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Crystal Forms and Optical Resolution by Preferential Crystallization of Calcium DL-Pantothenate

MASAHIRO INAGAKI

Research Institute, Daiichi Seiyaku Co., Ltd.[1]

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Five polymorphs (A-, B-, C-, D- and E-form), four solvates (monohydrate, a hydrate contained 3/4 molecule of water, methanol monosolvate and a solvate contained 4 molecules of methanol and 1 molecule of water) and an amorphous form of calcium DL-pantothenate were isolated. These crystal forms were characterized using thermal analysis, X-ray powder diffraction and infrared spectroscopy. In order to examine the resolution of calcium DL-pantothenate by preferential crystallization, the racemic structures of these crystal forms were investigated by comparing these physical properties with those of crystal forms of calcium D(+)-pantothenate.[2] As the results, C-form and the solvate contained 4 molecules of methanol and 1 molecule of water were found to be the racemic mixtures and then the optical resolutions of the two kinds of crystals were carried out.

Keywords——calcium DL-pantothenate; polymorph; solvate; amorphous form; thermogravimetry; differential thermal analysis; X-ray diffraction; IR spectrophotometry; racemic structure; optical resolution

Several kinds of crystals of calcium D(+)-pantothenate, *i.e.*, three polymorphs (α-, β- and γ-form), two solvates (monohydrate and D-PC·4MeOH·1H_2O) and an amorphous form, have been described in a recent paper from this institute.[2] Calcium DL-pantothenate has been reported to occur in different crystalline modifications such as two polymorphs,[3] a solvate[3,4] and an amorphous form.[3,5]

In the present study, ten kinds of crystalline modifications of calcium DL-pantothenate were isolated. Five were polymorphs (A–, B–, C–, D– and E–form), four were solvates (methanol monosolvate, monohydrate, a hydrate contained 3/4 molecule of water, and a solvate contained 4 molecules of methanol and 1 molecule of water) and one was amorphous. Thermal analysis, X-ray powder diffraction analysis and infrared spectrophotometry were performed with these crystals for identification. From these results and the results of crystal forms of calcium D-(+)-pantothenate already described,[2] the racemic structures of these crystals were examined, and it was revealed that C-form and the solvate contained 4 molecules of methanol and 1 molecule of water are recamic mixtures. Then, the optical resolutions of two kinds of the racemic mixtures were accomplished by prefential crystallization.

Experimental

Materials——Calcium DL-pantothenate used for the preparation of the various crystal forms and for the resolution of two kinds of the racemic mixtures was the amorphous form which was prepared by drying at 50—80° *in vacuo* the solvate recrystallized from 95% aqueous methanol at 0°. α-Form and D-PC·4MeOH·1H_2O of calcium D(+)-pantothenate were prepared by the method already described.[2] Calcium L(−)-pantothenate crystals which contained 4 molecules of methanol and 1 molecule of water of crystallization

1) Location: *Minamifunabori-cho, Edogawa-ku, Tokyo.*
2) M. Inagaki, H. Tukamoto, and O. Akazawa, *Chem. Pharm. Bull.* (Tokyo), **24**, 3097 (1976).
3) J.H. Ford, *J. Am. Chem. Soc.*, **68**, 1666 (1946).
4) S. Funabashi and K. Michi, *Bull. Inst. Phys. Chem. Research* (Japan), **22**, 681 (1943).
5) E.T. Stiller, S.A. Harris, J. Finkelstein, J.C. Keresztesy, and K. Folkers, *J. Am. Chem. Soc.*, **62**, 1785 (1940).

(hereinafter abbreviated as L-PC·4MeOH·$1H_2O$) were prepared in a similar method as for D-PC·4MeOH·$1H_2O$. All solvents used were reagent grade.

Instruments——Thermal analysis was carried out using TG–DTA (Rigaku Denki CAT .No. 8021). Measurement conditions adopted for this purpose were; heating rate, 1°/min; sensitivity of amplifier, ±50 μV; standard sample, Al_2O_3; atmosphere, air; weight, 100—150 mg. The X-ray powder diffraction patterns were obtained with a Rigakudenki X-ray diffractometer Type 2012 with; target, Cu; filter, Ni; voltage, 35 kV; current, 20 mA; count range, 2000 c.p.s; time constant, 1 sec; scanning speed, 2°/min; divergency, 1°; receiving slit, 0.15 mm. Infrared (IR) spectra were taken with a Hitachi Grating Infrared Spectrophotometer Model 215 using nujol mull. The gas-liquid chromatography was carried out with a Shimazu Gas Chromatograph Model GC-4BM equipped with a hydrogen flame ionization detector and a glass column (2.0 m×3.0 mm) packed with porapak. Q (80—100 mesh). Optical rotation measurements were carried out with an Applied Electric Lab., Ltd. Automatic Polarimeter Model MP-1T.

Preparation of Crystals of Calcium DL-Pantothenate——Amorphous calcium DL-pantothenate was dissolved in a suitable volume of appropriate solvents and allowed to stand for 2—10 days at a constant temperature. The deposited crystals were filtered and dried over silica gel at room temperature. The water contents of aqueous solvents used were less than 30%.

Solubility Determination——A sufficient amount of the crystals was placed in 20—100 ml of appropriate solvents and stirred at a constant temperature.

At appropriate time intervals, 5—20 ml aliquots of the solution were withdrawn and immediately filtered through 11G 4 galss filter. The filtrates were weighed and analyzed by the titration of Ca with 0.05 mol. ethylenediaminetetraacetic acid.

The Resolution of C-Form——Sixty grams of amorphous calcium DL-pantothenate were dissolved in a suitable volume of appropriate solvents (methanol, 95% methanol and 95% ethanol; 100 g, ethanol; 120 g, 90% isopropanol; 400 g) at 50° and these solutions were cooled at a desired temperature.

Into these supersaturated solutions 1.0 g of finely-pulverized α-form of calcium D(+)-pantothenate ($[\alpha]_D^{20}$ +28.0°, c=3, H_2O) was seeded and mixed.

Periodically, 10—20 ml aliquots of these systems were withdrawn, and the deposited crystals were filtered through 11G 4 glass filter, washed with the same solvent. The optical rotation (l=100 mm, at 20°) of the filtrate is shown in Fig. 5.

The Alternate Resolutions of DL-PC·4MeOH·$1H_2O$——One hundred grams of amorphous calcium DL-pantothenate were dissolved in 200 g of 95% methanol at 50° and the solution was cooled at 10—13°. Into this supersaturated solution 1.0 g of finely-pulverized D-PC·4MeOH·$1H_2O$ ($[\alpha]_D^{20}$ +21.2°, c=3, H_2O) was seeded and mixed. Periodically, the sample from the system was withdrawn directly with a cotton filter attached pipette and its optical rotation (l=100 mm, at 20°) was measured. After its optical rotation exceeded −1.0°, D-PC·4MeOH·$1H_2O$ which was precipitated was filtered, washed with 20 g of cold methanol and then was changed to the amorphous form by drying at 50—80° under an infrared lamp. The amorphous form obtained was weighed and its specific rotation was measured.

Next, the same amount of amorphous calcium DL-pantothenate as amorphous calcium D(+)-pantothenate obtained by the first resolution was dissolved in the filtrate at 50° and the solution was cooled at 10—13°. Into this supersaturated solution 1.0 g of finely-pulverized L-PC·4MeOH·$1H_2O$ ($[\alpha]_D^{20}$ −21.2°, c=3, H_2O) was seeded. Henceforth the alternate D- and L-resolutions were carried out by the same procedures.

Results and Discussion

Crystalline Modifications of Cacium DL-Pantothenate

Methanol Solvate and A-Form——The crystals obtained from aqueous methanol at above 30° or methanol are needles. Ford[3] and Funabashi, *et al.*[4] have reported that the crystals obtained from methanol were methanol solvate, but the composition of solvents of crystallization has not been investigated. The crystals readily lost methanol of crystallization upon allowing to stand at room temperature, and accordingly the composition of solvents of crystallization could not be determined by thermal analysis and elemental analysis. It was considered that there was a difference in the rate of liberation between adhered solvent and solvent of crystalization. Then the wet crystals obtained from methanol were dried over silica gel at 20° and the dry-weight was measured periodically. The weight loss curve obtained is shown in Fig. 1. It is apparent that the curve shows two inflection points and the weight loss of the first point to the second point agrees with the theoretical methanol content of methanol monosolvate (6.3%). From the results of gas-liquid chromatography, it was also found that the crystals at the first point contained 6.5% of methanol and the crystals at the second point were free from

methanol. On the basis of these facts, the crystals were determined as the solvate which contained 1 molecule of methanol of crystallization. The nonsolvated crystals obtained from the methanol solvate are named A-form.

DL-PC·4MeOH·1H_2O

The crystals obtained from aqueous methanol at low temperatures are hygroscopic prisms which have a melting point of 55—58°. These crystals were confirmed to be a solvate which contained 4 molecules of methanol and 1 molecule of water of crystallization by thermal analysis, gas-liquid chromatography, the Karl-Fisher method, weight-loss on drying and elemental analysis. These crystals are hereinafter abbreviated as DL–PC·4MeOH·1H_2O.

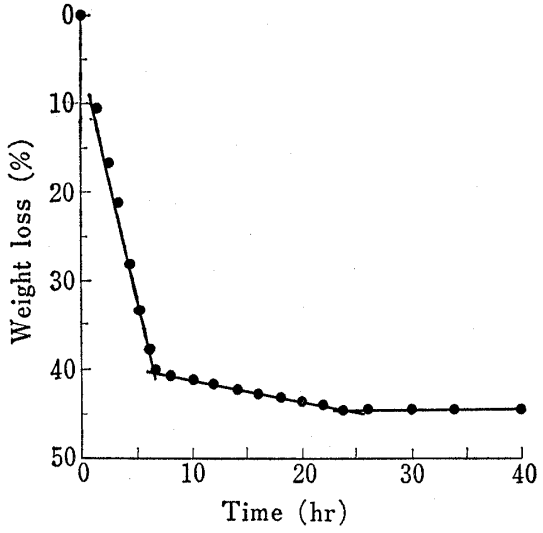

Fig. 1. Weight Loss Curve of the Wet Crystals obtained from Methanol by Drying over Silica gel at 20° and at Atmospheric Pressure

DL–PC·4MeOH·1H_2O is converted to amorphous form by drying at 50—80° and to methanol solvate by standing in a closed container for 1—2 weeks at 30—40°. It was also found that by allowing to stand in various relative humidities at 20° for 1—2 months, DL–PC·4MeOH·1H_2O is converted to amorphous form at below 60%, to monohydrate at 60—80% and deliquesced at above 80%.

B-Form

The crystals obtained from methyl cellosolve at below 20°, ethanol or aqueous ethanol containing less than 5% water are nonsolvated columns. These crystals are named B-form.

Monohydrate and 3/4 hydrate

The crystals obtained from aqueous ethanol were found to comprize two kinds of hydrated columns. These crystals were confirmed to be monohydrate and a hydrate which contained 3/4 molecule of water of crystallization (hereinafter abbreviated as 3/4 hydrate) by thermal analysis and the Karl-Fisher method. Monohydrate tends to be obtained from aqueous ethanol containing more than 20% water, while 3/4 hydrate tends to be obtained from aqueous ethanol containing less than 20% water. The crystals obtained from aqueous isopropanol containing more than 10% water are generally monohydrate, but rarely are 3/4 hydrate.

C-Form and D-Form

The crystals obtained from isopropanol and aqueous isopropanol containing less than 10% water are nonsolvated needles, and the crystals obtained from aqueous isopropanol by slow crystallization at above 30° are nonsolvated long columns. The former crystals are named C-form and the latter is named D-form.

E-Form

The crystals obtained from methyl cellosolve at above 20° are nonsolvated needles. These crystals are named E-form. E-Form is presumed to be equal to the crystals obtained from methyl cellosolve by Ford[3] because both of the crystals appear as needles under the microscope and have a same melting point of 170—172°.

Amorphous Form

The microcrystalline powders are precipitated from the methanol or ethanol solution by addition of acetone, ether, ethyl acetate, *etc.* These powders were proved to be amorphous form by X-ray diffraction analysis.

The crystals obtained from methyl cellosolve, methanol, ethanol, isopropanol and the corresponding aqueous alcohols in this studies described above are summarized in Fig. 2.

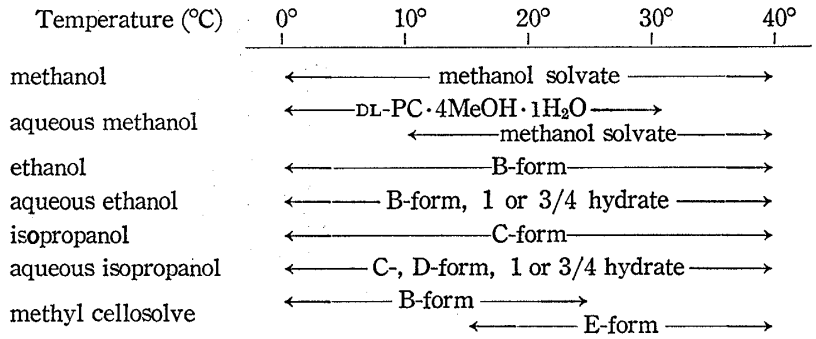

Fig. 2. The Crystal Forms of Calcium DL-Pantothenate obtained from Methyl cellosolve, Lower Alcohols and Aqueous Lower Alcohols

Thermal Analysis

The TG–DTA (thermogravimetry-differential thermal analysis) curves of the various forms of calcium DL-pantothenate are illustrated in Fig. 3. These thermograms differ clearly with each other in number and position of the endothermic peaks. DTA curves reveal that A–, B–, C–, D– and E-form are not solvated and melt without undergoing a polymorphic transformation. The thermograms of methanol solvate, monohydrate and 3/4 hydrate show an endothermic change and a weight loss at the same temperatures prior to melting.

These weight losses are nealy equal to the solvent content of these solvates. The melting point of methanol solvate is identical to that of A-form. The thermogram of DL-PC·4MeOH·$1H_2O$ suggests that the solvents of crystallization liberate through two steps.

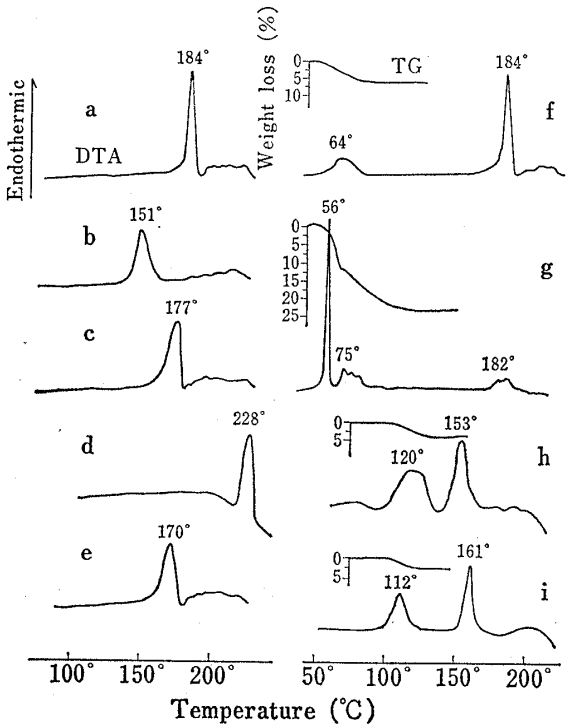

Fig. 3. TG-DTA Curves of Calcium DL-Pantothenate Crystal Forms

a: A-form b: B-form c: C-form d: D-form e: E-form f: methanol solvate g: DL-PC·4MeOH·$1H_2O$ h: monohydrate i: 3/4 hydrate

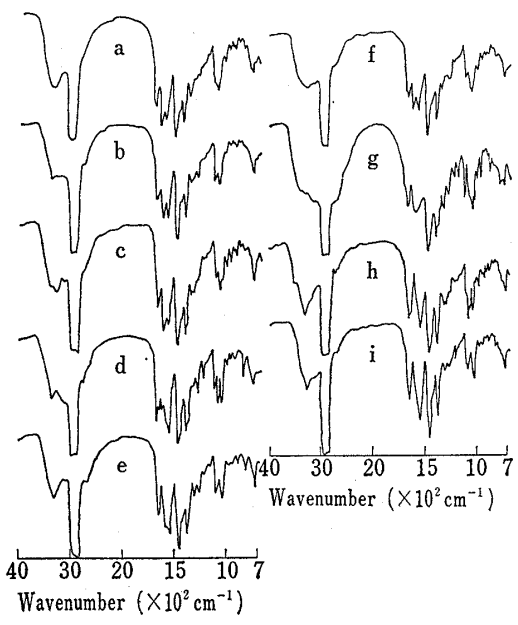

Fig. 4. Infrared Spectra of Calcium DL-Pantothenate Crystal Forms

a: A-form b: B-form c: C-form d: D-form e: F-form f: methanol solvate g: DL-PC·4MeOH·$1H_2O$ h: monohydrate i: 3/4 hydrate

When these solvates are packed into a capillary tube, methanol solvate, monohydrate and 3/4 hydrate melt at the temperatures of these melting points in Fig. 3, but DL–PC·4MeOH·$1H_2O$ melts at the first peak temperature (55—58°).

X-Ray Diffraction and IR Spectroscopy

The X-ray diffraction patterns and the infrared spectra for the various forms of calcium DL-pantothenate are shown in Table I and Fig. 4. These distinguishing patterns and spectra can be utilized for the identification of the various forms. X-ray diffractometry was also used successfully to measure the quantities of the various forms in mixtures.

TABLE I. X-Ray Powder Diffraction Patterns of Calcium DL-Pantothenate Crystal Forms

A–Form		B–Form		C–Form		D–Form		E–Form		Methanol solvate		DL-PC·4MeOH·$1H_2O$		Monohydrate		3/4 hydrate	
2θ	I/I_1	2θ	I/I_1	2θ	I/I_1	2θ	I/I_1	2θ	I/I_1	2θ	I/I_1	2θ	I/I_1	2θ	I/I_1	2θ	I/I_1
5.6	90	7.0	100	7.5	100	6.6	100	5.0	100	5.4	66	10.3	100	5.3	53	5.0	96
8.4	53	12.2	92	9.4	9	9.4	9	9.4	31	6.5	49	10.8	33	8.2	100	8.8	48
11.6	100	14.3	48	11.3	15	13.2	12	10.7	51	7.3	31	17.7	22	10.7	32	10.5	100
15.3	38	15.9	36	12.3	16	15.8	10	10.9	47	10.0	46	20.5	11	12.2	42	13.0	38
16.8	32	16.7	27	13.6	11	18.4	8	11.4	64	11.8	100	21.1	19	15.5	19	15.6	62
17.3	31	18.1	41	14.7	13	18.7	5	16.3	91	14.1	40	21.6	11	16.5	44	16.5	94
18.6	36	18.7	35	15.0	15	19.8	31	17.9	48	16.4	34			17.6	16	17.7	82
21.6	29	20.1	35	16.7	9	20.9	10	19.5	79	19.5	54			17.9	17	18.5	62
		20.8	21	17.5	9	21.7	16	20.3	43	21.1	29			18.2	18	22.0	30
		21.3	59	18.9	18	25.6	5	21.6	45	21.6	51			19.6	23	25.9	64
		25.6	19	20.1	11	26.5	5	26.8	37	22.4	29			21.6	26	27.5	33
		26.9	24	20.9	23					25.7	20			26.4	20		
				22.3	21					27.4	29			26.7	16		
				26.4	12					29.0	23			27.1	16		

2θ: degree, I/I_1: relative intensity

Racemic Structures

In gaseous or liquid states and in solution, a racemic modification is usually an ideal or nearly ideal mixture of equal numbers of enantiomeric molecules, but in the solid state, the in interrelation terrelation of D– and L–molecules is different.[6] As a result, a racemic modification in the solid state exists in three kinds of racemic structures, as racemic mixture, racemic compound and racemic solid solution. Optical resolution by preferential crystallization is applicable to racemic mixture among these.

In order to examine the optical resolution of calcium DL-pantothenate by preferential crystallization, the racemic structures of the various forms were examined by comparing the X-ray diffraction patterns and the IR spectra with those for crystal forms of calcium D(+)-pantothenate already described.[2] As the results, the patterns and the spectra for C-form and DL–PC·4MeOH·$1H_2O$ were found to be identical to those for α-Form and D–PC·4MeOH·$1H_2O$ respectively. It was also found that the melting points of C-form and DL–PC·4MeOH·$1H_2O$ are lower than those of α-form and D–PC·4MeOH·$1H_2O$ respectively, and the solubilities of two former crystals are higher than those of two latter crystals. On the basis of these facts, C-form and DL–PC·4MeOH·$1H_2O$ were proved to be racemic mixtures and the optical resolutions of the two racemic mixtures by preferential crystallization were examined. A part of the optical resolution of DL–PC·4MeOH·$1H_2O$ has been already reported from this institute.[7]

6) E.L. Eliel, "Stereochemistry of Carbon Compounds," Mcgraw-Hill Book Company, Inc., New York, 1962, pp. 43—44.
7) N. Okuda, I. Kuniyoshi, and H. Tukamoto, Japan Patent 49-27168(1974).

The patterns and the spectra for the other forms except C-form and DL–PC·4MeOH·$1H_2O$ are not agreed with those for crystal forms of calcium D(+)-pantothenate. Therefore, these forms are presumed to be racemic compounds.

Optical Resolution of C-Form

Optical resolutions of organic compounds by preferential crystallization have been studied by various authors.[8] The solvents used for this resolution are limited to the solvents from which seed crystals and the racemic mixture are crystallized ,and in order to obtain reproducible results, it is considered necessary that seed crystals and the racemic mixture are stable forms.

α-Form of calcium D(+)-pantothenate was crystallized from methyl cellosolve, methanol, ethanol, isopropanol or the corresponding aqueous alcohols, [2] but C-form of calcium DL-pantothenate was spontaneously crystallized only from isopropanol and aqueous isopropanol. Therefore, the crystallization of C-form from the solvents except isopropanol and aqueous isopropanol was examined by seeding the supersaturated solutions of calcium DL-pantothenate with C-form. As the result, C-form was found to be crystallized not only from isopropanol and aqueous isopropanol, but also from methanol, ethanol and the corresponding aqueous alcohols.

Next, in order to examine the relative physical stability of C-form and the other forms of calcium DL-pantothenate in these solvents, the solubilities of the various forms were determined. The results of the determination are shown in Table II. It was found from the data that C-form was not a stable form in the range of this experiment because it was more soluble form. In isopropanol calcium DL-pantothenate was slightly soluble and the supersaturated solution was labile, and hence isopropanol was also found to be not an appropriate solvent for this resolution.

Table II. Solubilities for the Various Crystal Forms of Calcium DL-Pantothenate

Solvent	Temperature (C°)	C–Form	Methanol solvate	DL-PC·4MeOH·$1H_2O$	B–Form	Monohydrate	3/4 Hydrate	D–Form
Methanol	0	5.78	0.95					
	20	8.39	1.32					
	40	12.1	6.32					
95% methanol	0	17.2	3.54	1.68				
	20	22.3	5.96	2.45				
	40	28.0	18.2					
Ethanol	0	0.32			0.05			
	20	0.44			0.07			
	40	0.67			0.11			
90% ethanol	0	37.4			4.33	0.87	0.82	
	20	58.2			8.96	1.85	1.42	
	40	78.5			12.37	4.37	3.52	
95% isopropanol	0	0.12				0.04	0.03	0.01
	20	0.14				0.06	0.05	0.02
	40	0.16				0.08	0.07	0.05
80% isopropanol	0	10.7				2.88	2.92	0.30
	20	16.0				6.26	6.45	0.48
	40	21.4				11.5	12.1	0.56

unit; $\times 10^{-3}$M/100 g

8) R.M. Secor, *Chem. Rev.*, **63**, 297 (1963).

From these results, the favorable condition for the resolution of C-form can not be found. But the supersaturated solutions except isopropanol of calcium DL-pantothnate are very stable, and accordingly the resolution of C-form was examined by seeding the supersaturated methanol, ethanol, aqueous methanol, aqueous ethanol and aqueous isopropanol solutions with α-form of calcium D(+)-pantothenate. The changes in the optical rotation of these solutions after seeding are shown in Fig. 5. As can be seen from Fig. 5, the resolution was accomplished with all of the solvents. But the optical purities of the crystals obtained were low and the reproducibility of the method was poor. It was found by X-ray diffraction analysis that this was because the stable forms of calcium DL-pantothenate were crystallized during the resolution.

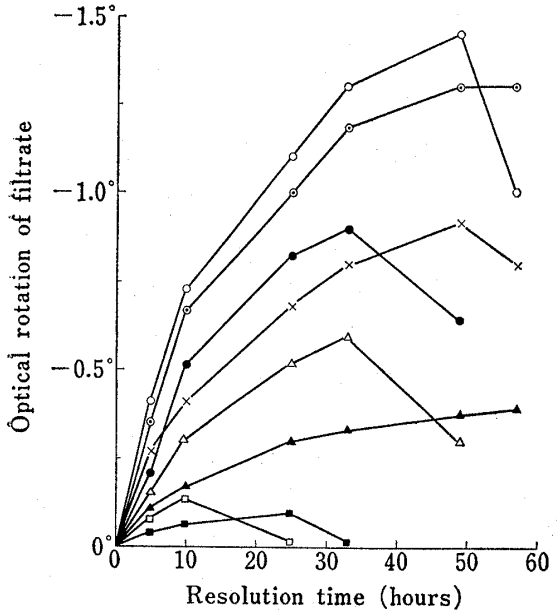

Fig. 5. The Resolution of C-Form of Calcium DL-Pantothenate

—○—: methanol at 40°, —●—: methanol at 20°,
—⊙—: 95% methanol at 30°, —△—: ethanol at 40°
—▲—: ethanol at 20°, —×—: 95% ethanol at 30°,
—□—: 90% isopropanol at 40°, —■—: 90% isopropanol at 20°

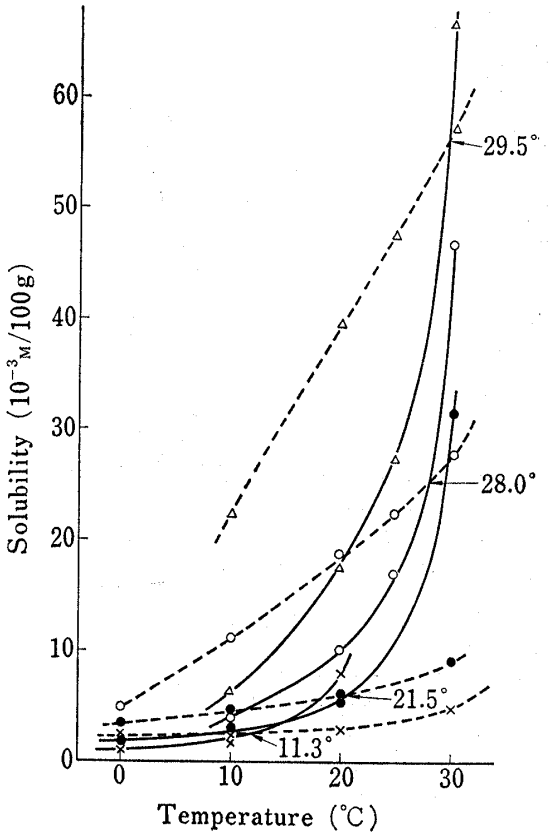

Fig. 6. Solubility Curves for DL-PC·4MeOH·$1H_2O$ and Methanol Solvate of Calcium DL-Pantothenate in Aqueous Methanol

——: DL-PC·4MeOH·$1H_2O$, ------: methanol solvate
×: 98% methanol, ●: 95% methanol, ○: 90% methanol, △: 85% methanol

Optical Resolution of DL-PC·4MeOH·$1H_2O$

The solvent for the resolution of DL–PC·4MeOH·$1H_2O$ is only aqueous methanol because DL– and D–PC·4MeOH·$1H_2O$ are not crystallized from solvents except aqueous methanol. From aqueous methanol, DL– and D–PC·4MeOH·$1H_2O$ are obtained at low temperatures but methanol solvate of calcium DL–pantothenate and α-form of calcium D(+)-pantothenate are crystallized at high temperatures. Then, in order to determine the transition temperatures between DL–PC·4MeOH·$1H_2O$ and the methanol solvate, and between D–PC·4MeOH·$1H_2O$ and α-form, the dissolution rates and solubilities of these forms were measured. The solubility curves of these crystals are shown in Fig. 6 and 7. The transition temperature corresponds to the temperature at which solubility curves of the two forms in

a given solvent cross and the less soluble form is stable.[9] It was found from the data that DL- and D-PC·4MeOH·1H_2O are stable forms at low temperatures, and the transition temperatures between each of the two forms for the two systems are shifted to higher temperatures with increase of water content of solvent. The transition temperature curves for the two systems are shown in Fig. 8.

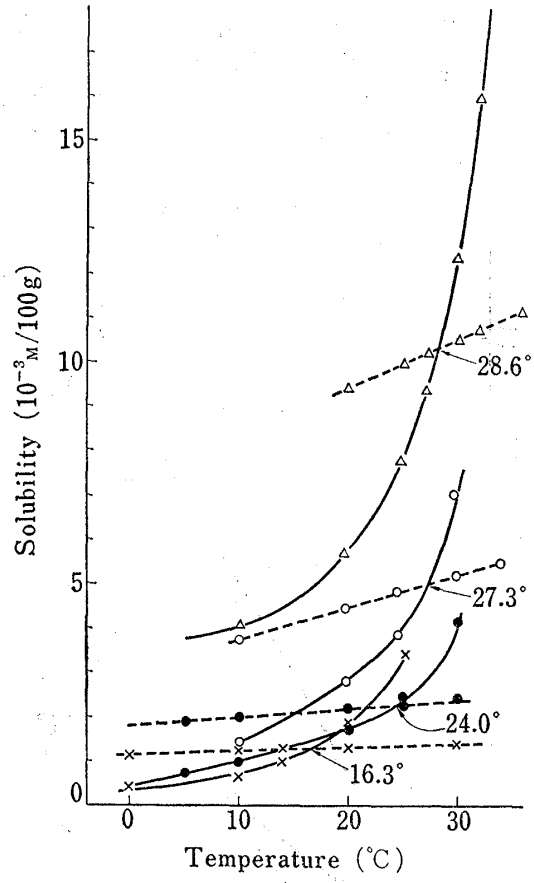

Fig. 7. Solubility Curves for D-PC·4MeOH·1H_2O and α-Form of Calcium D(+)-Pantothenate in Aqueous Methanol

——: D-PC·4MeOH·1H_2O, ------: α-Form
×: 98% methanol, ●: 95% methanol, ○: 90% methanol, △: 85% methanol

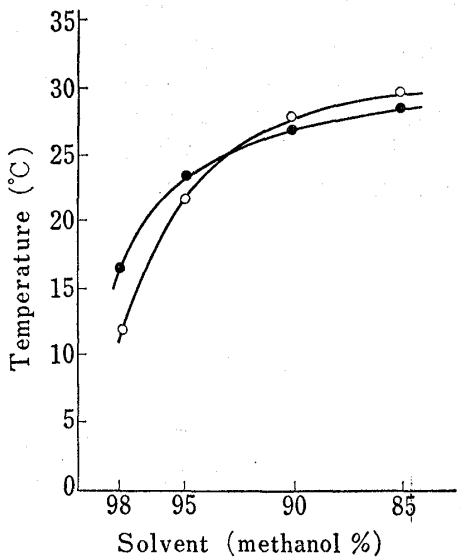

Fig. 8. Transition Temperatures between DL-PC·4MeOH·1H_2O and Methanol Solvate, and between D-PC·4MeOH·1H_2O and α-Form

—○—: DL-system, —●—: D-system

On the basis of the data, the optical resolution of DL-PC·4MeOH·1H_2O was carried out by seeding its supersaturated aqueous methanol solution with D-PC·4MeOH·1H_2O. As the results, the resolution was successfully carried out in the stable forms region of D- and DL-PC·4MeOH·1H_2O, but could not be accomplished in the stable forms region of the methanol solvate and α-form.

Table III. The Alternate Resolutions of DL-PC·4MeOH·1H_2O

	Seed	Yield(g)	$[\alpha]_D^{20}$ a)	Optical purity(%)
1 st	D	17.4	+26.0°	93
2 nd	L	34.1	−25.8°	92
3 rd	D	35.0	+25.4°	91
4 th	L	35.8	−25.6°	91
5 th	D	34.5	+26.1°	93

100 g of calcium DL-pantothenate was dissolved in 200 g of 95% methanol.
The alternate resolutions were accomplished by seeding of 1.0 g of D- and L-PC·4MeOH·1H_2O in the solution at 10—12°.
a) specific rotation of pure calcium D-pantothenate; $[\alpha]_D^{20}$ +28.0° (c=3, H_2O)

9) J. Haleblian and W. Mccrone, *J. Pharm. Sci.*, **58**, 911 (1969).

The resolution was also accomplished in the stable forms region of D–PC·4MeOH·1H_2O and the methanol solvate. This is considered to be because the supersaturated solution of the methanol solvate is stable and the rate of crystallization of it is very slower than that of D–PC·4MeOH·1H_2O in the region.

A result of the alternate D– and L–resolutions by this method is shown in Table III.

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