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Study of Crystalline Drugs by Means of a Polarizing Microscope. VI.[1] Polarizing Microscopy of Drugs Acting on the Nervous System and on Individual Organs Listed in the Japanese Pharmacopoeia X[2]

ATSUSHI WATANABE,*,[a] YUMIKO YAMAOKA,[a] EIICHI AKAHO,[a]
KOJI KURODA,[b] TERUYOSHI YOKOYAMA,[b]
and TSUNEO UMEDA[b]

Faculty of Pharmaceutical Sciences, Kobe-Gakuin University,[a] Arise, Ikawadani-cho, Nishi-ku, Kobe 673, Japan and Pharmacy, Kobe University Hospital,[b] Kusunoki-cho, Ikuta-ku, Kobe 673, Japan

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Twenty-six drugs acting on the nervous system and 30 drugs acting on the individual organs, all listed in the JPX, were investigated by polarizing microscopy using the improved immersion method. For 30 drugs having the form of plates, lamellar or scales, two key refractive indices were measured; these drugs were classified as group A. For 8 drugs having the form of elongated prisms, needles or rods showing always parallel extinction, one key refractive index along the direction of elongation was measured (classified as group B). For 18 other anisotropic crystalline drugs, two refractive indices were measured approximately at the most frequently observable positions; these drugs were classified as group C. It was found that drugs of groups A and B could be conveniently identified or analyzed by polarizing microscopy by measuring their key refractive indices, and these drugs accounted for 68% of the 56 drugs tested.

The correlation between refractive index and birefringence was plotted on rectangular coordinates; such a plot was also useful for the analysis of drugs by polarizing microscopy. Furthermore, the thicknesses of crystal sections might be easily estimated from the birefringence and interference colors, so polarizing microscopy could be useful for the quality control of slightly soluble drugs by estimating their specific surface areas.

Keywords——polarizing microscopy; key refractive index; drug analysis; drug identification; drug quality control; drug acting nervous system; drug acting individual organ; refractive index–birefringence relationship; Japanese Pharmacopoeia X

Polarizing microscopy using an immersion method (PM-method) should be a useful technic for pharmacists, considering that many drugs might be identified or analyzed simply and rapidly even when they exist in pharmaceutical preparations. For this purpose one of the authors, Watanabe, reported in 1939 the results of measurement of the refractive indices of 102 organic and inorganic crystalline drugs listed in the JP V,[3] and subsequently the optical crystallographic characteristics of 230 drugs listed in the National Formulary were measured and tabulated.[4] Nevertheless the PM-method has not been used in a practical sense either in our country[5] or in any other country in the world.

The authors have studied the practical use of the PM-method and have already reported an improved immersion method to minimize errors in the experimental process,[6,7] and defined key refractive indices which were measured from natural sections lying parallel to the microscope stage in the immersion process.[7] Watanabe also presented in a different paper a systematic method of polarizing microscopic observation of powdered drugs, which were classified into 4 groups mainly on the basis of measurable key refractive indices.[8]

In the previous paper[1] the authors reported the results of application of the PM-method to the antibiotic drugs listed in JP X, and it was found that many important antibiotics could be identified by measuring their key refractive indices. In the present works, the authors applied the PM-method to the drugs acting on the nervous system and on the individual organs listed in JP X.

Experimental

Instruments——A binocular polarizing microscope, Olympus BHA,[9] and a polarizing microscope, Nikon POH,[10] with photographic attachments were used. For the immersion method a commercial kit[11] of immersion oils covering the range of n_D^{20} from 1.47 to 1.73 at 0.005 intervals was used.

Materials——The JP X grade drugs shown in Table I were mainly used as samples for polarizing microscopy.

Classification of Solid Drugs into 4 Groups by the PM-Method[8]——Group A: Anisotropic crystals appearing as plates, lamellar or scales. When the crystals are suspended in an immersion oil on a slide glass and slightly pressed with a cover glass, wide planes of plates or thin plates are arranged parallel to the microscope stage and 2 definite key refractive indices can be measured correctly from the section.

Group B: Anisotropic crystals appearing as elongated prisms, needles or rods that always show parallel extinction. A definite key refractive index is measured along the elongated direction. However, the refractive index

Table I. Drugs Used in the Present Polarizing Microscopy Study

1. Drugs acting on the nervous system and the sense organs	11. Central nervous system drugs	No. 101 aspirin, No. 102 aspirin aluminium, No. 103 aminopyrine, No. 104 amobarbital, No. 105 amitriptyline hydrochloride, No. 106 imipramine hydrochloride, No. 107 chlorpromazine hydrochloride, No. 108 oxyphenbutazone, No. 109 chlordiazepoxide, No. 110 dimenhydrinate, No. 111 sulpyrine, No. 112 trimethadione No. 113 barbital, No. 114 haloperidol, No. 115 phenacetin, No. 116 phenytoin, No. 117 phenylbutazone, No. 118 phenobarbital, No. 119 bromvalerylurea I, No. 119′ bromvalerylurea II
	12. Peripheral nervous system drugs	No. 120 perphenazine, No. 121 chloral hydrate, No. 122 prochlorperazine maleate, No. 123 levomepromazine maleate, No. 124 trihexyphenidyl hydrochloride, No. 125 levodopa
2. Drugs acting on individual organs	21. Diuretics and cardiovascular drugs	No. 201 acetazolamide, No. 202 hydralazine hydrochloride, No. 203 procainamide hydrochloride, No. 204 propranolol hydrochloride, No. 205 caffeine, No. 206 digitoxin, No. 207 triamteren, No. 208 trichlormethiazide, No. 209 prenylamine lactate, No. 210 hydrochlorothiazide, No. 211 lanatsid C, No. 212 quinidine sulfate, No. 213 methyldopa
	22. Respiratory drugs	No. 221 aminophylline, No. 222 ethionamide, No. 223 epinephrine, No. 224 ethambutol hydrochloride, No. 225 ephedrine hydrochloride, No. 226 dimorpholamine, No. 227 dextromethorphan hydrochloride, No. 228 noscapine hydrochloride, No. 229 sodium cromoglicate
	23. Gastrointestinal drugs	No. 231 methylbenactyzium bromide No. 232 bethanecol chloride
	24. Hormones	No. 241 cortisone acetate, No. 242 triamcinolone acetonide, No. 243 betamethasone, No. 244 thiamazole, No. 245 norethisterone, No. 246 progesterone

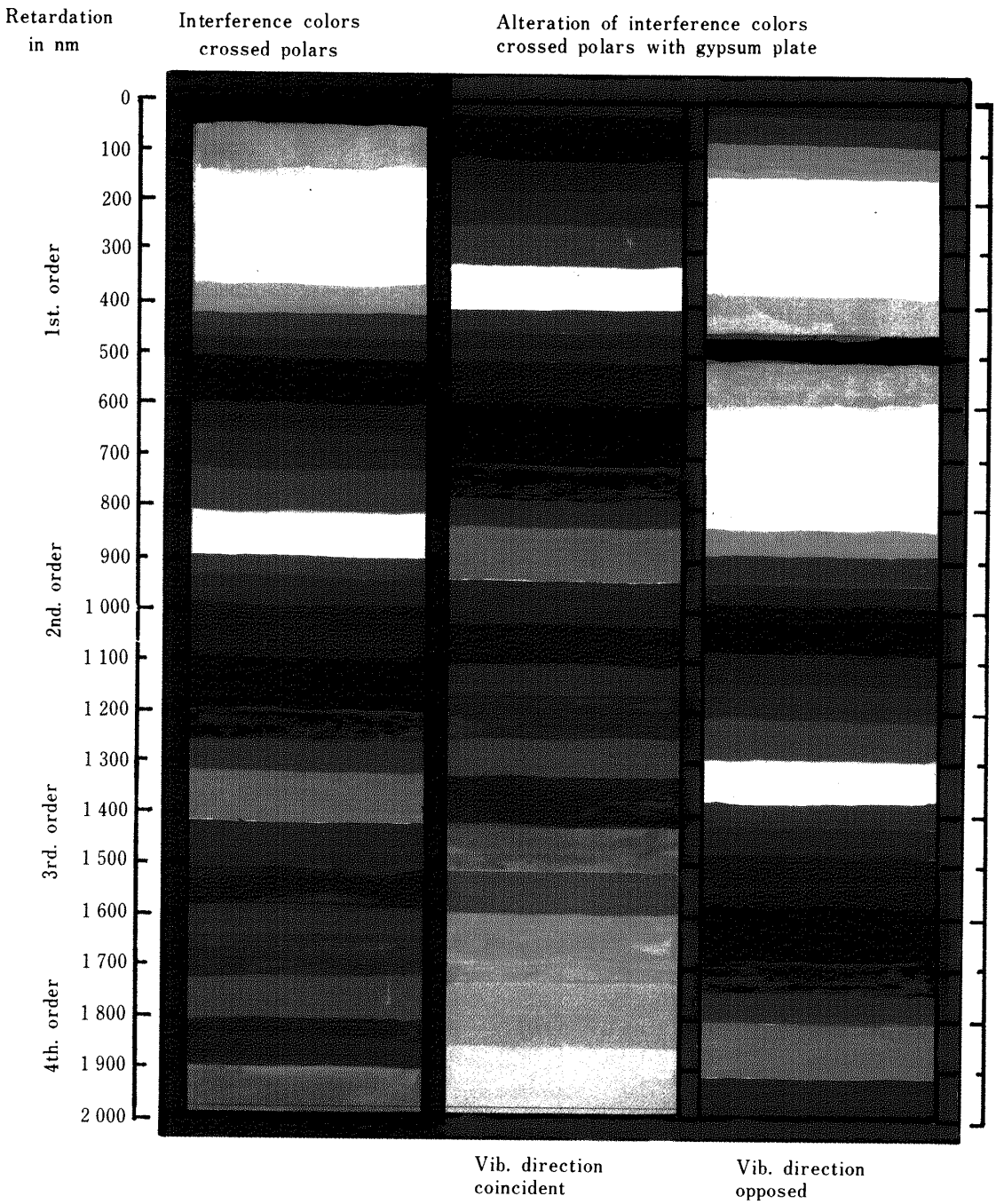

Fig. 1. Interference Colors under Crossed Polars and Their Alterations with a Gypsum Plate

observed at the perpendicular position to the elongated direction is indefinite as the crystals can rotate around the elongated axis. In such cases, the most frequently observable index is selected as a partner of the key refractive index on the basis of similar interference color and 'elongation.'

Group C: Anisotropic crystals which belong to neither group A nor group B. For instance, when elongated prisms show inclined extinction and rotate around the elongated axis in an immersion oil, it is difficult to measure definite refractive indices, and such crystals are classified as group C. In the case of group C crystals, a relatively frequently observable pair of refractive indices can be measured from crystals having similar shape, similar extinction and similar interference color.

Group D: Isotropic crystals or amorphous solids which do not show a double refraction under crossed polars. Isotropic crystals often exist in comparatively regular shape like sodium chloride crystals. On the other hand amorphous solids show irregular shape like pieces of broken glass. A unique and definite refractive index can usually be observed for the solids classified as group D. The refractive index which is observed for the isotropic crystals is a

key refractive index of the substance, but that which is observed for an amorphous solid is unlikely to be a key index, because it may change its value depending on the water content, as is characteristic of amorphous solids.

Measurement of Refractive Index——The measurement of refractive indices was performed by the improved immersion method, which has been developed by the authors.[6,7] In the cases of $n_D > 1.73$, approximate values were estimated by using freshly prepared immersion oils having n_D^{20}: 1.73—1.78 at about 0.01 intervals, made by adding suitable quantities of sulfur to methylene iodide.[5] The refractive indices of the newly made immersion oils as well as the oils of the unstable range in the kit (n_D^{20}: 1.66—1.73) were measured with a K-type refractometer.[12]

Calculation of the Thickness of the Crystal Section——The thickness of the section perpendicular to the microscope stage is easily estimated by observing the interference color and the birefringence of the section. The correlation between interference color and retardation in nm is shown in Fig. 1. Using this figure, the retardation (R) of the crystal section can be determined by finding a similar interference color under crossed polars; when the retardation is relatively small (less than about 250 nm) it would be better to observe the retardation by using a gypsum plate. Figure 1 shows the altered interference colors, one adding and the other subtracting the retardation of a gypsum plate, depending on the vibration direction when the gypsum plate is inserted. From the observed retardation (R) and the birefringence of the section ($n_2 - n_1$) the thickness (D) can be calculated as follows: $D = R/(n_2 - n_1)$. To calculate the thickness of the section it is convenient to estimate the shapes or habits of crystals and therefore to determine the classification in the PM-method. For instance, when the thickness (D) is less than $\sqrt{ab}/10$, where a and

TABLE II. Results of Polarizing Microscopy of Drugs Acting on the Nervous System

No.	Name	Refractive index n_1	Refractive index n_2	Group	Birefringence $(n_2 - n_1)$	Remarks Shape, extinction, elongation, literature, *etc.*
101	Aspirin	1.569	1.652[a]	A	0.083	Plates, parallel ext., elong. +, lit. 8
102	Aspirin aluminium	1.558	1.582	A	0.024	Lamellar, parallel ext., elong. +
103	Aminopyrine	1.512	1.662	C	0.150	Prisms, inclined ext. (13°),[b] lit. 4
104	Amobarbital	1.475	1.538	C	0.063	Lamellar, parallel or inclined ext. (2—5°), elong. +, lit. 4
105	Amitriptyline hydrochl.	1.57	1.67	C	0.100	Long prisms, inclined ext. (18°)
106	Imipramine hydrochl.	1.605	1.654	A	0.049	Plates, inclined ext. (6°), lit. 8
107	Chlorpromazine hydrochl.	1.59	1.78	C	0.190	Lamellar, inclined ext. (40°)
108	Oxyphenbutazone	1.564	1.619	A	0.055	Lamellar, inclined ext. (17°)
109	Chlordiazepoxide	1.69	1.76	C	0.070	Small rods and conglomerates
110	Dimenhydrinate	1.551	1.625	C	0.074	Plates, soluble in immersion oils
111	Sulpyrine	1.521	1.602	A	0.081	Lamellar, parallel ext., elong. +
112	Trimethadione	1.476	1.539	A	0.063	Hexagonal plates
113	Barbital	1.460	1.553	A	0.113	Plates, parallel or inclined ext. (4°), elong. +, lit. 4
114	Haloperidol	1.584	1.710	B	0.126	Lamellar, parallel ext., elong. −
115	Phenacetin	1.572	1.75	C	0.178	Prisms, inclined ext. (2°), lit. 3, 4
116	Phenytoin	1.606	1.631	A	0.025	Lamellar, parallel ext., elong. −
117	Phenylbutazone	1.600	1.616	A	0.016	Lamellar, parallel ext., elong. −
118	Phenobarbital	1.557	1.621	A	0.064	Plates (irregular), lit. 3, 4
119	Bromvalerylurea I	1.530	1.586	A	0.056	Parallelogramic scales, inclined ext. (6°), lit. 6
119′	Bromvalerylurea II	1.525	1.570	B	0.045	Needles, parallel ext., elong. −
120	Perphenazine	1.56	—	C	—	Rods, inclined ext. (19°), soluble in immersion oils
121	Chloral hydrate	1.58	1.617	A	0.037	Plates, lit. 3
122	Prochlorperazine maleate	1.673	1.704	A	0.031	Thin plates, inclined ext. (13°)
123	Levomepromazine maleate	1.61	1.64	C	0.030	Small rods
124	Trihexyphenidyl hydrochl.	1.567	1.589	A	0.022	Lamellar, inclined ext. (14°)
125	Levodopa	1.625	1.703	A	0.075	Plates, parallel ext., elong. −

a) Key refractive indices are underlined. *b*) Extinction angle. In the case of group C, extinction angle and refractive indices form one set.

b are parameters to obtain the area of a face parallel to the stage, the crystals might be called scales, and such crystals belong to group A. Furthermore, when D is less than $\sqrt{ab}/5$, the crystals might be called lamellar or thin plates, and they also belong to group A.

Results and Discussion

The results of the PM-method applied to 26 drugs acting on the nervous system and 30 drugs acting on the individual organs are listed in Tables II and III. Tables II and III show the

TABLE III. Results of Polarizing Microscopy of Drugs Acting on the Individual Organs

No.	Name	Refractive index n_1	Refractive index n_2	Group	Birefringence (n_2-n_1)	Remarks Shape, extinction, elongation, literature, *etc.*
201	Acetazolamide	1.532	1.701[a]	A	0.169	Hexagonal plates, lit. 8
202	Hydralazine hydrochl.	1.528	1.78	A	0.248	Lamellar, parallel ext., elong. − lit. 7
203	Procainamide hydrochl.	1.64	1.742	C	0.102	Thick plates
204	Propranolol hydrochl.	1.567	1.678	A	0.111	Plates, parallel ext., elong. +
205	Caffeine	1.470	1.688	A	0.218	Lamellar, inclined ext. (33°)[b] lit. 3
206	Digitoxin	1.522	1.557	B	0.035	Rods, parallel ext., elong. + lit. 4
207	Triamteren	1.522	1.78	C	0.330	Prisms, inclined ext.
208	Trichlormethiazide	1.534	1.76	A	0.226	Plates, inclined ext. (45°)
209	Prenylamine lactate	1.544	1.631	B	0.087	Lamellar, parallel ext., elong. −
210	Hydrochlorothiazide	1.498	1.653	A	0.155	Plates, parallel ext., elong. −
211	Lanatsid C	1.521	1.551	A	0.030	Plates, parallel ext., elong. − lit. 7
212	Quinidine sulfate	1.591	1.671	B	0.080	Lamellar, parallel ext., elong. + lit. 7, 8
213	Methyldopa	1.576	1.625	A	0.049	Plates, inclined ext. (14°)
221	Aminophylline	1.49	1.77	C	0.280	Small plates and their conglomerates
222	Ethionamide	1.57	1.79	C	0.220	Lamellar, inclined ext. (27°)[c]
223	Epinephrine	1.539	1.602	A	0.063	Plates, parallel ext., elong. −
224	Ethambutol hydrochl.	1.524	1.536	A	0.012	Lamellar, parallel ext., elong. + lit. 7
225	Ephedrine hydrochl.	1.602	1.64	B	0.038	Long plates, parallel ext., elong. −, lit. 3, 4, 7
226	Dimorpholamine	1.525	1.621	A	0.096	Plates, soluble in immersion oils
227	Dextromethorphan hydrochl.	1.574	1.618	A	0.044	Plates, parallel ext., elong. −
228	Noscapine hydrochl.	1.550	1.694	A	0.144	Hexagonal plates, parallel ext.
229	Sodium cromoglicate	1.541	1.598	B	0.057	Rods, parallel ext., elong. −
231	Methylbenactyzium bromide	1.606	1.609	A	0.003	Lamellar, parallel ext., elong. +
232	Bethanecol chloride	1.49	1.525	C	0.035	Rods, inclined ext. (18°)
241	Cortisone acetate	1.54	1.58	C	0.040	Small plates, inclined ext. (8°)
242	Triamcinolone acetonide	1.55	1.58	C	0.030	Rods, parallel ext., elong. − lit. 4
243	Betamethasone	1.55	1.662	B	0.112	Needles, parallel ext., elong. + lit. 3, 4
244	Thiamazole	1.69	1.717	C	0.027	Rods, parallel ext., elong. +
245	Norethisterone	1.567	1.624	A	0.057	Thin plates (irregular)
246	Progesterone	1.54	1.55	C	0.010	Soluble in immersion oils, lit. 3, 4

a) Key refractive indices are underlined. *b*) Extinction angle. *c*) In the case of group C, extinction angle and measured refractive indices form one set.

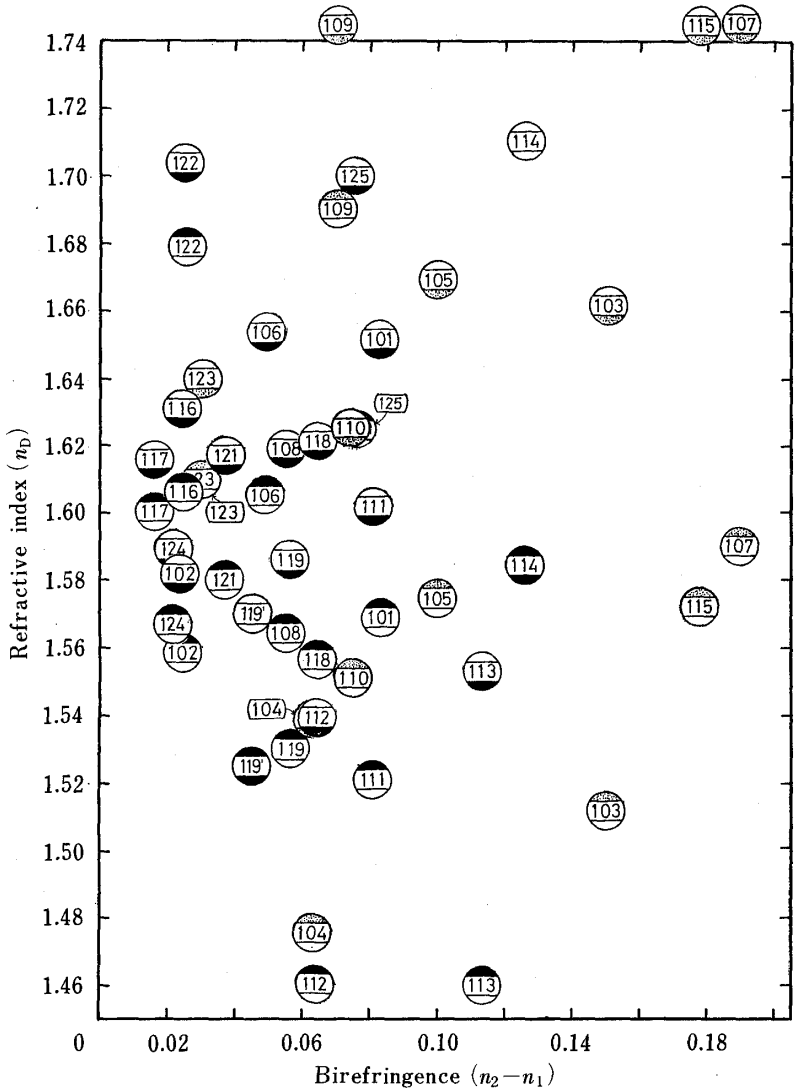

Fig. 2. Correlation between Refractive Indices and Birefringence of Drugs Acting on the Nervous System

Key refractive indices of group A: n_1, ⊖; n_2, ⊖. Key refractive index of group B: n_1 or n_2, ○. The numbers in the figure correspond to those in Table I or II.

measured refractive indices (key refractive indices are underlined), classification, birefringence (n_2-n_1) and remarks (shapes, extinction, elongation and literature). Among these data, the key refractive indices are the most important to identify or analyze drugs. In Table II, 17 drugs out of 26 are classified as group A or B, and in Table III, 21 drugs out of 30 are group A or B. Namely, about 68% of all the tested drugs could be identified or analyzed by the PM-method by measuring their key refractive indices.

The correlation between the refractive indices and the birefringences listed in Table II or III are shown in Fig. 2 or 3 on rectangular coordinates; the refractive indices are plotted on the ordinate and birefringences on the abscissa. Examination of the results shown in Figs. 2 and 3 indicates that these figures can be conveniently used to identify or analyze drugs on the basis of their refractive indices, as the values were sufficiently dispersed, though with some crowding around the birefringence of 0.01—0.05.

As described in the experimental section, birefringence is directly correlated with the interference colors of crystals. For instance, in the cases where birefringence is about 0.01—0.03 and the thickness of crystals is about 10 μm, the retardation is about 100—300 nm, and

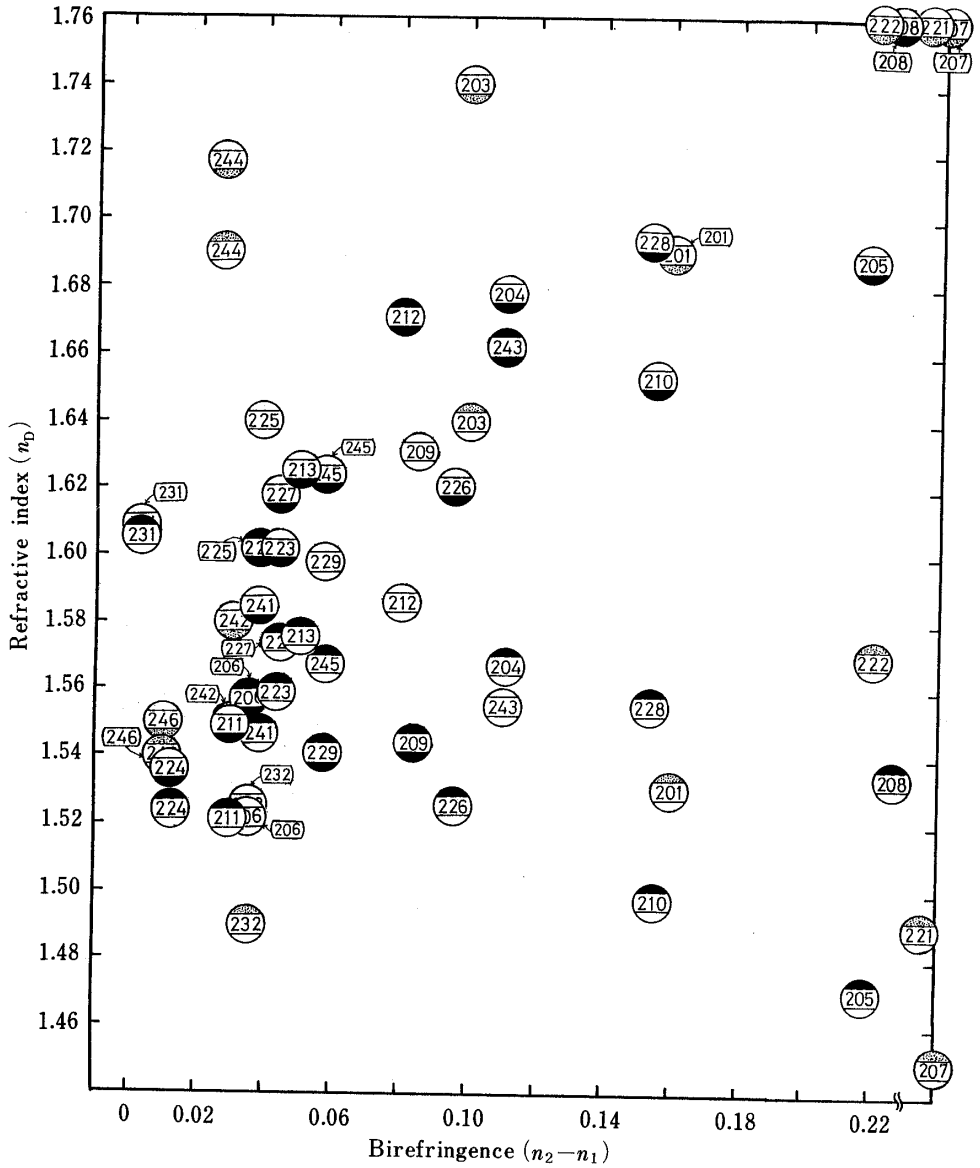

Fig. 3. Correlation between Refractive Indices and Birefringence of Drugs Acting on the Individual Organs

Symbols are the same as in Fig. 2. The numbers in the figure correspond to those in Table I or III.

the observable interference color is whitish-grey, which will alter to light blue (indigo) or light yellow (orange) on insertion of a gypsum plate. In Figs. 4 and 5 the polarizing photomicrographs of some of the drugs described in Table II or in Table III are shown in color. Photos No. 102, 122 and 124 in Fig. 4 and photos No. 211 and 231 in Fig. 5 are examples of crystals with birefringences of around 0.01—0.03. Generally in the PM-method it is important to observe carefully not only the shape and extinction of the crystals but also the pattern of interference colors as shown in Figs. 4 and 5. Tables II and III also listed the crystal shapes.

When the vibration direction of polarized light which is parallel to the elongated axis coincides with X' or Z' in the cases of group B crystals or elongated group A crystals showing parallel extinction, the elongation is defined as − (minus) or + (plus), respectively. In the remarks in Tables II and III measurable elongation data are shown. These are useful to predict the type of drug in question before actually carrying out the identification or analysis experiment.

Fig. 4. Polarizing Photomicrographs of Some Drugs Shown in Table II

Numbers of the photos correspond to those in Table II.

Under crossed polars: × 200, No. 116, 118; × 100, No. 104, 106, 111, 114, 119, 125; × 40, No. 121.

Under crossed polars with a gypsum plate: × 200, No. 107, 108, 115, 117; × 100, No. 102, 112, 113, 122, 124, 120.

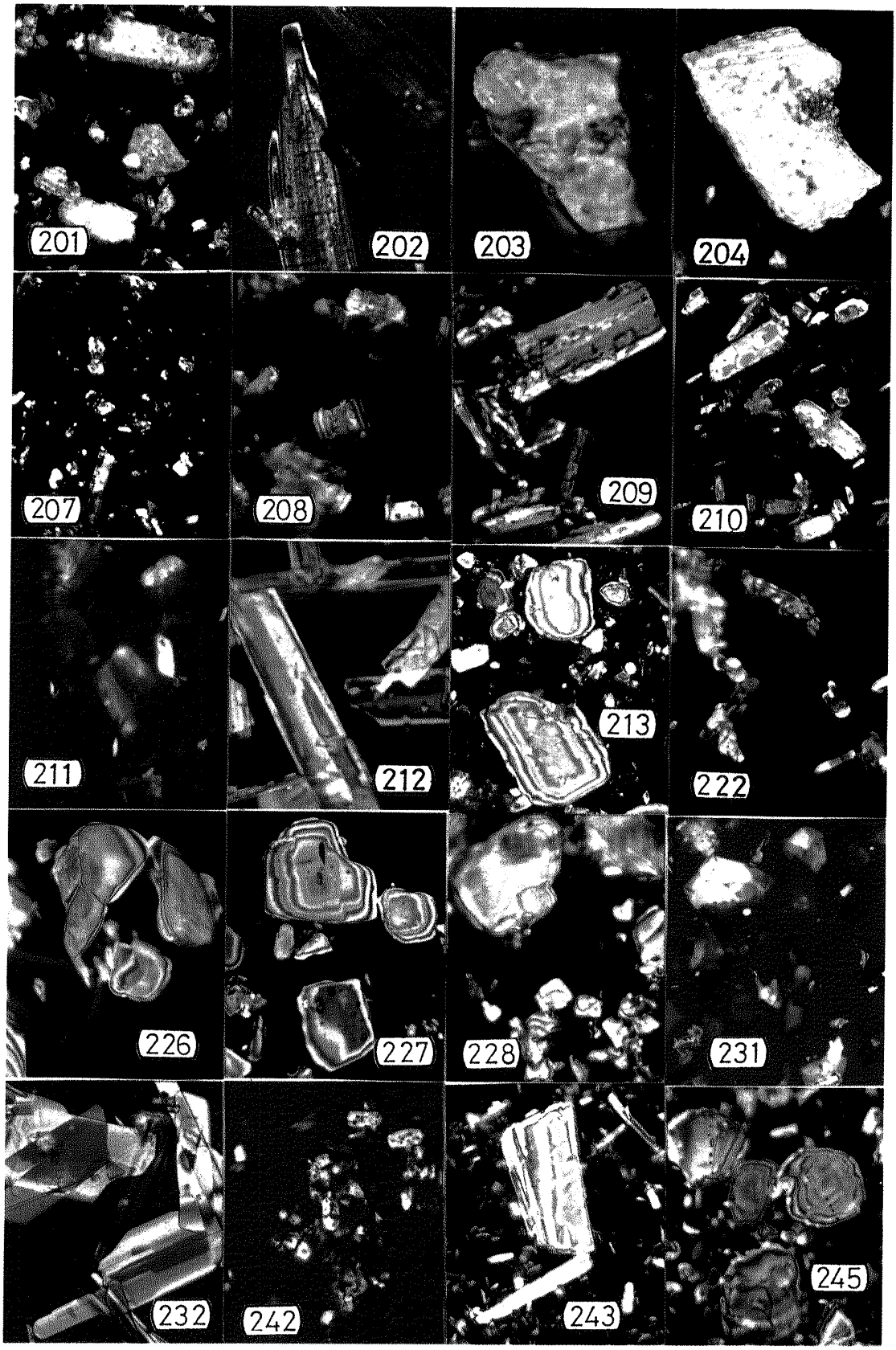

Fig. 5. Polarizing Photomicrographs of Some Drugs Shown in Table III

Numbers of the photos correspond to those in Table III.
Under crossed polars: ×200, No. 208, 222, 228, 243, 245; ×100, No. 201, 204, 207, 210, 212, 213, 226, 227, 232.
Under crossed polars with a gypsum plate: ×200, No. 242; ×100, No. 202, 203, 209, 211; ×40, No. 231.

In conclusion, it was found that the PM-method was applicable to some drugs acting on the nervous system or the individual organs; it should be possible to identify or analyze about 70% of them measuring their key refractive indices as well as observing their shapes or elongation. The correlations between refractive indices and birefringence shown in Figs. 2 and 3 are also useful for the same purpose. The values obtained by estimating the retardation of a crystal section from the interference color and by calculating the thickness are also useful, not only for the identification of the shape of a crystal quantitatively but also for the determination of the specific surface area using three dimensional parameters, *a*, *b* and *c* (thickness).[13] It is well known that the dissolution behavior of slightly soluble crystals depends upon the specific surface area. Therefore, the PM-method could be useful in the quality control of crystalline drugs.

There were a few drugs for which it was impossible to measure refractive indices, as they were soluble in the immersion oils or they existed as conglomerated masses of miscellaneous crystals. Further work is under way and will be reported shortly.

References and Notes

1) Part V: A. Watanabe, Y. Yamaoka, K. Kuroda, and T. Yokoyama, *Yakugaku Zasshi*, **104**, 896 (1984).
2) A part of this work was presented at the 98th Annual Meeting of the Pharmaceutical Society of Japan, Okayama, April 1978, and at the 31st General Meeting of the Kinki Branch, Pharmaceutical Society of Japan, Kobe Nov. 1981.
3) A. Watanabe, *Yakugaku Zasshi*, **59**, 30 (Ger.) and 131 (Jap.) (1939).
4) The National Formulary XIII, First Supplement, 1041—1056 (1970).
5) The following textbook deals with polarizing microscopy (in Japanese). S. Tsuboi, "Henkokenbikyo," 13th ed., Iwanami-Shoten, Tokyo, 1975.
6) A. Watanabe, Yu. Tanaka, and Yo. Tanaka, *Chem. Pharm. Bull.*, **25**, 2239 (1977).
7) A. Watanabe, Y. Yamaoka, and K. Kuroda, *Chem. Pharm. Bull.*, **28**, 372 (1980).
8) A. Watanabe, Proceedings of the 8th Asian Congress of Pharmaceutical Sciences and the 9th General Assembly, Kyoto, 1980, p. 59.
9) Olympus Kogaku Kogyo K. K., Ryumeikan-Bld., 3—4, Surugadai, Chiyoda-ku, Tokyo, Japan.
10) Nihon Kogaku Kogyo K. K., Fuji-Bld., 3–2–2, Chiyoda-ku, Tokyo, Japan.
11) K. K. Kenbikogaku-Kenkyusho, 7–4, Matsunouchi-cho, Ashiya-shi, Japan.
12) K-Type refractometer, RK-5, Nihon Chikagakusha K. K., Oike-agaru, Fuyamachi-dori, Nakagyo-ku, Kyoto, Japan.
13) A. Watanabe, Y. Yamaoka, and K. Takada, *Chem. Pharm. Bull.*, **30**, 2958 (1982).