Chem. Pharm. Bull. 28(2) 372—378 (1980)

Study of Crystalline Drugs by Means of Polarizing Microscope. III. Key Refractive Indices of Some Crystalline Drugs and Their Measurement using an Improved Immersion Method[1]

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(Received November 13, 1978)

When measurement of the refractive index of crystals is used for the identification of a drug or polymorphic form, it is more convenient to determine the "key refractive index" than the principal refractive index. Two characteristic key refractive indices can be measured for crystals in plate, bladed, or lamellar form, but for acicular or long prismatic crystals only one key refractive index can usually be measured along the direction of elongation when the crystals show parallel extinction.

Key refractive indices were measured for several drugs listed in J.P.IX. The relation between the key refractive indices and crystal symmetry, habit, and extinction is discussed.

The immersion method was improved to obtain more reliable results by using a commercial kit of immersion oils with refractive indices between 1.47 and 1.73 at 0.005 intervals.

Keywords——polarizing microscope; refractive indices of crystalline drugs; key refractive index; identification of crystalline drugs; immersion method; temperature coefficients of immersion media; key refractive indices of aspirin; key refractive indices of some J.P. IX drugs

As reported in our previous paper,[1b] measurement of refractive indices and related optical properties of crystalline drugs is useful for the identification of polymorphs or solvates which sometimes coexist in the active drugs and which may affect their bioavailability.

The refractive index of a crystalline drug is an important parameter for the identification of the drug or its polymorphic form. However, measurement of the principal refractive index is not easy, and may even be impossible, depending on the habit or the symmetry of crystals. However, for the purpose of identification it is not necessary to measure the principal refractive index. When a couple of refractive indices measured from the crystals under a microscope are unique and reproducible for a given drug, these data, which we will refer to as "key refractive indices," are sufficient to identify that drug whether they actually represent the principal refractive indices or not.

The present paper describes the correlation between the key refractive index and the crystal habit as well as the crystal symmetry, and discusses the use of the key refractive index as a tool for the identification of crystalline substances. The results of measurement of key refractive indices of sone drugs in J.P.IX, using an improved immersion method are also described.

Experimental

Materials——Some drugs listed in J.P.IX, aspirin, bromovalerylurea, chloramphenicol, and thiamine hydrochloride, were purchased and recrystallized when it was necessary. The following drugs were obtained

1) *a*) Presented at the 27th General Meeting of the Kinki Branch, Pharmaceutical Society of Japan, Kobe, November 1977, and at the 98th Annual Meeting of the Pharmaceutical Society of Japan, Okayama, April 1978; *b*) Part I: A. Watanabe, Y. Tanaka, and Y. Tanaka, *Chem. Pharm. Bull.*, **25**, 2239 (1977); Part II: T. Yokoyama, T. Umeda, K. Kuroda, and A. Watanabe, *ibid.*, **26**, 1044 (1978).

2) Location: *a*) *Arise, Ikawadani-cho, Tarumi-ku, Kobe 673, Japan*; *b*) *Kusunoki-cho, Ikuta-ku, Kobe 650, Japan.*

directly from the manufacturers: ethambutol hydrochloride, quinine hydrochloride, hydralazine hydrochloride, promethazine hydrochloride, digitoxine, sulfisomidine, cephalexin, pyrazinamide, prochlorperazine maleate, sodium cephalothin, lanatoside C, kanamycin sulfate, quinidine sulfate, and colistin sulfate. Sulfamethoxydiazine was the same as the material used in our previous paper.[1b]

Instruments——A binocular polarizing microscope, Olympus BHA, was used. Morphological and optical studies on the crystals were carried out using the uniaxial goniometer constructed by the firm of R. Fuess of Berlin, and a polarizing microscope, Nikon POH, with or without Fedrow's universal stage.

Measurement of the Refractive Index——A commercial kit of immersion oils (Kenbi Kogaku Kenkyusho, Ashiya) covering the range of n_D^{20} from 1.47 to 1.73 at 0.005 intervals was used. Refractive indices of crystals were determined essentially by the procedure described previously.[1b] The method consists of evaluation of the brightness of the Becke line by immersing the crystal in two immersion oils of the kit, one having the

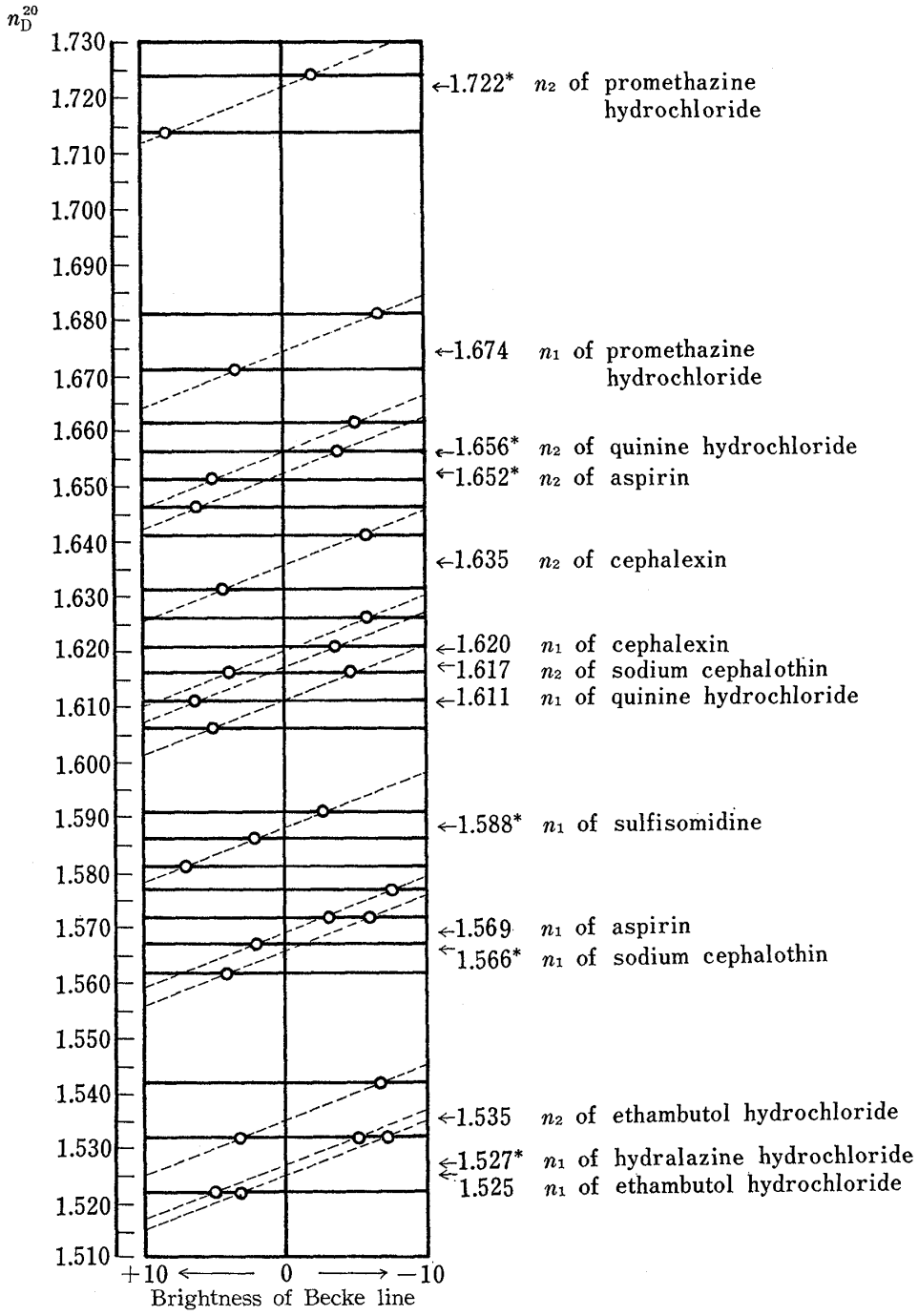

Fig. 1. Experimental Measurements of Key Refractive Indices of Some J.P.IX Drugs (Part 1)

next lower index and the other the next higher index compared with that of the corresponding oil, in which the Becke line becomes almost invisible. The brightness was evaluated on a scale between +10 and −10. The refractive indices of the crystals were obtained using the chart shown in Fig. 1. In the chart, the horizontal solid lines indicate the refractive indices of the immersion oils used. The values for the evaluated brightness were plotted (–o–) on the horizontal lines, and then an oblique line (dotted line) was drawn passing through the two points measured for the oils having higher and lower refractive indices. The precise refractive index was determined by interpolation at the center line.

Example: Plate Crystals of Aspirin——Measurement of n_2 (larger index): The Becke line became almost invisible in immersion oil No. 65-5 (n_D^{20} 1.655) at the extinction position (direction of elongation top and

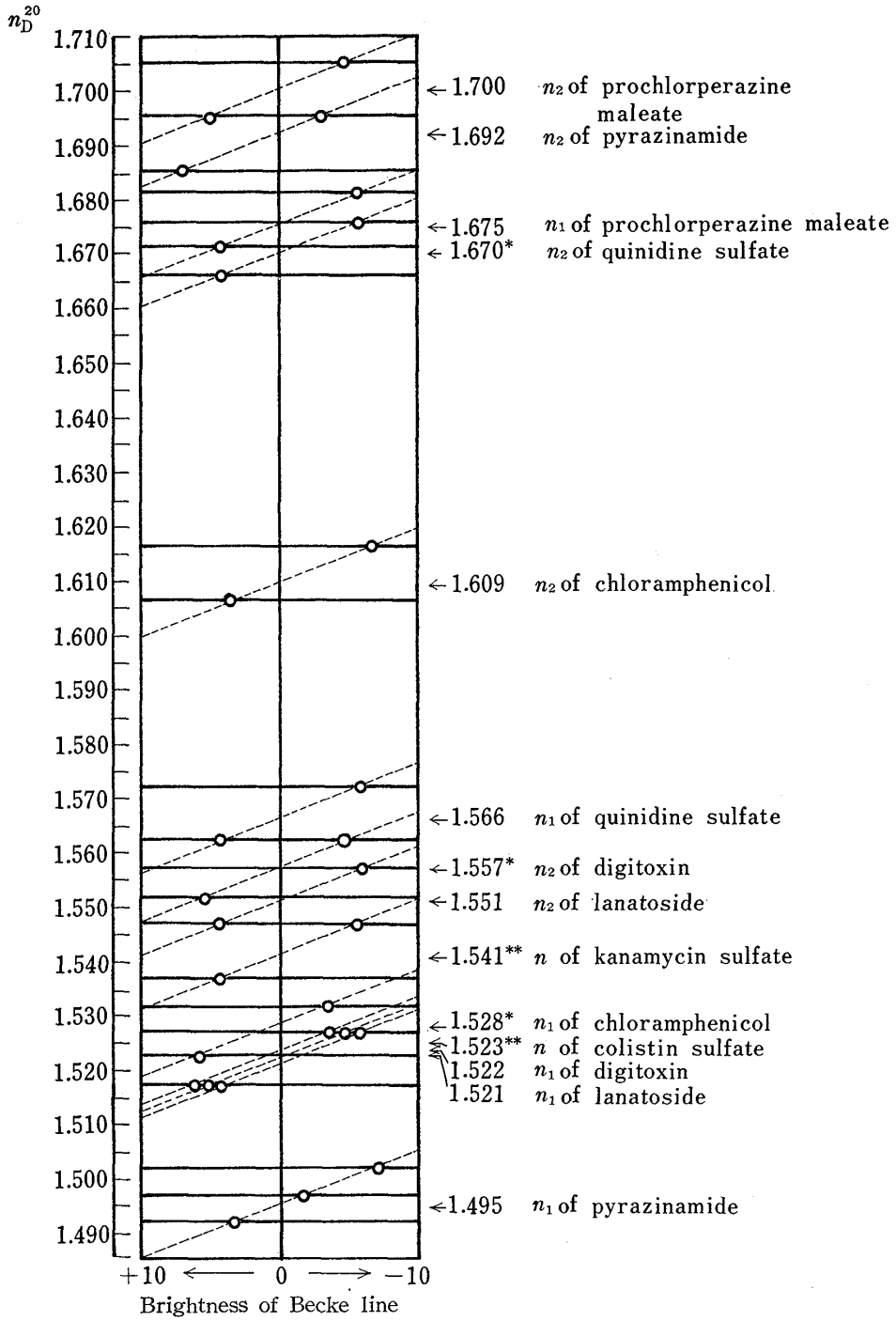

Fig. 2. Experimental Measurements of Key Refractive Indices of Some J.P. IX Drugs (Part 2)

bottom). The room temperature was 28°, and the temperature coefficient, $-dn/dt$, was 0.00047;[3] therefore, the calculated value of n_D^{28} was 1.651.[4] Next, the crystals were suspended in the two neiboring immersion oils as described previously, and the brightness of the Becke line was compared in the two suspensions at the same extinction position; the following values were obtained: in immersion oil No. 65-0 (n_D^{28} 1.646), the brightness was +6, and in immersion oil No. 66-0 (n_D^{28} 1.656), the brightness was −4. Horizontal lines were drawn at n_D^{28} 1.646 and 1.656 on the chart, and the evaluated brightness values were plotted on the lines. The refractive index of the drug, n_2=1.652, was determined from the intersection of the oblique line with the center line as shown in Fig. 1.

Measurement of n_1 (smaller index): The Becke line became extremely weak in immersion oil No. 57-0 (n_D^{20} 1.570) at the other extinction position (at right angles to the direction of elongation). The brightnesses of the Becke lines in suspensions in immersion oils No. 56-5 (the next lower index) and No. 57-5 (the next higher index) were as follows: in immersion oil No. 56-5 (n_D^{28} 1.562), the brightness was +7, and in immersion oil No. 57-5 (n_D^{28} 1.572), the brightness was −3. Lines parallel to the abscissa were drawn at n_D^{28} 1.562 and 1.572, and the evaluated brightness, +7 and −3, were marked on them as before. The refractive index, n_1=1.568, was determined from the intersection of the oblique line with the center line.

Results and Discussion

Results of Measurement of the Key Refractive Indices of Some J.P.IX Drugs

The results are listed in Table I, in which the measured key refractive indices are simply denoted by n_1 (smaller) and n_2 (larger) in place of the principal refractive indices. The refractive index for the light polarized along the direction of the long prismatic or acicular crystals is marked with an asterisk, as it was more reliable for the identification of the drugs than other indices. Among these J.P.IX drugs, kanamycin sulfate and colistin sulfate seemed to be quite different from the others. They did not show double refraction when observed with crossed nicols. Each of them had only one characteristic refractive index, as deter-

Table I. Key Refractive Indices of Some J.P.IX Drugs

Substance	Key refractive indices n_1	Key refractive indices n_2	Crystal shape and extinction
Aspirin	1.569	1.652*(n_γ)	Plates or prisms, parallel extinction
Ethambutol hydrochloride	1.525	1.535*	Elongated plates, parallel extinction
Quinine hydrochloride	1.611	1.656*	Long plates, parallel extinction
Hydralazine hydrochloride	1.527*	1.73<	Plates or rods, parallel extinction
Chloramphenicol	1.528*(n_α)	1.609(n_β)	Plate, parallel extinction
Promethazine hydrochloride	1.674	1.722*	Lamellar, parallel extinction
Digitoxin	1.522	1.557*	Acicular or bladed, parallel extinction
Sulfisomidine	1.588*	1.73<	Elongated plates, parallel extinction
Cephalexin	1.620*(n_α)	1.635(n_β)	Bladed, parallel extinction
Pyrazinamide	1.495*	1.692	Lamellar, parallel extinction
Prochlorperazine maleate	1.675	1.700	Bladed, inclined extinction
Sodium cephalothin	1.566*(n_α)	1.617	Thin plates, parallel extinction
Lanatoside C	1.521*	1.551	Bladed, parallel extinction
Kanamycin sulfate	1.541**		Amorphous
Quinidine sulfate	1.566	1.670*	Elongated plates, parallel extinction
Colistin sulfate	1.523**		Amorphous
Bromovalerylurea, form I	1.525	1.586	Plate, inclined extinction
form II	1.522	1.570*(n_γ)	Acicular, parallel extinction
Thiamine hydrochloride, form I	1.605(n_α)	1.689(n_γ)	Plate or lamellar, inclined extinction

3) A. Watanabe, *Yakugaku Zasshi*, **59**, 131 (1939). The temperature coefficients ($-dn/dt \cdot 10^4$) of these immersion media were 4.1 (1.470—1.480), 4.0 (1.485—1.505), 3.9 (1.515—1.530), 4.0 (1.535—1.545), 4.1 (1.550—1.560), 4.2 (1.565—1.575), 4.3 (1.580—1.590), 4.4 (1.595—1.610), 4.5 (1.615—1.625), 4.6 (1.630—1.645), 4.7 (1.650—1.655), 4.8 (1.660), 5.0 (1.665), 5.2 (1.670), 5.3 (1.675), 5.5 (1.680), 5.7 (1.685), 6.0 (1.690), 6.2 (1.695), 6.3 (1.700), 6.5 (1.710), 6.7 (1.720), 7.0 (1.730).

4) This value is also obtained from the table attached to the kit.

mined by the immersion method, this may be considered as a key refractive index of an isotropic substance.

Correlation between the Key Refractive Index and Known Morphological Properties of Some Drugs

Various shapes or habits of some crystalline drugs are shown in Fig. 3. The key refractive indices of these crystals measured by the immersion method are correlated with their shape or habit.

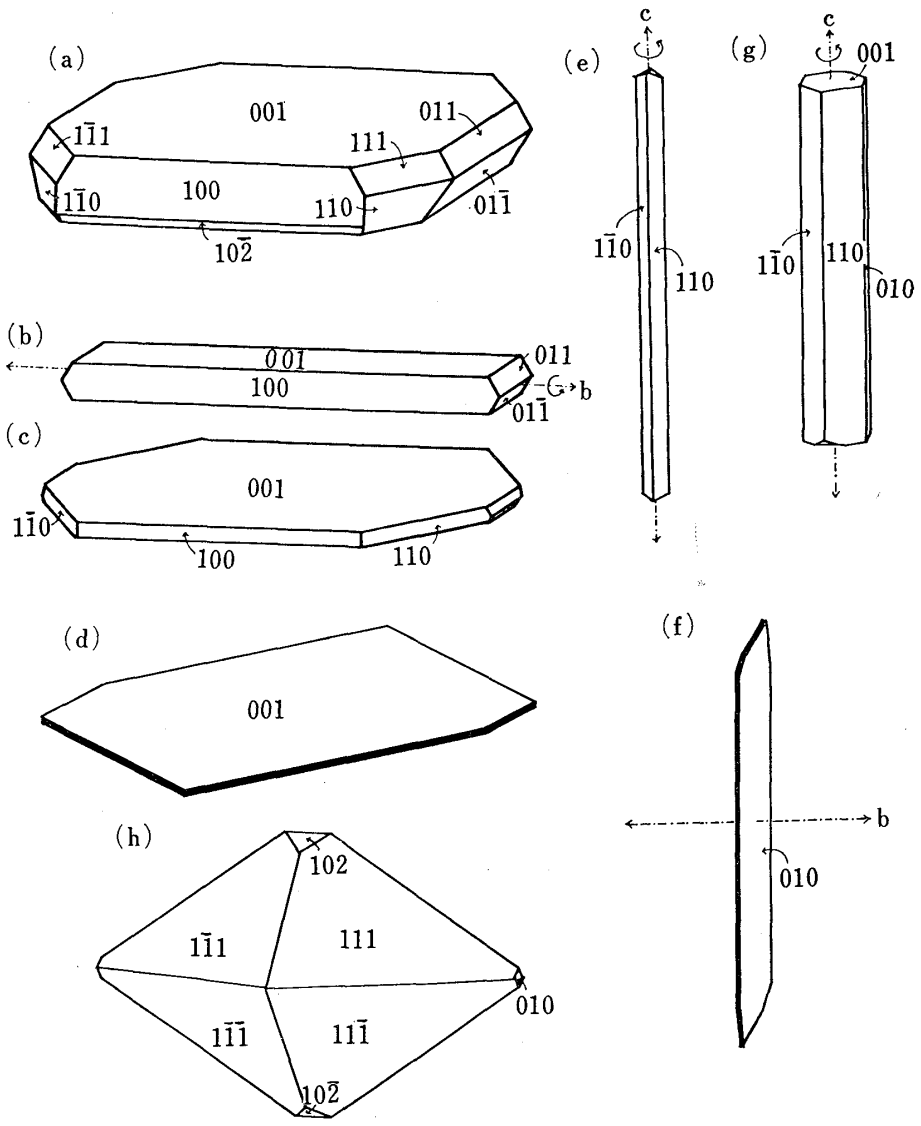

Fig. 3. Various Shapes or Habits of Crystals

(a), (b), and (c) Various habits of aspirin. (d) and (e) Bromovalerylurea, forms I and II. (f) Thiamine hydrochloride, form I. (g) Sulfamethoxydiazine, form I. (h) Bromodiethylacetylurea, form I.

Aspirin——The commercial aspirin usually exists as monoclinic tablets[5] as shown in Fig. 3(a), though they sometimes appear as elongated prisms or thin plates, as shown in Fig. 3(b) and (c), on recrystallization. Using plate crystals similar to those shown in (c), two key refractive indices were determined as shown in Table I, while from the long prismatic or acicular crystals such as (b) only one key refractive index along the direction of elongation

5) S. Watanabe and A. Watanabe, *Proc. Imp. Acad.* (*Tokyo*), **11**, 379 (1935).

was measured, and it was difficult to determine any other index because such crystals tended to rotate around the direction of elongation. When the commercial aspirin was crushed into a powder in an agate mortar, one key refractive index was measured as in case (b).

Bromovalerylurea——The commercial form of this polymorphic drug usually exists as thin triclinic plates or scales of form I as shown in Fig. 3(d).[6] Two characteristic refractive indices were measured from these plates as described in our previous paper.[1b] They were the key refractive indices, though they did not coincide with any of the principal refractive indices. On recrystallization or sublimation of form I, orthorhombic acicular crystals of form II were obtained as shown in Fig. 3(e).[6] Only one key refractive index, n_2, was measured along the direction of elongation as shown in Table I.

Thiamine Hydrochloride——The commercial product of form I of this polymorphic drug usually consisted of monoclinic plates or (010) blades as shown in Fig. 3(f). Two characteristic refractive indices were measured as shown in Table I. These were the key refractive indices, which coincided with the principal refractive indices n_α and n_γ.

Sulfamethoxydiazine——Form I of this polymorphic substance was crystallized in monoclinic (110) prisms elongated along the c-axis as shown in Fig. 3(g).[1b] These predominantly (110) monoclinic prisms showed inclined extinction, and the angle of extinction as well as the refractive index varied with rotation around the c-axis. Therefore, no characteristic refractive indices could be determined from these crystals. In such a case, a set of extinction angles and refractive indices observed for different positions may be useful for drug identification.

Bromodiethylacetylurea——The orthorhombic bipyramidal crystals of form I of this polymorphic substance, as shown in Fig. 3(h),[7] were crushed into a powder in an agate mortar to determine the refractive index. Again, it was very difficult to obtain a definite value, as the crystals did not have any distinctive cleavage.

Table II summarizes the mode of extinction and the nature of measurable key refractive indices observed for various crystal systems together with the crystal shape. It should be noted that key refractive indices measured for plate, lamellar, or bladed crystals, as shown

Table II. Correlation of Key Refractive Indices with Crystal System, Crystal Shape, and Extinction

Crystal system	Crystal shape	Extinction	Number of measurable key refractive indices	Number of measurable principal refractive indices
Triclinic	Plate, bladed, lamellar	Inclined	2	None
	Tabular	Inclined	2 or none	None
	Prismatic	Inclined	None	None
Monoclinic	Plate, bladed parallel to (001) or (100)	Parallel	2	1 (direction of b-axis)
	Plate, bladed parallel to (010)	Inclined	2	2
	Acicular, prisms elongated along b-axis	Parallel	1	1
	Long prisms, needles elongated along a- or c-axis	Parallel or inclined	1 or none	None
Orthorhombic	Plate, bladed	Parallel	2	2
	Long prisms, needles	Parallel	1	1
	Pyramidal	Inclined	None	None

6) A. Watanabe, *Yakugaku Zasshi*, **58**, 565 (1938).
7) A. Watanabe, *Yakugaku Zasshi*, **60**, 416 (1940).

in Fig. 3(c), (d), and (f), can be used for the identification, no matter what kind of symmetry they have. When the crystals are acicular of elongated prisms, as shown in Fig. 3(e), (b), and (g), it is only possible to observe the characteristic key refractive indices in the following limeted cases. (1) For orthorhombic crystals, the refractive index in the direction of elongation should be the key refractive index as in the case of Fig. 3(e), form II of bromovalerylurea. (2) For monoclinic crystals, when the elongation direction coincides with the crystallographic b-axis, the refractive index in the elongation direction should also be the key refractive index, as in the case of Fig. 3(b) for elongated prisms of aspirin. (3) When the extinction angle varies with the rotation around the elongation axis, characteristic key refractive indices cannot be obtained as in the case of Fig. 3(g) for form I of sulfamethoxydiazine.

When the crystal is extremely thick tabular, prismatic, or pyramidal, the usual immersion method cannot be applied. These crystals must be crushed into a powder in an agate mortar or recrystallized using a suitable solvent to obtain a suitable size for the immersion method, as in the case of aspirin.