ELSEVIER

International Journal of Pharmaceutics 146 (1997) 93–99

international journal of pharmaceutics

Solid-state investigation of the tautomerism of acetohexamide

Gregory A. Stephenson[a,*], Ralph R. Pfeiffer[b], Stephen R. Byrn[a]

[a]*Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, IN 47907, USA*
[b]*Solid-State Chemical Information, West Lafayette, IN 47906, USA*

Received 22 August 1996; revised 10 October 1996; accepted 10 October 1996

Abstract

Polymorphism of the anti-diabetic drug acetohexamide has been investigated by numerous techniques. On the basis of Fourier-transform infrared (FT-IR) data, one of the most common forms, form A, has been proposed to exist in the enol-tautomeric state, whereas form B has been proposed to be in the keto-tautomeric state. The following article examines the solid-state tautomerism of acetohexamide using the techniques of X-ray crystallography and ^{13}C solid-state nuclear magnetic resonance (NMR) spectroscopy. In the NMR spectra, resonances associated with the acetyl carbonyl and amide carbonyl groups are well resolved. By comparison of the spectra of acetohexamide with those of the related compounds chlorpropamide and tolbutamide, whose crystallographic structures have been determined, it is firmly established that both of the acetohexamide polymorphic forms are in the keto-form. The crystal structure of acetohexamide form A was solved and is reported herein. The structure not only shows the keto-tautomeric state of form A, but also confirms the NMR resonance assignments.

Keywords: Acetohexamide; Chlorpropamide; Tolbutamide; Polymorphic forms; Solid-state nuclear magnetic resonance; X-ray crystal structure

1. Introduction

Polymorphism is widespread among sulfonamides. The crystal structures of many of the forms have been determined and in most cases the conformation of the molecule is similar in each crystalline modification. Differences in crystal packing, rather than conformational differences, are mainly responsible for polymorphism among this class of compounds (Byrn, 1982), one notable exception being the conformational polymorphism of sulfapyridine (Bar and Bernstein, 1984).

Because of the poor water solubility of acetohexamide and the potential influence of polymorph-

* Corresponding author. Current address: Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, USA. E-mail: Stephenson_Gregory_A@lilly.com.

0378-5173/97/$17.00
PII S0378-5173(96)04775-8

ism on bioavailability (Yokoyama et al., 1979), many of its physical properties have been extensively examined. The techniques of X-ray powder diffraction, differential scanning calorimetry, infrared spectroscopy, dissolution rates, solubility studies, bioavailability and thermal microscopy have each been used to examine the polymorphic forms of acetohexamide and the related compounds, chlorpropamide and tolbutamide (Girgis-Takla and Chroneos, 1977; Burger, 1978; Yokoyama et al., 1979; Al-Saieq and Riley, 1982; Graf et al., 1984; Girgis-Takla and Dakas, 1989) (see Scheme 1).

The structures of tolbutamide form A and the only reported form of chlorpropamide have been determined (Koo et al., 1980; Donaldson et al., 1981). The structures of the polymorphic forms of acetohexamide, however, have not been solved even though crystallographic data would aid greatly in the interpretation of the spectroscopic data. It has been proposed, on the basis of infrared spectra, that forms A and B of acetohexamide might not be true polymorphs (same compound in different crystalline environments), but instead different tautomeric forms (Girgis-Takla and Chroneos, 1977, Girgis-Takla and Dakas, 1989; Graf et al., 1984). The purpose of the following study is to demonstrate the use of solid-state nuclear magnetic resonance (NMR) for the study of keto–enol tautomerism in solid pharmaceuticals, a use for which the technique is extremely well suited. The data presented herein, indicate that acetohexamide does not exist in an enol-tautomeric state in either crystal form. The combination of solid-state NMR spectroscopy and X-ray crystallography provide strong evidence that both forms exist in the keto-tautomeric state and are truly polymorphic.

2. Materials and methods

2.1. Materials

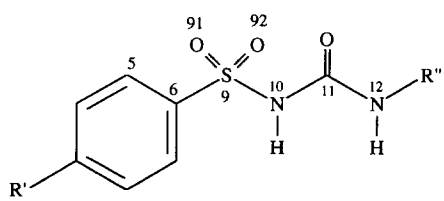
Acetohexamide was a gift from Eli Lilly. Chlorpropamide and tolbutamide were purchased from Sigma. Crystalline forms A and B of acetohexamide were produced by recrystallization from methanol and chloroform, respectively. The polymorphic form of the resulting solid was determined by comparison with the X-ray powder diffraction patterns of published reference patterns (Al-Saieq and Riley, 1982). High-quality single crystals of acetohexamide were obtained by slow evaporation of an ethanol–water solution.

2.2. Single crystal X-ray diffraction

Crystals of acetohexamide ($SO_4N_2C_{15}H_{20}$) form A crystallized as colorless needles of dimensions $0.25 \times 0.22 \times 0.15$ mm from an ethanol–water solution. The cell constants and orientation matrix for data collection were obtained from least-squares refinement, using the setting angles of 25 reflections in the range of $46 < \theta < 48°$. The structure was solved by direct methods using SHELX-86 (Sheldrick, 1986) and refined by full-matrix least-squares methods to a final R-value of 0.048 and R_w-value of 0.081 using 2649 unique reflections collected on an Enraf-Nonius CAD4 computer-controlled kappa axis diffractometer equipped with a Cu Kα radiation source and a graphite crystal incident beam monochromator. All atoms were located in succeeding Fourier syntheses and their positions and isotropic thermal parameters were refined. All nonhydrogen atoms were refined anisotropically and hydrogen atoms isotropically to convergence.

2.3. X-ray powder diffraction

Diffraction patterns were acquired using a Siemens D500 diffractometer equipped with a Cu



	R'	R"
Acetohexamide	acetyl	cyclohexyl
Chlorpropamide	Cl	*n*-propyl
Tolbutamide	methyl	*n*-butyl

Scheme 1.

Kα X-ray source and a Kevex liquid-nitrogen-cooled solid-state detector. Theoretical X-ray powder diffraction patterns were calculated using atomic coordinates from the single crystal X-ray data and the POW12 program (Smith, 1989). The powder diffraction patterns of acetohexamide, chlorpropamide and tolbutamide were calculated to ensure that the crystal form for which the structure was determined was consistent with the bulk material being analyzed spectroscopically.

2.4. ^{13}C CP/MAS solid-state NMR

Cross-polarized magic angle spinning (CP/MAS) ^{13}C solid-state NMR spectra were recorded at 50.19 MHz on a Chemagnetics M200 FT-NMR spectrometer. Approximately 250–300 mg of powdered sample were placed in a Kel-F rotor and spun at approximately 3.3 kHz. Free induction decays were defined typically by 8000 data points over a 3 KHz sweep width and were accumulated over 300–3000 transients with a recycle delay of 5 s for an acceptable signal-to-noise ratio. A proton decoupling field of 199.58 MHz and a contact time of 2.00 ms were used. Chemical shifts were measured relative to a hexamethylbenzene external standard with a methyl resonance at 17.36 ppm relative to tetramethylsilane.

3. Results and discussion

3.1. Crystallography

Acetohexamide form A molecules crystallize in the triclinic space group $P\bar{1}$ with $Z = 2$. The unit cell parameters are $a = 5.0990(3)$ Å, $b = 9.9863(4)$ Å, $c = 16.552(1)$ Å, $\alpha = 106.600(5)°$, $\beta = 96.009(5)°$, $\gamma = 97.2(2)°$. The calculated density is 1.359 g cm^{-3} with a formula weight of 324.40 g mol^{-1}. The atomic coordinates are provided in Table 1. Additional structural details are available in supplemental material.

An ORTEP diagram of acetohexamide is provided in Fig. 1; no unusual structural features were indicated by the thermal ellipsoids (Johnson, 1976). Comparison of the torsional angles of acetohexamide, chlorpropamide and tolbutamide molecules indicate that there is conservation of the geometry of the sulfonamide moiety. Selected torsion angles are provided in Table 2. In the case of tolbutamide form A, there are differences in the torsion angles of its aromatic ring with respect to the sulfonyl group, as compared with acetohexamide or chlorpropamide. The bond angles and lengths (Table 2) are consistent in each of the structures, with all molecules being in the keto-

Table 1
Positional parameters of nonhydrogen atoms for acetohexamide form A

Atom	x	y	z	B (Å^2)
S9	0.2898(1)	0.39 448(6)	0.79 132(3)	3.97(1)
O2	0.8059(4)	1.0096(2)	1.0888(1)	6.50(6)
O11	0.3599(3)	0.5849(2)	0.6858(1)	4.89(4)
O91	0.1432(4)	0.2909(2)	0.8203(1)	4.76(4)
O92	0.5175(4)	0.3618(2)	0.7502(1)	5.04(4)
N10	0.0683(4)	0.4314(2)	0.7256(1)	4.11(4)
N12	−0.0804(4)	0.5792(2)	0.6526(1)	4.70(5)
C1	0.4463(6)	0.9566(3)	1.1587(2)	5.98(7)
C2	0.6099(5)	0.9276(3)	1.0885(2)	4.60(6)
C3	0.5302(5)	0.7935(2)	1.0153(1)	4.11(5)
C4	0.6773(5)	0.7701(3)	0.9484(2)	4.45(6)
C5	0.6071(5)	0.6492(3)	0.8795(2)	4.46(5)
C6	0.3893(5)	0.5513(2)	0.8777(1)	3.84(5)
C7	0.2431(6)	0.5734(3)	0.9439(2)	5.00(6)
C8	0.3126(6)	0.6939(3)	1.0124(2)	5.03(6)
C11	0.1289(5)	0.5383(3)	0.6869(1)	3.91(5)
C13	−0.0556(5)	0.6966(3)	0.6169(1)	4.23(5)
C14	−0.1010(7)	0.8323(3)	0.6796(2)	6.13(8)
C15	−0.0732(8)	0.9536(3)	0.6419(3)	7.50(9)
C16	−0.2631(7)	0.9171(3)	0.5585(3)	7.52(9)
C17	−0.2198(8)	0.7831(4)	0.4968(2)	7.15(9)
C18	−0.2448(7)	0.6615(3)	0.5344(2)	5.35(7)

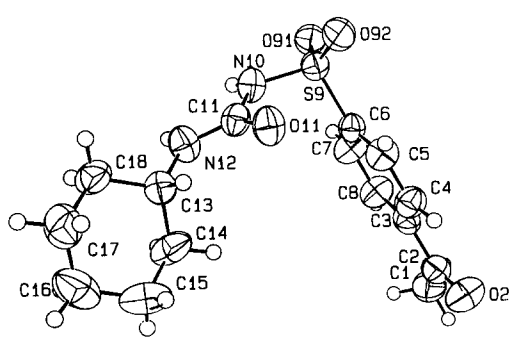


Fig. 1. ORTEP diagram of acetohexamide molecular conformation in polymorphic form A crystals, 50% probability.

Table 2
Comparison of selected torsion angles, bond angles and distances of acetohexamide form A (AHA), chlorpropamide (CPA), and tolbutamide form A (TBA)

Atoms	AHA: angle (°)	CPA: angle (°)	TBA: angle (°)
C5–C6–S9–N10	−87	−81	−143
C6–S9–N10–H101	−97	−99	−101
C6–S9–N10–C11	61	76	53
S9–N10–C11–O11	17	9	26
S9–N10–C11–N12	−164	−170	−153
N10–C11–N12–H121	3	−11	−7
Bond	AHA: angle (°)	CPA: angle (°)	TBA: angle (°)
S9–N10–H101	112	116	117
S9–N10–C11	122	126	121
H101–N10–C11	122	119	117
N10–C11–N12	115	113	115
N10–C11–O11	120	121	120
O11–C11–N12	125	126	124
C11–N12–H121	119	129	115
Bond	AHA: length (Å)	CPA: length (Å)	TBA: length (Å)
N10–C11	1.412(3)	1.380(9)	1.394(9)
C11–O11	1.215(3)	1.221(8)	1.262(8)
C11–N12	1.321(4)	1.318(9)	1.323(8)
N12–C13	1.454(4)	1.380(9)	1.468(9)

tautomeric state. A complete listing of bond lengths, bond angles, torsion angles, atomic coordinates and atomic displacement parameters are available in the supplementary materials. A search of the Cambridge Structural Database indicates that the average C–O bond length for N=C–OH groups is 1.30 Å (Cambridge Crystallographic Data Centre, 1996). This distance is far greater than the 1.22 Å measured in the structure of polymorphic form A.

3.2. Hydrogen bonding

In acetohexamide, the hydrogen-bonding network forms a continuous chain of hydrogen-bonded molecules by translation parallel to the *a*-axis. In chlorpropamide and tolbutamide form A, the hydrogen-bonded molecules form a continuous chain in which the molecules making up the network are related by two fold axes. Fig. 2 shows a molecular modeling representation of the hydrogen bonding networks of acetohexamide form A, chlorpropamide, and tolbutamide form A.

3.3. X-ray powder diffraction

The X-ray powder diffraction pattern calculated from the single crystal data of acetohexamide compares favorably with the experimentally obtained powder pattern of polymorphic form A (Fig. 3). Similarly, powder patterns for chlorpropamide and tolbutamide form A were calculated and compared to their experimentally obtained patterns; again there was good agreement (data provided in supplement). It should be noted that calculated patterns assume that the samples are randomly oriented (a situation which may not always be obtained experimentally, thus leading to differences in relative intensities between the calculated and observed patterns).

3.4. ^{13}C solid-state NMR

As shown in Fig. 4, the ^{13}C solid-state NMR spectra of acetohexamide form A and B are substantially different. The resonances associated

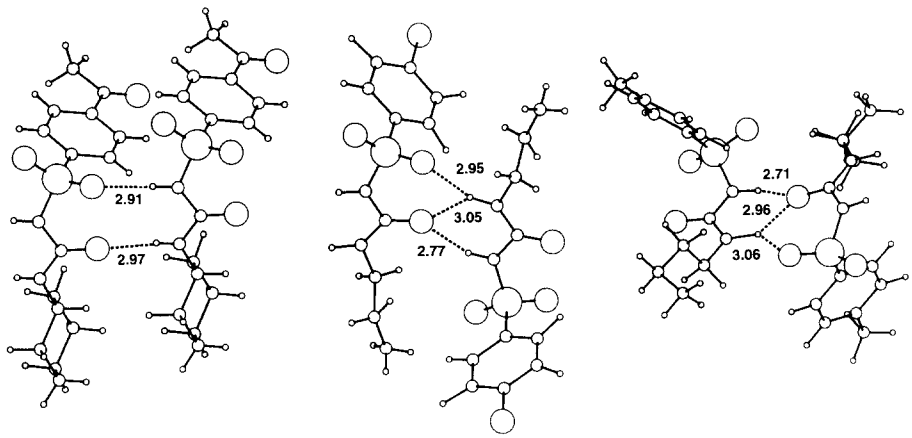


Fig. 2. Molecular modeling representation of the hydrogen-bonding networks of acetohexamide form A (left), chlorpropamide (middle), and tolbutamide form A (right). The heteroatom-to-heteroatom distance(s) for the hydrogen bonds to the amide oxygen are 2.97 Å; 3.05 and 2.77 Å; and 2.96 and 3.06 Å, respectively.

with the acetyl–carbonyl and amide–carbonyl groups are well resolved in the ^{13}C spectra—a distinct advantage which this technique has over Fourier-transform infrared (FT-IR) spectroscopy. The spectra of the acetohexamide forms show resonances associated with the acetyl–carbonyl at 197.2 and 196.7 ppm, form A and B, respectively. The chemical shift of the urea–amide carbonyl resonance in both polymorphs is consistent with that of chlorpropamide and tolbutamide form A, resonating at approximately 154 ppm (spectra provided in supplement). Carbonyl chemical shifts generally move up field by 10–15 ppm when in the enol-state, relative to the keto-state (Etter et al., 1990). Such a difference in chemical shift is not observed in polymorphic forms of acetohexamide. Amide–carbonyl carbon resonances are often broadened or split in solid-state NMR spectra because of bonding to the quadrupolar nitrogen nucleus. This broadening effect is observed in each of the solid-state NMR spectra, providing confirmation of the assignment of the amide–carbonyl resonances. The solid-state NMR data and the crystallographic data are consistent for form A, confirming that it exists in the keto-tautomeric state. The solid-state NMR data provides substantiating evidence that acetohexamide form B, whose crystal structure has not been solved, is also in the keto-tautomeric state. Enolization would result in a more pronounced change in the chemical shift for the amide carbonyl carbon.

3.5. Infrared spectroscopy

As already mentioned, acetohexamide has both an acetyl–carbonyl and an urea–amide carbonyl. In the infrared spectrum of form A, a single absorption is observed at 1685 cm^{-1}, whereas in form B two carbonyl absorptions are observed, 1690 and 1660 cm^{-1}, respectively. Because of the infrared data, it was earlier concluded that molecules in acetohexamide form B have their urea–amide group in the keto-state, whereas form A molecules are in the tautomeric enol-state (Girgis-Takla and Dakas, 1989). Chlorpropamide and tolbutamide molecules have only one carbonyl group and have a single absorption at 1660 cm^{-1} in their infrared spectra. In light of the present evidence provided by solid-state NMR and X-ray crystallography, the observation of a single infrared absorption in acetohexamide form A must be attributed to overlap of acetyl and urea–amide carbonyl group absorptions. The shift of the urea–amide carbonyl-stretching frequency (to 1685 from 1660 cm^{-1}) in form A with respect to form B can be explained on the basis of hydrogen bonding. In the structurally similar molecules chlorpropamide and tolbutamide form A, the

urea–amide carbonyl oxygen has two hydrogen bonds (and infrared absorptions at 1660 cm^{-1}), whereas in acetohexamide form A there is a single hydrogen bond to its carbonyl (Fig. 2). The magnitude and direction of the shift in infrared absorption frequency is consistent with the difference in hydrogen bonding to this group (Bellamy, 1980). Furthermore, since the urea carbonyl-stretching frequency in acetohexamide form B is equal to that of chlorpropamide and tolbutamide form A, it is likely that its hydrogen-bonding network resembles that found in their crystal structures.

4. Conclusions

The data presented herein clearly indicate that acetohexamide is polymorphic and that the molecules in each crystal form are in the keto-tautomeric state. This conclusion is supported by the solution of the crystal structure for polymorphic form A. By comparing the ^{13}C solid-state NMR amide carbonyl resonance values of the two crystalline forms, one may infer that polymorphic form B is also in the keto-state, since a far greater change in chemical shift is expected for keto–enol tautomerism than is observed in the solid-state NMR spectra of the two forms.

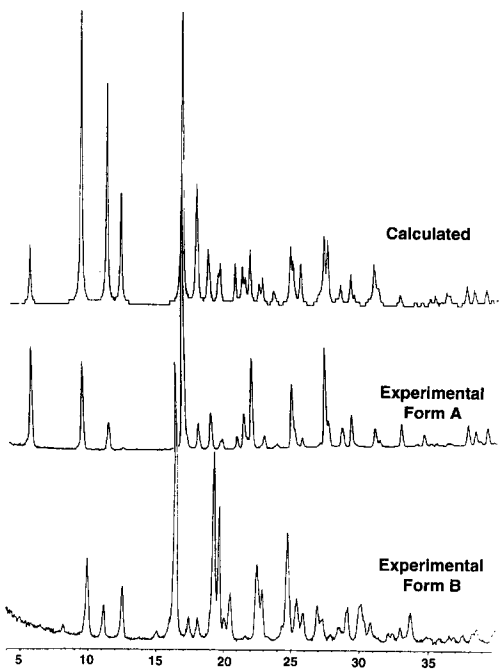


Fig. 3. Acetohexamide X-ray powder diffraction patterns: calculated pattern using single crystal data (top), experimentally obtained polymorphic form A (middle) and experimentally obtained polymorphic form B (bottom).

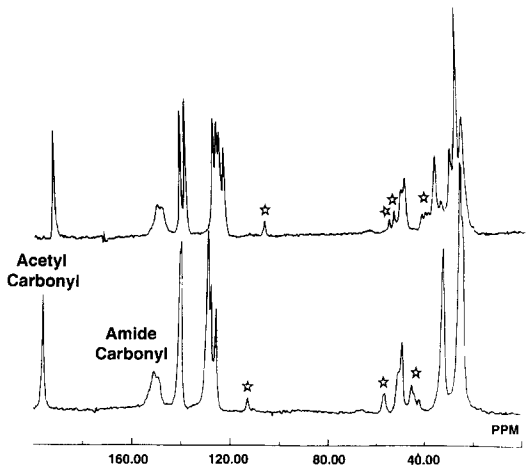


Fig. 4. ^{13}C CP/MAS solid-state NMR spectra of acetohexamide polymorphs: form B (top); form A (bottom). * Indicates spinning sidebands.

Acknowledgements

We express our appreciation to Ann McKenzie for running X-ray powder diffraction patterns of the samples, Pascal H. Toma for assisting in the structural solution and Joe Stowell for assistance in proof-reading this manuscript. We also thank G. Cooke and Eli Lilly and Company for providing the acetohexamide. We acknowledge the Byrn/Zografi Solid-state Joint-project Consortium for its contributions to our group's research. Greg Stephenson expresses his appreciation to the United States Pharmacopeial Convention for its funding through the USP-NF research fellowship.

References

Al-Saieq, S.S. and Riley, G.S., Polymorphism in sulphonylurea hypoglycaemic agents: III. Acetohexamide. *Pharm. Acta. Helv.*, 57 (1982) 43–46.

Bar, I. and Bernstein, J., Conformational polymorphism VI. The crystal and molecular structures of form II, form III, and form V of 4-Amino-*N*-2-pyridinylbenzenesulfonamide (sulfapyridine). *J. Pharm. Sci.*, 74 (1984) 255–263.

Bellamy, L.J., In *The Infrared Spectra of Complex Molecules*, 2nd edn., Vol. 2, Chapman and Hall, New York, 1980, pp. 155–157.

Burger, A., Acetohexamide: thermodynamics and biopharmaceutical aspects. *Sci. Pharm.*, 46 (1978) 207–222.

Byrn, S.R., *Solid-State Chemistry of Drugs*, Academic Press, New York, 1982.

Cambridge Crystallographic Data Centre, *The Cambridge Structural Database*, University Chemical Laboratory, Cambridge, UK, 1996.

Donaldson, J.D., Leary, J.R., Ross, S.D. and Thomas, M.J.K., The structure of the orthorhombic form of tolbutamide. *Acta Cryst. B*, 37 (1981) 2245–2248.

Etter, M.C., Reutzel, S.M. and Vojta, G.M., Analysis of isotropic chemical shift data from high-resolution solid-state NMR studies of hydrogen-bonded organic compounds. *J. Mol. Struct.*, 237 (1990) 165–185.

Graf, E., Beyer, C. and Abdallah, O., On the polymorphism of acetohexamide. *Pharm. Ind.*, 46 (1984) 955–959.

Girgis-Takla, P. and Chroneos, I., The polymorphism of acetohexamide. *J. Pharm. Pharmacol.*, 29 (1977) 640–642.

Girgis-Takla, P. and Dakas, C.J., An infrared study of tautomerism in acetohexamide polymorphs. *J. Pharm. Pharmacol.*, 41 (1989) 227–230.

Johnson, C.K., *ORTEPII: Report ORNL-5138*, Oak Ridge National Laboratory, Oak Ridge, TN, 1976.

Koo, C.H., Cho, S.I. and Yeon, Y.H., The crystal and molecular structure of chlorpropamide. *Arch. Pharmacal. Res.*, 3 (1980) 37–49.

Sheldrick, G.M., *SHELX 86: Program for the solution of crystal structures*. Institut für Anorganische Chemie der Universität Göttingen, Göttingen, Germany, 1986.

Smith, D.K., *POW12*. Penn State University, University Park, PA, 1989.

Yokoyama, T., Umeda, T., Kuroda, K., Sato, K. and Takagishi, Y., Studies on drug nonequivalence. VII. Bioavailability of acetohexamide polymorphs, *Chem. Pharm. Bull.*, 27 (1979) 1476–1478.