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X-Ray Diffraction Patterns and Crystal Structures of Riboflavin Tetrabutyrate

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The X-ray diffraction patterns of two polymorphic forms of riboflavin tetrabutyrate (RTB) powders were examined. The orange powder showed sharp peaks suggesting a well-crystallized material, while the yellow powder showed broad peaks suggesting a polymer-like material. By using the cell constants of RTB crystal, all peaks in the Debye-Sheller powder patterns of RTB were assigned. Increase of the ratio of H_2O in the recrystallization solvent (MeOH–H_2O) decreased the peak intensities of X-ray patterns corresponding to specific faces of RTB crystals, reflecting the direction of growth of RTB crystals in the presence of H_2O.

Keywords riboflavin tetrabutyrate; polymorph; single crystal; X-ray powder analysis; stacking; hydrogen bond; IR; ^{1}H-NMR

The conformation of riboflavin tetrabutyrate (RTB) in solution[1)] and in the solid state has been extensively studied in relation to that of flavin-adenine dinucleotide (FAD). RTB is widely used as a food additive[2)] and as a drug for treating hyperlipemia.[3)]

Two types of RTB, orange fine crystals and yellow powder, are available in the market. Both afforded essentially the same analytical results (elemental analyses, thin layer chromatography (TLC), and high performance liquid chromatography (HPLC)).[4)] The yellow RTB dissolved in MeOH gave an orange solution at high concentration.

Previously, one of the authors has prepared three types of RTB powders, orange, yellow, and brown, by changing the composition of MeOH–H_2O used for recrystallization[4)] or by heating RTB in isopropyl alcohol.[5)] Infrared (IR), electron spin resonance (ESR), reflection, emission, and X-ray diffraction spectra of these three types of RTB in the solid state were examined. We suggested that the difference in color of these RTB powders seems to arise from differences in the mode of binding in the solid state.[4,5)] The X-ray diffraction pattern of the orange RTB powder appeared to be that of well-crystallized material, while those of the yellow and brown RTB powders were suggestive of polymer-like materials.[4,5)]

Since the crystal structure of RTB is not available at present, we have estimated the structure of RTB on the basis of X-ray powder analysis, IR, and nuclear magnetic resonance (NMR) spectra of RTB in connection with the colors.

Results and Discussion

X-Ray Powder Patterns of RTB Crystallized from MeOH–H_2O Solvents RTB was prepared from riboflavin, *n*-butyric acid, and $POCl_3$ in pyridine and recrystallized from MeOH as transparent red crystals (plates). As the ratio of H_2O content to MeOH was increased in the recrystallization procedure, the color of RTB powders changed from orange to yellow. A yellow powder of RTB was obtained by pouring an MeOH solution of RTB directly into H_2O. A series of RTB samples thus obtained was subjected to X-ray diffraction analyses. X-Ray diffraction patterns of RTB from MeOH : H_2O, (a) 85 : 15; (b) 70 : 30; (c) 55 : 45 and (d) 45 : 55, are shown in Fig. 1.

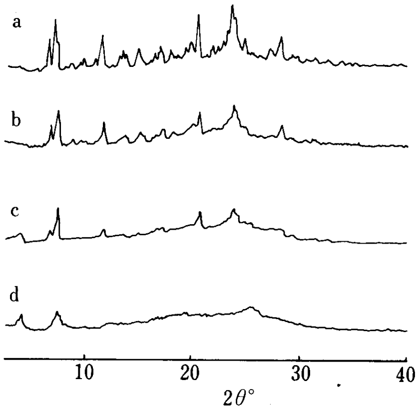


Fig. 1. X-Ray Diffraction Patterns of RTB Powders

a, recrystallized from MeOH–H_2O (85 : 15, v/v); b, recrystallized from MeOH–H_2O (70 : 30, v/v); c, recrystallized from MeOH–H_2O (55 : 45, v/v); d, recrystallized from MeOH–H_2O (45 : 55, v/v).

As can be seen from Fig. 1a and c, increase in the H_2O content in MeOH–H_2O mixture used for recrystallization resulted in a decrease in the intensities of sharp peaks of the orange RTB powder. In the case of d in Fig. 1 (yellow RTB powder), almost all the peaks had disappeared and a broad halo and two new peaks at lower angle appeared. These results suggest that increase in the ratio of H_2O changes the well-crystallized structure into a loosely bound polymer-like yellow solid. For example, the peak intensities at 7.0°, 14.0°, 14.3°, 16.9°, and 21.1° shown in the diffraction pattern in Fig. 1a decreased to those in Fig. 1b. Therefore, we thought that the assignment of these peaks might give us some information about the direction of decay or growth of RTB crystals in the MeOH–H_2O solvent system. The cell constants of the RTB crystal, which are necessary to assign the Miller indices for each reflection, were determined using a single crystal.

Unit Cell Constants of RTB Crystal and Assignment of X-Ray Powder Patterns Crystal data of an RTB single crystal are as follows: triclinic, space group $P1$, $a=19.629$ Å, $b=15.453$ Å, $c=11.814$ Å, $\alpha=94.99°$, $\beta=91.76°$, $\gamma=83.86°$, volume$=3548.6$ Å^3, $Z=4$, $D_c=1.229$, $D_m=1.214$ g·cm^{-3} (floating method, glycerine/H_2O). Using these unit-cell parameters, theoretical values of reflection angles (2θ) were calculated for all possible combinations of Miller indices (h, k, l) from 0 to 5. Table I shows the calculated and the observed values of d.

Again, the peak intensities in Fig. 1a and b are compared. As can be seen from Table I, the peaks at 7.0°, 12.0°, 14.0°, 14.3°, and 21.1°, which remarkably decreased in intensi-

Table I. *dhkl* Millar Indeces of the Diffraction Peaks of Powder RTB

Observed d(Å)	I/I_0	h	k	l	Calcd d(Å)
12.62	60	1	$\bar{1}$	0	12.71
11.55	90	1	1	0	11.48
9.82	20	2	0	0	9.76
9.02	20	0	1	1	8.97
8.93	20	1	$\bar{1}$	1	8.89
8.84	25	2	$\bar{1}$	0	8.65
8.58	25	1	1	$\bar{1}$	8.55
7.85	25	2	1	0	7.86
7.37	65	1	$\bar{2}$	0	7.39
6.80	15	2	1	$\bar{1}$	6.73
6.51	30	1	$\bar{2}$	1	6.48
6.32	40	3	$\bar{1}$	0	6.23
6.23	30	0	2	1	6.18
6.21	30	1	2	$\bar{1}$	6.17
5.75	40	2	$\bar{2}$	1	5.73
5.37	20	0	1	2	5.34
5.34	20	2	2	$\bar{1}$	5.32
5.24	30	$\bar{1}$	1	2	5.23
5.09	30	3	1	1	5.08
5.06	45	2	2	1	5.01
4.82	35	1	$\bar{3}$	1	4.79
4.78	35	1	$\bar{2}$	2	4.78
4.64	25	1	3	$\bar{1}$	4.60
4.46	40	4	$\bar{1}$	$\bar{1}$	4.43
4.36	40	2	3	0	4.34
4.35	50	4	$\bar{2}$	0	4.32
4.21	100	2	3	$\bar{1}$	4.18
4.06	30	0	3	$\bar{2}$	4.03
3.99	40	2	3	1	3.97
3.95	45	0	0	3	3.92
3.88	45	1	0	$\bar{3}$	3.86
3.80	50	4	0	$\bar{2}$	3.80
3.74	70	0	1	3	3.73
3.69	100	4	2	1	3.66
3.63	90	2	0	3	3.61
3.57	45	0	4	1	3.55
3.52	60	4	1	2	3.51
3.51	60	5	$\bar{2}$	1	3.49
3.42	30	3	0	$\bar{3}$	3.39
3.22	35	3	$\bar{2}$	3	3.20
3.20	30	4	2	2	3.18
3.12	45	3	3	2	3.10
3.01	25	3	4	1	2.99
		0	3	3	
2.96	25	0	0	4	2.94
2.88	20	4	4	0	2.87
		2	4	2	
2.81	20	2	0	4	2.80

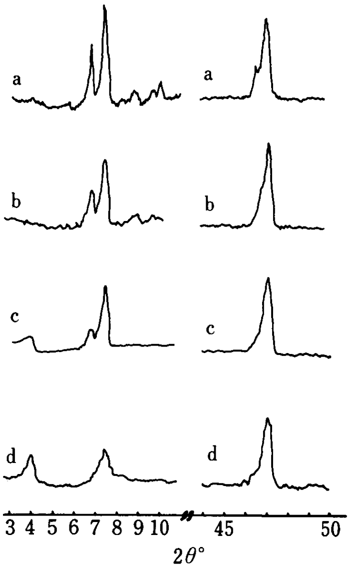


Fig. 2. X-Ray Diffraction of RTB at $2\theta = 7°$ with Powdery Silicone (10 wt%) as an Internal Standard

The letters have the same meaning as in Fig. 1. The peak at $2\theta = 47.3°$ is assigned to silicone used as an internal standard.

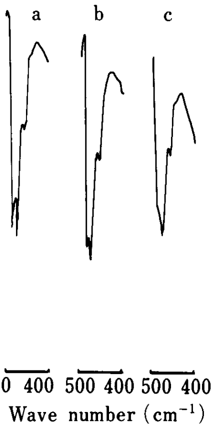


Fig. 3. IR Spectra of RTB Powder

The letters have the same meaning as in Fig. 1.

ty, are assigned to the crystal planes of $(1\bar{1}0)$, $(1\bar{2}0)$, $(3\bar{1}0)$, $(12\bar{1})$, and $(23\bar{1})$, respectively. Further, the addition of more H_2O to the recrystallization solvent decreases the peak intensities, as can be seen from Fig. 1a and d. From these results, it can be suggested that in an H_2O-rich solvent RTB crystals could not grow along these specific crystal planes. As shown in Fig. 1, the peak at the lowest $2\theta = 7.0°$, $(1\bar{1}0)$ decreased in intensity remarkably. The quantitative analysis of the diffraction peaks was carried out with powdery silicone as an internal standard. The results for RTB powders from MeOH–H_2O (a) 85 : 15 (sample a), (b) 70 : 30 (sample b), (c) 55 : 45 (sample c), and (d) 45 : 55 (sample d) are illustrated in Fig. 2. The peak at $2\theta = 47.3°$ shown in Fig. 2 is assigned to silicone used as an internal standard. The peak intensity at $2\theta = 7.0°$ of sample b was about 45% of that of sample a, while the relative peak intensities at $2\theta = 7.65°$ and 12.0° are 67% and 58%, respectively. With increase in the ratio of H_2O to MeOH, as can be seen from Fig. 1b and c, the peak at $2\theta = 7.0°$ disappeared. Therefore, we can propose that growth along the $(1\bar{1}0)$ crystal plane is strongly inhibited with increase in the ratio of H_2O in the solvent used for the recrystallization. This is in accord with the IR and ^{1}H-NMR data reported previously.[4,6)]

IR spectra of RTB powders recrystallized from MeOH–H_2O in various ratios are shown in Fig. 3. The intensity of the spectral band at 465 cm^{-1}, which is assigned to hydrogen bonding between the isoalloxazine groups of RTB, was decreased by the addition of H_2O. The ^{1}H-NMR spectra of sample a are shown in Fig. 4. This figure shows that the proton chemical shift values of the 6 and 9 positions (see Chart 1) on the isoalloxazine group are 468.6 and 463.8 Hz for 0.1 M MeOH solution and are shifted to 472.8 and 462.6 Hz for MeOH–H_2O (80 : 20) solution. These changes are considered to reflect the separation between stacked molecules.[6)]

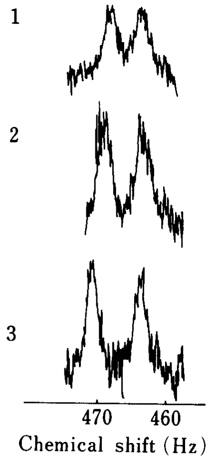
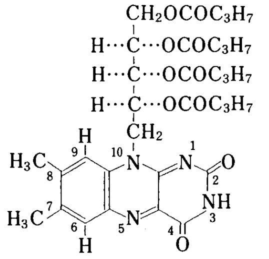


Fig. 4. ^{1}H-NMR Spectra of 0.1 M RTB in MeOH–H_2O

1, MeOH–H_2O (100 : 0); 2, MeOH–H_2O (92 : 8, v/v); 3, MeOH–H_2O (80 : 20, v/v).



riboflavin tetrabutylate (RTB)

Chart 1

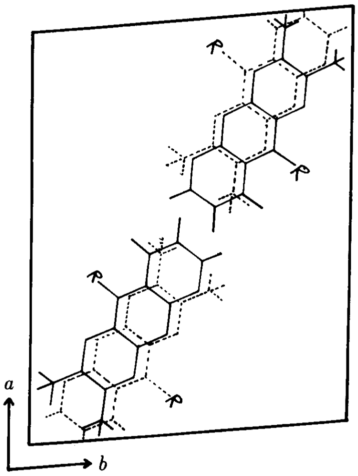


Chart 2

Therefore, the decrease in X-ray reflection intensity at $(1\bar{1}0)$ plane seems to accompany the breakage of hydrogen bonds and the separation of stacked RTB molecules. The peak at $2\theta=4.1°$ shown in c or d of Fig. 1 or 2, which appeared at high ratios of H_2O to MeOH, was considered to be due to another crystal form (polymorph) of yellow RTB.

From these results, a possible packing mode of the RTB crystal having four molecules in the unit cell is postulated in Chart 2. Two RTB molecules are associated with each other by hydrogen bonds, forming a coplanar arrangement and two other molecules of RTB are stacked on them. In the presence of H_2O, the hydrogen bonding and the stacking are presumed to be broken to give a sheet-like structure.

Table II. Melting Point (mp) and Elemental Analysis of RTB Powder Recrystallized from Mixed Solvent

MeOH–H_2O (v/v)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N
		$C_{33}H_{44}N_4O_{10}$	60.35	6.75	8.53
85 : 15	149.2		(60.29	6.80	8.67)
70 : 30	149.4		(60.30	6.80	8.60)
55 : 45	149.3		(60.32	6.78	8.50)
45 : 55	149.5		(60.14	6.74	8.71)

Experimental

IR spectra were recorded with a Hitachi 260-50 spectrophotometer. KBr tablets were prepared by mixing RTB powder (2 mg) and KBr (200 mg) and compressing the mixture. ^{1}H-NMR spectra were measured on a Hitachi R-24B spectrometer. Chemical shifts are given in hertz (Hz) with tetramethylsilane as an internal standard.

Preparation of Crude RTB According to the previous paper,[5] a mixture of *n*-butyric acid (100 g), pyridine (120 g), $POCl_3$ (20 g) and riboflavin (22 g) was stirred at 70—75 °C for 5 h. After the removal of the solvent by evaporation, the residue was dissolved in $CHCl_3$ and the solution was dried at 20 °C over anhydrous Na_2SO_4. The $CHCl_3$ layer was evaporated. The residue was recrystallized from MeOH–H_2O (85 : 15). Crude RTB was filtered off and dried at 60 °C for 7 h *in vacuo*. mp 149 °C. *Anal.* Calcd for $C_{33}H_{44}N_4O_{10}$ (RTB): C, 60.35; H, 6.75; N, 8.53. Found: C, 60.23; H, 6.85; N, 8.78.

Recrystallization of RTB Powder from MeOH–H_2O Mixed Solvents The crude RTB (5 g) was recrystallized from various compositions of MeOH–H_2O mixed solvent. The fine crystals were filtered off and dried at 20—60 °C for 2 d *in vacuo*. Results are shown in Table II.

Preparation of RTB Single Crystal The fine RTB crystals obtained from (a) (5 g) were dissolved in EtOH–H_2O (85 : 15, 80 ml). After standing at 20°C for several weeks, single crystals suitable for X-ray analysis were obtained.

X-Ray Powder Diffraction Analysis Debye-Sheller patterns of RTB powder were measured on an X-ray diffractometer (Geigerflex D-8C, Rigaku Co.) with Ni-filtered Cu K_α radiation. Conditions: voltage, 30 kV; current, 20 mA; time constant, 1 s; scan speed, 2° min; divergency, 1°, receiving slit, 0.4 mm; and detector, GMC. The diffracted peaks were measured from 60° to 5°. Relative peak intensity of RTB powder was measured with powdery silicone (10 wt %) as an internal standard.

Millar Indices of the Diffracted Peaks of Powder RTB Using a single crystal of RTB, the unit-cell parameters were determined by an automated four-circle X-ray diffractometer (Rigaku AFC-5) with graphite-monochromated Cu K_α radiation. The parameters were refined by the least squares method using 2θ angles for 25 reflections ($30°<2\theta<60°$). The interplanar spacing d of a series of $(h\,k\,l)$ indices in a triclinic lattice was calculated according to the following equation.

$$dhkl=\frac{b_0}{\sqrt{\dfrac{h/a\begin{vmatrix}h/a & \cos\gamma & \cos\beta\\ k & 1 & \cos\alpha\\ l/c & \cos\alpha & 1\end{vmatrix}+k\begin{vmatrix}1 & h/a & \cos\beta\\ \cos\gamma & k & \cos\alpha\\ \cos\beta & l/c & 1\end{vmatrix}+l/c\begin{vmatrix}1 & \cos\gamma & h/a\\ \cos\gamma & 1 & k\\ \cos\beta & \cos\alpha & l/c\end{vmatrix}}{\begin{vmatrix}1 & \cos\gamma & \cos\beta\\ \cos\gamma & 1 & \cos\alpha\\ \cos\beta & \cos\alpha & 1\end{vmatrix}}}}$$

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