Chem. Pharm. Bull.
30(10)3685—3694(1982)

Studies on the Characteristics of Carbochromen Hydrochloride Crystals. I. Polymorphism and Behavior of Water of Crystallization

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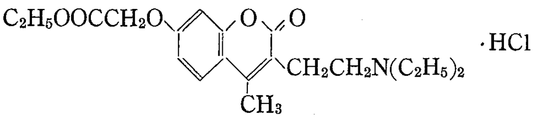
(Received August 24, 1981)

To clarify the cause of cracking of carbochromen hydrochloride tablets on exposure to moisture, crystal modifications of the drug were investigated in detail. At least two hydrate forms (I and II) and three anhydrate forms (I′, II′ and III) were found. Analyses of X-ray powder diffraction patterns, infrared spectra, the activation energy of dehydration, and other data showed that water of crystallization contained in forms I and II has different roles in the crystals. Transformation from form I to form I′ or *vice versa* produced a mosaic structure of the crystals, whereas mutual transformation between form II and form II′ did not affect the appearance of the crystal, only causing contraction or expansion of the crystal in a certain direction. These differences are believed to be closely related to phenomena occurring in tablets prepared from these crystal forms.

Keywords——carbochromen hydrochloride; cracking of tablet; water of crystallization; X-ray powder diffraction; IR spectra

Carbochromen hydrochloride [7-(ethoxycarbonylmethoxy)-3-(2-diethylaminoethyl)-4-methylcoumarin hydrochloride], hereafter abbreviated as Carb·HCl, has been clinically used as a coronary vasodilator in the treatment of angina pectoris, myocardial infarction and coronary thrombosis. It is obtained in the form of colorless crystals with a melting point of 159°C to 161°C, and has been sold as a soft capsule preparation for clinical use. One of the reasons for this may be that Carb·HCl tablets prepared by the wet granulation method tend to crack on exposure to moisture. The susceptibility to cracking clearly differs among different lots. By comparative examination of the physical and chemical characteristics of both cracked and non-cracked tablets, we found that the difference resides in the crystal form of Carb·HCl contained in the tablet.[1] This finding was confirmed by an experiment described in the following paper,[2] in which tablets were formed with various ratios of two different crystal forms of Carb·HCl and their cracking was observed for one month.



Several reports have been published with respect to the influence of the crystal form on some properties of the tablet.[3] In particular, the content of water of crystallization is often a critical factor. However, no report has yet appeared on the relationship between the cracking phenomenon of the tablet and the crystal form contained therein. Thus, to investigate why the difference of Carb·HCl crystal form affects cracking, we studied the polymorphism of Carb·HCl crystals.

Experimental

1) **Preparation of Crystal Forms**——As starting material, Carb·HCl from Cassela Co., Ltd. was used. Basic carbochromen (Carb) was obtained by neutralization of an aqueous Carb·HCl solution, followed by extraction with Et_2O.

Form I: Carb·HCl, 2 g, was dissolved in 2 ml of H_2O at 30°C and the solution was cooled to 5°C to afford needles of trihydrate (1.2 g, form I). *Anal.* Calcd for $C_{20}H_{27}NO_5 \cdot HCl \cdot 3H_2O$: C, 53.15; H, 7.58; N, 3.10; water, 11.96. Found: C, 53.63; H, 7.46; N, 3.11; water, 12.0 (determined by thermogravimetry).

Form I′: Crystals were prepared by the follwing three methods. The products obtained were identified by their infrared (IR) spectra and X-ray powder diffraction patterns.

Method A: A solution of Carb·HCl (10 g) in isopropyl alcohol (100 ml) was cooled with ice to yield 8 g of form I′. *Anal.* Calcd for $C_{20}H_{27}NO_5 \cdot HCl$: C, 60.37; H, 7.09; N, 3.52. Found: C, 60.40; H, 6.99; N, 3.65.

Method B: Form I was dried at 23°C under reduced pressure (2 mmHg) for 2 h.

Method C: Dry HCl gas was passed through a solution of Carb in 100 ml of dry Et_2O. The mixture was cooled to 5°C for 2 h. Fine crystals that precipitated were collected by filtration and dried, giving 2 g of form I′.

Form II: A solution of Carb·HCl (6 g) in 2 ml of water, prepared at 60°C, was cooled to room temperature, affording dihydrate prisms (3 g). *Anal.* Calcd for $C_{20}H_{27}NO_5 \cdot HCl \cdot 2H_2O$: C, 55.36; H, 7.43; N, 3.22; water 8.30. Found: C, 55.67; H, 7.41; N, 3.15; water, 7.8 (determined by thermogravimetry).

Form II′: Form II was dried by heating it gradually up to 40°C under reduced pressure, 0.2 mmHg, for 5 h to give form II′.

Form III: Carb·HCl (2 g) was dissolved in MeOH (1 ml) or in dioxane (1 ml) at 60°C and the solution was cooled to room temperature to obtain solvates. Solvates showed crystal forms different from form III and other forms described above by X-ray analysis. These solvates were collected and dried *in vacuo* at 25°C to give form III. *Anal.* Calcd for $C_{20}H_{27}NO_5 \cdot HCl$: C, 60.37; H, 7.09; N, 3.52. Found: C, 60.94; H, 6.98; N, 3.51.

2) **X-Ray Analysis**——Powder diffraction patterns were recorded using a Rigakudenki RU-3 X-ray diffractometer with CuK_α radiation (40 kV, 80 mA). Unit cell dimensions and the space group were determined from oscillation and Weissenberg photographs, which were taken on a Nenius Weissenberg goniometer (57.3 mmϕ) with CuK_α radiation (40 kV, 40 mA).

3) **Infrared (IR) Spectroscopy**——IR spectra were recorded as nujol mulls on a Hitachi 215 infrared spectrophotometer.

4) **Thermal Analysis**——Differential scanning calorimetry (DSC) was carried out with a Perkin Elmer DSC-1B calorimeter in a dry nitrogen atmosphere at the scanning rate of 2.5°C/min. In the case of hydrate crystals, holes were made in the sample pan cover to facilitate the evaporation of water of crystallization. Thermogravimetry (TG) was conducted with a Perkin Elmer TGA-1 thermobalance in the dry state or under a relative fumidity (RH) of 48% in a nitrogen atmosphere[4] at the scanning rate used for DSC. In the determination of the activation energy for dehydration, a slower scanning rate (1.25°C/min) was chosen. At scanning rates faster than 2.5°C/min, form I was found to be transformed to form II′ by X-ray analysis.

5) **Measurement of Moisture Equilibrium**——Precisely weighed samples of about 300 mg in weighing bottles were stored for one month under controlled relative humidities utilizing the saturated salt solutions given in Table I. The weight change was measured until equilibrium was attained.

TABLE I. Relative Humidities over Saturated Solutions of Various Salts

Relative humidity (%)		Salt
at 25 °C	at 40 °C	
0	0	P_2O_5
22	20	$CH_3COOK \cdot 1.5H_2O$
33	31	$MgCl_2 \cdot 6H_2O$
44	43	$K_2CO_3 \cdot 2H_2O$
51	46	$Ca(NO_3)_2$
57	53	$NaBr \cdot 2H_2O$
68	68	$CuCl_2 \cdot 2H_2O$
75	75	NaCl
85	85	KCl
93	91	$(NH_4)H_2PO_4$

6) **Microscopic Analysis**——Optical microscopy was carried out with a No. 51692 apparatus (Union Co.). Each crystal form was kept in a sealed sample tube containing $CaCl_2$ or a saturated solution of NaCl at 25°C in order to observe the structural change accompanying the hydration or dehydration process. Each sample was observed periodically to follow the change. Electron microscopy was done with a JEM-6 instrument (JEOL). Carbon replicas were prepared by the conventional method.

7) **Measurement of the Rates of Hydration and Dehydration**——A CAHN electrobalance was used. The sample (100 mg) was kept at 25°C, RH 75% or 11%. The weight change was measured at appropriate intervals.

Results and Discussion

Physicochemical Properties of Each Crystal Form

Crystalline Carb·HCl was found in at least five crystal forms, two hydrated and three anhydrous. Elemental analysis and thermogravimetry indicated that form I was a trihydrate, form II a dihydrate and forms I′ and III anhydrates. Form II′ was also considered to be an anhydrate since it was produced from form II by the loss of two mol equivalents of water on drying. Differences among these samples in crystal form were clearly indicated by their X-ray powder diffraction patterns, shown in Fig. 1. The unit cell constants of the single crystal of form II are shown in Table II.

In the IR spectra (Fig. 2), absorption bands due to ν_{OH} were observed at 3300 cm^{-1} in the case of the hydrates, forms I and II, while they were lacking in the anhydrous forms.

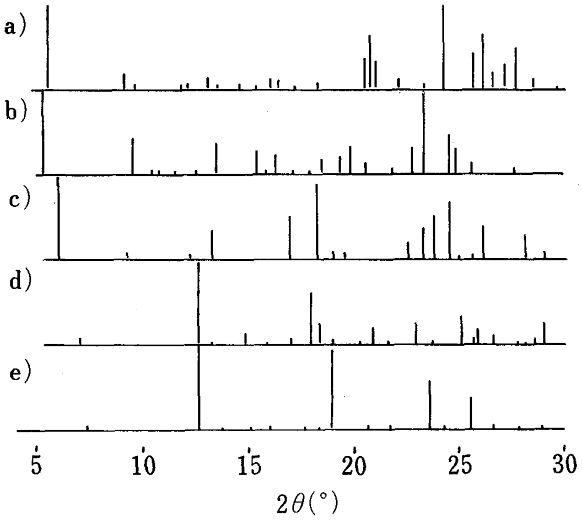


Fig. 1. X-Ray Powder Diffraction Patterns of Various Crystal Forms of Carbochromen Hydrochloride

a) form I, b) form I′, c) form III, d) form II, e) form II′.

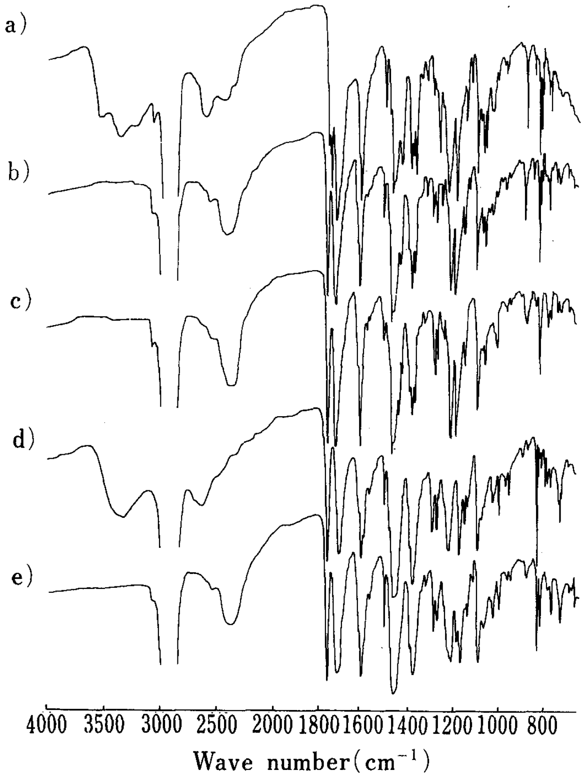


Fig. 2. Infrared Spectra of Various Forms of Carbochromen Hydochloride

a) form I, b) form I′, c) form III, d) form II, e) form II′.

TABLE II. Crystal Data for Form II of Carbochromen Hydrochloride

Crystal system	Monoclinic
a-axis	29.4 Å
b-axis	6.4 Å
c-axis	14.2 Å
β	122.8°
Unit cell volume	2351.2 Å^3
Number of molecules in unit cell	4
Space group	$P2_1/C$
Systematic absences	$h0l: l=2n+1$ $0k0: k=2n+1$

Ammonium bands were located at 2600 cm^{-1} in the hydrates and at 2350 cm^{-1} in the anhydrates. Both ν_{OH} and ammonium bands of form I were split into two or three peaks while those of form II appeared as single broad bands. This difference suggests different association modes of water molecules in the crystal lattices of forms I and II. The absorption due to $\nu_{C=O}$ of the lactone appears at a lower wave number in the IR spectra of forms II and II′ (1710 cm^{-1}) than in those of forms I and I′. Other characteristic bands were rather similar in all five crystal forms. Thus, the ν_{C-O} of the ester, ether and lactone groups overlapped at around 1300 to 1000 cm^{-1}, the ν_{N-C} appeared at around 1100 cm^{-1} and the out-of-plane C–H vibrations of the 1,3,4-trisubstituted benzene ring appeared at 880 cm^{-1} and 820 to 800 cm^{-1}. These data further suggest a difference in the hydrogen bonding in the molecular construction of the crystal structure.

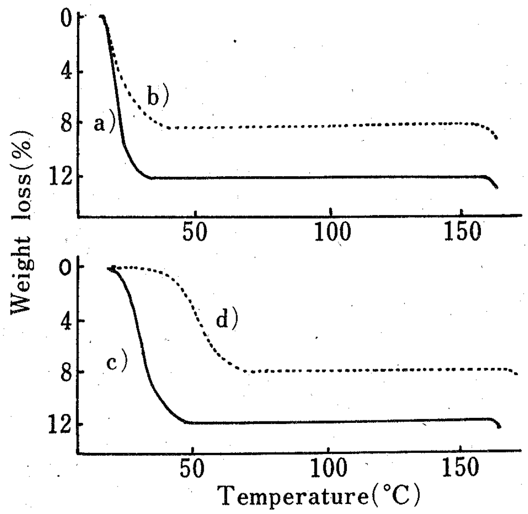


Fig. 3. TG Curves of Two Hydrates of Carbochromen Hydrochloride

a) form I (the heating cell was cooled with dry ice before heating), b) form II. The measurement was carried out in a stream of dry N_2 gas (heating rate: 2.5°C/min), c) form I, d) form II. The measurement was carried out in an N_2 gas stream of RH 48 % (heating rate: 2.5°C/min).

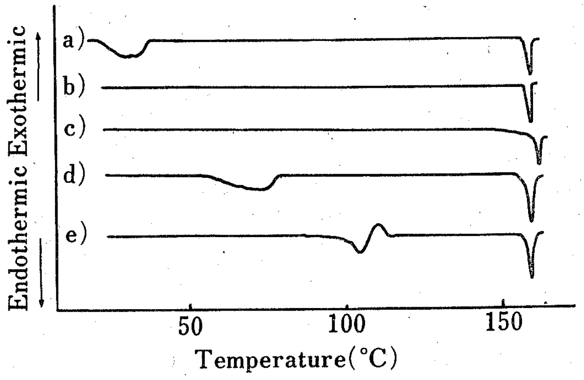


Fig. 4. DSC Curves of Various Crystal Forms of Carbochromen Hydrochloride

a) form I (the heating cell was cooled with dry ice before heating), b) form I′, c) form III, d) form II, e) form II′.

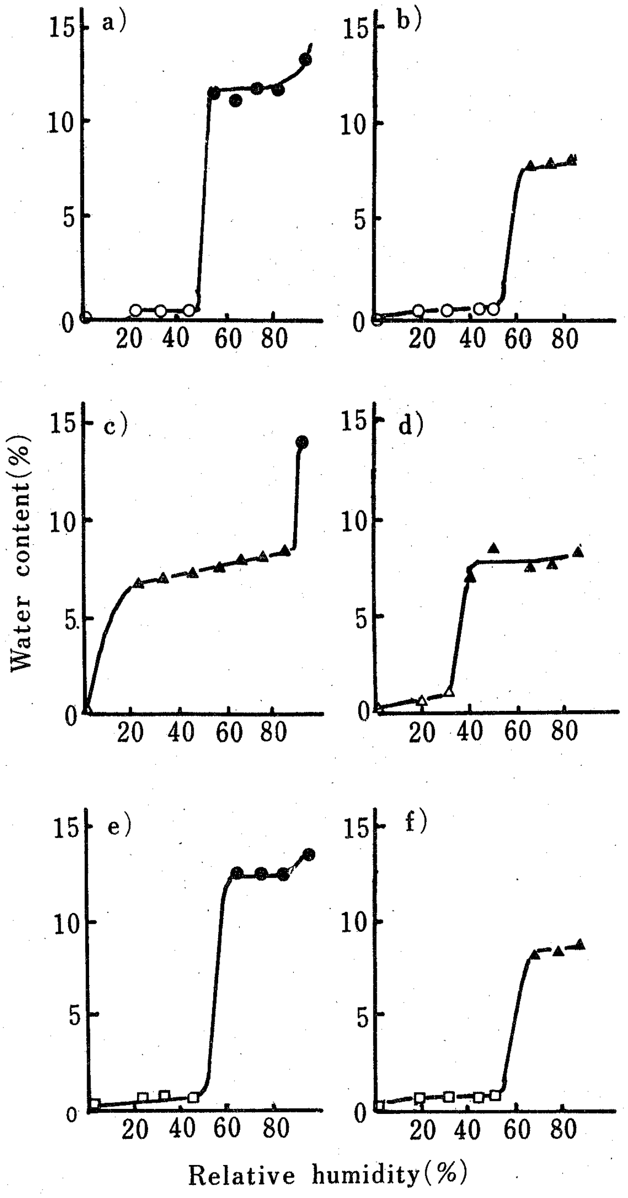


Fig. 5. Moisture Equilibrium Study of Carbochromen Hydrochloride

Starting material: a) form I′ (25°C), b) form I′ (40°C), c) form II′ (25°C), d) form II′ (40°C), e) form III (25°C), f) form III (40°C). ○, form I′; ●, form I; △, form II′; ▲, form II; □, form III.

Phase Transformation

Form I was transformed into form I′ in a stream of dry nitrogen at room temperature by losing 3 mol of water (Fig. 3a). Two endothermic peaks of form I at 30 and 160°C in DSC correspond to this transformation and to fusion, respectively (Fig. 4a). Similar loss of water was seen under RH 48% (Fig. 3c). Form II also released its water molecules and was transformed into form II′ in a stream of dry or RH 48% nitrogen at room temperature or at temperatures between 50 and 70°C, respectively (Figs. 3b and 3d). However, form II was found to be transformed into form I′ in DSC, probably due to the prevention of water diffusion by the pan cover, even in a stream of dry nitrogen (Fig. 4d). These results were confirmed by X-ray analysis. Endothermic and exothermic peaks appearing in succession between 100 and 115°C in DSC of form II′ indicate the transformation into form I′ (Fig. 4e). Form III gave a straight line up to its fusion peak at 162°C (Fig. 4c). Above the melting temperature, each form of Carb·HCl showed weight loss due to decomposition (Fig. 3).

Figure 5 shows the summarized results of moisture equilibrium studies at 25 and 40°C under controlled relative humidities. At 25°C, form I′ absorbed 3 mol of water under RH higher than 51% and was transformed into form I, which gave form I′ in a reversible manner on dehydration under RH lower than 44% . Form II′ was converted into form II under RH 22 to 85%, and this in turn reverted to form II′ by dehydration under RH about 0%. Under RH 91%, form II was transformed into form I by further hydration. Form III was stable under RH below 44% but changed into form I under RH 57 to 93%. At 40°C, form II′ changed into form II under RH higher than 43%, while forms I′ and III also changed into form II under RH higher than 68%. In this case, form I′ transformed to form I within 10 min, then gradually to form II. This process was confirmed by the changes of X-ray diffraction patterns. The mutual transformations among these crystal forms at various temperatures and humidities are schematically illustrated in Fig. 6.

Properties of Water of Crystallization in Each Crystal Form

At 25°C and RH 75%, form II′ was transformed into form II within 1 h. Under the same conditions the transformation of form I′ into form I took more than 10 h. Under RH 11%,

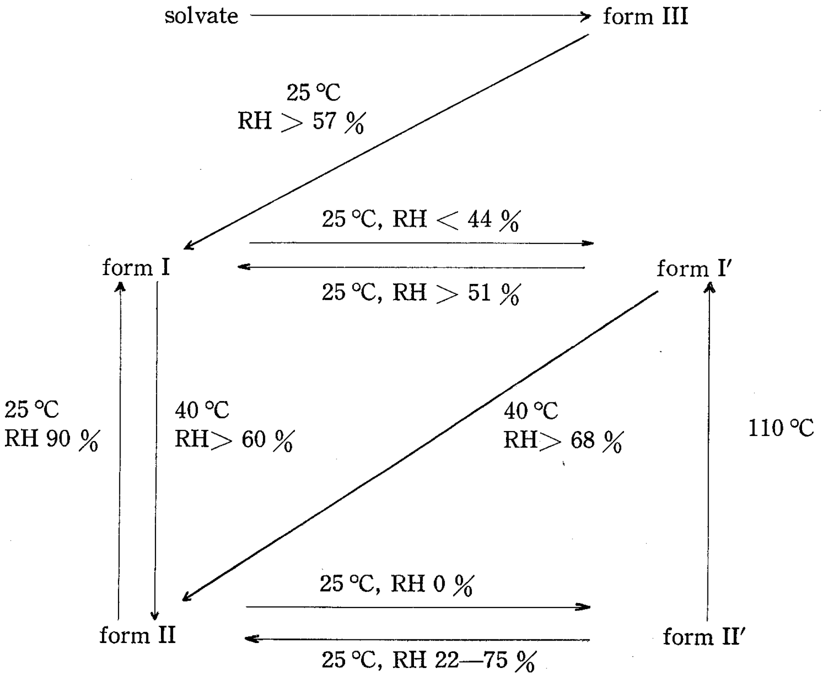


Fig. 6. Schematic Diagram of Mutual Transformations of Crystals of Carbochromen Hydrochloride

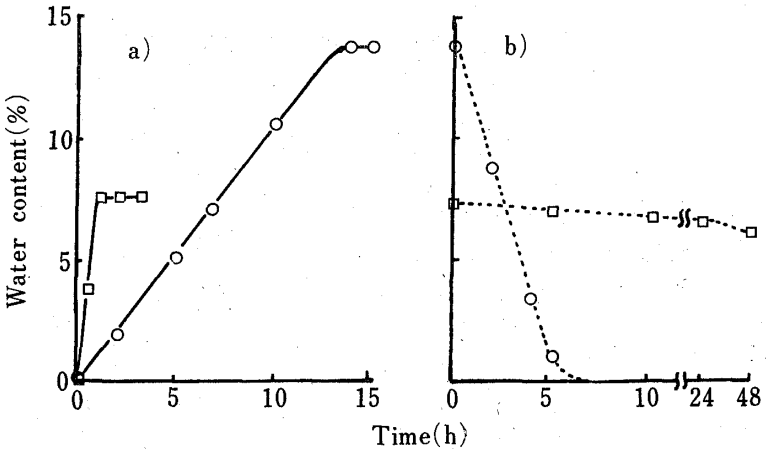


Fig. 7. Hydration and Dehydration Rates of Crystals of Carbochromen Hydrochloride

a) Hydration at RH 75%, 25°C; —○—, form I′; —□—, form II′.
b) Dehydration at RH 11%, 25°C; ···○···, form I; ···□···, form II.

form II retained most of its water after 48 h, whereas form I was dehydrated almost completely to the anhydrate in 6 h (Fig. 7).

The activation energies for the dehydration of forms I and II were estimated from the results of the thermogravimetric analyses according to the Freeman–Carroll method (eq. 1).[5)]

$$\frac{\frac{-E}{2.3R}\cdot\Delta\left(\frac{1}{T}\right)}{\Delta\log W_r} = -n + \frac{\Delta\log dw/dt}{\Delta\log W_r} \qquad \text{eq. 1}$$

E: activation energy
R: gas constant
T: absolute temperature
W_r: residual weight loss
$\frac{dw}{dt}$: rate of change in sample weight
n: order of reaction

The results are shown in Table III and Fig. 8. The activation energy for the dehydration of form I was similar in both dry and RH 48% atmospheres, while that of form II under RH 48% was twice as large as that in dry nitrogen. These results together with those described in the preceding paragraph indicate that in the case of form II, the mobility of the water of crystallization depends on the environmental humidity. As the cracking phenomenon of Carb·HCl tablets was believed to be related to the transformation of crystal forms on hydration and dehydration,[2)] changes of the crystal shape accompanying the transformation were carefully examined microscopically. On dehydration of form I, slight shrinkage along the short axis (Fig. 9b) was observed as well as mosaic formation all over the crystal surface (Fig. 9b).

Table III. Activation Energy of Dehydration of Carbochromen Hydrochloride Forms I and II

Condition	Activation energy (kcal/ml)	
	Form I	Form II
In dry N_2 gas	47.5	27.4
In wet N_2 gas (RH 48%)	50.8	50.8

Reabsorption of water led to recovery of the whole crystal size but the mosaic appearance remained unchanged (Fig. 9c). The X-ray diffraction patterns of form I′ obtained by dehydration of form I showed broad lines as compared with those of the same form directly obtained by recrystallization. The broadening reflects the mosaic formation described above. Hydra-

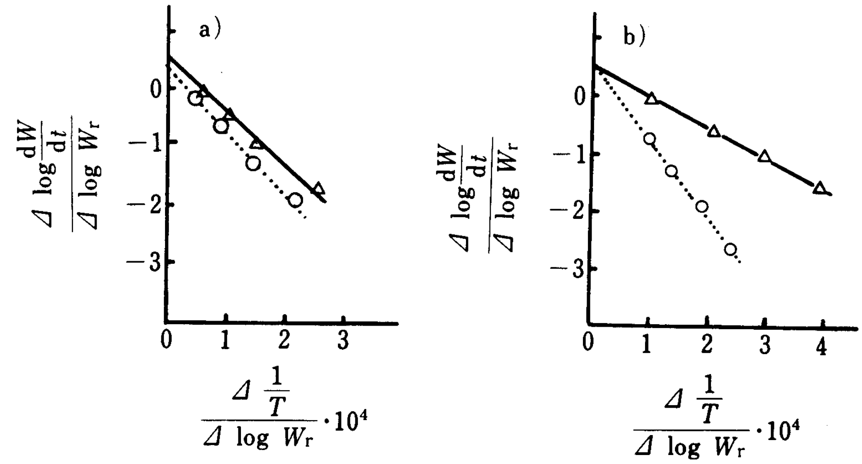


Fig. 8. Freeman–Carroll Plots for Dehydration of Hydrates of Carbochromen Hydrochloride

a) form I; b) form II; △, in dry N_2 gas; ○, in N_2 gas of RH 48% $W_r = W_c - W$, W; total weight loss up to time t, W_c; weight loss at completion of reaction.

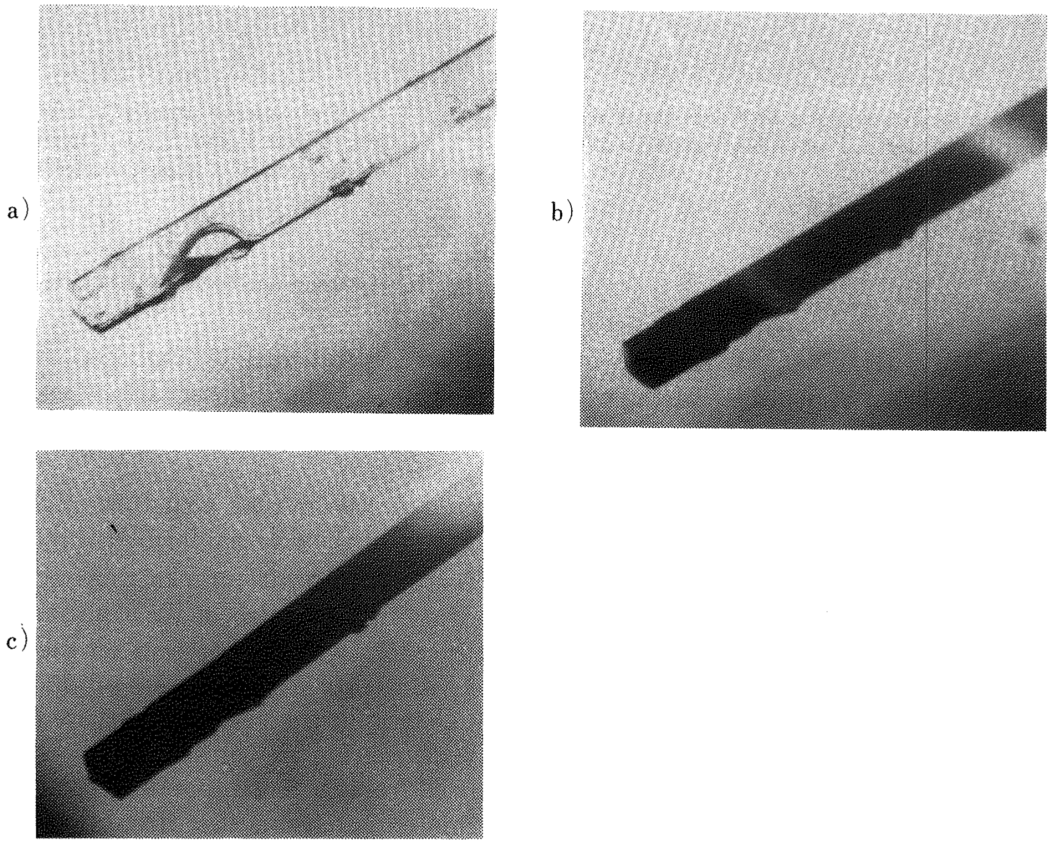


Fig. 9. Microphotographs of Carbochromen Hydrochloride Form I at Various Stages of Dehydration and Moisture Reabsorption (×80)

a) initial stage.
b) after dehydration at RH 0% for 10 h.
c) after moisture reabsorption at RH 75% for 10 h.

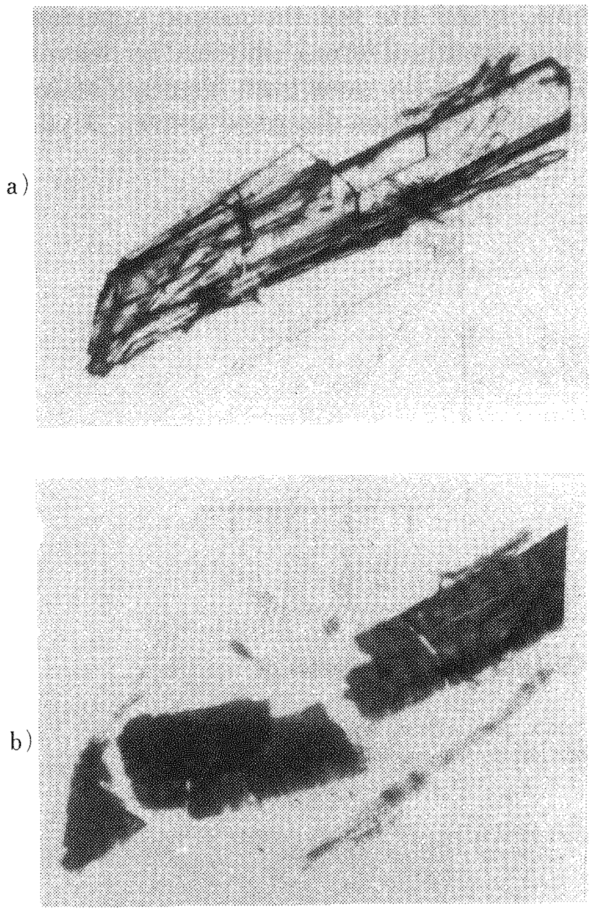


Fig. 10. Photomicrographs of Carbochromen Hydrochloride Form I′ upon Hydration (×40)

a) initial stage.
b) after hydration at RH 75% for 10 h.

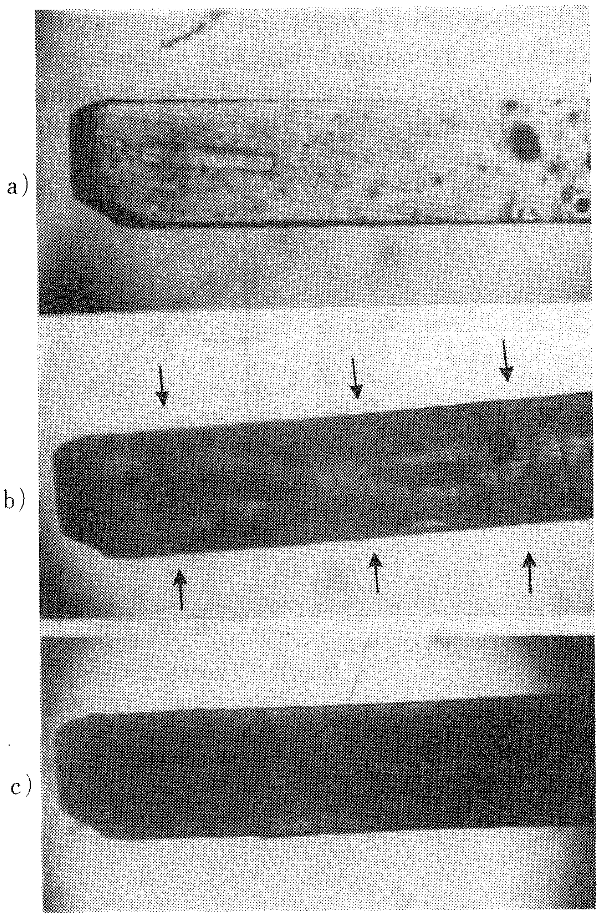


Fig. 11. Photomicrographs of Carbochromen Hydrochloride Form II at Various Stages in Dehydration and Moisture Reabsorption (×70)

a) initial.
b) after dehydration at RH 0% for 10 h.
c) after moisture reabsorption at RH 75% for 10 h.

tion, at 25°C and RH 75%, of form I′ obtained by recrystallization (Fig. 10a) also caused cracking and mosaic appearance of the crystal (Fig. 10b). These observations suggest that a significant alteration in the crystal structure took place in the course of the interconversion between forms I and I′.

In contrast, dehydration of form II did not produce the mosaic structure on the crystal and reabsorption of water under RH 75% led to recovery of the original form II (Fig. 11).

These changes were studied by electron microscopy. Minute cracks were observed on the surface of form I′ crystals obtained by dehydration. The size of each mosaic cell was estimated to be less than 10 μm (Fig. 12a, b). Such cracks were not present on the crystal of form II (Fig. 12d). Parallel lines in Fig. 12d merely illustrate steps of layers produced crystal growth. Thus, there seemed to be no remarkable change in the crystal structure during the conversion from form II to form II′. The X-ray diffraction pattern of form II′ may be explained as being composed of lines which had shifted from the corresponding positions in the X-ray pattern of form II (Fig. 13). Based on this explanation, the diffraction lines were indexed as shown in Table IV using the unit cell dimensions of form II (Table II). From the Table, the *a*-axis of form II′ was calculated to be contracted by 4.7%.

The evidence obtained by the various techniques shows a clear difference in the character of the water of crystallization of forms I and II. The former seems to play a role as a bridge for the construction of the molecular arrangement in the crystal, whereas the latter seems to be a kind of clathrate. This difference may affect the behavior of Carb·HCl tablet on storage in an ordinary atmosphere, as further discussed in the following paper.

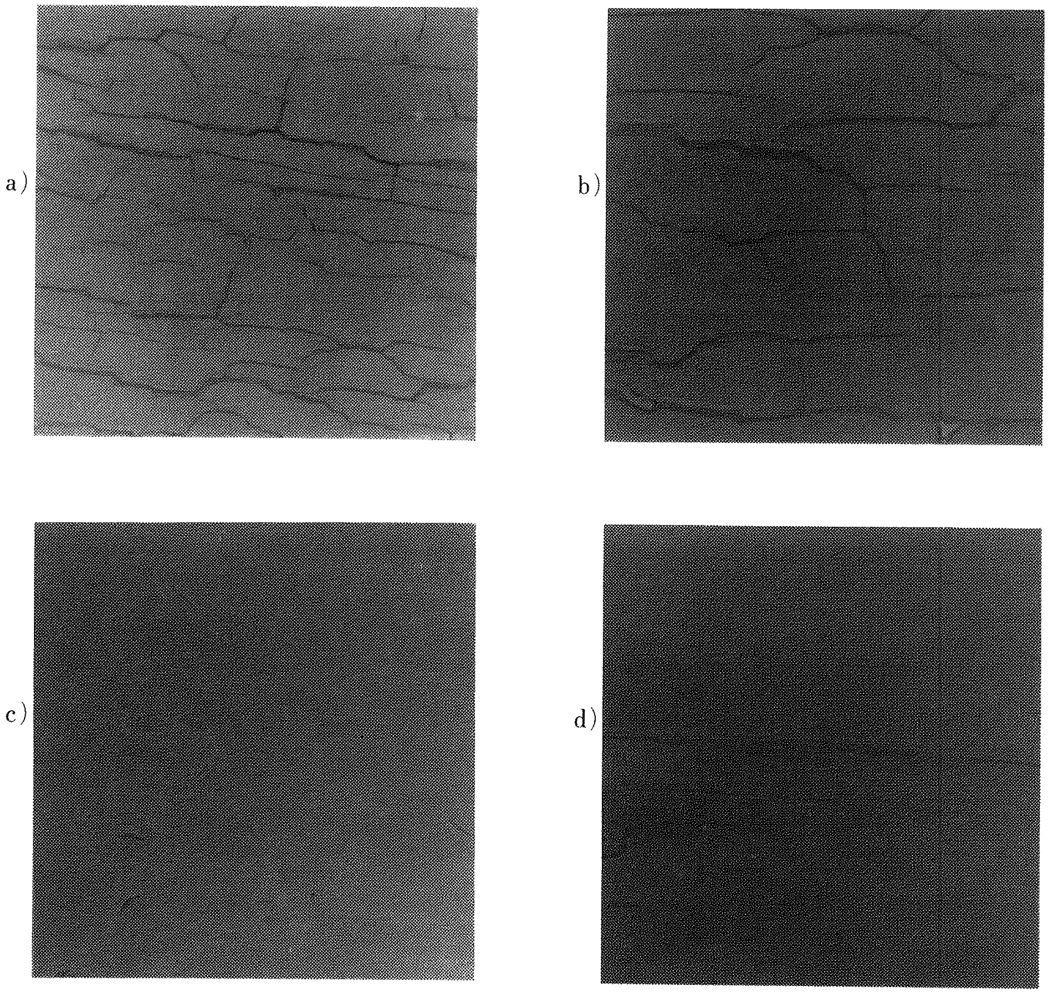


Fig. 12. Electron Microscopic Photographs of Carbochromen Hydrochloride

a, b) microstructure of form I′ produced by dehydration of form I, a) ×6000, b) ×30000.
c) surface of form I′ produced by recrystallization (×6000).
d) layer along the *b*-axis of form II′ produced by dehydration of form II (×30000).

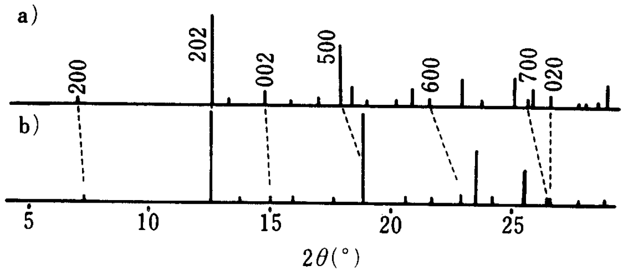


Fig. 13. Correspondence of the X-Ray Diffraction Patterns of Forms II′ and II

a) form II, b) form II′.

TABLE IV. Comparison of Corresponding *d*-Values of Forms II and II′

hkl	Form II (Å)	Form II′ (Å)	Difference (%)
200	12.45 (12.4)	11.86	4.7
202	7.132 (7.1)	6.964	2.5
002	5.969 (6.0)	5.862	2.8
500	4.870 (4.9)	4.667	4.7
600	4.073 (4.1)	3.881	4.7
700	3.490 (3.5)	3.326	4.7
020	3.323 (3.2)	3.325	—

( ): calculated from the cell dimensions.

Acknowledgement The authors thank Drs. E. Ohmura, H. Mima and M. Nishikawa for their helpful advice and encouragement.

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