Ritonavir: An Extraordinary Example of Conformational Polymorphism

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Purpose. In the summer of 1998, Norvir semi-solid capsules supplies were threatened as a result of a new much less soluble crystal form of ritonavir. This report provides characterization of the two polymorphs and the structures and hydrogen bonding network for each form.

Methods. Ritonavir polymorphism was investigated using solid state spectroscopy and microscopy techniques including solid state NMR, Near Infrared Spectroscopy, powder X-ray Diffraction and Single crystal X-ray. A sensitive seed detection test was developed.

Results. Ritonavir polymorphs were thoroughly characterized and the structures determined. An unusual conformation was found for form II that results in a strong hydrogen bonding network A possible mechanism for heterogeneous nucleation of form II was investigated.

Conclusions. Ritonavir was found to exhibit conformational polymorphism with two unique crystal lattices having significantly different solubility properties. Although the polymorph (form II) corresponding to the "cis" conformation is a more stable packing arrangement, nucleation, even in the presence of form II seeds, is energetically unfavored except in highly supersaturated solutions. The coincidence of a highly supersaturated solution and a probable heterogeneous nucleation by a degradation product resulted in the sudden appearance of the more stable form II polymorph.

KEY WORDS: polymorphism; crystal forms; ritonavir; Norvir; carbamate; AIDS drug.

INTRODUCTION

Polymorphism is the ability for a compound to exist in more than one crystal form with different unit cell parameters. These individual crystal forms or polymorphs can exhibit differences in physical properties reflective of the crystal lattice. For example, the solubility of different polymorphs of the same compound reflect the differences in free energy between their respective crystalline states, which are different for each polymorph, and the solvated state. Thus a large range in equilibrium solubilities can exist for the various crystal forms of a compound. Differences in solubility between crystal forms of a pharmaceutical can lead to differences in bioavailability of solid dosage forms if the bioavailability is dissolution limited. Such differences can also lead to crystallization from solution, ointment or suppository formulations. The phenomenon of polymorphism is very common among pharmaceuticals and has been widely reported in the literature (1–11). However, rarely has the existence of multiple crystal forms had such a dramatic effect on commercial pharmaceuticals as in the case of the antiviral compound ritonavir. Ritonavir (I), [5S-(5R*,8R*,10R*,11R*)]-10-Hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-2,4,7,12-tetraazatridecan-13-oic acid, 5-thiazolylmethyl ester, is a novel protease inhibitor (12–14) marketed in 1996 as Norvir oral liquid and Norvir semi-solid capsules for treatment of Acquired Immunodeficiency Syndrome (AIDS). Since ritonavir is not bioavailable from the solid state, both formulations contained ritonavir in ethanol/water based solutions. Therefore no crystal form control was required (15). The ICH (International Committee on Harmonization) guideline states "For a drug product that is a solution, there is little scientific rationale for polymorph control"

Only one crystal form of ritonavir was identified during development of the compound and 240 lots of Norvir capsules were produced with no stability problems.

In mid-1998, however, several lots of capsules failed the dissolution requirement and when capsule contents were examined using microscopy and X-ray powder diffraction, a new polymorph was identified that had greatly reduced solubility compared to the original crystal form. This new form, referred to as form II, is an example of conformational polymorphism, which occurs when different conformational isomers of a compound crystallize as distinct polymorphs (16). Within weeks this new polymorph began to appear throughout both the bulk drug and formulation areas. Since the manufacture of Norvir semi-solid capsules formulation involved the preparation of a hydroalcoholic solution of ritonavir which although not saturated with respect to form I was 400% supersaturated with respect to form II, the sudden appearance and dominance of this dramatically less soluble crystal form made this formulation unmanufacturable. Additionally Norvir oral solution could no longer be stored at 2–8°C without the risk of crystallization. These factors combined to limit inventory and seriously threatened the supply of this life saving treatment for AIDS. It was necessary to immediately reformulate Norvir. Form II of ritonavir was found to be both unusually stable and at the same time unusually difficult to crystallize. This report provides characterization of the two polymorphs and explanation of the unusual properties of form II.

EXPERIMENTS

Reagents

Ritonavir was synthesized at Abbott Laboratories, North Chicago IL. [4S-[4', 5'[R*9R*0]]]-3-methyl-2-[[[methyl[[2-1-methylethyl-0-4-thiazoyl]methyl]amino]-carbonyl]amino]-N-[1-[[2-oxo-4-(phenylmethyl)-5-oxazolidinyl]-2-phenylethyl]-butanamide was synthesized at Abbott Laboratories, North Chicago IL. Seed detection test solution was prepared by dissolving 160 milligrams of Ritonavir form I in 10 mL of 200 proof ethanol.

Microscopy

Microscopic examination and photomicroscopy were performed using a Nikon Microphot FXA polarized light mi-

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croscope equipped with a Hitachi HV-C20 camera. Capsule fills were examined directly and by mounting in a drop of mineral oil.

Infrared Spectroscopy

Near infrared (NIR) spectra of solid ritonavir were generated using a Nicolet model 750 Magna-IR spectrometer with a CaF_2 beam splitter, and with a Nicolet SabIR near-infrared (NIR) diffuse reflectance fiber optic probe accessory equipped with a PbS detector. Sixteen scans were run using a resolution of 8 cm^{-1} over a range of 1.0 to 2.4 micrometers Mid-infrared spectra of solid ritonavir were also acquired using a Nicolet model 750 Magna-IR spectrometer with a DTGS detector and sixteen scans at a resolution of 4 cm^{-1}. The spectrum was collected from 4000 to 400 cm^{-1}. Solution mid-infrared spectra were obtained on a thin film using a Nicolet Magna 750

X-Ray Diffraction

X-ray powder diffraction patterns were obtained using a Nicolet I-2 X-ray powder diffractometer using Cu Kα(λ = 1.54178 angstroms) radiation and a scan rate of 2 degrees per minute. Samples were ground to a fine powder with a mortar and pestle. Single crystal X-ray data was collected using both a Bruker SMART diffractometer at ambient temperature using Mo Kα (λ = 0.7107 angstroms) radiation and a $2\Theta_{max}$ of 46.6° as well as a Rigaku AFC5R diffractometer using Cu Kα (λ = 1.54178 angstroms) radiation and a $2\Theta_{max}$ of 120.2°. Both instruments used a Lorentz-polarization correction and a total of 14960 and 6520 reflections were measured respectively. Crystal density was calculated from the single crystal data and the hydrogen bonding assignments were confirmed using Cerius software version 3.7

Solid State NMR

Solid-state ^{13}C NMR spectra were obtained on a Bruker AMX-400 instrument operating at a carbon frequency of 100.6 MHz. The ^{13}C spectra were collected using the Variable Amplitude Cross-Polarization Magic-Angle Spinning pulse sequence (VA-CP2LEV) in 7-mm sample rotors, which were spun at a 7 kHz spin rate. The Hartmann-Hahn matching condition for the VA-CP2LEV pulse sequence was calibrated using an external sample of hexamethylbenzene (HMB). The ^{13}C spectra were collected using 3000 scans, with a contact time of 2.5 msec and a recycle delay time of 5 sec. The carbon chemical shifts were measured relative to an external HMB sample, using the methyl resonance at 17.3 ppm as the reference signal Solution proton NMR was performed in d6-DMSO using an AMX Bruker 400 MHz instrument.

Thermal Analysis

Differential Scanning Calorimetry (DSC) and Thermal Gravimetric Analysis (TGA) were performed using a Thermal Analysis Instruments controller Model 5100 in conjunction with a DSC model 2910 module and a TGA model 2950 module. DSC data was obtained in a loosely covered aluminun pan using a sample weight of 10 mg and a heating rate of 5°C per minute and a nitrogen flow of 40 mL/min. TGA data was collected using a sample weight of 15 mg, heating rate of 50°C per minute and a nitrogen flow of 40 mL/min.

Solubility Studies

Solubility studies were performed by slowly adding portions of the appropriate crystal form to approximately 100 mL of solvent or formulation and stirring at controlled temperature (5°C or 25°C) in a temperature controlled room, until completely dissolved by visual inspection. Portions were added until residual undissolved solid remained. Stirring was continued and periodically aliquots were taken and filtered. The supernatants were analyzed by HPLC versus an external standard until a concentration plateau was reached.

Seed Detection Test

A solution of ritonavir form I was prepared in absolute ethanol at 100 mg/mL. Samples were tested by either weighing a portion of bulk drug (500 mg) into 10 mL of test solution or placing the equipment part into the solution that was then stored at 5°C for 12 h. The test sample was then warmed to room temperature (approximately 22°C).

Preparation of Form I and Form II Ritonavir

Approximately 40 g of ritonavir bulk drug (form I) was dissolved in approximately 250 mL of absolute ethanol (solubility = 165 mg/mL at 25°C) at approximately 40°C. Half of the solution was filtered warm into a flask containing crystals of form II isolated from Norvir Semi-solid capsule fill. Crystallization occurred immediately upon contacting the form II seed crystals and after cooling to room temperature (22–24°C) without stirring a significant amount of crystals precipitated. After standing unstoppered overnight (12 h) approximately 20 g of pure form II were isolated. The other half of the solution also containing 267 mg/mL ritonavir was not seeded and was allowed to cool to room temperature. No crystallization of ritonavir occurred. The ethanol from this portion was slowly evaporated to dryness under a nitrogen stream . The solid isolated from the unseeded portion was identified as form I.

Solubility Studies with Mixtures of Forms

4.0 grams of Form I was slurried in 16 grams of formulation placebo (117% saturation). The slurry was continually stirred on a mechanical stirrer at 5°C for seven days. The slurry was then filtered using a 0.45 micron millipore filter and 100 mg of form II was added. In a separate vessel 2.0 grams of form II was slurried in 16 grams of formulation placebo (125% saturation). The slurry was continually stirred on a mechanical stirrer at 5°C for seven days. The slurry was then filtered using a 0.45 micron millipore filter and 2.5 grams of form I was added.

CHARACTERIZATION OF RITONAVIR POLYMORPHS

When Norvir semi-solid capsules first failed the dissolution requirement, the contents of the capsules were examined by polarized light microscopy. Crystals were detected in the capsule fill with a distinctly different crystal habit than bulk ritonavir (Fig. 1). Solution NMR and IR confirmed the crys-

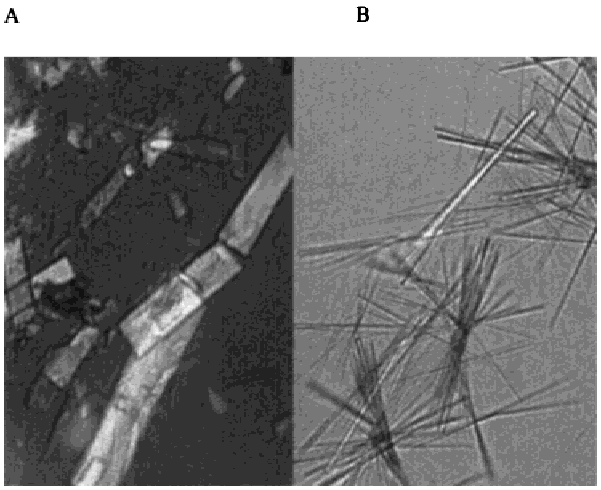


Fig. 1. Photomicrographs for ritonavir polymorphs. (A) Form I, (B) form II.

tals were ritonavir and X-ray powder diffraction Fig. 2) indicated they were a new heretofore unknown polymorph.

Differential scanning calorimetry and thermal gravimetric analysis did not show any significant differences between the two polymorphs. Neither form gave a weight loss below the melting and the two forms melted within 0.5°C of each other at approximately 122°C. Carbon-13 solid state NMR, proton solid state NMR, solid state mid and near infrared spectroscopy, and X-ray powder diffraction were used to distinguish form II ritonavir from form I ritonavir (17). A solubility profile of the two forms in a range of ethanol water ratios is presented in Table I. Due to limited solution stability, ritonavir formulations must be stored at 5°C, therefore the solubility was determined at this temperature. As can be seen, form II has solubility much lower than that of form I throughout the solvent range.

In a search for the source of form II ritonavir, bulk drugs were examined using X-ray powder diffraction and near-infrared spectroscopy, which have detection limits for form II in bulk form I of approximately 3% and 1% respectively. No evidence of form II was found at these levels so a more sensitive and specific detection test was developed. Fig. 3 shows the result of a solubility experiment in which each polymorph was equilibrated with the hydroalcoholic based formulation solvent system (approx. 92% ethanol plus other proprietory solvating agents) by stirring at 25°C until saturation was achieved, i.e., there was no increase in concentration although the form I solution concentration did begin to decrease at approximately 7 days. At this point the form I solution was approximately 210% supersaturated with respect to form II. After 7 days each solution was filtered and on day 8 the other polymorph was added in excess. As shown in Fig. 5, when polymorph I is present an initial high solubility is achieved but in the presence of form II the ritonavir concentration rapidly decreases to the equilibrium solubility of form II. As can be seen from Fig. 5, a solution which is saturated with respect to form II can still dissolve a significant amount of form I ritonavir as long as no solid form II is present. However if any form II is present the equilibrium solubility of form II will be re-established. This phenomenon lead to the development of a Seed Detection Test wherein a solution of form I is prepared at a concentration significantly above (110% or greater) the equilibrium solubility of form II but which is still undersaturated with respect to form I. This solution was then used to detect low amounts of form II either in ritonavir bulk drug samples or on equipment parts. Any form I present dissolves; however, if any seeds of form II are present they seed the highly supersaturated solution and form II crystallizes. This detection test was demonstrated to be sensitive to 1 ppm form II. Seeding this solution with other insoluble compounds, which might be present in the formulation (i.e., saturated fatty acids), did not evoke crystallization.

STRUCTURES OF RITONAVIR POLYMORPHS

The structures of both polymorphs were determined from single crystal X-ray data (Tables IIA, B) and are shown

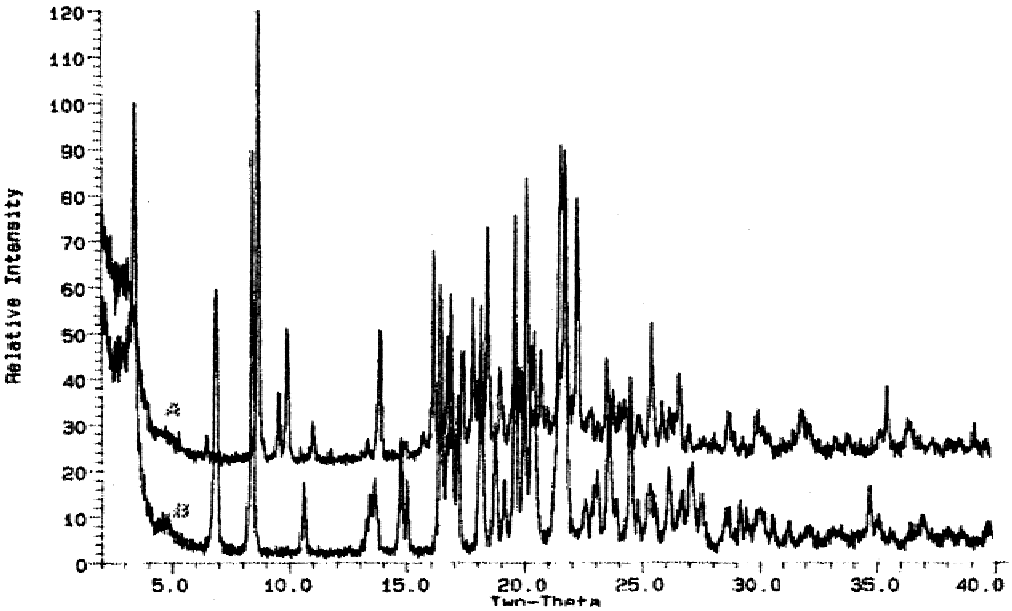


Fig. 2. X-ray powder diffraction patterns for forms I and II of ritonavir. (A) Form II, (B) form I.

Table I. Solubility Profile in Various Hydroalcoholic Solvent Systems at 5°C

Ethanol/Water	99/1	95/5	90/10	85/15	80/20	75/25
Form I	90 mg/mL	188	234	294	236	170
Form II	19 mg/mL	41	60	61	45	30

in Figs. 4, 5. The three torsion angles (A, B, C) labeled in the Scheme 1 indicate areas with significant differences between the different polymorphs.

A search of the Cambridge Structure Database (CSD) for acyclic N-methyl urea groups did not yield enough structures to provide the data for a meaningful comparison, therefore the chemically similar fragment (acyclic N-methyl amides) was chosen. A search of the CSD, shows that acyclic N-methyl amide torsional angles are *trans* in 60% and *cis* in 40% of the recorded crystal structures containing this type of linkage. In contrast to the N-methyl urea however, the form II carbamate torsion angle is atypical of this type of bond. A search of the CSD for acyclic carbamates shows that this conformation is only present in about 2% of the reported crystal structures. Since the *cis* carbamate conformation is not the one typically observed in solution and very few other carbamate linkages have been found in this conformation, it would not be expected to be a more stable (i.e., less soluble) crystal form. In order for the crystal to grow, the molecule attaching to the crystal face is required to alter the conformation of its carbamate linkage. This requires energy and would not seem to be favored. However, as described below, the increase in hydrogen bonding strength attained in form II appears to compensate for this unusual arrangement.

HYDROGEN BONDING COMPARISON

The total number of hydrogen bonds is the same in both forms, however the hydrogen bond network is different.

Table II. Single Crystal X-Ray Data

A: Comparison of Torsion Angles in Ritonavir Forms		
Torsion Angle	Form I	Form II
A (N-Methyl Urea)	–5 (cis)	–179 (trans)
B	72	–77
C (carbamate)	–178 (trans)	–8 (cis)
B: Single Crystal X-ray Data for Form I and Form II Ritonavir		
Parameter	Form I	Form II
Crystal system	monoclinic	orthorhombic
Space Group	$P2_1$ (#4)	$P2_1P2_1P2_1$ (#19)
Z value	2	4
Dcalc	1.28 g/cm^3	1.25 g/cm^3
Lattice Parameters	a = 13.433 (1) Å	a = 10.0236 (3) Å
	b = 5.293 (2) Å	b = 18.6744 (4) Å
	c = 27.092 (4) Å	c = 20.4692 (7) Å
	β = 103.102 (9) Å	
	V = 1876.0 (8) Å^3	V = 3831.5 (2) Å^3

Form I

The hydrogen bonding follows two basic patterns in form I. First the amide linkages of one molecule line up transitionally with the same amide in the next molecule to form a continuous beta-like stack parallel to the short crystallographic axis (Fig. 4a). These stacks are then paired up by a hydrogen bond between the alcohol of one molecule and the thiazole ring of a second molecule related by a 2-fold screw axis (Fig. 4b).

Form II

The hydrogen bonding follows only one basic pattern in form II (Fig. 5). In form II all the strong hydrogen bond donors and acceptors have been satisfied. In both forms there are eight hydrogen bonds connecting each molecule to the adjoining molecules in the crystal lattice. The hydrogen bond

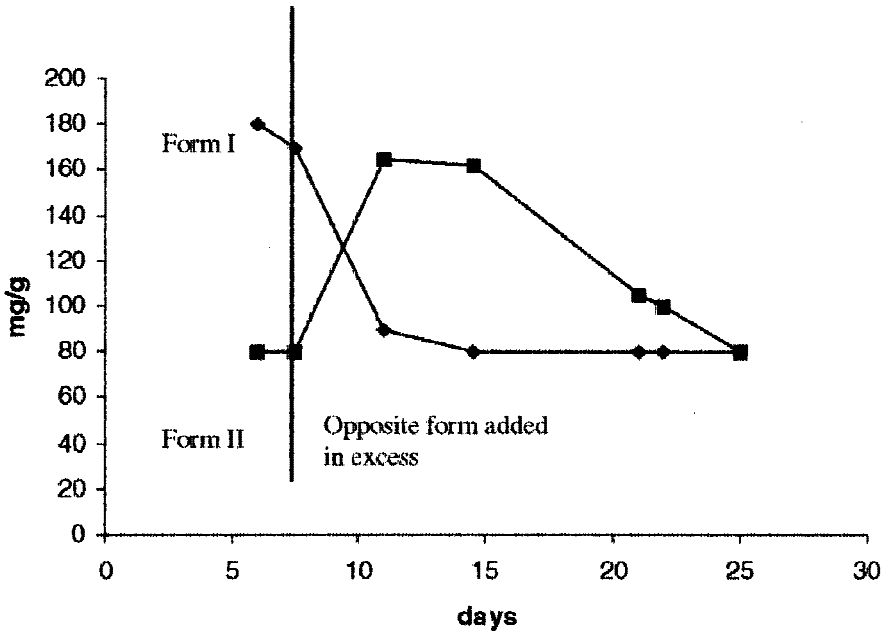


Fig. 3. Solubility effect of mixed suspensions of ritonavir crystal forms.

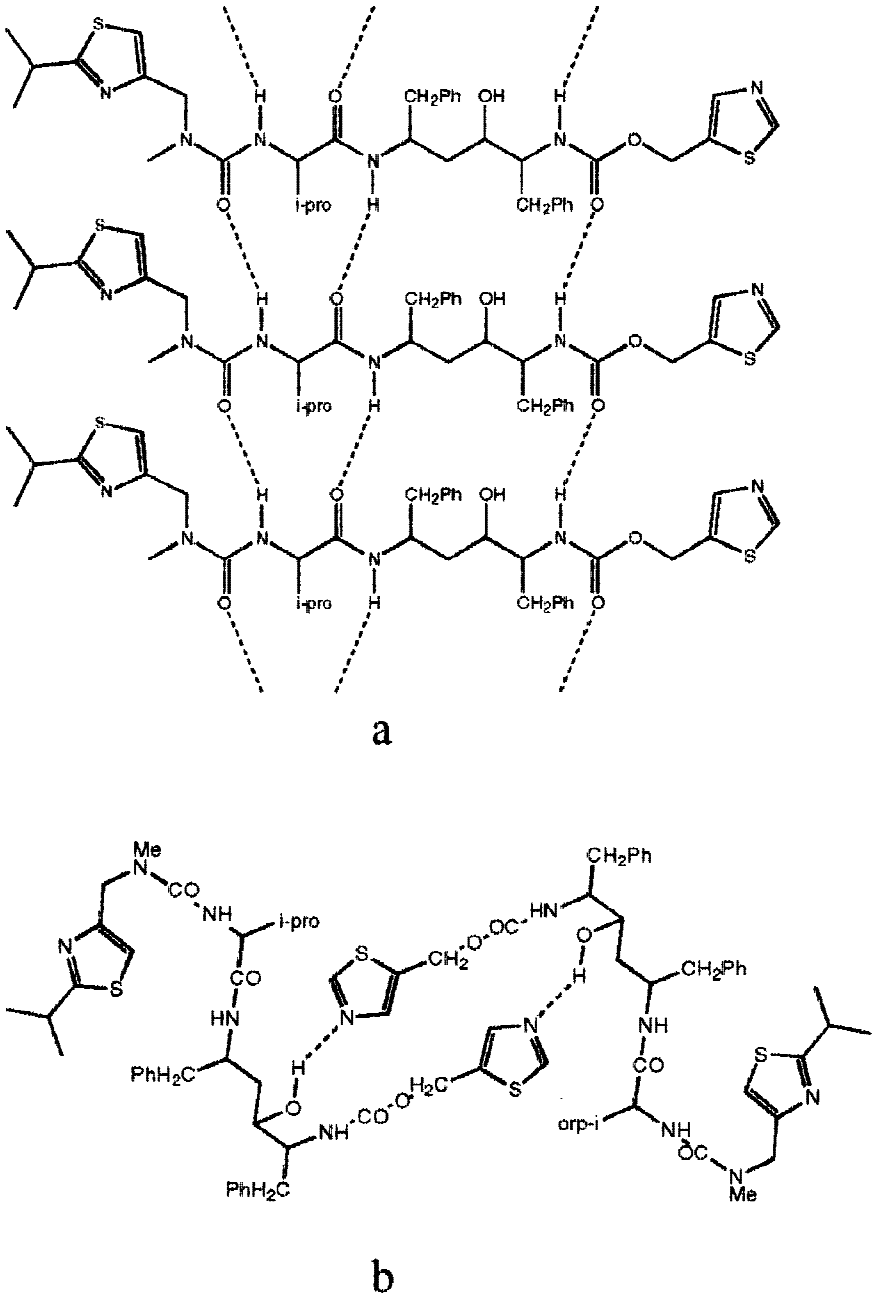


Fig. 4. Hydrogen bonding network for ritonavir form I. (A) Beta like stacks, (B) 2-fold screw axis.

between the alcohol of one molecule and the thiazole ring of another molecule, which exists in form I, and not form II is a weaker hydrogen bond than the other hydrogen bonds. In form II the alcohol group is acting as both a hydrogen donor and acceptor. This results in a synergistic effect, which makes both hydrogen bonds stronger.

The primary mechanism involved in both the solubilization and crystal growth for ritonavir involves hydrogen bond formation, either new or exchanged. The crystals of form I have a much greater percentage of their surface area with exposed hydrogen bond donors and acceptors than crystals of form II. Since the rates of crystallization and dissolution are proportional to the summation of the corresponding rates for each crystal face times the area of that face, the bulk crystallization and dissolution rates are greater for form I in hydrogen bonding solvents (water, alcohols, etc.) than for form II.

This indicates that although the solubility of form II is less than that of form I, primary nucleation from a solution of ritonavir would follow Ostwald's progression, (18) and crystallize the polymorph with the minimal energy barrier to surmount ie the crystal form whose structure is closest in energy and arrangement to the molecules in solution. Therefore form I would crystallize preferentially.

It is generally found that the polymorph with the highest density is the more stable crystal form (19,20) however, in the case of ritonavir the gain in stability due to very strong hydrogen bonds overcomes the stability benefit of denser pack-

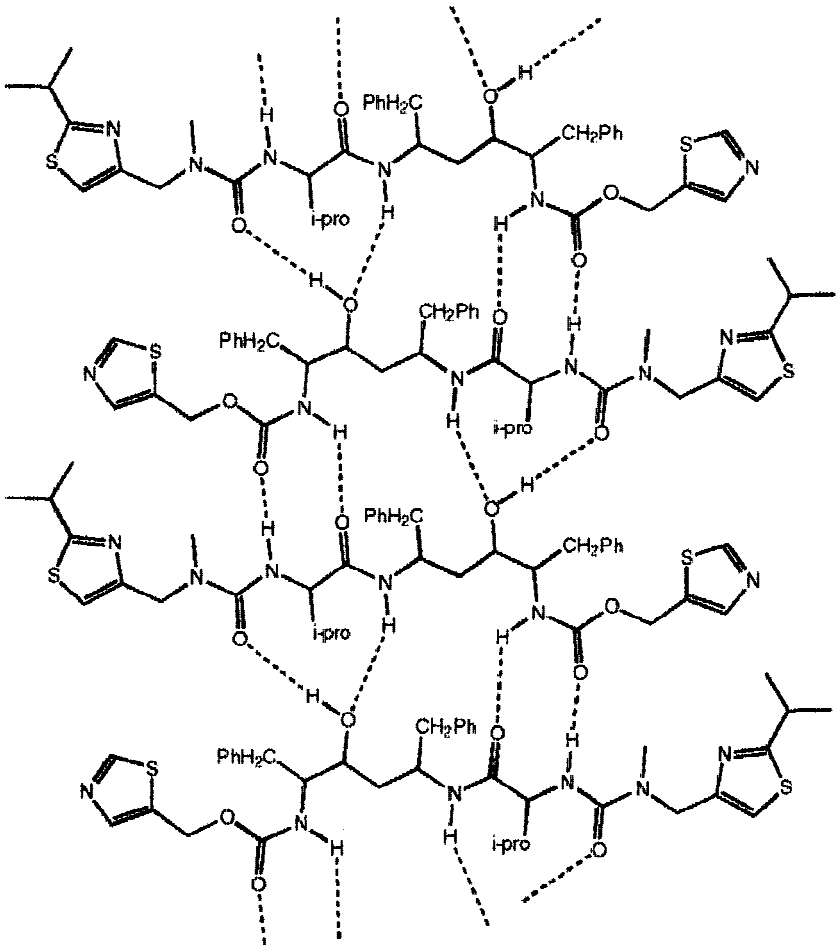


Fig. 5. Hydrogen bonding network for ritonavir form II (needle growth is aligned with hydrogen bonding).

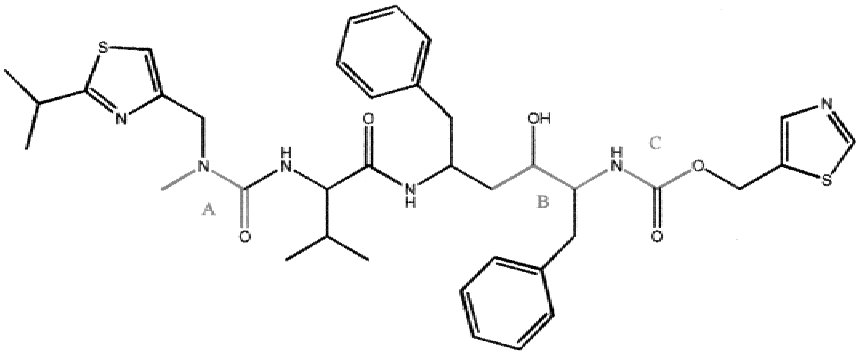
ing, and form I (1.28 g/cc) is slightly more dense than form II (1.25 g/cc)

TENDENCY TO CRYSTALLIZE AS FORM II

A solution, which is supersaturated with respect to both crystal forms of ritonavir, would be expected to follow Ostwald's rule and initially crystallize as form I. However, due to the drastic difference in solubilities between the polymorphs, it is possible, as in the Seed Detection Test described above, to have a solution which is supersaturated with respect to form II but does not approach saturation with respect to form I solubility. Norvir formulations, both semi-solid and oral liquid dosage forms, which were manufactured prior to the appearance of form II, showed no evidence of crystallization even after 24 months at 2–8°C storage. Four-year-old semi-solid capsules, which had lost all the ethanol from the fill due to lack of banding at the capsule joint, were examined and the ritonavir, which had crystallized, was form I.

In addition to the above observations, two series of experiments were performed in which ritonavir was isolated from 15 different solvents by slow and fast evaporation. One set of experiments occurred prior to introduction of form II to the laboratory, and the second was performed using form II as the starting form. Form II crystallized in only one of the experiments using form II and in a case where the form II may not have been completely dissolved. Additional experiments were performed in which solutions (in Norvir formulation) that were supersaturated (111% and 125%) with respect to form II were seeded with form II and stored at 5°C (required storage temperature for Norvir formulations) for up to 24 months. No evidence of crystallization was seen (i.e., only the seed crystals were detected). This is because any crystal growth that occurs in form II ritonavir is restricted to the thin pointed faces of the needle crystals. As shown in Fig. 5 there are no binding sites along the sides of the needle. As long as the degree of supersaturation with respect to form II is not extensive (e.g., 125% or less) crystal growth even in seeded solutions is greatly restricted and limited to submicroscopic seeds. The same lack of hydrogen bonding sites along the major faces of the form II crystal is thought to account for the low solubility of form II.

The difficulty in rearranging from one conformation to the other even in solution has been investigated using NMR. As expected a solution of ritonavir in DMSO produces the same NMR spectrum regardless of the polymorph dissolved; however the spectra show the existence of two conformations of ritonavir in solution in a ratio of approximately 99 to 1. Heating experiments indicate that this ratio does not change even at elevated temperatures, where the interconversion of



Scheme 1.

conformers in solution is generally faster than the NMR time frame. The fact that the two conformers remain visible in the solution NMR even at elevated temperatures indicates that the interconversion of the two conformers requires significant energy and would not be expected to occur spontaneously. The two conformers detected by NMR have not been unequivocally identified as form I and form II ritonavir, although a 99 to 1 relationship of form I to form II is reasonable and the difficulty in interconversion parallels the reluctance to nucleate form II.

POSSIBLE SOURCE OF FORM II

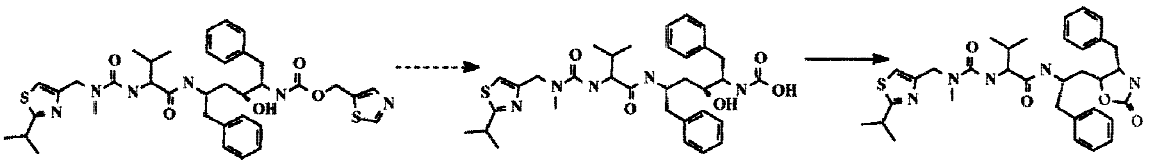
In light of this reluctance of form II to nucleate, a question arises as to how it was generated for the first time. Highly supersaturated solutions will crystallize form II but only if seeded with form II, so this could not be the original source of this polymorph. A possible source of form II was investigated which involves heterogeneous nucleation by a related compound with structural similarities able to act as a template for form II. The most reasonable possibility is [4S-[4α, 5β [R*(R*)]]]-3-methyl-2-[[[methyl [(2-1-methylethyl)-4-thiazoyl]methyl]amino]carbonyl]amino]-N-[1-[[2-oxo-4-(phenylmethyl)-5-oxazolidinyl]methyl]-2-phenylethyl]butanamide which can be formed by the base catalyzed degradation of ritonavir shown in Scheme 2.

The compound formed contains a cyclic carbamate linkage, which as a result of the ring formation, is forced into a "cis"-like conformation. This "cis" carbamate could act as a template or seed to facilitate nucleation of form II. This hypothesis was tested by using the Seed Detection Test. A portion (~100 μg) of [4S-[4α, 5β [R*(R*)]]]-3-methyl-2-[[[methyl [(2-1-methylethyl)-4-thiazoyl]methyl]amino]carbonyl]amino]-N-[1-[[2-oxo-4-(phenylmethyl)-5-oxazolidinyl]methyl]-2-phenylethyl]butanamide prepared by an unambiguous route that could not produce ritonavir, was used to seed a portion of the Seed Detection Test. The seeded solution was stored at 5°C overnight, as directed in the seed detection test and crystallization of form II ritonavir occurred. It is therefore possible that solid cyclic carbamate impurity was responsible for seeding (i.e., facilitating and directing to produce form II) either enough bulk drug to create a small number of form II seeds which later seeded the formulation manufacturing equipment or for seeding the manufacturing lines directly. The cyclic carbamate is less soluble than ritonavir and is formed very rapidly when catalytic amounts of base are present. Bulk ritonavir invariably contains small quantities of this degradate. Loss of solvent from a solution of ritonavir could result in solid residual cyclic carbamate occurring in numerous places during the manufacturing cycle.

CONCLUSION

Ritonavir was found to exhibit conformational polymorphism with the "cis" and "trans" conformations around the carbamate linkage leading to two unique crystal lattices with significantly different solubility properties. Although the polymorph (form II) corresponding to the "cis" conformation is a more stable packing arrangement, nucleation of the polymorph requires the formation of a less stable conformation in solution, clustering of this less stable conformation and subsequent crystal growth. This nucleation process, even in the presence of form II seeds, is energetically unfavored except in highly supersaturated solutions. For this reason form II may never have been identified except for the coincidence of a solution which was very highly supersaturated (~ 400%) with respect to the polymorph and an unknown nucleation enhancer, possibly a related compound capable of heterogeneously seeding the solution.

No control of crystal form was required for ritonavir's



ritonavir base hydrolyzed carbamate cyclic carbamate

Scheme 2.

original formulation as directed in the International Conference on Harmonization (ICH) Guidelines (15). In view of the phenomenon observed with ritonavir over time, this guideline must be re-evaluated to state that no control is necessary providing the solubilities of all the crystal forms of a drug are essentially equivalent.

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