Pergamon

Tetrahedron Vol. 50, No. 46, pp. 13125-13134, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4020/94 $7.00+0.00

0040-4020(94)00858-2

Detection and Characterization of Polymorphic Modifications of the Anxiolytic Drug ABECARNIL

Martin Bock, Gisbert Depke, Ursula Egner, Anke Müller-Fahrnow* and Gabriele Winter

Research Laboratories of Schering AG, 13342 Berlin, Germany.

Abstract: Polymorphism is of special interest in pharmaceutical research since the stability and the biological activity of a drug substance are influenced by its solid state form. For the anxiolytic β-carboline compound ABECARNIL (isopropyl 6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate, $C_{24}H_{24}N_2O_4$) three different modifications have been observed.

Single crystal X-ray structure analyses and a variety of physicochemical methods as well as energy minimizations have been carried out in order to characterize the three solid state forms and to find at least one method capable of differentiating between the polymorphic forms. Due to nearly identical conformations and very similar packings of the molecules within their crystal lattices, polymorphs A and B show a pronounced resemblance in most physicochemical properties. Nevertheless, both forms can be distinguished by X-ray powder diffraction and differential scanning calorimetry.

In the crystal, molecules belonging to polymorph C show altered conformation and packing characteristics compared to forms A and B. Polymorph C can therefore be distinguished from the other solid state forms by means of X-ray powder diffraction, differential scanning calorimetry and by IR spectroscopy. The relative energy of C in the crystalline environment is significantly lower than for the other two modifications.

INTRODUCTION

Exactly 100 years have passed since Emil Fischer has published his well known work on the "lock and key system"[1] concerning the mutual recognition of complementary molecular surfaces of biologically active molecules and their protein targets. He was one of the first scientists to realize the importance not only of the chemical constitution of a compound but also of its three-dimensional structure. Since that time, the development of powerful computer hard- and software and the rapidly increasing number of advanced structure determination techniques have enabled chemists to investigate structure-activity relationships resulting in a rational approach to drug design. Unless the three-dimensional structure of the enzyme or receptor protein is known, the crystal structures of active compounds often serve as a basis for computer aided molecular design.

Nevertheless, the conformations of the molecules may not correspond to the biologically active form but might be influenced by the crystal packing. Furthermore, conformational differences are not only likely to occur in solution, but due to varying intermolecular interactions substances can also exist in multiple solid state forms called polymorphs or - if solvent is included in the crystal lattice - solvates. Slight variations of crystallization conditions or altered ambient conditions like temperature and air humidity can give rise to the growth of crystals belonging to a new solid state form or to the transformation of already grown crystals.

Many physical and chemical properties as well as the energy content of a given substance depend on its solid state form[2,3], e.g. the melting point, the dissolution behaviour (and thus the bioavailability), the stability against air oxidation and hydrolysis, and the behaviour during processing. A substance intended for use as a pharmaceutical agent has to be thoroughly characterized concerning its physicochemical properties and its biological effect in order to get admission by the authorities. As the behaviour of a drug substance depends on its solid state form, normally only preparations containing one single polymorph are accepted. If a mixture of different forms is to be applied as therapeutical agent it has to be well defined and each component has to be analyzed.

The recognition of multiple solid state forms and the characterization of their properties are prerequisites in the development of drugs with high and constant quality. The optimal polymorphic form for an intended use should be sufficiently stable. Under production conditions, its preparation, quality and processing to a drug product should be reproducible and easily achieved by a low-cost procedure.

ABECARNIL (isopropyl 6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate, $C_{24}H_{24}N_2O_4$) is a typical example for polymorphism. The β-carboline compound is a partial agonist at the benzodiazepine receptor acting as a anxiolytic. It has been found to exist in three different solid state forms arbitrarily named **A**, **B** and **C**. In the following sections, the results of several physicochemical methods as well as molecular mechanics techniques applied to ABECARNIL will be presented and their capability of differentiating between the polymorphic forms and detecting small amounts of admixtures will be discussed.

RESULTS AND DISCUSSION

Single crystal X-ray structure determination[4]. Figure 1 shows the molecular conformation of polymorph **A** together with the atomic numbering scheme valid for all three structures. The bond angles and distances for the β-carboline moieties of the three crystal structures are comparable to those found in other determinations of this unsaturated skeleton[5,6,7] and are indicative of the aromatic nature of the ring system which is planar with root-mean-square deviations of 0.035 Å (**A**), 0.028 Å (**B**) and of 0.033 Å (**C**), respectively.

Taking the high standard deviations of the atomic positions of forms **A** and **B** into consideration, the molecular conformations of both species are nearly identical. A superposition of the respective β-carboline moieties results in an almost perfect fit of the substituents, the main difference being an angle of about 5° between the planes through the phenyl rings. The significance of this small difference is lowered further by the high atomic displacement coefficients of atoms C65 through C68 of both polymorphic forms. The overall conformations can be characterized as compact with all substituents pointing into the same directions. The planes between the phenyl rings and the β - carboline moieties are almost perpendicular to each other.

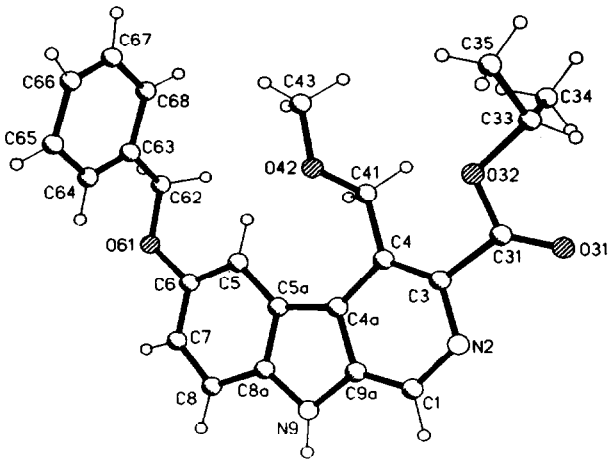

Figure 1. Molecular conformation and atomic numbering scheme for polymorph **A** of ABECARNIL.

Table 1. Crystal Data for Polymorphs **A**, **B** and **C** of ABECARNIL.

	A	**B**	**C**
Crystal habit	thin needle	thin platelet	compact
Space group	$P2_12_12$	$P2_1$	$Pca2_1$
cell constants			
a [Å]	11.49(1)	5.249(5)	12.697(2)
b [Å]	35.31(3)	11.51(2)	11.638(2)
c [Å]	5.224(5)	17.93(4)	14.656(3)
α [°]	90°	90°	90°
β [°]	90°	96.7(1)°	90°
γ [°]	90°	90°	90°
Volume [Å^3]	2119(3)	1076(3)	2165.6(7)
Z	4	2	4
D_C [g/cm^3]	1.27	1.25	1.24
Reflections measured	12510	2965	3524
Independent reflections	3249	1504	1848
R_{int}	9.87%	7.25%	1.89%
Observed reflections	1686	983	1591
	F > 3 s (F)	F > 2.5 s (F)	F > 4 s (F)
Final R - factor	9.09%	8.56%	2.95%
Final R_W - factor	10.52%	8.89%	2.92%
Data-to-parameter ratio	6.2 : 1	3.6 : 1	5.9 : 1

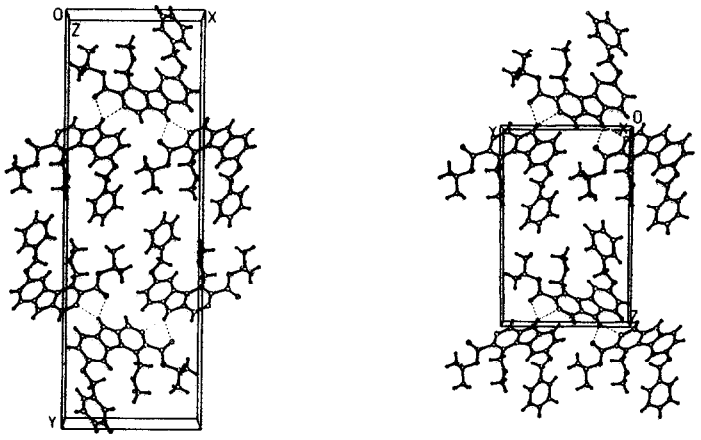

Figure 2. Comparison of the packings within the unit cell of polymorphs **A** (left) and **B** (right). Hydrogen bonds are indicated by dashed lines.

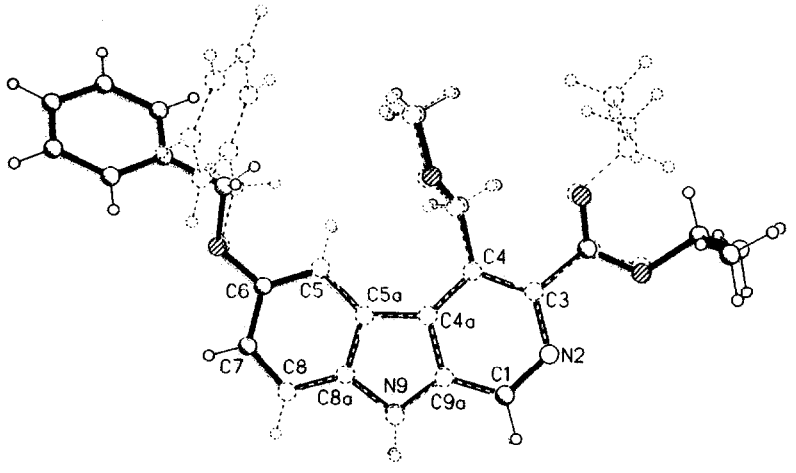

Figure 3. Superposition of the β-carboline moieties of polymorphs **C** (solid lines) and **B** (dashes).

Not only the molecular conformations of modifications **A** and **B** are similar but also the unit cell constants and the packing characteristics. The *a* - and *b* - axes of polymorph **A** correspond to the *b* - and *a* - axes of form **B** and the *b* - axis of **A** has about twice the length of the *c* - axis of **B** (see Table 1). Even the monoclinic angle $\beta = 96.7°$ of polymorph **B** is close to the 90° angles of polymorph **A**. A comparison of corresponding projections of the crystal packing of forms **A** and **B** is shown in Figure 2. The relative orientations of the three molecules in

the upper part of the figure are almost identical, even the hydrogen bondings are conserved within similar geometries. The three molecules in the lower part are rotated in the paper plane by 180° with respect to each other due to the 2_1 screw axis in **A**, which is replaced by a pure translation in case of **B**.

A superposition of the β - carboline moieties of polymorphs **B** and **C** is shown in Figure 3. Besides the rigid β - carboline ring only the conformation of the methoxymethyl group has remained unchanged. With respect to forms **A** and **B** the isopropyloxycarbonyl group of **C** has been rotated by 180° so that the oxygens O31 and O32 have traded places. The estercarbonyl group is now in close vicinity to the methoxymethyl group. In **C**, the angle between the planes through the phenyl- and the β - carboline rings is 46.5°. In contrast to the other two solid state forms, the three-dimensional structure of **C** can be described as extended. It lacks the continuous hydrophobic region created by the close vicinity of the substituents in forms **A** and **B**.

In spite of the changed conformation of **C** and a totally different packing within the unit cell with respect to **A** and **B**, a weak hydrogen bond between H9A and O31 of a symmetry related molecule is formed in all the structures. The distance between H9A and O31 varies between 2.11 Å and 2.20 Å and the angle N9 - H9A - O31 between 131.7° and 136.6°. Only in **A** and **B**, H9A is also in interaction with N2 of a related molecule. The distances between H9A and N2 are 2.33 Å and 2.37 Å and the angles are 149.1° and 152.9°, respectively.

X-ray powder diffraction. The diffraction patterns of polymorphs **A** and **B** are very similar but they can easily be distinguished from that of form **C**. Based on the crystal structures, X-ray powder diffractograms have been calculated. A comparison of theoretical and experimental diffraction patterns which are in good agreement is presented in Figure 4. It should be noted that the intensity distributions in experimental X-ray powder diffractograms especially of polymorph **B** may differ from the one shown in Figure 4 due to strong ordering effects.

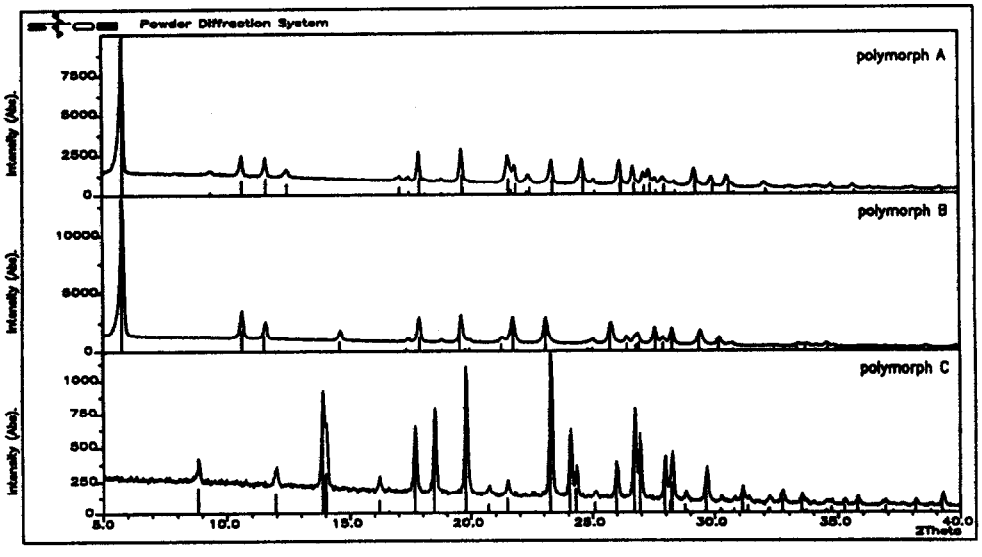

Figure 4. Comparison of measured and calculated X-ray powder diffractograms of polymorphs **A**, **B** and **C** of ABECARNIL.

Differential scanning calorimetry. The melting behaviours measured by differential scanning calorimetry are depicted in Figure 5. It shows that the three solid state forms can be distinguished with the help of this method. The average melting points and corresponding melting enthalpies of the polymorphs are 148 °C (89 J/g), 150 °C (87 J/g) and 151 °C (96 J/g), respectively. The melting endotherm of polymorph A in Figure 5 exhibits a shoulder at higher temperature due to the melting of polymorph B.

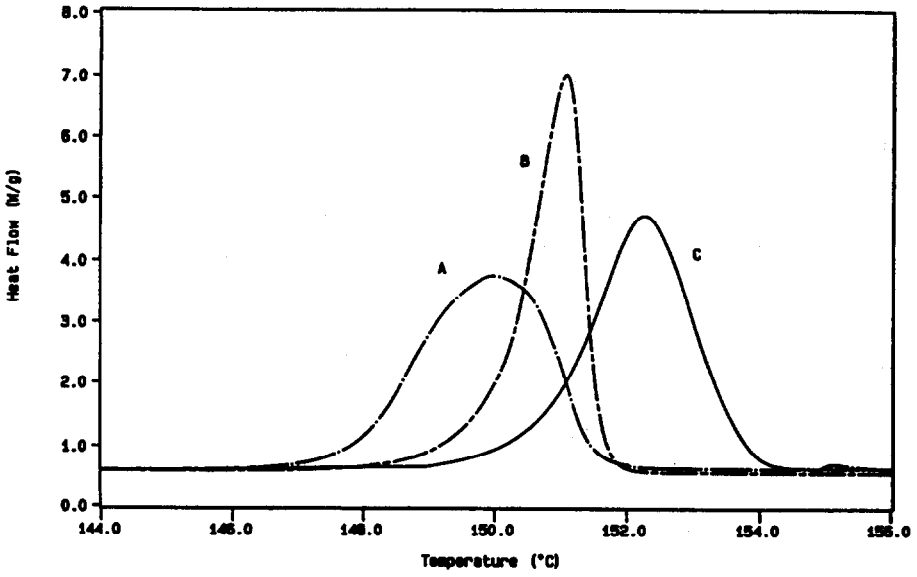

Figure 5. DSC thermograms of polymorphs A, B and C of ABECARNIL.

As microscopic hot stage investigations show, polymorph B may crystallize simultaneously during the melting of polymorph A. In some cases even small amounts of polymorph C may occur. Therefore, the detection of small admixtures of polymorph A in samples of B and *vice versa* is complicated. Because of the small difference between the melting temperatures of both polymorphs a small heating rate seems to be convenient to detect a low polymorph A content in a B sample. On the other hand, Figure 6 shows the heat flow of the melting endotherm decreasing with decreasing heating rate and the occurrence of an increasing polymorph B formation during the melting of polymorph A due to the prolonged scan time. At 5 K/min the formation of polymorph B may be neglected and a detection limit of approx. 5% polymorph A in B is found for the micronized drug substance. For reasons discussed above, the detection of polymorph B in A is much less sensitive and X-ray diffraction is more suitable.

IR spectroscopy. Figure 7 shows photoacoustic spectra of the three polymorphic forms. This method was chosen because it requires no special preparation of the samples which could effect a transformation of one polymorph into another. However, polymorphs A and B cannot be distinguished by this method, whereas the spectrum of form C is quite different from the others.

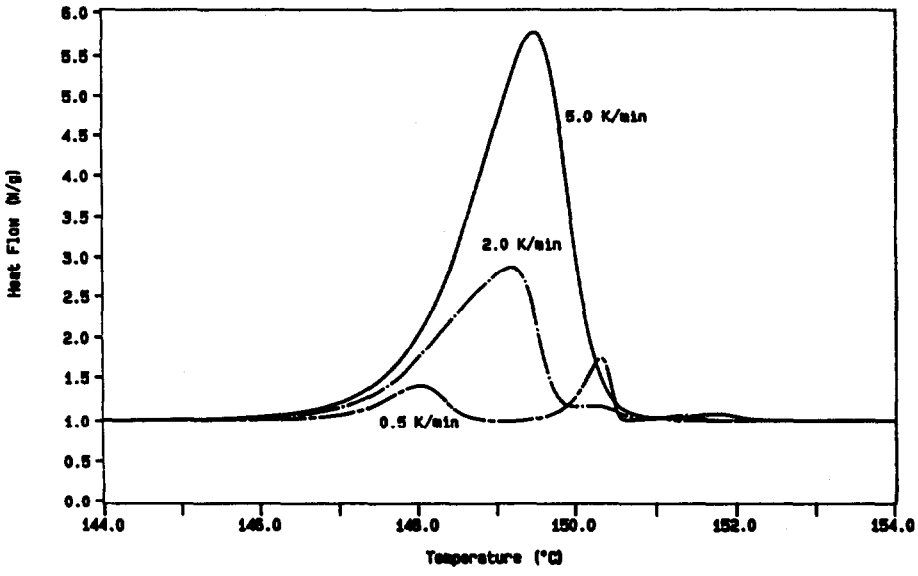

Figure 6. DSC thermograms of polymorph A at different heating rates.

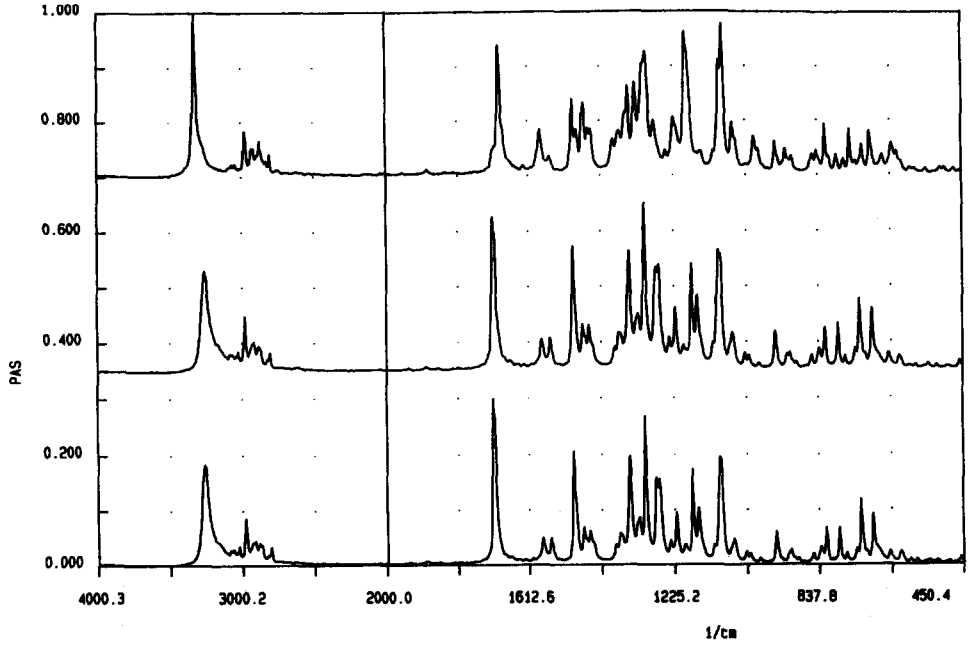

Figure 7. Photoacoustic spectra of polymorphs A (lower trace), B (middle trace) and C (upper trace) of ABECARNIL (PAS = Photo Acoustic Spectrometry units).

Computer simulations. The force field parameters reproduce sufficiently well the bond lengths and angles observed in the X-ray crystal structures of ABECARNIL. The relative energies of the molecules in the different solid state forms are given in Table 2. Within the approximations of the method (omission of polarization interactions, anisotropy), the lattice energy calculations of the three polymorphs yielded similar energies for forms **A** and **B** and a lower energy for polymorph **C**. The intermolecular energies seem to be dominated by van der Waals interactions as the van der Waals energy of form **C** is more favourable by 2.5 and 1.3 kcal/mol in comparison to forms **A** and **B**. The intermolecular Coulomb energies of all three forms are nearly identical and seem to have a minor impact on the packing mode. In contrast to this result, the intramolecular energy is dominated by the Coulomb interactions contributing largely to the lowest intramolecular energy of form **C**. The results of the energy minimizations are in agreement with the experimentally determined melting points of the crystals (average melting points for polymorphs **A**, **B** and **C** are 148°C, 150°C and 151°C, respectively).

Table 2. Relative Energies [kcal/mol] of the Three Solid State Forms of ABECARNIL.

Polymorph	Intramolecular energy			Intermolecular energy		Total energy
	Valence	VDW[1]	Coulomb	VDW	Coulomb	
A	80.6	95.1	-6.9	-59.8	-4.3	104.8
B	81.6	94.8	-6.9	-61.0	-4.7	103.8
C	79.9	95.8	-8.3	-62.3	-4.7	100.4

[1] VDW = van der Waals

Lattice energy calculations are valuable in understanding the basis of the energetic preference of a given molecule for its packing motif. The method would be even more helpful if it could be used in the prediction of crystal structures, but this application is hampered by the fact that it is in general difficult to know in advance what packing modes are available to the conformational isomers.

CONCLUSIONS

The anxiolytic compound ABECARNIL has been found to exist in three polymorphic forms, arbitrarily named **A**, **B** and **C**. Due to almost identical conformations and a striking resemblance in the crystal packing, most physicochemical properties of forms **A** and **B** are very similar. Form **C** differs in both aspects from the other two polymorphic forms and can therefore be easily distinguished by most methods applied throughout this study. Admixtures of polymorph **A** in preparations of form **B** can be detected most efficiently by differential scanning calorimetry and small amounts of **B** in samples of **A** by X-ray diffraction.

EXPERIMENTAL SECTION

Single crystal X-ray structure determination. Single crystal X-ray structure analyses were carried out for all three polymorphs. Crystals of polymorph **A** were needle-shaped (0.6 x 0.08 x 0.08 mm³), crystals of **B** grew as thin platelets (0.6 x 0.25 x 0.08 mm³) and only those of **C** had a compact form (0.5 x 0.5 x 0.25 mm³). The crystallographic data are given in Table 1. The X-ray intensity data were collected up to $2\theta = 47.5°$ ($2\theta = 45.0°$ for **B**) on a Siemens P4 four-circle diffractometer using monochromatized MoKα radiation from a fine focus sealed tube (λ = 0.71073 Å), and corrected for Lorentz and polarisation effects. Three standard reflections measured every 97 reflections revealed no decay due to radiation damage.

The structures were solved by direct methods[8] which proved to be difficult for **A** and **B** but finally resulted in providing the coordinates of all non-hydrogen atoms. After the full matrix least-squares refinement had converged, the hydrogen atoms were included at calculated positions and a common isotropic temperature factors was refined for them. The final R-factors for **A** and **B** (see Table 1) are relatively high because of the small diffracting volumes and the resulting poor quality of the intensity data.

X-ray powder diffraction. The samples were introduced between two polyacetate films held together by double-sided adhesive tape. Data collection was carried out in transmission mode on the automated STOE Powder Diffractometer STADI P using germanium-monochromatized CoKα_1-radiation (λ = 1.78897 Å). The 2θ-scans were performed using the small linear position-sensitive proportional counter between 5° and 40°. All calculations were carried out using parts of the STOE software.

Differential scanning calorimetry. DSC thermograms were recorded using a Perkin Elmer DSC 7 at a heating rate of 5 K/min between 140°C and 160°C. Samples comprising of 1.0 to 4.6 mg ABECARNIL were sealed in aluminium pans, inserted at room temperature and heated quickly to the starting temperature of the scan.

IR spectroscopy. IR spectra were measured on a NICOLET 710 FTIR spectrometer using MTEC's model 100 photoacoustic cell. The measuring cell was flushed with helium. For each of the three polymorphs, 100 scans recorded with a velocity of 10 were averaged. In order to remove moisture from the gas phase, P_2O_5 was placed under the samples, which were used without any prior preparation.

Computer simulations. The molecules within the unit cell were generated with SHELXTL+[8]. All further calculations were carried out on a Siemens Nixdorf S200 supercomputer using DISCOVER 2.8 (Biosym Technologies, Inc.). Energy minimizations were performed in space group P1 with periodic boundary conditions, a cutoff radius of 16 Å and with recalculation of the nearest neighbour list every 10 cycles. The conjugate gradient algorithm was applied until the maximum derivative was less than 0.1 kcal mol^{-1} Å^{-1} with a root-mean-square derivative less than 0.2 kcal mol^{-1} Å^{-1}. A value of $\varepsilon = 1.0$ was used for the dielectric constant.

The force field parameters for ABECARNIL were assigned with Insight II (Biosym Technologies, Inc., consistent valence force field[9]). To examine the accuracy of the force field used in the calculations, the minimized structure of ABECARNIL was compared with its X-ray structure. The charge distribution was calculated with the AM1[10] formalism as implemented in the program MOPAC[11].

ACKNOWLEDGEMENT. The work of D. Wiele, who first prepared the three polymorphic forms of ABECARNIL and helped with their characterization, is gratefully acknowledged. We are also grateful to M. Vlassis, TU Berlin and to Dr. W. Beckmann, Schering AG for kindly providing the single crystals used in this study and to Drs. F. Lopez and P.J. Weining from the working group Calorimetry and Kinetics of Schering AG for experimental support. We would like to thank Drs. E. Eckle, G.-A. Hoyer, P.W. Schneider and C. Wienhold for fruitful discussions.

REFERENCES

1. Fischer, E. *Ber. Dtsch. Chem. Ges.* **1894**, 27, 2985-2993.
2. Bernstein, J.; Hagler, A.T. *J. Am. Chem. Soc* **1978**, 100, 673-681.
3. Leonidov, N.B.; Zorkii, P.M.; Masunov, A.E.; Gladkikh, O.P.; Bel'skii, V.K.; Dzyabchenko, A.V.; Ivanov, S.A. *Russ. J. Phys. Chem.* **1993**, 67, 2220-2223.
4. The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.
5. Codding, P.W. *Can. J. Chem.* **1982**, 61, 529-532.
6. Muir, A.K.S.; Codding, P. W. *Can. J. Chem.* **1984**, 62, 1803-1806.
7. Bertolasi, V.; Ferretti, V.; Gilli, G.; Borea, P.A. *Acta Cryst.* **1984**, C40, 1981-1983.
8. SHELXTL+ , Siemens Analytical X-Ray Instruments, Inc.
9. Hagler, A.T.; Huler,E.; Lifson, S. *J. Am. Chem. Soc.* **1974**, 96, 5319-5327.
10. Dewar, M.J.S.; Zoebisch, E.G.; Healy, E.F.; Stewart, J.J.P. *J. Am. Chem. Soc.* **1985**, 107, 3902-3909.
11. Stewart, J.J.P.; Seiler, F.J. Program MOPAC in QCPE 455, University of Indiana.

(*Received in Germany* 5 *September* 1994; *accepted* 30 *September* 1994)