PNA resides in the system of hydrogen bonds. The molecules are held together in the crystal by two hydrogen bonds, O(3) · · O(2) (2.77 Å) and N(2) · · · O(1) (2.92 Å) (Table III), and the linear and effective hydrogen-bond formation involving the hydroxyl group is probably responsible for the different conformations of the pyrrolidine ring found in 2-PNA and 4H-2-PNA. Figure 4 shows the molecular packing, including the hydrogen-bonding scheme in the crystal.

Theoretical Calculations

Table VI summarizes the results of conformational energy calculations using the *ab-initio* method. A single energy minimum, among the conformational energies calculated for 4H-2-PNA as an isolated molecule, is found for the conformation with $\varphi = 270°$ and $\omega = 300°$ [hereafter denoted as (270, 300)].

For comparison, the corresponding calculation was carried out for 2-PNA in which, due to the symmetry about the pentatomic ring plane, two equivalent minima were observed at (90, 60) and (270, 300).

In both molecules, the preferred twisted conformation of the acetamide fragment appears to be determined by: i) steric effects which, as far as the N(1)–C(5) bond is concerned, are minimal in a perpendicular arrangement of the pyrrolidone ring and the N(1)–C(5)–C(6) plane; ii) intramolecular hydrogen-bond formation between O(1) and H(2). Additional steric interactions involving the 4-hydroxy group decrease (by 2.222 kcal mol^{-1}) the relative stability of the conformation (90, 60) which corresponds to an equivalent arrangement of the acetamide fragment on the opposite plane of the pyrrolidone ring.

The calculated conformational energies indicate, in general, as found for 2-PNA, the conformationally flexible nature of 4H-2-PNA. In fact, the contour map of theoretical conformational energy of this molecule, which was obtained in the range $180 \leqslant \varphi, \omega \leqslant 360°$, reproduces the contours and relative energies of the corresponding energy-conformation surface of 2-PNA.[1] Therefore, in solution, several conformations can be populated in a wide range around the minimum. The conformation found in the solid also cannot be excluded, even if energetically unfavored. These findings indicate that for the molecule of 4H-2-PNA, as in the case of 2-PNA, the packing forces in the crystal and effective intermolecular hydrogen-bonds can be the determining factors of the adopted conformation in the solid.[14]

In conclusion, it appears that the conformational profile of 2-PNA does not change in the 4-hydroxy substituted derivative. This result suggests that any difference found in pharmacological action and potency between these two closely related nootropics is likely to be

attributable to properties other than conformation.

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