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Research Article

Solid-State Phase Transitions Initiated by Water Vapor Sorption of Crystalline L-660,711, a Leukotriene D_4 Receptor Antagonist

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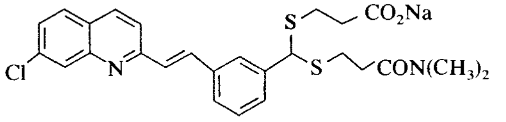
Significant water vapor sorption at room temperature by the crystalline and lyophilized forms of L-660,711, a potent, selective leukotriene D_4 receptor antagonist, has been measured and shown to produce increasingly more nonflowing, semisolid masses with increasing relative humidity. Thermal analysis, SEM, powder X-ray diffraction, solid-state NMR, and thermomicroscopic measurements reveal that water vapor sorbed at room temperature converts the crystalline form to a noncrystalline form resembling the lyophilized sample. Evidence is presented to indicate that L-660,711 has surface active properties with a critical micellization concentration of approximately 1×10^{-4} *M* and an ability to form thermotropic and lyotropic mesomorphic phases when the crystal is heated above 80°C in the anhydrous state; it is lyophilized from aqueous solution, and it is exposed to relative humidities at and above 12%, at room temperature.

KEY WORDS: leukotriene D_4 receptor antagonist; water vapor sorption; solid-state transitions; surface activity; mesomorphic phases.

INTRODUCTION

This paper is concerned with the solid-state properties of the compound, L-660,711, the sodium salt of [3-(((3-(2-(7-chloro-2-quinolinyl)ethenyl)phenyl)((3-(dimethylamino)-3-oxo-propyl)thio)methyl)thio)propanoic acid], first synthesized by Zamboni *et al.* (1). The substance is a potent, selective leukotriene D_4 receptor antagonist whose *in vitro* and *in vivo* pharmacological profile was described by Jones *et al.* (2).



Scheme 1

Of particular interest in this study is the ability of this compound, in both crystalline and lyophilized forms, to take up large amounts of water vapor from the atmosphere with marked changes in solid-state properties. The major goal was to understand better the mechanisms of water vapor uptake and the effects of such water on the physical stability of L-660,711.

MATERIALS AND METHODS

Materials

L-660,711, synthesized large scale by J. M. McNamara *et al.* (3), and recrystallized from acetone, is an off-white to pale yellow free-flowing powder. The sample used in this study had a specific surface area of 5.1 m^2/g. Figure 1A shows a scanning electron micrograph of the crystalline material, while Fig. 1B shows a micrograph of the same material after lyophilization from aqueous solution. The latter substance, a fluffy off-white to pale yellow material, exhibits no distinctive features in the SEM, typical of most lyophilized materials. The substance exhibits a high solubility in water at room temperature.

Methods

Water Vapor Sorption. Samples of L-660,711 in both the crystalline and the lyophilized forms were stored at room temperature (22°C) in sealed jars containing saturated salt solutions equilibrated to various relative humidities. Crystalline samples were pretreated prior to moisture exposure by drying at 50°C under vacuum overnight. Lyophilized samples were used directly from the lyovials. After the period of time required for apparent sorption equilibrium, as evidenced by constant sample weight, about 48 hr, the weight of water sorbed per gram of initially dried solid was determined by measuring the loss of water thermogravimetrically, using

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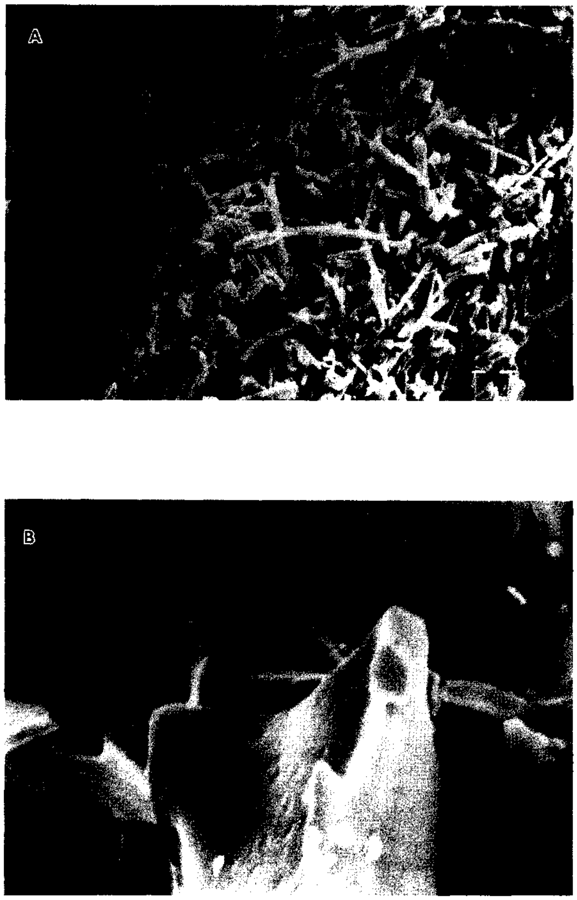

Fig. 1. Scanning electron micrographs for (A) crystalline and (B) lyophilized substance. 2000×; reduced 40% for reproduction.

a Perkin–Elmer TGS-2 in conjunction with a System 4 and TADS 3600 data station. Scans were taken over the temperature range of 30–150°C at a heating rate of 10°C/min, under a nitrogen purge.

Differential Scanning Calorimetry. A Perkin–Elmer DSC-4 with a System 4 and TADS 3600 data station was used to obtain thermal curves, under nitrogen purge, from 30 to 150°C at a scanning rate of 10°C/min. Sealed volatile aluminum pans were used in all experiments unless otherwise specified.

Scanning Electron Microscopy. All observations were made on an International Scientific Instruments ISI-40 electron microscope. Samples were gold sputter-coated prior to examination to render them electrically conductive. All images presented here were obtained using secondary electron detection.

Thermomicroscopy. A Karl Zeiss universal microscope with a Mettler FP-82 hot stage was used to visualize samples heated over the range of 30–250°C at a heating rate of 10°C/min.

X-Ray Powder Diffractometry. A Philips compact X-ray powder diffractometer system, Model PW1840, was used. Crystalline samples were ground with a mortar and pestle to obtain uniform and small particle size. An initial powder diffractogram, with a dried sample, was generated for each relative humidity to which a sample was to be exposed. Then the samples, contained in the X-ray sample holder, were placed in the sealed jars containing saturated salt solutions equilibrated at various relative humidities. Powder diffractograms were taken at various time points to evaluate potential changes in the crystallinity of the solid upon exposure to water vapor. By using the same sample at a particular relative humidity for the different time periods, it was felt that changes in the powder patterns due to differences in the sample surface could be minimized.

Surface Area Measurements. Surface area was determined by the BET method using the Quantasorb surface area analyzer and nitrogen gas. The material was purged with dry nitrogen for 4 hr prior to the measurement, which was carried out in triplicate. The determination was a single-point one with the N_2/He partial pressure maintained at 0.33.

Solid-State NMR. The solid-state ^{13}C-NMR spectra were obtained on a Chemagnetics A-220S spectrometer in the cross polarization/magic-angle spinning (CP/MAS) mode. About 250 mg of sample was placed in a KEL-F rotor and spun at 3300 rps. The CP contain time was 2 msec and the repetition time was 3 sec. Six thousand acquisitions were collected over 20 kHz and zero-filled to 16 K. Tetramethylsilane was used as the external standard, with hexamethylbenzene as a secondary standard (methyl signal at 17.35 ppm).

Surface Tensiometry. Surface tension was measured using a Wilhelmy plate attached to a Cahn Model 2000 recording electrobalance. The crystalline compound was dissolved either in triply distilled water or in 1.25% $NaHCO_3$ solution. The measurements were carried out at 25°C in triplicate.

RESULTS

Water Vapor Sorption

Equilibrium moisture uptake was achieved after approximately 2 days of exposure to moisture even at relative humidities as low as 12%. Figure 2 presents the grams of water sorbed per gram of dried solid vs relative humidity at 22°C for both the crystalline and the lyophilized samples. Note that the extent of sorption is very similar for both solid forms. Taking into account the specific surface area of 5.1

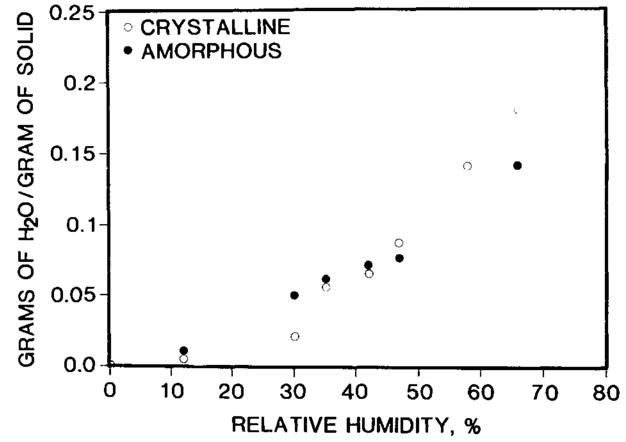

Fig. 2. Water vapor sorption (g/g) vs relative humidity (%) at 22°C for (○) crystalline and (●) lyophilized compound.

m^2/g for the crystalline sample, and assuming a minimal cross-sectional area of a water molecule to be about 12.5 Å, we estimate that the level of water sorbed is two to three orders of magnitude greater than could occur by adsorption to the available surface. Hence, it appears most likely that water vapor is absorbed primarily into the bulk structure of the solid.

Scanning Electron Micrographs

Figures 3A–E indicate the significant change in morphology of the crystalline material as the relative humidity to which it is exposed is raised above 30% at room temperature. This corresponds to the marked increase in water vapor sorption above 30% relative humidity. The loss of distinctive morphology of the needle-like particles above 30% to give structures more like the lyophilized sample (see Fig. 1B) strongly suggests that the presence of water at room temperature is causing a loss in crystallinity. This change in morphology also was observed by optical microscopy, where at low relative humidities a rounding-off of the sharp crystal ends was noted. At higher relative humidities individual crystals fused into a featureless mass. A similar change from a crystalline to a featureless morphology also occurred when the anhydrous crystalline sample was heated from 30 to 150°C and then cooled to room temperature. This is seen in Fig. 4.

X-Ray Powder Diffractometry

Figures 5A and B show the diffractograms for the initially crystalline and lyophilized samples, respectively. Clearly, the lyophilization process leads to a loss in crystallinity. Figures 6A–C illustrate typical changes in crystallinity

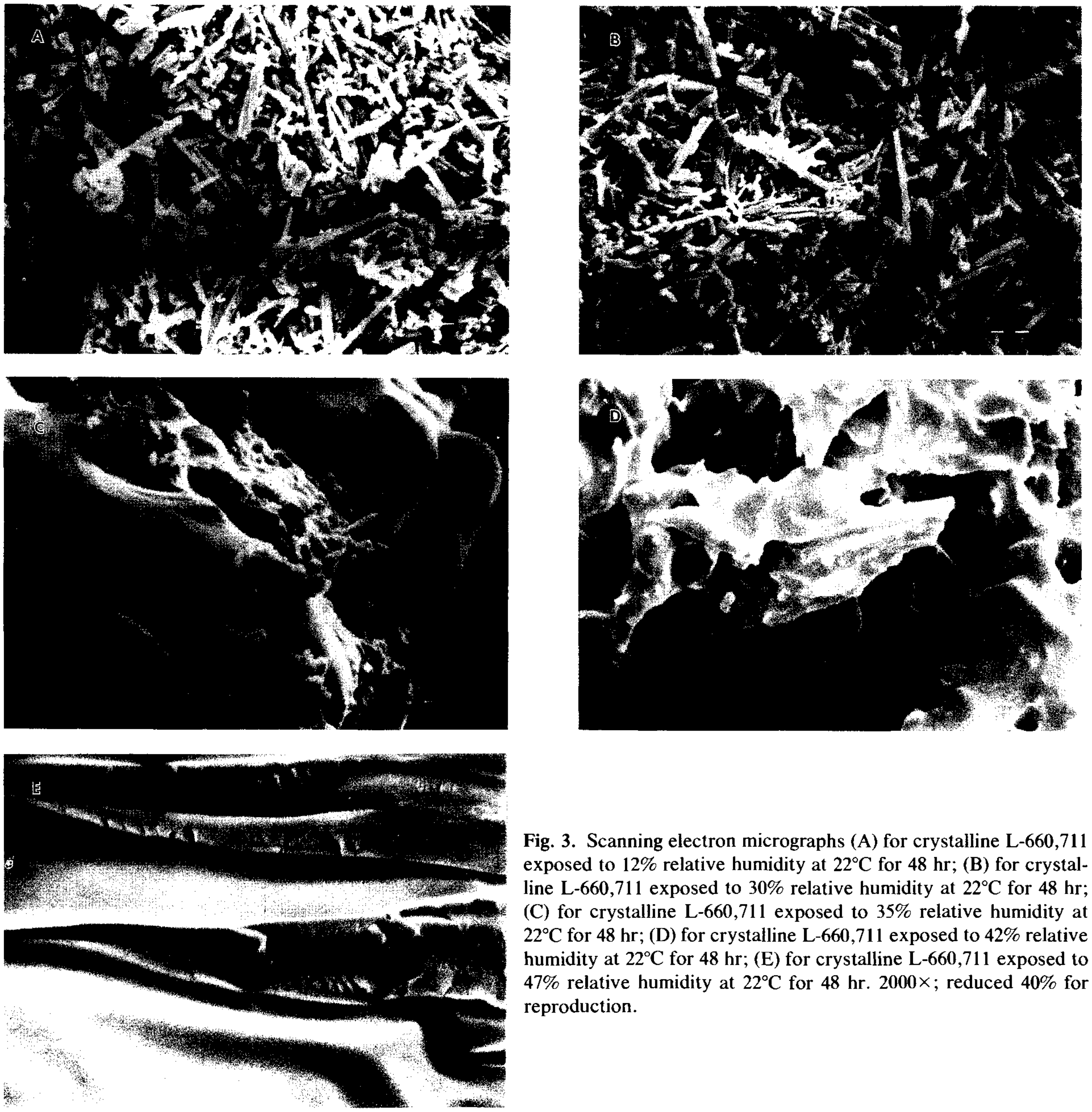

Fig. 3. Scanning electron micrographs (A) for crystalline L-660,711 exposed to 12% relative humidity at 22°C for 48 hr; (B) for crystalline L-660,711 exposed to 30% relative humidity at 22°C for 48 hr; (C) for crystalline L-660,711 exposed to 35% relative humidity at 22°C for 48 hr; (D) for crystalline L-660,711 exposed to 42% relative humidity at 22°C for 48 hr; (E) for crystalline L-660,711 exposed to 47% relative humidity at 22°C for 48 hr. 2000×; reduced 40% for reproduction.

Fig. 4. Scanning electron micrograph for crystalline compound heated to 150°C and cooled to 30°C. 2000×; reduced 40% for reproduction.

relative to that shown in Fig. 5A, upon exposure to various relative humidities. These figures indicate two important points: extended exposure to relative humidities as low as 12% can reduce crystallinity of L-660,711, and the time scale to reach about the same level of loss in crystallinity changes significantly as one increases relative humidity from 12% (1 week) to 35% (1 day) to 58% (1 hr).

Although using the same sample at a given relative humidity should have reduced any variability which might have occurred with sample preparation, it was not possible to eliminate completely such differences from sample to sample since the physical nature of the solid surface was altered by water to different extents at different relative humidities and time periods. For example, at the higher relative humidities the particles appeared to fuse, resulting in sintering and shrinking of the entire sample. Thus the X-ray results, with regard to the effects of moisture on crystallinity, can only be qualitative. They do, however, clearly show that a loss of crystallinity occurs for L-660,711 upon exposure to water.

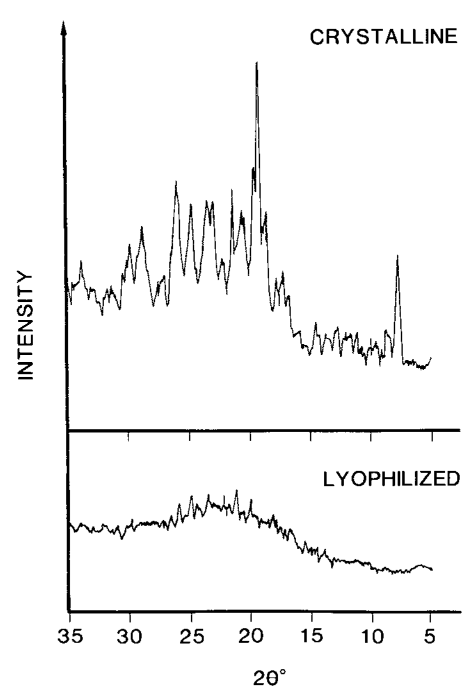

Fig. 5. X-ray powder diffractograms for (A) crystalline and (B) lyophilized substances.

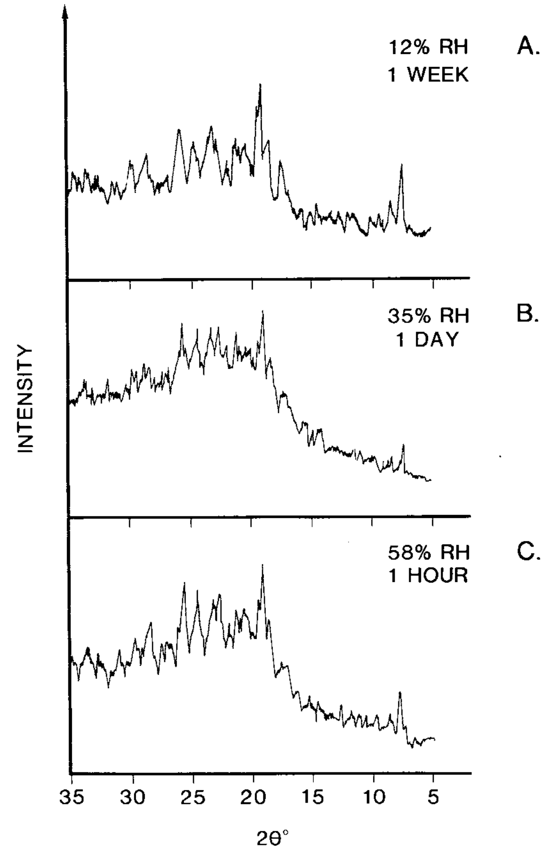

Fig 6. X-ray powder diffractograms for the crystalline compound exposed to various relative humidities for various times: (A) 12% relative humidity for 1 week; (B) 35% relative humidity for 1 day; (C) 58% relative humidity for 1 hr.

Thermomicroscopy

Direct observation of the crystalline sample, heated from 30 to over 200°C, indicated that the sharp edges of the particles, as seen in Fig. 3A, began to round off at about 80°C. These particles remained immobile until about 130°C, where they appeared to slowly coalesce. Complete liquefaction did not occur until over 200°C, accompanied by decomposition. At about 80°C shimmering colors appeared under crossed polarizers. These tended to disappear about 110°C. Exposure of the crystalline material to increasing relative humidities progressively lowered the temperature at which rounding and coalescence of particles occurred. For example, coalescence had begun to occur at 60°C when the samples were exposed to 58% relative humidity. Birefringent colors under crossed polarizers were observed also for the dried lyophilized sample in the temperature range of 35–60°C.

Differential Scanning Calorimetry

Figures 7A and B depict the thermal curves for the crystalline and lyophilized forms of L-660,711, respectively. Note in particular the significant endotherm for the crystalline form, i.e., about 8 cal/g, over the range of about 80–120°C. Note also the absence of any endotherm for the lyophilized sample. The range of temperatures over which the endotherm occurs seems to correspond to the region between rounding and coalescence of particles, as observed with the hot-stage microscope. Upon rescanning the sample depicted in Fig. 7A, an endotherm of only about 1–2 cal/g is observed in the range of 35–55°C as shown in Fig. 8. Three repeated heating and cooling scans on this sample, in sealed and open pans, produced no further changes in this endotherm. DSC scans and rescans also were run with the crystalline form after exposure to various relative humidities. As seen in Table I, the transitional enthalpies for the initial scan are reduced significantly upon exposure to water vapor, reaching essentially 0 cal/g following exposure to 42% relative humidity. The endotherm for the rescanned samples seems to disappear at 35%. The lyophilized samples exhibited no endotherms upon scanning and rescanning.

In order to rule out degradation upon scanning and rescanning, samples were rapidly cooled to room temperature either after the initial scans or after the rescans in a manner similar to that described by Cotton *et al.* (4). The cooled samples were analyzed by HPLC for degradation products. No degradation was observed either during the initial scan or during rescan.

DISCUSSION

It appears from the DSC, X-ray, SEM, and thermomicroscopic measurements that the initially anhydrous form of L-660,711 can undergo a transition to a less-ordered solid state upon being heated to about 80°C and that this solid becomes more liquid-like as the temperature is increased. Upon cooling and reheating, this less-ordered state does not revert to the crystalline form. However, upon repeated heating and cooling in the range of 30–150°C in the DSC experiments, a smaller endotherm appears with a constant transition enthalpy for each cycle. Although the latter endotherm could be reflecting a transition from some residual crystalline material, its tendency to persist upon repeated scanning and its appearance in the range of 35–55°C, rather than 80–120°C, suggests that it may reflect a lower-energy order–disorder transition between two different states of the noncrystalline material formed by heating and cooling.

The exposure of the crystalline form of L-660,711 to water vapor at room temperature appears to facilitate its conversion to a noncrystalline form. Observations with SEM and thermomicroscopy indicate no major changes in morphology at 12 and 30% relative humidity but do indicate very significant changes at higher relative humidities. This is also true for the lyophilized sample, but the tendency at any particular relative humidity to form masses with semisolid to

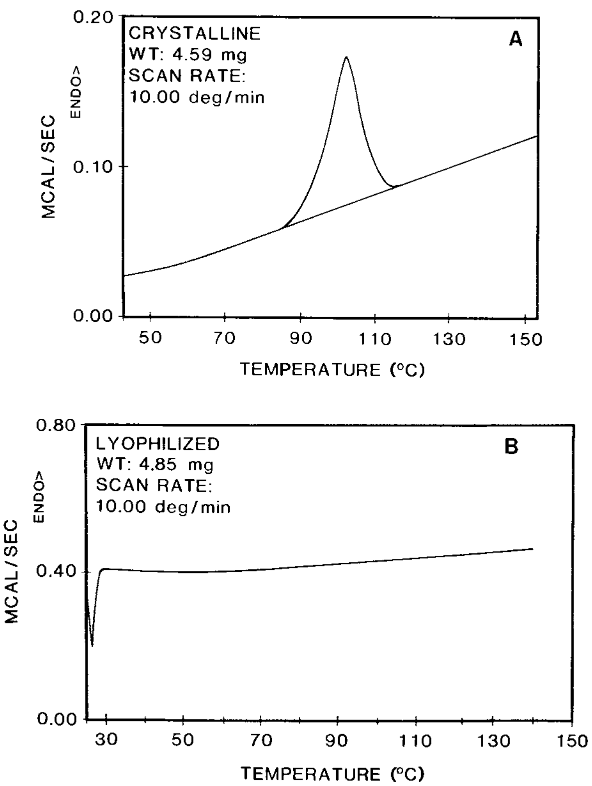

Fig. 7. DSC thermal curves, 10°C/min, (A) for the crystalline form of L-660,711, initial scan; (B) for the lyophilized form, initial scan.

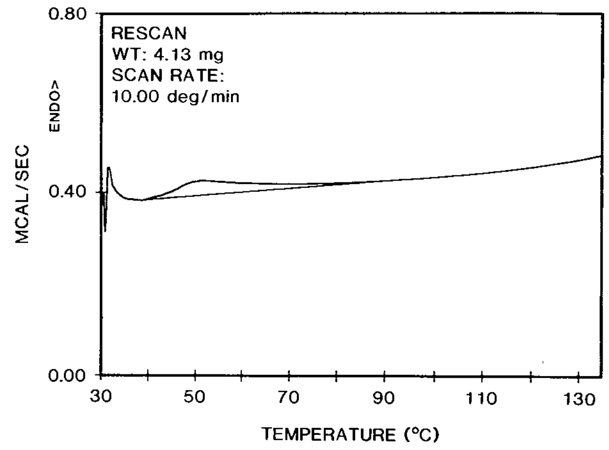

Fig. 8. DSC thermal curve—rescan of a crystalline sample.

Table I. Transition Enthalpies for Samples of L-660,711 Exposed to Various Relative Humidities[a]

Relative humidity (%)	Initial scan (cal/g)	Rescan (cal/g)
	Crystalline samples	
0	8.0	1.4
12	6.8	0.7
30	3.9	1.7
35	2.3	0
42	0.4	0
47	0.5	0
58	0	0
66	0	0
	Lyophilized sample	
0	0	0

[a] See text for details of the DSC experiments.

liquid-like properties is much greater than with the crystalline sample. X-ray powder diffraction and DSC measurements indicate that such a conversion is also occurring at 12 and 30% relative humidity, but very slowly. These observations, along with the similar water vapor sorption isotherms obtained for the crystalline and lyophilized samples, suggest that water, absorbed into the bulk structure of L-660,711 makes it possible for a phase change to occur at room temperature, rather than at 80°C, as with the anhydrous crystalline material. This appears to be the same process as that which occurs when an aqueous solution of L-660,711 is lyophilized. These conclusions are supported by preliminary data obtained using ^{13}C solid-state NMR. Figures 9A and B, for example, show differences in chemical shifts for the crystalline sample prior to and following exposure to 12% relative humidity for only a few hours. Note in particular the loss of doublets at around 180, 170, 140, 130, and 55 ppm upon exposure to water vapor, reflective of enhanced mobility of the carbon atoms corresponding to these peaks. Presumably this enhancement of molecular mobility, triggered by the presence of water molecules, provides the energy required to bring about the order–disorder transition.

The formation of amorphous forms of organic solids upon lyophilization or heating and then cooling is a very common phenomenon, e.g., sugars (5), cephalosporins (6), and peptides (7). However, with such systems reexposure to moisture or raising the temperature above the glass transition temperature of the amorphous solid generally triggers recrystallization (5,7). The lack of any apparent glass transition temperature in the range 30–150°C for the lyophilized sample and the spontaneous formation of a less-ordered solid state, even at 12% relative humidity, strongly suggest a very different type of noncrystalline phase or phases for L-660,711 compared to most amorphous organic solids.

In considering possible reasons for such phase transitions, one must examine the apparent similarity of this behavior and that of many surfactants and surfactant–water mixtures. Most surface active agents undergo a variety of phase transitions with increasing temperature and moisture content to form physical states intermediate between the highly ordered crystalline and the isotropic liquid phases. These have been termed thermotropic mesomorphic or liquid crystalline phases (8). Sodium laurate, for example, exists in the anhydrous state in a variety of phases, depending on the temperature, e.g., subwaxy I and II, waxy I and II, superwaxy, subneat, and neat (9). Each transition, with increasing temperature, leads to a progressively less-ordered state, until the isotropic liquid phase is reached at about 300°C, with transitional enthalpies ranging from about 1 to 10 cal/g. The presence of water for such systems causes lyotropic liquid crystalline phases to occur at greatly reduced temperatures relative to the thermotropic phases formed with anhydrous samples (8). It is well established that some water-soluble drugs are capable of acting as surface active agents (10). Mlodozeniec has demonstrated that nafoxidine HCl, with surface active properties, also exhibits thermotropic and lyotropic mesomorphic phases (11). Sodium cromolyn also has been shown to exhibit liquid-crystalline behavior (12).

That L-660,711 might have surface active properties in common with surfactants is suggested by an examination of its chemical structure, which appears to exhibit a concentration of polar and nonpolar groups at opposite ends of the molecule. To test for possible surface activity, the crystalline sample was dissolved in triply distilled water, pH 5.4, and in 1.25% $NaHCO_3$ solution, pH 8.4, up to concentrations of 1×10^{-3} *M*, and surface tensions were measured at 25°C using the Wilhelmy plate method. The apparent pK_a of L-660,711 was determined from solubility measurements of the free acid to be about 6.7. In Fig. 10 it can be seen that L-660,711 is capable of lowering the surface tension of water

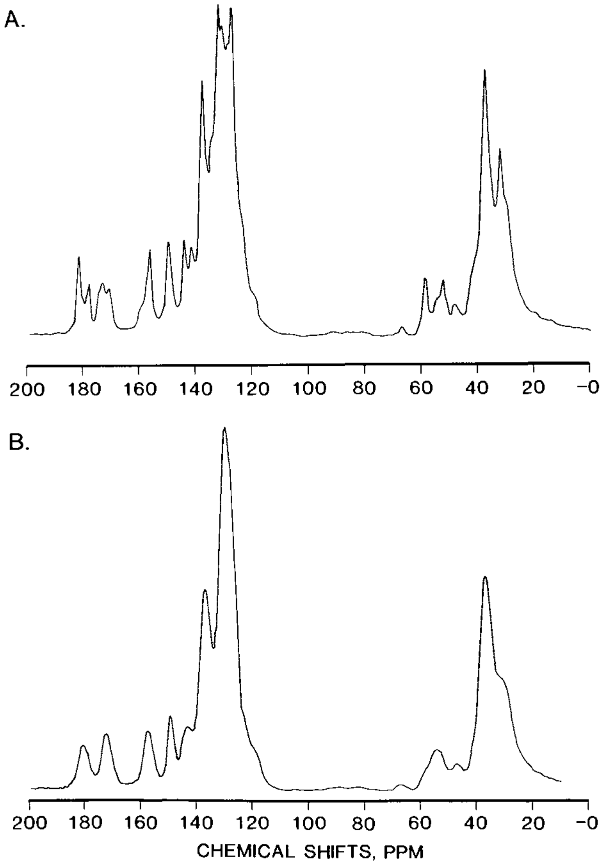

Fig. 9. Solid-state NMR: crystalline compound (A) prior to and (B) after exposure to 12% relative humidity.

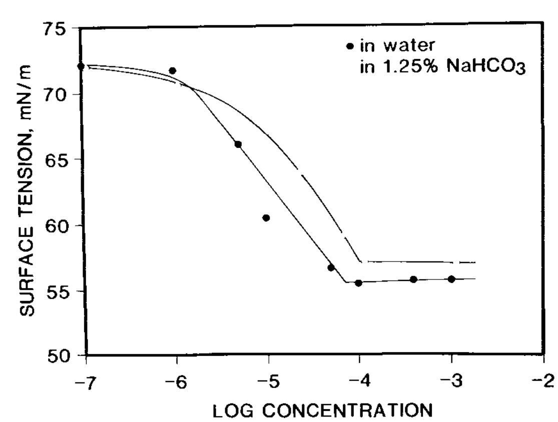

Fig. 10. Surface tension vs molar concentration for L-660,711 in (●) water and (○) 1.25% $NaHCO_3$ solution, at 25°C.

significantly at very low concentrations with an apparent critical micellization concentration (cmc) of about 1×10^{-4} *M* for both solutions, with the more ionized system at pH 8.4 having a slightly higher cmc as expected (10). This relatively low cmc for a highly water-soluble compound (the cmc for sodium lauryl sulfate in water at 25°C, for example, is 8.3×10^{-3} *M*) reveals the strong tendencies of L-660,711 to self-associate in water and the possibility that it could form various types of mesomorphic phases at higher aqueous concentrations and in the solid state. This strong tendency to self-associate in water may be brought about by two specific structural features of this molecule. First, the hydrophobic portion of the molecule consists of a planar aromatic system which could facilitate self-association through the stacking of the aromatic groups. Second, the close proximity of the carboxylate ion and the amide group could allow intramolecular interactions to occur, with a net decrease in the effective negative charge of the polar group. This, in turn, could result in a decrease in the ionic repulsion between molecules organized into the micelle and a lowering of the cmc. The apparent pK_a of 6.7 for this compound, as compared to values closer to 5 for most carboxyl groups, could arise from the orientation of molecules in the micelle and the effect of the electrical double layer on the surface pH (13).

Further support for the ability of L-660,711 to exist as one or more mesomorphic forms comes from some observations made with the hot-stage microscope. As previously indicated, we noted shimmering birefringent colors (8) under cross polarizers, under conditions where the compound was assumed to have had fully or partially lost its crystallinity, i.e., for the initially crystalline sample in the temperature range of 80–120°C, for the initially lyophilized sample in the temperature range of 35–55°C, and for both samples when small amounts of water were allowed to penetrate under the coverslip and then to evaporate slowly. Figures 11A and B represent typical views of the initially crystalline and lyophilized solids, respectively, subjected to the third treatment. Here we clearly see patterns reflective of the presence of mesomorphic phases (8) and good evidence that L-660,711 is indeed behaving in a manner expected of substances with surface active properties.

We propose, therefore, the following model for the solid-state behavior of L-660,711 in the absence and presence of water. The anhydrous crystalline form undergoes a major phase change from a crystalline to a mesomorphic phase upon heating to about 80°C. With further heating the apparent viscosity of this new phase progressively decreases until about 130°C, where it appears to liquefy. Upon cooling the sample to 30°C the metastable mesomorphic phase persists. However, at the lower temperature it retains some level of molecular order relative to the lyophilized sample, which had been previously exposed to water, since a small endotherm continues to exist in the range 35–55°C. This may reflect a transition between noncrystalline solid phases, e.g., subwaxy, waxy, etc. (9). The exposure of the crystalline form of L-660,711 at room temperature to water vapor at high relative humidities, i.e., >30%, before any heating or cooling occurs, produces material with the same properties as the lyophilized material. This includes the tendency to form semisolid, paste-like masses upon exposure to high relative humidities. Exposure at lower relative humidities, i.e., 12 and 30%, slowly converts the crystalline material to the mesomorphic phase, although macroscopically such changes are not readily apparent over extensive time periods. More definitive studies of the temperature vs composition phase behavior of L-660,711 and water will be necessary to establish the exact nature of these mesomorphic phases and the conditions under which they arise.

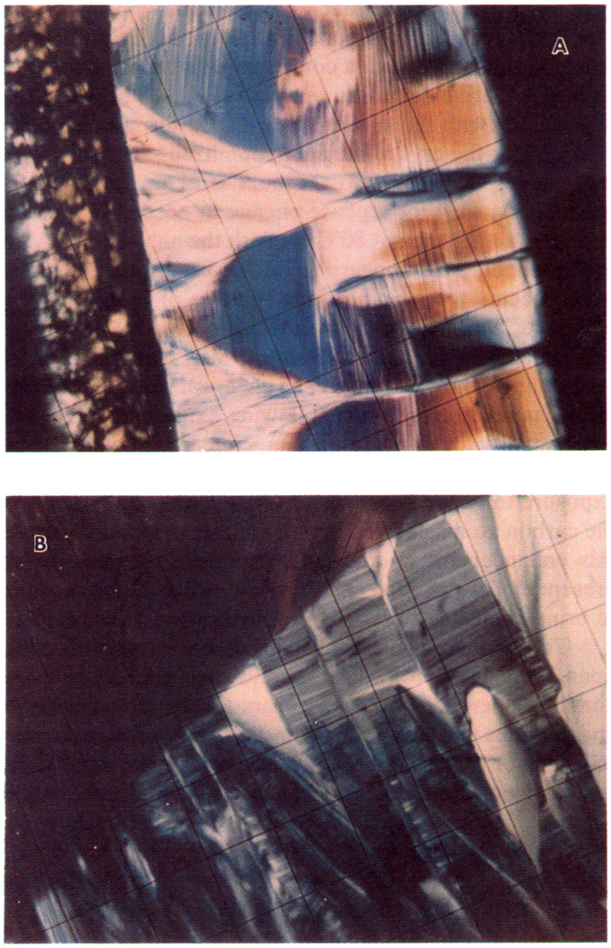

Fig. 11. Microscopic appearance of L-660,711 after hydration and drying on the sample slide: (A) initially crystalline; (B) initially lyophilized. 100×; reduced 30% for reproduction.

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REFERENCES

1. R. Zamboni, T. R. Jones, M. Belley, E. Champion, L. Charette,

R. deHaven, R. Frenette, J.-Y. Gauthier, S. Leger, P. Masson, C. S. McFarlane, S. S. Pong, H. Piechuta, J. Rokach, M. Thérien, H. W. R. Williams, and R. N. Young. *J. Med. Chem.* (in press).

2. T. R. Jones, R. Zamboni, M. Belley, E. Champion, L. Charette, A. W. Ford-Hutchinson, R. Frenette, J.-Y. Gauthier, S. Leger, P. Masson, C. S. McFarlane, H. Piechuta, J. Rokach, H. Williams, R. N. Young, R. N. deHaven, and S. S. Pong. *Can. J. Physiol. Pharmacol.* **67**:17–28 (1989).
3. J. M. McNamara, J. L. Leazer, Jr., M. Bhupathy, J. S. Amato, R. A. Reamer, P. J. Reider, and E. J. J. Grabowski. *J. Org. Chem.* **54**:3718–3721 (1989).
4. M. L. Cotton, D. W. Wu, and E. B. Vadas. *Int. J. Pharm.* **40**:129–142 (1987).
5. H. Levine and L. Slade. *Carbohydr. Poly.* **6**:213–244 (1986).
6. M. J. Pikal, A. L. Lukes, J. E. Lang, and K. Gains. *J. Pharm. Sci.* **67**:767–773 (1978).
7. T. W. Chan and A. Becker. *Pharm. Res.* **5**:523–527 (1988).
8. F. B. Rosevear. *J. Soc. Cosmet. Chem.* **19**:581–594 (1968).
9. C. Madelmont and R. Perron. *Colloid Polym. Sci.* **254**:581–595 (1976).
10. D. Attwood and A. T. Florence. In *Surfactant Systems: Their Chemistry, Pharmacy and Biology*, Chapman and Hall, London, 1983, p. 124.
11. A. R. Mlodozeniec. *J. Soc. Cosmet. Chem.* **29**:659–683 (1978).
12. J. S. G. Cox, G. D. Woodward, and W. C. McCrone, *J. Pharm. Sci.* **60**:1458–1465 (1971).
13. S. H. Yalkowsky and G. Zografi. *J. Colloid Interface Sci.* **34**: 525–533 (1970).