

X-Ray Crystallographic and ^1H NMR Spectroscopic Investigations of (3*RS*, 4*SR*)-4-Hydroxypiperidine-3-carboxylic Acid, an Inhibitor of Neuronal and Glial GABA Uptake

LOTTE BREHM,^a KJELD SCHAUMBURG^b and POVL KROGSGAARD-LARSEN^a

^a Royal Danish School of Pharmacy, Department of Chemistry BC, DK-2100 Copenhagen Ø, Denmark and ^b University of Copenhagen, Chemical Laboratory V, DK-2100 Copenhagen Ø, Denmark

The conformations of (3*RS*,4*SR*)-4-hydroxypiperidine-3-carboxylic acid in the crystalline state and in aqueous solution have been investigated by X-ray crystallographic methods and by 270 MHz ^1H NMR spectroscopy, respectively. (3*RS*,4*SR*)-4-Hydroxypiperidine-3-carboxylic acid exists in the crystalline state in the chair conformation with the carboxylate group in an equatorial position and the hydroxy group in an axial position. The measured coupling constants indicate that the compound adopts predominantly a chair conformation in aqueous solution.

The central GABA (γ -aminobutyric acid) mediated neurotransmission appears mainly to be terminated by uptake of GABA into glial and presynaptic neuronal tissue elements.¹ Inhibitors of these uptake systems, which have different substrate specificities,² have pharma-

cological interest. (3*RS*,4*SR*)-4-Hydroxypiperidine-3-carboxylic acid [(\pm)-*cis*-4-hydroxynipecotic acid] inhibits the two uptake processes effectively and to approximately the same extent.² Furthermore (\pm)-*cis*-4-hydroxynipecotic acid appears to be a substrate for the neuronal transport carrier.³ These findings make (\pm)-*cis*-4-hydroxynipecotic acid an important tool for pharmacological studies of the GABA system. This paper describes the conformations of (\pm)-*cis*-4-hydroxynipecotic acid in the examined crystal and in aqueous solution.

The coupling constants between the C3 proton and the two C2 protons (J_{ax3a} 4.55 Hz, J_{ax3e} 11.15 Hz) in the ^1H NMR spectrum of (\pm)-*cis*-4-hydroxynipecotic acid are typical for equatorial-axial and axial-axial configurations of these protons (Fig. 1). This is consistent

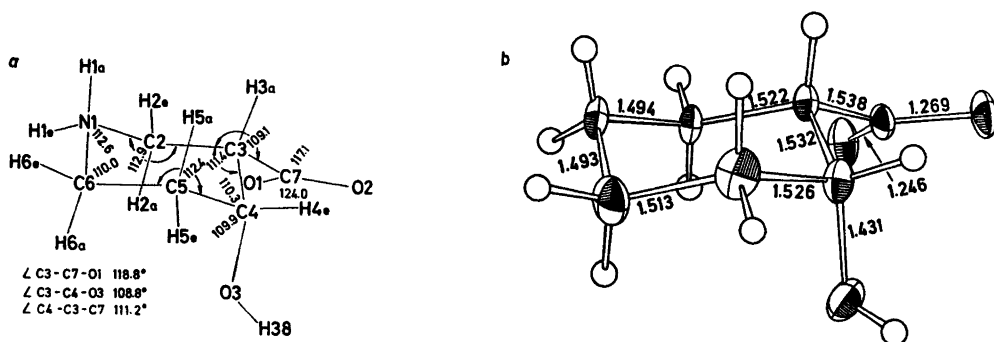


Fig. 1. Perspective drawings of *cis*-4-hydroxynipecotic acid. a, The numbering of the atoms and bond angles involving only non-hydrogen atoms; e.s.d.'s are 0.2–0.3°. b, The thermal ellipsoids for the non-hydrogen atoms, scaled to 50% probability; hydrogen atoms are represented as spheres of arbitrary radius. Bond lengths between non-hydrogen atoms; e.s.d.'s are 0.003–0.005 Å.

with a predominantly equatorial orientation of the carboxylate group at C3. Furthermore the coupling constant for the C3 and C4 protons (J_{3a4e} 2.50 Hz) unequivocally indicates axial-equatorial orientation of these protons and consequently a 3,4-*cis* configuration. The sum of the vicinal coupling constants for the C4 and C5 protons (7.0 Hz) reflects the presence of an axial-equatorial and an equatorial-equatorial coupling in agreement with an axial orientation of the hydroxy group.

The values of J_{2e3a} and of J_{3a4e} were calculated according to the method of Forrest,⁴ assuming dihedral angles of 60° and taking into consideration the electronegativity⁵ differences between the substituents and hydrogen. The differences between these calculated values, 3.98 and 1.84, and the respective observed values for J_{2e3a} (4.55) and for J_{3a4e} (2.50) might reflect a more or less distorted chair conformation of the piperidine ring of (\pm)-*cis*-4-hydroxynipecotic acid. To assess the degree of distortion the dihedral angles H2e-C2-C3-H3a and H3a-C3-C4-H4e were calculated. Using the method of Forrest⁴ the angles were found to be 59 and 57°, respectively. These results indicate that the piperidine ring of (\pm)-*cis*-4-hydroxynipecotic acid predominantly adopts a conformation in aqueous solution close to the ideal chair conformation.

As anticipated from its pK_A values (3.42, 10.02)⁶ (\pm)-*cis*-4-hydroxynipecotic acid crystallizes in its zwitterionic form. The conforma-

Table 1. Selected torsion angles (°). The two signs refer to the *SR*- and *RS*-forms, respectively.

C6-N1-C2-C3	∓	54.2(3)
N1-C2-C3-C4	±	51.8(3)
C2-C3-C4-C5	∓	51.8(3)
C3-C4-C5-C6	±	55.4(3)
C4-C5-C6-N1	∓	56.9(3)
C5-C6-N1-C2	±	55.7(3)
C2-C3-C7-O1	±	3.0(4)
C4-C3-C7-O2	∓	52.6(3)
C7-C3-C2-N1	±	174.7(2)
C7-C3-C4-C5	∓	173.6(2)
C7-C3-C4-O3	∓	52.9(3)
O3-C4-C5-C6	∓	64.6(3)
O3-C4-C3-C2	±	68.9(3)
C5-C4-O3-H38	∓	86(3)
H2e-C2-C3-H3a	±	59(3)
H3a-C3-C4-H4e	∓	48(3)

tion of the molecule found in the crystalline state, the numbering of the atoms, and the most significant bond distances and valency angles are shown in Fig. 1. Some torsion angles are shown in Table 1. The piperidine ring is in the chair form with the carboxylate group equatorial and the hydroxy group axial. The best least-squares plane defined by the four atoms C2, C3, C5 and C6, the standard deviation of the atoms from the plane is 0.02 Å, makes angles of 130° and 132° with the planes defined by the atoms C6, N1, C2 and C3, C4, C5, respectively. Except for a difference in the C7-O2 bond the bond distances found in (\pm)-*cis*-4-hydroxynipecotic acid are not signi-

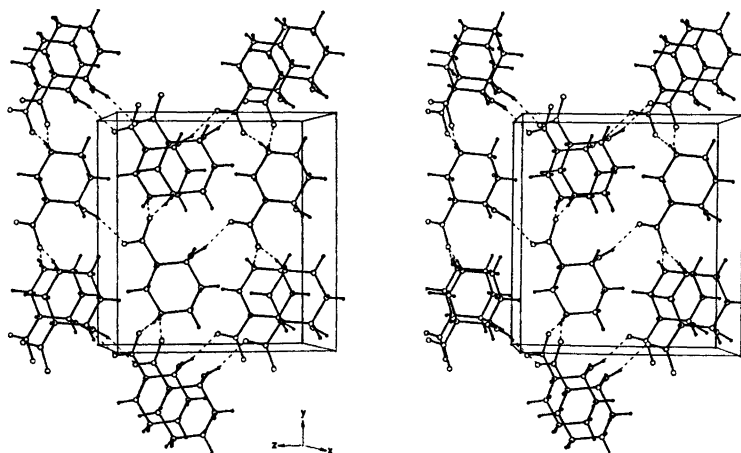


Fig. 2. Stereo diagram illustrating the molecular packing of (\pm)-*cis*-4-hydroxynipecotic acid. Hydrogen bonds are drawn with broken lines.

Table 2. Hydrogen bond distances and angles. Symmetry code: (i), $x, 1-y, z-\frac{1}{2}$; (ii), $x+\frac{1}{2}, y-\frac{1}{2}, z$; (iii), $x-\frac{1}{2}, y-\frac{1}{2}, z$.

A-H...B	A-H (Å)	A...B (Å)	H...B (Å)	∠AHB (°)
O3-H38...O1 ⁱ	0.85(4)	2.757(4)	1.93(4)	164(4)
N1-H1a...O2 ⁱⁱ	0.88(4)	2.840(4)	1.99(4)	163(3)
N1-H1e...O2 ⁱⁱⁱ	0.83(4)	2.736(4)	1.93(4)	164(4)

ificantly different from the equivalent distances found in (*RS*)-piperidine-3-carboxylic acid (nipecotic acid).⁷ The lengthening of the C7-O2 bond may be attributed to the fact that O2 of (\pm)-*cis*-4-hydroxynipecotic acid is acceptor for two hydrogen bonds, whereas O1 of (\pm)-*cis*-4-hydroxynipecotic acid and O1 and O2 of nipecotic acid are acceptors for only one hydrogen bond.

The equivalent bond angles between non-hydrogen atoms in the two compounds agree to within 2.1°.

The *intra*-molecular distances N1...O1, N1...O2 and N1...O3 are 4.150(3), 4.890(3) and 3.543(3), respectively.

The packing of the molecules in the crystals of (\pm)-*cis*-4-hydroxynipecotic acid is shown in Fig. 2. The crystal structure is stabilized by hydrogen bonds, one for each hydrogen atom covalently bonded to nitrogen or oxygen. The *c* glide-plane-related molecules are connected by the hydrogen bond O3-H38...O1ⁱ, thereby forming infinite zig-zag chains of molecules in the *c* direction. These chains are interlinked by the hydrogen bonds N1-H1a...O2ⁱⁱ and N1-H1e...O2ⁱⁱⁱ. Thus, each molecule is involved in six hydrogen bonds to six different neighbouring molecules. Hydrogen bond distances and angles are given in Table 2. All other *inter*-molecular contacts correspond to van der Waals' interactions.

Like (\pm)-*cis*-4-hydroxynipecotic acid, nipecotic acid is a substrate-competitive inhibitor of the neuronal GABA transport-carrier.⁸ In the solid state and in aqueous solution (*RS*)-nipecotic acid also exists predominantly in a chair conformation with the carboxylate group equatorial.⁷ It might be assumed that (\pm)-*cis*-4-hydroxynipecotic acid and nipecotic acid have similar conformations when interacting with the GABA transport carrier.

EXPERIMENTAL

The computations in connection with the X-ray structure determination were performed on an IBM 370/165 computer using *INDIFF*,⁹ a local version of the *NRC 2A Picker Data Reduction Program*,¹⁰ *MULTAN* (1974 version),¹¹ *The X-Ray System* (1972 version)¹² and *ORTEP II*.¹³ The X-ray atomic scattering factors used were those of Cromer and Mann¹⁴ for O, N and C, and of Stewart, Davidson and Simpson¹⁵ for H.

The 270 MHz ¹H NMR spectra were obtained on a Bruker HX 270 S instrument. Recording of the spectra and decoupling experiments were performed as described in a previous paper.⁷ The computations in connection with the analyses of the spectra were performed on a Varian 620/I computer using the *SIMEQ*¹⁶ program and on a Nicolet 1180 computer using the *ITRCAL* program.¹⁷

(\pm)-*cis*-4-Hydroxynipecotic acid was synthesized as earlier described.⁶ ¹H NMR data for (\pm)-*cis*-4-hydroxynipecotic acid (270 MHz, D₂O): δ_{2a} 3.31, δ_{2e} 3.33, δ_{3a} 2.73, δ_{4e} 4.40, δ_{5a} ca. 2.0, and δ_{5e} ca. 2.0. Analysis of the H6 decoupled spectrum yields an upper limit on $\Delta\delta_{ae}$ for the C5 protons of 0.04 ppm. δ_{6a} 3.23 and δ_{6e} 3.23. Analysis of the H5 decoupled spectrum yields an upper limit on $\Delta\delta_{ae}$ for the C6 protons of 0.03 ppm. J_{2a2e} -13.4 Hz, J_{2a3a} 11.15 Hz, J_{2e3a} 4.55 Hz, J_{3a4e} 2.50 Hz, and ($J_{4e5a} + J_{4e5e}$) 7.0 Hz.

X-Ray analysis

The colourless prismatic crystals used for the X-ray examination were crystallized from a water-*N,N*-dimethylformamide solution.

Preliminary film investigations revealed monoclinic symmetry. Systematically absent reflections are *hkl* when *h+k* odd and *h0l* when *l* odd; the space group is thus *Cc* or *C2/c*. The unit-cell parameters were determined by a least-squares refinement of the 2θ angles of 38 reflections measured on the diffractometer.

Table 3. Crystal data.

(3 <i>RS</i> ,4 <i>SR</i>)-4-Hydroxypiperidine-3-carboxylic acid [(\pm)- <i>cis</i> -4-hydroxynipecotic acid], C ₈ H ₁₁ NO ₃	
	M.p. = 253–255°C (decomp.)
	M.W. = 145.16
Monoclinic	<i>V</i> = 638.0 Å ³
<i>a</i> = 4.9097(4) Å	<i>Z</i> = 4
<i>b</i> = 12.057(2) Å	<i>D_m</i> = 1.52 g cm ⁻³
<i>c</i> = 11.082(1) Å	<i>D_x</i> = 1.511 g cm ⁻³
β = 103.45(1)°	μ = 1.30 cm ⁻¹ (MoK α)
Space group: <i>Cc</i> (No. 9)	

Table 4. Final positional and thermal ($\times 10^3 \text{ \AA}^2$) parameters for the non-hydrogen atoms. The temperature factor is of the form: $\exp\{-2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + l^2c^{*2}U_{33} + 2hka^*b^*U_{12} + 2hla^*c^*U_{13} + 2klb^*c^*U_{23})\}$.

Atom	<i>x</i>	<i>y</i>	<i>z</i>	<i>U</i> ₁₁	<i>U</i> ₂₂	<i>U</i> ₃₃	<i>U</i> ₁₂	<i>U</i> ₁₃	<i>U</i> ₂₃
N1	.7439(6)	.1580(1)	.7747(3)	2.48(9)	.79(7)	2.50(9)	.09(7)	.63(7)	.06(6)
C2	.6664(7)	.2607(2)	.8344(3)	2.41(10)	.80(8)	1.89(9)	.02(7)	.76(7)	.02(6)
C3	.8113(6)	.3640(2)	.8010(3)	1.93(9)	.88(7)	1.89(9)	-.02(6)	.59(7)	-.10(6)
C4	.7705(-)	.3760(2)	.6602(-)	2.24(10)	1.36(8)	2.07(10)	-.15(7)	.86(8)	.33(7)
C5	.8529(7)	.2684(2)	.6049(3)	2.77(12)	2.10(11)	2.17(10)	.31(8)	1.14(9)	-.12(7)
C6	.6962(7)	.1688(2)	.6371(3)	2.99(11)	1.54(9)	2.42(10)	.05(8)	.74(9)	-.68(8)
C7	.6983(6)	.4663(2)	.8564(3)	2.12(10)	.87(8)	2.30(10)	-.17(7)	.47(7)	-.27(7)
O1	.5253(6)	.4526(1)	.9214(3)	4.20(11)	1.57(7)	3.37(10)	-.25(7)	2.13(8)	-.72(7)
O2	.7853(6)	.5604(1)	.8306(3)	2.95(9)	.91(7)	4.38(11)	-.28(6)	1.29(8)	-.15(6)
O3	.4827(6)	.4007(2)	.6069(3)	2.68(8)	2.02(8)	2.47(8)	.39(6)	.45(6)	.86(6)

The crystal density was measured by flotation in a bromobenzene–methyl iodide mixture. Cell constants and other crystal data are given in Table 3.

Three-dimensional diffraction intensity data were measured at room temperature on a NONIUS three-circle automatic diffractometer using graphite monochromated MoK α radiation ($\lambda = 0.71069 \text{ \AA}$). The ω -scan technique was employed, and background counts were taken for half the scanning time at each of the scan range limits. All the data were measured from a single crystal with approximate dimensions $0.30 \times 0.44 \times 0.20 \text{ mm}$. The crystal was sealed in a glass capillary and orientated with the *b* axis parallel to the ϕ axis of the goniostat. The one standard reflection sampled after every 50 intensity measurements showed no significant intensity variation over the period of data collection. Of the 937 independent reflections measured in the range $2.5 \leq \theta \leq 30.0^\circ$, 874 had net intensities greater than $3.0\sigma(I)$, where $\sigma(I)$ is the estimated standard deviation of an intensity as calculated from counting statistics. These were regarded as observed reflections and used in the refinement procedure. Lorentz and polarization corrections were applied, but no absorption corrections were made.

Structure solution and refinements. Normalized structure amplitudes ($|E|$'s) were calculated. The distribution of $|E|$'s indicated the non-centrosymmetric space group *Cc*, and this was confirmed by the successful solution and refinement of the structure in this space group. The phases for 100 individual $|E|$'s with $|E| \geq 1.55$ were derived with the program MULTAN¹¹ leading to an *E*-map which revealed the positions of all non-hydrogen atoms. Anisotropic least-squares refinement, followed by a difference synthesis, gave positions for all hydrogen atoms ($0.7\text{--}0.4 \text{ e\AA}^{-3}$). In subsequent full-matrix least-squares calculations, an overall

scale factor, atomic coordinates for all atoms and anisotropic thermal parameters for the non-hydrogen atoms were refined. The thermal parameters for the hydrogen atoms were fixed at isotropic values corresponding to those of the non-hydrogen atoms to which they are bonded. The quantity minimized was $\sum w(|F_o| - |F_c|)^2$; the weighting scheme was $w = 1/[1 + (|F_o| - 6.5)/7.1]^2$. On the last cycle of least-squares refinement the values of maximum and average shift/error were 0.1 and 0.01, respectively. The final *R* value is 0.043 and $R_w = 0.052$ for 874 unique reflections. Tables 4 and 5 list the final positional and thermal parameters of the non-hydrogen and hydrogen atoms, respectively. The terminal set of structure factors, listed with the observed data is available on request.

Acknowledgements. This work was supported by the Danish Medical Research Council and the 270 MHz NMR Spectrometer was made available by the Danish Research Council (SNF).

Table 5. Final positional and thermal (\AA^2) parameters for the hydrogen atoms.

Atom	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i> _{iso}
H1a	.920(8)	.140(3)	.802(3)	1.9
H1e	.625(8)	.118(3)	.794(3)	1.9
H2a	.483(8)	.268(3)	.804(3)	1.8
H2e	.710(7)	.251(3)	.921(3)	1.8
H3a	1.019(7)	.359(3)	.831(3)	1.6
H4e	.892(7)	.432(3)	.638(4)	1.9
H5a	1.055(8)	.258(3)	.632(3)	2.4
H5e	.820(7)	.274(3)	.520(4)	2.4
H6a	.504(8)	.171(3)	.605(4)	2.3
H6e	.772(8)	.098(3)	.605(3)	2.3
H3B	.505(8)	.435(3)	.543(4)	2.4

REFERENCES

1. Martin, D. L. In Roberts, E., Chase, T. N. and Tower, D. B., Eds., *GABA in Nervous System Function*, Raven, New York 1976, p. 347.
2. Schousboe, A., Krogsgaard-Larsen, P., Svenneby, G. and Hertz, L. *Brain Res.* 153 (1978) 623.
3. Krogsgaard-Larsen, P. In Fonnum, F., Ed., *Amino Acids as Neurotransmitters*, Plenum, New York 1978, p. 305.
4. Forrest, T. P. *J. Am. Chem. Soc.* 97 (1975) 2628.
5. Huggins, M. L. *J. Am. Chem. Soc.* 75 (1953) 4123.
6. Krogsgaard-Larsen, P., Thyssen, K. and Schaumburg, K. *Acta Chem. Scand. B* 32 (1978) 327.
7. Brehm, L., Krogsgaard-Larsen, P., Johnston, G. A. R. and Schaumburg, K. *Acta Chem. Scand. B* 30 (1976) 542.
8. Johnston, G. A. R., Stephanson, A. L. and Twitchin, B. *J. Neurochem.* 26 (1976) 83.
9. Sørensen, A. M. INDIFF, *A Fortran Nonius Three-Circle Diffractometer Input Data Program*, Department of Chemistry BC, Royal Danish School of Pharmacy, DK-2100 Copenhagen, Copenhagen 1968.
10. Ahmed, F. R. *NRC Crystallographic Program System*, National Research Council, Ottawa (Canada) 1968.
11. Main, P., Woolfson, M. M., Lessinger, L., Germain, G. and Declercq, J. P. *MULTAN 74*, University of York, York 1974.
12. *X-Ray System 72*, Technical Report TR-192, Computer Science Center, University of Maryland, College Park 1972.
13. Johnson, C. K. *ORTEP II*, Report ORNL-3794, revised 1971, Oak Ridge National Laboratory, Oak Ridge 1971.
14. Cromer, D. T. and Mann, J. B. *Acta Crystallogr. A* 24 (1968) 321.
15. Stewart, R. F., Davidson, E. R. and Simpson, W. T. *J. Chem. Phys.* 42 (1965) 3175.
16. Kort, C. W. F., *SIMEQ*, Spectrometry Dept., Laboratory of Organic Chemistry, University of Amsterdam, Holland.
17. *ITRCAL*, Nicolet Instrument Corporation 5225 (Copyright), Verona Road, Madison, Wisconsin 53711, USA.

Received January 19, 1979.