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Solid-State Conformations of Aminosuccinyl Peptides: Structure of *tert*-Butyloxycarbonyl-L-prolyl-L-aminosuccinyl-glycyl-L-alanine Methyl Ester (Boc-L-Pro-L-Asu-Gly-L-Ala-OMe). A Case of Pseudo-Translational Symmetry

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Abstract

$C_{20}H_{30}N_4O_8$, $M_r = 454.48$, monoclinic, $P2_1$, $a = 13.411$ (2), $b = 12.592$ (2), $c = 14.710$ (1) Å, $\beta = 104.30$ (1)°, $V = 2407$ (6) Å^3, $Z = 4$, $D_x = 1.254$ Mg m^{-3}, λ(Cu $K\alpha$) = 1.5418 Å, $\mu = 0.783$ mm^{-1}, $F(000) = 968$, room temperature, final $R = 0.086$, $wR = 0.080$ for 4055 observed reflections. The title compound is a model for the intermediate formed in the deamidation reaction of porcine adrenocorticotropin hormone. The structure presents a pseudo-translational symmetry and was solved by using a modified version of the *SIR*88 package. In the refinement, few stereochemical restraints were needed to handle the static disorder shown by the C-terminal fragment of one molecule in the asymmetric unit. The conformation of the two independent molecules is almost identical and is a II′ β-bend, stabilized by an intramolecular hydrogen bond. In the crystal, screw-related molecules are linked by hydrogen bonds. The two molecules in the independent unit are related by the translation vector $\mathbf{u} = 0.4962\ (2)\mathbf{a} + 0.7310\ (2)\mathbf{b} + 0.5075\ (2)\mathbf{c}$.

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Introduction

In protein chemistry the non-enzymatic deamidation of the asparaginyl side chain is a well-characterized process, which transforms specific asparagines into aspartyl and isoaspartyl residues (Clarke, 1987). In particular, porcine adrenocorticotropin hormone (ACTH) has been shown to deamidate easily at Asn in position 25 (Graf, Bajusz, Patthy, Barart & Cseh, 1971). During the deamidation process an aminosuccinyl (Asu) residue is formed as an intermediate.

As this residue significantly restricts the available range of backbone conformations, we are currently investigating structural and conformational properties of model Asu-containing peptides, with the aim of correlating the tertiary structure of the polypeptide chain with the propensity of the deamidation reaction.

Previous investigations in solid and solution states have been focused on Asu-*X*-containing di- and tripeptides, studying the influence of the bulkiness and nature of the side chain of the *X* residue (Capasso, Mattia, Mazzarella & Zagari, 1984*a*,*b*; Capasso, Mazzarella, Sica & Zagari, 1984, 1987, 1989*a*). They indicate that a type II′ β-bend, with the Asu residue in the second position of the turn, is strongly preferred in non-proton acceptor solvents and in the solid state, when the *X* residue does not have a branched side chain.

In this paper, the crystal structure of Boc-L-Pro-L-Asu-Gly-L-Ala-OMe, solved by a non-standard procedure, is presented and discussed in comparison with related reports. This peptide, which has the same sequence as ACTH 24–27, with an Asu in place of an Asn residue, is a model of the intermediate formed in the spontaneous deamidation reaction of this hormone.

Fig. 1 shows the structural formula together with the atom numbering; the numbering scheme is consistent with those adopted for other Asu peptide crystal structures (Capasso *et al.*, 1989*a*; and references therein).

Experimental

The peptide was synthesized by standard procedures (Capasso, Mazzarella, Sica & Zagari, 1989*b*) and fully characterized. Colorless crystals were grown from aqueous acetone, 0.80 × 0.50 × 0.20 mm. Data were collected in $\omega/2\theta$ mode on an Enraf–Nonius CAD-4F diffractometer with Ni-filtered Cu $K\alpha$ radiation; reflections $0k0$ with k odd were systematically absent; in addition, the intensities of reflections with $2h + k + 2l = 4n$ were markedly stronger; lattice parameters were determined by a least-squares analysis of 23 reflections ($17 \le \theta \le 28°$); a total of 4559 independent reflections were measured with $2\theta \le 140°$ ($-17 \le h \le 17$, $0 \le k \le 16$, $0 \le l \le 18$); no systematic fluctuations greater than 2% were observed in the intensities of two regularly monitored reflections; a Lorentz–polarization correction was applied, absorption and extinction effects were ignored.

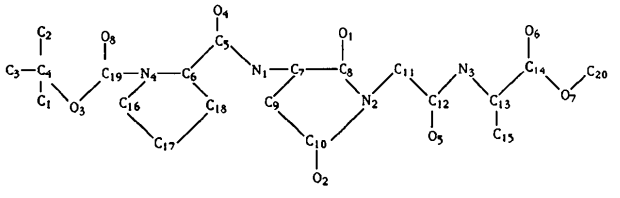

Fig. 1. Structural formula and numbering scheme of Boc-Pro-Asu-Gly-Ala-OMe.

Various attempts at solving the structure with *MULTAN*80 (Main, Fiske, Hull, Lessinger, Germain, Declercq & Woolfson, 1980), *SHELX*86 (Sheldrick, 1986) and *SIR*88 (Burla, Camalli, Cascarano, Giacovazzo, Polidori, Spagna & Viterbo, 1989) were unsuccessful. Trials with *SIR*88 revealed the presence of a pseudo-translational vector $\mathbf{u} = \mathbf{a}/2 + \mathbf{b}/4 + \mathbf{c}/2$. The calculated mean fractional scattering power of the electron density affected by the pseudo-translational symmetry was 16%: significant displacive-type deviations from ideal pseudosymmetry were also suggested.

All subsequent trials by *SIR*88 aiming at solving the structure by exploiting pseudo-translational symmetry as prior information (Cascarano, Giacovazzo & Luic, 1989*a*,*b*) were also unsuccessful. A Patterson map was then calculated which showed the following three largest peaks:

u	*v*	*w*	Intensity
0.00	0.00	0.00	1000
0.50	0.27	0.50	429
0.10	0.00	0.02	106

whose coordinates and positions agree with the *SIR*88 indications of pseudosymmetry. The presence of a very large peak at (0.50, 0.27, 0.50) suggested the idea of assuming the Patterson map as prior information in the probabilistic procedure for phase estimation. The theoretical approach [Giacovazzo, 1991, equations (18)–(20)] suggested the estimation of triplet-phase invariants according to a reliability parameter depending on the intensity and on the coordinates of the Patterson peak (0.50, 0.27, 0.50). The application of the conclusive formulas was successful and immediately provided a quasi-complete crystal structure.

Difference maps, interspersed with least-squares cycles, revealed all 64 non-H independent atoms. At this stage peaks as high as 2.6 e Å^{-3} were present in the difference synthesis in the region of the C-terminal Ala-OMe fragment of the unprimed molecule. Furthermore, at the end of the refinement these atoms showed large oscillations and the fragment had unrealistic geometrical parameters. An inspection of the electron density, using the program *FRODO* running on an Evans & Sutherland PS390 graphic system, revealed a disordered region embodying the atoms C(13), C(14), C(15), O(6), O(7) and C(20). A satisfactory fit to the electron density was found when two alternative orientations were allowed for the C-terminal fragment, with partial

occupancy set at 0.5. A stereochemically restrained isotropic least-squares procedure was performed on this model using the program *PROLSQ* (Hendrickson & Konnert, 1981). The function minimized was a sum of two residuals R_C and R_S. R_C represents the usual crystallographic residual $\sum w(|F_o| - |F_c|)^2$, where $w = 1/\sigma(F_o)^2$. R_S is the weighted residual with respect to ideal values of bond lengths, 1–3 distances and planarity of groups for the disordered fragment. To maintain the geometrical parameters close to ideal values, a large weight was applied to the stereochemical equations, $w = 1/\sigma^2$, $\sigma = 0.001$. The R index changed from 0.203 to 0.170. The refinement also indicated the occupancy factors of the two alternative fragments to be close to 0.5 and this value was finally adopted. The structure was then refined by standard full-matrix least-squares iterations, in which positional parameters of the disordered group were kept fixed and only isotropic temperature factors were allowed to vary. Positional and anisotropic thermal parameters for all remaining non-H atoms were refined. H atoms were stereochemically positioned and were not refined; they were included in structure-factor calculations with the same isotropic B_{eq} as the atoms to which they are bonded. The calculated H-atom parameters were regularly updated during the refinement. The weighting scheme was $w = 1$ for $F_o < 0.8F_o(\max)$ and $w = 0.8\ F_o(\max)/(F_o)^2$ for $F_o \geq 0.8F_o(\max)$. Final $R = 0.086$ and $wR = 0.080$ for 4055 reflections with $I > 2.5\sigma(I)$.

In the final cycle of the refinement, all parameter shifts/e.s.d.'s were smaller than 0.03. The final value of the standard deviation of an observation of unit weight was 3.17. The final electron-density difference map showed only random fluctuations, all smaller than 0.38 and higher than -0.36 e Å^{-3}. Scattering factors were taken from *International Tables for X-ray Crystallography* (1974, Vol. IV). Standard crystallographic computations were performed on a VAX 750, using the Enraf–Nonius (1979) *Structure Determination Package*.

Results and discussion

Structure solution

Owing to the large fraction of independent atoms related by the pseudo-translational symmetry, the application of standard direct packages failed, and the information contained in the largest non-origin Patterson peak was essential for the crystal structure solution. A *post-mortem* analysis of the triplet-phase distribution showed an unusually high percentage of negative triplets incorrectly designated as positive (Altomare, Cascarano & Giacovazzo, 1991). The use of the Patterson information led to more realistic concentration parameters of the triplet-invariant distributions and to the identification of some negative peaks which were therefore excluded from any active use.

Molecular geometry

Final parameters for both independent molecules are listed in Table 1.* Most bond lengths and angles are in the expected ranges and agree with average values recently derived for peptides (Ashida, Tsunogae, Tanaka & Yamane, 1987) and the Asu moiety (Capasso *et al.*, 1989*a*, and references therein). In particular, the distances N(2)—C(8) and N(2)—C(10) in the succinimide ring are equal within experimental error, and their average value of 1.38 Å is larger than the C—N distance usually found for a peptide linkage. Deviations from expected values of some bond lengths and angles are probably related to thermal motion. The highest discrepancies involve the atoms C(17) and C(17)′, which show substantial thermal motion, with a large component normal to the plane of the pyrrolidine ring.

Molecular conformation

Fig. 2 illustrates the conformation of the unprimed molecule. With the exception of the C-terminal fragment Ala-OMe (see below), the corresponding φ, ψ dihedral angles (Table 2) in the two molecules do not differ by more than 6°, indicating that the conformations are almost identical. Larger differences are only observed for the torsion angles of Pro rings.

All the peptide bonds are *trans*, whereas the urethane bond is *cis*, so that the Boc group is folded back on the peptide. This is usually observed when *tert*-Boc is followed by a Pro residue (Blessing & Smith, 1982; Benedetti, Bavoso, Di Blasio, Pavone, Pedone, Toniolo & Bonora, 1983).

* Lists of structure factors, anisotropic thermal parameters and H-atom parameters have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 54786 (27 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

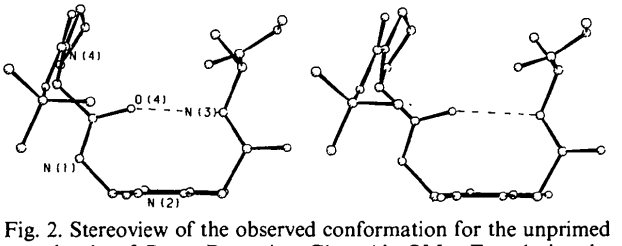

Fig. 2. Stereoview of the observed conformation for the unprimed molecule of Boc-L-Pro-L-Asu-Gly-L-Ala-OMe. For clarity the alternative conformation of the C-terminal fragment is not shown. Dotted lines indicate the internal hydrogen bond.

Table 1. *Fractional coordinates and equivalent isotropic temperature factors, with e.s.d.'s in parentheses*

Starred atoms were refined isotropically. $B_{eq} = (4/3)\sum_i\sum_j b_{ij}\mathbf{a}_i.\mathbf{a}_j$.

	x	y	z	B_{eq} (Å^2)
O(1)	0.6813 (4)	1.0187 (7)	0.3992 (4)	8.1 (2)
O(2)	0.5472 (4)	1.0313 (7)	0.6526 (4)	8.1 (2)
O(3)	0.9804 (4)	1.0842 (4)	0.4444 (3)	4.9 (1)
O(4)	0.8327 (3)	1.0820 (4)	0.6031 (4)	5.0 (1)
O(5)	0.5075 (5)	1.3117 (6)	0.5185 (6)	9.5 (2)
O(6)	0.752	1.385	0.459	11.2 (5)*
O(7)	0.705	1.520	0.535	7.8 (3)*
O(8)	1.0524 (4)	1.2415 (5)	0.5007 (4)	6.3 (1)
N(1)	0.8341 (4)	0.9129 (5)	0.5538 (5)	4.7 (1)
N(2)	0.6020 (5)	1.0422 (6)	0.5169 (4)	5.1 (2)
N(3)	0.6703 (5)	1.2472 (6)	0.5731 (6)	6.7 (2)
N(4)	1.0364 (4)	1.1086 (5)	0.5952 (4)	3.9 (1)
C(1)	1.0355 (7)	1.149 (1)	0.3072 (6)	7.6 (3)
C(2)	0.9040 (7)	1.0096 (9)	0.3011 (6)	6.8 (2)
C(3)	0.8657 (8)	1.1962 (8)	0.3351 (7)	7.2 (3)
C(4)	0.9465 (6)	1.1142 (8)	0.3471 (6)	5.5 (2)
C(5)	0.8795 (5)	1.0036 (6)	0.5877 (5)	4.2 (2)
C(6)	0.9973 (5)	1.0029 (6)	0.6095 (4)	3.8 (1)
C(7)	0.7211 (5)	0.9054 (7)	0.5357 (6)	5.0 (2)
C(8)	0.6726 (5)	0.9924 (8)	0.4745 (5)	5.3 (2)
C(9)	0.6820 (6)	0.9106 (7)	0.6245 (6)	5.5 (2)
C(10)	0.6008 (6)	1.0004 (8)	0.6041 (6)	5.8 (2)
C(11)	0.5369 (6)	1.1326 (9)	0.4760 (7)	7.1 (3)
C(12)	0.5700 (7)	1.2383 (9)	0.5260 (7)	7.1 (3)
C(13)	0.713	1.353	0.607	10.4 (7)*
C(14)	0.728	1.418	0.527	5.1 (3)*
C(15)	0.817	1.336	0.680	6.4 (4)*
C(16)	1.0840 (6)	1.1600 (7)	0.6835 (6)	5.5 (2)
C(17)	1.064 (1)	1.087 (1)	0.7573 (7)	15.1 (5)
C(18)	1.0426 (7)	0.9841 (8)	0.7159 (6)	6.4 (2)
C(19)	1.0246 (5)	1.1506 (6)	0.5125 (5)	4.3 (2)
C(20)	0.702	1.586	0.452	9.8 (7)*
O(6)″	0.680	1.528	0.606	8.8 (4)*
O(7)″	0.715	1.433	0.490	7.0 (3)*
C(13)″	0.709	1.341	0.629	5.2 (3)*
C(14)″	0.700	1.443	0.575	6.6 (4)*
C(15)″	0.821	1.324	0.686	18 (1)*
C(20)″	0.709	1.529	0.432	8.6 (6)*
O(1)′	0.1841 (4)	0.2695 (6)	−0.1078 (4)	7.1 (2)
O(2)′	0.0591 (5)	0.3259 (6)	0.1460 (4)	8.2 (2)
O(3)′	0.4860 (4)	0.3487 (4)	−0.0616 (3)	5.0 (1)
O(4)′	0.3374 (4)	0.3557 (4)	0.0896 (4)	5.2 (1)
O(5)′	0.0046 (5)	0.5743 (6)	−0.0134 (6)	8.8 (2)
O(6)′	0.2135 (6)	0.6709 (7)	−0.0838 (5)	9.5 (2)
O(7)′	0.1946 (5)	0.7934 (5)	0.0148 (5)	6.9 (2)
O(8)′	0.5563 (4)	0.5090 (5)	−0.0040 (4)	6.5 (1)
N(1)′	0.3374 (4)	0.1807 (5)	0.0548 (5)	5.0 (1)
N(2)′	0.1046 (5)	0.3099 (5)	0.0078 (4)	4.9 (1)
N(3)′	0.1712 (5)	0.5191 (5)	0.0413 (5)	5.9 (2)
N(4)′	0.5436 (4)	0.3716 (5)	0.0901 (4)	4.6 (1)
C(1)′	0.5345 (7)	0.424 (1)	−0.1997 (6)	7.4 (3)
C(2)′	0.4033 (8)	0.282 (1)	−0.2073 (7)	8.3 (3)
C(3)′	0.3648 (7)	0.466 (1)	−0.1630 (6)	7.2 (3)
C(4)′	0.4460 (6)	0.3824 (8)	−0.1589 (5)	5.4 (2)
C(5)′	0.3846 (5)	0.2745 (6)	0.0809 (5)	4.5 (2)
C(6)′	0.5017 (5)	0.2712 (7)	0.1039 (5)	4.5 (2)
C(7)′	0.2247 (5)	0.1737 (6)	0.0388 (6)	5.0 (2)
C(8)′	0.1739 (5)	0.2548 (7)	−0.0294 (5)	5.2 (2)
C(9)′	0.1873 (5)	0.1950 (7)	0.1274 (6)	5.3 (2)
C(10)′	0.1102 (6)	0.2847 (7)	0.0991 (6)	5.5 (2)
C(11)′	0.0379 (6)	0.3934 (8)	−0.0421 (7)	6.4 (2)
C(12)′	0.0689 (6)	0.5058 (7)	−0.0027 (6)	6.1 (2)
C(13)′	0.2051 (7)	0.6239 (7)	0.0751 (7)	6.9 (2)
C(14)′	0.2046 (6)	0.6951 (7)	−0.0092 (6)	5.1 (2)
C(15)′	0.318 (1)	0.6149 (9)	0.131 (1)	13.3 (4)
C(16)′	0.5884 (6)	0.4278 (8)	0.1790 (6)	6.3 (2)
C(17)′	0.583 (1)	0.346 (1)	0.2490 (7)	13.7 (6)
C(18)′	0.5433 (8)	0.253 (1)	0.2106 (6)	8.3 (3)
C(19)′	0.5288 (5)	0.4148 (7)	0.0068 (6)	4.9 (2)
C(20)′	0.1971 (8)	0.8686 (9)	−0.0615 (8)	8.3 (3)

Table 2. *Torsion angles* (°)

		Unprimed unit		Primed unit
ω_0	O(3)—C(19)—N(4)—C(6)	−4.3 (9)		−9.9 (10)
φ_1	C(19)—N(4)—C(6)—C(5)	−70.1 (8)		−65.8 (9)
ψ_1	N(4)—C(6)—C(5)—N(1)	148.6 (6)		154.1 (6)
ω_1	C(6)—C(5)—N(1)—C(7)	176.8 (6)		174.9 (6)
φ_2	C(5)—N(1)—C(7)—C(8)	54.2 (9)		54.2 (9)
ψ_2	N(1)—C(7)—C(8)—N(2)	−128.9 (7)		−129.1 (7)
ω_2	C(7)—C(8)—N(2)—C(11)	−179.1 (7)		−177.6 (7)
φ_3	C(8)—N(2)—C(11)—C(12)	−107.5 (9)		−108.7 (9)
ψ_3	N(2)—C(11)—C(12)—N(3)	25.0 (12)		26.9 (11)
ω_3	C(11)—C(12)—N(3)—C(13)	168.1	−175.6	177.0 (8)
φ_4	C(12)—N(3)—C(13)—C(14)	−74.5	−61.7	−71.8 (10)
ψ_T	N(3)—C(13)—C(14)—O(7)	141.7	−36.6	155.2 (7)

The most interesting feature of the molecular conformation is the presence of a II′ β-turn (Venkatachalam, 1968) in which Asu occupies the second position. This structure is stabilized by a 4→1 intramolecular hydrogen bond. The N(3) of Ala donates a proton to the carbonyl O(4) atom of Pro [N(3)···O(4) 2.966 (9), N(3)′···O(4)′ 2.988 (8) Å]. This conformation has been observed in the crystal structures of many Asu peptides (Capasso *et al.*, 1984, 1987, 1989*a*; Mazzarella, Schön, Sica & Zagari, 1988) and has also been predicted by theoretical calculations to be the most stable one *in vacuo* for the sequences Asu-*X*, when *X* does not have a branched side chain (Capasso, Mattia, Mazzarella, Sica & Zagari, 1990). Moreover, spectroscopic data in solution indicate that the II′ β-bend conformation is the predominant one in non-polar solvents (Capasso *et al.*, 1989*b*).

The succinimide ring is essentially planar, as no atom deviates more than 0.040 (8) Å out of the best plane of the five-membered ring. This result is consistent with previous observations (Capasso *et al.*, 1989*a*) that a flat ring provides a better orientation of the groups involved in the 4→1 intramolecular hydrogen bond. Larger deviations from planarity were observed for Asu peptides which do not present an internal hydrogen bond (Capasso *et al.*, 1984*a*,*b*).

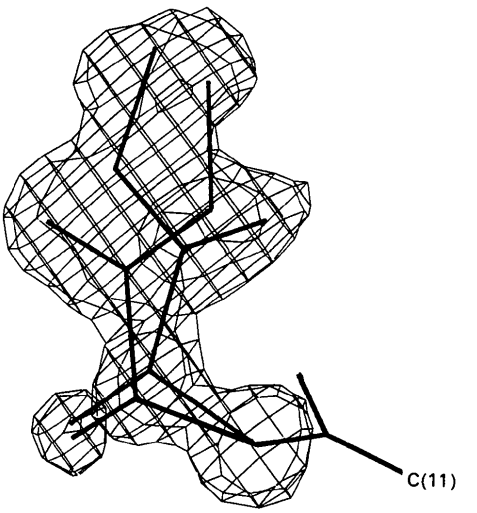

Fig. 3. Electron-density map with the two fitted models of the C-terminal region of the unprimed molecule.

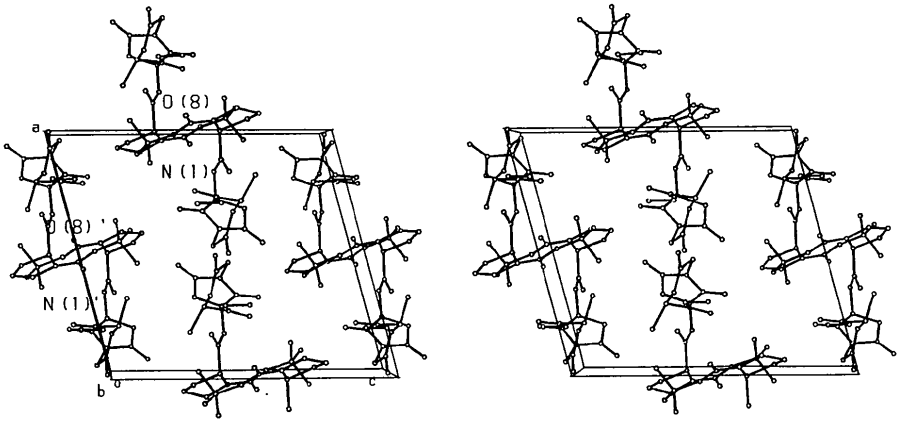

Fig. 4. Stereo packing diagram on the *ac* plane. For clarity the alternative conformation of the C-terminal fragment of the unprimed molecule is not shown.

In the present structure the Pro residue is in the *F* conformational state (Zimmermann, Pottle, Némethy & Scheraga, 1977), which is most frequently observed in the crystal structures of Pro-containing peptides (Ashida *et al.*, 1987; Benedetti *et al.*, 1983). However, the pyrrolidine ring conformation is slightly different in the two molecules: it is almost a 'twist' conformation with the axis through N(4) in the unprimed molecule and an 'envelope' with the apex at C(6) in the primed molecule (Cromer & Pople, 1975).

The electron density of the terminal region of the unprimed molecule is shown in Fig. 3. The two alternative models, which give the best fit to the observed electron density, differ by a rotation of $\sim 180°$ around the bond C(13)—C(14), flanked by a small rotation around bonds C(12)—N(3) and N(3)—C(13). This results in a replacement of the carbonyl O(6) atom by O(7). All intermolecular distances from atoms of this fragment are longer than 3.1 Å.

Crystal packing

The two molecules in the independent unit are related by a translational vector $\mathbf{u} = 0.4962\,(2)\mathbf{a} + 0.7310\,(2)\mathbf{b} + 0.5075\,(2)\mathbf{c}$. These values were obtained by the best superposition of 25 atoms, with the exclusion of seven atoms of Ala-OMe fragment, which is disordered in the unprimed molecule. The r.m.s. deviation between corresponding atoms was 0.17 Å, the largest deviations (0.3–0.7 Å) were observed for atoms not included in the fitting procedure.

The crystal packing is shown in Fig. 4. Molecules related by the crystallographic screw axis are stacked in columns and linked by intermolecular hydrogen bonds [N(1)···O(8) 2.866 (9), N(1)′···O(8)′ 2.792 (9) Å]. The columns form sheets of primed or unprimed molecules which alternate along the **c** direction.

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1,2-Naphthalenedicarboxylic Acid: Structures of Channel Clathrates and an Unsolvated Crystalline Phase

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Abstract

(1) 1,2-Naphthalenedicarboxylic acid–diethyl ether, $C_{12}H_8O_4.nC_4H_{10}O$, $M_r = 216.19$, tetragonal, $I4_1/a$, $a = 22.086$ (3), $c = 9.463$ (3) Å, $V = 4616$ (2) Å^3, $Z = 16$, $F(000) = 1792$ (without ether), $F(000) = 1873$ (with 2.7 molecules of ether per unit cell), $D_x = 1.24$ g cm^{-3} (without ether), $D_x = 1.31$ g cm^{-3} (with 2.7 molecules of ether per unit cell), λ(Mo $K\bar{\alpha}$) = 0.71073 Å, $T = 296$ K, $\mu = 0.88$ cm^{-1}, $R = 0.052$ for 1130 unique reflections having $I > 3\sigma_I$. (2) 1,2-Naphthalenedicarboxylic acid, $C_{12}H_8O_4$, $M_r = 216.19$, triclinic, $P\bar{1}$, $a = 9.027$ (1), $b = 9.234$ (1), $c = 7.256$ (1) Å, $\alpha = 106.08$ (1), $\beta = 90.79$ (1), $\gamma = 111.80$ (1)°, $V = 535.0$ (1) Å^3, $Z = 2$, $F(000) = 224$, $D_x = 1.34$ g cm^{-3}, λ(Mo $K\bar{\alpha}$) = 0.71073 Å, $T = 296$ K, $\mu = 0.95$ cm^{-1}, $R = 0.043$ for 1216 unique reflections having $I > 3\sigma_I$. (3) 1,2-Naphthalenedicarboxylic acid–dimethoxyethane, $C_{12}H_8O_4.nC_4H_{10}O_2$, $M_r = 216.19$, tetragonal, $I4_1/a$, $a = 22.067$ (2), $c = 9.465$ (2) Å, $V = 4609$ (1) Å^3, $Z = 16$, $D_x = 1.25$ g cm^{-3} (without dimethoxyethane), $D_x = 1.39$ g cm^{-3} (with 4.4 molecules of dimethoxyethane per unit cell), λ(Mo $K\bar{\alpha}$) = 0.71073 Å, $T = 296$ K. (4) 1,2-Naphthalenedicarboxylic acid–ethyl acetate, $C_{12}H_8O_4.nC_4H_8O_2$, $M_r = 216.19$, tetragonal, $I4_1/a$, $a = 22.043$ (2), $c = 9.489$ (2) Å, $V = 4611$ (2) Å^3, $Z = 16$, $D_x = 1.25$ g cm^{-3} (without ethyl acetate), $D_x = 1.37$ g cm^{-3} (with 4.1 molecules of ethyl acetate per unit cell), λ(Mo $K\bar{\alpha}$) = 0.71073 Å, $T = 296$ K. Crystalline 1,2-naphthalenedicarboxylic acid has been found in tetragonal and triclinic phases at room temperature. The tetragonal phases are solvent clathrates, unit cells having been determined for crystals grown from three solvents: diethyl ether (1), dimethoxyethane (3), and ethyl acetate (4). These three unit cells are quite similar but have statistically different edge lengths. Proton NMR was employed to confirm the presence of solvent in each tetragonal phase and to determine the solvent content of these phases. Triclinic unit cells were determined for two samples, one crystallized from anisole (2), the other from water; these cells agreed with each other to within their combined e.s.d.'s. Data sets were collected from a tetragonal diethyl ether clathrate crystal and from a triclinic crystal grown from anisole. For the tetragonal phase, intermolecular hydrogen bonding by the carboxylic acid groups at the C(1) positions forms infinite helical chains about each fourfold screw axis, with H···O(acceptor) distances of 1.52 (6) Å. These chains are interconnected *via* cyclic dimer hydrogen bonding of the carboxylic acid groups at the C(2) positions, with H···O(acceptor) distances of 1.74 (6) Å. This framework results in open channels, parallel to the z direction, in which disordered solvent molecules are located. The electron density of the disordered diethyl ether molecules was treated by a model with uncorrelated C atoms with fixed displacement parameters but variable populations. In the triclinic phase of this acid, intermolecular hydrogen bonding occurs in cyclic dimer fashion for the carboxylic acid groups both at the C(1) and at the C(2) positions. For these hydrogen bonds the H···O(acceptor) distances are 1.53 (5) and 1.71 (4) Å, respectively. This arrangement of hydrogen bonds produces an infinite ribbon of molecules connected in a zigzag pattern.

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