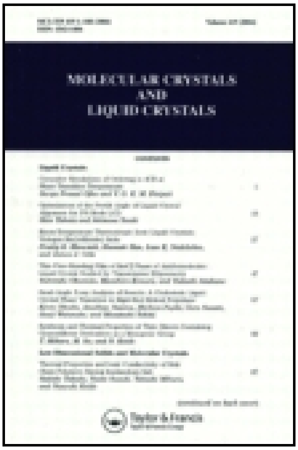

Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

Photochemical Behaviors of Freeze-Drying Crystals Containing Nucleic Acid Bases

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Version of record first published: 04 Oct 2006

To cite this article: Hideko Koshima, Keiko Yamashita & Teruo Matsuura (1998): Photochemical Behaviors of Freeze-Drying Crystals Containing Nucleic Acid Bases, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 313:1, 303-308

To link to this article: http://dx.doi.org/10.1080/10587259808044291

Mol. Cryst. Liq. Cryst., 1998, Vol. 313, pp. 303–308
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Photochemical Behaviors of Freeze-Drying Crystals Containing Nucleic Acid Bases

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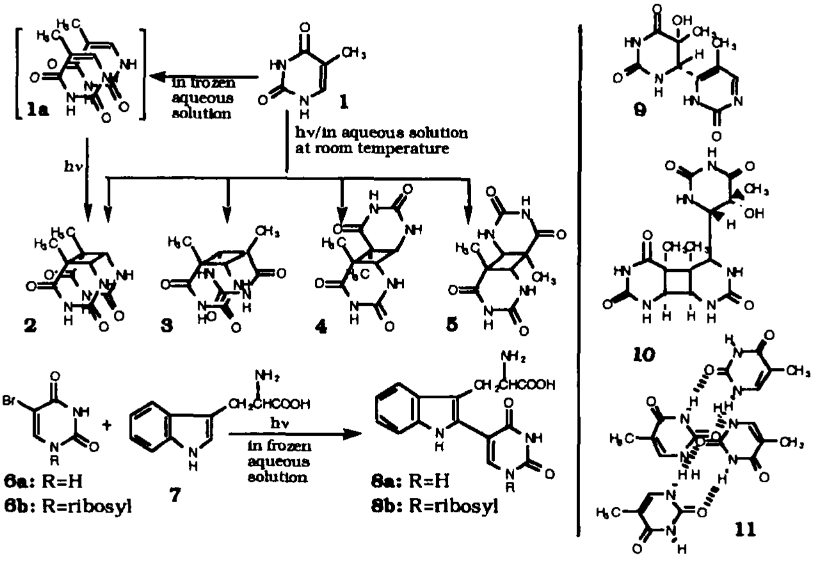
Freeze-drying crystals and mixed crystals were prepared from the frozen aqueous solutions of thymine (**1**) and of a mixture of 5-bromouracil (**6a**) and L-tryptophan (**7**). Their spectral and photochemical behaviors were compared to those of crystals and mixed crystals obtained by the evaporation of the aqueous solutions at room temperature. The freeze-drying thymine crystals underwent nonselective photodimerization to give a mixture of photodimers **2**, **3** and **5**, while the thymine crystals obtained at room temperature showed no photoreactivity. The freeze-drying crystals from **6a** and **7** were a simple mixture of component crystallites.

Keywords Freeze-drying crystals, photocemistry, nucleic acid bases.

INTRODUCTION

Much attention has been drawn to the photochemistry of nucleic acid bases, nucleosides and nucleotides.[1] Among them pyrimidine bases, nucleosides and nucleotides are known to undergo selective photodimerization in aqueous frozen solutions[2] or in thin solid films:[3] e.g. a syn-cis dimer **2** from thymine (**1**),[2] whereas thymine gives a mixture of four stereoisomers **2** - **5** by direct UV irradiation[4] or triplet sensitization[5] in an aqueous solution. In a frozen solution thymine molecules are believed to aggregate as **1a**. Wang has shown that the photodimerization of thymine in a thin film is greater as the humidity increases.[3] The result indicates that the hydrated thymine crystals have a crystal structure suitable for the photodimerization to **2**, but that the dehydrated thymine crystals have no photoreactivity.[3] Saito et al. have shown that the photocoupling of 5-bromouracil (**6a**) or 5-bromouridine (**6b**) to tryptophan (**7**) to form **8a** or **8b** occurs in aqueous frozen solutions, but hardly in an aqueous solution.[6] This was also interpreted by the formation of aggregates between **6** and **7** in ice. We were interested to see whether crystals or mixed crystals being obtained by the freeze-drying of such aqueous frozen solutions still keep the same aggregated form as that in the frozen solution to give the selective photoproducts. We were also interested to throw a light on the elucidation of the above Wang's finding.[3] In the present study we employed spectral characterization and photoirradiation methods, which have been proved to be useful as tools for the analysis of solid-state photochemistry.[7,8]



MATERIALS AND METHODS

General procedure.

Melting points are uncorrected. HPLC[8] was carried out with a Waters 600E system controller and a 991J photodiode array detector on a Bondasphere 5μm C18-100A (3.9 x 150 or 19 x 150mm) column. FT-IR[8], IR, UV and fluorescence spectra were taken on a JASCO FT/IR-8300, a Shimadzu IR-470, a Shimadzu UV-3100 and a Shimadzu RF-5000 spectrophotometers. PXD[8] was measured on a Rigaku ROTAFLEX PU-200B diffractometer.

Preparation of thymine crystals.

(1) Crystal I: Thymine (**1**) dissolved in water was evaporated in vacuo at room temperature to give colorless crystals. Anal. calcd. for $C_5H_6N_2O_2$: C, 47.62; H, 4.80; N, 22.22 %. Found: C, 47.45; H, 4.68; N, 21.78 %.

(2) Crystal II; An 8 mM aqueous solution of thymine (**1**) placed in a Pyrex tube was bubbled with argon for 30 min. After the deaerated solution was frozen at -25 °C to form a thin ice layer on the wall, it was freeze-dried in vacuo at -5 °C to give colorless crystals, Anal. calcd. for $C_5H_6N_2O_2$: C, 47.62; H,4.80; N, 22.22. Found: C, 47.18; H, 4.54; N, 21.81.

Irradiation of thymine in solid state and frozen state.

(1) In the solid state: Crystals I and II (20 mg each) pulverized were placed between two quartz plates (3 mm thickness) and irradiated with a 10 W low-

pressure mercury lamp under argon in a quartz apparatus[9] at 15 °C. The irradiated mixtures were dissolved in water and analyzed by HPLC.

(2) In the frozen state: An aqueous solution (8 mM, 100 ml of **1** was placed in a Pyrex tube of 5 cm diameter, degassed by bubbling with an argon stream and frozen at a given temperature (Table 1) to form a thin layer of the frozen solution and internally irradiated at a given temperature with a 10 W low-pressure mercury lamp which was covered with a Vycor tube. The irradiated frozen solution was defrozen and the solution was evaporated in vacuo to give colorless fine crystals, which were submitted to HPLC analysis (95 : 5 water-methanol).

Preparation of 5-bromouracil-L-tryptophan mixed crystals.

(1) Crystal III: An aqueous solution of a 1 : 1 molar mixture of 5-bromouracil (**6a**) and L-tryptophan (**7**) was evaporated in vacuo at room temperature to give colorless crystals.

(2) Freeze-dried crystals: A 10.5 mM aqueous solution of a 1 : 1 molar mixture of **6a** and **7** was deaerated by bubbling with argon and frozen at a given temperature and the frozen solution was freeze-dried at -5 °C to give colorless crystals. Crystal IV: -25 °C. Crystal V: -5 °C. Crystal VI: Gradually frozen by repeating freeze and defreeze at -5 to 0 °C.

Irradiation in solid state and frozen state.

(1) In the solid-state: Crystals I - VI were irradiated as above. The irradiated mixtures were analyzed by HPLC.

(2) In the frozen state: An aqueous solution of the substrates, **1** or mixed crystal **6a/7** of a given molarity was placed in a Pyrex tube, frozen at a given temperature and irradiated as above. The irradiated frozen solution was treated as above and analyzed for the product.

RESULTS AND DISCUSSION

Two samples of thymine crystals were prepared: (1) by evaporating an aqueous solution at room temperature (Crystal I) and (2) by freese-drying an aqueous frozen solution at -5 °C (Crystal II). The elemental analysis of these thymine crystals showed that both crystalscontain no water of crystallization. They were submitted to the characterization by PXD (Fig. 1) and IR spectroscopy showing that Crystal I and Crystal II are dimorphic.

Crystal I and Crystal II were irradiated with a low-pressure mercury lamp through quartz filter and the product distribution were compared with those for the photolysis of thymine in frozen aqueous solutions under different conditions. The results are shown in Table 1. In accordance with the Wang's finding that thymine monohydrate crystals showed less photoreactivity under more anhydrous conditions, Crystal I was found to be photochemically quite inert, while irradiation of Crystal II gave photodimers **2**, **3** and **5** in addition to

9, showing no selectivity. The results indicate that the geometrical arrangement of thymine molecules in the frozen aqueous solution was altered during freeze-drying contrary to our expectation that the aggregated form like **1a** may be kept in this process. The inertness of Crystal I can be interpreted by the crystal structure (**11**) of thymine anhydrate in which the geometrical arrangement is unfavorable forphotodimerization.[10] It should be noted that the crystal structure of thymine anhydrate is different from that of thymine monohydrate which undergoes photodimerization to the *syn-cis* dimer.[3,11]

In the case of the irradiation of the frozen solutions of thymine (Table 1), photodimer **2** was selectively produced, but no other photodimers (**3**, **4** and **5**) were deteced. In some cases a small amount of unidentified products **9** and **10** were deteced by HPLC. They are possibly photodimer **9**[13] and phototrimer **10**[14,15] from their UV spectra (**9**, λ_{max} 315 nm;[12] **10**, end absorption).

Next we prepared several crystal samples from an aqueous solution of the 1 : 1 mixture of 5-bromouracil (**6a**) and *L*-tryptophan (**7**) under various conditions: Crystal III was obtained by the vacuum evaporation at room temperature; Crystal IV by freeze-drying at -5 °C after freezing at -25 °C; Crystal V by freeze-drying at -5 °C after freezing at -5 °C; Crystal VI by freeze-drying at -5 °C after freezing at -5 - 0 °C with repeating freeze and defreeze. The conditions for Crystal VI were chosen in order to obtain the aggregated form in a better yield than that for quick freezing (Crystals IV and V). The PXD measurement of Crystals III, V and VI (Fig. 2) showed essentially the same PXD patterns which were found to be also same as that of 1 : 1 mechanical mixture of **6a** and **7** crystals. The results indicate that Crystal III, V and VI are substantially the simple mixture of the component crystals. The PXD pattern of Crystal IV (Fig. 2c), which prepared at the fastest freezing rate at -25 °C, showed the amorphous nature of Crystal IV.

The results of irradiation experiments for Crystals III - VI are summarized in Table 2. In all cases, the photocoupling product **8a** and uracil were obtained, although the product ratios were not always the same. The photocoupling product **8a** was identified by its characteristic fluorescence maximum at 440 nm (excited at 360 nm).[6] The photochemical formation of uracil from 5-bromouracil is known.[6]

The photochemical formation of **8a** from Crystals III - VI are interpreted by the photocoupling at the interface between 5-bromouracil and L-Tryptophan crystals. Some differences in the product ratios may be due to those in the size of the mixed crystals. We have reported various solid-state photoreactions occurring at the interface of two different organic crystals.[16] In some case the smaller size of the mixed crystals results in the faster photoreaction due to the wider interface of component crystallites.[17,18]

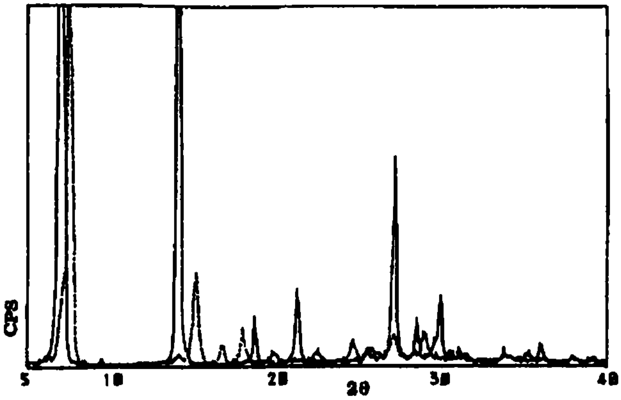


Figure 1. The PXD patterns of thymine crystals:
——— Crystal I; ········ Crystal II

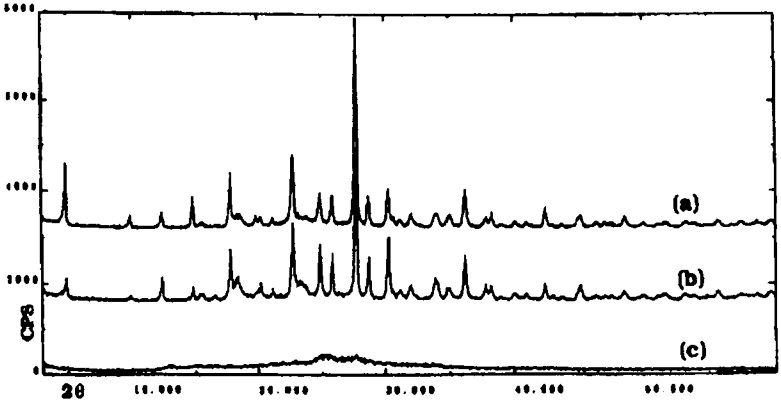


Figure 2. The PXD patterns of the mixed crystals of 5-bromouracil (6a) and *L*-tryptophan (7): (a) Crystal III; (b) Crystal V = Crystal VI; (c) Crystal IV.

Table 1. Photochemical reactions of Crystal I, Crystal II and frozen aqueous solutions of thymine.

Conditions	Yields of thymine photodimers (Relative to **2**)				Other products	
	2	**3**	**4**	**5**	**9**	**10**
Crystal I	–	–	–	–	–	–
Crystal II	1.0	0.2	0	0.7	+	–
Froz. soln. I	1.0	0	0	0	++	+
Froz. soln. II	1.0	0	0	0	+	+
Froz. soln. III	1.0	0	0	0	+	–

Table 1 footnote. Froz. soln. I: 8 mM aqueous solution frozen at –5°C and irradiated at –5°. Froz. soln. II: 8 mM aqueous solution frozen at –25°C and irradiated at –5°C. Froz. soln. III: 8 mM aqueous solution frozen at –25°C and irradiated at -25 °C.

Table 2. Photochemical reactions of mixed crystals prepared from 5-bromouracil and L-tryptophan

Substrate	Yields of photoproducts (%) **8**	 Uracil
Crystal III	4.3	1.6
Crystal IV	5.1	5.5
Crystal V	4.4	5.6
Crystal VI	5.2	4.3

Acknowledgement

This work was partly supported by a Grant-in-Aid for Scientific research from the Ministry of Education, Science and Culture, Japan.

References and Footnotes

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§ Abbreviations: HPLC, high performance liquid chromatography; FT-IR, Fourier-transformed infrared spectroscopy; PXD, powder X-ray diffractometry.

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