Mechanochemical Racemization of L-Leucine

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Synopsis On treating a ball-milled mixture of L-leucine and such diluents as silicone dioxide in hydrochloric acid, a part of L-leucine was transformed into D-leucine.

Racemization of optically active amino acids is of great interest with regard to the origin of optical activity. Mechanochemical racemization has not been reported yet, though several studies have been reported concerning racemization on γ-irradiation,[1] and acid-catalyzed[2,3] or base-catalyzed racemization of α-amino acids[4] under drastic conditions. It is known that little racemization occurs on treating a 6 M hydrochloric acid solution (1M=1 mol dm^{-3}) of α-amino acids at about 100 °C for 20 h.[3] In this paper, racemization of L-leucine on mechanochemical treatment was investigated.

A 10-g portion of a sample was inserted in a stainless-steel mill (in diameter of 10 cm and in capacity of 900 cm^3) containing ten stainless-steel balls (with a density of 8.2 g/cm^3 and in diameter of 2.5 cm) and thirty balls in diameter of 1.9 cm. The mill was rotated at a velocity of 120 min^{-1}. This procedure is called "ball-milling" hereafter. The ball-milled samples were L-leucine, silicone dioxide, talc, alminium oxide, titanium dioxide, microcrystalline cellulose (MCC), ethyl cellulose (EC), and a mixture of L-leucine and one of the other powders (diluents).

After a suspension of various samples containing L-leucine in a 6 M hydrochloric acid solution had been set aside for 30 min at room temperature, the suspension was centrifuged. The degree of optical rotation, $[\alpha]_D$, of the supernatant was measured with a Yanaco automatic polarimeter (Model OR-50).

N-(trifluoroacetyl)leucine isopropyl esters were derived as follows. Approximately 100 mg of leucine obtained by evaporating the supernatant was dissolved in 6.9 cm^3 of a saturated solution of hydrogen chloride in isopropyl alcohol and the solution was heated under reflux for 3 h. The solvent was removed under vacuum with a rotary evaporator. After washed by adding 5 cm^3 of dichloromethane and vacuum evaporating twice, the residue was dissolved in 2 cm^3 of dichloromethane and treated with 3 cm^3 of trifluoroacetic anhydride. The solution was heated under reflux for 15—30 min, and then the solvent was removed under vacuum. The residue was washed twice as before.[5] A dichloromethane solution of these derivatives was analysed by gas chromatography using a 30 m×0.24 mm fused-silica capillary column coated with OA-201 (optically active liquid of Shimadzu Seisakusho) as a stationary phase. This analysis was carried out at 110 °C with an inlet nitrogen pressure of 1 kg/cm^2. The IR spectra were measured by the use of Nujol method with a JASCO diffraction granting infrared spectrophotometer (Model IRA-2).

Results and Discussion

L-Leucine was ball-milled with diluents and treated in hydrochloric acid. The $[\alpha]_D$ value of the supernatant decreased by ball-milling with diluents, though the values for L-leucine ball-milled for 60 h without any diluent (b-L-leucine) or a mixture of b-L-leucine and a diluent ball-milled without leucine (b-diluent) were nearly identical with the value for original L-leucine.

N-(Trifluoroacetyl)leucine isopropyl esters, derived from leucine[5] obtained by evaporating the supernatant, were analyzed by gas chromatography. Hatano *et al.* found that keto acids and ammonia were formed on γ-ray irradiation in aqueous solutions of amino acids or oxidation of them in alkaline solutions.[6] On the other hand, Arakawa *et al.* showed that, on oxidation of amino acids in acidic or neutral solutions, both deamiation and decarboxylation occurred with subsequent formation of the coresponding aldehydes.[7] A large amount of bubbles were generated on treating the mixtures of L-leucine and diluents in hydrochloric acid; from this fact, decarboxylation is suggested to occur. These facts suggest that the peak "P" in Fig. 1 is due to 3-methylbutanal formed by deamination and decarboxylation of leucine. A peak of D-leucine was observed for the ball-milled mixture with talc, silicone dioxide, alminium oxide or titanium dioxide. In contrast, no peak of D-leucine was observed for b-L-leucine, a mixture of b-L-leucine and a b-diluent, or a ball-milled mixture with MCC or EC. Racemization percentage shown in Fig. 2 is twice the percentage of D-leucine in all the leucine, obtained from the peak areas of D- and L-leucine in the chromatogram. After keeping the mixture for some days in a stoppered bottle at room temperature after the end of ball-milling, the racemization percentage was determined. As is shown in Fig. 2, racemization percentage decreased gradually with the lapse of the time after the end of ball-milling. These results show that racemization of L-leucine takes place while the surface of the diluent in the mixture is active.

Figure 3 shows the effect of ball-milling with talc on IR spectra of L-leucine. The spectra of the mixtures of L-leucine and talc were nearly identical with those of

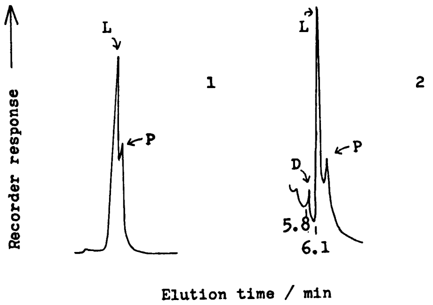

Fig. 1. Gas chromatogram of leucine derivatives. Sample 1: L-leucine, 2: a mixture with silicone dioxide ball-milled together for 18 h. (content of L-leucine; 10 w/w %). P; A peak of a degradation product.

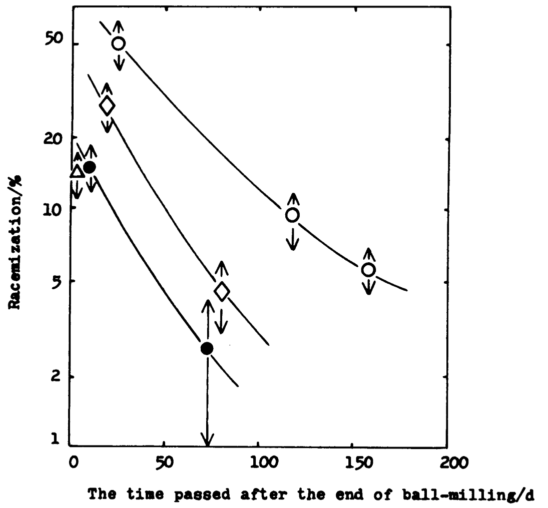

Fig. 2. Decrease in racemization percentage with the lapse of the time after the end of ball-milling.

Ball-milling conditions (content of L-leucine; 10 w/w %).

	diluent	ball-milling time/h
○	talc	60
◇	silicone dioxide	18
●	titanium dioxide	18
△	alminium oxide	3

the mixtures of b-L-leucine and b-talc. It is considered from this fact that the changes in the range of 1600—1640 cm^{-1} and 3000—3400 cm^{-1} regions are not due to the adsorption of water. In this case the effect was as follows; (1) the absorbance at 3000—3400 cm^{-1} region (assigned to the NH$_2$ stretching vibration) increased, (2) the absorbance at 1600—1640 cm^{-1} region (assigned to the COO$^-$ asymmetric stretching vibration) increased, and (3) the band at 1410 cm^{-1} (assigned to the COO$^-$ symmetric stretching vibration) and the band at 1510 cm^{-1} (assigned to the NH$_3^+$ symmetric stretching vibration) disappeared. The similar effect was also observed upon ball-milling with silicone dioxide, MCC, or EC. Jackovitz and Walter reported an analogous change in the IR spectra of leucine on complexation with such metals as palladium. They considered that leucine in the metal complex was in the state of $NH_2CH(R)COO^-$ ($R=(CH_3)_2CHCH_2-$) where N and O binded with metals,8) though leucine is in the state of $NH_3^+CH(R)COO^-$ in crystal powders.9) These facts suggest that leucine in the ball-milled mixture with talc or silicone dioxide is also in the state of $NH_2CH(R)COO^-$ where N and O interact with these diluents. According to Jackovitz and Walter, the band in the range of 1550—1600 cm^{-1} due to the NH$_2$ scissors vibration is at a higher frequency, when the N-metal bond is stronger. The band in the range of 1570—1640 cm^{-1} due to the COO$^-$ asymmetric stretching vibration is also at a higher frequency, when the O-metal bond is stronger.8) The IR spectra suggested that the interaction force of L-leucine with talc in the ball-milled mixture was stronger than the force with silicone dioxide. A remarkable change was not observed in the spectra of the mixture with titanium dioxide or alminium oxide. This result shows that leucine in the ball-milled mixture with these diluents is in the state of $NH_3^+CH(R)COO^-$.

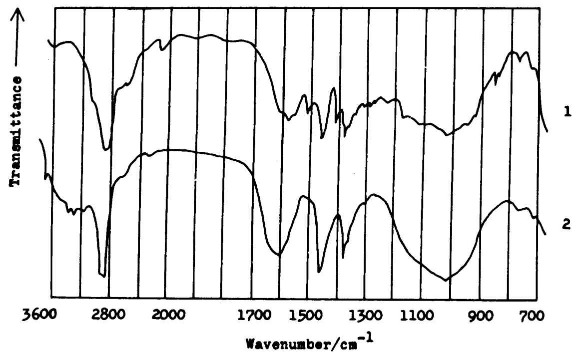

Fig. 3. Influence of ball-milling on IR spectra of the mixtures of L-leucine with talc. Content of L-leucine; 30 w/w %, ball-milling time 1: 0 h, 2: 60 h.

Bonner *et al.* found an approximately linear relationship between the extent of racemization and that of degradation upon γ-irradiation for the aqueous sodium salts of leucine.1) The $[\alpha]_D$ values for the samples within 25 d from the end of ball-milling were nearly identical, and it is not considered from this fact that the degradation percentage for the mixture with talc or silicone dioxide is larger than the percentages for the other mixtures. The racemization percentages within 25 d from the end of ball-milling shown in Fig. 2 are large when leucine is in the state of $NH_2CH(R)COO^-$. It is also shown that racemization of L-leucine takes place more easily when the interaction force with a diluent is stronger. Bonner *et al.* irradiated γ-ray on aqueous solutions of sodium salts or hydrochloride salts of amino acids and found that $NH_2CH(R)COO^-$ suffered 5—11% of racemization and that $NH_3^+CH(R)COOH$ racemized little.1)

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