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GABA Agonists. X-Ray Crystallographic and ^{1}H NMR Spectroscopic Investigations of (3*RS*,4*RS*)-3-Hydroxypiperidine-4-carboxylic Acid

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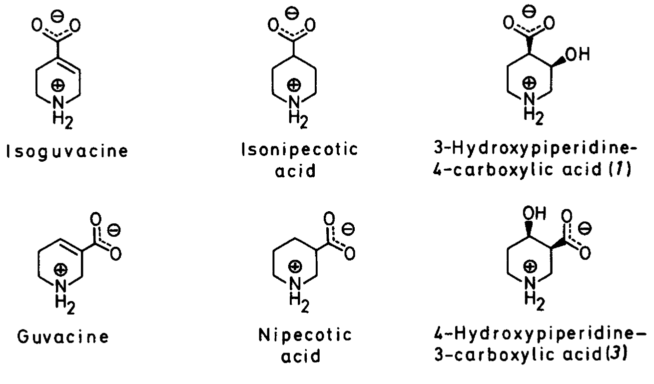
The conformations of (3*RS*,4*RS*)-3-hydroxypiperidine-4-carboxylic acid (*1*) in the crystalline state and in aqueous solution have been determined by X-ray crystallography and by ^{1}H NMR spectroscopy at 270 MHz, respectively. The experimental results show that in the crystal *1* exists in the chair conformation with the carboxylate group in an equatorial position and the hydroxy group in an axial position. Based on an analysis of the coupling constants in the ^{1}H NMR spectrum of *1*, the compound seems to adopt predominantly a similar conformation in aqueous solution.

The development of compounds with specific effects on the GABA (γ-aminobutyric acid) receptors and transport systems have disclosed remarkable substrate specificities of these central synaptic functions. The different structural requirements for interaction with these functions are illustrated by a structure-activity analysis of the conformationally restrained GABA analogues depicted in Fig. 1. While isoguvacine, isonipecotic acid, and *cis*-3-hydroxyisonipecotic acid (*1*) are specific GABA receptor agonists,[1,2] the respective isomeric compounds guvacine, nipecotic acid, and *cis*-4-hydroxynipecotic acid (*3*) have no significant receptor affinity, but these compounds are potent GABA transport inhibitors.[3,4] Within both groups of cyclic GABA analogues even minor structural changes typically result in pronounced loss of activity.

The present study of the conformations of (3*RS*,4*RS*)-3-hydroxyisonipecotic acid (*1*) is part of a series of X-ray and ^{1}H NMR spectroscopic investigations of this class of cyclic amino acid. Earlier studies include nipecotic acid[5] and *cis*-4-hydroxynipecotic acid (*3*).[6]

X-Ray crystallographic investigations provide a



Isoguvacine

Isonipecotic acid

3-Hydroxypiperidine-4-carboxylic acid (*1*)

Guvacine

Nipecotic acid

4-Hydroxypiperidine-3-carboxylic acid (*3*)

Fig. 1. Schematic drawings of some heterocyclic GABA receptor agonists and GABA uptake inhibitors.

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very accurate picture of the molecular structure in the crystal and of the *inter*molecular contacts, which stabilize the crystal structure. ^{1}H NMR analysis provides, through chemical shifts and particularly through the vicinal coupling constants, valuable information concerning the predominant conformation of the compound in solution.

The biologically active compounds may interact with the receptors and carrier-macromolecules under conditions, which may be regarded as intermediary between a solution and a solid state. It will, however, remain an open question whether the "active conformations" of the pharmacologically active compounds can be represented by the structure obtained in the crystals and by the average conformation in solution.

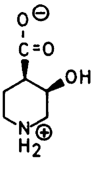
RESULTS AND DISCUSSION

The ^{1}H NMR data obtained for (3*RS*, 4*RS*)-3-hydroxypiperidine-4-carboxylic acid (*1*) are shown in Table 1. Data for dimethyl (3*RS*,4*RS*)-3-hydroxypiperidine-1,4-dicarboxylate (*2*),[13] (3*RS*,4*SR*)-4-hydroxypiperidine-3-carboxylic acid (*3*),[6] dimethyl (3*RS*,4*SR*)-4-hydroxypiperidine-1,3-dicarboxylate (*4*),[14] and nipecotic acid (*5*)[5] have been included for reference in the following discussion.

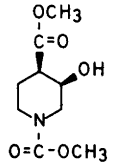
In piperidines[7,8] and *N*-substituted piperidines[7,9] the equatorial protons adjacent (α) to nitrogen are found to have chemical shifts downfield from their axial counterparts. In low temperature studies these chemical shift differences $\delta_{ea}(\alpha)$ in piperidine have been found to be *ca.* 0.5 ppm, and in *N*-substituted piperidines *ca.* 1 ppm, depending on the solvent used.[7] The origin of the $\delta_{ea}(\alpha)$ has been related[10] to the lonepair and bond anisotropies, and it has been suggested that the observed $\delta_{ea}(\alpha)$ values may serve as an estimate of the extent to which the ring inversion (which tends to equalize the chemical shifts) is restricted or biased towards one conformation.

The overall effect of protonation has been found[11] to be a low field shift of the α protons, while the $\delta_{ea}(\alpha)$ value is almost unchanged. Obviously care should be exerted in drawing conclusions concerning the relative stability of conformers related by ring inversion, when partial zwitterion formation can be expected to take place. In the present case the pK_A values determined for the three compounds are very similar (*1*: 3.7, 10.0; *3*: 3.4, 10.0; 5: 3.9, 10.3) thus adding significance to the conclusions reached on basis of the observed data.

Table 1. Some chemical shifts (δ ppm) and coupling constants (*J* Hz) from the ^{1}H NMR spectra of *1*–*4* (270 MHz) and *5* (100 MHz).



1



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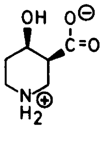

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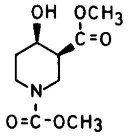

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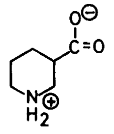

5

	1	*2*	*3*	*4*	*5*
δ_{2a}	3.16	3.00	3.31	3.43	3.58
δ_{2e}	3.40	4.18	3.33	4.05	3.85
δ_{3a}	–	–	2.73	2.64	3.09
δ_{3e}	4.413	4.18	–	–	
δ_{4a}	2.546	2.57	–	–	
δ_{4e}	–		4.40	4.32	
δ_{5a}	2.13	2.05	2.0	1.66	
δ_{5e}	1.96	1.76	2.0	1.84	
δ_{6a}	3.029	2.87	3.2	3.30	
δ_{6e}	3.44	4.0	3.2	3.79	
$\delta_{CCOOCH3}$		3.695			
$\delta_{NCOOCH3}$		3.734			
J_{2a2e}	−13.42	−13.5	−13.4	−13.2	−12.78
J_{2a3a}	–	–	11.15	10.7	9.32
J_{2a3e}	1.65	2.5	–	–	–
J_{2e3a}	–	–	4.55	4.5	3.94
J_{2e3e}	3.	3.0	–	–	
J_{3a4a}	–	–	–	–	
J_{3a4e}	–	–	2.50	2.5	
J_{3e4a}	2.48	2.5	–	–	
J_{3e4e}	–	–	–	–	
J_{4a5a}	12.2	12.0	–	–	
J_{4a5e}	4.3	4.2	–	–	
J_{4e5a}	–	–	7.0[a]	3.0	
J_{4e5e}	–	–		4.4	
J_{5a5e}	−14.5	−13.5		−13.6	
J_{5a6a}	12.87	12.0		11.7	
J_{5a6e}	4.41	4.3		4.8	
J_{5e6a}	3.68	3.5		3.2	
J_{5e6e}	2.20	2.8		3.4	
J_{6a6e}	−12.9	−13.5		−13.4	

[a] $(J_{4e5a}+J_{4e5e})$ 7.0 Hz.

The protons at C6 may serve as the most accurate probe, since the substituent effects can be neglected. It is observed in Table 1 that the average chemical shifts for the two protons in α positions are coinciding in *1* and *3*. Since the value of $\delta_{ea}(\alpha)$ for the protons at C6 found in *1* is 0.41 ppm,[7] a strongly favoured conformation probably is present in *1*, whereas *3* [$\delta_{ea}(\alpha)=0.0$ ppm] does not display a similar pronounced preference for one conformation. Although $\delta_{ea}(\alpha)$ for the protons at C2 serves as a less reliable source of information, due to the proximity of the substitution, the tendency found in the results in Table 1 is consistent with the above interpretation. A further support for the interpretation can be found in the vicinal coupling constants between the protons at C5 and C6. In *1* the largest vicinal coupling constant is 12.9 Hz, and this predominantly axial-axial coupling constant indicates a strongly biased conformation in *1*. The chemical shifts observed in the positions 2 and 3 are within the ranges predicted for piperidine[11] combined with standard increments.[12] For the protons at C5 the observation of the axial proton at low field must be related to the influence of the substituents, since this finding is the reverse of the sequence reported for piperidine itself.[10]

The vicinal coupling constants observed in *1* between the two C2 protons and the C3 proton clearly determine the hydroxyl group as an axial substituent. In *3* and *5* the presence of one large vicinal coupling between the C2 and C3 protons proves the preference of the carboxylate group in the equatorial position. With a proton at C3 of *1* in an equatorial position, little information is contained in the measured vicinal coupling constant to the proton at C4. To determine the orientation of the proton at C4 it is necessary to consider the coupling constants from the proton at C4 to the protons at C5. In *1* they are $J_{4a5a}=12.2$ Hz and $J_{4a5e}=4.3$ Hz, being typical for the coupling of an axial proton to its neighbouring CH_2 group. The data are therefore consistent with a predominantly equatorial orientation of the carboxylate group, and consequently with a 3,4-*cis* configuration.

In the study of similar compounds[13-15] it has frequently been a problem to analyze the spectra of the zwitterions in sufficient detail. In such cases, derivatives like *2* and *4* have often provided a source of information. The question has been raised as to what extent the data for these derivatives do reflect the data for the corresponding zwitterions. As can be seen in Table 1, differences occur primarily in the chemical shifts of the H2e and H6e protons. As expected from the general discussion the $\delta_{ea}(\alpha)$ values in the *N*-substituted compounds *2* and *4* have increased relative to *1 viz.* *3* by approximately 0.6 ppm. This increase is consistent with a predominantly equatorial orientation of the urethane group in the two compounds. Aside from these effects, only minor differences are found. This would support the use of compounds like *2* and *4* in conformational studies, where the spectra of the corresponding zwitterions cannot be analyzed.

As anticipated from its pK_A values, compound *1* crystallizes in its zwitterionic form, as previously found in studies of the compounds *3*,[6] *5*,[5] and piperidine-2-carboxylic acid (*6*).[16] The conformation of *1* found in the crystalline state, the numbering of the atoms, and the most significant bond distances and valency angles are shown in Fig. 2. Some torsion angles are given in Table 2. The values of equivalent bond lengths and angles obtained for the four molecules (*1*, *3*, *5*, *6*) are in general agreement. The piperidinium ring of *1* 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 being 0.02 Å, makes angles of 131 and 130° with the planes defined by the atoms C6, N1, C2 and C3, C4, C5, respectively. The mean torsion angle is 54.5 ° (Table 2), the mean torsion angles for the compounds *3*, *5*, and *6* are 54.3, 56.1, and 57,3 °, respectively.

Table 2. Selected torsion angles (°).[a]

C6 – N1 – C2 – C3	∓52.5(2)[b]
N1 – C2 – C3 – C4	±51.2(2)
C2 – C3 – C4 – C5	∓53.6(2)
C3 – C4 – C5 – C6	±57.2(2)
C4 – C5 – C6 – N1	∓57.5(2)
C5 – C6 – N1 – C2	±55.1(2)
C5 – C4 – C7 – O1	∓ 0.9(3)
C3 – C4 – C7 – O2	∓56.7(2)
C7 – C4 – C5 – C6	∓177.7(2)
C7 – C4 – C3 – C2	±179.7(2)
C7 – C4 – C3 – O3	∓58.1(2)
O3 – C3 – C2 – N1	∓70.8(2)
O3 – C3 – C4 – C5	±68.6(2)
C2 – C3 – O3 – H33	∓82(2)

[a] The two signs refer to the (3*S*,4*S*)- and (3*R*,4*R*)-forms, respectively. [b] Numbers in parentheses here and throughout this paper are the estimated standard deviations of the last significant digits.

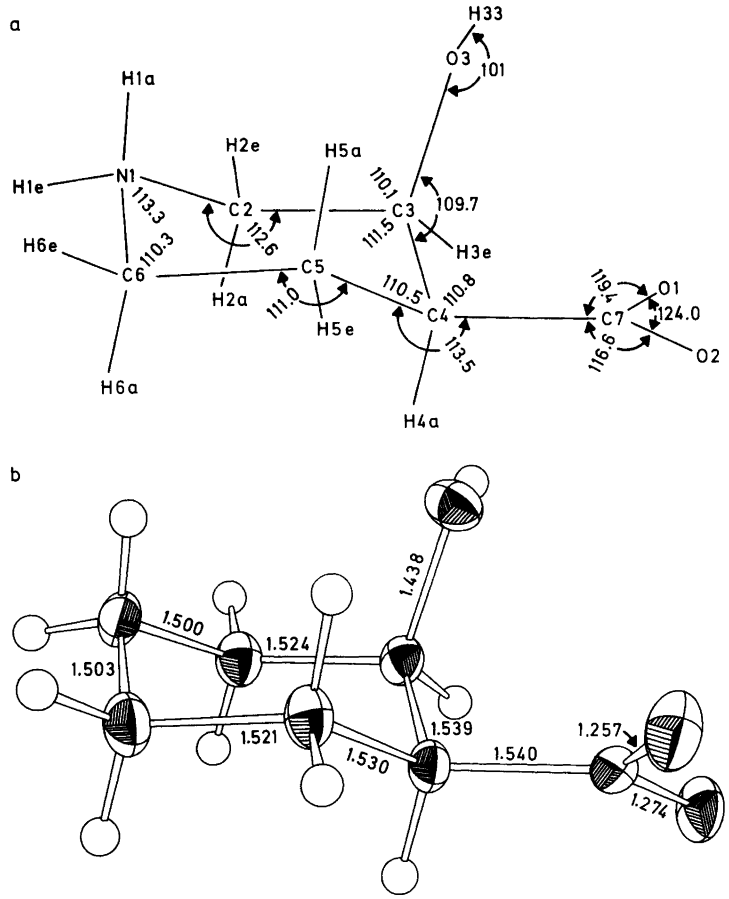

Fig. 2. Perspective drawings of the (3*S*,4*S*)-form of 3-hydroxypiperidine-4-carboxylic acid. a. The numbering of the atoms and bond angles (°) involving only non-hydrogen atoms; e.s.d.'s are 0.2 °. b. The thermal ellipsoids for the non-hydrogen atoms, scaled to 50 % probability; hydrogen atoms are represented as spheres of arbitrary radius. Bond lengths (Å) between non-hydrogen atoms; e.s.d.'s are 0.003 Å. The C – H bond distances are found within the range 0.92(3) – 1.01(3) Å. The *intra*-molecular distances from N1 to O1, O2 and O3 are 4.969(3), 5.181(3) and 3.039(3) Å, respectively.

The shapes of ring systems can be evaluated from ^1H NMR coupling constants using the *R* value method.[7] By application to the CH_2-CH_2 system formed by C5 and C6, the value is determined to be 1.86 for *1*. The *R* value determined for piperidine is 2.07.[7] These values, calculated from NMR data, indicate the substituted system *1* to be less puckered than that of piperidine in solution. On the basis of the NMR data for *2* and *4* *R* values of 1.90 and 1.89 are calculated, indicating *2* and *4* to be less puckered than *N*-methylpiperidine, $R=2.06$,[7] in solution.

The molecular packing of *1* is illustrated in Fig. 3. The crystal structure is stabilized by hydrogen bonds, one for each hydrogen atom covalently bonded to nitrogen or oxygen. Hydrogen bonds are formed between molecules related by a *c*-glide-plane [$O3^{i}-H33\cdots O1^{iv}$], thereby forming infinite zigzag chains in the *c*-direction. These chains are interlinked in the *a* direction by hydrogen bonds [$N1^{i}-H1e\cdots O2^{ii}$ and $N1^{i}-H1a\cdots O2^{iii}$] formed between molecules related by a 2-fold screw axis. Hydrogen bond distances and angles are given in Table 3. All other *inter*molecular contacts correspond to van der Waals' interactions.

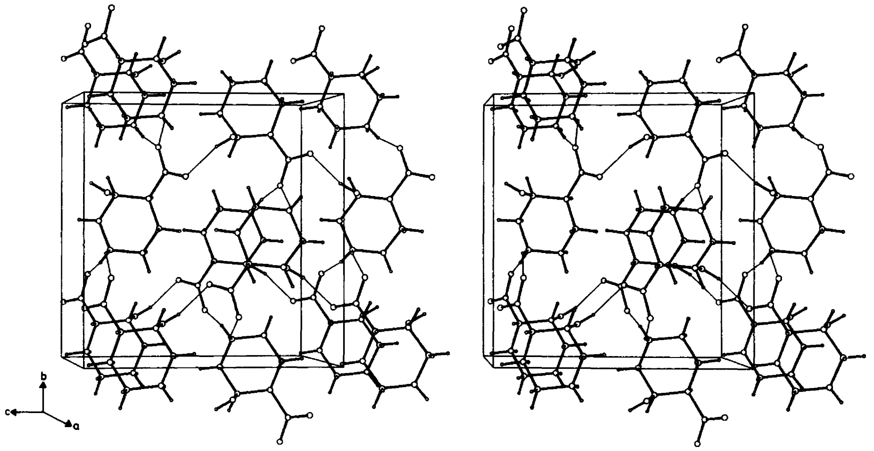

Fig. 3. Stereo view of the molecular packing of (3*RS*,4*RS*)-3-hydroxypiperidine-4-carboxylic acid. Hydrogen bonds are drawn with solid lines.

EXPERIMENTAL

^{1}H NMR analysis

The 270 MHz NMR spectra were obtained on a Bruker HX 270 S instrument at a probe temperature of 300 K. An undegassed sample was prepared as a *ca.* 5 % solution in D_2O and placed in a 5 mm tube. Chemical shifts are reported relative to the internal standard TSP. To assign the transitions in the observed spectra and obtain reliable coupling constants homo decoupling experiments were performed. The spectra were obtained in FT mode using 16 K data points for a spectral width of 2000 Hz. The chemical shift separation of the individual protons makes the spectrum weakly coupled. The deduced NMR parameters have been checked by simulation using the SIMEQ programme.[17]

The obtained ^{1}H NMR data for compound *1* are shown in Table 1.

X-Ray analysis

The crystals used for the X-ray examination were grown from a water-ethanol solution.

The space group, $P2_1/c$, and preliminary cell parameters were determined from photographs. A crystal with approximate dimensions 0.4 × 0.2 × 0.4 mm was used for both cell refinement and data collection at room temperature on a NONIUS CAD-3 diffractometer with graphite monochromated MoK_α radiation ($\lambda = 0.71069$ Å). For the determination of cell parameters the θ angles for 31 reflections were measured. The crystal density was measured by flotation in a bromobenzene/methyl iodide mixture. Cell parameters and other crystal data are given in Table 4.

Intensity data were measured in the ω scan mode. 2043 reflections in one quadrant of reciprocal space in the range $3.0 \leq \theta \leq 31.0°$ were collected. Three reference reflections measured, one after every 20 intensity measurements, showed no significant intensity variation over the period of data collection.

Table 3. Hydrogen-bond distances (Å) and angles (°).

Symmetry code:
(i) x,y,z
(ii) $-x,-\frac{1}{2}+y,1\frac{1}{2}-z$
(iii) $1-x,-\frac{1}{2}+y,1\frac{1}{2}-z$
(iv) $x,1\frac{1}{2}-y,\frac{1}{2}+z$

A−H···B	A−H	H···B	A···B	∠AHB
$N1^i-H1e\cdots O2^{ii}$	0.88(3)	1.99(3)	2.791(3)	151(3)
$N1^i-H1a\cdots O2^{iii}$	0.87(3)	2.09(3)	2.888(3)	152(3)
$O3^i-H33\cdots O1^{iv}$	0.84(3)	1.92(3)	2.749(3)	166(3)

Table 4. Crystal data.

(3*RS*,4*RS*)-3-Hydroxypiperidine-4-carboxylic acid (*1*),	
$C_6H_{11}NO_3$	M.p. 250 – 253 °C (decomp.)
Monoclinic	M.W. 145.16
a = 5.047(3)	V 642.4 (Å)^3
b = 11.760(5)	Z 4
c = 11.145(6)	D_m 1.51 g cm^{-3}
β = 103.78(5)	D_x 1.501 g cm^{-3}
Space group: $P2_1/c$ (No. 14)	μ(Mo$K\alpha$) 1.29 cm^{-1}

Table 5. Final positional and thermal (× 10⁴ Å²) parameters for the non-hydrogen atoms. The anisotropic temperature parameters are of the form: $T = \exp[-2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + l^2c^{*2}U_{33} + 2hka^*b^*U_{12} + 2hla^*c^*U_{13} + 2klb^*c^*U_{23})]$.

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N1	.2034(4)	.4248(1)	.8196(2)	209(8)	143(8)	294(10)	4(6)	66(8)	49(7)
C2	.1144(5)	.5384(2)	.8569(2)	227(10)	175(9)	270(11)	1(8)	88(8)	−8(8)
C3	.2455(4)	.6371(2)	.8037(2)	179(8)	132(8)	249(10)	23(7)	61(7)	2(7)
C4	.2101(4)	.6244(2)	.6633(2)	183(9)	121(8)	224(9)	−6(7)	50(7)	12(7)
C5	.3139(4)	.5082(2)	.6328(2)	236(10)	133(8)	243(10)	−14(7)	71(8)	−25(7)
C6	.1673(5)	.4126(2)	.6824(2)	254(10)	143(8)	301(12)	−24(8)	62(9)	−20(8)
C7	.3424(4)	.7250(2)	.6110(2)	185(9)	148(8)	222(10)	−6(7)	−7(7)	33(7)
O1	.5197(4)	.7058(1)	.5513(2)	318(9)	204(8)	384(10)	26(6)	165(8)	58(7)
O2	.2597(3)	.8241(1)	.6307(2)	275(8)	126(7)	398(10)	17(6)	103(7)	27(6)
O3	.5308(3)	.6441(1)	.8634(2)	196(7)	227(7)	269(8)	−3(6)	20(6)	−61(6)

1300 reflections had net intensities greater than 2.0 σ(I), where σ(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 refinement. The structure was solved by direct methods with the MULTAN programme.[19] Least-squares refinement of the non-hydrogen atoms, first with isotropic, then anisotropic thermal parameters converged at an *R*-value = 0.093. The quantity minimized was $\Sigma w(|F_o| - |F_c|)^2$, $w = 1$. A difference map then revealed the positions of all the hydrogen atoms (0.6 – 1.0 e Å^{-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 weights used in the final cycles of refinements were given by $w^{-1} = 1 + ((F_o - a)/b)^2$, where $a = 6.2$ and $b = 8.5$, determined empirically by analysis of $\Sigma w(|F_o| - |F_c|)^2$ averaged over ranges of $|F_o|$ and sin θ. On the last cycle of least squares refinement the values of maximum and average shift/error were 0.02 and 0.004, respectively. The final *R*-value is 0.058 and $R_w = 0.067$ for 1300 unique reflections. Tables 5 and 6 list the final positional and thermal parameters of the non-hydrogen and hydrogen atoms, respectively. The terminal set of structure factors

Table 6. Final positional and thermal (Å²) parameters for the hydrogen atoms.

Atom	x	y	z	B_{iso}
H1a	.375(6)	.413(2)	.853(3)	1.7
H1e	.102(6)	.375(3)	.848(3)	1.7
H2a	−.074(6)	.541(2)	.833(3)	1.8
H2e	.149(6)	.543(2)	.942(3)	1.8
H3e	.147(6)	.707(2)	.824(2)	1.5
H4a	.006(6)	.629(2)	.628(3)	1.4
H5a	.516(6)	.498(2)	.665(2)	1.7
H5e	.305(5)	.498(2)	.547(3)	1.7
H6a	−.029(6)	.414(2)	.646(3)	1.8
H6e	.234(6)	.340(3)	.664(3)	1.8
H33	.526(5)	.680(2)	.928(3)	1.5

listed with the observed data are available on request.

The computations were performed on an IBM 370/165 computer using *The X-Ray System* (version 1972),[18] MULTAN (version 1977),[19] and *ORTEP II*.[20] The X-ray atomic scattering factors used were those of Cromer and Mann for O, N, and C,[21] and of Stewart, Davidson and Simpson for H.[22]

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