Thermodynamic Relationship between α- and β-Forms of Crystalline Progesterone

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Abstract □ Thermodynamic properties such as the latent heat of fusion, aqueous solubility, heat of solution, and interchange energy with water were determined for single crystals of progesterone in α- and β-forms. The differences in the properties between the polymorphic crystals arise from a dissimilar mode of molecular packing, rather than of molecular conformation, in the unit cell, as reflected in the IR spectral and X-ray crystallographic results.

Keyphrases □ Thermodynamic properties, various—determined for α- and β-forms of crystalline progesterone □ Progesterone—α- and β-forms of crystals, various thermodynamic properties determined □ Crystals—α- and β-forms of progesterone, various thermodynamic properties determined □ Steroids—progesterone, α- and β-forms of crystals, various thermodynamic properties determined

In addition to its pharmaceutical and pharmacological role (1, 2), the importance of crystalline polymorphism has been recognized in understanding thermodynamic properties of solid substances (3). Some thermodynamic differences between polymorphic crystals are revealed in orientational changes of the molecules in insoluble monolayers at air–solution interfaces when polymorphic transformation is associated essentially with a conformational change of the molecules in the two-dimensional film (4–6).

Progesterone has at least two polymorphic forms in its crystalline state (7–13). Little has been reported on the physicochemical properties to clarify the thermodynamic relationship between them. In the present experiments, an attempt was made to provide information about the thermodynamic and relevant properties of the steroid in different polymorphic states. Such information is necessary not only for establishing criteria of crystallographical and pharmacological purities for quality control of the steroid but also for the quantitative understanding of its surface chemical properties.

EXPERIMENTAL

Identification of the polymorphic crystals was achieved with an X-ray diffraction unit[1], an IR spectrometer[2] (resolving power of 0.5 cm^{-1}), and a differential scanning calorimeter[3] with a temperature range from −150 to +300° (accuracy of ±2.5 mJ/sec). For all experiments, a commercially available sample (pure grade) of crystalline progesterone[4] was used after repeated recrystallization from aqueous ethanol (1:1 v/v).

The solubility in water was determined by the radiotracer method (14) with a radiochemically pure sample of 1,2-^{3}H-progesterone[5] (specific activity of 410 μCi/mmole). The techniques used were similar to those reported previously (6).

RESULTS AND DISCUSSION

Single Crystals of Progesterone—The sample, when recrystallized without seeding from an ethanolic mixture (1:1 v/v) with water or *n*-hexane, was identified as the α-form (9, 10) or Form A (12, 13), which gave only one peak at 128° on the differential scanning calorimetric chart. After equilibrium melting in air at 85 ± 2° for a few hours, it had turned to the 122° melting crystal, which corresponded to the β-form (9, 10) or Form B (12, 13). When such a crude sample of the α- or β-form was used for seeding, upon slow cooling of a progesterone solution, a single crystal (~1 mm × ~2 mm average) was always obtained and was identified as the α-crystal or a mixture of α- and β-crystals, respectively; the latter permitted manipulative separation with the unaided eye.

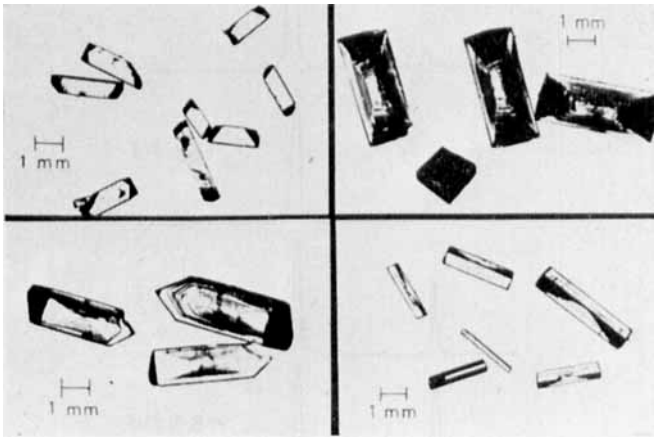

Figure 1—*Single crystals in α- (left) and β- (right) forms obtained by seeding–recrystallization from ethanolic mixtures (1:1 v/v) with water (top) and n-hexane (bottom).*

Figure 1 shows the microphotographs of these single crystals. Those in the left figures were identified as pure samples of the α-form from crystallographical (15, 16) and IR spectral (12, 13) aspects. No definitive data have been established for identification of the β-form.

Crystalline Transformation—When molten liquid underwent equilibrium melting at 85° in air, nitrogen, or helium, β-crystals always were formed; in a vacuum (2–3 mm Hg), α-crystallization took place. At room temperature, both crystals seemed to be so stable that apparently no polymorphic transformation occurred for several months or longer. At a higher temperature, a unilateral transformation, $\beta \rightarrow \alpha$, occurred spontaneously (Fig. 2). The β-form apparently is thermodynamically unstable. Heating at 122 and 128° returned the β- and α- forms, respectively, to the molten state.

Differential scanning calorimetry (−100 ⇄ +150°) of the steroid in air showed that the molten specimen was crystallized at a cooling rate of −1°/min; −10°/min was so fast that the specimen remained supercooled throughout the cooling processes. In such a case, crystallization

Table I—Aqueous Solubilities of Progesterone in α- and β-Forms

Temperature	Solubility, 10^{-5} M	
	α-Form	β-Form
21.7°	2.60	—
24.0°	—	3.72
25.3°	2.81	—
30.2°	3.80	—
30.5°	—	4.52
35.5°	—	5.39
36.4°	4.69	—
40.7°	—	6.75
41.3°	6.37	—
46.1°	6.50	—

[1] Rigaku Denki model 2001.
[2] Hitachi Perkin-Elmer model 225.
[3] Shimadzu model DSC-30 equipped with model LTC-30 cooling unit.
[4] Nakarai Pharmaceutical Co., Kyoto, Japan.
[5] The Radiochemical Centre, Amersham, England.

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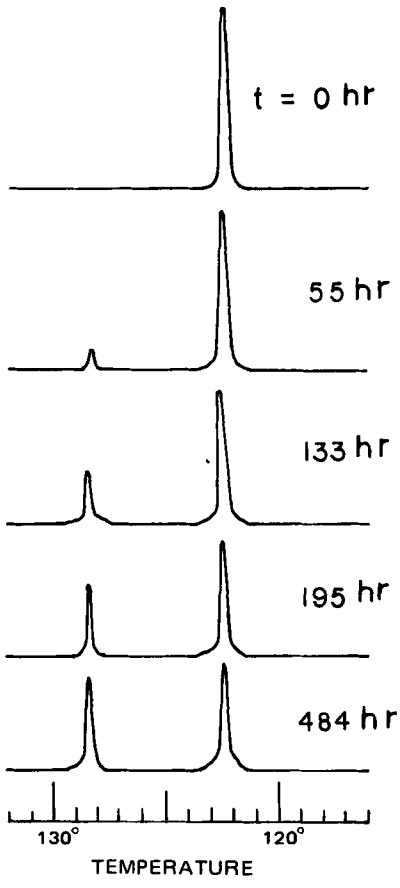

Figure 2—*Differential scanning calorimeter diagrams for β-crystal kept standing in a vacuum (2–3 mm Hg) at 99 ± 1° for the indicated time. Five samples of single β-crystals were split into two half-portions for the high temperature and control tests. No change was observed with the control portion, which had been kept in a vacuum at room temperature. With the α-crystal, no change was observed on the differential scanning calorimetric chart throughout several weeks, regardless of the pressure (2–760 mm Hg) and temperature (25–99°).*

usually took place in the subsequent heating processes. In all cases, the crystallization temperature was from 37 to ~120°, depending on the experimental conditions. All of these crystals proved to be of the β-form regardless of the solidification conditions. Forms III–V, reported by Brändstatter-Kuhnert and coworkers (9–11), seem to represent metastable states that change commonly to the β-form.

Recrystallization from an organic solvent such as ethanol, benzene, acetone, or ether always yielded the α-form, as did any binary mixture of these solvents.

Thermodynamic Relationship—The dissolution processes of progesterone in water obey the first-order rate law (6, 14). The solubility, C_s, at a temperature, T, was determined by extrapolating the rate equation to infinite time (Table I). Table II gives the heat of solution, ΔH_s, based on the linearity of the $C_s \sim 1/T$ relationship, the latent heat

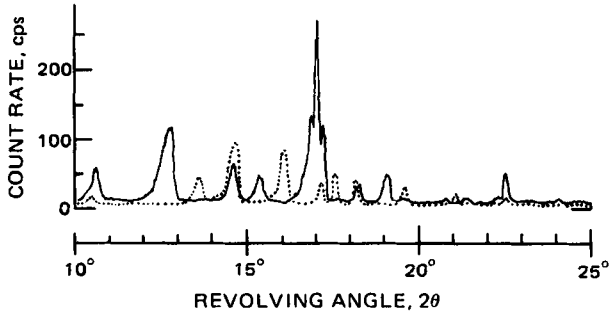

Figure 3—*X-ray diffraction patterns for pulverized samples in α- (—) and β- (. . . .) forms.*

Table II—Heat of Solution, ΔH_s, Latent Heat of Fusion, ΔH_f, and Molar Interchange Energy, Φ, for Progesterone in α- and β-Forms

Parameter, kcal/mole	α-Form	β-Form	Difference
ΔH_s	8.41	7.27	$\delta\Delta H_s = 1.14$
ΔH_f	6.68	5.60	$\delta\Delta H_f = 1.08$
Φ	+1.73	+1.67	$\delta\Phi = 0.06$

Table III—Lattice Constants of Progesterone in α- and β-Forms (Orthorhombic Crystalline System)

Sample	α-Form, Å	β-Form, Å
a	12.62	12.65
b	13.84	22.65
c	10.38	6.36

of fusion, ΔH_f, obtained from the differential scanning calorimetric chart with crystalline potassium nitrate as the reference, and the molar interchange energy, Φ, defined as:

$$\Phi = \Delta H_s - \Delta H_f \qquad \text{(Eq. 1)}$$

This term can, in turn, be expressed (6) as:

$$\Phi = pzN\phi \qquad \text{(Eq. 2)}$$

where N is Avogadro's number, p is the fraction of progesterone–water linkage to all z linkages around a progesterone molecule in the solution, and ϕ is the pair hydrophilicity defined as:

$$\phi = \epsilon_{12} - (\epsilon_{11} + \epsilon_{22})/2 \qquad \text{(Eq. 3)}$$

in terms of the bond energies for the pairs of progesterone–water (ϵ_{12}), water–water (ϵ_{11}), and progesterone–progesterone (ϵ_{22}).

The interchange energy, Φ, or the pair hydrophilicity, ϕ, is rationalized under the assumption that the molecular conformation in a polymorphic crystal remains unchanged throughout the dissolution processes since the solubility is determined by extrapolating the rate equation to infinite time. In Table II, therefore, the small $\delta\Phi$ value is indicative of almost the same conformation of the progesterone molecules in between α- and β-crystals. The $\delta\Delta H_s$ is contributed largely by $\delta\Delta H_f$ and negligibly by $\delta\Phi$, suggesting that the crystalline polymorphism is characterized by the dissimilarity in the packing mode of the molecules without a significant difference in their conformation. In fact, the IR spectral difference appeared only as the shift at ~870 cm^{-1}, which Mesley (13) ascribed to the difference in CH out-of-plane vibration. The X-ray diffraction patterns for pulverous samples (Fig. 3) and the lattice constants thereby obtained (Table III) are in good agreement with the reported values (15–18).

Campsteyn *et al.* (17) reported that the intermolecular cohesion in α-crystalline progesterone is due mainly to van der Waals attraction. Foresti-Serantoni *et al.* (18) indicated that the molecular conformation in the α-crystal is similar to that in the β-crystal and, accordingly, that the packing forces in these crystals have little correlation to the molecular conformation. All these aspects are in accord with the consideration that the thermodynamic difference comes solely from a different mode of molecular packing, rather than the molecular conformation, in the unit cell.

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New *In Vitro* Dissolution Test Apparatus

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Abstract □ A new *in vitro* dissolution test apparatus was designed and evaluated. Compressed tablets of drugs representing different solubility characteristics were tested at various air pressures and compared to dissolution patterns of similar tablets by the Levy beaker and USP methods. Air pressure of 46 mm generally was suitable for determining the dissolution rates of tablets. This new dissolution tester possibly can be useful in determining drug release from solid dosage forms and correlating it with *in vivo* bioavailability because dissolution rate can be controlled easily with the adjustment of air pressure without complicated changes in the apparatus, there is no excessive settling of particles, and complete drug dissolution can be achieved with no clogging of the screen.

Keyphrases □ Dissolution test apparatus—designed, evaluated with various drugs at various air pressures, compared to other methods □ Apparatus, dissolution test—designed, evaluated with various drugs at various air pressures, compared to other methods

Determination of *in vitro* dissolution rates is important in the design, evaluation, and quality control of solid dosage forms. The USP and NF dissolution tests suffer from a number of technical problems (1, 2).

Various other methods generally involve induced agitation. The Levy beaker method (3) is the most commonly used method and is generally recommended as a standard. Other reported methods (4–7) have the commonly encountered problem of mound formation of particles at the bottom of the container due to poor particle dispersion. Mound formation may affect apparent dissolution characteristics (8). In this study, a better correlation was observed with a rotating flask that allowed good dispersion at low agitation. Another problem is the degree of agitation, which is usually greater than what the dosage form will encounter in the GI tract.

The USP and NF methods also are subject to poor dispersion. In these methods, the screen acts as an interfacial barrier at moderate stirring rates. In addition, the fine mesh screen is clogged by undissolved particles (9).

An apparatus will be most suitable for *in vitro* testing of solid dosage forms if it achieves a thorough dispersion of particles with minimum agitation, similar to the agitation in the GI tract. With these factors under consideration, an *in vitro* dissolution tester was designed and evaluated.

EXPERIMENTAL

Description of Apparatus—The apparatus (Figs. 1 and 2) consists of two cylindrical glass tubes: tube A, 28.4 cm long × 3.4 cm i.d.; and tube B, 29.4 cm high × 2.8 cm i.d. Tube B is connected to tube A at the base and at a length of 17.3 cm by tubes with an identical diameter of 1.0 cm and a length of 4.7 cm.

A stainless steel basket and an air tube are suspended in tube A. The bottom of the basket is approximately 15.7 cm from the bottom of tube A, and the air tube just protrudes from the stopper.

The basket consists of a solid metal top with a small vent and is fitted

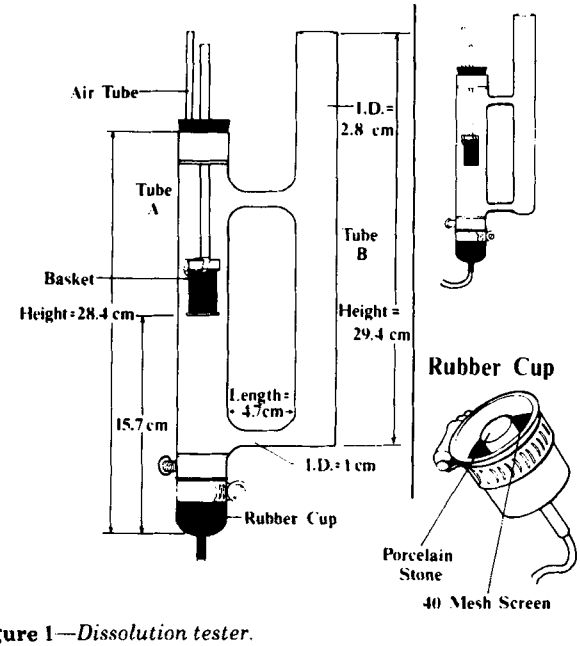

Figure 1—*Dissolution tester.*