Characterization and Crystal Structure of Two Polymorphic Forms of Racemic Thalidomide

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There are two polymorphic forms of racemic thalidomide. The α-polymorph was formed by crystallization from 2-ethoxyethanol, methanol or dichloromethane, while the β-polymorph was formed by crystallization from a supersaturated solution in refluxing 2-ethoxyethanol. The two polymorphs were characterized by IR spectra, differential scanning calorimetry (DSC), melting points, powder X-ray diffraction patterns, and X-ray crystallography. They were easily differentiated by IR (KBr) in which the α polymorph absorbed at 3196, 3098 and 859 cm^{-1} and the β at 3276 and 755 cm^{-1}. DSC scans of the α and β forms showed endothermic peaks at 272.3 and 275.7 °C, respectively. During this measurement the α form was partially or fully converted into the β form if the rate of temperature change was slow or if the α form contained some β which provided seed crystals for the interconversion. Upon remelting both forms gave one peak corresponding to the β polymorph. The powder X-ray diffraction patterns for the two forms differ significantly. The crystal structures of the two polymorphs differ primarily in their mode of hydrogen bonding. In the α polymorph the molecules form dimers, while in the β the dimers form infinite linear strings linked by bifurcated hydrogen bonds along the *b*-axis.

The tragic history of thalidomide as a teratogenic agent is well known. Since it was withdrawn from the market in 1961, thalidomide has been used on a limited basis primarily as an antiinflammatory or immunosuppressant agent.[1] Thalidomide is the drug of choice for the treatment of erythema nodosum leprosum (ENL), an inflammatory condition commonly seen in leprosy patients.[1,2] In recent years thalidomide has been used experimentally to treat or prevent graft-*versus*-host disease (GVHD) in bone marrow transplantation,[3] to prolong cardiac graft survival in rats,[4] and to inhibit the activation of human-immunodeficiency-virus type 1 (HIV-1).[5]

Several years ago our laboratory developed monographs for the quality control of thalidomide tablets, capsules and pure drug substances. One procedure in the monographs called for the identification of thalidomide by solid-state (KBr) IR spectroscopy. While conducting this test on samples from various manufacturers and on batches of thalidomide synthesized in our laboratory, we observed significant differences in the region near 3350–3050 cm^{-1} and 880–750 cm^{-1} and attributed these differences to the presence of two polymorphic forms of thalidomide. Polymorphism is important in pharmaceuticals because it may influence drug bioavailability. This paper discusses the preparation, characterization and crystal structure of the two polymorphic forms of racemic thalidomide.

Experimental

Materials.—Racemic thalidomide was synthesized in three steps according to known procedures by condensation of phthalic anhydride with glutamic acid to form *N*-phthalylglutamic acid,[6] conversion of this product into its anhydride,[6] and fusion of the anhydride with urea to give thalidomide.[7] Certified reference materials of carbazole, anthraquinone and 2-chloroanthraquinone were obtained from the National Physical Laboratory, Teddington, England. Other chemicals were obtained from commercial sources.

Preparation of α and β Polymorphic Forms of Racemic Thalidomide.—The α polymorph of racemic thalidomide was formed by crystallization of 2 g of thalidomide from 50 cm^3 of 2-ethoxyethanol (Cellosolve); crystal formation began at 90 °C as the solution was allowed to cool. When β-thalidomide was dissolved in methanol (30 mg in 50 cm^3) or dichloromethane (100 mg in 70 cm^3) and the solution concentrated to 3 cm^3 on a rotary evaporator in a bath kept at room temperature, white crystals of α-thalidomide were formed. The β polymorph was formed by crystallization from a supersaturated solution in refluxing 2-ethoxyethanol generated by Soxhlet extraction of the α polymorph. Thus, 28 grams of the α form were placed in a Soxhlet thimble and extracted with 180 cm^3 of refluxing Cellosolve continuously for 16 h. The crystalline solid was collected by filtration, washed with water, and dried to give 23.9 g of the β polymorph. The β form can also be generated by melting the α form under nitrogen (see below), although the resultant product is less pure than the product recrystallized from refluxing 2-ethoxyethanol.

IR Spectroscopy.—Each polymorph sample was prepared as a KBr disk or Nujol mull and spectra were recorded from 4400 to 450 cm^{-1} on a Perkin-Elmer, model 1600, Fourier transform spectrophotometer.

DSC and Melting Point.—DSC thermograms were recorded on a Dupont differential scanning calorimeter, model 910, with a 9900 computer/thermal analyser and a modular interface (MIM). The DSC temperature scale was calibrated using the extrapolated onset temperatures on the fusion endotherms of indium and zinc standards heated at a rate of 1 °C min^{-1}. The accuracy of this dual point calibration was verified by conducting DSC scans on a lead standard and on two organic standards, anthraquinone and carbazole, the melting points of which bracket those of the two thalidomide polymorphs. Samples were heated in a sealed pan at a rate of 10 °C min^{-1} (exploratory) from 30 to 300 °C and at 1 °C min^{-1} from 260 to 285 °C under a nitrogen flow. DSC temperatures cited in this paper correspond to peak maxima.

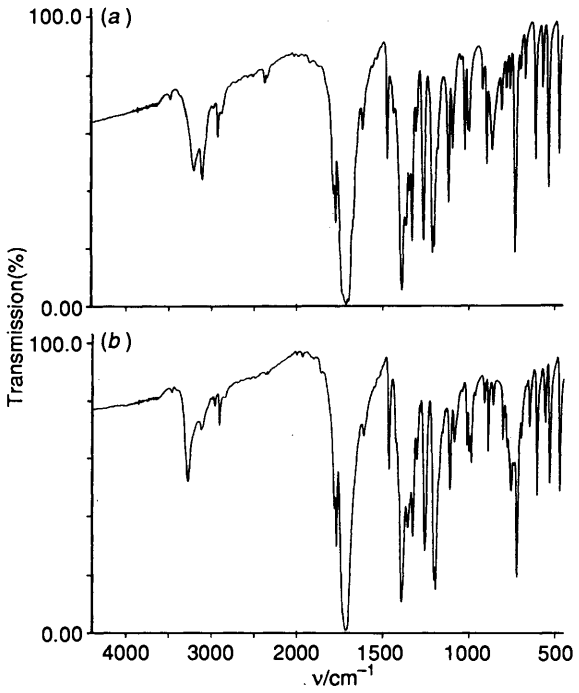

Fig. 1 IR spectra of racemic thalidomide prepared as KBr disks: (*a*) α-polymorph; (*b*) β-polymorph

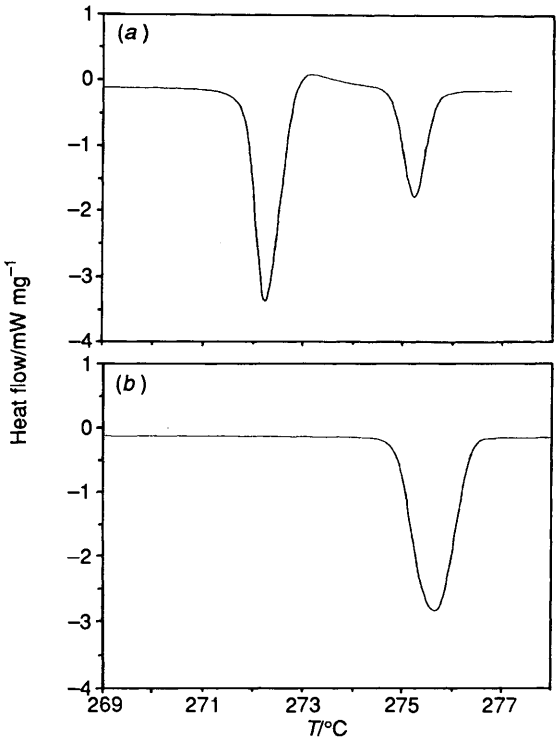

Fig. 2 DSC thermograms of racemic thalidomide generated at a rate at 1 °C min^{-1}: (*a*) α-polymorph; (*b*) β-polymorph. The α-polymorph is partially converted into the β-polymorph during this measurement. Upon remelting, both produced a single endothermic peak corresponding to the β-polymorph.

Melting points were measured at 1 °C min^{-1} on a Thomas Hoover capillary melting point apparatus with a Valley Forge Instrument Company temperature programmer. The temperature scale of the apparatus was calibrated against three high melting standards: 2-chloroanthraquinone, carbazole and anthraquinone. Thalidomide melting points were corrected.

Powder X-Ray Diffraction.—Each polymorph and a mixture of equal amounts of each polymorph were gently ground, then sieved between 60 and 200 mesh screens to give a particle size range of 75–250 μm. The powder diffraction pattern was recorded with a Rigaku D/MaxIIV automated vertical diffractometer with graphite-monochromated Cu-Kα radiation over a range where 2θ = 4–45° in steps of 0.048°.

Elemental Analysis.—C, H, N and O analyses were performed by Galbraith Laboratories, Knoxville, Tennessee.

HPLC and UV.—A solution of each polymorph at a concentration of 0.1 mg cm^{-3} was injected consecutively onto a Novapak C-18 column, 4 μm, 150 × 3.9 mm, and eluted with MeCN–0.1% aqueous H_3PO_4 (15:85) at 1.0 cm^3 min^{-1}. Ultraviolet spectra were recorded as the compounds eluted. The chromatographic system consisted of a Spectra Physics 8800 pump, 8880 autosampler, scanning SpectraFOCUS detector, and a computer controller with SpectraSYSTEM software.

NMR Spectroscopy.—Proton NMR spectra of each polymorph in [$^2H_6$]DMSO were recorded on an Hitachi R-1200 60 MHz NMR.

Single-crystal X-Ray Diffraction.—Crystal data. $C_{13}H_{10}N_2O_4$, M = 258.24. T = 23 ± 1 °C. Monoclinic, a = 20.741(2), b = 8.072(1), c = 14.216(1) Å, β = 102.78(1)°, V = 2321.1 Å^3 (by least squares refinement on 15 automatically centred reflections, Cu-Kα radiation λ = 1.541 84 Å), space group $C2/c$, Z = 8, D_x = 1.48 g cm^{-3}, μ = 9.0 cm^{-1}. Crystal dimensions: 0.2 × 0.2 × 0.05 mm.

Data collection and processing. Enraf–Nonius CAD4 diffractometer,[8] scan type: $\theta/2\theta$. Scan rate: 1–20° min^{-1} (in omega). Scan width: 1.0 + 0.350 tan θ°. Cu-Kα radiation. Monochromator: graphite crystal, incident beam. Attenuator: Ni foil, factor 26.5. Detector aperture: 1.3 horizontal, 4.0 mm vertical. Crystal-detector distance: 17.3 cm. Maximum 2θ: 148.0°. No. of refl. measured: 2350 (unique set). Corrections: Lorentz polarization. Linear decay (from 1.000 to 1.008 on I). Extinction (coefficient = 0.000 001 4).[9] F(000) = 1072.

Structure analysis and refinement. Direct methods (MITHRIL[10]). Refinement: full-matrix least-squares. Minimization function: $\Sigma w(|F_o| - |F_c|)^2$. Least-squares weights: $4F_o^2/\sigma(F_o^2)$. Scattering factors (ref. 11). Anomalous dispersion for all non-hydrogen atoms.[12] Reflections included: 1700 with $I > 3.0\sigma(I)$. Parameters refined: 173. R: 0.057. R_w: 0.081. Esd of obs. of unit weight: 2.71. Largest shift at convergence: 0.03σ. High peak in final difference map: 0.48(6)[13] e Å^{-3}. Low peak in final difference map: −0.28(6) e Å^{-3}. Computer hardware: VAX Station 3520. Computer software except for direct methods (including figures): MolEN (Enraf–Nonius).[14]

Observed and calculated structure factors (available from the authors) were submitted to the referees and full dimensional tables and coordinates have been deposited with the Cambridge Crystallographic Data Centre. For details of the CCDC deposition scheme see 'Instructions for Authors (1994),' *J. Chem. Soc., Perkin Trans. 2*, 1994, issue 1.

Results and Discussion

The IR spectra of the α and β polymorphs are shown in Fig. 1. The α form produced distinguishing absorption bands at 3196, 3098 and 859 cm^{-1}, while the β form produced distinguishing bands at 3277 and 755 cm^{-1}. Other regions of the spectra show a few subtle differences. Similar spectral differences were obtained for samples prepared as Nujol mulls.

DSC thermograms for the two thalidomide polymorphs are shown in Fig. 2. The β form has one endothermic peak due to fusion with a maximum at 275.7 °C; the standard deviation of

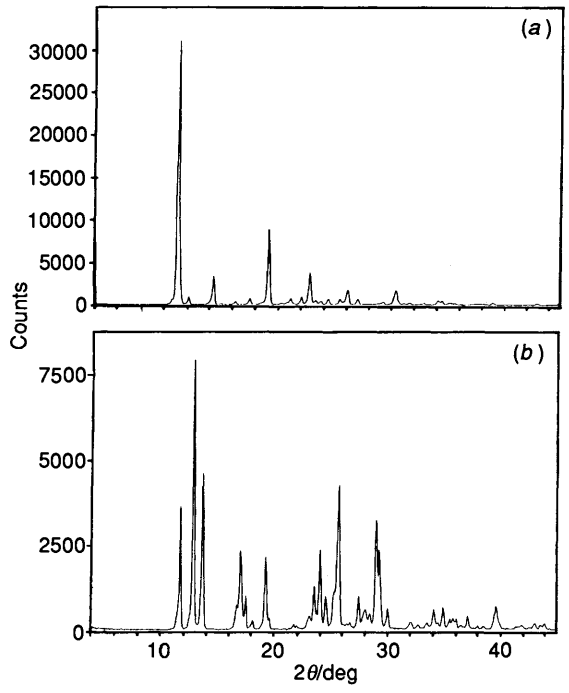

Fig. 3 Powder X-ray diffraction patterns of racemic thalidomide: (*a*) α-polymorph; (*b*) β-polymorph

this temperature for consecutive scans on one day of five samples of the polymorph was 0.1 °C. One sample of the β-polymorph was melted and cooled repeatedly on the DSC. With successive cycles, the fusion peak generally broadened, decreased in intensity, and shifted to a lower temperature maximum, which signified an increase in the level of impurities due to decomposition. When heated at 1 °C min^{-1}, the α form gave two endothermic peaks, one with a maximum at 272.3 °C and the other, corresponding to the β form, with a maximum at 275.2 °C. When this sample was cooled until it resolidified, then remelted, the thermogram showed a single peak at 275.0 °C. A remelt of the β form similarly gave a peak at 275.1 °C. As indicated above, the endothermic peak maximum of a remelted thalidomide sample is slightly lower than that of the original sample showing some decomposition occurs with melting. A 3:1 or 1:3 mixture of α and β gave a single endothermic peak each at 275.3 °C, corresponding to the β polymorph.

Evidently, the α form is partially or fully converted into the β form during DSC measurements if the rate of temperature change is slow or if the α form contains some β form which provides seed crystals to enhance the interconversion. The melted α or β samples resolidify as the β polymorph. There is an exotherm following the 272.3 °C endotherm on the DSC scan of the α polymorph [Fig. 2(*a*)]. This exotherm is due to a crystallization to the β polymorph which subsequently melts at 275.2 °C. This crystallization to the β form must be incomplete because the enthalpy of fusion for the 275 °C endotherm is lower in the initial melt than in a remelt.

When heated at 10 °C min^{-1} the α form gave a single slightly distorted endothermic peak at 274.3 °C. This peak actually corresponded to the lower-melting α form. A heating rate of 10 °C min^{-1} typically gave an endothermic peak at a temperature of about 2 °C higher than one produced at a rate of 1 °C min^{-1} for these samples due to the lag time at the rapid heating rate. On remelting at 10 °C min^{-1} this sample gave a peak at 276.2 °C (transformed into the β form upon recrystallization after the first melt). At 10 °C min^{-1} the β form gave a peak at 278.0 °C and, on remelting, at 276.0 °C.

To demonstrate that α was converted into β during measurements by DSC, a sample of the α form was melted and remelted at 1 °C min^{-1}. The product in the metal pan was examined by IR spectroscopy and found to be the β polymorph. For additional configuration, samples (900 mg each) of α, β and a 1:1 mixture of the two, were placed in glass tubes, flushed with nitrogen, sealed tightly, and heated in a muffle furnace preheated to 300 °C. The samples, which melted within 8 min, were held at 300 °C for an additional 8 min and allowed to cool and crystallize. IR spectra of all three samples were practically identical and matched the IR spectra of the β polymorph.

When the two polymorphs were heated simultaneously in a melting point apparatus, the β form melted at 275.0–276.5 °C and most of the α form melted at 271.5–273.0 °C. However, a few large white translucent crystals, presumably crystals of the β-polymorph formed by recrystallization, were suspended in the α melt and finally melted at 274.0–275.5 °C. The melts were cooled to 165 °C and then remelted. Both compounds remelted at 275.0–276.0 °C. The process observed in the melting tube capillary correlates well with the DSC data.

Elemental analyses of the α and β polymorphs were within 0.3% of the calculated values for $C_{13}H_{10}N_2O_4$ which indicated that neither polymorph was a hydrate or solvate [Found: C, 60.5; H, 4.2; N, 10.8; O, 24.6% (α form). Found: C, 60.4; H, 4.2; N, 10.7; O, 24.6% (β form). Calc. for $C_{13}H_{10}N_2O_4$: C, 60.5; H, 3.9; N, 10.8; O, 24.8%].

The identical appearance of the two substances once in solution provided additional proof that they were polymorphs. Proton NMR spectra of the α and β forms in [$^2H_6$]DMSO were superimposable. The α and β polymorphs had retention times of 7.47 and 7.46 min, respectively, and assay values of 100.0% (taken as standard) and 99.8%, respectively by HPLC. Their UV spectra were also superimposable.

The α and β polymorphs are readily distinguished by their powder X-ray diffraction patterns as shown in Fig. 3. Strong diffraction peaks for the α form occurred where 2θ (intensity counts) = 11.33 (30 675), 14.32 (3287), 19.20 (8799), 22.77 (3734), 26.12 (1740) and 30.36 (1697), and for the β form where 2θ (intensity counts) = 11.78 (3458), 12.96 (7843), 13.75 (4446), 17.06 (2256), 19.26 (2088), 24.06 (2214), 25.73 (4095), 29.05 (3146) and 29.29 (2149).

Allen and Trotter[15] determined the crystal structure of racemic thalidomide in 1971. The cell dimensions of the crystalline material examined in that study match those of the polymorph which we have defined as α-thalidomide. The X-ray crystallography data presented here is for β-thalidomide. Table 1 shows a comparison of the crystallographic data for the polymorphs. An ORTEP[17] drawing of the crystal conformation of the β-polymorph is given as Fig. 4 and the atomic numbering is also indicated there. The numbering is related to

Table 1 Comparison of structural parameters of the α- and β-forms of racemic thalidomide

	Polymorph	
	α	β
Reference	Allen and Trotter[15]	This investigation
a/Å	8.233(1)	20.741(2)
b/Å	10.070(2)	8.072(1)
c/Å	14.865(2)	14.216(1)
β/°	102.53(2)	102.78(1)
V/Å^3	1203.0	2321.1
Z	4	8
D_x/g cm^{-3}	1.425	1.48
Space group	$P2_1/n$	$C2/c$
Hydrogen-bonding	Discrete dimers	Bifurcated infinite stacks
Structure-solution	Direct[16]	Direct (MITHRIL[10])
R	0.052	0.057
R_w	NA	0.081

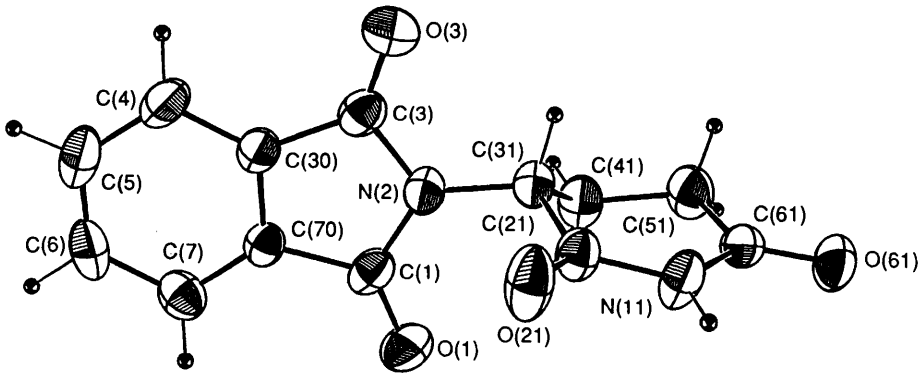

Fig. 4 ORTEP drawing for β-polymorph showing 50% probability ellipsoids for heavy atoms. Hydrogen atoms are arbitrary.

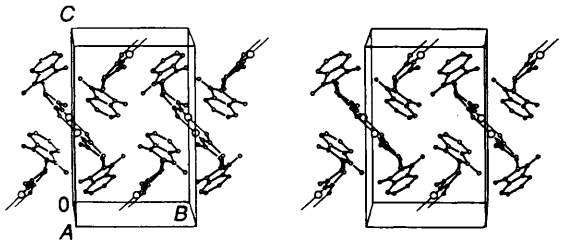

Fig. 5 Stereoscopic view of the packing of α-thalidomide as determined by Allen and Trotter drawn for this paper by MolEN. The projection is approximately along *a*. The hydrogen atoms involved in hydrogen bonding are depicted larger than the heavier atoms and the lines radiating from atoms indicate incompletely depicted further hydrogen bonds.

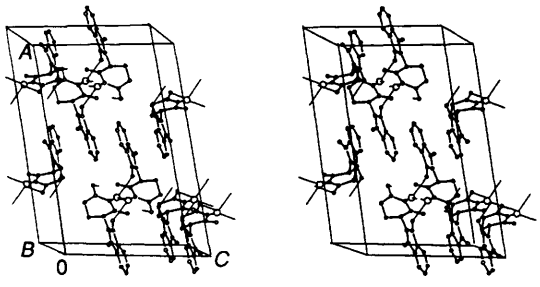

Fig. 6 Stereoscopic view of the packing of β-racemic thalidomide. Note the parallel stacking of the aromatic moieties. The view is approximately along *b* but with a rotation of 10° horizontally and 20° vertically. Hydrogen atoms and bonds are depicted as in Fig. 5.

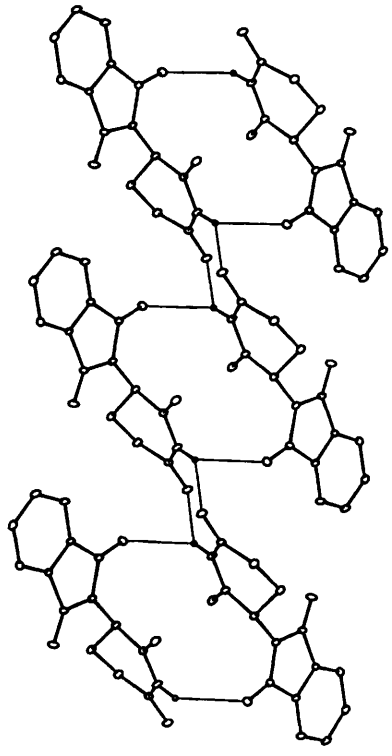

Fig. 7 ORTEP drawing showing the linking of *R*/*S* dimers along the *b* axis by means of bifurcated hydrogen bonds

the usual chemical designations with some modifications to allow only numbers in the atomic designators.

The observed bond lengths in the α- and β-polymorphs sometimes differ by about three standard deviations but the differences are small and probably of no biological significance (numerical values for the atomic parameters and derived distances and angles for β-thalidomide were submitted and may be obtained from the Cambridge Crystallographic Data Centre). The molecular conformations in the two polymorphs are also very similar indeed. The rings are essentially identical and the torsion angles between the two linked ring systems are 81.3° for the α-form and 86.1° for the β-form.

The major difference lies in the hydrogen bonding patterns. In the α-polymorph as shown in Fig. 5, the molecules form dimers around centres of symmetry, *i.e.*, N(11) is associated with O(61) in the same ring of another molecule by a bond of dimensions N ··· O, 2.928(3) Å; O ··· H, 2.10 Å and N–H–O, 171°. There are thus two identical hydrogen bonds linking the dimers and the crystal density is relatively high: 1.425 g cm^{-3} (Table 1). Given the presence of the centre of symmetry, the dimers are *R*/*S* pairs. In the β-polymorph, a similar hydrogen bond about a centre of symmetry is present but the molecules are rotated so that the hydrogen atom forms a bifurcated bond and thus is associated with O(3) in a molecule related by the *b* translation giving infinite stacks of dimers extending along the *b* axis. A stereoscopic drawing is given as Fig. 6 which shows another significant packing motif in that the aromatic groups are arranged in infinite parallel stacks along the *c*-axis. The distance between the ring systems, *ca.* 3.3 Å, is normal for such packing and must contribute to the stabilization of the polymorph and, despite the weaker bifurcated bonds, the calculated density of the crystal is a little higher than that of the α form. For clarity, another picture of the hydrogen-bonding pattern is given in Fig. 7. The dimer pairs, as in the α polymorph, are formed by linkages between the glutarimide N–H atoms and glutarimide carbonyl oxygen atoms in adjacent molecules. The same glutarimide hydrogen atom is linked along the *b* axis to a phthalyl carbonyl oxygen atom in an adjacent molecule. As a consequence of the symmetry operations involved, the *R*/*S* dimer pairs are linked so that all stereoisomers on one side of the infinite stack are *R* and those on the other are *S*. Geometric details of the hydrogen bonding are as follows: N(11) ··· O(61), 3.098(3); H(11) ··· O(61), 2.330(2) Å; N(11)–H(11)–O(61), 141.9(2)°; N(11) ··· O(3), 3.143(3); H(11) ··· O(3), 2.418(2) Å; N(11)–H(11)–O(3), 136.6(2)°. (Formal estimated standard deviations involving H atoms are given but the hydrogen atoms

were constrained to ride on the heavier atoms and thus the values are calculated with only heavier atom positional standard deviations.)

Acknowledgements

We thank James M. Timper, Jr. for preliminary DSC investigations.

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Paper 4/00175C
Received 11*th January* 1994
Accepted 21*st June* 1994