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Physico-chemical Analysis of 3,3,5-Trimethylcyclohexyl DL-Mandelate

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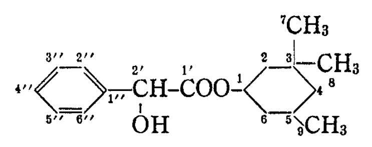
The diastereomeric isomer ratio of 3,3,5-trimethylcyclohexyl DL-mandelate (cyclandelate) was examined by various physico-chemical methods. Capillary gas chromatography and ^{13}C nuclear magnetic resonance spectroscopy showed that the bulk of cyclandelate which had been determined by proton magnetic resonance to have an isomer ratio of 73:27 was actually a mixture of nearly equal amounts of the two isomers. The reason for the difference in the results was studied in detail by an analysis of the physical properties of solid solutions of the isomers.

Keywords——3,3,5-trimethylcyclohexyl DL-mandelate diastereomeric isomer; separation of diastereomers; glass capillary gas chromatography; liquid chromatography; X-ray analysis; CMR; PMR; solid solution

Cyclandelate, 3,3,5-trimethylcyclohexyl DL-mandelate, is a vasodilator which improves blood flow in peripheral tissues.[1] It has three asymmetric carbon atoms in its chemical structure, one in the mandelic acid moiety and the other two in the trimethylcyclohexanol moiety. As a consequence, eight isomers are theoretically expected to exist. However, since the drug is manufactured from *cis*-3,3,5-trimethylcyclohexanol with DL-mandelic acid, only the four isomers listed in Table I are contained in cyclandelate in bulk. Of these, A and C are the optical antipodes of B and D, respectively. Ernsting and Nauta[2] have synthesized these four isomers in pure form by the reaction of optically pure D- or L-mandelic acid with optically pure D- or L-*cis*-3,3,5-trimethylcyclohexanol. The physicochemical properties and biological activity of the isomers were reported in their paper. They also described a method of separating a racemic pair, A and B, from the other pair, C and D, out of a reaction mixture obtained by using racemic starting materials. Recrystallization of the reaction mixture from 90% ethanol afforded crystals with a melting point of 84°. From the mother liquor, crystals melting at 55—56° were recovered. By comparison of the melting points and solubilities in ethanol

Table I. Isomers of *cis*-3,3,5-Trimethylcyclohexyl Mandelate



Absolute confirguration of mandelic acid moiety		Absolute configuration of cyclohexanol moiety[a)] Position 1	Position 5
A	*S*	*R*	*R*
B	*R*	*S*	*S*
C	*R*	*R*	*R*
D	*S*	*S*	*S*

a) The cyclohexanol moieties of A, C and B, D are levorotatory and dextrorotatory, respectively.[2] The absolute configuration of (−)-*cis*-3,3,5-trimethylcyclohexanol is assigned as R on the basis of its chemical correlation with pulegone.[3]

with those of the synthetic A, B, C, and D, they proposed that the former crystals were the A, B antipode pair and the latter, the C, D pair.

Vlies *et al.*[4] measured the proton magnetic resonance spectra of these so-called A, B and C, D antipode pairs and found that their spectra showed different relative heights of the resonance peaks at $\delta=0.94$ and 0.88. Based on this observation, they proposed a method of determining the isomer ratio AB/CD in bulk material manufactured for medical use. Since then, the method has been widely used and most of the bulk materials were estimated to be composed of 20—30% AB pair and 70—80% CD pair.[5] We examined a cyclandelate bulk sample on a capillary gas chromatograph and unexpectedly found a noticeable discrepancy between the mixture ratios determined by this method and by Vlies' method. The present paper describes a careful study of this discrepancy along with an intensive investigation into the physico-chemical properties of the AB and CD pair crystals and of various mixtures of them. Several methods for mixture ratio analysis are also proposed.

Experimental

Nuclear Magnetic Resonance Spectra——Proton magnetic resonance spectra were measured on a Varian EM-390 spectrophotometer (90 MHz) with $CDCl_3$ as a solvent at a sample concentration of about 10%. Carbon spectra (^{13}C) were obtained with a Varian XL-100-12 spectrometer (25.2 MHz) under the following conditions; sweep width, 200 ppm; data points, 8192; accumulation, 5000—50000 times. $CDCl_3$ was used as an internal standard, and by assigning its peak as 76.9 ppm, the shifts of the sample peaks from the TMS peak were calculated in ppm.

X-Ray Powder Diffraction Patterns——A Rigaku Denki Rotaflex RU-3 diffractometer (CuKα) was used. Samples were powdered in an agate mortar and loaded on a glass plate.

Infrared Spectra——A Hitachi model 260-10 spectrometer was used. Spectra in the solid state were obtained by the Nujol mull method. To measure solution spectra, 10% CS_2 and CCl_4 solutions were prepared and the spectrum of each solution was determined in a fixed liquid cell of 0.5 mm thickness.

Differential Thermal Analysis——Experiments were performed on a Perkin Elmer DSC-1B in a stream of dry N_2 gas. The temperature was raised at a rate of 0.625°/min.

Capillary Gas Chromatography——A Shimadzu GC-5A (FID) was used. The conditions were as follows.
Column: FFAP glass capillary column (0.28 mm i.d., 30 m in length)
Carrier gas: He, 6 ml/min
Make-up gas: He, 50 ml/min
Split ratio: 10: 1
Column temperature: 170°
Injection port and detector temperature: 230°
Chart speed: 5 mm/min
Samples were dissolved in *n*-hexane to make an about 1% solution, and 0.5 μl was injected into the port.

Column Chromatography——A column of 25 mm i.d. and 310 mm in length was packed with Merck Lobar column LiChroprep Si 60, size B. A mixture of *n*-hexane and isopropyl ether (4: 1) was used as the eluting solvent. The flow rate was adjusted to 6 ml/min by using a Kyowa Seimitsu KHD-94 minimicropump. An LKB Uvicord II (254 nm) was used as the detector.

Results and Discussion

Fig. 1-a shows a capillary gas chromatogram of a cyclandelate bulk sample, the composition of which had been determined to be AB/CD =27: 73 by Vlies' NMR method. The peaks at $t_R=21$ and 22 min are nearly the same in both height and width. The peaks are sharp and symmetrical and have no tailing or leading characteristic of thermal degradation products in the port or column. Each of the peaks was, thus, tentatively attributed to either the AB or CD pair. As the sensitivity of AB and CD pair to the flame ionization detector is considered to be the same, this chromatogram suggests that the mixing ratio of AB and CD pairs is 1: 1. To clarify this discrepancy, an attempt was made to isolate pure AB and CD pairs according to the procedure described by Ernsting and Nauta.[2] After several recrystallizations from 90% ethanol, crystals having a sharp melting point at 84° were obtained in exact accordance with their results. The capillary gas chromatography of the crystals afforded a

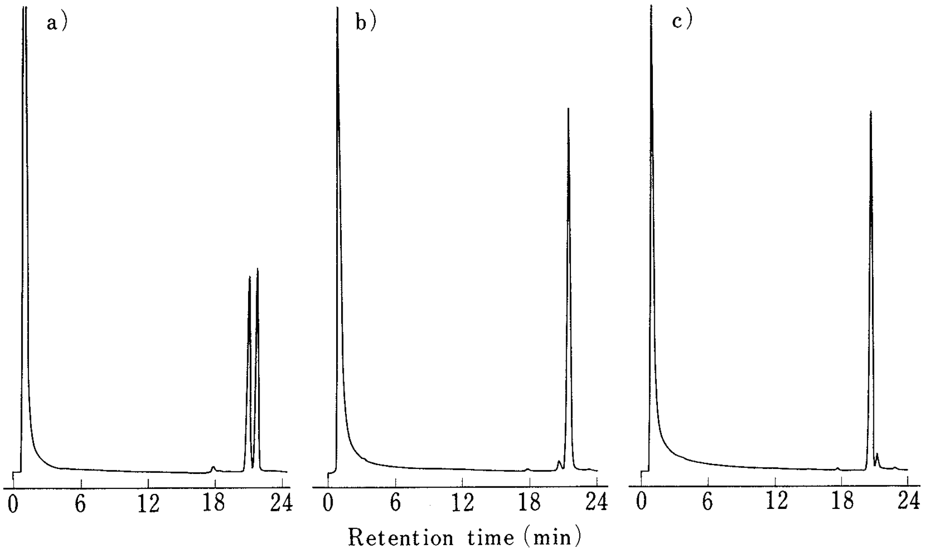


Fig. 1. Glass Capillary Gas Chromatograms of *cis*-3,3,5-Trimethylcyclohexyl Mandelates

a) Cyclandelate,
b) AB Antipode pair,
c) CD Antipode pair.

strong peak, which corresponded to the longer t_R peak, (t_R=22 min) accompanied by a very small one at t_R=21 min (Fig. 1-b). From the relative peak height, the content of the major component was calculated to be 98.2%.

Crystals recovered from the mother liquor were also purified by recrystallization from the same solvent. The melting point of the product converged to 56°, which coincided with the description in the literature.[2] The gas chromatogram of the crystals, however, showed two peaks of medium height, the relative height being 7: 3 for the peaks at t_R=21 min and 22 min. Although the melting point was fairly sharp, we assumed that the crystals were still a rough mixture and attempted separation by column chromatography. After elaborate selection of absorbent and eluting solvent, the chromatogram shown in Fig. 2 was obtained on loading about 450 mg of the sample. Even in this case, clear separation was not the achieved and the two components heavily overlapped, failing to afford two maxima. We, therefore, collected ten percent portions of eluate from the shortest and the longest retention time fractions, as shown by dot-dashed lines in Fig. 2.

Capillary gas chromatographic determination revealed that the CD content of the longest t_R fraction, indicated by b) in Fig. 2, was about 75%. More than ten repetition of this process afforded about 450 mg of CD-rich fraction. The pooled CD-rich fraction was rechromatographed and the longer t_R fractions, about 20 to 30%, were collected and found to have a CD content of about 90%. Through two more consecutive column chromatographic separations, about 50 mg of CD fraction of 97.9% purity was obtained. The capillary gas chromatogram of this final product is shown Fig. 1-c.

Proton magnetic resonance spectra of the purified AB and CD pairs thus obtained are shown in Figs. 3-b and c, together with the spectrum (Fig. 3-a) of the initial bulk sample which gave the gas chromatogram shown in Fig. 1-a. Fig. 3-b coincides with the PMR spectrum of the purified AB pair obtained by Vlies *et al.*,[4] whereas Fig. 3-c is clearly different from the reported spectrum of their purified CD pair. The relative heights of the peak at δ=0.94 to that at δ=0.88 were 2.8 in Fig. 3-b and 0.35 in Fig. 3-c. According to Vlies *et al.*, the relative peak heights for their purified AB and CD pairs were 2.8 and 0.65, respectively. Simple calculation from these values showed that Vlies' purified CD pair contained about 30% AB pair. The analytical results hitherto obtained on the basis of this impure CD standard sample should thus be revised in view of our present work.

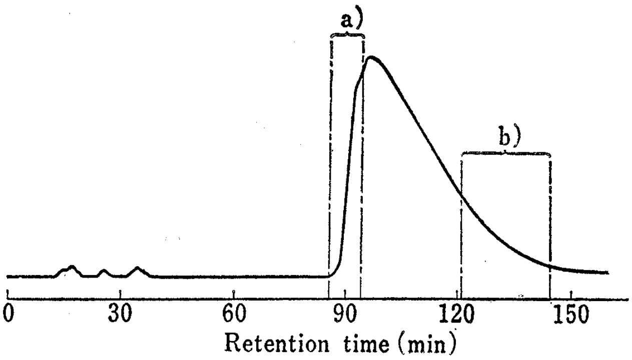


Fig. 2. Column Chromatograms of Cyclandelate (or *cis*-3,3,5-Trimethylcyclohexyl (+)-Mandelate)

a) Cut collected for isolation of AB or A (*ca.* 10% of total peak area. AB or A content: 77%).
b) Cut collected for isolation of CD or D (*ca.* 10% of total peak area. CD or D content: 75%)

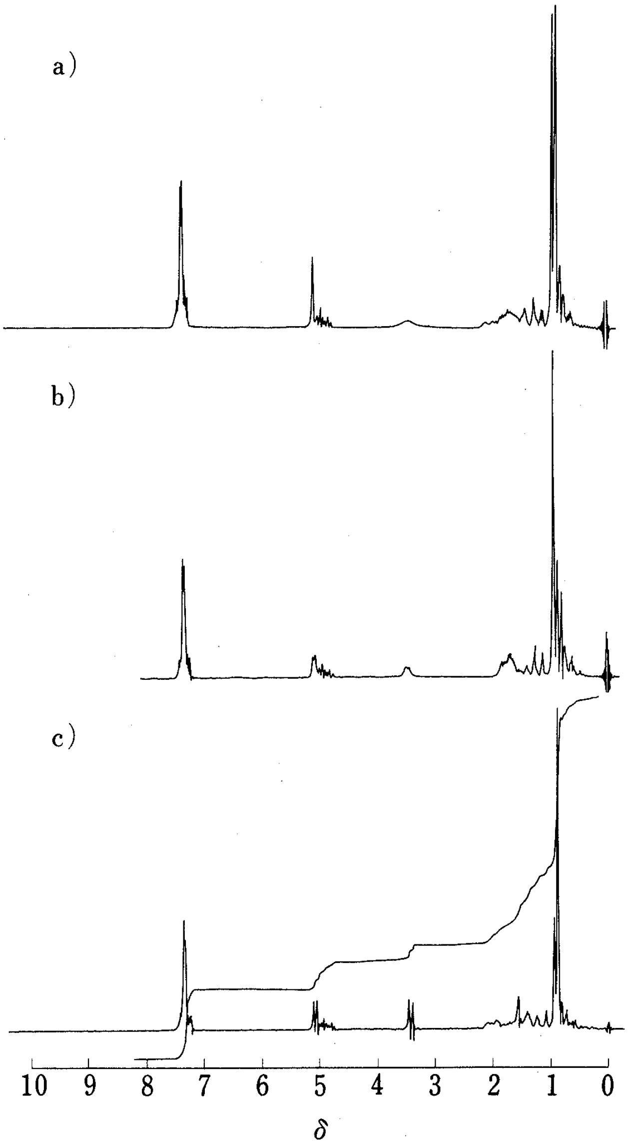


Fig. 3. Proton Magnetic Resonance Spectra of *cis*-3,3,5-Trimethylcyclohexyl Mandelates (90 MHz, in $CDCl_3$)

a) cyclandelate,
b) AB antipode pair,
c) CD antipode pair.

One of the reasons for such erroneous results is presumably the complexity of the proton magnetic resonance peaks used for the ratio analysis. The *gem*-dimethyl group at position 3 of the cyclohexane ring in the A*B* and CD pairs gives peaks at $\delta=0.94$ and $\delta=0.88$ respectively. However, the doublet due to the 5-methyl group in the AB and CD pairs (AB: $\delta=0.84$, $J=6$ Hz, CD: $\delta=0.91$, $J=6$ Hz) overlaps these two peaks. Thus, even the pure form of the AB pair has a moderately high peak indistinguishably close to $\delta=0.88$, which is the lower field component of the doublet ($\delta=0.87$ and 0.81) due to the 5-methyl group. Similarly, the pure CD pair shows a peak of medium intensity at $\delta=0.94$ at the foot of the strong peak at $\delta=0.88$, the former being the lower field component of the doublet ($\delta=0.94$ and 0.88) due to the 5-methyl group and the latter being the overlapped peak of the singlet due to the 3-*gem*-dimethyl group with the higher field component of the above doublet. Consequently, a purity check based on these NMR peaks leaves room for improvement.

In contrast, we could obtain two completely separated peaks for AB and CD pairs by capillary gas chromatography, and interpretation of the analytical results was easier and much more accurate. Another method for isomer ratio analysis is provided by ^{13}C nuclear magnetic resonance spectroscopy. Fig. 4 shows the spectra of three samples corresponding to those which gave the PMR spectra in Fig. 3. Though the overall patterns of the three ^{13}C spectra are similar, the carbon at positions 2 and 6 of the cyclohexanol ring resonate at slightly different frequencies in the AB and CD pairs. The chemical shifts of the two carbons in the AB pair were 43.8 and 39.7 ppm whereas in the CD pair they were 43.2 and 40.1 ppm. These small differences, 0.6 ppm for the carbon at position 2 and 0.3 ppm for that at position 6, are large enough to afford two pairs of completely separated peaks. The isomer ratio determined by measuring the intensities of those peaks should be reliable. As is clear from Fig. 4-a, the

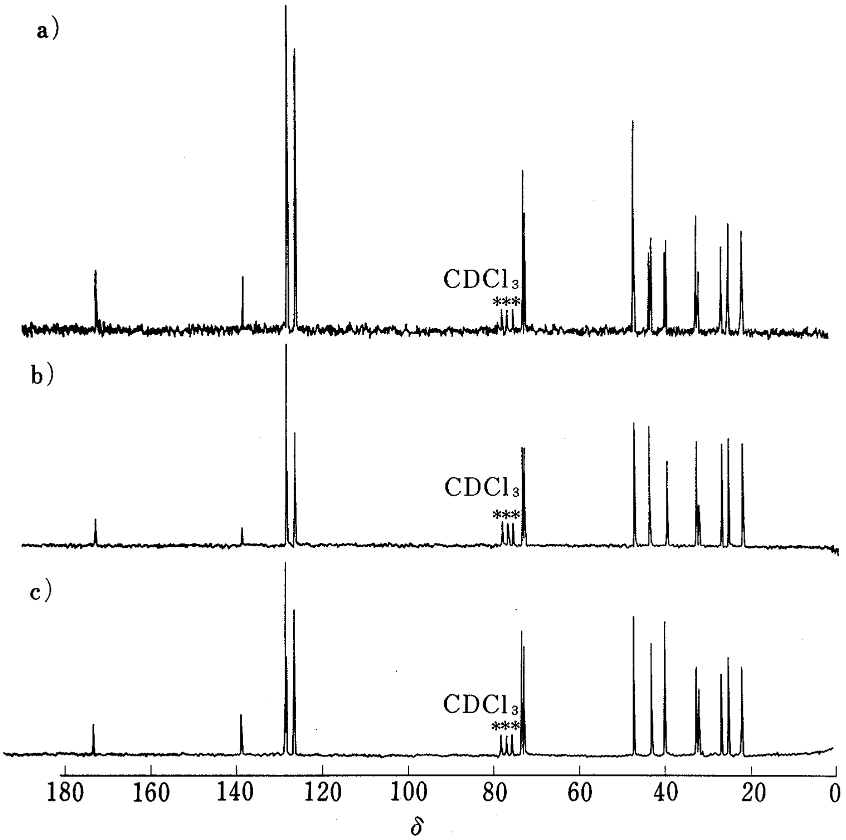


Fig. 4. ^{13}C-Nuclear Magnetic Resonance Spectra of *cis*-3,3,5-Trimethylcyclohexyl Mandelates (25.2 MHz, in $CDCl_3$)

a) cyclandelate,
b) AB antipode pair,
c) CD antipode pair.

Table II. ^{13}C-Chemical Shifts of *cis*-3,3,5-Trimethylcyclohexyl Mandelates

Carbon[a]	Cyclandelate	AB antipode pair	CD antipode pair
1	73.3(d)	73.4	73.3
2	43.3(t), 43.8(t)	43.8	43.2
3	32.2(s)	32.2	32.1
4	47.4(t)	47.3	47.3
5	26.9(d)	27.0	27.0
6	39.7(t), 40.2(t)	39.7	40.1
7 (eq.)	32.8(q)	32.9	32.8
8 (ax.)	25.4(q)	25.4	25.3
9 (eq.)	22.1(q)	22.0	22.1
1′	172.9(s)	172.9	172.9
2′	72.8(d)	72.8	72.8
1″	138.5(s)	138.5	138.4
2″, 6″	126.2(d)	126.3	126.2
3″, 5″	128.2(d)	128.3	128.2
4″	128.0(d)	128.0	128.0

a) Numbering of carbons is shown in Table I.

bulk sample of cyclandelate, which was determined by Vlies′ method to be composed of 27% AB and 73% CD pairs, exhibited two doublets at about 43 and 40 ppm consisting of two peaks with nearly the same intensity. This indicates that the isomer ratio obtained by capillary gas chromatography is correct. To make the results clearer, assignment of each peak in Fig. 4

was carried out by the off-resonance and the selective proton decoupling methods. The assignments are listed in Table II.

Using the 98% pure AB and CD pairs obtained in this work as starting material, crystals of mixtures containing both pairs in various ratios were prepared by crystallization from concentrated solutions, and their X-ray powder diffraction patterns and differential scanning calorimetric thermograms were obtained. As can be seen in Fig. 5, the pure AB and CD antipode pairs gave distinctly different powder diffraction patterns. Mixtures containing less than 20% of the CD pair showed patterns which were not very different from that of the pure AB pair. At a higher CD pair content, the X-ray pattern became complicated and peaks due to the CD pair crystals were observed at various places. On the other hand, in mixtures containing more than 50% of the CD pair, the peaks due to the AB pair disappeared almost completely, and therefore the pattern of an equimolar mixture of the AB and CD pairs was difficult to distinguish from that of the pure CD pair. This evidence suggests that the lattice point of the pure AB or CD crystals can be replaced by the molecules of the counter isomer without causing significant distortion of the lattice. In other words, AB pair molecules can form a solid solution with CD pair molecules up to 20% CD content in the AB crystal lattice and CD pair molecules can form a solid solution with AB pair molecules up to 50% content of the AB pair in the CD crystal lattice. The changes of the lattice space *d*-values accompanying changes of the constitution were obtained by analyzing diffraction patterns of mixtures having various isomer ratios and the results were plotted against the isomer ratio, as shown in Fig. 6. Although slight changes of *d*-values were seen on increasing the content of the counter isomer, they did not exceed 2% within the same crystal form (solid solution).

In accordance with the X-ray results shown above, the infrared spectrum of the cyclandel-

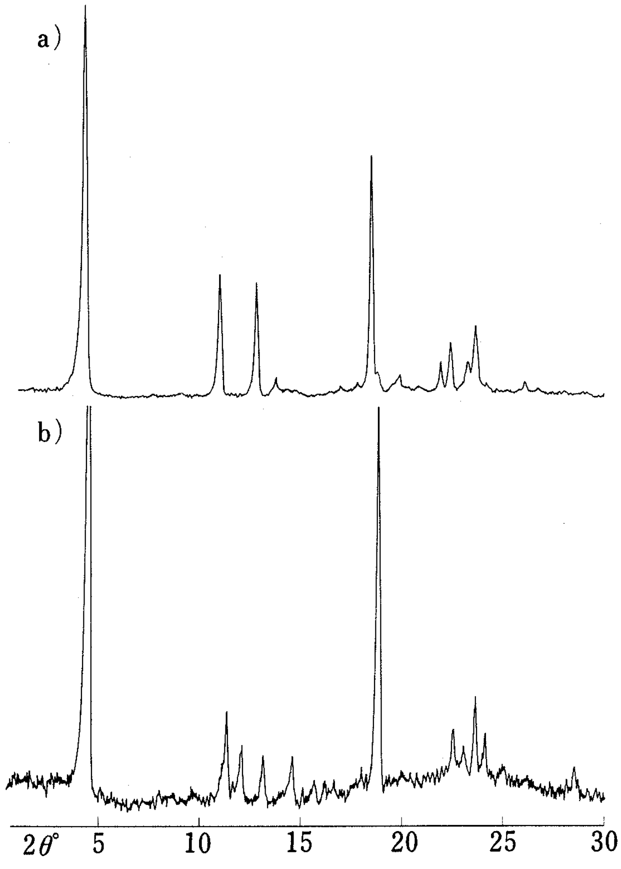


Fig. 5. X-Ray Powder Diffraction Patterns of *cis*-3,3,5-Trimethylcyclohexyl Mandelates

a) AB antipode pair,
b) CD antipode pair.

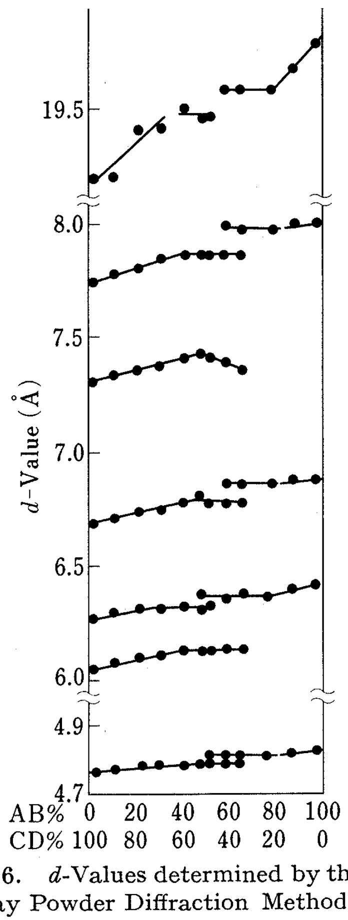


Fig. 6. *d*-Values determined by the X-Ray Powder Diffraction Method *versus* Compositions of Diastereoisomeric Pairs, AB and CD

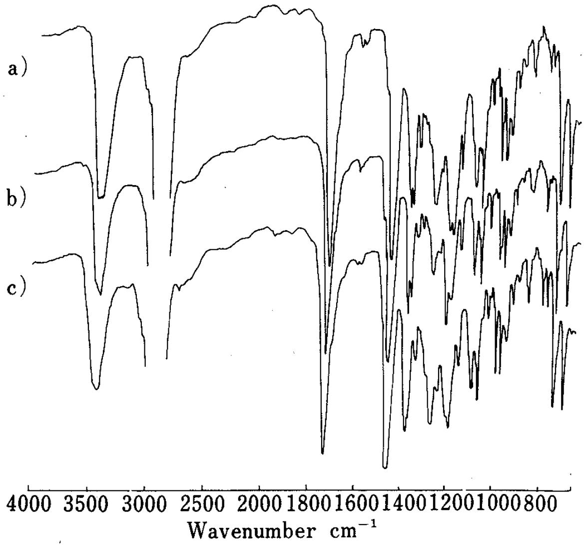


Fig. 7. Infrared Spectra of *cis*-3,3,5-Trimethylcyclohexyl Mandelates (Mull in Nujol)

a) Cyclandelate,
b) AB antipode pair,
c) CD antipode pair.

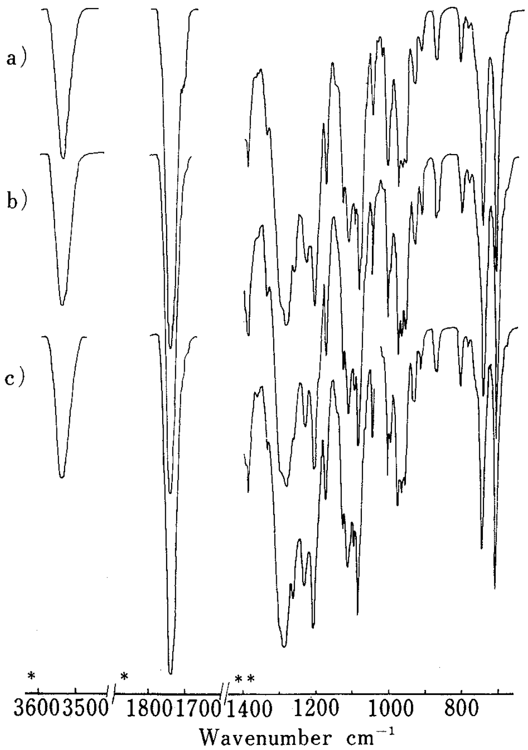


Fig 8. Infrared Spectra of *cis*-3,3,5-Trimethylcyclohexyl Mandelates (*in CCl_4, **in CS_2)

a) Cyclandelate,
b) AB antipode pair,
c) CD antipode pair.

ate bulk sample, which was determined to have an isomer ratio of 1:1 in the present work, closely resembled that of the pure CD sample, whereas the pure AB crystals gave a distinctly different infrared spectrum (Fig. 7).

In solution in carbon tetrachloride or in carbon disulfide, all three samples afforded indistinguishable spectra, as shown in Fig. 8.

Differential scanning calorimetric diagrams revealed another interesting feature of these solid solution (Fig. 9). Crystals of pure AB pair showed a sharp melting peak at 84°. Contamination with about 13% CD pair lowered the melting point by about 7° and the melting peak broadened considerably. Pure CD pair crystals exhibited a sharp peak at 58°. However, crystals containing 32.0% AB pair and those containing even 49.3% AB pair showed comparably sharp melting peaks at about 55° and 53°, respectively. These results imply that although the solid solution of the AB pair with the CD has usual thermographic properties, the solid solution of the CD pair with the AB behaves like single-component crystals over a very wide range of AB content. Because of this unusual property, mere recrystallization failed to give pure CD crystals, as described in a preceding section.

The phase diagram (Fig. 10) of the diastereomeric mixture of cyclandelate was drawn on the basis of the above thermographic results.

In the figure, the α-phase represents the solid solution of the AB pair in the CD pair. In this region, a sharp melting point is obtained with every composition of diastereomers. The solid solution of the CD pair in the AB is designated as the β-phase. Between these two phases, there is a region in which both crystal phases coexist.

A mixture belonging to this region begins to melt at about 53°. First, α-phase crystals having the isomer ratio of about 1 begin to melt and the content of β-phase in the remaining solid gradually increases as the temperature rises. The temperature at which melting is completed varies from 60° to 80° depending on the initial amount of β-phase crystals in the mixture. Thus, while contamination of the CD pair in the pure AB pair is readily detectable by a decrease in melting point, contamination of the AB pair in the pure CD pair cannot be

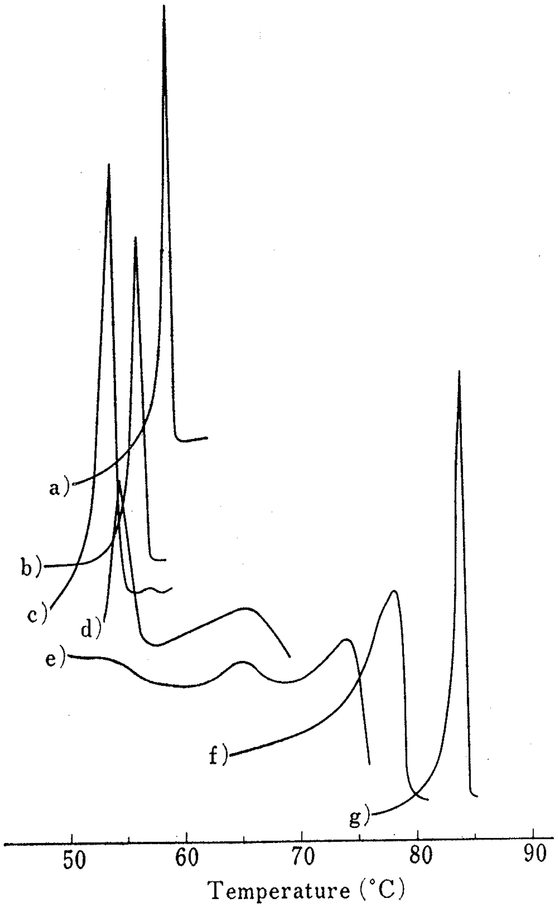


Fig. 9. Differential Scanning Calorimetric Thermograms of Diastereoisomeric Mixtures of AB and CD

	AB%	CD%		AB%	CD%
a)	2.1	97.9	e)	77.5	22.5
b)	32.0	68.0	f)	87.1	12.9
c)	49.3	51.7	g)	98.2	1.8
d)	65.8	34.2			

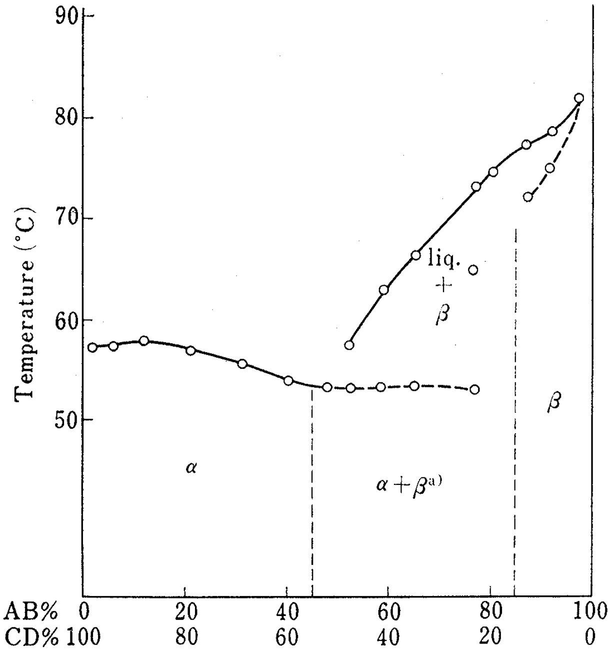


Fig. 10. Phase Diagram of Diastereoisomeric Mixture of AB and CD

○——○ melting point,
○----○ starting point of melting peak.
Eutectic phase may exist in the range of about AB 40 to 50% and about 80 to 90%.

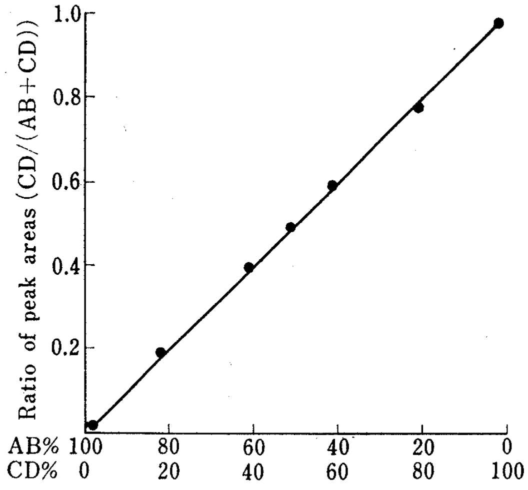


Fig. 11. Ratios of Gas Chromatographic Peak Areas (Glass Capillary Column) *versus* Composition of Diastereoisomeric Mixtures of AB and CD

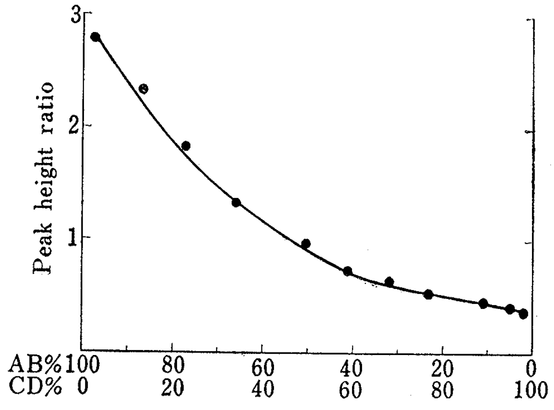


Fig. 12. Composition Calibration Curve for Diastereoisomeric Mixtures of AB and CD Based on the Peak Height Ratio of the Two PMR Peaks ($\delta=0.94/\delta=0.88$)

detected by measurement of melting point alone.

Finally, we prepared calibration curves for isomer ratio analysis by capillary gas chromatography and proton magnetic resonance spectrometry. The former curve is superior to the latter in that it gives a linear relationship in the whole range of the isomer ratio (Fig. 11). The slope of the latter curve is shallow in the range of AB content from 0 to 40%, resulting in relatively poor accuracy of analysis in this range (Fig. 12).

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