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Effects of Grinding and Humidification on the Transformation of Conglomerate to Racemic Compound in Optically Active Drugs

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Abstract

The effects of grinding and humidification on the transformation of conglomerate to racemic compound have been investigated by X-ray powder diffraction (XPD), differential scanning calorimetry (DSC) and infrared (IR) spectroscopy for leucine, norleucine, valine, serine, tartaric acid and malic acid.

Racemic physical mixtures were prepared by physical mixing of equimolar quantities of D and L crystals using a mortar and pestle. Ground mixtures were obtained by grinding the physical mixtures with a vibrational mill. Humidification was performed by storing the physical mixtures and the ground mixtures in a desiccator containing saturated aqueous salt solutions at 40°C.

When physical mixtures of malic acid, tartaric acid and serine were ground, the XPD peaks of the racemic compounds were observed. The XPD patterns of humidified physical mixtures of these compounds also showed the formation of the racemic compounds. This indicated that grinding or humidification of malic acid, tartaric acid and serine induced the transformation of conglomerate to racemic compound crystals. When, on the other hand, the physical mixtures of valine, leucine and norleucine were ground, peaks of racemic compounds were not detected in the XPD pattern. After humidification of the ground mixtures of valine, leucine and norleucine, however, the XPD peaks of racemic compounds were observed. DSC and IR studies revealed consistent results.

We concluded that grinding or humidification of malic acid, tartaric acid and serine could induce the transformation of a conglomerate to racemic compound. In contrast, humidifying after grinding was needed to bring about the transformation in leucine, norleucine and valine.

Drug stereoisomerism is increasingly being recognized as an issue with clinical, research and regulatory implications in drug development (Sørensen 1990). The different pharmacodynamic and pharmacokinetic properties of stereoisomers, molecules differing only in the spatial arrangement of certain atoms or groups at asymmetrical centres, is well documented (Levy & Boddy 1991). In the field of pharmaceutical technology, investigations concerning the stereoselectivity of enantiomeric drugs have also been reported, for example the stereoselective dissolution of propranolol hydrochloride from some chiral pharmaceutical excipients (Duddu et al 1993) and studies of stereoselective complex formation of some optically active substances (Vakily et al 1995). In pharmaceutical manufacturing, moreover, differences in significant physicochemical properties, e.g. melting temperature and solubility, between the optically active and racemic forms are currently of interest to pharmaceutical scientists (Dwivedi et al 1992). Many reports clearly reveal that ignoring the issue can lead to a useless drug product or even toxicity (Repta et al 1976; Lambert & Timmer 1991; Fassihi 1993; Touitou et al 1994). More basic research into physicochemical aspects are, however, needed for insight into chiral drug systems. Because many drugs are administered as racemates, racemic modifications in the solid crystalline state or in racemates is an issue of interest.

A racemate is defined as an equimolar mixture of two enantiomers that can belong to one of three different classes depending on its crystalline arrangement (Jacques et al 1991).

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The first is a racemic compound in which the two enantiomers are present in equal quantity in a well-defined arrangement within the same unit cell. Mandelic acid and benzylidenecamphor are examples of this category (Jacques et al 1991). The second type of a crystalline racemate is a racemic mixture or conglomerate, that is, a separable mechanical mixture of the two pure enantiomers, for examples, *m*-tyrosine and 3-chloro-2-decalone (Jacques et al 1991). The third class, called the pseudo-racemate or the racemic solid solution, is a solid solution formed by the two enantiomers co-existing in an unordered manner in the crystal. Camphor oxime when crystallized above 103°C was found to be in this class (Eliel 1962). In some systems, the temperature at which crystallization of the racemic substance occurs might determine the nature of the crystalline solid obtained. For instance, rubidium tartrate forms a racemic mixture above 40°C but a racemic compound below that temperature. These three classes of crystalline system are different in the relative strength, or affinity, between the intermolecular forces of like-like and like-unlike molecules in the crystal. For this reason differences in important physicochemical behaviour, for example, melting point, and the solubility, were recognized.

Knowledge about crystalline racemates has been used to explain many phenomena, for example the limitation of optical resolution and the separation of the two enantiomers that constitute a racemate by preferential crystallization (Inagaki 1977). Studies aimed at deeper understanding of the crystallization of a racemate to a conglomerate or racemic compound have also been performed (Bernal & Cetrullo 1988; Kimoto et al 1989; Kozma et al 1994; Böcskei et al 1995). Duddu & Grant (1992) have investigated the formation of the racemic compound of ephedrine base from its conglomerate in the

solid, liquid, solution and vapour states. Although grinding and humidification have been known to induce solid-state crystalline rearrangement of drugs in many systems, for example, polymorphic and hydrate compounds (Pirttimäki et al 1993; Ketolainen et al 1995), little such research has been conducted on optically active compounds.

This study was an attempt to investigate the effects of grinding and humidification on the transformation of conglomerate into racemic compound. Serine, leucine, norleucine and valine were chosen as model drugs because the majority of amino acids are optically active compounds and play an important role in many biological substances. The basic biochemical substances tartaric acid and malic acid were also used as drug samples.

Materials and Methods

Chemicals

D-, L- and DL- leucine, norleucine, valine, tartaric acid, malic acid and serine of reagent grade were purchased from Nacalai Tesque (Tokyo, Japan) and used as received. Magnesium chloride hexahydrate, ammonium nitrate and potassium chloride were also obtained from Nacalai Tesque and used as received. KBr powder was used for infrared (IR) spectroscopic measurements.

Preparation of physical mixture

Equimolar physical mixtures were prepared by physical mixing of 1.0 g each of the D and L forms by use of a mortar and pestle.

Preparation of ground mixture

Equimolar ground mixtures were obtained by grinding 2·0 g of the physical mixture by use of a vibrational mill (Heiko TI-200, Tokyo, Japan).

Humidification

Humidification was performed by storage of samples in desiccators containing saturated aqueous solutions of $MgCl_2 \cdot 6H_2O$, NH_4NO_3 and KCl for 31·5, 53·0 and 82·0 percent relative humidity, respectively, at 40°C.

X-ray powder diffraction

X-ray powder diffraction was performed with a Rigaku Denki 2027 diffractometer (Tokyo, Japan). Measurements were performed at 30 kV voltage, 5 mA current and a scanning speed of 4° min^{-1} with a CuKα radiation source.

Infrared spectroscopy

IR spectra of the serine, valine, leucine and norleucine systems were measured by the KBr disc method using a Nicolet ZDX Fourier-transform infrared (FTIR) spectrometer (WI, USA). The scanning range was 4000–400 cm^{-1}.

Differential scanning calorimetry (DSC)

DSC study of the tartaric acid and malic acid systems was performed with a DuPont model TA9900 instrument under nitrogen gas flow (60 mL min^{-1}). The sample (3 mg) was sealed in an aluminium pan and heated at 10° min^{-1}.

Results and Discussion

Malic acid, tartaric acid and serine systems

Crystals of the D and L isomers gave identical powder X-ray diffraction patterns, whereas the diffraction patterns of the racemic compounds were distinctly different. The physical mixtures of all compounds studied showed X-ray diffraction patterns identical with those of the corresponding enantiomers. Fig. 1 shows the changes in the powder X-ray diffraction pattern of the physical mixture of malic acid during the process of grinding. The physical mixture showed characteristic X-ray diffraction peaks of enantiomeric crystals at $2\theta = 7{\cdot}1°$, 19·0° and 29·0°. When the physical mixture was ground, the intensity of these peaks (some of which are indicated by arrows) gradually decreased with grinding time, whereas the characteristic peaks of the racemic compound (indicated by asterisks) were observed in the mixture ground for 3 min. After grinding for 5 min, no peak arising from the enantiomeric crystals could be observed and the X-ray diffraction pattern was similar to that of the racemic compound.

Similar results were observed for the tartaric acid and serine systems, as is shown in Figs 2 and 3, respectively. In the tartaric acid and the serine systems, ground mixtures gave diffraction patterns similar to those of the racemic compounds, even though it took some time for serine system to reach the grinding equilibrium.

The thermal behaviour of the physical mixture and the ground mixtures of malic acid are presented as DSC thermograms in Fig. 4. In the physical mixture, subsequent thermal events were observed: an endotherm at 106°C (peak a), an exotherm at 110°C (peak b) and an endotherm at 133°C (peak c). These were considered to correspond to melting of the

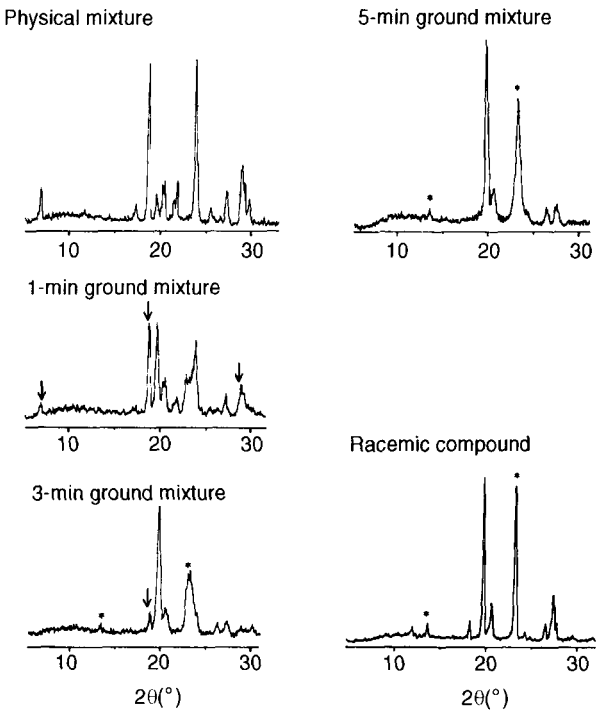


FIG. 1. Changes in X-ray powder diffraction pattern of the physical mixture of malic acid upon grinding (↓ enantiomer, * racemic compound).

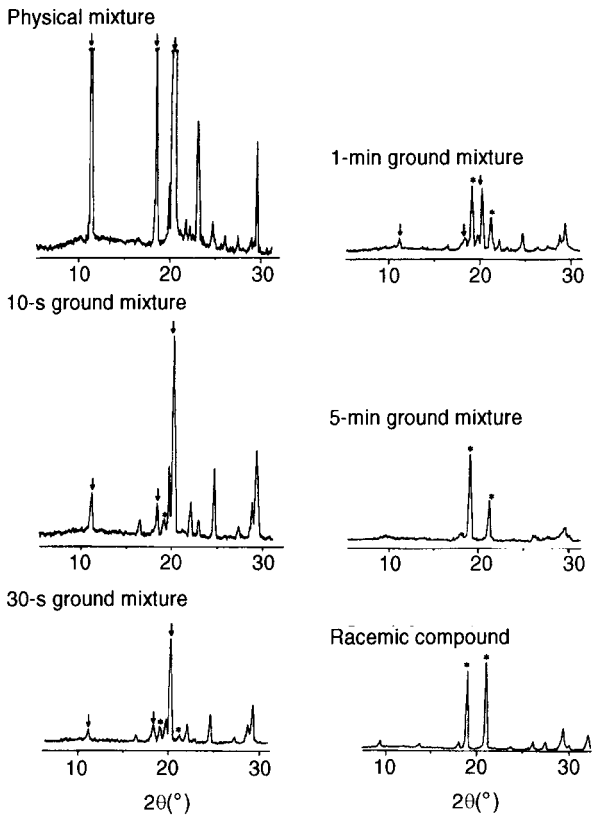


FIG. 2. Changes in X-ray powder diffraction pattern of the physical mixture of tartaric acid upon grinding (↓ enantiomer, * racemic compound).

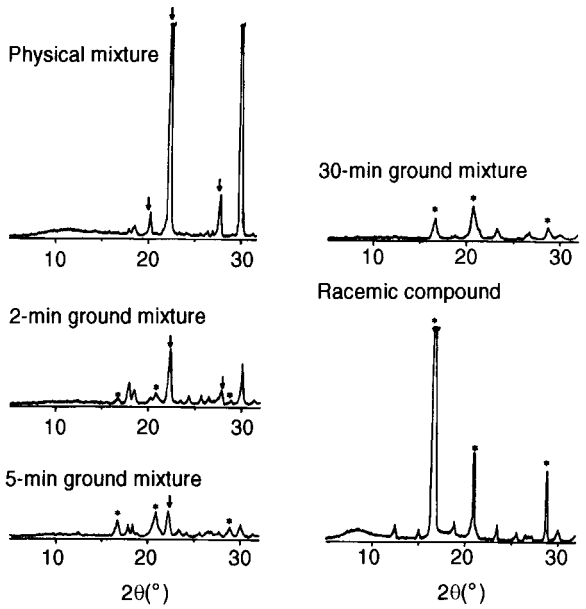


FIG. 3. Changes in X-ray powder diffraction pattern of the physical mixture of serine upon grinding (↓ enantiomer, * racemic compound).

eutectic, crystallization of the racemic compound and melting of the racemic compound, respectively. Upon grinding, the areas of peaks a and b decreased with grinding time. It was considered that conversion of the enantiomers into the racemic

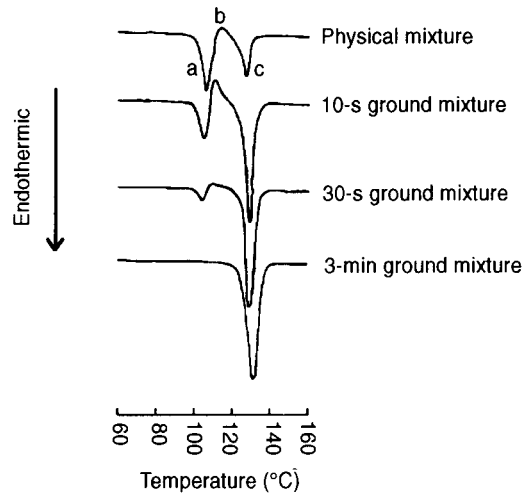


FIG. 4. Changes in DSC curve of the physical mixture of malic acid upon grinding. a. Melting of eutectic mixture, b. crystallization of racemic compound, c. melting of racemic compound.

compound progressed during grinding. After grinding for 3 min, peaks a and b disappeared and only peak c was observed, that is, no endothermic peak arising from the enantiomer was observed in the DSC curve, although X-ray diffraction still showed peaks arising from the enantiomers. This result could be explained by the transformation of some portions of the conglomerate to the racemic compound during DSC measurement. The tartaric acid system showed similar thermal behaviour. Consequently, the grinding process induced conversion of conglomerate to racemic compound crystals in the malic acid, tartaric acid and serine systems.

Fig. 5 shows the X-ray diffraction patterns of the physical mixture of malic acid after storage at 40°C under 31·5, 53·0 and 82·0 percent relative humidity for 3 days. Storage under 31·5 percent relative humidity caused no change in the X-ray diffraction pattern. Storage under 53·0 percent relative humidity, on the other hand, induced a decrease in the intensity of X-ray diffraction peaks arising from the enantiomers (indicated by arrows) and the growth of the X-ray diffraction peak arising from the racemic compound (indicated by an asterisk). Further, the sample stored under 82·0 percent relative humidity gave the same X-ray diffraction pattern as the racemic compound. Similar results were obtained for the physical mixtures of tartaric acid and serine. This suggested that humidification, as well as grinding, could bring about the transformation of conglomerates to racemic compounds in the malic acid, tartaric acid and serine systems. It was considered that in samples containing a sufficient amount of moisture as a result of storage at high humidity, the molecules had more mobility which enabled easier rearrangement to the racemic compound.

Valine, leucine and norleucine systems

The changes in the X-ray diffraction pattern arising as a result of grinding of the physical mixture of valine are shown in Fig. 6. The intensities of the diffraction peaks arising from the enantiomers were reduced. In contrast with the malic acid, tartaric acid and serine systems, no X-ray diffraction peaks from the racemic compound were observed. The ground

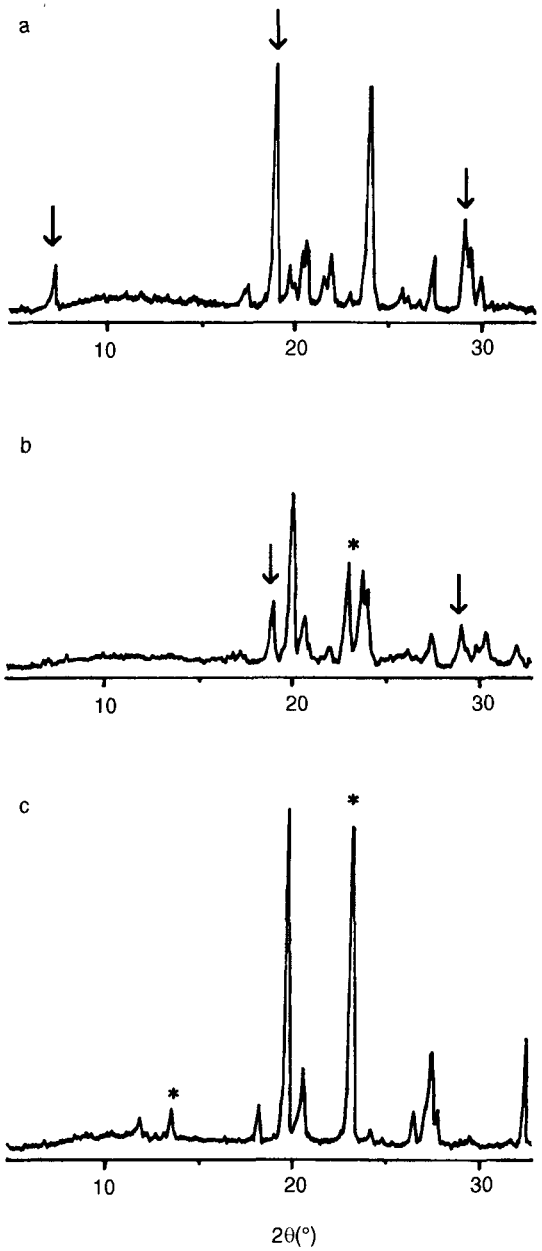


FIG. 5. Changes in X-ray powder diffraction pattern of the physical mixture of malic acid after storage at 40°C and relative humidity 31·5 (a), 53·0 (b) and 82·0 % (c) for 3 days (↓ enantiomer, * racemic compound).

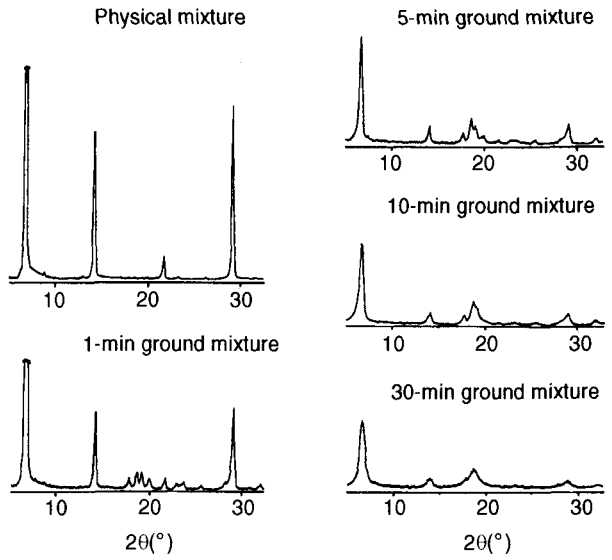


FIG. 6. Changes in X-ray powder diffraction pattern of the physical mixture of valine upon grinding.

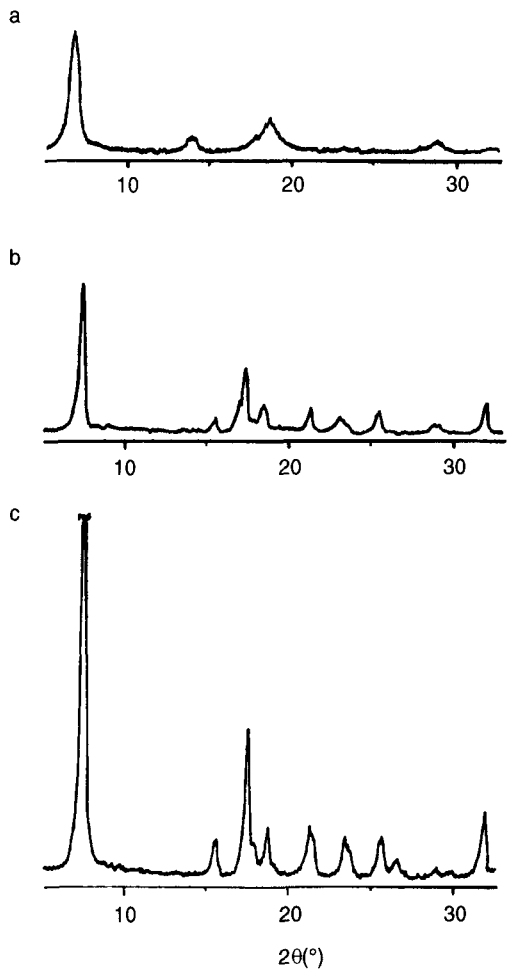


FIG. 7. X-ray powder diffraction patterns of valine. a. 30-min ground mixture of valine, b. 30-min ground mixture stored at 40°C under 82·0 % relative humidity for 5 days. c. DL-valine ground for 3 min.

mixture was then stored under 82 percent relative humidity at 40°C for 5 days.

Fig. 7 shows the changes in the diffraction pattern of the valine ground mixture after humidification (b), in comparison with that of the racemic compound after grinding for 3 min (c). After humidifying, the diffraction pattern was changed to that of the racemic compound, which was characterized by the diffraction peaks at 7·6°, 15·6° and 32·0°. On the other hand, even when the physical mixture of valine was humidified at 82 percent relative humidity at 40°C for 30 days, no change in powder X-ray diffraction pattern was observed.

To investigate the molecular state, IR spectroscopy was performed on the physical mixture and ground mixture of

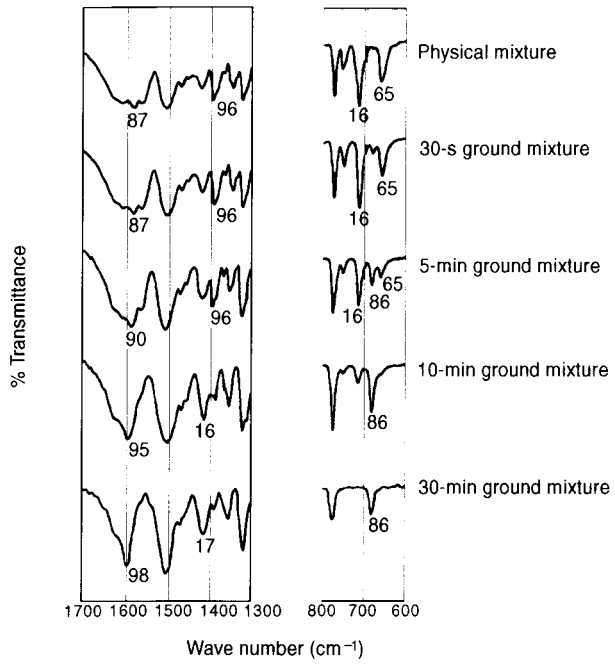
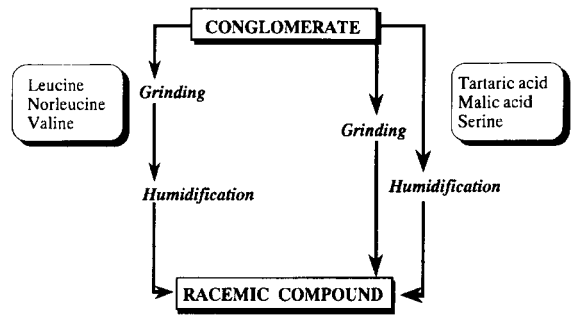


FIG. 8. Changes in the IR spectrum of the physical mixture of valine upon grinding.

valine. IR absorption bands corresponding to COO^- asymmetric and symmetric stretching were observed at approximately 1598 and 1417 cm^{-1}, respectively, for the racemic compound and 1587 and 1396 cm^{-1}, respectively, for the enantiomers. In addition, bands assigned as COO^- bending vibration were observed at 686 cm^{-1} for the racemic compound and at 716 and 665 cm^{-1} for the enantiomers. Fig. 8 shows the changes in the IR spectrum of the physical mixture upon grinding of the valine systems. The physical mixture gave IR bands at 1587, 1396, 716 and 665 cm^{-1}, similar to those of the enantiomers. With prolongation of the grinding time, IR bands of the COO^- stretching vibration shifted to higher wave numbers, and the intensities of the bands at 665 and 716 cm^{-1} decreased, accompanied by an increase in the intensity of the band at 686 cm^{-1}. After grinding for 30 min, strong IR bands were observed at 1598, 1417 and 686 cm^{-1} these were identical with those of the racemic compound.

IR measurement showed that the racemic compound was formed after grinding, although no peaks arising from crystals of the racemic compound were observed in X-ray powder diffraction pattern. We may say that grinding caused the formation of the racemic compound in the amorphous part of the ground mixture, and subsequent humidification induced crystallization of the racemic compound. Similar X-ray diffraction and IR results as a consequence of grinding and humidification were found for the leucine and norleucine systems.



SCHEME 1. Transformation of a conglomerate into a racemic compound.

These results are summarized in Scheme 1, which shows the effect of the processes of grinding and humidification on the transformation of the conglomerate to the racemic compound. For the malic acid, tartaric acid and serine systems either grinding or humidification induced the transformation whereas grinding then humidification was required for the transformation in the valine, leucine and norleucine systems. For comparison, the melting points and crystal structures of malic acid (Van der Sluis & Kroon 1984), tartaric acid (Stern & Beevers 1950; Okaya & Stemple 1966), serine (Shoemaker et al 1953; Benedetti et al 1972; Kistenmacher et al 1974), leucine (di Blasio et al 1975; Harding & Howieson 1976), norleucine (Torii & Iitaka 1973) and valine (Tsuboi et al 1959; Torii & Iitaka 1970) are presented in Table 1. Interestingly, crystals of the enantiomers of leucine, norleucine and valine were reported to contain hydrogen bonds to form a specific type of crystal structure –'a double-layer structure'. In contrast, crystals of the enantiomers of malic acid, tartaric acid and serine do not have such a specific structure. Owing to this difference, leucine, valine and norleucine crystals are considered to be stabilized and show remarkably higher melting points than those of malic acid, tartaric acid and serine. It can be concluded that because of the stability in the crystals, the transformation of leucine, valine and norleucine was not easy as that of malic acid, tartaric acid and serine.

Acknowledgements

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Table 1. Melting points and crystal structures of malic acid, tartaric acid, serine, leucine, norleucine and valine.

Compound	Melting point (°C)		Crystal structure	
	Enantiomer	Racemic compound	Enantiomer	Racemic compound
Malic acid	100–101	131–132	Planar	–
Tartaric acid	168–170	206	Planar	–
Serine	228 (dec)*	246 (dec)	TD†	TD
Leucine	293–295 (dec)	332 (dec)	HDL‡	HDL
Norleucine	301	327 (dec)	HDL	HDL
Valine	315	298 (dec)	HDL	HDL

*dec = decomposes. †Three-dimensional network of intermolecular hydrogen bonds. ‡Hydrogen-bonded double layers.

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